Handbook of Analytical Instruments

Third Edition

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PREFACE TO THE THIRD EDITION

I am delighted to place before you the third and enlarged edition of my popular book *Handbook of Analytical Instruments*. This edition is totally revised and updated based on the technical advances which have penetrated the field of analytical sciences during the last decade. Each chapter has been revisited, obsolete material deleted and latest material based on new developments included at various places, so that the reader gets the best from the new edition.

Three new chapters have been added to the book. For observing and analysing the surface micro-structure of a sample, very sophisticated imaging instruments have become available. These instruments enable the users to look into and work at atomic level. The new topics which have been included as separate chapters are: Scanning Electron Microscope and Scanning Probe Microscope (Scanning Tunneling Microscope and Atomic Force Microscope). Particle size analysers play an important role in research and development and for quality control in many industries such as pharmaceuticals, ceramics, cement, paints and emulsions etc. Realizing the need of the users in these areas, a new chapter on particle size analysers and counters has been included.

Several new topics have been incorporated in the revised text, some of which are:

- Brief introduction to the use of MEMS in analytical instrumentation, leading to Lab-on-a-Chip technology.
- Rising trends in portable spectrophotometers using fibre optics.
- Use of Fourier transform with conventional instrumentation such as Infrared Spectroscopy, Raman Spectrometry, NMR Spectrometry, Mass Spectrometry etc.
- Neutron Activation Analysis Technique.
- Liquid Chromatography-Mass Spectrometry.
- Flow injection automated analysis system, semi-automated clinical chemistry analyser and point of care instrumentation.
- Thermo-mechanical analysis instrumentation.
- Capillary electrophoresis instrumentation including DNA sequencer.
- Integrated chips for modern pH metres.
- Process gas analysers including online quantitative analysis of industrial gases.
- Various levels of power supplies.
- Embedded systems and digital signal processors.
- Popular software packages which find applications in analytical instrumentation, interfacing techniques, computer networking and smart laboratory concepts.

While the basic principles of instrumentation and techniques are generated by researchers, their practical utilisation gets established when they are commercially produced by various manufacturers. Therefore, it is more pertinent for the users to understand the way an instrument is designed, the placement of the various subsystems inside it and the precautions needed during its operation. Inclusion of information from the manufacturers on the typical instruments thus, indicates the practical aspects of the technology utilised in the instruments. It is for this reason that efforts have

been made to include information on some of the popular commercially available instruments. It, however, does not imply that the instrument described is superior to the competing ones. The motive is to illustrate the instruments typical of their class or possessing features of special interest for intended applications.

A distinctive feature of the third edition is the improved visual impact of the illustrations and photographs of latest commercial equipment. The list of references has been greatly expanded in the book, which will be found useful by those who would like to know more about the latest research in the field.

The book continues to enjoy an enviable position in the field of analytical instrumentation both in India and abroad for which I feel obliged to the students and the teachers for patronising the book. I, on my part, have endeavoured to make the text more lucid and illustrations more meaningful.

I thank the readers once again for sending me the feedback and suggestions, formally and informally, which I have tried to incorporate in the revised edition. I do hope that the present book would be found even more useful to students and professionals in the field of analytical instrumentation.

I am grateful to my wife Ramesh Khandpur for constant encouragement and support during the period the book has been under revision. When the first edition of this book was published in 1989, my children Vimal, Gurdial and Popila were all studying. By the time the second edition was out in 2006, they were all married, well employed and had their own children. Today, my two granddaughters Ravleen and Harsheen are in prestigious professional colleges, making me proud of their achievements. The other three grandchildren Manmeet, Aashna and Gurtej, all millennium children born in the year 2000, are school going. Each one of them has been getting motivated with my keen interest in their studies and I, in turn with their inquisitive enquiries about what I do on my computer. Thanks are also due to McGraw Hill Education (India) Pvt. Ltd. for bringing out an excellent publication.

R S Khandpur

PREFACE TO THE FIRST EDITION

Instruments used for analysis constitute the largest number of instruments in use today. Their range is spectacular and variety baffling. It is difficult to imagine a field of activity where analytical instruments are not required and used. They are used in hospitals for routine clinical analysis, drugs and pharmaceutical laboratories, oil refineries, food processing laboratories and above all for environmental pollution monitoring and control. This book has been designed to cater to the needs of a wide variety of readers working in these areas. The postgraduate students of chemistry and physics undergoing courses on instrumental analysis will find the book a useful text. It is also intended as a guide for the scientific investigator who wishes to acquire knowledge about the more widely used analytical instruments. The availability of a bewildering array of instrumental techniques and a large variety of commercially available equipment have provided several courses of action to the investigator. The student who undergoes this course will be in a position to select instruments for a particular problem with some idea of their merits, demerits and limitations. The treatment of the subject is designed to be sufficient to give the reader the necessary background to fruitfully discuss the more esoteric details of an instrument with an expert in this area.

With the widespread use of analytical instruments, it has now become essential to have qualified and sufficiently knowledgeable service and maintenance engineers. Besides having a basic knowledge of the principle of operation, it is important for them to know the details of commercial instruments from different manufacturers. A concise description of instruments of each type from leading manufacturers has, therefore, been provided. Since rapid changes and improvements in instruments usually make some of the description obsolete, an attempt has been made to describe the principles that are basic to the various types of analytical instruments. The principles learned would enable the service engineers to carry over to new equipment as it appears in the market.

The area of analytical instrumentation involves a multidisciplinary approach, with electronics and optics forming the major disciplines. Highly populated printed circuit boards often have application-specific integrated circuits mounted on them. This has necessitated a new approach to repair and servicing involving replacement of the printed circuit card without having to repair the instrument at the component level. Work of this nature requires a knowledge of the various building blocks of an instrument, and this has been the approach in the preparation of the text. This approach will also be advantageous to students of chemistry, physics, chemical engineering, instrumentation and electronics, etc.

The book starts with an explanation of basic concepts in electronics and optics, with special reference to operational amplifiers, digital integrated circuits and microprocessors. The chapter also covers various types of display systems and laboratory recorders. The information in this chapter would serve as a base for the description in the rest of the book.

The modern laboratory has a large number of analytical instruments churning out information. Electronic procedures for handling the huge amount of data have become imperative. The marriage of computers and instruments has offered tremendous possibilities of easing the burden on the scientists, as well as optimising the performance of analytical instruments. Many of the leading

instrument manufacturers are producing systems for use in the laboratory, both for data acquisition and for control purposes. The personal computer has been virtually responsible for a revolution in the methodology, quantity, economy and quality of experiments performed. Taking into consideration the impact of computers on analytic instruments, the topic is covered in the second chapter itself.

Colorimetry and spectrophotometry have become the central techniques of analytical chemistry. Spectrophotometry, particularly in the ultraviolet region, offers a sensitive, precise and non-destructive method of analysis of biologically important substances such as proteins and nucleic acids. The introduction of spectrophotometers, especially in the visible region of the spectrum, led directly to the explosion of analytical methodology, characterised by the use of an ever decreasing amount of sample size and multi-sample analytical methods. The use of the double-beam principle helped in the development of infrared spectroscopy, where direct recording of spectra is now routine. Similarly, flame photometers and atomic absorption spectrophotometers have become almost indispensable tools in clinical and research laboratories. These topics, along with fluorimeters and phosphorimeters, photoacoustic and photothermal spectrometers, mass spectrometers, and Raman spectrometers, are covered in the next part of the book.

The most important spectroscopic techniques for structural determination are nuclear magnetic resonance and electron spin resonance spectrometry. These topics are covered in two chapters. Fourier Transform NMR and spin decouplers are also concisely dealt with. A separate chapter on electron and ion spectroscopy, important analytical tools for surface analysis, has been included.

Qualitative and quantitative analyses have been greatly facilitated by the increasing availability of isotopically labelled compounds. The measurement of these has required the development of increasingly complex equipment, usually capable of measuring and printing out the radioactive counts from 100 or more samples wholly automatically. Multichannel counters, scanners and gamma cameras are representative of the highly sophisticated equipment and richly deserve their place in the book. X-ray spectrometers based on diffraction, absorption and fluorescence are also covered in detail. Analysis procedures have been automated, with the result that a very large number of samples can be handled per hour for a multiplicity of tests. Systems like auto-analysers have brought about tremendous procedural and conceptual innovations. A complete description of the automated analysis systems has been provided in a separate chapter.

Chromatography offers a unique method of separation of closely similar substances. Various methods like liquid-liquid partition, gas-liquid partition, ion-exchange, molecular sieve, etc. have all provided a basis for the same. Gas-liquid chromatography is perhaps the most widely used than any other single analytical method for small- to medium-sized molecules. However, a liquid mobile phase possesses certain advantages over a gaseous mobile phase since it can contribute to the separation achieved through the specificity of its interaction with solutes. This advantage has resulted in the development of high-pressure, high-performance liquid chromatography, using specialised column packing of very small size. Two chapters are devoted to the different types of chromatograph instruments. Perhaps the most sophisticated ultra-micro-scale analytical instrument is the combination of the gas chromatograph with the mass spectrometer, and the same has been well illustrated in the chapter on mass spectrometry. A separate chapter on thermo-analytical methods gives a brief introduction to these techniques.

Electrophoresis, as a medium-scale method for the separation of proteins and its development into an ultra-micro method with the introduction of zone electrophoresis, is covered in the next

chapter. The continuous improvements in staining and optical visualisation methods have led to this technique being applicable to the microgram level or even less. The chapter also covers spectrodensitometers including those based on microprocessors.

Electrochemical instruments, based on a variety of principles of operation, have undergone considerable improvements in terms of accuracy, speed and automation. While the chapter on electrochemical instruments covers various types of instruments like conductivity metres and polarographs, the chapter on pH metres includes the latest in electronic circuitry, ion-selective electrodes and bio-sensors. An exhaustive treatment has been given to blood gas analysers and industrial gas analysers.

Awareness and concern about the deteriorating environment is increasing the world over. It is necessary to monitor changes taking place in the quality of the environment for initiating efforts to accomplish environmental pollution control. Many of the analytical tools used elsewhere in other areas of applications could be profitably utilised for air and water pollution monitoring purposes. However, a special chapter on this subject illustrates specific techniques employed for monitoring different pollutants in air and water.

This book has a sufficient degree of comprehensiveness and depth to give the reader the most important information without having to delve in more specialised books on the subject. I hope that the material in the book would be found useful by a large number of readers working in different disciplines.

I would also like to add that mention of the products of industrial manufacturers does not necessarily imply that I consider them superior to competing items. The aim is to describe instruments typical of their class, possessing some special features of interest as an illustration of the indicated principles and intended application, rather than writing a catalog of analytical equipment.

I am indebted to the Director, CSIO, for his kind permission to publish the book and to Tata McGraw-Hill Publishing Company Limited for excellent editing and printing. I am thankful to TAB Books, USA, for their kind permission to reproduce some parts of my earlier book published by them. I am also grateful to my wife Mrs Ramesh Khandpur and children Vimal, Gurdial and Popila for the help they extended me during preparation of the manuscript and proofreading.

R S Khandpur

1

FUNDAMENTALS OF ANALYTICAL INSTRUMENTS

1.1 TYPES OF CHEMICAL ANALYSIS

Basically, chemical analysis can be divided into four broad categories as given below, which are generally applied in chemical laboratories:

Qualitative Analysis: Chemical analysis which just identifies one or more species present in a sample.

Quantitative Analysis: Chemical analysis which finds out the total amount of the particular species present in a sample.

Structural Analysis: Chemical analysis which helps in finding the spatial arrangement of atoms in a molecule and the presence or position of certain organic functional groups in a given compound.

Surface Analysis: Analysis which helps to obtain information regarding surface-related physical properties such as topography, depth profiling, orientation of molecules, etc.

To carry out various types of analysis, there is a need for sophisticated analytical instruments. It has been a vast expanding area of knowledge as the instrument and computer manufacturers are producing analytical machines, which are providing an ever-increasing power and scope. Consequently, all the manual techniques in the field of the analytical studies had steadily been transferred to the instrumental techniques.

In order to effectively and efficiently carry out the analysis task, some basic knowledge of instruments and analytical techniques is required. This may give the person the ability, with some confidence, to choose and operate a varied range of instruments which would be required as a routine in the chemical laboratories.

1.2 ELEMENTS OF AN ANALYTICAL INSTRUMENT

Analytical instruments are used to provide information about the composition of a sample of matter. They are employed, in some instances, to obtain qualitative information about the presence or absence of one or more components of the sample, whereas in other instances quantitative data are sought from them. In the broadest sense, any analytical instrument (Figure 1.1) consists the following four basic units:

Chemical information source, which generates a set of signals containing necessary information. The signal may be generated from the sample itself. For example, the yellow radiation emitted by heated sodium atoms constitutes the source of the signal in a flame photometer.

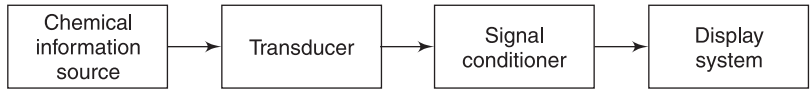


Figure 1.1 *Elements of an analytical instrument*

Transducer, which converts the signal to one of a different nature. Because of the familiar advantages of electric and electronic methods of measurement, it is the usual practice to convert into electrical quantities all non-electrical phenomena associated with the analysis of a sample. For example, a photocell and a photomultiplier tube are transducers that convert radiant energy into electrical signals.

Signal conditioner, that converts the output of the transducer into an electrical quantity suitable for operation of the display system. Signal conditioners may vary in complexity from a simple resistance network or impedance-matching device to multi-stage amplifiers and other complex electronic circuitry. They help in increasing the sensitivity of instruments by amplification of the original signal or its transduced form.

Display system, which provides a visible representation of the quantity as a displacement on a scale, or on the chart of a recorder, or on the screen of a cathode ray tube or in numerical form.

Thus, the instrument can be considered in terms of flow of information, where operation of all parts is essentially simultaneous.

The first two blocks constitute the *characteristic* module, whereas the last two blocks form the *processing* module of an instrument (Strobel, 1984 a). The characteristic module in a pH metre consists of the glass membrane pH electrode and reference electrode immersed in the cell solution. Similarly, constituents of the characteristic module in an UV-Vis spectrophotometer are a source of radiant energy, a monochromator, the sample holder and photodetector. Each one of the components of the characteristic module contributes to the performance specifications of an instrument. For example, by choosing a photomultiplier tube with the broadest spectral response available, good sensitivity of detection is ensured from 190 nm to beyond 800 nm. The higher its gain and the lower its noise, the better the possibility of working at trace concentration levels. The transducer also determines the limit of detection of measurement. Similarly, the monochromator fixes the resolution, signal-to-noise ratio and level of stray light.

The signal amplitude is produced by a transducer is processed in the processing module. After adequate amplification, the processing can be carried out with the signal still in analog form (a signal of varying amplitude) or to convert it to digital form (a series of pulses whose number indicates the signal amplitude) by use of an analog-to-digital (A/D converter). This conversion ordinarily gives higher precision and accommodates use of a microprocessor or computer for processing steps and instrument control.

The results of a measurement in analytical instruments are usually displayed either on analog metres or digital displays. Digital displays present the values of the measured quantities in numerical form. Instruments with such a facility are directly readable and slight changes in the parameter being measured are easily discernible in such displays, as compared to their analog counterparts. Because of their higher resolution, accuracy and ruggedness, they are preferred for display over conventional analog moving coil indicating metres. Different types of devices such as light emitting diodes (LEDs) and liquid crystal displays (LCDs) are available for display in the digital or numerical form.

Since computers are used increasingly to control the equipment and to implement the man-machine interface, there is a growing appearance of high resolution colour graphic screens to display the course of analytical variables, laboratory values, machine settings or the results of image processing methods The analog and digital displays have been largely replaced by video display units, which present information not only as a list of numbers but as elegant character and graphic displays and sometimes as a 3-Dimensional colour display. Visual display units (VDUs) are usually monochrome as the CRTs in these units are coated with either white or green phosphors. Coloured video display units are also becoming popular.

A key board is the most common device connected into almost all form of data acquisition, processing and controlling functions in analytical instruments. A key board can be as simple as a numeric pad with function keys, as in a calculator or complete alphanumeric and type writer key board with associated group of control keys suitable for computer data entry equipment. Most available keyboards have single contact switches, which are followed by an encoder to convert the key closures into American Standard Code for Information Interchange (ASCII) code for interfacing with the microprocessor.

All analytical instruments can thus be split (Strobel 1984b) into the above indicated four subsystems. Some examples of the instruments along with their sub-systems are given in Table 1.1

The progress in instrumental methods of analysis has closely paralleled developments in the field of electronics, because the generation, transduction, amplification and display of signals can be conveniently accomplished with electronic circuitry. All electronic circuits are constructed with the help of some basic components and circuit blocks, which are broadly described in Chapters 29 and 30 of this book.

Table 1.1 *Examples of instrument sub-systems*

Instrument	Characteristic Module		Processing Module	
	Analytical Signal	**Input Transducer and Output**	**Signal Processor**	**Readout**
Colour comparator	Colour	Eye (Optic nerve signal)	Brain	Visual colour response
Photometer	Attenuated light beam	Photodetector (Electrical current)	Amplifier	Analog/digital display
Atomic emission spectrometer	UV or visible radiation	Photomultiplier tube (Electrical current)	Amplifier A/D converter	Analog/digital display, chart recorder
Coulometer	Cell current	Electrodes (Electrical current)	Amplifier	Chart recorder
pH metre	Hydrogen ion activity	Glass-calomel electrodes (Electrical potential)	Amplifier, A/D converter	Digital display
X-Ray powder diffractometer	Diffracted x-radiation	Photographic film (Latent image)	Chemical developer	Black images on film

1.3 SENSORS AND TRANSDUCERS

Transducers are the devices, which convert one form of energy into another. Numerous methods have since been developed for this purpose and basic principles of physics have extensively been employed. Variation in electric circuit parameters like resistance, capacitance and inductance in accordance with the events to be measured, is the simplest of such methods. Piezoelectric and photoelectric transducers are also very common. Chemical events are detected by measurement of current flow through the electrolyte or by the potential changes developed across the membrane electrodes. A number of factors decide the choice of a particular transducer to be used for the study of a specific phenomenon, which are given below:

- The magnitude of quantity to be measured
- The order of accuracy required
- The static or dynamic character of the process to be studied
- The type of application in vitro or in vivo
- Economic considerations

1.3.1 Classification of Transducers

Many physical, chemical and optical properties and principles can be applied to construct transducers for applications in the analytical field. The transducers can be classified in many ways, as given below:

(i) By the process used to convert the signal energy into an electrical signal. For this, transducers can be categorised as:

Active Transducers – A transducer that converts one form of energy directly into another (e.g. photovoltaic cell in which light energy is converted into electrical energy).

Passive Transducers – a transducer that requires energy to be put into it in order to translate changes due to the parameter under measurement. They utilise the principle of controlling a DC excitation voltage or an AC carrier signal. For example, a variable resistance (e.g. a thermistor) placed in a Wheatstone bridge in which the voltage at the output of the circuit reflects the physical variable (temperature). Here, the actual transducer is a passive circuit element but requires to be powered by an AC or DC excitation signal.

(ii) By the physical or chemical principles used (e.g. variable resistance devices, Hall effect devices and optical fibre transducers).

(iii) By the application for measuring a specific analyte variable (e.g. flow transducers, pressure transducers, temperature transducers, etc.).

1.3.2 Performance Characteristics of Transducers

A transducer is normally placed at the input of a measurement system, and therefore, its characteristics play an important role in determining the performance of the system. Ideally, a transducer should have a high-level output, zero source impedance, low noise and be relatively linear; however, they are not. Some of the important parameters which characterise the transducers are as follows:

- Input and output impedance
- Common mode rejection ratio
- Overload range

- Recovery time after overload
- Excitation voltage
- Shelf life
- Reliability
- Size and weight
- Response time: to a step change in the input (measured) and includes rise time, decay time and time constant.

However, manufacturers typically specify detectors by the noise-equivalent power (NEP), detectivity (D), responsivity (R) and time constant (t). These factors are described below.

Noise Equivalent Power (NEP) is the root mean square (rms) value of sinusoidally modulated radiant power falling upon a detector that gives an rms signal voltage equal to the rms noise voltage from the detector.

Detectivity is defined as the inverse of NEP.

Responsivity of a detector specifies its response to unit irradiance.

Time constant of a detector is the measure of a detectors ability to respond to a rising or falling optical signal.

A majority of the transducers used in analytical instrumentation are analog devices. They measure continuous physical or chemical properties and normally give continuous electrical outputs. For example, changes in ion activity in a solution are measured with specific ion electrode (transducer) whose output is available in terms of changes in voltages. Similarly, a polarographic cell measures concentration of electroactive species and gives current as the output.

The three terms, viz, detectors, transducers and sensors are often used interchangeably. However, there is a fine difference in their definition and scope, which is explained below:

Detector: This is a device that indicates change in environment (e.g. a smoke detector or gas detectors).

Transducer: This is a device that converts one form of energy to another, specifically from non-electrical to electrical data (e.g. a thermocouple which converts temperature into voltage).

Sensor: This is a device that converts chemical to electrical data (e.g. a biosensor which converts biological activity to an electrical signal).

The action of transducers is based on some property of the analyte on the basis of which the measurements are done and instruments are constructed. Some of the properties of the analytes and the techniques employed are given in Table 1.2.

The choice of an instrumental method and the equipment to be used for the same would depend upon the following performance requirements:

Accuracy:	How close to the true value?
Precision:	How reproducible?
Sensitivity:	How small a difference can be measured?
Selectivity:	How much interference?
Dynamic Range:	What range of amounts?

Section 1.11 details the performance requirements of instruments and the methods employed for analysis purposes.

Table 1.2 *Property of analytes and techniques used in analytical instruments*

Category	Property	Technique Used
Radiation	Radiation absorption	Absorption spectroscopy – Photometry – Spectrophotometry – Nuclear magnetic resonance – Electron spin resonance
	Radiation emission	Emission spectroscopy – Fluorescence – Phosphorescence – Luminescence
	Radiation scattering	– Raman spectrometry – Turbidity measurement
	Radiation refraction	– Refractometry – Interferometry
	Radiation diffraction	– X-ray spectroscopy – Electron spectroscopy
	Radioactivity	– Activation analysis – Isotope dilution
Electrical	Electrical potential	Potentiometry
	Electrical current	Amperometry
	Electrical charge	Polarography
	Electrical resistance	– Coulometry – Conductometry
Mass	Mass	Gravimetry
	Mass-to-charge ratio	Mass spectrometry
Thermal	Temperature change	– Thermal gravimetry – Calorimetry

1.3.3 Smart Sensors

Any analytical measurement primarily involves two steps: the *reaction step* with which involves sampling, sample transport and processing, separation and reaction. The *measurement step* involving transduction, signal acquisition and processing. An area of intense research and study at present is as to how these two steps can be combined to get integrated analytical systems. This is possible by having a *chemical sensor* having two integrated parts which are:

- Receptor, which is a highly selective recognition material.
- Transducer, which is a material that converts the recognition signal (be it optical, electrochemical, mass or thermal) into a signal, usually electrical in nature.

The recent designs of such chemical sensors which combine recognition and transduction have led to the development of new analytical instrumentation resulting in simplification, miniaturisation, robustness, speed, mobility and cost (Alegret, 2003).

The working of these chemical sensors depends to a large extent on the availability of highly selective recognition materials, which are of the following two types:

- Synthetic materials such as a cyclic or macro-cyclic ligands, cyclodextrines, molecularly imprinted polymers.
- Natural compounds such as enzymes, micro organisms, animal or plant tissues, antibodies, DNA, etc.

The chemical sensors that integrate synthetic recognition materials are known as *chemosensors* and when they integrate biomaterials are known as *Biosensors* (Diamond, 1998). Figure 1.2 shows the constituent elements of a biosensor.

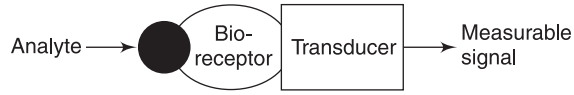


Figure 1.2 *Principle of a biosensor*

In most cases, sensors are used to detect a single analyte. By integrating several sensing devices within the same platform, it is possible to have an array of micro-fabricated sensors with different selectivity for multi-component analysis simultaneously. For example, gas-sensor arrays enable to measure concentrations of different gases in multi-component gas samples.

Rapid and continuous developments in the micro-electronics industry have resulted in their influence in different fields such as chemistry and life sciences. The objective is to have the common laboratory instruments, devices and machines down to a global size of a credit card. Examples of current research in this area are for the development of micro-sensors and micro-electromechanical systems (MEMS). These are integrated devices that combine both electrical and mechanical components, within a micro-meter dimension of fabricated in glass, quartz or plastic (Figeys and Pinto, 2000).

The integration of fluidic micro-structures with other analytical micro-structures such as micro-reactors, micro-sensors and micro-actuators, has led to the development of 'Lab-on-chip' on a commercial scale. The technology includes any type of laboratory operation related to liquid handling, chemistry reactor technology and biotechnology, amplification, etc. The main advantages of these chips (micro-laboratories) is improved analytical performance in respect of higher throughputs, increased mechanical stability and reduced resource consumption (Manz and Becker, 1998).

The sensors which have a tight coupling between sensing and computing elements are also known as smart sensors. Their characteristics would normally include: temperature compensation, calibration, amplification, some level of decision making capability, self-diagnostic and testing capability and the ability to communicate interactively with external digital circuitry. Currently available smart sensors are actually hybrid assemblies of semiconductor sensors plus other semiconductor devices. In some cases, the coupling between the sensor and computing element is at the chip level on a single piece of silicon in what is referred to as an integrated smart sensor. In other cases, the term is applied at the system level. The important roles of smart sensors are as follows:

Signal Conditioning: The smart sensor serves to convert from a time-dependent analog variable to a digital output. Functions such as linearisation, temperature compensation and signal processing are included in the package.

Tightening Feedback Loops: Communication delays can cause problems for systems that rely on feedback or that must react/adapt to their environment. By reducing the distance between sensor and processor, smart sensors bring about significant advantages to these types of applications.

Monitoring/Diagnosis: Smart sensors that incorporate pattern recognition and statistical techniques can be used to provide data reduction, change detection and compilation of information for monitoring and control purposes.

Smart sensors divert much of the signal processing workload away from the general purpose computers. They offer a reduction in overall package size and improved reliability, both of which are critical for in situ and sample return applications. Achieving a smart sensor depends on integrating the technical resources necessary to design the sensor and the circuitry, developing a manufacturable process and choosing the right technology. A typical example of a smart sensor is the ion-sensitive field-effect transistor.

1.4 SIGNAL PROCESSING IN ANALYTICAL INSTRUMENTS

The information from the transducer is often obtained in terms of current intensity, voltage level, frequency or signal phase relative to a standard. Voltage measurements are the easiest to make, as the signal from the transducer can be directly applied to an amplifier having a high input-impedance. Usually, the input device in the amplifiers in modern equipment is FET or MOSFET. The voltage signal source can be connected directly to a sensitive current-measuring device (moving coil metre), but this is usually not done, because of inadequate sensitivity and low input-impedance of the metre.

Most of the transducers produce signal in terms of current, which in many cases, is too small and direct connection between the transducer and the metre is difficult. However, some of the earlier colorimeters and flame photometers used this arrangement. In modern instruments, the most widely used current-measuring arrangement is that of placing a resistor in series with the current source and measuring the voltage drop across it, which is subsequently measured by observing the current that it will drive into an amplifier. When impedance-matching problems preclude the use of dropping resistors, low input-impedance current-to-voltage conversion can be achieved by using operational amplifiers with appropriate feedback.

In order to make an accurate measurement of voltage, it is necessary to ensure that the input impedance of the measuring device is large as compared to the output impedance of the signal source. This is done to minimise the error that would occur, if an appreciable fraction of the signal source were dropped across the source impedance. Conversely, the need for an accurate measurement of current source signals necessitates that the source output impedance to be larger than the receiver input-impedance. Ideally, a receiver that exhibits a zero input-impedance would not cause any perturbation of the current source. Therefore, high-impedance current sources are more easily handled than low-impedance current sources.

In general, the frequency response of the system should be compatible with the operating range of the signal being measured. A signal conditioner accepts information from a detector and presents it in a convenient form to the user. To process the signal waveform without distortion, the bandpass of the system must encompass all of the frequency components of the signal that contribute

significantly to signal of interest. The range can be determined quantitatively by obtaining a Fourier analysis of the signal. The *bandpass* of an electronic instrument is usually defined as the range between the upper and lower half-power frequencies.

Electrical signals are invariably accompanied by components that are unrelated to the phenomenon being studied. Spurious signal components, which may occur at any frequency within the bandpass of the system, are known as noise. The instruments are designed in such a way that the noise is minimised to facilitate accurate and sensitive measurement. An important factor which characterises a signal conditioner is the signal-power to noise-power (S/N). For extraction of information from noisy signals, it is essential to enhance S/N, for which several techniques have been put in practice. The simplest method is that of bandwidth reduction. The ratio S/N is improved by eliminating unused segments of the spectrum. If it is possible to exclude especially noisy regions, such as 50 Hz, by bandwidth reduction technique, the result will be very good. However, if the reduced bandwidth extends to low frequencies, the results may not be satisfactory, because of the presence of drift and flicker noise.

Signal averaging is another noise reduction technique, in which the signal is averaged over a period that is long compared with the periods of interfering noise components. The average of the noise signal will tend towards zero, while that of non-random information will be its actual value, even though signal and noise may have the same frequencies.

The limitation of using long observing time as compared to the period of the lowest frequency signal components of interest in signal averaging technique can be avoided by use of signal modulation and phase-sensitive detection. In *modulation*, the information-bearing signal is used to modify a second signal, which is termed the carrier. The modification may involve encoding of information in terms of carrier amplitude (amplitude modulation) or in terms of carrier frequency (frequency modulation). Certain frequency ranges are generally very troublesome, particularly below 1 Hz, in which drift and flicker noise cause difficulties, because AC-coupled amplifiers cannot be used. Frequency modulation of the signal with a higher frequency conveniently solves this problem.

Phase sensitive detection is a demodulation technique, by which only signals that are coherent (have a fixed phase relationship) with a reference signal are extracted from a modulated signal. This technique makes it possible to eliminate even those noise components which occur at signal frequency, as noise is not coherent with other signals.

The recent progress of digital technology, in terms of both hardware and software, makes digital processing more efficient and flexible than analog processing. Digital techniques have several advantages. Their performance is powerful as they are able to easily implement even complex algorithms. Their performance is not affected by unpredictable variable such as component ageing and temperature which can normally degrade the performance of analog devices. Moreover, design parameters can be more easily changed through digital techniques because they mostly involve software rather than hardware modifications.

The designs of analytical instruments have undergone tremendous changes during the last decade with the use of micro-computers, resulting in replacement of a large number of hardware components with software. Many circuit functions previously performed by hard wired electronics are elegantly executed by the software. This has resulted not only in greater reliability with reduced maintenance costs but also improved performance and extended applications.

1.5 READ OUT (DISPLAY) SYSTEMS

The results of a measurement in modern analytical instruments are usually displayed in the following forms:

- Analog metres
- Digital displays
- Laboratory recorders
- Video display units

1.5.1 Analog Meters

Moving coil metres are used in instruments which employ barrier-layer type photocells. Meters used in such instruments when connected directly should have very good current sensitivity. This is achieved by using strong permanent magnets and phosphor bronze suspension wire. Current sensitivity of the order of 0.5 μA (full-scale deflection) has been employed in several direct reading commercially available colorimeters. Analytical instruments which incorporate electronic amplifiers and use analog metres for display do not require such sensitive metres. Generally metres used in such instruments have sensitivity of either 50 μA, 100 μA or 1 mA. They often use shunt or series resistors to suit the amplifier output.

1.5.2 Digital Displays

Different types of devices are available for display of the measured quantities in numerical form. However, the seven-segment display arrangement has become quite popular in digital displays. The numerical indicator consists of seven bars positioned in such a way that the required figure can be displayed by selection of the appropriate bars. For example, when the decimal digit 4 has to be shown, bars f.g.b and c are illuminated. A special decoder is required for driving such an indicator from a BCD code. The seven segments can be illuminated by incandescent lamps, neon lamps and directly heated filaments.

Light emitting diodes (LEDs) arranged in seven-segment format are used in small-sized displays. These semiconductor diodes are made of gallium arsenide phosphide and are directly compatible with 5 V supplies typically encountered in digital circuitry. LEDs are very rugged and can withstand large variations in temperature. LEDs are available in almost all colours in the visible range.

Liquid crystal displays (LCDs) are currently preferred devices for displays as they require very low current for their operation. LCDs with large screen sizes and full colour display capabilities are available commercially and are finding extensive and preferable applications in laptop computers and many portable analytical instruments.

The outputs from most of the transducers are analog signals. For digital operations which may include computations and display, these analog signals are converted to a digital representation. Several types of A/D converters have been developed. They are commercially available in the form of a single monolithic chip. They even include features like auto-zero and auto-polarity, so that they can be readily used for constructing digital panel metres. Three digit A/D converter LD 130 from M/S Siliconix U.S.A. is a typical example.

1.5.3 Laboratory Recorders

Every modern laboratory possesses recorders of different varieties for recording various types of analog signals available from analytical instruments. Recorders are available in a large range of sizes, speeds, sensitivities and prices.

The most elementary electronic recording system consists of three important components, namely the signal conditioner, the writing part and the chart drive mechanism. The signal conditioners usually consist of a pre-amplifier and the main amplifier. Both these amplifiers have to satisfy specific operating requirements, such as input impedance, gain and frequency response characteristics. For recording analog signals, the writing part may be direct writing galvanometric type or null balancing potentiometric type. The dot matrix printer and the laser printer are normally used with the personal computer (PC)-based instruments.

1.5.4 Video Display Units

Among the indicating and display devices, the cathode ray oscilloscope occupies an important place in analytical instrumentation. Like a pen recorder, the oscilloscope presents a 2-Dimensional graphical display. In recorders, a mechanical device does the writing, while in the oscilloscope, an electronic beam does the same job, with speed and elegance unthinkable in other types of display devices.

The oscilloscope produces a visual display of electrical phenomenon. Its chief advantage is that, it produces visual representation directly, with extremely high speed. This is made possible because of the very high velocity with which the electrons can move.

With the incorporation of micro-computers in analytical instruments, the display systems for results and processed data in these instruments have undergone a complete change. The analog and digital displays have been replaced by LCD video display units, which present information not only as a list of numbers but as elegant character and graphic displays and sometimes as a 3D colour display.

1.6 INTELLIGENT ANALYTICAL INSTRUMENTATION SYSTEMS

Most of the signals which are required to be processed in analytical instruments are analog in nature. The development of electronic A/D converters connected to computers has changed all that; data acquisition provides the measurements to be displayed in the graphical and numerical form and also provides the ability to process it for deriving the parameters of interest from the raw signal. This simple step is the basis of the modern laboratory's ability to handle everything from complex experiments, analytical techniques, and automated sample preparation, with almost every piece of lab equipment now fitted with a computer chip. Figure 1.3 shows a various subsystems in a typical intelligent digital instrument

Intelligent technology is pervading every area of modern society, from satellite communications to washing machines. The analytical instrumentation field is no exception from this reality. This has become possible with the availability of high-performance microprocessors, micro-controllers and PCs.

The application of microprocessors in analytical instrumentation has matured following a series of stages. In the first stage, the microprocessors simply replaced conventional electronic

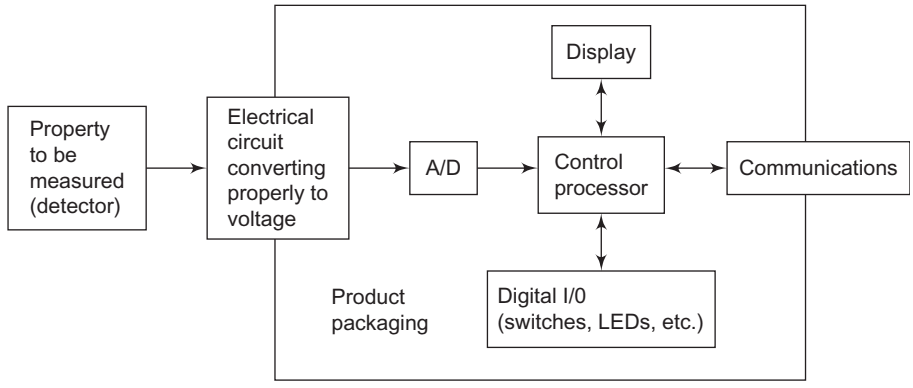


Figure 1.3 *Various sub-systems in a digital instrument*

systems that were used for processing data. This resulted in more reliable and faster data. This was followed soon by use of the microprocessor to control logic sequences required in instrumentation. Thus, the microprocessor replaced programming devices as well as manual programming, making possible digital control of all of the functions of the analytical instruments. With the availability of more powerful microprocessors and large data storage capacity, it has become possible to optimise the analytical conditions as the analysis is proceeding. They can be used to replace the complicated instructional procedures that are now required in several analytical techniques.

Extensive use has been made of microprocessors in analytical instruments designed to perform routine measurements, particularly in those situations where data computing and processing could be considered as a part of measurement and diagnostic procedure. The incorporation of microprocessors into instruments enables to have a certain amount of intelligence or decision making capability. The decision making capability increases the degree of automation of the instrument and reduces the complexity of the man-machine interface. Systems have been designed with numerous safety back-up features and real-time self-diagnostics and self-repair facilities. The reliability of many transducers has been improved and many measurements can now be made in situ because of the added computational ability of microprocessors. The computational capability makes possible features such as automatic calibration, operator guidance, trend displays, alarm priority and automated record keeping. Use of microprocessors in various instruments and systems has been explained at various places in the text.

Microprocessor-based instrumentation is enabling to incorporate the ability to make intelligent judgement and provide diagnostic signals in case of potential errors, provide warnings or preferably make appropriate corrections. Already, the microprocessors are assisting in instruction-based servicing of equipment. This is possible by incorporating monitoring circuits that will provide valuable diagnostic information on potential instrumentation failure modes and guide the operator in their correction. The instrument diagnostic microprocessor programs would sense such a potential failure of the unit and switch on the stand-by unit. The operator is informed to remove and service the defective part while the analytical work proceeds uninterrupted. Modern analytical instruments have been greatly affected by the use of microprocessors and have provided various degrees of control. The types of controls that are exercised for operation of various instruments are described below:

Manual Operation: In this case, the operator is provided with sufficient information in the operating or instruction manual to permit him to operate the instrument. The operator is expected to set the operating conditions to achieve optimum performance. For example, to operate a spectrophotometer, the settings relating to source intensity, monochromator slit width, scanning speed and range, amplifier gain and system time constant, etc. are made by the operator. If performance varies, the values are required to be reset appropriately by the operator.

Automatic Operation: In automatic operation, after the device is started, stable operating conditions and an appropriate signal-to-noise ratio is insured, even though conditions may change during a measurement or from sample to sample. Here, the system constituents are self-standardising and/or operate automatically during unattended operation.

Automatic Control: For complete control and automation, one or more microprocessors (micro-computers) are wired into an instrument. Here, programs for operation are entered by the manufacturer into the read-only-memory incorporated into the micro-computer. (A micro-computer has a microprocessor, read-only-memory and random-access-memory.) The programs also include provision for the user to interact with the computer. The system is menu-driven and the computer prompts the user to supply information about the kind of measurements to be made. The programs indicated by these data provide for appropriate operation of sub-systems and data acquisition. The computer processes the data and extracts chemical information.

The incorporation of computer control necessitates some redesign of the instrument. For example, sensing devices such as position encoders must be inserted to furnish data to the computer. Devices that the computer can actuate, such as stepping motors are required to be added to allow program control of scanning and other operations.

1.7 PC-BASED ANALYTICAL INSTRUMENTS

The popularity of the so called personal computers (PCs) or home computers has fuelled intense commercial activity in the field of computers. Hardware is a comprehensive term for all of the physical parts of a computer, as distinguished from the data it contains or operates on, and the software that provides instructions for the hardware to accomplish tasks. Typically, the PC contains in a desktop or tower case and the following parts:

- Motherboard, which holds the CPU, main memory and other parts, and has slots for expansion cards
- Power supply is a case that holds a transformer, voltage control and fan
- Storage controllers of IDE, SCSI or other type that control hard disk, floppy disk, CD-ROM and other drives; the controllers sit directly on the motherboard (on board) or on expansion cards.
- Graphics controller that produces that output for the monitor
- The hard disk, floppy disk and other drives for mass storage
- Interface controllers (parallel, serial, USB, Firewall) to connect the computer to external peripheral devices such as printers or scanners.

The PC has made many advances in analytical instrumentation possible. The large volumes of data generated by instruments were once evaluated on paper, but have become much easier to

deal with once desktop PCs are available to researchers. As PC's became faster and more capable of handling larger volumes of data, they have taken over the job of instrument control and data acquisition. Furthermore, many software packages now come with interactive, multimedia tutorials that aid in learning the software and the instrument. The novice instrument user can now focus more attention on learning the fundamentals of the instrument operation.

The low cost and increasing power of PCs are making them popular in the analytical field (Chu and Zilora, 1986) as they provide tremendous computational and data handling capabilities. These capabilities make possible the routine use of techniques that would have been impractical because of their large computational time requirements. The important among such applications are Fourier Transform (FT) calculations, signal averaging and correlation techniques in spectrophotometry. Also, software for PCs is largely commercially available and users can purchase and use it conveniently. Personal computers (PCs) are now well established and widely accepted in analytical field for data collection, manipulation and processing and are emerging as complete workstations for a variety of applications. A PC becomes a workstation with the simple installation of one or more "instruments-on-a-board" in its accessory slots, and with the loading of the driver software that comes with each board. The concept has proven to be ideal instrument, providing a low cost yet highly versatile computing platform for the measurement, capture, analysis, display and storage of data derived from a variety of sources.

Figure 1.4 illustrates the typical configuration of a PC-based workstation. It is obvious that the system is highly flexible and can accommodate a variety of inputs, which can be connected to a PC for analysis, graphics and control. The basic elements in the system include sensors or transducers that convert physical or chemical phenomena into a measurable signal, a data acquisition system (a plug-in instrument/acquisition board), an acquisition/analysis software package or programme and computing platform. The system works totally under the control of software. It may operate from the PC's floppy and/or hard disk drive. Permanent loading or unloading of driver files can be accomplished easily. However, for complex applications, some programming in one or several of the higher level programming languages such as 'C language' may be needed. Data received from the measurements can be stored in a file or output to a printer, plotter or other device via one of the ports on the computer.

Voltage can be digitised into computer memory through plug-in data acquisition DAQ boards that contain A/D converters or stand-alone interfaces that transfer data to the computer through a serial port. Many of the commercial DAQ boards also support digital and trigger inputs, as well

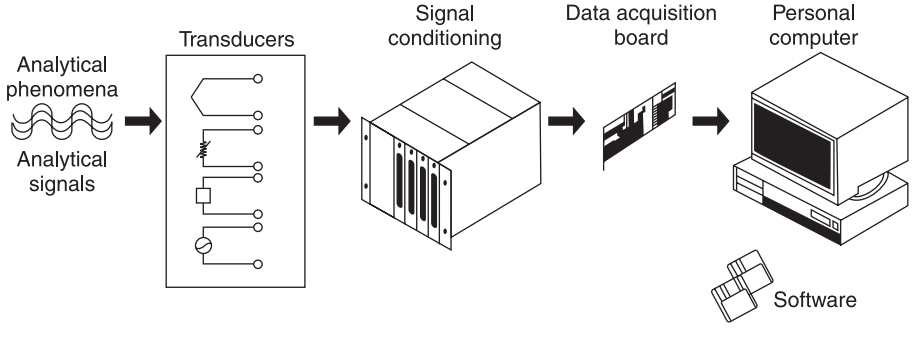


Figure 1.4 *Typical configuration of PC-based analytical instrument*

as analog and digital outputs for integrated data acquisition and instrument control. A variety of software packages support the commercially available DAQ boards for data acquisition and instrument control. National Instruments, Inc. (http://www.natinst.com) sells a wide range of DAQ boards and LabView software for sophisticated instrument control, data acquisition, and data analysis. Vernier Software, Inc. (http://www.vernier.com) sells a variety of probes for biological, chemical, and physical measurements, computer and calculator interfaces, and data logging software.

PC-based analytical instruments are gaining in popularity for several reasons including price, programmability and performance specifications they offer. Software development, rather than hardware development, increasingly dominates new product design cycles. Therefore, one of the most common reasons why system designers are increasingly choosing PC and PC architecture is the PC's rich and cost effective software tool set. This includes operating systems, device drivers, libraries, languages and debugging tools. Several examples of PC-based analytical instruments can be found at various places in the book.

1.8 MEMS IN ANALYTICAL INSTRUMENTS

Micro-Electro-Mechanical Systems (MEMS) are the integration of mechanical elements, sensors, actuators, and electronics on a common silicon substrate made using micro-fabrication technology. Thus, MEMS are a broad set of technologies developed with the goal of miniaturising systems through the integration of functions into small packages. Micro-fabricated components are small size, light weight, rugged and consume low power.

MEMS find a number of applications in the analytical instrumentation field for making in vivo measurements. Some of the devices in which MEMS have been developed are shown in Figure 1.5 are as follows:

- Micro-pump – used to pump small amounts of fluid, all the way down to picolitres. Figure 1.5a is a sketch of cross section of a micro-pump. Alternating voltage causes the Lead Zirconate Titanate (PZT) component to expand and contract along the horizontal direction. This induces a bending stress on the diaphragm, which in turn pumps the fluid through the chamber. The overall dimensions of this pump are $6{,}000 \times 6{,}000 \times 1{,}000$ μm for the length, width and height, respectively. The two orifices serve as channels for the inlet and outlet valves. The entire housing of the pump is composed of Silicon Nitride (Si_3N_4), with the diaphragm having a thickness of 10 μm (Linnemann, 1998).
- Micro-gear – having each tooth of about 8 μm or the size of a human red blood cell (Figure 1.5b).
- Micro-mirror – used in optical systems and also in displays.
- Fluid Channel – for a micro-pump.
- Micro-sensors (transducers) – converting a physical property into an electrical property (force to voltage) (Figure 1.5c).
- Micro-actuators – to actuate mechanical devices (switches, mirrors, etc.).

Integrated Circuits (ICs) are fabricated using micro-fabrication techniques. However, they involve electrons and move them about, amplify, attenuate, etc. ICs are based on the transistor – a basic unit or building block of ICs. Most ICs are silicon based, depositing a relatively small set of materials. On the other hand, MEMS do not have a basic building block (i.e. there is no MEMS

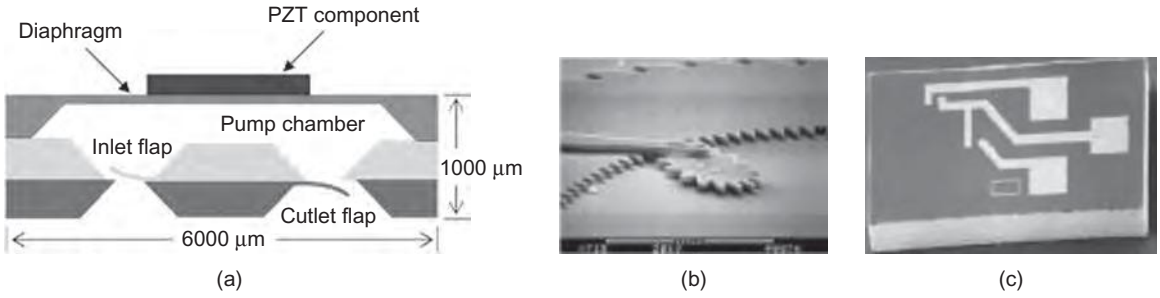


Figure 1.5 *Typical examples of MEMS (a) Micro-pump (b) Micro-gear (c) Micro-sensor*

equivalent of a transistor). However, Some MEMS are silicon based and use surface micro-machining (CMOS based) technology.

What differentiates many MEMS devices and products from ICs is that the processes used to fabricate can be radically different and non-compatible. So, MEMS is far more complex than ICs, and hence, these applications need to draw from a large variety of technologies to be successful. ICs and MEMS can be integrated on one chip if the processes are compatible.

The advantages of MEMS are low cost, low power consumption, miniaturisation, integration and high performance. MEMS technology has been rapidly growing since the last two decades and now they are not just restricted to electrical and mechanical systems but are widely being used in physics, chemistry, biology, medicine, optics and aerospace. MEMS technology is thus diverse and includes many sub-fields such as Optical MEMS, RF MEMS, BioMEMS, etc.

1.9 MICRO-FLUIDICS IN ANALYTICAL INSTRUMENTS

Micro-fluidics is a multi-disciplinary field concerning the behaviour and the manipulation of small amounts of fluids with characteristic length scales from nano-metres to hundreds of micro-metres (Sin et al., 2011). The field has been under intensive research for over two decades as a result of the emergence of MEMS. From a technological point of view, micro-fluidics offers many advantages including low fluid volumes (less reagents and lower cost), short assay time, low power consumption, rapid generation of small liquid compartments and high degree of parallelisation.

In the past decade, micro-fluidics has undergone rapid development with numerous new fabrication techniques and device designs. There are a large number of publications and patents on micro-fluidic devices functioning as pumps, mixers, concentrators, and valves, which are the building blocks for creating functional bioreactors and lab-on-a-chip systems. Nevertheless, a major hurdle for transforming micro-fluidics into practical applications is the integration of these components into a fully automated platform that can be conveniently accessed by the end users. This is primarily due to the complexity of combining various components including bulky supporting equipments (e.g. pressure sources and cell culture modules), detection components (e.g. optics and engineering interfaces), and sample preparation modules (e.g. mixers and concentrators) into a single platform (Mariella, 2008).

Manipulating fluids in microchips remains a persistent challenge in the development of inexpensive and portable point-of-care diagnostic tools (Collino, 2013). Figure 1.6 shows the construction of a typical microchip based on the micro-fluidic principle. Flow in micro-fluidic chips can be controlled *via* frequency tuning, wherein the excitation frequency of a pressure source is matched

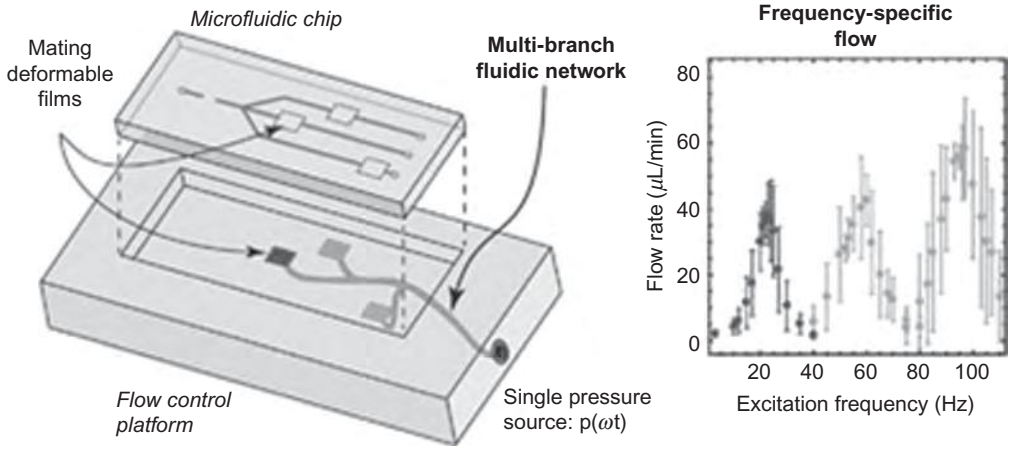


Figure 1.6 *Typical layout of micro-fluidic chip*

with the characteristic frequencies of network branches. The characteristic frequencies of each branch arise from coupling between fluid in the channels and passive deformable features, and can be programmed by adjusting the dimensions and stiffness of the features. In contrast to quasi-static 'on–off' valves, such networks require only a single active element and relatively small dynamic displacements. To achieve effective flow switching between different pathways in the chip, well-separated peak frequencies and narrow bandwidths are required.

1.10 METHODS OF ANALYSIS

Each analysis system is quite different from the other. However, they do share several primary characteristics. The parameters that effect instrument control, data acquisition, and data analysis are typically combined into what is referred to as a *method*. Methods are used repeatedly by setting up an operational *sequence*. For example, a sequence entry tells the instrument to evaluate Sample A using Method B.

Sequences: Most instruments now have robotic auto-samplers that introduce a specified volume of a specified sample at a specified time. The auto-samplers allow the instrument to run 24 hours a day, thus speeding up the routine and research work. Auto-samplers are controlled by the commands entered into a sequence table. The sequence is written by the instrument users and consists of a table of sequence entries. A sequence entry typically tells the instrument to inject Vial A, evaluate it using Method B, and store the data in File C. The sequence is usually customised for a given series of samples. However, they can be saved and used repeatedly to evaluate the same sample set.

Methods: Since there are a large number of combinations of samples that instruments must evaluate, they are designed to handle a large range of operating options. Instrument parameters such as temperature, flow rates, injection volumes, etc. are adjusted to fit specific sample evaluating applications. Computers can store the operating parameters so that they can be used repeatedly to evaluate samples, thus assuring reproducibility. Once an optimal method is developed, it is given a computer file name for storage and retrieval.

1.10.1 Types of Instrumental Methods

An analytical instrument converts chemical or physical characteristics of the analyte to a form that can be manipulated and understood by a human being. To obtain desired information from the analyte, it is necessary to provide some type of stimulus which is usually light, heat, current or voltage, as shown in Figure 1.7. The system under study responds to the stimulus as a result of the interaction of the stimulus with the analyte. A typical example is that of a colorimeter in which a narrow band of wavelengths of visible light pass through a sample and the extent of absorption of light by the analyte is measured. The intensity of light before and after its interaction with the analyte is determined, and the ratio of the two intensities gives a measure of the concentration of the analyte in the sample. The measurement process involves a suitable transducer to convert the information of interest into an electrical signal, a signal processor and a readout device.

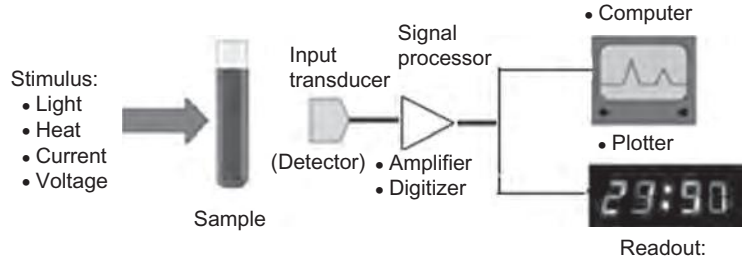


Figure 1.7 *Basic building blocks of an analytical instrument*

1.10.2 Classification of Analytical Instruments

Analytical instruments can be classified in the following categories:

Spectroscopy: Spectroscopy measures the interaction of the molecules with electromagnetic radiation. Spectroscopy consists of many different applications such as

- Ultraviolet-Visible Spectroscopy,
- Infrared Spectroscopy,
- Atomic Absorption Spectroscopy,
- Atomic Emission Spectroscopy,
- Photoemission Spectroscopy,
- Raman Spectroscopy
- Nuclear Magnetic Resonance Spectroscopy,
- X-Ray Fluorescence Spectroscopy,
- Mössbauer spectroscopy

The application of computers to individual instruments has led to the widespread development of methods such as FT to produce new and more precise analytical techniques, such as Fourier transform infrared spectroscopy.

Mass Spectrometry: Mass spectrometry measures mass-to-charge ratio of molecules using **electric** and **magnetic fields**. Mass spectrometry is categorised by the principles used in mass analysers:

- Magnetic-Sector,
- Quadrupole Mass Analyser,

- Quadrupole Ion Trap,
- Time-of-flight,
- Fourier transform ion cyclotron resonance

Crystallography: Crystallography is a technique that characterises the chemical structure of materials at the atomic level by analysing the **diffraction** patterns of usually **x-rays** that have been deflected by atoms in the material. From the raw data, the relative placement of atoms in space may be determined.

Separation Techniques: Separation processes are used to decrease the complexity of material mixtures. **Chromatography** and **electrophoresis** are representative of this field. Chromatography is a physical method of separation of the components of a mixture by distribution between two phases, of which one is stationary bed of a large surface area and the other a fluid phase that percolates through or along the stationary phase.

Electrochemical Analysis: Electroanalytical methods measure the **potential** (**volts**) and/or **current** (**amps**) in an **electrochemical cell** containing the analyte. These methods can be categorised according to which aspects of the cell are controlled and which are measured. The three main categories are:

- Potentiometry (measurement of the difference in electrode potentials),
- Coulometry (measurement of the cell's current over time),
- Voltammetry (measurement the cell's current while actively altering the cell's potential).
- Amperometry (measuring the current passing through the cell while applying a fixed voltage across the electrodes

Thermal Analysis: Analytical instruments in which some property of the system is measured as a function of the temperature. Calorimetry and thermogravimetric analysis are examples of techniques which measure the interaction of a material and heat.

Hybrid Techniques: Hybrid or Hyphenated separation techniques refers to a combination of two (or more) above techniques to detect and separate chemicals from solutions. Several examples are in popular use today and new hybrid techniques are under development. For example,

- **Gas chromatography** – M**ass spectrometry**,
- Liquid Chromatography – Mass spectrometry (LC-MS),
- Gas Chromatography – Infrared Spectroscopy (GC-IR),
- Liquid Chromatography – Nuclear Magnetic Resonance (LC-NMR),
- Liquid Chromatography – Infrared Spectroscopy (LC-IR),
- Capillary Electrophoresis – Mass Spectrometer (CE-MS), and so on.

Microscopy: The visualisation of single molecules, single cells, biological tissues and nano-micro-materials is very important and attractive approach in analytical science. Also, hybridisation with other traditional analytical tools is revolutionising analytical science. **Microscopy** can be categorised into the following three different fields:

- Optical Microscopy,
- Electron Microscopy,
- Scanning Probe Microscopy.

Lab-on-a-chip: Devices that integrate (multiple) laboratory functions on a single chip of only a few millimetres in size and that are capable of handling extremely small fluid volumes down to less than picolitres.

The book details out the principles, the applications and the performance parameters of various types of analytical instruments as listed above.

1.11 PERFORMANCE REQUIREMENTS OF ANALYTICAL INSTRUMENTS

1.11.1 Errors in Chemical Analysis

Many times, measurement systems consist of several elements and total control of a number of experimental variables is usually difficult. These variables include sampling methods, analytical technique and instrument-related errors. Where a measurement consists of a single reading on a simple equipment, like taking a reading of temperature on a thermometer, the number of variables contributing to uncertainties in that measurement are fewer than a measurement involving a multi-step process, like use of a variety of reagents. It is thus important to be able to estimate the uncertainty in any measurement because not doing so would mean as if the person making measurement does not know anything about the variability of the measurement leading to erroneous results. Statistical methods are often employed as means for objectively evaluating the source and amount of error in analytical methods.

An *'error'* by definition is the difference between the measured value and the true value.

$$\text{Error } e = V_{\text{measured}} - V_{\text{true}}$$

However, we normally do not know the true value, otherwise there would be no reason to make the measurement. Therefore, we estimate the likely upper bound of an error, expressed as the uncertainty. Therefore,

$$-u \le e \le u$$

or

$$-u \le V_{\text{true}} \le V_{\text{meas}} + u$$

where u = uncertainty

Errors arise from many sources and therefore, it is necessary to determine their likely dominant sources. Armed with this knowledge, anyone involved in measurement would be in a position to control the errors wherever possible and consider the effects of the error on the results.

1.11.2 Types of Errors

Errors are broadly classified into three types, viz. random, systematic or gross.

Random errors: These are the result of intrinsically uncertain nature of the measurement technique and are invariably present in every measurement. For example, thermal, shot or flicker noise are sources of random errors. Their magnitude is usually small. They can be minimised usually by filtering techniques through hardware or software implementation.

Systematic errors: They are procedural errors and arise from incorrect calibration of the instrument, its improper operation and impure reagents. These are identifiable errors and can mostly be eliminated by modification of the analytical procedure.

Another type of error is *bias* which represents a systematic distortion in a measurement. It is a non-compensating error and arises due to

- flaw in measurement instrument
- flaw in the method of selecting sample
- flaw in the technique of estimating a parameter
- subjectivity of operators

The only practical way to minimise systematic or bias errors is by continual check of instruments and assumptions, care in the use of instruments and application of methods and meticulous training of the instrument operator. It is essential to check all instruments before commencement of any important measuring session and re-check periodically during the course of the project.

Gross errors: The common sources of gross errors include mistakes made in analytical techniques, improper reading or recording of results and data and errors in calculations. Gross errors are usually irregular in nature and are characterised by large amplitude.

Mistakes are caused by human carelessness, casualness or fallibility. Even though there is no excuse for mistakes, but we all make them. The advice, therefore, is to never be satisfied with a single reading, no matter what is being measured. Repeating the measurement will also improve the precision of the final result.

In addition, *accidental errors* take place due to uncontrolled environmental conditions, limitations or deficiencies of instruments, assumptions and methods. Accidental errors can be reduced by using more accurate and precise equipment, but this can be expensive. Therefore, it is advisable to make an approximate choice from the available equipment so that you may obtain results of an accuracy sufficient for the task in hand.

For many systems, the error in a measurement is not isolated to one source but rather is due to several different individual errors. A simplistic method of calculating the total error is simply to add the errors from each source together. The key is to make certain that the error is expressed in terms of the final measurement quantity.

In many instances, a measurement will be taken many times to improve the overall quality of the measurement. This averaging improves error levels if the noise is random. For example, if a measurement '*M*' is taken three times, the average value for *M* is found by taking the sum of the three measurements and dividing by three. Similarly, the net error in the average is found by taking the total error for the three measurements and dividing by three.

Hysteresis: This is a specific type of error that is very common in mechanical systems. This error appears as a different output value for the same input value, with the difference due to the manner in which the input

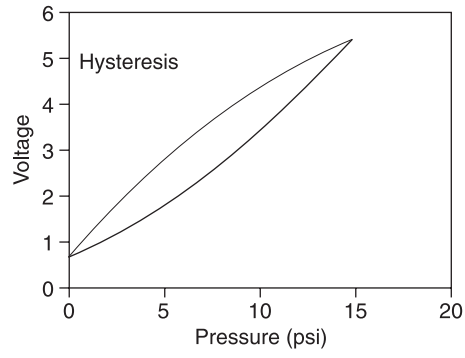


Figure 1.8 *Hysteresis error*

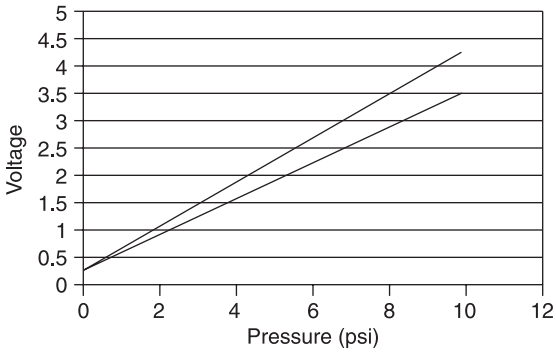


Figure 1.9 *Repeatability error*

value was set. Specifically, if the input value was set by increasing the physical parameter, it will have a different output value than if the input value was set by decreasing the input value. This is illustrated in Figure 1.8.

Repeatability Error: Repeatability is similar to hysteresis except that it refers to the difference in output values for the same input values when the input value is produced in the same fashion. For example, a repeatability error would be the difference in output voltage for a pressure transducer when an identical pressure is placed on the sensor with the pressure being either increased or decreased each time. Figure 1.9 illustrates the concept of repeatability error.

As one can see, hysteresis and repeatability are both errors that quantify the difference in output for identical input; the difference between the two is how the input quantity is applied to the transducer or sensor.

1.11.3 Accuracy and Precision

The *accuracy* of a measurement is how close a result comes to the true value. Broadly speaking, accuracy is the degree to which a statement or quantitative result approaches the true value. Accuracy refers to the size of the total error and this includes the effects of systematic errors. An estimated value may be inaccurate because of one or more kinds of error. Determining the accuracy of a measurement usually requires calibration of the analytical method with a known standard

Accuracy includes the effects of repeatability, hysteresis, and linearity in a single term. It is exactly as it sounds and is a statement of the relationship between input parameter and output signal. Accuracy is typically specified as ± a fraction of either the full-scale reading of the instrument or of the value that is being measured. For example, if a pressure transducer is specified as ±1% accurate of its full-scale value and the transducer is used at pressures from zero to 50 psi, the error in any measurement will be ±0.5 psi.

Precision: Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. Precision has several variants depending on use, such as:

- The fitness of a single measurement, which refers to the resolving power of the measuring device and is ordinarily indicated by the number of decimal places in the measurements made with the device.
- The degree of agreement in a series of measurements
- The clustering of sample values about their own average.
- The reproducibility of an estimate in repeated sampling

Accuracy and precision are not synonymous. Accuracy signifies freedom from all types of errors, whereas precision implies freedom from variation – one does not imply the other

(i.e. precise measurements may not be accurate as illustrated in Figure 1.10). An accurate measurement is one in which the systematic and random errors are small, whereas precise measurement is one in which the random errors are small.

The uncertainties in measurement generally emanate from two common causes which are bias and precision as shown in Figure 1.11. An example of a bias (measure of systematic or determinate error of a method) error is poor calibration. Because of the poor calibration, all measurements are incorrect by the same amount say 5%, whereas precision errors are variable and random. An example of precision error is if repeated measurements are supposed to be the same, but they are not. You can see from Figure 1.11 that a measurement with a small range of precision errors could be called precise. However, the average value of measurements might have a large bias error, so the measurement would not be accurate (i.e. it does not represent the true value).

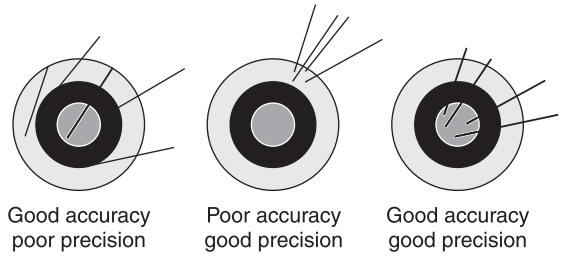


Figure 1.10 *Concept of accuracy and precision (after Tissue, 2004a)*

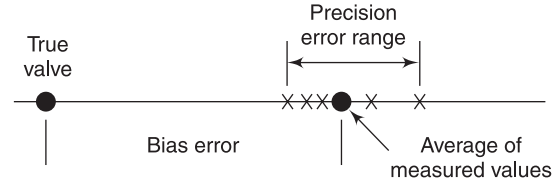


Figure 1.11 *Bias and precision error (after Wheeler and Ganji, 1996)*

Bias errors are usually caused by:

- Incorrect calibration – a system may give values that err by a fixed percentage.
- Systematic human errors – not synchronising readings between two experimenters.
- Defective equipment – incorrect scale graduations.
- Loading – the thermistor inserted into an ice water bath might change the temperature of the bath.

When plotting data on a graph, bias errors usually shift the total data set away from the true line, or change its slope.

Precision errors reflect that instrumentation or measurement devices are both imperfect and of finite resolution. They cause scatter about the true line. While it is easier to spot precision errors, identification of bias errors requires comparison with an independent model or data set.

1.11.4 Significant Figures

All measurements have some degree of uncertainty. The degree of uncertainty depends on both the accuracy of the measuring device and the skill of its operator. For example, in a digital balance, the mass of a sample substance can be measured to the nearest 0.1 g (i.e. mass differences less than this cannot be detected on this balance). We might therefore, indicate the mass of a substance measured on this balance as 3.2 ± 0.1 g; the ±0.1 (read plus or minus 0.1) is a measure of the accuracy of the measurement. It is important to have some indication of how accurately any measurement is made; the ± notation with the understanding that there is uncertainty of at least one unit in the last digit of

the measured quantity. In general, the measured quantities are reported in such a way that only the last digit is uncertain. All of the digits, including the uncertain one, are called significant digits or, more commonly, significant Figures. The number 3.2 has two significant figures, while the number 3.2405 has five significant figures.

In order to determine the number of significant digits in a measurement or number, the following rules may be apply:

- If there is no decimal point, the right-most non-zero digit is the least significant digit.
- For numbers that include a decimal point, the right-most digit is the least significant digit regardless of its value.

Significant digits should not be considered the same as uncertainty in measurement. They are used in manipulating experimental data, but not in expressing uncertainty. When calculating results from the measurement, if your calculator displays too many significant digits, round it to the nearest proper number.

Significant figures have economic significance simply because more the significant numbers expected from the measurement, the more expensive equipment and higher quality reagents will be required. Care should therefore, be taken in properly defining the optimum number of significant figures when attempting an analysis.

1.11.5 Application of Statistical Methods

A single measurement often does not have a high degree of validity. All measurements include some degree of bias and random variation due to noise, resulting in significant errors. The precision of results always should be estimated by performing multiple measurements and then calculating the average and the standard deviation of the average. The standard deviation is an indicator of the validity of a measurement. Monitoring of changes in the standard deviation of results over time can be a useful diagnostic for many measurement problems.

Statistical methods of data analysis are used to provide the analyst with a systematic and objective approach to the problems involved in error analysis. We know that the precision of an analytical method is usually indicated by the range of values about the mean that includes a specified percentage of the total observation. When many independent random factors act in an additive manner to create variability, data will follow a bell-shaped distribution called the 'Gaussian Distribution'. This distribution is also called 'Normal Distribution' which is shown in Figure 1.12. Gaussian distribution has some special mathematical properties that form the basis of many statistical tests. However, the catch is that the sample number has to be reasonably large, normally more than 30.

For computing normal probabilities for any normal distribution, it can be characterised as follows:

$$Z = x - \mu/\sigma$$

where μ = the population mean of the distribution. Because of symmetry, this occurs at the peak of the distribution.

σ = the population standard deviation of the distribution
Z = standard normal random variable
x = Value of a given measurement

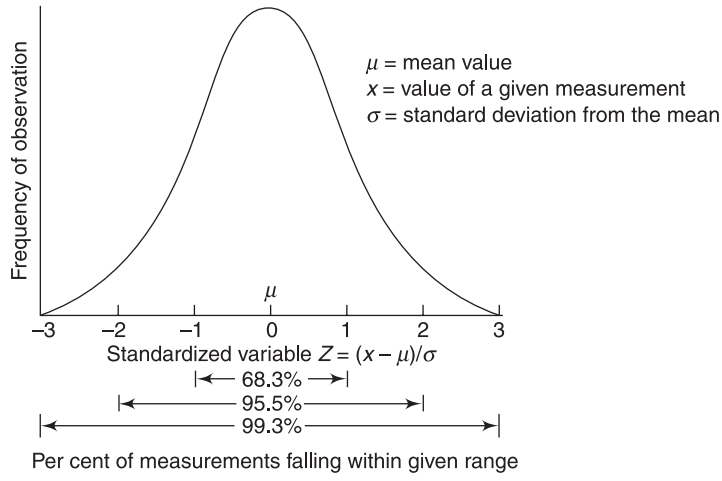


Figure 1.12 *Normal Gaussian distribution (adapted from Willard et al, 1988)*

The Gaussian distribution, as shown in the Figure 1.12, is a continuous function, and is normalised so that the sum over all values of x gives a probability of 1. The nature of Gaussian gives a probability of 0.683 of being within one standard deviation of the mean. The mean value is $a = np$ where n is the number of events and p the probability of any integer value of x. Similarly, the percentage measurements falling within two standard deviations is 95.5% and it is 99.3% for three standard deviations.

Confidence Interval is defined as the probability that the data point is found in certain neighbourhood of the mean. Three different confidence intervals are commonly used. They are usually called one sigma, two sigma and three sigma intervals. The probability that the data point is within one sigma interval around the mean is 0.6828. In other words, approximately 68.28% of data will be found within one sigma confidence interval around the average. The two sigma and the three sigma confidence intervals are interpreted exactly the same way, where two sigma has 95.5% and three sigma as 99.3% confidence intervals. One sigma also indicates one standard deviation from the mean value.

As is evident from the Figure 1.12, precision increases with decreasing value of standard deviation. It also indicates that the distribution of measurement results carries wealth of information which can be used to improve the measurements and the results. For example, the results of individual samples falling outside the desired confidence levels may be rejected. It also helps to establish trends in results to enable taking corrective measures.

1.11.6 Signal-to-Noise Ratio

The term signal-to-noise ratio, often abbreviated SNR or S/N represents the ratio between maximum possible signal (meaningful information) and the background noise,

$$\text{S/N} = \frac{\text{Average signal amplitude}}{\text{Average noise amplitude}}$$

In other words, S/N expresses the ability of an instrument to discriminate between signals and noise. Since many signals have a very wide dynamic range, S/N is often expressed in terms

of the logarithmic decibel scale. Due to the definition of decibel, the S/N gives the same result, independent of the type of signal which is evaluated (i.e. power, current, voltage). Often the signals being compared are electromagnetic in nature, though it is also possible to apply the term to sound and light stimuli. The S/N in decibels is 20 times the base-10 logarithm of the amplitude ratio, or 10 times the logarithm of the power ratio.

It may be observed that higher the signal-to-noise ratio, the better is the measurement result. The higher S/N is possible only if the noise value is reduced. Higher levels of amplification do not improve S/N because increase in the magnitude of the signal will be accompanied by a proportional increase in the value of noise.

1.11.7 Other Performance Parameters

1.11.7.1 Sensitivity

The sensitivity of an instrument is the change in output signal for a change in the physical parameter being measured. For linear systems, this is simply the proportionality constant used to relate output to input.

The sensitivity of a measurement system is determined from the calibration curves. The sensitivity is constant over the entire range in case of a linear response system as illustrated in Figure 1.13a. However, from the slopes of curves '*A*' and '*B*' it is evident that the sensitivity of the method is much greater in case of substance '*B*' than the substance '*A*' but constant for the same substance. In case, the response curve is non-linear (Figure 1.13b) the sensitivity is found to be changing with respect to the concentration. Evidently, the sensitivity for substance '*C*' decreases with an increase in concentration.

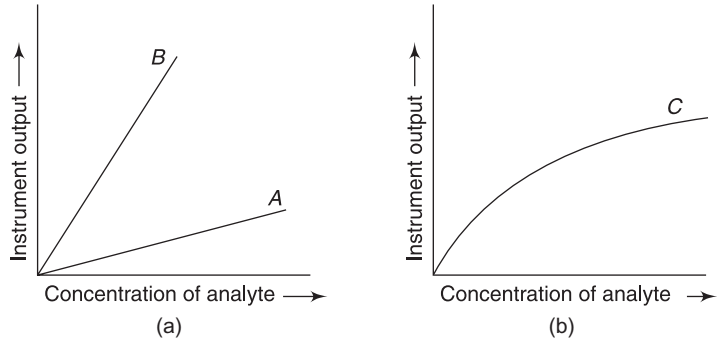


Figure 1.13 *Determination of sensitivity from calibration curve (a) Linear response (b) non-linear response*

In case of analytical instruments, sensitivity is usually expressed as the concentration of analyte required to cause a given instrument response.

1.11.7.2 Selectivity

The selectivity of an analytical method is defined as its ability to accurately measure an analyte in the presence of interference that may be expected to be present in the sample matrix.

1.11.7.3 Specificity

The term *specificity* generally refers to a method that produces a response for a single analyte only.

1.11.7.4 Resolution

Resolution is the smallest amount of input signal change that the instrument can detect reliably. This term is determined by the instrument noise which could be either circuit or quantisation noise.

1.11.7.5 Range

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the specified method. The range is normally expressed in the same units as the test results (e.g. percentage, per parts million) obtained by the analytical method.

1.11.7.6 Limit of detection

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. For example, in chromatography the detection limit is the injected amount that results in a peak with a height at least twice or three times as high as the baseline noise level.

Detection limits are generally defined at 95% confidence level, which means that in 95 out of 100 measurements, the measurements can be done reliably. However, concentration values smaller than the detection limit may be qualitatively detected but will be quantitatively unreliable.

1.11.7.7 Linearity

The linearity is the ability of the method to produce test results that are proportional, either directly or by a well-defined mathematical transformation, to the concentration of analyte in samples within a given range. Frequently, the linearity is evaluated graphically in addition or alternatively to mathematical evaluation. The evaluation is made by visual inspection of a plot of signal height or peak area as a function of analyte concentration. Because deviations from linearity are sometimes difficult to detect, two additional graphical procedures can be used.

The first procedure involves plotting the deviations from the regression line versus the concentration or versus the logarithm of the concentration, if the concentration range covers several decades. Regression analysis, a statistical technique, provides the means for objectively obtaining a linear line with minimum deviation between the plot and data. Many computer spreadsheet programs have built in functions which can do linear regression analysis. For linear ranges, the deviations should be equally distributed between positive and negative values.

The second method is to divide signal data by their respective concentrations yielding the relative responses. A graph is plotted with the relative responses on the *Y*-axis and the corresponding concentrations on the *X*-axis on a log scale. The resultant plot should be horizontal over the full linear range. At higher concentrations, there will typically be a negative deviation from linearity. Parallel horizontal lines are drawn in the graph corresponding to, for example, 95% and 105% of the horizontal line. The method is linear up to the point where the plotted relative response line intersects the 95% line. Figure 1.14 shows a comparison of the two graphical evaluations on the example of caffeine using high-performance liquid chromatography.

The range within which a linear relationship exists between a measured value and the corresponding concentration is called the *working range*. The minimum concentration for the working range is known as limit of detection, whereas the maximum concentration is called the limit of linearity. It is generally attempted to adjust the concentration by dilution or volume reductions to bring it within the working range.

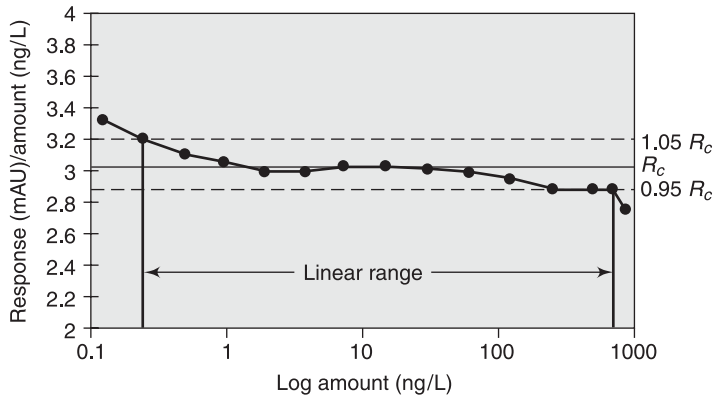


Figure 1.14 *Graphical presentation of linearity plot of a caffeine sample using HPLC. Plotting the amount (concentration) on a logarithmic scale has a significant advantage for a wide linear ranges Rc = line of constant response (www.labcompliance.com/methods/meth-val.html/ linearity and calibration curve)*

It is not always possible to obtain a linear graph due to interferences, signal noise, sample matrix or deviations from Beer's Law. However, several programs are commercially available for computing the best 'fit' curve for the data set.

1.12 INSTRUMENT CALIBRATION TECHNIQUES

Taking measurements with any analytical method or instrument necessitates calibration to ensure the accuracy of the measurement. The process of quantitatively defining the system responses to known, controlled signal inputs is known as calibration. Three commonly used methods to calibrate the instruments are as follows:

- Calibration Curve Method
- Standard Addition Method
- Internal Standard Method.

The choice of calibration technique from the above methods is determined by the instrumental method, instrument response, number of samples to be analysed and the interferences present in the sample. A brief description of each of the methods is given below:

1.12.1 Calibration Curve Method

In the calibration or working curve method, a series of standard solutions containing known non-concentrations of the analyte, is prepared. These solutions should cover the concentration range of interest. A blank solution containing only the solvent matrix is also analysed. The net readings – standard solution minus blank (background) – are plotted versus the concentrations of the standard solutions to obtain the working calibration curve. Figure 1.15 shows

a typical calibration curve. The non-linearity in the curve is usually corrected by computer software techniques.

The chief advantage of the working curve method is its speed as in it a single set of standards can be used for the measurement of multiple samples.

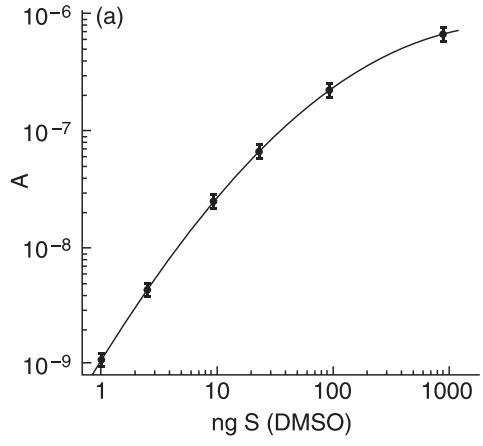


Figure 1.15 *Illustration of analytical (working) curve method for calibration*

1.12.2 Standard Addition Method

Due to matrix effects the analytical response for an analyte in a complex sample may not be the same as for the analyte in a simple standard. In such a case, calibration with a working curve would require standards that closely match the composition of the sample. For routine analyses it is feasible to prepare or purchase realistic standards (e.g. NIST standard reference materials). However, for diverse and one-of-a-kind samples, this procedure is time consuming and often impossible.

An alternative calibration procedure is the standard addition method. An analyst usually divides the unknown sample into two portions, so that a known amount of the analyte (a spike) can be added to one portion. These two samples, the original and the original plus spike, are then analysed. The sample with the spike will show a larger analytical response than the original sample due to the additional amount of analyte added to it.

The difference in analytical response between the spiked and unspiked samples is due to the amount of analyte in the spike. This provides a calibration point to determine the analyte concentration in the original sample.

Let us assume that with an unknown concentration '*X*' the instrument gives a reading R_x. If a known concentration '*Y*' is added to the sample solution to give a reading R_y, then '*X*' can be calculated from the equation:

$$X/X + y = R_x/R_y$$

The principle of standard addition is shown in Figure 1.16

The concentration scale is on the *X*-axis. When the analyte is added to the sample solution, the unknown concentration can be determined by the point at which the extrapolated line intersects the concentration axis.

The method of standard addition gives more accurate results than the calibration curve method. It is particularly used in electroanalytical chemistry wherein the unknown and standard solutions are measured under identical conditions. These conditions exist in matrix sensitive volumetric techniques such as anode stripping voltammetry. The method also provides a systematic means of identifying sources of error in analyses which could be due to defective instrument, incorrect internal standard or depletion of test reagents.

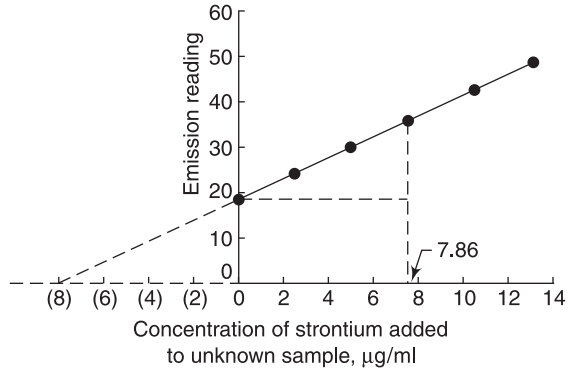


Figure 1.16 *Standard addition method of calibration*

1.12.3 Method of Internal Standard

An internal standard is a known amount of a compound, different from analyte that is added to the unknown. Signal from the analyte is compared with signal from the internal standard to find out how much analyte is present. This known substance must not be present in the original unknown substance.

Thereafter, the response of the analyte and internal standard are determined and the ratio of the two responses is calculated. The response ratio of analyte to internal standard would depend only on the analyte concentration because the internal standard and analyte get affected generally if one or more of the parameters affecting the measured response varies. The calibration curve is generated by plotting the response ratio as a function of analyte concentration. The method is used in emission spectroscopy, gas chromatographic analysis and improved spectroscopy. While using the internal standard method, the following points should be taken into consideration:

- The internal standard should be a substance similar to the analyte.
- The internal standard should have an easily measured signal that does not interfere with the response of the analyte
- The response of the internal standard should not be affected by other components of the sample.
- The concentration of the internal standard should be of the same order of magnitude as that of the analyte in order to minimise error in calculating the ratio.

When used in spectroscopy, the internal standard method requires instruments with a special capability of determining intensity or absorbance at two wavelengths simultaneously – one for the standard and the other for the unknown. The output of two detectors (known vs. standard) is read out as a ratio, which is then plotted against concentration and the analyte in the unknown is determined from the graph.

In order to illustrate the method for internal standard when determining sodium concentration in a solution, Li is added to each of the series of known and standard solutions. Figure 1.17 shows a plot of intensity ratio vs. concentration of Na from which the concentration of Na an unknown solutions can be calculated. The graph is non-linear and therefore the determination is made graphically. For example, the unknown '*A*' has a concentration of 3.2 ppm Na, whereas unknown '*B*' has a concentration of 6.9 ppm Na. If the graph were linear, then a linear equation can be developed from the calculation of the slope and *Y*-intercept.

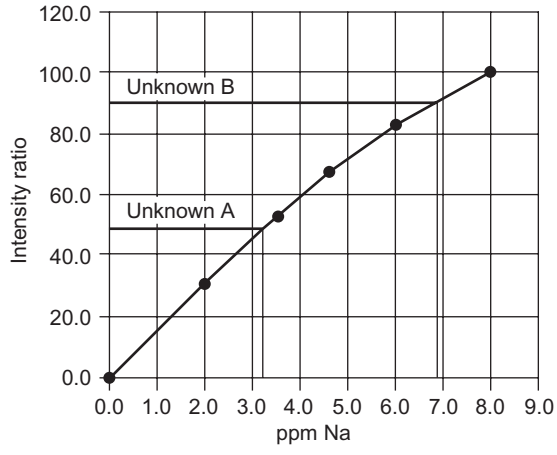


Figure 1.17 *Internal standard method of calibration (Moore, 2000)*

Internal standards are especially useful for analyses in which the quantity of sample analysed or the instrument response varies, slightly from run to run. Internal standards, therefore are widely used in chromatography in which small quantity of sample solution injected into the chromatograph is not very reproducible in some experiments.

1.13 VALIDATION

The process of assessing, by independent means, the quality of the data products derived from the system outputs is termed as validation. As regards analytical instruments/equipment, method validation is usually resorted to in order to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Every analytical method need to be validated/revalidated:

- before its introduction into routine use.
- whenever the conditions change for which the method has been validated (i.e. instrument with different characteristics).
- whenever the method is changed.

The validity of a specific method is closely related to the type of equipment and its location where the method will be run. Before an instrument is used to validate a method, its performance should be verified using generic standards. Satisfactory results for a method can only be obtained with well performing equipment. Special care should be taken regarding characteristics that are critical for the method. For example, if detection limit is critical for a specific method, it is advisable to verify the instruments specification for baseline noise and the response of the detectors to specified compounds.

Method validation has received considerable attention from several organisations and regulatory agencies. For example, the guidance on the Interpretation of the EN45000 series of standards and ISO/IEC (International Standards Organization/International Electrotechnical Commission), (Eurachem Guide 25, 1993) includes a chapter on the validation of analytical methods.

2

COLORIMETRES AND SPECTROPHOTOMETERS (VISIBLE – ULTRAVIOLET)

2.1 ABSORPTION SPECTROSCOPY

The most important of all the instrumental methods of analysis are the methods based on the absorption of electromagnetic radiation in the visible, ultraviolet (UV) and infrared ranges. According to the quantum theory, the energy states of an atom or molecule are defined and for any change from one state to another, would, therefore, require a definite amount of energy. If this energy is supplied from an external source of radiation, the exact quantity of energy required to bring about a change from one given state to another will be provided by photons of one particular frequency, which may thus be selectively absorbed. The study of the frequencies of the photons which are absorbed would thus indicate a lot about the nature of the material. Also, the number of photons absorbed may provide information about the number of atoms or molecules of the material present in a particular state. It thus provides us with a method to have qualitative and quantitative analysis of a substance.

Molecules possess three types of internal energy – electronic, vibrational and rotational. When a molecule absorbs radiant energy, it can increase its internal energy in a variety of ways. The various molecular energy states are quantised and the amount of energy necessary to cause any change in any one of the above energy states would generally correspond to specific regions of the electromagnetic spectrum. Electronic transitions correspond to the UV and visible regions, vibrational transitions to the near infrared and infrared regions and rotational transitions to the infrared and far infrared or even microwave regions. This is shown in Figure 2.1.

The method based on the absorption of radiation of a substance is known as Absorption Spectroscopy. The main advantages of spectrometric methods are speed, sensitivity to very small amounts and a relatively simple operational methodology. The time required for the actual measurement is very short and most of the analysis time, in fact, goes into preparation of the samples. Absorption spectroscopy has a tremendously wide range of analytical applications and is proving extremely useful for analysis even at trace levels.

2.1.1 Electromagnetic Radiation

Electromagnetic radiation is a type of energy that is transmitted through space at a speed of approximately 3×10^{10} cm/s. Such a radiation does not require a medium for propagation and can readily travel through vacuum. Electromagnetic radiation may be considered as discrete packets of energy

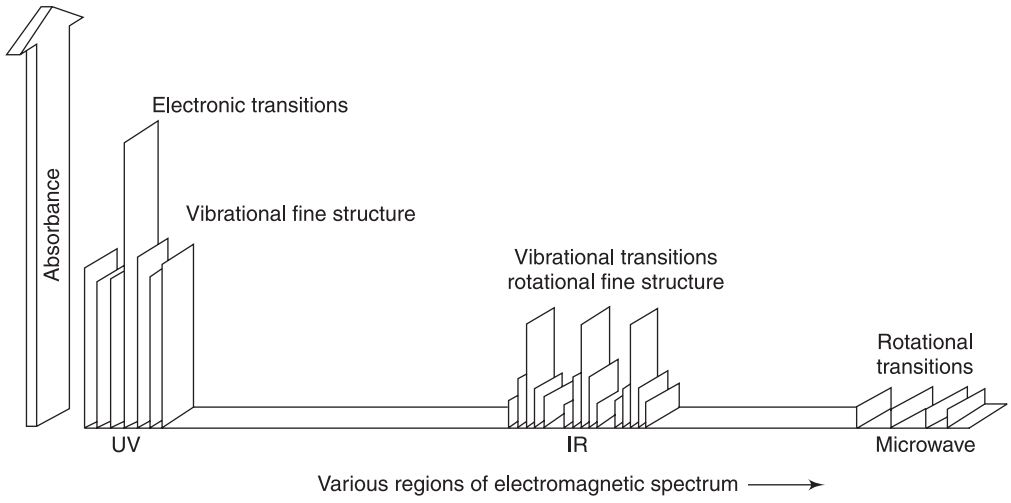


Figure 2.1 *Relationship of wavelength and energy induced transitions (after Human, 1985)*

called photons. A photon consists of an oscillating electric field component (E) and an oscillating magnetic field component (M). The electric and magnetic fields are perpendicular to each other (Tissue, 1996). These fields are in turn, perpendicular (orthogonal) to the direction of propagation of the photon. The electric and magnetic of a photon flip direction as the photon travels. The number of flips or oscillations of a photon that occur in 1 s represents the frequency, expressed in Hertz or units of oscillations per second. This is shown in Figure 2.2. The relation between the energy of a photon and the frequency of its propagation is given as follows:

$$E = h\upsilon$$

where E is energy in ergs, υ is frequency in cycles and h is Plank's constant (6.626×10^{-34} joules. sec.)

For some purposes, electromagnetic energy can be more conveniently considered as a continuous wave motion in the form of alternating electric field in space. The electric field produces magnetic field at right angles to its own direction. These fields in turn are mutually perpendicular to the direction of propagation. If λ is the wavelength (interval between successive maxima or minima of the wave), then

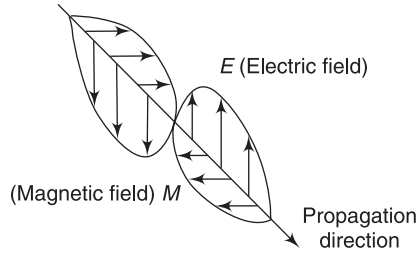


Figure 2.2 *Propagation of electromagnetic radiation*

$$C = \upsilon\lambda$$

where C is the velocity of propagation of radiant energy in vacuum and λ is the frequency in cycles. The practical units employed to express wavelength are

nm (nano-metres) or mμ (millimicron) = 10^{-9} metres
μ mt. (micro-metre) or μ (micron) = 10^{-4} cm
Å (Angstrom) = O.1 nm = 10^{-8} cm

The wavelength is now commonly expressed in nano-metres. The other units Angstrom (Å) and millimicron (mμ) are discouraged.

$$1 \text{ nano-metre} = 1 \text{ nm} = 1 \text{ m}\mu = 10 \text{ Å}$$

Wave number: Wave number is defined as the number of waves per centimetre.

2.1.2 The Electromagnetic Spectrum

Figure 2.3 shows the various regions in the electromagnetic spectrum which are normally used in spectroscopic work. For convenience, we photons in all of these regions have the same electromagnetic nature, but because of their very different energies they interact with matter very differently. However, some of the boundaries between spectral regions are not well-defined as between UV and visible radiation. Visible light represents only a very small portion of the electromagnetic spectrum and generally covers a range from 380 to 780 nm. The short wavelength cut-off (380 nm) is due to absorption by the lens of the eye and the long wavelength cut-off (780 nm) due to the decrease in sensitivity of the photoreceptors in the retina for longer wavelength. The UV region extends from 185 nm to the visible. Shorter wavelengths lie in the far UV region, which overlaps the soft X-Ray part of the spectrum. Infrared region covers wavelengths above the visible range. Table 2.1. shows the frequencies and wavelengths of different spectral regions.

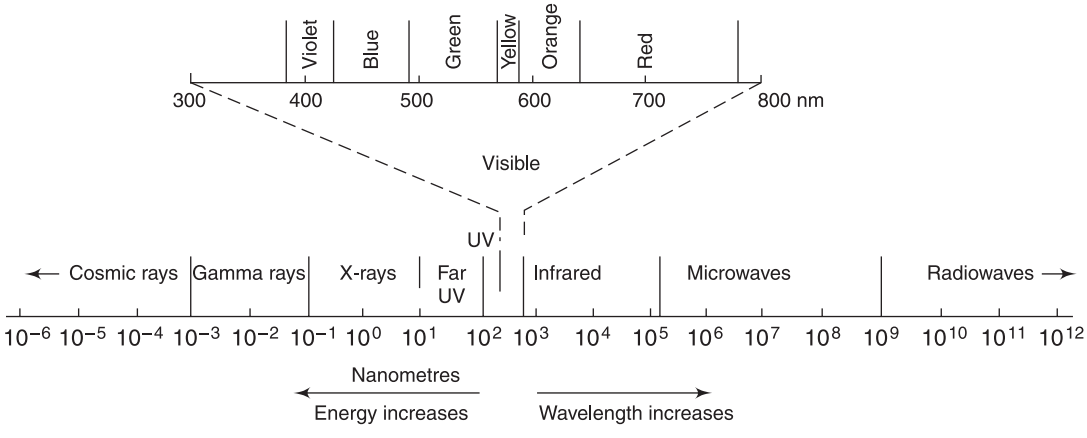


Figure 2.3 *Electromagnetic spectrum*

2.1.3 Interaction of Radiation with Matter

When a beam of radiant energy strikes the surface of a substance, the radiation interacts with the atoms and molecules of the substance or molecular ions or solids. The radiation may be transmitted, absorbed, scattered or reflected, or it can excite fluorescence depending upon the properties of the substance. The interaction, however, does not involve permanent transfer of energy.

Table 2.1 *Electromagnetic spectrum*

Type of radiation	Wavelength range	Frequency range (Hz)
Gamma-rays	<1 pm	10^{20}–10^{24}
X-rays	1 nm–1 pm	10^{17}–10^{20}
Ultraviolet	400 nm–1 nm	10^{15}–10^{17}
Visible	750 nm–400 nm	$4–7.5 \times 10^{14}$
Near infrared	2.5 μm–750 nm	1×10^{14}–4×10^{14}
Infrared	25 μm–2.5 μm	10^{13}–10^{14}
Microwaves	1 mm–25 μm	3×10^{11}–10^{13}
Radio waves	>1 mm	$< 3 \times 10^{11}$

The velocity at which radiation is propagated through a medium is less than its velocity in vacuum. It depends upon the kind and concentration of atoms, ions or molecules present in the medium. Figure 2.4 shows various possibilities which might result when a beam of radiation strikes a substance. These are:

(a) The radiation may be transmitted with little absorption taking place, and therefore, without much energy loss.
(b) The direction of propagation of the beam may be altered by reflection, retraction, diffraction or scattering.
(c) The radiant energy may be absorbed in part or entirely by the substance.

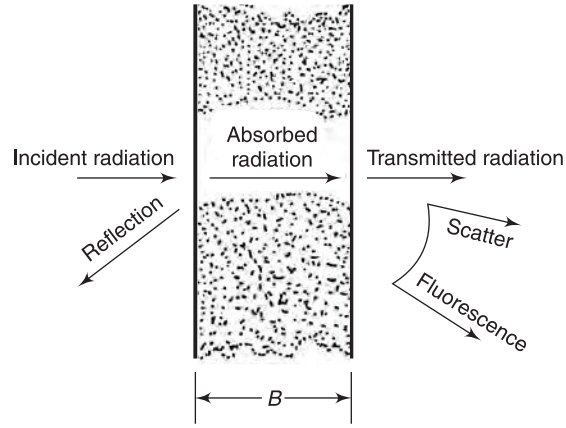


Figure 2.4 *Interaction of radiation with matter*

In absorption spectrophotometry, we are usually concerned with absorption and transmission. Generally, the conditions under which the sample is examined are selected to keep reflection and scattering to a minimum.

Absorption spectrophotometry is based on the principle that the amount of absorption that occurs is dependent on the number of molecules present in the absorbing material. Therefore, the intensity of the radiation leaving the substance may he used as an indication of the concentration of the material. The sample is usually examined in solution.

2.2 LAWS RELATING TO ABSORPTION OF RADIATION

2.2.1 Lambert's Law

Lambert's Law states that each layer of equal thickness of an absorbing medium absorbs an equal faction of the radiant energy that traverses it.

The proportion of incident light absorbed by a given thickness of the absorbing medium is independent of the intensity of the incident light, provided that there is no other physical or chemical change to the medium.

Let us suppose, I_o is the incident radiant energy and I is the energy which is transmitted. The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample is known as the transmittance. Lambert's Law is expressed as:

$$\text{Transmittance } T = I/I_o$$

It is customary to express transmittance as a percentage

$$\% \text{ Transmittance} = I/I_o \times 100$$

The logarithm to the base of the reciprocal of the transmittance is known as absorbance.

$$\text{Absorbance} = \log_{10}(1/T) = \log_{10}(I_o/I)$$
$$\text{Optical density} = \log_{10}(100/T)$$

It can be immediately seen that to determine the concentration of an unknown sample the percentage transmittance of a series of solutions of known concentration or 'standards' can be plotted and the concentration or the unknown read from the graph. It will be found that the graph is an exponential function which is obviously inconvenient for easy interpolation.

2.2.2 Beer's Law

This law states that the absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium in the light path.

2.2.3 The Beer-Lambert Law

A combination of the two laws, known jointly as the Beer-Lambert Law defies the relationship between absorbance (A) and transmittance (T). It states that the concentration of a substance in solution is directly proportional to the 'absorbance', A, of the solution.

$$\text{Absorbance } A = \varepsilon\, c\, b$$

Where

A = absorbance (no unit of measurement)
ε = molar absorptivity ($dm^3\ mol^{-1}\ cm^{-1}$)
c = molar concentration ($mol\ dm^{-3}$)
b = path length (cm)

It may be noted that ε is a function of wavelength. So, the Beer-Lambert Law is true only for light of a single wavelength or monochromatic light. Absorptivity is a constant, depending upon the wavelength of the radiation and nature of the absorbing material. Absorptivity is also sometimes referred to as specific extinction and absorbance as 'Optical Density'. Absorbance is the property of a sample, whereas absorptivity is the property of a substance and is a constant.

Mathematically, absorbance is related to percentage transmittance T by the expression:

$$A = \log_{10} (I_o/I) = \log_{10} (100/T) = \varepsilon\, b\, c$$

The relationship between energy absorption and concentration is of great importance to analysis. The amount of monochromatic radiant energy absorbed or transmitted by a solution is an exponential function of concentration of the absorbing substance present in the path of radiant energy. This means that successive equal thickness of a homogenous absorbing medium will reduce the intensity by successive equal fraction and, therefore, radiant energy will diminish in geometric or exponential progression. In other words, if a particular thickness absorbs half the radiant energy, the thickness which follows the first and is equal to it will not absorb the entire second half, but only a half of this half and will consequently reduce it to one quarter.

Consider a condition when three samples (standard solutions) having identical absorption are introduced in a beam of monochromatic light. Each of the samples is chosen so that precisely one-half of the intensity of the incident radiation is transmitted (T = 50%). If the intensity of the incident radiation is 100% T, then their intensity after each sample will be:

After sample $S_1 = 1 \times 0.5 = 50\%T$
After sample $S_2 = 50\% \times 0.5 = 25\%T$
After sample $S_3 = 25\% \times 0.5 = 12.5\ \%T$.

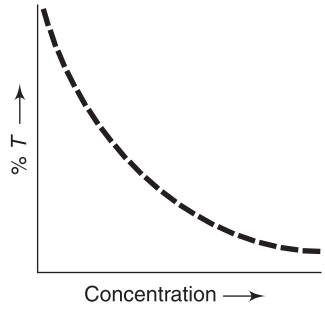


Figure 2.5 *Transmission plotted against concentration*

If we plot the concentration against transmission for the above samples, it will found that the resultant graph is exponential as illustrated in Figure 2.5.

However, the expression relating to absorbance 'A' to transmittance 'T' (A = log 100/T) shows that the absorbance after each sample will be

After sample $S_1 = 0.301$
After sample $S_2 = 0.602$
After sample $S_3 = 0.903$

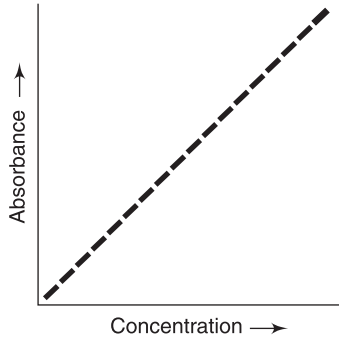


Figure 2.6 *Absorbance plotted against concentration*

By plotting the absorbance against concentration, it will be seen that the plot is linear (Figure 2.6). It is, therefore, more convenient to express results in absorbance rather than transmission when measuring unknown concentrations since linear calibration plots will be available.

An alternative to plotting calibration curves is to make use of the relationship:

$$C = k\, A$$

Where C = the concentration of the unknown
A = The measured absorbance of the unknown
And k = is factor derived from the reference or standard solution

In order to determine k, the absorbance of a standard solution of known concentration is measured and the concentration is divided by the absorbance.

$$K = \frac{\text{Concentration}\,(\text{standard})}{\text{Absorbance}\,(\text{standard})}$$

The factor k may be applied to a series of absorbance measurements on similar solution measured in the same conditions to give results directly in concentration.

In modern instruments, the output electronics provide the means of entering the concentration value of the standard or the factor to the calculation so that instrument readings are directly in concentration units.

$E^{1\%}_{1\text{ cm}}$ = The value of ε for a 1% sample concentration of 1 cm thickness.
ε = The molar extinction coefficient (i.e. the value of ε for a sample concentration of 1 g molecule per litre and 1 cm thickness).

The relationship between transmittance and absorbance as marked on the scales of anlog indicating metres is shown in Figure 2.7.

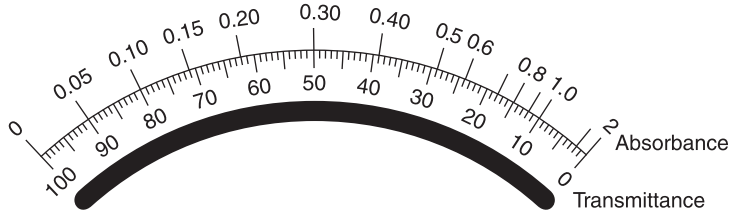


Figure 2.7 *Absorbance and transmittance scale*

2.2.4 Deviations from Beer's Law

Beer's Law only describes the relationship between absorbance, thickness and concentration. It does not imply that these are the only factors that affect absorbance. The direct, linear relationship between absorbance and concentration is used as a fundamental test of a system's conformity to the combined laws. A straight line passing through the origin indicates conformity to the Beer's Law. Discrepancies are usually found when the absorbing solute dissociates or associates in solution.

Beer's Law is derived by assuming that the beam of radiation is monochromatic. However, in all photometers and most of the spectrophotometers, a finite bandwidth of frequencies is always present. The wider the bandwidth of radiation passed by the filters or dispersing device, the greater is the deviation of a system from adherence to Beer's Law.

Beer's Law is a limiting law and should be expected to apply only at low concentrations. It has been observed that the deviation becomes evident at higher concentrations (Figure 2.8) on an absorbance versus concentration plot, when the curve bends towards the concentration axis.

The linearity of the Beer-Lambert Law is limited by chemical and instrumental factors. Causes of non-linearity include the following:

- Deviations in absorptivity coefficients at high concentrations (>0.01 M) due to electrostatic interactions between molecules in close proximity.
- Scattering of light due to particulates in the sample.
- Fluorescence or phosphorescence of the sample.
- Changes in refractive index at high analyte concentration.
- Shifts in chemical equilibrium as a function of concentration.
- Non-monochromatic radiation, deviations can be minimised by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band.
- Stray light.

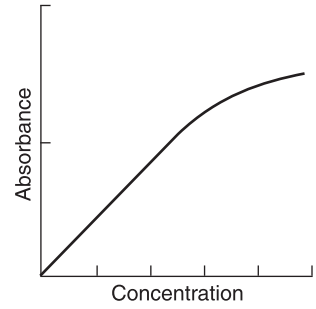


Figure 2.8 *Representation of Beer's Law and deviations*

2.2.5 Quantitative Analysis

The most usually employed quantitative method consists of comparing the extent of absorption or transmittance of radiant energy at a particular wavelength by a solution of the test material and a series of standard solutions. It can be done with visual colour comparators, photometers or spectrophotometers.

For quantitative analysis, it is normally chosen to use radiation of a wavelength at which k, the extinction coefficient, is a maximum (i.e. at the peak of the absorption band) for the following reasons:

- The change in absorbance for a given concentration change is greater, leading to greater sensitivity and accuracy in measurement
- The relative effect of other substances or impurities is smaller.
- The rate of change of absorbance with wavelength is smaller, and the measurement is not so severely affected by small errors in the wavelength setting

In order to carry out an analysis of an unknown material, it should be possible in theory to measure the absorption of a sample at its absorption maximum, measure the thickness, obtain values of ε from tables and calculate the concentration. In practice, values of ε depend on instrumental characteristics, so published values from tables are not accurate enough, and a reliable analyst will usually plot the absorbance values of a series of standards of known concentration; the concentrations of actual samples can then be read directly from the calibration graph.

Using today's modern software driven instruments, the analyst will usually only require one standard which can be used to calibrate the instrument so that it displays the concentration of subsequent samples directly in the required units. If the calibration curve is non-linear due to either instrumental or chemistry considerations, then some instruments will automatically construct a 'best fit' calibration when presented with a number of standards.

2.2.6 Choice of Wavelength

The selection of a suitable wavelength in the spectrum for quantitative analysis of a sample can he made during the course of preparing the calibration curve for the unknown material. The calibration curve is plotted to show the absorbance values of a series of standards of known concentration

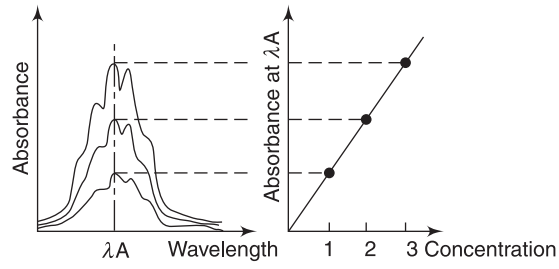


Figure 2.9 *Absorption spectra and Beer-Lambert calibration*

and the concentrations of actual samples can then be read directly as shown in Figure 2.9. A series of standard solutions is prepared along with a blank. Using one filter at a time, calibration curves are plotted in terms of absorbance versus concentration. The filter which provides closest adherence to linearity over the widest absorbance interval and which yields the largest slope with a zero intercept will constitute the best choice for analysis. In a spectrophotometer, the wavelength of maximum absorbance is readily ascertained from the absorbance wavelength curve for the material.

2.2.7 Simultaneous Spectrophotometric Determination

It is often found in practice that when there are several components which absorb radiation of the same wavelength, their absorbances add together and it would no longer be true that the absorbance of the sample is proportional to the concentration of one component. If there is no reaction or interaction between the different solutes, the absorbances are additive of all the components at a given frequency. The Beer-Lambert Law can then be written as follows:

$$A = a_1bc_1 + a_2bc_2 + a_3bc_3 + \ldots + a_nbc_n$$

where the subscripts refer to the respective components. While determining the various values for a, if the cell thickness b is held constant, the b may be included in the a. Hence this is an equation with *nc's*, when n compounds are present. If n such equations were determined from values of the absorbance at n different frequencies, n simultaneous linear equations would result, which may be solved to find the required concentrations. Certainly, the procedure is difficult, and in the presence of many components, one would really need a computer to solve the equations. However, much of the classical work in the analysis of petroleum fractions was carried out in this way. The method is not preferred if there are more than two or three components absorbing radiation of the same wavelength.

The multi-wavelength or multi-components analysis technique has been a resurgence of interest over the last few years. This has been due to data processing techniques. A variety of algorithms is available and the analyst is generally required to input the number of components, measurement wavelengths and concentration values of the standards. Having measured the standards, samples can be processed and results presented appropriately. Programs of this type, including measurement parameters, can then be stored on disk and recalled and run with a minimum of operator intervention, providing results on complex mixtures containing up to ten components.

A simplified and more common method is to convert the component under analysis, by adding a chemical reagent which specifically reacts with it to form a highly absorbing compound. The addition of this reagent to the mixture would result in change of the wavelength of the absorption maxima, so that there is no longer interference among the components. The analysis then becomes very simple.

2.3 ABSORPTION INSTRUMENTS

Figure 2.10 shows an arrangement of components of an absorption instrument. These essential components are:

- A source of radiant energy, which may be a tungsten lamp, xenon-mercury arc, hydrogen or deuterium-discharge lamp, etc.
- Filtering arrangement for selection of a narrow band of radiant energy. It could be a single wavelength absorption filter, interference filter, a prism or a diffraction grating.
- An optical system for producing a parallel beam of filtered light for passage through an absorption cell (cuvette). The system may include lenses, mirrors, slits, diaphragm, etc.
- A detecting system for measurement of unabsorbed radiant energy, which could be human eye, barrier-layer cell, phototube or photomultiplier tube.
- A readout system or display, which may be an indicating metre or numerical display.

Generally, the components are selected in consonance with their intended use. Figure 2.11 shows optical characteristics of various optical components and their range of suitability in the electromagnetic spectrum.

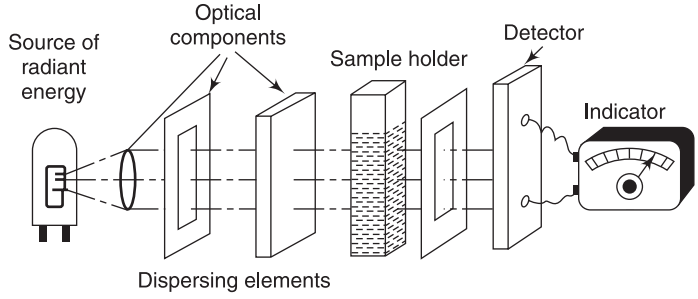


Figure 2.10 *Various components of an absorption instrument*

2.3.1 Radiation Sources

The function of the radiation source is to provide sufficient intensity of light suitable for making a measurement. The most common and convenient source of light is the tungsten lamp. Lamps convert electrical energy into radiation. Different designs and materials are needed to produce light in different parts of the electromagnetic spectrum. The following sections describe several different types of lamps that are useful in spectroscopy.

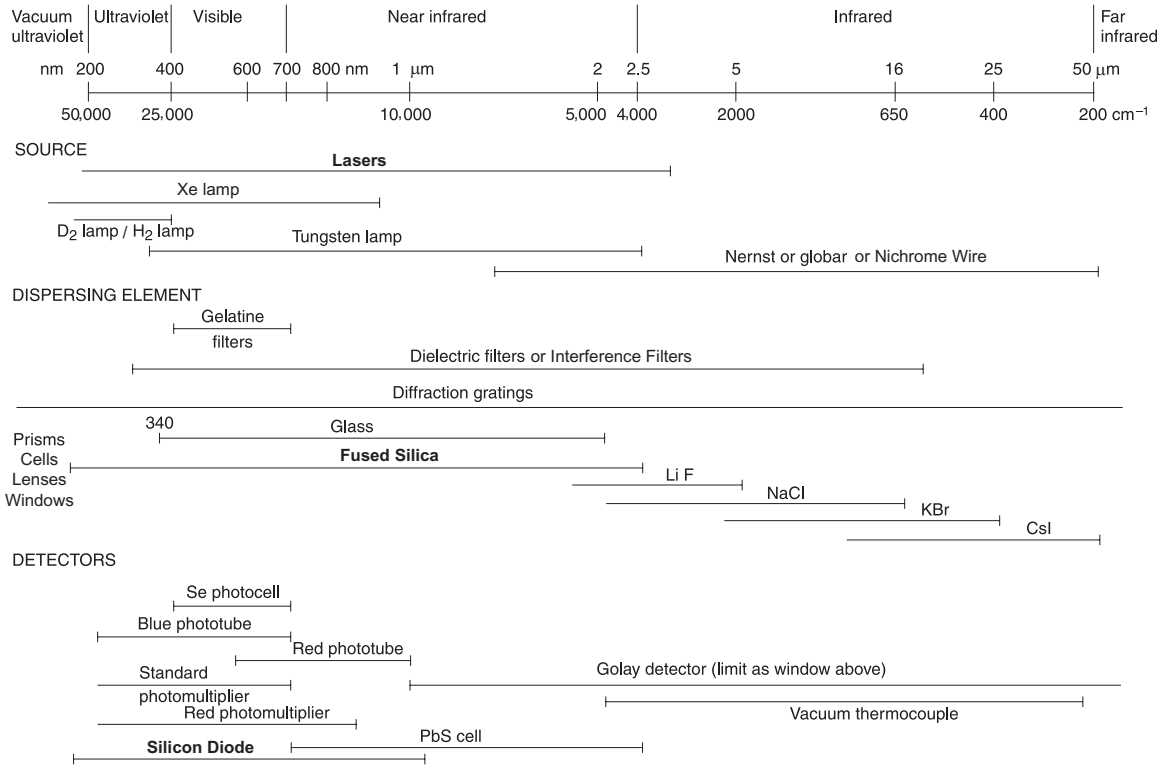


Figure 2.11 *Spectral characteristics of various optical components and their range of suitability in the electromagnetic spectrum*

2.3.1.1 Blackbody sources

A hot material, such as an electrically heated filament in a light bulb, emits a continuum spectrum of light. The spectrum is approximated by Planck's radiation law for blackbody radiators:

$$B = \{2h\upsilon^3/c^2\}\{1/\exp(h\upsilon/kT)-1\}$$

Where h is Planck's constant, υ is frequency, c is the speed of light, k is the Boltzmann constant, and T is temperature in K.

The most common incandescent lamps and their wavelength ranges are:

Tungsten filament lamps	: 350 nm–2.5 μm
Glowbar	: 1–40 μm
Nernst glower	: 400 nm–20 μm

Tungsten lamps are used in visible and near infrared (NIR) absorption spectroscopy and the glowbar, and Nernst glower are used for infrared spectroscopy.

This lamp consists of a tungsten filament (Figure 2.12a) enclosed in a glass envelope. It is cheap, intense and reliable. A major portion of the energy emitted by a tungsten lamp is in the visible

region and only about 15–20% is in the infrared region. When using a tungsten lamp, it is desirable to use a heat absorbing filter between the lamp and the sample holder to absorb most of the infrared radiation without seriously diminishing energy at the desired wavelength. For work in the UV region, a hydrogen or deuterium-discharge lamp is used.

In these lamps, the envelope material of the lamp puts a limit on the smallest wavelength, which can be transmitted. For example, quartz is suitable only up to 200 nm and fused silica up to 185 nm. The radiation from the discharge lamps is concentrated into narrow wavelength regions of emission lines. Practically, there is no emission beyond 400 nm in these lamps. For this reason, spectrophotometers for both the visible and UV regions always have two light sources, which can be manually selected for appropriate wavelength.

For work in the infrared region, a tungsten lamp may be used. However, due to high absorption of the glass envelope and the presence of unwanted emission in the visible range, tungsten lamps are not preferred. In such cases, Nernst filaments or other sources of similar type are preferred. They are operated at lower temperatures and still radiate sufficient energy.

The radiation from hot solids is made up of many wavelengths and the energy emitted at any particular wavelength depends largely on the temperature of the solid and is predictable from probability theory. The curves in Figure 2.12b show the energy distribution for a tungsten filament at three different temperatures. Such radiation is known as 'black body radiation'. Note how the emitted energy increases with temperature and how the wavelength of maximum energy shifts to shorter wavelengths. More recently it has become common practice to use a variant of this – the tungsten-halogen lamp. The quartz envelope transmits radiation well into the UV region. For the UV region itself, the most common source is the deuterium lamp and a UV-Visible spectrophotometer will usually have both lamp types to cover the entire wavelength range.

Figure 2.12a *A tungsten lamp of the type used in absorption instruments (Courtesy: M/s Thomson Higher Education)*

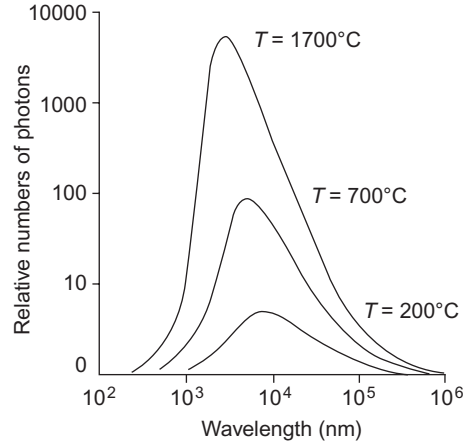


Figure 2.12b *Tungsten filament radiation characteristics*

The introduction of the tungsten-halogen light source has a higher intensity output than the normal tungsten lamp in the changeover region 320–380 nm used in colorimetry and spectrophotometry. It also has a larger life and does not suffer from blackening of the bulb glass envelope. In the UV region of the spectrum, the deuterium lamp has superseded the hydrogen discharge lamp as a UV source. The radiation sources should be highly stable and preferably emit out a continuous spectrum.

A combination of deuterium tungsten-halogen light source combines the continuous spectrum of a deuterium UV light source and a tungsten-halogen VIS/Shortwave NIR light source in a single optical path. The combined light source produces a powerful, stable output from ~200–1,100 nm. It

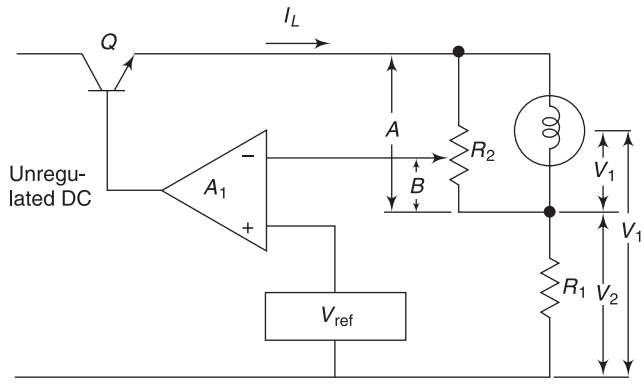


Figure 2.13 *Schematic diagram of a lamp regulator*

however, requires a highly stabilised microprocessor-based power supply designed for optimum stability.

***Lamp Regulator*:** A simplified circuit diagram of a lamp regulator that delivers constant power to a tungsten lamp is shown in Figure 2.13. A part of the voltage across the lamp is summed up with a voltage proportional to the current through the lamp. This total voltage V_f is compared with a reference voltage V_{ref} by operational amplifier A_1. A_1 controls the voltage at the emitter of transistor Q, such that V_f will always be equal to V_{ref}.

$$V_f = V_{ref} = V_1 + V_2$$

The voltage V_f can be written in terms of lamp current (I_L) and filament resistance R_f.

$$V_f = B/A.\ I_L.\ R_f + I_L.\ R_1$$

where B/*A* is the ratio corresponding to the setting of potentiometer R_2.R_1 is the current sensing resistor.

The ratio B/*A* can be selected, so that constant power is delivered for a particular type of lamp. The power delivered to the lamp can be varied by adjusting the reference voltage.

2.3.1.2 Discharge lamp

Discharge lamps, such as neon signs, pass an electric current through a rare gas or metal vapour to produce light. The electrons collide with gas atoms, exciting them to higher energy levels which then decay to lower levels by emitting light. Low pressure lamps have sharp line emission characteristic of the atoms in the lamp, and high pressure lamps have broadened lines superimposed on a continuum spectrum.

Common discharge lamps and their wavelength ranges are given below:

Hydrogen or deuterium: 160–360 nm
Mercury: 253.7 nm, and weaker lines in the near UV and visible
Ne, Ar, Kr, Xe discharge lamps: many sharp lines throughout the near UV to near-lR
Xenon arc: 300 – 13 nm

Deuterium lamps (Figure 2.14a) are the UV source in UV-Vis absorption spectrophotometers. The sharp lines of the mercury and rare gas discharge lamps are useful for wavelength calibration of optical instrumentation. For fluorescent work, an intense beam of UV light is required. This requirement is met by a Xenon arc or a mercury vapour lamp. Cooling arrangement is very necessary when these types of lamps are used.

Mercury lamps are usually run direct from the AC power line via a series ballast choke. This method gives some inherent lamp power stabilisation and automatically provides the necessary ionising voltage. The ballast choke is physically small and a fast warm-up to the lamp operating temperature is obtained.

A deuterium arc lamp provides emission of high intensity and adequate continuity in the 190–380 nm range. A quartz or silica envelope is necessary not only to provide heat shield, but also to transmit the shorter wavelengths of the UV radiation. The limiting factor is normally the lower limit of atmospheric transmission at about 190 nm. Figure 2.14b shows the energy output as a function of wavelength in case of deuterium arc lamp and tungsten-halogen lamp. Wilson (1985) describes on circuit for a stable constant current – supply for a deuterium lamp.

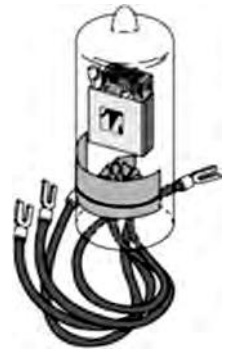


Figure 2.14a *A deuterium lamp of the type used in spectrophotometers (Courtesy : M/s Thomson Higher Education)*

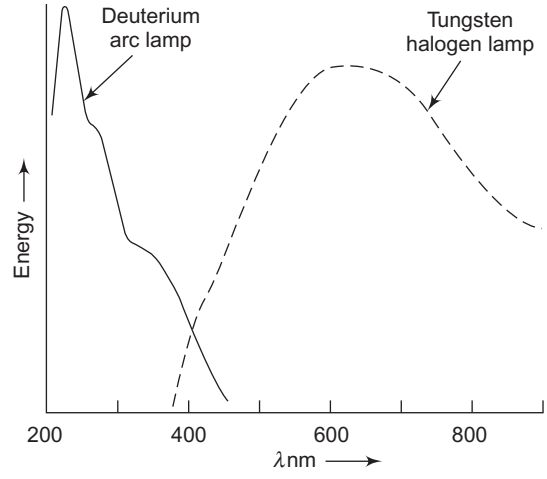


Figure 2.14b *Energy output as a function of wavelength for deuterium arc lamp and tungsten halogen lamp*

In the modern spectrophotometers, the power supply arrangements including any necessary start-up sequences for arc lamps, as well as changeover between sources at the appropriate wavelength are automatic mechanical sequences. Lamps are generally supplied on pre-set focus mounts or incorporate simple adjustment mechanisms for easy replacement.

2.3.1.3 Lasers

The term laser has been coined by taking the first letters of the expression 'Light amplification by simulated emission of radiation'. Although an amplifier, as suggested by the abbreviation, the laser is invariably used as a generator of light. But its light is quite unlike the output of conventional source of light. The laser beam has spatial and temporal coherence, and is monochromatic (pure wavelength). The beam is highly directional and exhibits high density energy which can be finely focused.

The laser is acquiring an increasingly important role in analytical chemistry and it is important for the practicing analyst to have an appreciation of both its capabilities and its problems (Wright and Wirth, 1980a).

Lasers have a number of advantages over conventional sources such as glowbars, continuous discharges, pulsed discharges, and X-ray tubes that make them useful for analytical chemistry applications (Morris, 1992). These advantages include higher intensity, monochromaticity, low beam divergence, the availability of short and ultrashort pulses for studies of transient phenomena, and coherence (well-defined phase).

Lasers have been used in many spectroscopic applications such as Raman Spectroscopy (West, 1984). However, there is little the laser can offer for UV/visible spectrophotometry. Perhaps, a high peak power will allow a measurable amount of light to get through an optically dense sample so high absorbance can be measured.

Another advantage of the highly collimated beam of a laser is to measure absorption over a very long path length. Both of these situations are specialised. Wright and Wirth (1980b) explain the principles of working of different types of layer such as solid, gas and liquid lasers.

2.3.1.4 Light Emitting Diodes (LEDs)

The development of LEDs has resulted in the appearance of a new optical light source in analytical instrumentation. The first LED was developed in 1962 based on GaAsP layers, which emitted red light. Significant advances in III-V nitride manufacturing processes have resulted in high power commercially available LEDs in the region of 247–1,550 nm, covering UV, visible and near infrared regions.

LEDs offer a number of advantages compared to existing light sources in optoelectronic applications. These include increased lifetime, low cost, reduced power consumption, higher brightness, rugged construction, flexible configuration, enhanced spectral purity, small size, and breadth of spectral range.

The most popular type of tri-colour LED has a red and a green LED combined in one package with three leads. When both the red and green LEDs are turned on, the LED appears to be yellow.

Figure 2.15 shows the construction of a tri-colour LED. The centre lead (K) is the common cathode for both LEDs, the outer leads (A_1 and A_2) are the anodes to the LEDs allowing each one to be lit separately, or both together to give the third colour. The use of bi-/tri- colour LEDs can provide a compact rugged multi-wavelength photometer source that can facilitate multi-component analysis (O'Toole and Diamond, 2008).

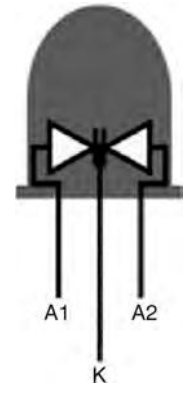


Figure 2.15 *Schematic of Tri-colour LED*

2.3.2 Optical Filters

A filter may be considered as any transparent medium which by its structure, composition or colour enables isolation of radiation of a particular wavelength. For this purpose, ideal filters should be monochromatic (i.e. they must isolate radiation of only one wavelength). A filter must meet the following two requirements:

(a) high transmittance at the desired wavelength and
(b) low transmittance at other wavelengths

However, in practice, the filters transmit a broad region of the spectrum. Referring to Figure 2.16, they are characterised by the relative light transmission at the maximum of the curve T_λ the width of the spectral region transmitted (the half-width – the range of wavelength between the two points on the transmission curve at which the transmission value equal $1/2\ T_\lambda$) and T_{res}, (the residual value of the transmission in the remaining part of the spectrum). The ideal filter would have the highest value of T_λ and the lowest values for the transmission half-width and T_{res}.

Filters can be broadly classified as absorption filters and interference filters.

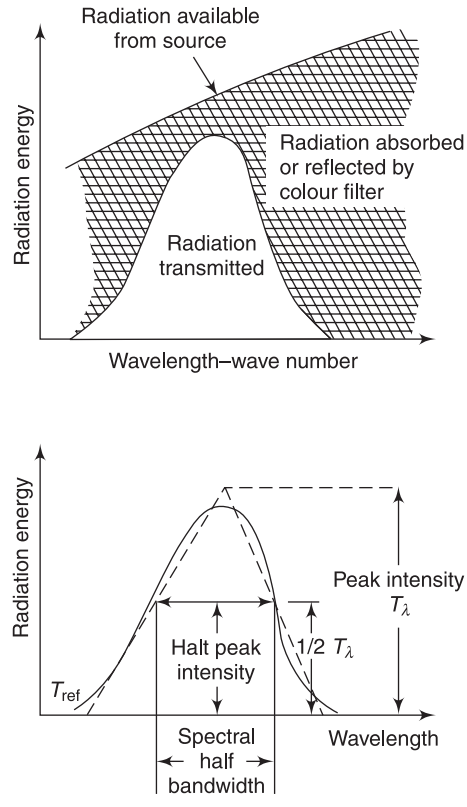


Figure 2.16 *Optical properties of a light filter*

2.3.2.1 Absorption filters

The absorption type optical filter usually consists of colour media: colour glasses, coloured films (gelatine, etc.), and solutions of the coloured substances. This type of filter has wide spectral bandwidth, which may be 40–50 μ in width at one-half the maximum transmittance. Their efficiency of transmission is very poor and is of the order of 5–25%.

It is possible to obtain more selective light filters from coloured media by increasing their thickness two, three or more times. Here the transmission of the filter for light of the wavelength isolated is decreased, but there is a simultaneous increase in the selectivity. By using this technique, it is theoretically possible to achieve a very good selectivity, but the fall in transmission efficiency would have to be compensated by suitable amplification of the photocurrent. As absorption type filters do not provide a high degree of monochromaticity required for isolating complex systems, their use is restricted to only very simple type of photometers.

Composite filters consisting of sets of unit filters are often used. In the combination, one set consists of long wavelength, sharp cut-off filters and the other of short wavelength, cut-off filters; combinations are available from about 360–700 nm.

The glass filter consists of a solid sheet of glass that has been coloured with a pigment, which is either dissolved or dispersed in glass; whereas the gelatine filter consists of a layer of gelatine impregnated with suitable organic dyes and sandwiched between two sheets of glass. Gelatine filters are not suitable for use over long periods. With the absorption of heat, they tend to deteriorate due to changes in the gelatine and bleaching of the dye.

2.3.2.2 Interference filters

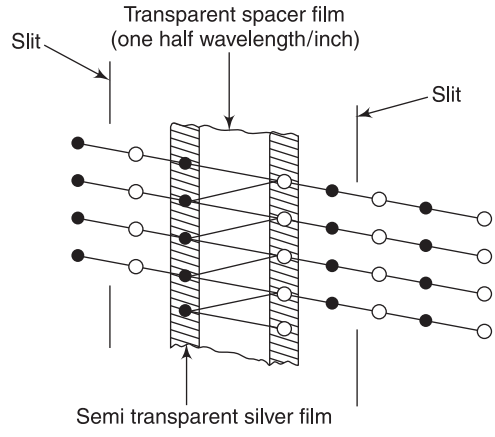


Figure 2.17 *Path of light rays through an interference filter*

Interference filters usually consist of two semi-transparent layers of silver, deposited on glass by evaporation in vacuum and separated by a layer of dielectric (ZnS or MgF_2). In this arrangement, the semi-transparent layers are held very close. The spacer layer is made of a substance which is of low refractive index. The thickness of the dielectric layer determines the wavelength transmitted. Figure 2.17 shows the path of light rays through an interference filter. Some part of light that is transmitted by the first film is reflected by the second film and again reflected on the inner face of the first film, as the thickness of the intermediate layer is one-half a wavelength of a desired peak wavelength. Only light which is reflected twice will be in phase and come out of the filter, other wavelengths with phase differences would cause destructive interference. Constructive interference between different pairs in superposed light rays occurs only when the path difference is exactly one wavelength or some multiple thereof. The relationship expressing a maximum for the transmission of a spectral band is given by

$$m\lambda = 2d(n) \sin \theta$$

when light is incident normally, $\sin \theta = 1$

$$m\lambda = 2d(n)$$

where d is the thickness of the dielectric spacer, whose refractive index is n. The multiple of frequencies harmonically related to the wavelength of the first order rays is the order *(m)* of the interference.

Interference filters allow a much narrower band of wavelengths to pass and are similar to monochromators in selectivity. They are simpler and less expensive. However, as the selectivity increases, the transmittance decreases. The transmittance of these filters varies between 15 and 60% with spectral bandwidth of 10–15 nm

One type in interference filters is the continuous wedge filter, which permits a continuous selection of different wavelengths. The continuity of an interference filter is achieved by using a spacer film of graded thickness between the two semi-transparent layers of silver. They usually have a working interval of 400–700 nm and a dispersion of 5.5 nm/mm. Transmittance is usually not more than 35%. With less transmittance, the sensitivity gets lower, which may be compensated by using electronic amplifiers after the photodetectors.

For efficient transmission, multi-layer transmission filters are often used. They are characterised by a bandpass width of 8 nm or less and a peak transmittance of 60–95%. Interference filters can be used with high intensity light sources, since they remove unwanted radiation by transmission and reflection, rather than by absorption.

2.3.3 Monochromators

Monochromators are the optical systems, which provide better isolation of spectral energy than the optical filters, and are therefore preferred where it is required to isolate narrow bands of radiant energy. Monochromators usually incorporate a small glass of quartz prism or a diffraction grating system as dispersing media. The radiation from a light source is passed either directly or by means of a lens or mirror into the narrow slit of the monochromator and allowed to fall on the dispersing medium, where it gets isolated. The efficiency of such monochromators is much better than that of filters and spectral half-bandwidths of 1 mμ or less are obtainable in the UV and visible regions of the spectrum.

2.3.3.1 Prism monochromators

Isolation of different wavelengths in a prism monochromator depends upon the fact that the refractive index of materials is different for radiation of different wavelengths. If a parallel beam of radiation falls on a prism, the radiation of two different wavelengths will be bent through different angles. The greater the difference between these angles, the easier it is to isolate the two wavelengths. This becomes an important consideration for selection of material for the prisms, because only those materials are selected whose refractive index changes sharply with wavelength.

Figure 2.18 shows the use of a prism as a monochromator. Light from the source S is made into a parallel beam and made to fall on a prism after it is passed through entrance slit S_1 and mirror M_1. The entrance silt is at the focus of mirror M_1. The prism disperses the light and photons of different wavelengths are deflected at different angles. If the dispersed beam is again refocused, the focal point for photons of one wavelength will be displaced from that for photons of a different wavelength. The light of any one wavelength can be selected by moving a slit across the focal plane. The required wavelength passes through the slit, the other wavelengths are blocked. The optical arrangement used in practice may differ from that illustrated in this figure, but the principle remains the same. In most of the cases, the prism is moved to shift the spectrum across the exit slit, rather than the slit being moved across the focal plane. The same collimating mirror is used for both M_1 and M_2 to save costs of high grade optical components. The instrument manuals can be consulted to know the alternative systems.

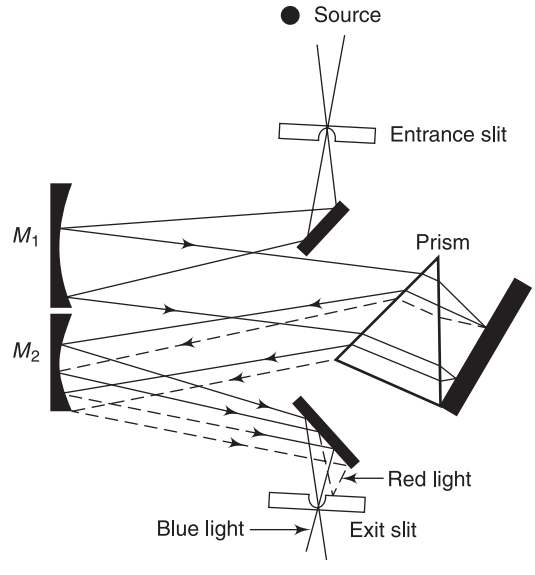


Figure 2.18 *Prism monochromator*

Prism may be made of glass or quartz. The glass prisms are suitable for radiations essentially in the visible range whereas the quartz prism can cover the UV spectrum also. It is found that the dispersion given by glass is about three times that of quartz. However, quartz shows the property of double refraction. Therefore, two pieces of quartz, one right handed and one left handed are taken and cemented back to back in

the construction of 60° prism (Cornu mounting), or the energy must be reflected and returned through a single 30° prism, so that it passes through the prism in both directions (Littrow mounting). The two surfaces of the prism must be carefully polished and optically flat. Prism spectrometers are usually expensive, because of exacting requirements and difficulty in getting quartz of suitable dimensions.

There are several ways of selecting a particular wavelength in prism monochromators. It may be chosen by local selection with movable exist slits, or by local selection with fixed slits, behind which are placed as many photosensitive elements as there are slits. The selection can also be achieved by prism rotation, in which all the lines of the spectrum are passed through a fixed slit one after the other. The wavelength scale in this case is non-linear.

2.3.3.2 Diffraction gratings

Monochromators may also make use of diffraction gratings as dispersing medium. A diffraction grating consists of a series of parallel grooves ruled on a highly polished reflecting surface. When the grating is put into parallel radiation beam, so that one surface of the grating is illuminated, this surface acts as a very narrow mirror. The reflected radiation from this grooved mirror overlaps the radiation from neighbouring grooves as shown in Figure 2.19.

The waves would, therefore, interfere with each other. On the other hand, it could be that the wavelength of radiation is such that the separation of the grooves in the direction of the radiation is a whole number of wavelengths. Then the waves would be in phase and the radiation would be reflected undisturbed. When this is not a whole number of wavelengths, there would be destructive interference and the waves would cancel out and no radiation would be reflected. By changing the angle at which the radiation strikes the grating, it is possible to alter the wavelength reflected.

The expression relating the wavelength of the radiation and the angle (θ) at which it is reflected is given by

$$m\,\lambda = 2d \sin\theta$$

where d is the distance separating the grooves and is known as the grating constant and m is the order of interference.

when $m = 1$, the spectrum is known as first order and with $m = 2$, the spectrum is known as second order.

The resolving power of a grating is determined by the product mN, where N is the total number of grooves or lines on the grating. Higher dispersion in the first order is possible when there are a larger numbers of lines. When compared with prisms, the gratings provide much higher resolving powers and can be used in all spectral regions. Gratings would reflect, at any given angle, radiation of wavelength λ and also $\lambda/2$, $\lambda/3$, etc. This unwanted radiation must be removed with filters or pre-monochromators, otherwise it will appear as stray light.

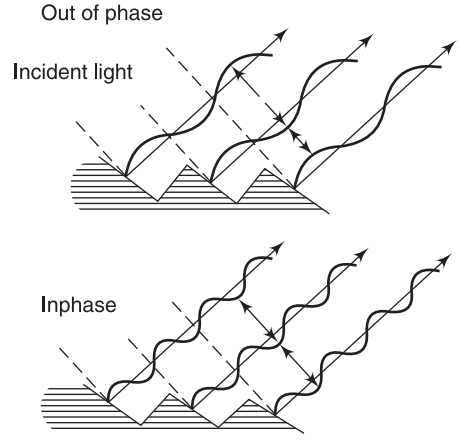


Figure 2.19 *Dispersion phenomenon in diffraction gratings*

Figure 2.20 shows a typical diffraction grating monochromator. The entrance slit is illuminated by the light source, and light from the slit is focused to a parallel beam by the collimating mirror, this beam being incident on the grating. The grating is rotated to diffract light of the required wavelength on to the focusing mirror, which in turn focuses it on to the exit slit.

Good monochromator design depends on ensuring that the mirrors and dispersing elements are of high quality with little scatter and that scattered light is minimised by baffling, so that it cannot reach the exit slit. In addition the light can be diffracted twice or more by the grating on reflection from the mirrors. Higher order spectra are usually removed by suitable filters.

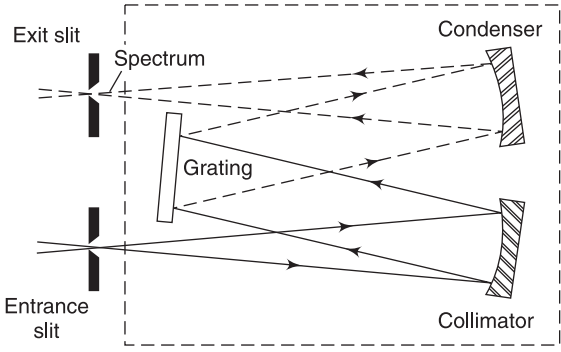


Figure 2.20 *Use of diffraction grating in a monochromator*

Most modern instruments now use a diffraction grating as a dispersing element in the monochromator, as prisms in general have a poorer stray-light performance and require complex precision cams to give a linear wavelength scale. Replica gratings can even be produced more cheaply than prisms and require only a simple sine-bar mechanism for the wavelength scale.

A typical reflection grating may have 1,200 grooves/mm, which means the grooves are spaced at about 800 nm intervals. The grating may have a width of 20 mm or more, giving a total of at least 24,000) grooves. To obtain constructive interference across this number of grooves with little light scattering, the spacing and form of the grooves must be accurate to within a few nano-metres to give a high quality grating. Mechanical diamond ruling engines to give a high quality grating near to this accuracy is one of the difficult technological problems.

2.3.3.3 Holographic gratings

Precision spectrophotometers use holographic or interference gratings, which have superior performance in reducing stray light as compared to diffraction gratings. Holographic gratings are made by first coating a glass substrate with a layer of photo resist, which is then exposed to interference fringes generated by the intersection of two collimated beams of laser light. When the photo resist is developed, it gives a surface pattern of parallel grooves. When coated with aluminium, this becomes diffraction gratings.

As compared to ruled gratings, the grooves of a holographic grating are more uniformly spaced, smoothly and uniformly shaped. These characteristics result in much lower stay light levels. Moreover, the holographic gratings can be produced in much less time than the ruled grating. Holographic gratings used in commercial spectrophotometers are either original master gratings produced directly by an interferometer or replica gratings. Replica gratings are reproduced from a master holographic grating by moulding its grooves onto a resin surface on a glass or silica substrate. Both types of gratings are coated with an aluminium reflecting surface and finally with a protective layer of silica or magnesium fluoride. Replica gratings give performance, which are as good as master gratings. The holographic process is capable of producing gratings that almost reach the theoretical stray-light minimum.

2.3.4 Optical Components

Several different types of optical components are used in the construction of analytical instruments based on the radiation absorption principle. They could be windows, mirrors and simple condensers. The material used in the construction of these components is a critical factor and depends largely on the range of wavelength of interest. Normally, the absorbance of any material should be less than 0.2 at the wavelength of use.

The following factors need to be considered while selecting optical components:

- Ordinary silicate glasses are satisfactory from 350 to 3,000 nm.
- From 300 to 350 nm, special corex glass can be used
- Below 300 nm, quartz or fused silica is utilised, the limit for quartz is 210 nm
- From 180 to 210 nm fused silica can be used, provided the monochromator is flushed with nitrogen or argon to eliminate absorption by atmospheric oxygen.

Reflections from glass surfaces are reduced by coating these with magnesium fluoride, which is one-quarter wavelength in optical thickness. With this, scattering effects are also greatly reduced. However, using a layer of magnesium fluoride over aluminium coating does not offer a satisfactory solution, as the layer is soft and has poor chemical resistance (Sharpe, 1984). It cannot be easily cleaned. A better solution is to use a silica or synthetic quartz coating, which is hard and chemically resistant. If they become dirty, they can be washed with a mild detergent and distilled water to restore the original high reflectance. The use of silica-coated aluminium mirrors ensures long *mirror* life with enhanced reflectance in the UV region and minimum deterioration of stray-light performance. Table 2.2 provides data on materials that are used for optical components in different parts of the electromagnetic spectrum.

Table 2.2 *Materials for optical components*

Region of the spectrum	Mirrors	Lenses	Windows
X-ray	–	–	Beryllium
Ultraviolet	Aluminium	Fused silica (synthetic quartz), sapphire	Fused silica, sapphire
Visible	Aluminium	Glass, sapphire	Glass, sapphire
Near infrared	Gold	Glass sapphire	Glass, sapphire
Infrared	Copper, gold	CaF_2, ZnSe	NaCl, BaF_2, CaF_2, SnZe

In order to reduce the beam size or render the beam parallel, condensers are used. These condensers operate as simple microscopes. To minimise light losses, l*enses* are sometimes replaced by front-surfaced mirrors to focus or collimate light beam in absorption instruments. Mirrors are aluminised on their front surfaces. With the use of mirrors, chromatic aberrations and other imperfections of the lenses are minimised.

Beam splitters are used in double-beam instruments. These are made by giving a suitable multilayer coating on an optical flat. The two beams must retain the spectral properties of the incident

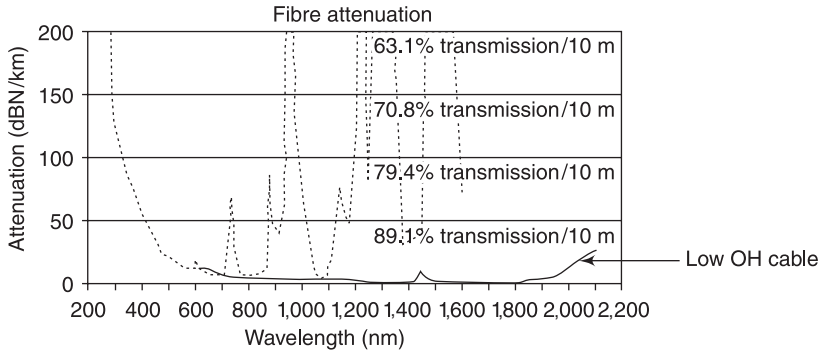


Figure 2.21 *Attenuation versus wavelength of light in a fibre optic cable*

beam. Half-silvered mirrors are often used for splitting the beam. However, they absorb some of the light in the thin metallic coating. Beam splitting can also be achieved by using a prismatic mirror or stack of thin horizontal glass plates, silvered on their edges and alternatively oriented to the incident beam.

Fibre Optics: Optical fibres are now being extensively used in spectroscopic applications to transmit light for quantitative measurements. The fibres used in most of the analysers have core diameters of 200–600 microns. They come in two types based upon their hydroxyl content.

Figure 2.21 shows fibre attenuation with respect to wavelength. Fibre attenuation is typically specified in decibels (dB) per kilometre. Most on-line applications use fibres less than 50 m; and 10 m is more typical.

Low-OH fibres (shown by the solid transmission spectrum) are used for visible and NIR applications from 400 to 2,500 nm. The transmittance of a typical 10 m Low-H fibre is 99.77%. Thus, for most applications, loss of light in the fibre optic cables is not a limiting factor. Other transmission values for 10 m fibres are also shown in the figure.

High-OH fibres are primarily used in the UV region, but can also be used in the visible and up to about 800 nm in the NIR. The high-OH content produces too much absorption of light at longer NIR wavelengths.

The fibre is protected inside the cable with polypropylene or Teflon tubing, which in turn is surrounded by a braided Aramid fibre mesh for strength. The protected fibre is often placed in PVC-covered monocoil jacket, which is a strip-wound metal tube that adds protection for the assembly.

Spectroscopic applications generally require a large diameter optical fibre to transmit sufficient amount of light for quantitative measurement. The fibres used most analysers have core diameters of 200–600 microns. Most analysers, including on-line instruments, use fibre less than 50 m, and 10 m is more typical. The transmittance of a typical 10 m cable used in visible and near infrared applications is 99.77%. Thus, for most applications, loss of light in the fibre optic cables is not a limiting factor.

2.3.5 Photosensitive Detectors

After isolation of radiation of a particular wavelength in a filter or a monochromator, it is essential to have a quantitative measure of their intensities. This is done by causing the radiation to fall on a photosensitive element, in which the light energy is convened into electrical energy. The electric

current produced by this element can be measured with a sensitive galvanometer directly or after suitable amplification.

Any type of photosensitive detector may be used for detection and measurement of radiant energy, provided it has a linear response in the spectral band of interest and has a sensitivity which is good enough for the particular application. There are two types of photoelectric cells: photovoltaic cells and photo-emissive cells.

2.3.5.1 Photovoltaic or barrier layer cells

Photovoltaic or barrier-layer cells usually consist of a semiconducting substance, which is generally selenium deposited on a metal base which may be iron and which acts as one of the electrodes. The semiconducting substance is covered with a thin layer of silver or gold deposited by cathodic deposition in vacuum. This layer acts as a collecting electrode. Figure 2.22 shows the construction of the barrier-layer cell. When radiant energy falls upon the semiconductor surface, it excites the electrons at the silver-selenium interface. The electrons are thus released and collected at the collector electrode.

Photovoltaic cells are very robust in construction and need no external electrical supply to work. The typical photocurrents produced by these cells are as high as 120 μA/lumen. At constant temperature, the current set up in the cell usually shows a linear relationship with the incident light intensity.

Selenium cells are sensitive to almost the entire range of wavelengths of the spectrum. However, their sensitivity is greater within the visible spectrum and highest in the zones near to the yellow wavelengths. Figure 2.23 shows spectral response of the selenium photocell and the human eye. The human eye can only detect radiation that is in the visible region of the spectrum, and hence the name.

Selenium cells have a high temperature coefficient and therefore, it is very necessary to allow the instrument to warm-up before the readings are commenced. They also show fatigue effects. When illuminated, the photocurrent rises to a value several percentages above the equilibrium value and then falls off gradually. Apart from selenium, photocells may be made of some other materials. The spectral sensitivity is different for different types of cells and should be chosen in accordance with the wavelength of the radiation to be measured.

Selenium cells are not suitable for operations in instruments where the levels of illumination change rapidly, because they fail to respond

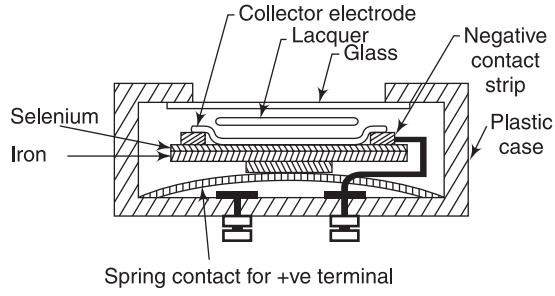


Figure 2.22 *Construction of a barrier layer cell*

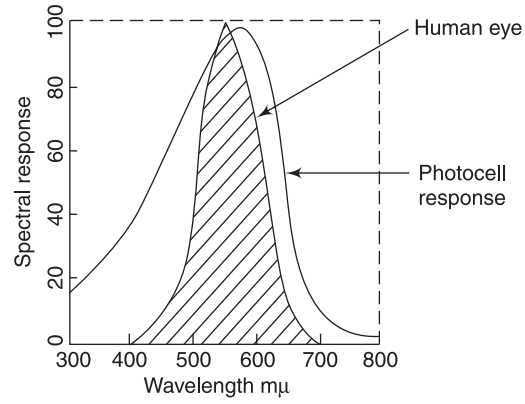


Figure 2.23 *Spectral response of a selenium photocell and the human eye*

immediately to those changes. They are thus not suitable where mechanical choppers are used to interrupt light 15–60 times a second.

2.3.5.2 Photo-emissive cells

Photo-emissive cells are of three types: (a) high vacuum photocells, (b) gas-filled photocells and (c) photomultiplier tubes. All of these types differ from selenium cells, in that they require an external power supply to provide a sufficient potential difference between the electrodes to facilitate the flow of electrons generated at the photosensitive cathode surface. Also, amplifier circuits are invariably employed for the amplification of this current.

2.3.5.3 High vacuum photo-emissive cells

The vacuum photocell consists of two electrodes, cathode having a photosensitive layer of metallic caesium deposited on a base of silver oxide and the anode is either an axially centred wire or a rectangular wire that frames the cathode. The construction of the anode is such that no shadow falls on the cathode. The two electrodes are sealed within an evacuated glass envelope.

When a beam of light falls on the surface of the cathode, electrons are released from it, which are drawn towards the anode which is maintained at a certain positive potential. This gives rise to photocurrent, which can be measured in the external circuit. The spectral response of a photo-emissive tube depends on the nature of the substance coating the cathode, and can be varied by using different metals or by variation in the method of preparation of the cathode surface. Caesium-silver oxide cells are sensitive to the near infrared wavelengths. Similarly, potassium-silver oxide and caesium-antimony cells have maxima of sensitivity in the visible and UV regions. The spectral response also depends partly on the transparency to different wavelengths of the medium to be traversed by the light before reaching the cathode. For example, the sensitivity of the cell in the UV region is limited by the transparency of the wall of the envelope. For this region, the use of quartz material can be avoided by using a fluorescent material like sodium salicylate, which when applied to the outside of the photocell, transforms the ultra violet into visible radiations.

Figure 2.24 shows current-voltage characteristics of vacuum photo-emissive tube at different levels of light flux. They show that as the voltage is increases, the point is reached where all the photoelectrons are swept to the anode as soon as they are released and result in saturation photocurrent. It is not desirable to apply very high voltages, as they would result in excessive dark current without any gain in response.

Figure 2.25 shows a typical circuit configuration usually employed with photo-emissive tubes. Large values of phototube load resistor are employed to increase the sensitivity up to the practical limit. Load resistances as high as 10,000 MΩ have been used. This, however, almost puts a limit, as further increase of sensitivity induces difficulties in the form of noise, non-linearity and slow response. At these high values of load resistors, it is very essential to shield the circuit from moisture and electrostatic effects. Therefore, special type of electrometer tubes, carefully shielded and with grid cap input, are employed in the first stage of the amplifier.

2.3.5.4 Gas-filled photo-emissive cells

This type of cell contains small quantities of inert gas like argon, whose molecules can be ionised when the electrons present in the cell possess sufficient energy. The presence of small quantities of this gas prevents the phenomenon of saturation current, when higher potential differences are

applied between the cathode and anode. Due to repeated collisions of electrons in the gas-filled tubes, the photoelectric current produced is greater even at low potentials.

2.3.5.5 Photomultiplier tubes

Photomultiplier tubes (PMTs) convert photons to an electrical signal. They have a high internal gain and are sensitive detectors for low-intensity applications such as fluorescence spectroscopy.

Photomultiplier tubes are used as detectors when it is required to detect very weak light intensities. The tube consists of a photosensitive cathode and has multiple cascade stages of electron amplification; in order to achieve a large amplification of primary photocurrent within the envelope of the phototube itself. When a photon of sufficient energy strikes the photocathode, it ejects a photoelectron due to the photoelectric effect. The photocathode material is usually a mixture of alkali metals, which make the PMT sensitive to photons throughout the visible region of the electromagnetic spectrum. The photocathode is at a high negative voltage, typically –500 to –1,500 V. The photoelectron is accelerated towards a series of additional electrodes called dynodes. These electrodes are each maintained at successively less negative potentials. Additional electrons are generated at each dynode. There may be 9–16 dynodes (Figure 2.26). This cascading effect creates 10^5 to 10^7 electrons for each photoelectron that is ejected from the photocathode. The amplification depends on the number of dynodes and the accelerating voltage. This amplified electrical signal is collected at an anode at ground potential, which can be measured.

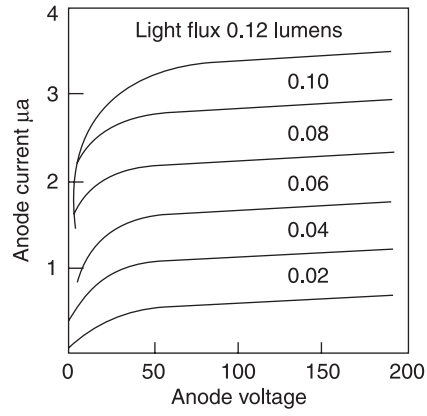


Figure 2.24 *Current-voltage characteristics of vacuum*

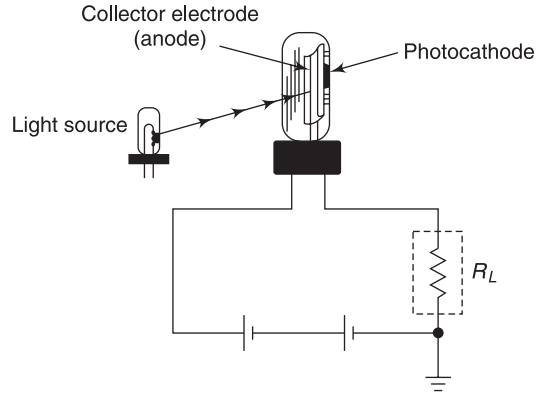


Figure 2.25 *Typical circuit configuration employed with photo-emissive tubes*

The sensitivity of the PMT can be varied by regulating the voltage of the first amplifying stage. Because of the relatively small potential difference between the two electrodes, the response is linear. The output of the PMT is limited to an anode current of a few milliamperes. Consequently, only low-intensity radiant energy can be measured without causing any appreciable heating effect on the electrode surface. They can measure light intensities about 10 times weaker than those measurable with an ordinary phototube. For this reason, they should be carefully shielded from stray light. The tube is fairly fast in response to the extent that they are used in scintillation counters, where light pulses as brief as 10^{-9} s duration are encountered. A direct current (DC) power supply

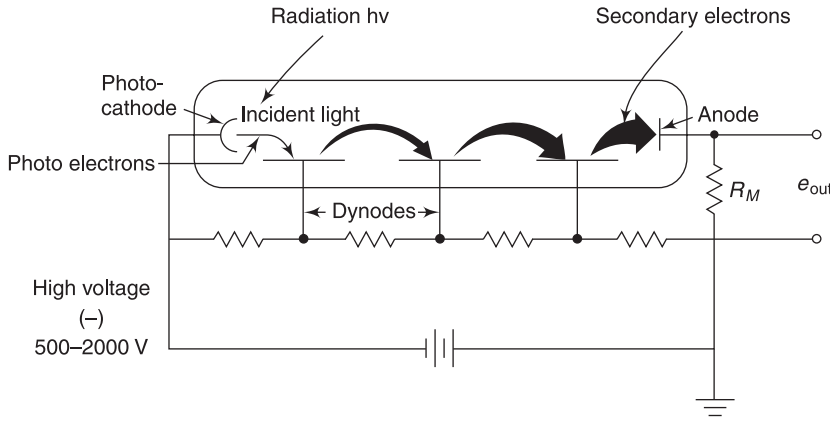


Figure 2.26 *Schematic of a photomultiplier tube*

is required to operate a photomultiplier, the stability of which must be at least one order of magnitude better than the desired precision of measurement (e.g. to attain precision of 1%, fluctuation of the stabilised voltage must not exceed 0.1%).

Fatigue and saturation can occur at high illumination levels. The devices are sensitive to electromagnetic interference, and they are also more costly than other photoelectric sensors. Photomultipliers are not uniformly sensitive over the whole spectrum and in practice; manufacturers incorporate units best suited for the frequency range, for which the instrument is designed. In the case of spectrophotometers, the photomultipliers normally supplied cover the range of 185–650 nm. For measurements at longer wave lengths, special red-sensitive tubes are offered. They cover a spectral range from 185 to 850 nm, but are noticeably less sensitive at wavelengths below 450 nm, than the standard photomultipliers. Photomultiplier tubes show a quantum efficiency of 1–10%, with a response time of 1–20 ns.

Photomultiplier tubes may be damaged if excessive current is drawn from the final anode. Since accidental overload may easily occur in a laboratory and tubes are expensive to replace, it is advisable to adopt some means of protection from overloads. Generally, the circuits are so designed that they automatically cut-off the EHT supply to a PMT if accidental overload of the tube should occur. The EHT once cut off, has to be reset manually.

2.3.5.6 Silicon diode detectors

The photomultiplier which is large and expensive and requires a source of stabilised high voltage can be replaced by a photodiode. This diode is useable within a spectral range of 400–1,050 nm, in a number of instruments (spectrophotometers, flame photometers). The photodiode can be powered from low voltage source. The signal is amplified by a low noise op-amp.

When a photon strikes a semiconductor, it can promote an electron from the valence band (filled orbital) to the conduction band (unfilled orbital) creating an electron (–) – hole (+) pair. The concentration of these electron-hole pairs is dependent on the amount of light striking the semiconductor, making the semiconductor suitable as an optical detector. There are two ways to monitor the concentration of electron-hole pairs. In photodiodes, a voltage bias is present and the concentration of

light-induced electron-hole pairs determines the current through semiconductor. Photovoltaic detectors contain a *p-n* junction that causes the electron-hole pairs to separate to produce a voltage that can be measured. Figure 2.27 shows a schematic diagram of a semiconductor photovoltaic detector.

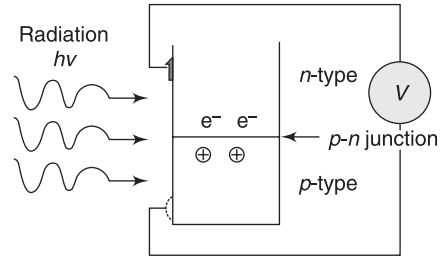


Figure 2.27 *Schematic of a semiconductor detector*

However, photodiode detectors are not as sensitive as PMTs but they are small and robust. The wavelength range for different solid-state detectors is given in Table 2.3.

Table 2.3 *Wavelength range*

Detector type	λ (μ)
Si	0.2–1.1
Ge	0.4–1.8
InAs	1.0–3.8
InSb	1.0–7.0
InSb (77K)	1.0–5.6
HgCdTe (77K)	1.0–25.0

Silicon diode detectors (when integrated with an operational amplifier) have performance characteristics which compare with those of a photomultiplier over a similar wavelength range. Figure 2.28 shows spectral response of silicon diode detectors. The devices being solid state are mechanically robust and consume much less power. Dark current output and noise levels are such that they can be used over a much greater dynamic range.

Diode detectors are extremely versatile and have been employed in various configurations, such as flow through detectors in flow injection analysis (FIA), separation systems and probe photometers. Diode detectors are popular because of their rapid response and wide linear range, which is typically three and four orders of magnitude better than phototubes.

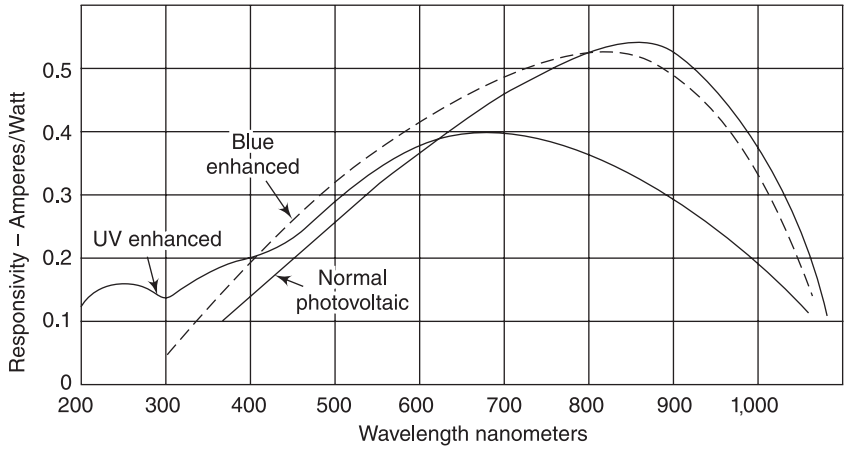


Figure 2.28 *Spectral response of silicon diode detectors*

2.3.5.7 Photodiode arrays (PDA)

Diode arrays are assemblies of individual detector elements in linear or matrix form, which in a spectrophotometer can be mounted so that the complete spectrum is focused on to an array of appropriate size. The arrangement does not require any wavelength selection mechanism and the output is instantaneously available. However, resolution in diode arrays is limited by the physical size of individual detector elements.

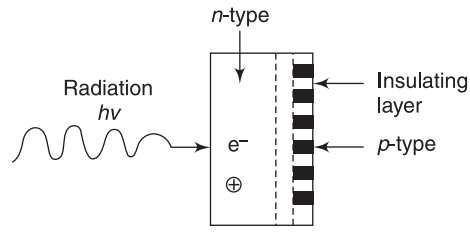


Figure 2.29 *Schematic of a photodiode array*

A photodiode array consisting of discrete photodiodes is available on an integrated circuit (IC) chip. For spectroscopy it is placed at the image plane of a spectrometer to allow a range of wavelengths to be detected simultaneously. In this regard it can be thought of as an electronic version of photographic film. Array detectors are especially useful for recording the full uv-vis absorption spectra of samples that are rapidly passing through a sample flow cell, such as in HPLC detectors. PDAs are available with 512, 1,024, or 2,048 elements with typical dimensions of 25 μm wide and 1–2 mm high. A schematic diagram of a photodiode array is shown in Figure 2.29.

Light creates electron-hole pairs and the electrons migrate to the nearest PIN junction. After a fixed integration time the charge at each element is read with solid-state circuitry to generate the detector response as a function of linear distance along the array.

Knud et al. (1980) described the details of diode array photodetectors used in the Hewlett Packard spectrophotometer Model 8450A. The detector consists of two silicon integrated circuits, each containing 211 photosensitive diodes and 211 storage capacitors. The photodiode array is PMOS (p-channel metal-oxide semiconductor) integrated circuit that is over 1.25 cm long. Each photosensitive diode in the array is 0.05 by 0.50 mm and has a spectral response that extends well beyond the 200–800 nm range.

A functional block diagram of the diode array chip is shown in Figure 2.30. In parallel with each of the 211 photodiodes is an 10pF storage capacitor. These photodiode capacitor pairs are sequentially connected to a common output signal line through individual MOSFET switches. When a FET

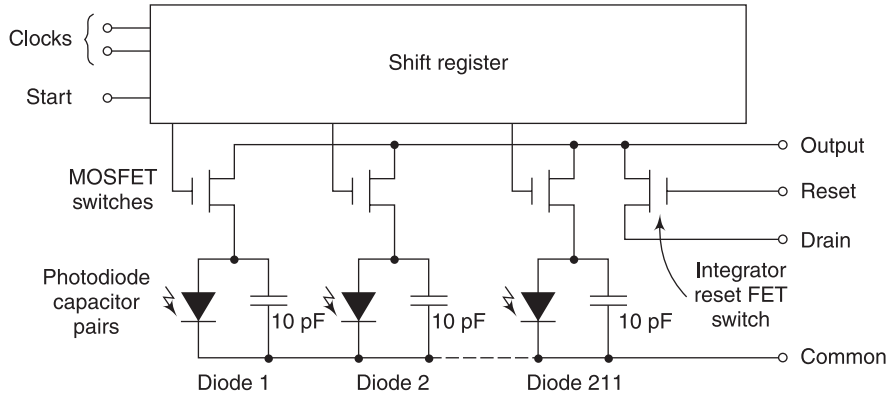


Figure 2.30 *Functional diagram of the photodiode array chip*

switch is closed, the preamplifier connected to this signal line forces a potential of – 5V on to the capacitor-diode pair. When the FET switch is opened again, the photocurrent causes the capacitor to discharge towards zero potential. Serial read-out of the diode array is accomplished by means of a digital shift register designed into the photodiode array chip.

The diode arrays typically exhibit a leakage current less than 0.1 pA. This error term increases exponentially with temperature, but because the initial leakage value is so low, there is no need to cool the array at high ambient temperatures.

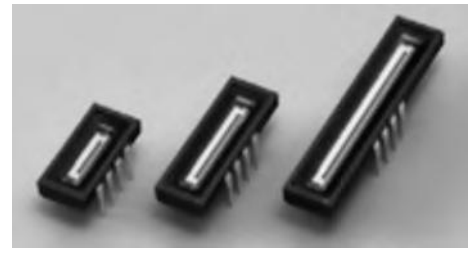


Figure 2.31 *Diode arrays of various sizes*

The obvious advantage of using photodiode array is the short amount of time required for making a measurement. The measurement time in the spectrophotometers using photodiode array is hardly 5 s as compared to more than 5 min in the usual scanning monochromator-based instruments.

The major disadvantage to the diode array instrument is the limited resolution. Scanning instruments, depending on the resolution of the excitation monochromator, are able to easily achieve resolutions of the order of 0.1 nm, whereas in photodiode array instruments, resolution is 1 nm, and it cannot be changed.

The array typically comprises between 200 and 1,000 elements, depending on the instrument and its intended application (Figure 2.31). For example, the diode array of the Agilent 8,453 spectrophotometer comprises 1,024 detector elements, and the photosensitive area measures approximately 25 × 0.5 mm. The readout cycle, which corresponds to the illumination time, is 100 ms.

2.3.6 Slit Width

The resolution of a spectrophotometer is usually limited by the spectral purity and intensity of the monochromator light output and the detector sensitivity at that wavelength. In some instruments, the control of the energy level reaching the detector is obtained by adjusting the aperture of the slit at the monochromator exit. More than one slit width, when available, gives the user a means of trading energy against spectral sensitivity. However, most instruments using diffraction gratings tale advantage of the linear dispersion and provide fixed slit widths to give known and controlled bandwidth at the exit slit of the monochromator.

Where it is necessary to determine accurately the absorbance at λ_{max}, it is better first to plot apparent absorbance against slit width. Figure 2.32 shows that the slit width greater than about 0.75 mm may result in significant error in the measurement of the absorbance concerned.

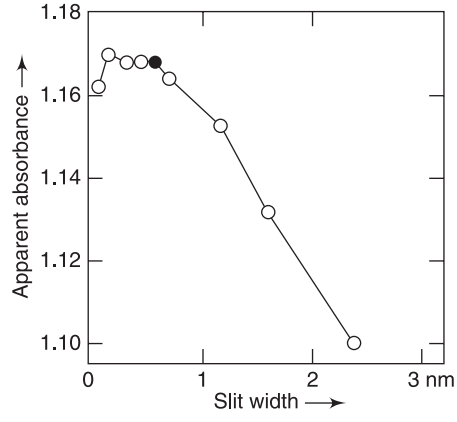


Figure 2.32 *Effect of slit width on absorbance*

2.3.7 Sample Holders

Liquids may be contained in a cell or cuvette made of transparent material such as silica, Perspex. The faces of these cells through which the radiation passes are highly polished to keep reflection and scatter losses to a minimum. Solid samples are generally unsuitable for direct spectrophotometry. It is usual to dissolve the solid in a transparent liquid. Gases may be contained in cells which are scaled or stoppered to make them air-tight. Sample holder is generally inserted somewhere in the interval between the light source and the detector.

For the majority of analyses, 10 mm path length rectangular cell is usually satisfactory. For the far UV region below 210 mμ, 10 mm path length rectangular cell made from special silica that has better transmission characteristics at shorter wavelengths than does the standard cell is recommended. However, some samples, such as turbid or densely coloured solutions, may absorb so strongly that shorter path lengths are necessary for the sample to transmit sufficiently. Rectangular liquid cells are commercially available in both 5 and 1 mm path lengths, while the standard 10 mm path length cell can be reduced to 1 mm path length by using a silica spacer. These shorter path length cells have lesser volumes – of the order of 0.43 ml for a 1 mm path length – which are necessary for some studies.

In analyses where only minimal volumes of liquid samples are practical, micro-cells, which have volumes as small as 50 μl can be employed. Studies of dilute, weakly absorbing liquid samples or of samples where trace components must be detected require a cell with a long path length. For such applications, a 50 cm path length with about 300 ml volume cell is employed. Figure 2.33 shows a selection of typical sample cuvettes.

The cells lowest in cost are made of plastic, usually an acrylic. These cells are not resistant to all solvents and absorb strongly below 300 nm, making them unsuitable for measurements in this region. Also, consistency (absorbance and path length) can vary from cell to cell, depending on the manufacturer.

Glass cells are slightly more expensive than plastic cells but are more durable and, with proper care, can provide years of use. Glass absorbs strongly below 320 nm and thus is not suitable for measurements in the UV region.

Fused quartz cells are reasonably transparent down to 210 nm. The best cells are made of high-purity fused synthetic silica and are reasonably transparent down to 190 nm. Figure 2.34 shows the absorption characteristics of cells of different materials. Note that all materials exhibit at least an approximately 10% loss in transmission at all wavelengths.

Gas cells are available in both rectangular and cylindrical configurations. Both configurations incorporate glass stopcocks. Two stopcocks are installed on the cylindrical cells to permit connecting it into a flow system for dynamic measurements. Rectangular gas cells usually have path lengths of 2 and 10 mm, while cylindrical gas cells have a path length of 100 mm.

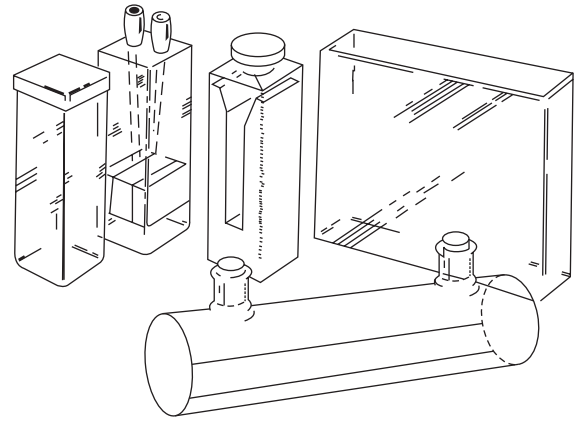


Figure 2.33 *Selection of sample cuvettes*

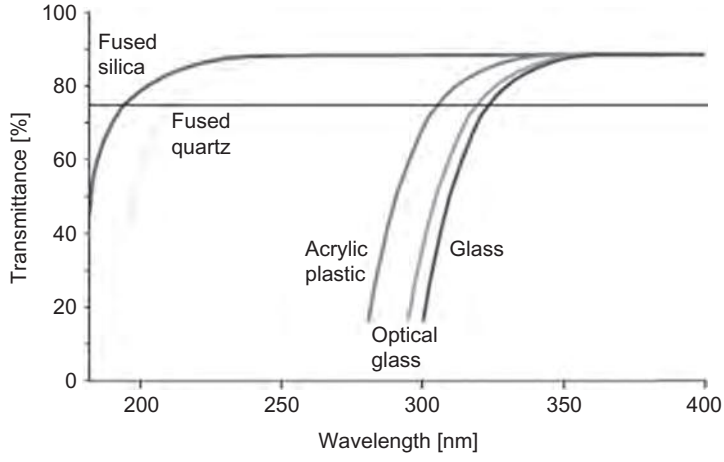


Figure 2.34 *Shows the absorption characteristics of cells of different materials. (Courtesy : M/s Agilent Technologies)*

2.4 ULTRAVIOLET AND VISIBLE ABSORPTION SPECTROSCOPY

Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface in the UV-Vis range of the electromagnetic spectrum. Absorption measurements can be at a single wavelength or over an extended spectral range. UV-Vis light are energetic enough to promote outer electrons to higher energy levels, UV-Vis spectroscopy is usually applied to molecules or inorganic complexes in solution. The UV-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law.

Since the UV-Vis range spans the range of human visual acuity of approximately 400–750 nm, UV-Vis spectroscopy is useful to characterise the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings, windows, and filters. This more qualitative application usually required recording at least a portion of the UV-Vis spectrum for characterisation of the optical or electronic properties of materials (Manning, 1969).

The UV-Vis spectral range is approximately 190–900 nm, as defined by the working range of typical commercial UV-Vis spectrophotometers. The short-wavelength limit for simple UV-Vis spectrometers is the absorption of ultraviolet wavelengths less than 180 nm by atmospheric gases. Purging a spectrometer with nitrogen gas extends this limit to 175 nm. Working beyond 175 nm requires a vacuum spectrometer and a suitable UV light source. The long-wavelength limit is usually determined by the wavelength response of the detector in the spectrometer. High-end commercial UV-Vis spectrophotometers extend the measurable spectral range into the NIR region as far as 3,300 nm.

The light source is usually a deuterium-discharge lamp for UV measurements and a tungsten-halogen lamp for visible and NIR measurements. The instruments automatically swap lamps when scanning between the UV and visible regions. The wavelengths of these continuous light sources are

typically dispersed by a holographic grating in a single or double monochromator or spectrograph. The spectral bandpass is then determined by the monochromator slit width or by the array-element width in array-detector spectrometers. Spectrometer designs and optical components are optimised to reject stray light, which is one of the limiting factors in quantitative absorbance measurements. The detector in single-detector instruments is a photodiode, phototube or PMT. UV-Vis-NIR spectrometers utilise a combination of a PMT and a Peltier-cooled PbS IR detector. The light beam is redirected automatically to the appropriate detector when scanning between the visible and NIR regions. The diffraction grating and instrument parameters such as slit width can also be change.

Most commercial UV-Vis absorption spectrometers use one of three overall optical designs given below:

- A fixed or scanning spectrometer with a single light beam and sample holder,
- A scanning spectrometer with dual light beams and dual sample holders for simultaneous measurement of P and P_o, or
- A non-scanning spectrometer with an array detector for simultaneous measurement of multiple wavelengths.

In single-beam and double-beam spectrometers, the light from a lamp is dispersed before reaching the sample cell. In an array-detector instrument, all wavelengths pass through the sample and the dispersing element is between the sample and the array detector.

2.4.1 Types of Absorption Instruments

The absorption instruments are defined by different nomenclatures. The common nomenclatures are given below:

Colorimeter: A colorimeter is an instrument for absorption measurements in which the human eye serves as the detector using colour comparison standards.

Photometer: The photometer is primarily an electronic equipment which contains a source of light, a filter, a photosensitive detector, signal processor and display/readout system. They are also sometimes referred to as photoelectric colorimeters or simply as colorimeters.

Spectrometer: This is an instrument that provides information about the intensity of radiation as a function of wavelength or frequency.

Spectrophotometer: This is an instrument that is equipped with one or more exit slits and photoelectric detectors that permit the determination of the ratio of the radiant power of two beams as a function of wavelength.

Spectroscope: A spectroscope is an optical instrument used for the visual identification of atomic emission lines.

2.5 COLORIMETERS/PHOTOMETERS

A colorimetric method in its simplest form uses only the human eye as a measuring instrument. This involves comparison by visual means of the colour of an unknown solution, with the colour produced by a single standard or a series of standards. The comparison is made by obtaining a match between the colour of the unknown and that of a particular standard by comparison with a

series of standards prepared in a similar manner, to the unknown. Errors of 5–20% are not uncommon, because of the relative inability of the eye to compare light intensities.

In the earlier days, visual methods were commonly employed for all colorimetric measurements, but now photoelectric methods have largely replaced them and are used almost exclusively for quantitative colorimetric measurements. These methods are more precise and eliminate the necessity of preparing a series of standards every time a series of unknowns is run.

Strictly speaking, a colorimetric determination is one that involves visual measurement of colour; and a method employing photoelectric measurement is referred as a photometric or spectrophotometric method. However, usually any method involving measurement of colour in the visual region of the electromagnetic spectrum (400–700 mμ) is referred to as the colorimetric method.

In a colorimeter, the sample is normally a liquid. The sample compartment of a colorimeter is provided with a holder to contain the cuvette, in which the liquid is examined. Usually this holder is mounted on a slide with positions for at least two cuvettes, so that sample and reference cuvettes are measured first and a shutter is moved into or out of the light beam until the micro-ammeter gives a full-scale deflection (100% T-scale reading). The sample is then moved into the beam and the light passing through it is measured as a percentage to the reference value.

$$\text{Sample concentration} = \text{Standard concentration} \times \frac{\text{Sample reading}}{\text{Reference reading}}$$

Colorimeters are extremely simple in construction and operation. They are used for a great deal of analytical work, where high accuracy is not required. The disadvantage is that a range of filters is required to cover different wavelength regions. Also the spectral bandwidth of these filters is large in comparison with that of the absorption band being measured.

2.5.1 Single-beam Filter Photometers

Figure 2.35 illustrates the basic components of a filter photometer. The source of light is a tungsten filament lamp, which is held in a reflector and throws light on the sample holder through a filter. The filter may be either of absorption or interference type. The sample holder is a cuvette with parallel walls or may be a test tube. The light, after passing through the sample holder, falls on the surface of the photocell. The output of the cell is measured on a micro-ammeter. The lamps must be energised from a highly stabilised DC source, or by the output of a constant voltage transformer.

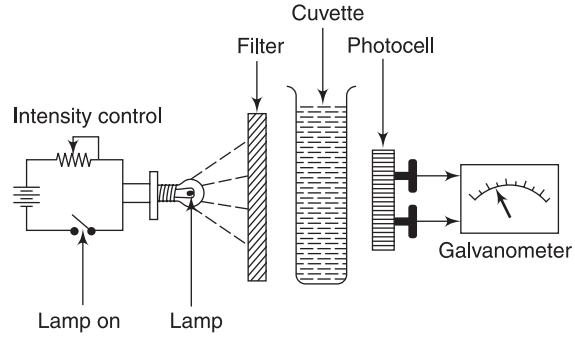


Figure 2.35 *Basic components of a filter photometer*

2.5.2 Double-beam Filter Photometer

In double-beam filter photometers, two photocells are normally employed. The two photocells are connected to two potentiometers R_1 and R_2 in Figure 2.36. Each of the two

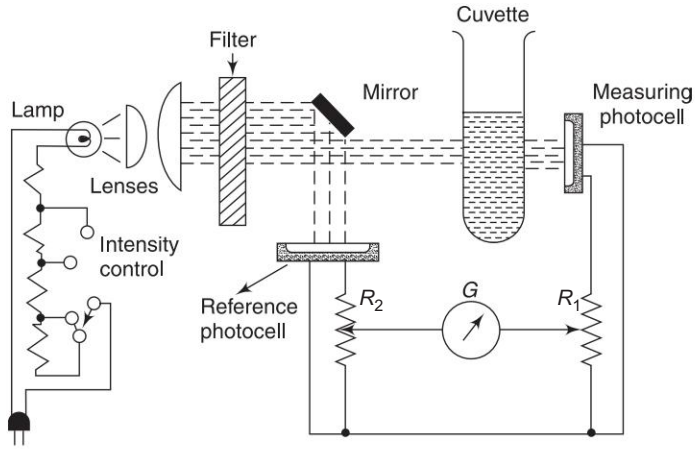


Figure 2.36 *Schematic of a double-beam filter photometer*

potentiometers is of low resistance and is wound linearly. Light from the source lamp is made to pass through the filter and is then divided into two parts, one part passing through the solution in the cuvette before falling on the measuring photocell, and the other part passing directly on to the reference photocell. The galvanometer *G* receives opposing currents through it. The potentiometer R_1 is graduated in transmittance and absorbance units.

The operation of the instrument is very simple. With the lamp off, the galvanometer zero is adjusted mechanically. The potentiometer R_1 is set to $T = 1$ or $A = 0$. Then with the lamp on, the blank solution is placed in the light path of the measuring cell. Potentiometer R_2 is adjusted until galvanometer *G* reads zero. The solution to be analysed is then substituted for the blank and R_1 is adjusted until the current through the galvanometer is zero, the setting of R_1 remaining unchanged. The absorbance or transmittance can then be read directly on the scale of potentiometer R_1.

In two-cell photometers, the errors resulting from the fluctuations of the lamp intensity are minimised. The scale of potentiometer R_1 (transmittance scale) can be made much larger in size than the scale of the metre in single-cell instruments. The modern instruments no longer use analog galvanometers. They directly display the absorbance values on direct-reading digital display.

2.5.3 Probe Type photometer

Dipping probe-type photometers use fibre optics to transport light. The light from a tungsten lamp travels down a fibre optic cable which is dipped into the solution of interest (sample). The light then passes through the solution and a mirror reflects the light back to a return fibre optics cable. The arrangement is shown in Figure 2.37.

The cell path length is two times the distance between the ends of the optical cable and the mirror. Interference filters are provided to select a wavelength. The reflected light is then passed through a photodiode with an amplifier and an electronic chopper which is synchronised with the lamp. This results in the detector not responding to extraneous light. A commercial probe-type photometer is shown in Figure 2.38.

2.5.4 Miniature Fibre Optic Spectrometer

Miniature fibre optic spectrometer, the S2000 (Figure 2.39), from M/s Ocean Optics couples a low-cost, high-performance 2048-element linear CCD-array detector with an optical bench that's small enough to fit into the palm of your hand. This is a high-sensitivity, low-cost UV-VIS NIR spectrometer for low light level applications that demand high detector sensitivity. It accepts light energy transmitted through optical fibre and disperses it via a fixed grating across the detector, which is responsive from 200–1,100 nm. Up to seven spectrometer channels can be added to expand wavelength range, perform multiple tasks or provide reference monitoring.

The total system comprises five basic elements: the S2000 Miniature Fibre Optic Spectrometer, an A/D converter, operating software, a light or excitation source, and sampling optics. The light or excitation source sends light through an optical fibre to the sample. The light interacts with the sample. Then the light is collected and transmitted through another optical fibre to the spectrometer. The spectrometer measures the amount of light and the A/D converter transforms the analog data collected by the spectrometer into digital information that is passed to the software, providing the user with application-specific information.

The software has the ability to perform spectroscopic measurements such as absorbance, reflectance and emission; control all system parameters; collect data from up to eight spectrometer channels simultaneously in real time, display the results in a single spectral window; and perform reference monitoring and time acquisition experiments.

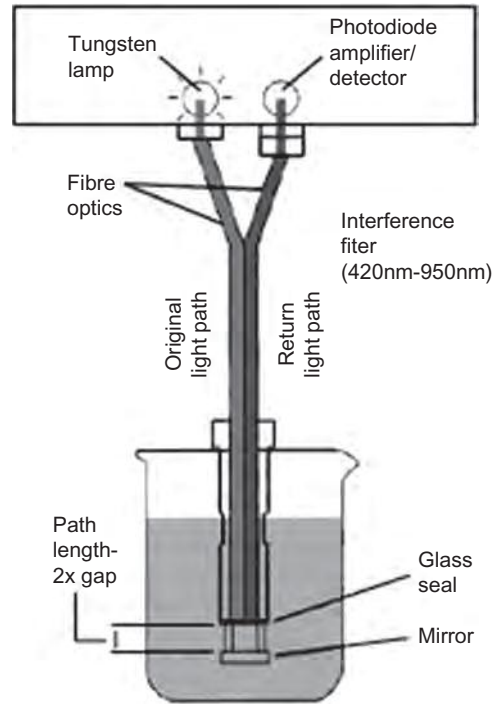


Figure 2.37 *Schematic diagram of a probe-type photometer (Courtesy : M/s STH Company USA)*

Figure 2.38 *Photograph of a probe-type photometer. (Courtesy : M/s Metrohm USA)*

The optical system has two identical fibres in a bifurcated assembly. A plano-convex lens shapes the light coming out of the illumination fibre. The light is transmitted through the sample, hits the mirror, reflects off the mirror, and interacts with the sample again before being transmitted back

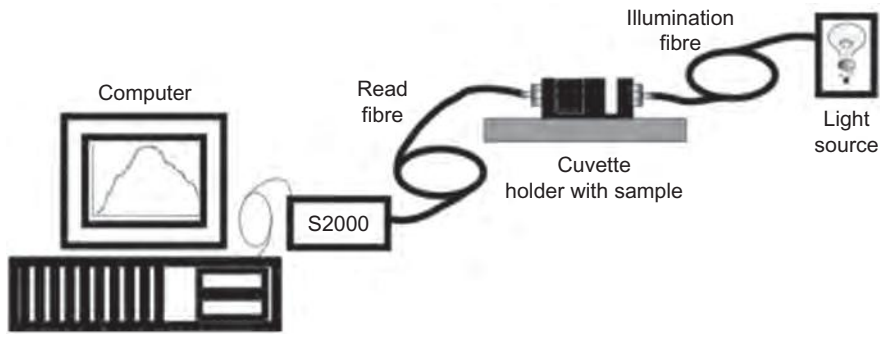


Figure 2.39 *Absorbance/Transmission Setup (Courtesy : M/s Ocean Optics Inc.)*

through the probe via the read fibre. Because the light travels through the sampling region twice, the optical path length is actually twice the length of the sample aperture. The transmission cell is used to measure absorbance of the fluid that fills the sample compartment between the fibres and the mirror.

The silica-core and silica-clad optical fibres are optimised for the UV-VIS (200–750 nm) or VIS-NIR (450–1,000 nm) range. Standard assemblies are 2 m in length, and are available in sizes ranging from 8–1,000 μm in diameter.

2.5.5 Multi-channel Photometer

An increasing number of chemical analyses are carried out in the laboratories of industry and hospitals, and in most of these the final measurement is performed by a photometer. Obviously, it is possible to increase the capacity of the laboratory by using photometers, which have a large measuring capacity. One of the limitations for rapid analyses is the speed at which the samples can be transferred in the light path.

In a multi-channel photometer, instead of introducing one sample at a time into a single light path, a batch of samples is introduced and measurements carried out simultaneously, using a multiplicity of fibre optic light paths (Figure 2.40) and detectors, and then scanned electronically instead of mechanically. The 24 sample cuvettes are arranged in a rack in a three key eight matrix. The 25th channel serves as a reference beam and eliminates possible source and detector drifts. The time required to place the cuvette rack into the measuring position corresponds to the amount of time necessary to put one sample into a sample changer.

The light source is a 50 W tungsten-halogen lamp, driven from a precisely controlled voltage source. The light is chopped by means of a mechanical rotating chopper. A lens focuses the light on the end of a bundle of fibre optic elements. The output of the detectors is amplified and displayed on digital volt-metre. The whole operation is synchronised with digital logic circuits.

2.5.6 Pocket Colorimeter

Figure 2.41 shows a typical Pocket Photometer from M/s Hach Lange. It is lightweight, battery operated, waterproof and ideal for field work. It has accuracy comparable to a lab instrument, yet it remains simple to use. It operates on fixed wavelengths 420, 450, 476, 500, 550, 580, 600, 650 nm

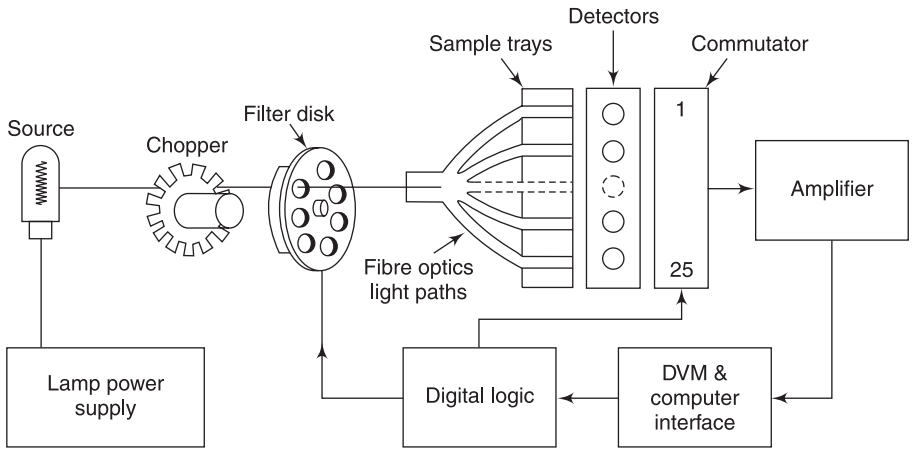


Figure 2.40 *Schematic of a multi-channel photometer*

with bandwidth of 15 nm. They use a silicon detector, fixed bandwidth of ±2nm, absorbance range of 0–2.5Abs and sample cell path lengths of 1 cm and/or 22 mm.

Each instrument measures 1–2 parameters, with a total of 35 parameters available. These portable units are factory-calibrated for Hach chemistry. Wavelength-specific 'generic' instruments are also available, allowing users to enter their own methods using from two up to ten standards.

The use of multi-LED photometers allows a wide range of the electromagnetic spectrum to be covered simultaneously or individually without manually changing the LEDs. Hauser et al. (1995) employed a fibre optic coupler to guide the light from up to 7 LEDs into a single measuring cell. This photometer could be configured to detect Al, Cu, NH_3, Cu, Ca, chromium, phosphate and nitrite using colorimetric methods. A coupler was used to merge the light from one of the seven input channels into two output fibres. One was brought to the measuring photodiode and the other was brought to the reference photodiode. This is shown in Figure 2.42. The photometer features a data logging function that allows users to store and recall the ten most recent data points, eliminating the need to manually record data.

Figure 2.41 *Photograph of a simple LED-based colorimeter. (Courtesy : M/s HACH Lange, UK)*

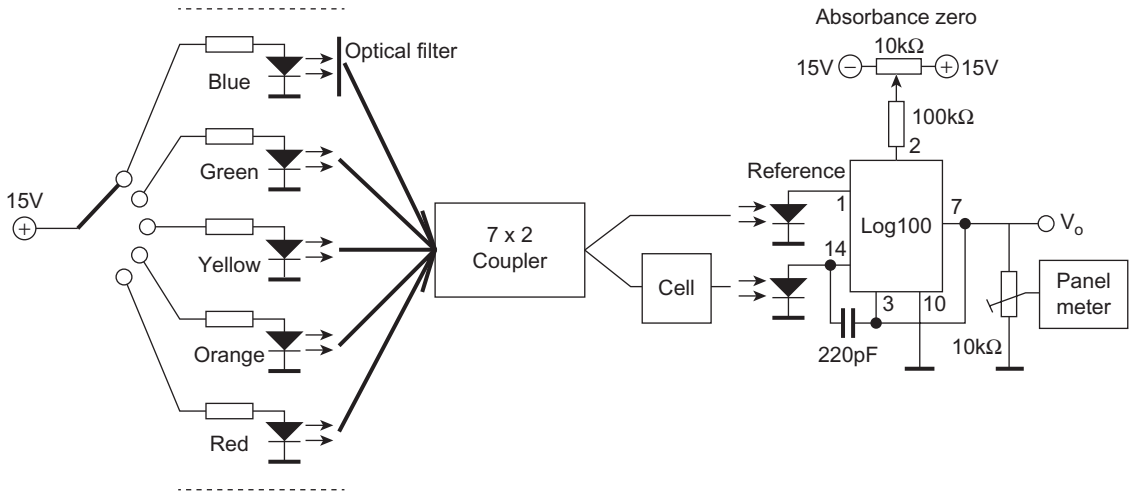


Figure 2.42 *The circuit diagram for the multi-LED photometer (Adapted from Hauser et al. 1995)*

2.5.7 Process Photometers

If we need to monitor an analyte's concentration over time, it may not be possible to physically remove samples for analysis. This is often the case, for example, when monitoring industrial production lines or waste lines, when monitoring a patient's blood, or when monitoring environmental systems. With a fibre-optic probe, we can analyse samples in situ. An example of a remote sensing fibre-optic probe is shown in Figure 2.39. The probe consists of two bundles of fibre-optic cable. One bundle transmits radiation from the source to the probe's tip, which is designed to allow the sample to flow through the sample cell. Radiation from the source passes through the solution and is reflected back by a mirror. The second bundle of fibre-optic cable transmits the non-absorbed radiation to the wavelength selector. Another design replaces the flow cell as shown in Figure 2.43 with a membrane containing a reagent that reacts with the analyte. When the analyte diffuses across the membrane, it reacts with the reagent, producing a product that absorbs UV or visible radiation. The non-absorbed radiation from the source is reflected or scattered back to the detector. Fibre optic probes that show chemical selectivity are called optrodes.

For on-line applications, M/s Optical Solutions, USA have introduced process photometers, known as ChemView, which allow measurement from 250 to 2,150 nm without using any moving parts, this enable lower maintenance problems. The photometer uses a

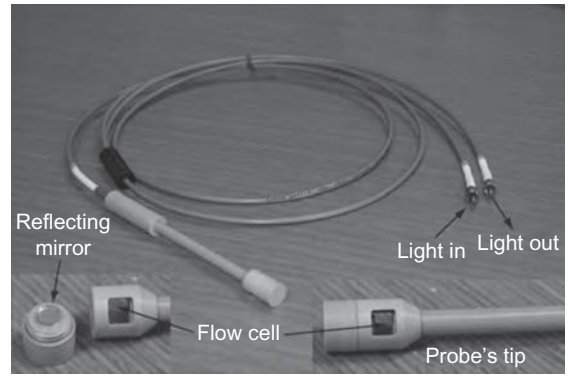


Figure 2.43 *Example of a fibre-optic probe. The inset photographs provide a close-up look at the probe's flow cell and the reflecting mirror (Courtesy : UC Davis, ChemWiki)*

low-power tungsten lamp in the visible and NIR region. By continuously adjusting its brightness with a detector in a feedback circuit, very low optical drift is achieved. The company calls this 'StabLamp'. In the UV, a pulsed neon source is used.

Figure 2.44 shows a block diagram of a single probe instrument. Light is sent to the fire optic probe and returned to the analyser. Beam splitters divide the light and a portion passes through narrow-band wavelength filters into each detector. Up to six detectors, one or more of which are references may be used. Any combination of NIR (In GaAs) and UV-Vis (Si) detectors can be placed in any position, providing flexibility in operation.

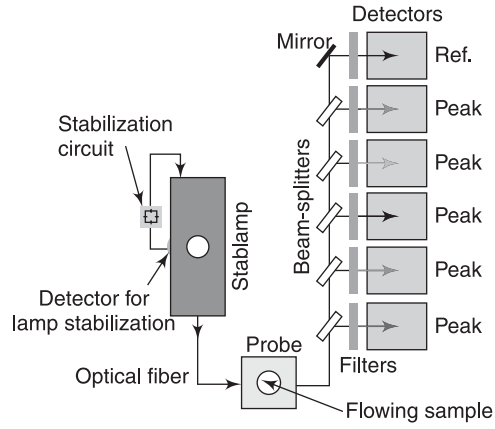


Figure 2.44 *Optical diagram of a single channel uv-vis and NIR process photometer*

Voltage from each detector is logarithmically converted, and then the signals from each peak and its reference are subtracted, producing a voltage equivalent to optical absorbance units (Au). A microprocessor converts the signals into chemical units for display and transmits them as 4–20 mA signals to the process computer.

The instrument uses reference voltage (i.e. the signal strength at the reference wavelength) to identify fibre breakage, probe fouling or intermittent cloudiness due to filter break through. It additionally uses an internal circuit to indicate if the lamp is about to burn out or if it has burnt out.

Multi-probe analysers for applications un UV-Visible (200–800 nm) and near infrared (1,100–2,150) using diode array detectors are also available from the some company. The arrangement is shown in Figure 2.45. Compatible with PC and using popular GRAMS spectral software to operate the system, the instrument enables to control the diode array settings, screen displays and referencing. The software also enables to perform virtually any manipulation of the spectra, including derivatives, peak areas, base line corrections, smoothing, subtraction, etc.

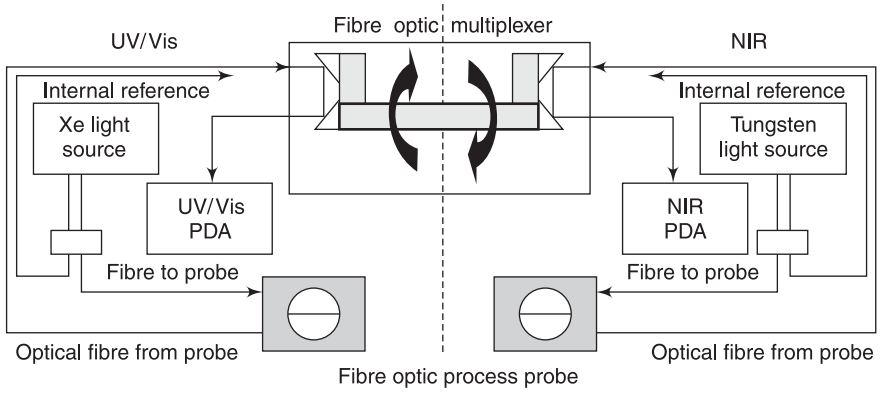


Figure 2.45 *Operating principle of a multi-probe portable spectrophotometer*

2.6 SPECTROPHOTOMETERS

A spectrophotometer is an instrument which isolates monochromatic radiation in a more efficient and versatile manner than colour filters used in filter photometers. In these instruments, light from the source is made into a parallel beam and passed to a prism or diffraction grating, where the light of different wavelengths is dispersed at different angles.

The amount of light reaching the detector of a spectrophotometer is generally much smaller (Figure 2.46) than that available for a colorimeter, because of the small spectral bandwidth. Therefore, a more sensitive detector is required. A photomultiplier or vacuum photocell is generally employed. The electrical signal from the photoelectric detector can be measured by using a sensitive microammeter. However, it is difficult and expensive to manufacture a metre of the required range and accuracy. To overcome this problem, either of the following two approaches is generally adopted:

(a) The detector signal may be measured by means of an accurate potentiometric bridge. A reverse signal is controlled by a precision potentiometer, until a sensitive galvanometer shows that it exactly balances the detector signal and no current flows through the galvanometer. This principle was adopted in earlier Beckman Model DU single-beam spectrophotometer.
(b) The detector current is amplified electronically and displayed directly on an indicating metre or in digital form. These instruments have the advantage in speed of measurement. As in the case of colorimeters, the instrument is adjusted to give a 100% transmission reading, with the reference sample in the path of the light beam. The sample is then moved into the beam and the percentage transmission is observed.

Modern commercial instruments are usually double beam, direct digital reading and/or recording instruments, which can provide absorbance, concentration, per cent transmission and differential absorbance readings. It is also possible to make reaction rate studies. They can be used to include specialised techniques, such as automatic sampling and batch sampling, with the addition of certain accessories. The measurements can be made generally with light at wavelengths from 340 to 700 nm and from 190 to 700 nm with deuterium source. In variable-slit type of instruments, the slit can be made to vary from 0.05 to 2.0 mm. The wavelength accuracy is +0.5 nm. The recorders are usually single channel, strip-chart potentiometric recorders. They are calibrated from 0.1 to 2.0 A or 10 to 200% T full-scale. The recorder used with spectrophotometers have four wavelength scanning speeds (100, 50, 20 and 5 nm/mm) and seven chart speeds (10, 5, 2, 1, *0.5*, 0.2 and 0.1 inch/min). It has a sensitivity of 100 mV absorbance units or 100 mV/100% T.

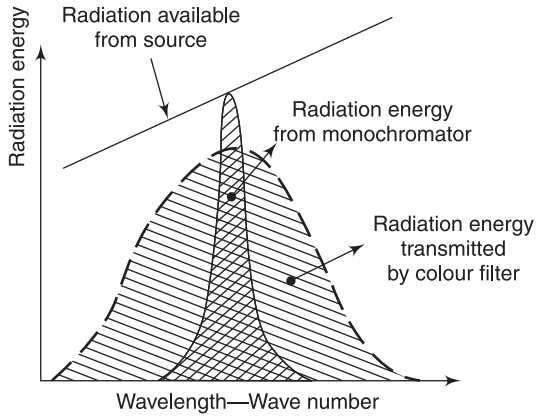


Figure 2.46 *Comparison of radiation energy from a colour filter and monochromator*

When scanning narrow wavelength range, it may be adequate to use a fixed slit width. This is usually kept at 0.8 mm. In adjustable slit-width instruments, it should be so selected that the resultant spectral slit width is approximately 1/10 of the observed

bandwidth of the sample (i.e. if the absorption band is 25 nm wide at half of its height, the spectral slit width should be 2.5 nm). This means that the slit width set on the instrument should be 1.0 mm. This is calculated from the dispersion data, as the actual dispersion in grating instruments is approximately 2.5 nm/mm slit width.

The tungsten lamp used in spectrophotometers generally operates on 6 V and emits radiation in the wavelength region of visible light. Typically, it is 32 candle power. These lamps should preferably be operated at a potential of say 5.4V, when its useful life is estimated at 1,200 h. The life is markedly decreased by an increase in the operating voltage. With time, the evaporation of tungsten produces a deposit on the inner surface of the tungsten lamp and reduces emission of energy. Dark areas on the bulb indicate this condition. It should then be replaced.

The useful operating life of the deuterium lamp normally exceeds 500 h. under normal conditions. The end of the useful life of this lamp is indicated by failure to start or by a rapidly decreasing energy output. Ionisation may occur inside the anode rather than in a concentrated path in front of the window. Generally, this occurs when the lamp is turned on while still hot from previous operation. If this occurs, the lamp must be turned off and allowed to cool before restarting.

Spectrophotometers should be placed in an area which is reasonably free of dust and excessive moisture and not subject to significant temperature variations. As they are sensitive instruments, their performance is likely to be affected by strong electromagnetic fields, as would exist in proximity to diathermic machines or large electric motors. Disturbances of this nature should be avoided when determining location. The surface on which the instrument is to be placed must be stable and free from vibrations.

Harris (1982) states that instrumental design for quantitative analysis by solution spectrophotometry has remained conceptually stable since the early part of the last century. However, the advent of stable electronics has spawned a group of techniques for measuring absorbance that provides a quantum leap in spectrophotometric sensitivity. Modern Spectrophotometers are mostly designed with the following configurations:

- Single-beam
- Split-beam
- Double-beam, single-detector
- Double-beam, dual-detector

2.6.1 Single-Beam Spectro-Colorimeters/Spectrophotometers

Direct reading instruments offer greater speed in operation and convenience. However, they have lower accuracy and precision than the null-balance type instruments. Figure 2.47 shows a schematic of a conventional single-beam spectrophotometer. Polychromatic light from the source is focused on the entrance slit of a monochromator, which selectively transmits a narrow band of light. This light then passes through the sample area to the detector. The absorbance of a sample is determined by measuring the intensity of light reaching the detector without the sample (the blank) and comparing it with the intensity of light reaching the detector after passing through

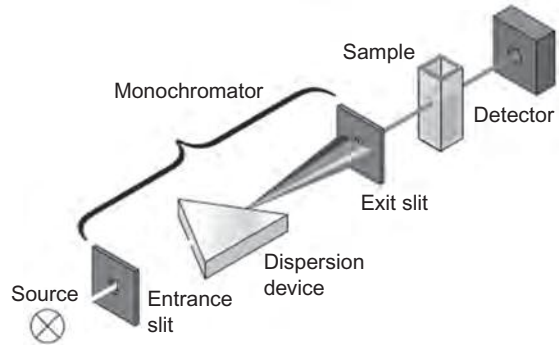


Figure 2.47 *Schematic of a single-beam direct reading spectrophotometer (courtesy: M/s agilent technologies)*

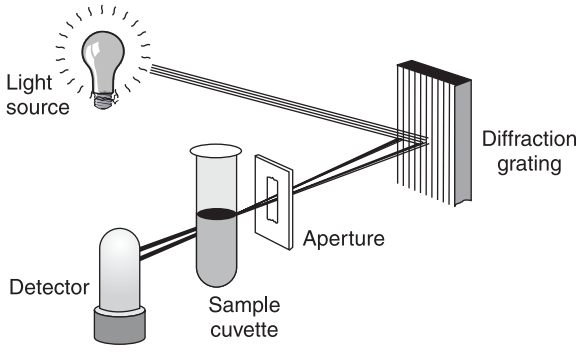


Figure 2.48 *Optical diagram of spectrocolorimeter using diffraction grating as a monochromator*

the sample. As explained earlier, most spectrophotometers contain two source lamps, a deuterium lamp and a tungsten lamp, and use either PMTs or, more recently, photodiodes as detectors.

This design is well-suited for measuring absorbance at a single point in the spectrum. It is less appropriate, however, for measuring different compounds at different wavelengths or for obtaining spectra of samples. To perform such tasks with a conventional spectrophotometer, parts of the monochromator must be rotated, which however may introduce the problem of mechanical irreproducibility into the measurements.

The optical path of single-beam direct-reading instrument of using a reflection grating is shown in Figure 2.48. The instrument makes use of a grating monochromator and can be used with tungsten lamp or arc stabilised mercury vapour lamp. A colour filter is used to block the higher ordered wavelengths reflected from the grating.

In such type of instruments, two interchangeable phototubes are employed, which are mounted on a sliding carriage. For the visible and near ultraviolet range (220–625 nm), a blue sensitive tube is used. It has caesium-antimony photosensitive surface and an insert of fused silica in the envelope. In the range 625–1,000 nm, a red-sensitive phototube having caesium-oxide coated photocathode is utilised. When photomultipliers are used for increased sensitivity or for operation at smaller slit widths, they usually replace the blue sensitive tube. The slit width is often marked in millimetres to serve as a performance check and allow resetting a previous slit opening.

2.6.1.1 Spectronic 20 spectrocolorimeter/spectrophotometer

Bausch and Lomb Spectronic 20 is a direct-reading grating spectrophotometer/spectrocolorimeter. In this instrument, normal range of operation is 350–650 nm and can be extended up to 900 nm by the use of a red-sensitive phototube. The monochromator comprises of a reflection grating, lenses and a pair of fixed slits as shown in Figure 2.49. The standard Spectronic 20 phototube is a type S-4 caesium-antimony tube, which is most sensitive to light in the blue region. A red-sensitive phototube is used above 625 nm.

The curved arm of metal at the bottom of the sample chamber is the occluder, which mounted on a pivot, opens and closes the shutter gate which allows light into the sample. It is automatically pushed aside by the cuvette when a reading is taken.

Continuous light over the entire visible spectrum is emitted from the tungsten lamp. This light is collected by the field lens and collimated (made parallel) by objective lens. The white light next falls upon the diffraction grating, which disperses it horizontally into the familiar sequence of spectral colours. The grating is ruled at 600 lines per millimetre. The dispersed light next passes through the light control, the occluder and the exit slit. Only when a cuvette is inserted into the sample chamber is the occluder moved out of the way. The width of the

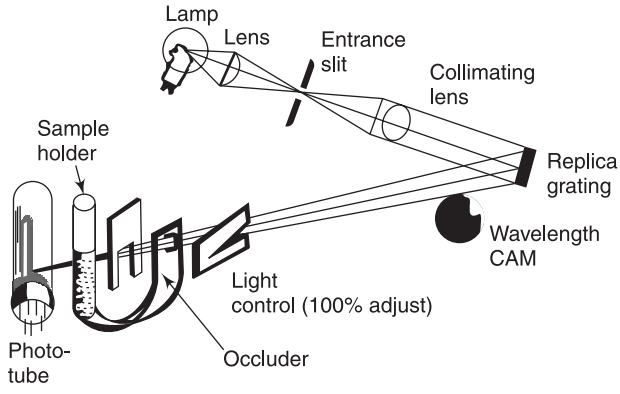


Figure 2.49 *Optical arrangement of spectronic-20 spectro-colorimeter*

exit slit and the spread or dispersion of the light from the diffraction grating together determine the spectral bandwidth, which is 20 nm. Thus, for example, if the instrument is set at 600 nm, light having wavelengths in the range 590–610 nm will be passed to the sample and phototube.

Only that portion of the light dispersed by the grating which falls on the exit slit is passed to the sample. Selection of the desired portion of the spectrum to be passed is accomplished by adjusting the angle of incidence between the source ray and the diffraction grating. The control on the instrument performs this task by physically turning the grating and is known as the wavelength control knob. Attached to this control is a dial calibrated in nano-metres, which indicates the setting.

As the grating produces a dispersion that is independent of wavelength, a constant bandwidth of the order of 20 μm can be obtained throughout the operating range. The electrical components include the power supply, the amplifier which strengthens signals coming from the phototube, and the readout device which in this case is a voltmeter calibrated in both absorbance and per cent transmittance units. In the earlier versions of this instrument, a differential amplifier was used to amplify the photocurrent from the detector. Since the amplifier current is proportional to the radiant power, the scale of the metre can be calibrated to read transmittance and absorbance. The amplifier is so constructed that the current through the metre is zero under no signal (dark current) conditions. With the detector input fed to the amplifier, the unbalance current as indicated in the metre is proportional to the radiant energy falling on the detector tube. The design of the amplifier is such that electrical fluctuations get cancelled out. The wavelength scale is linear and is coupled to the grating with a sine-bar drive.

In quantitative spectrophotometry, one is interested not in the absolute intensity of light passing through a sample, but rather in the relative intensity of such light with respect to the intensity of light passing through a reference or blank solution. It is thus necessary to set the 0 and 100% transmittance limits between which the transmittance of samples will be measured. The 0% T is set with no light reaching the phototube that is, with no cuvette in the sample chamber and all light blocked by the occluder. This setting is performed with the amplifier control on the instrument. This control adjusts the gain, or sensitivity of the amplifier, and the offset. This determines how much needle deflection is caused by a specific intensity of light striking the phototube. The 100% T is set with the light control, usually with a cuvette of water or another blank solution in the sample chamber.

Adjusting the light control knob causes a V-shaped slit behind the knob to move into or out of the light beam. It is thus a purely mechanical control which simply physically blocks out more or less of the diffracted light.

2.6.1.2 Spectronic 21

Spectronic 21 is an advanced version of a single-beam spectrophotometer from Bausch and Lomb. The instrument is designed to provide a wide wavelength range (200–1,000 nm), a narrow spectral width (10 nm) and more accuracy due to very low stray radiant energy. Figure 2.50 shows the optical diagram of the Spectronic 21. The system has two lamps mounted in an exterior lamp house. Light from the proper lamp is selected by appropriate positioning of the lamp interchange mirror, to either pass the deuterium light or reflect the white light from the tungsten lamp. A silica relay lens system focuses the light near the chopper. The light is chopped and focused by a condenser lens onto the entrance slit of the monochromator after passing through the appropriate filter. The optical arrangement used in the monochromator is called a folded crossover Czerny-Turner configuration, which provides higher quality optical characteristics over the wider wavelength range. After passing through the slit, the light is incident on a collimating mirror, which collimates the light onto the diffraction grating. The light dispersed from the grating is picked up by the focusing mirror, which focuses the light onto the exit slit. The exiting light then passes through the sample and is incident on the photodiode.

Via the rigidly built-in condenser and the mirror, an image of the radiation source is produced on the entrance slit. The light converted into parallel rays by a collimator objective strikes the diffraction grating and is spectrally dispersed by the achromat (An achromat is a lens that is designed to limit the effects of chromatic and spherical aberration.). The diffracted light is focused in the plane of the exit slit. Monochromatic radiation separated from the spectrum through the slit passes the measurement sample and strikes the radiation detector. The photocurrent produced is amplified in the amplifier and fed to the indicating instrument. The wavelength is set by turning the grating by means of the drum provided with 1 nm divisions in the range from 330 to 850 nm.

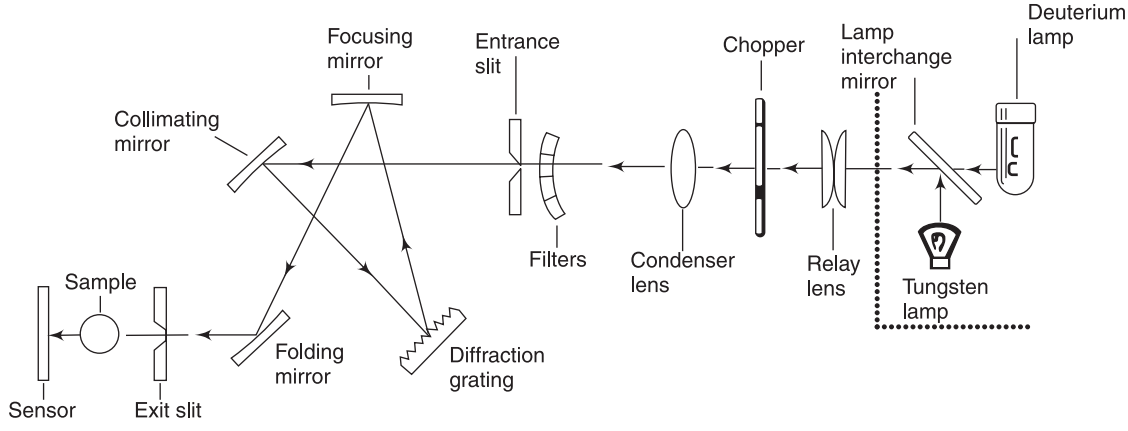


Figure 2.50 *Optical diagram of the SPECTRONIC 21 Spectrophotometer (Courtesy Bauch and Lomb, USA.*

2.6.1.3 Signal processing in direct reading spectrophotometers

In single-beam direct-reading instruments, use is made of operational amplifiers for handling signals from the detectors, which could be a vacuum phototube, photodiode, photomultiplier or photocell.

Figure 2.51 shows a typical circuit diagram for amplification and measurement of current from the phototube. The phototube anode is given 90 V from a stabilised power supply and its cathode is connected to ground through a high resistance R_1. Operational amplifier A_1. is of very high input impedance, low drift and low noise. The op-amp offers a very high input impedance, as the input is connected to the non-inverting input. The inverting input terminal is connected to a variable supply voltage (+15 V). This control is provided on the front panel to set zero, initially when light is blocked from falling on the phototube. This control not only balances the dark current, but also nullifies offset voltage of the op-amp.

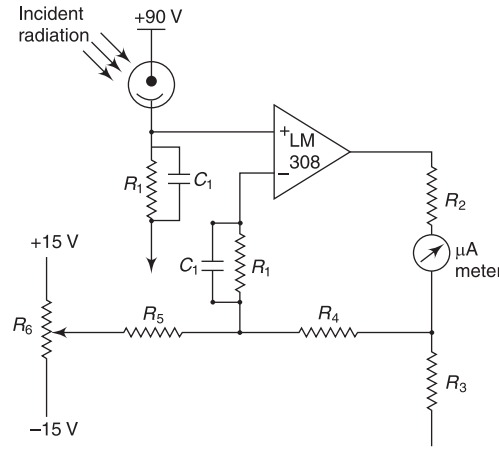


Figure 2.51 *Typical circuit used with direct-reading spectrocolorimeter/ spectrophotometer*

The feedback resistance uses a *T*-resistor network, instead of a single resistor. This arrangement gives a very high effective feedback resistance and at the same time, makes possible the use of resistors of lower values, which at the required precision of 0.1% are substantially cheaper and stable. The output of the amplifier gives linear readings on the transmittance scale. If absorbance is to be displayed, the output of the transmittance stage is given to a logarithmic converter, whose output is equal to the minus log of transmittance.

$$A = -\log_{10} T$$

The logarithmic modules are available in encapsulated form and can be directly used.

Currents from photomultipliers are usually very small. Therefore, considerable amplification is necessary before the current can be suitably recorded.

2.6.1.4 Spectrophotometer using diode array detector

The optical system of the spectrophotometer Model 8453 from M/s Agilent is shown in Figure 2.52. Its radiation source is a combination of a deuterium-discharge lamp for the ultraviolet (UV) wavelength range and a tungsten lamp for the visible and short wave NIR wavelength range. The image of the filament of the tungsten lamp is focused on the discharge aperture of the deuterium lamp by means of a special rear-access lamp design which allows both light sources to be optically combined and share a common axis to the source lens. The source lens forms a single, collimated beam of light. The beam passes through the shutter/stray-light correction filter area then though the sample of the spectrograph lens and slit. In the spectrograph, light is dispersed onto the diode array by a holographic grating. This allows simultaneous access to all wavelength information. The result is a fundamental increase in the rate at which spectra can be acquired.

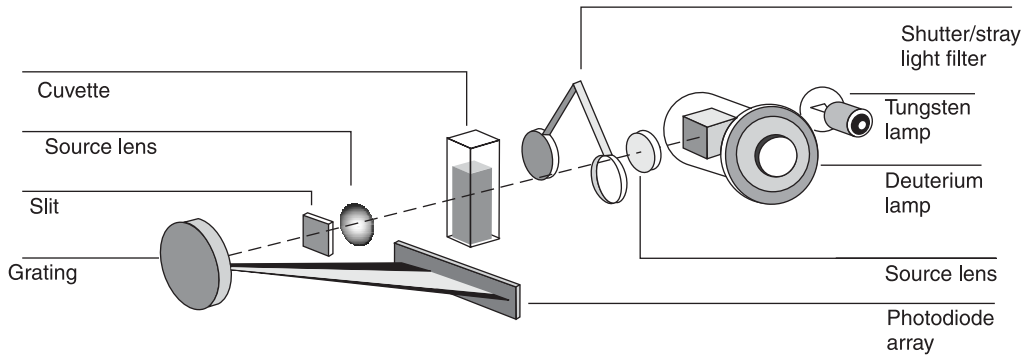


Figure 2.52 *Optical system of a spectrophotometer using diode array (Courtesy M/s Agilent)*

As a result of plasma discharge in a low pressure deuterium gas, the deuterium lamp emits light over the 190 nm to approximately 800 nm wavelength range. Similarly, the low-noise tungsten lamp emits light over the 370–1,100 nm wavelength range. The source lens receives the light from both lamps and collimates it. The collimated beam passes through the sample in the sample compartment.

The shutter is electromechanically actuated. It opens and allows light to pass through the sample for measurements. Between sample measurements, it closes to limit exposure of sample to light. If the measurement rate is very fast, you can the shutter can be commanded to remain open (Agilent ChemStation software) or it stays open automatically.

In a standard measurement sequence, reference or sample intensity spectra are measured without and then with the stray-light filter in the light beam. Without the filter the intensity spectrum over the whole wavelength range from 190 to 1,100 nm is measured. The stray-light filter is a blocking filter with 50% blocking at 420 nm. With this filter in place any light measured below 400 nm is stray light. This stray-light intensity is then subtracted from the first spectrum to give a stray-light corrected spectrum. Depending on the software, you can switch off the stray-light correction in case you want to do very fast repetitive scans or it is switched off automatically.

The spectrophotometer has an open sample compartment for easier access to sample cells. Because of the optical design a cover for the sample area is not required. The spectrophotometer is supplied with a single-cell holder already installed in the sample compartment. This can be replaced with the Peltier temperature control accessory, the thermostatable cell holder, the adjustable cell holder, the long path cell holder or the multi-cell transport. All of these optional cell holders mount in the sample compartment using the same quick, simple mounting system. An optical filter wheel is also available for use with the spectrophotometer and most of the accessories.

The spectrograph housing material is ceramic to reduce thermal effects to a minimum. It main components of the spectrograph are the lens, the slit, the grating and the photodiode array with front-end electronics. The mean sampling interval of the diode array is 0.9 nm over the wavelength range 190–1,100 nm. The nominal spectral slit width is 1 nm. The spectrograph lens refocuses the collimated light beam after it has passed through the sample.

The slit is a narrow aperture in a plate located at the focus of the spectrograph lens. It is exactly the size of one of the photodiodes in the photodiode array. By limiting the size of the incoming

light beam, the slit makes sure that each band of wavelengths is projected onto only the appropriate photo. The combination of dispersion and spectral imaging is accomplished by using a concave holographic grating. The grating disperses the light onto the diode array at an angle proportional to the wavelength.

The photodiode array is the heart of the spectrograph. It is series of 1,024 individual photocopies and control circuits etched onto a semiconductor chip. With a wavelength range from 190 to 1,100 nm the sampling interval is nominal 0.9 nm. The electrical signal from the diode arrays in their amplified and displayed in the system electronics.

Absorbance is a ratiometric measurement, which requires the measurement of the ratio of the intensity of light transmitted through the sample to the intensity of light transmitted through a 'blank'. With a double-beam instrument, this ratio is measured directly as the user installs both a blank and a sample into the instrument. Diode array instruments are single-beam instruments, and the blank and sample must be measured sequentially. With the HP 8453 photodiode array spectrophotometer, when the blank is measured, it is stored in a register which identifies it as the blank. The most recently measured blank is always used to calculate the absorbance spectrum of all subsequently measured samples.

2.6.2 Double-Beam Spectrophotometers

In a conventional single-beam spectrophotometer, the blank and the sample are measured consecutively, with an interval of several seconds for a single wavelength measurement and up to several minutes for a full spectrum measurement with a conventional instrument. Lamp drift can result in significant errors over long time intervals.

The double-beam spectrophotometer compensates for these changes in lamp intensity between measurements on blank and sample cuvettes. In this configuration, a chopper is placed in the optical path, near the light source. The chopper switches the light path between a reference optical path and a sample optical path to the detector. It rotates at a speed such that the alternate measurements of blank and sample occur several times per second, thus correcting for medium- and long-term changes in lamp intensity (drift).

Figure 2.53 shows a schematic of a double-beam spectrophotometer. Compared with single-beam designs, double-beam instruments contain more optical components, which reduces throughput

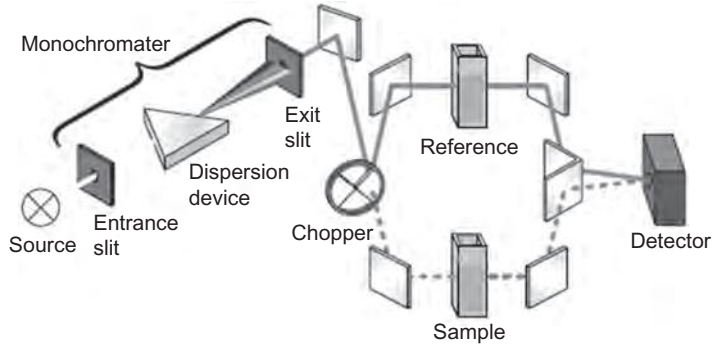


Figure 2.53 *Optical system of a double-beam spectrophotometer(Courtesy: M/s Agilent Technologies)*

and sensitivity. For high sensitivity, long measurement times may be required. In addition, the more complex mechanical design of the double-beam spectrophotometer may result in reduced reliability.

Most modern spectrophotometers are double-beam instruments. These instruments are configured to allow the automatic and simultaneous (or near simultaneous) measurement of the sample and reference beam intensities. This can be accomplished using a beam splitter to divide the beam into the reference beam and sample beam. In the double-beam-in-space configuration, the intensities of the split beams are measured simultaneously after passing through the sample and reference cells. Alternately, in a double-beam-in-time spectrophotometer, the beams pass through a modulator which allows the detector to see either the reference beam or the sample beam. Since the source beam is directed through a reference cell part of the time and through the sample cell the rest of the time, the term 'double-beam-in-time' is used.

The main disadvantage of single-beam spectrophotometer is that the instrument settings have to be adjusted to give a 100% reading with the reference in the beam, before the sample is examined. This drawback is overcome by using a double-beam instrument, wherein the arrangement is such that the radiation beam is shifted automatically to pass alternately through the sample and reference cuvettes. The cuvettes themselves are not shifted and remain in their fixed position. There are several ways by which this can be achieved. Most commonly, a single rotating sector mirror is used. Monochromators used in these instruments are similar to those used in single-beam instruments.

Figure 2.54 shows the signals in a ratio-recording double-beam spectrophotometer. The signals are in the form of pulses, which are directly proportional to intensities of radiation passing through the sample and reference. These two signals are resolved electronically into two DC voltages corresponding to the sample and reference beams, and the latter is used as the standardising potential on a potentiometric recorder. Double-beam instruments are also constructed on the optical-null photometer system (Figure 2.55). In these instruments, the alternating signal from the reference and sample is not separated into its components, but is used directly to drive a servomotor. The motor drives an optical attenuator or wedge into or out of the reference beam of the spectrophotometer, until it has the same intensity as the sample beam. The electrical signals produced at the detector from reference and sample sides are equal and there is no signal to drive the servomotor and it stops. The optical attenuator arrangement is such that the distance it is driven into the reference beam is proportional to either the transmittance or absorbance. Therefore, the distance it has moved when the signals become equal and the motor stops, is proportional to the

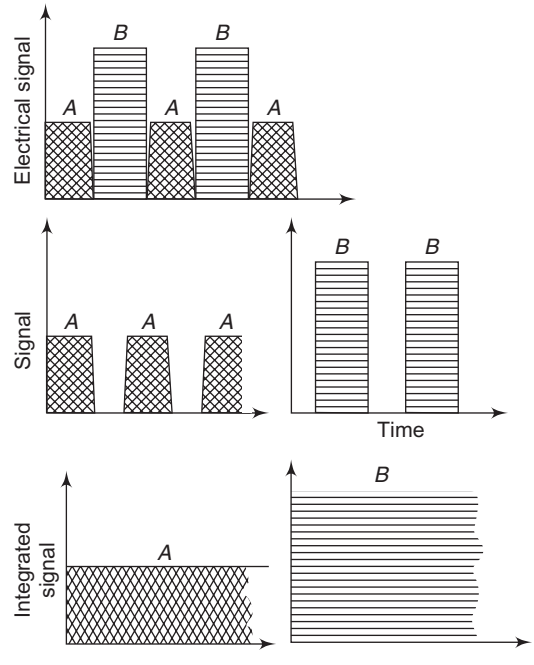


Figure 2.54 *Principle of ratio-recording spectrophotometric system*

relative intensifies of the sample arid reference beams. The absorbance or transmittance can be recorded by mechanically coupling the pen of a recorder to the attenuator.

Figure 2.56 shows optical arrangement of the double-beam spectrophotometer from M/a Varian. Either tungsten or a deuterium lamp can be switched in the system to cover an operating range from 200 to 700 nm, to cover the UV-Vis regions. The monochromator comprises of a diffraction grating and two narrow slits, one of which serves as an entrance, and the other as an exit. It is possible to set the spectral bandwidth at 2, 4, 8 or 16 nm. The larger bandwidths are used for increased light output; the smaller is used where spectral resolution is of importance. A four-segment

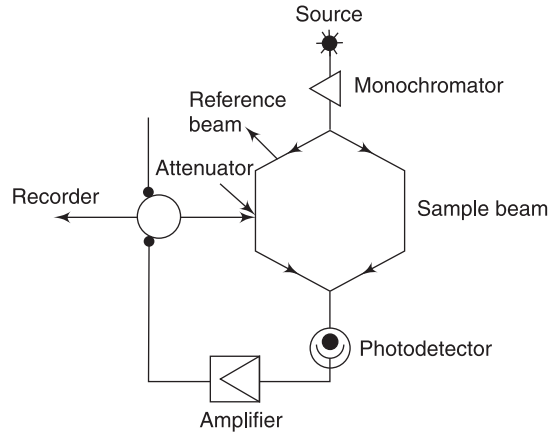


Figure 2.55 *Principle of optical-null spectrophotometric system*

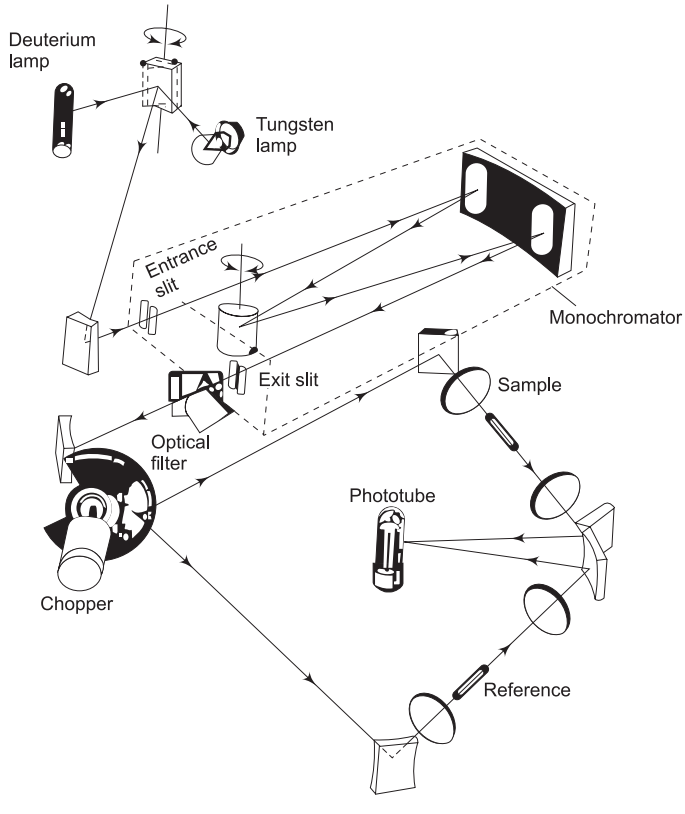


Figure 2.56 *Optical arrangement of a double-beam spectrophotometer (Courtesy M/S Varian, USA)*

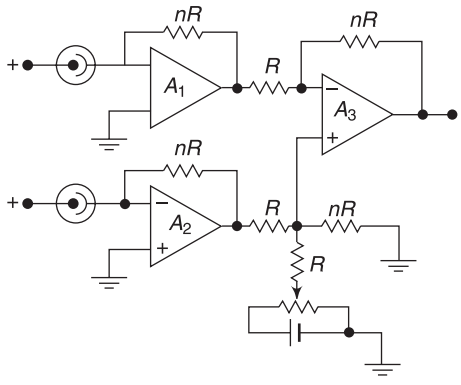


Figure 2.57 *Basic measuring circuit in double-beam spectrophotometer*

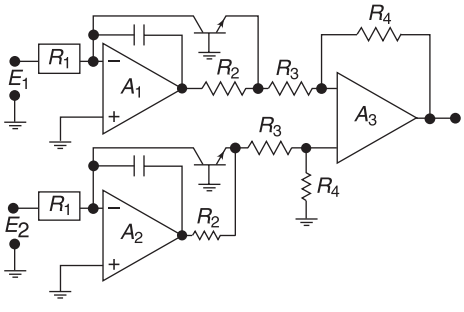


Figure 2.58 *Circuit for measuring logarithm of a ratio of two currents or voltages used in double-beam spectrophotometers*

filter automatically performs order sorting and rejects stray light from the monochromator, holding it to 0.1% at 340 nm, giving low noise over the entire wavelength range.

The instrument chopper is so synchronised that light passes alternately through the sample and the reference cells. In this arrangement, the entire light passes through a given cell when the transmission of that cell is being measured. This offers a distinct advantage over beam splitter arrangements, where only 50% of the available light passes through each cell during measurement.

Figure 2.57 shows the arrangement employed in measurement of transmittance in a double-beam instrument. The measuring process consists basically of comparing the photocurrents from two photocells or phototubes. The input circuits are current followers and their output signals are compared in a differential amplifier. If it is required to measure absorbance *A*, the output signals from a current follower are given to logarithmic amplifiers. The logarithms of the signals are subtracted in a differential amplifier. The output of the amplifier is given by

$$E_0 = \mathrm{K}.\log I_o/I = \mathrm{K}A$$

where K is constant and I_0 and I are the incident and transmitted light intensities respectively. The circuit used for this purpose is shown in Figure 2.58.

This circuit enables to take the logarithm of a ratio of the two voltages or currents, equalling the difference of the logarithms of the input signals. The outputs of the logarithmic amplifiers are brought to the inputs of a differential amplifier. This circuit is used mainly in spectrophotometric measurements, when the logarithm of the ratio of the radiant power of the incident radiation to that of the radiation after passage through the absorbing layer is determined. In the circuits described above, both transistors should have identical properties, and matched temperature dependent resistors should be employed. Integrated circuit logarithmic amplifiers are used in the most instruments.

The optical system as shown in Figure 2.59 is that of Model 24–25 from M/s Beckman Instruments double-beam instruments. Energy of the appropriate wavelengths is produced by the appropriate source lamp. This energy is converted to monochromatic light by using filter-grating optical system. The grating has 1,200 lines/mm and is blazed at 250 nm. The filters are necessary to eliminate unwanted orders from the grating. Six different filters cover the wavelength regions from 300 to 900 nm.

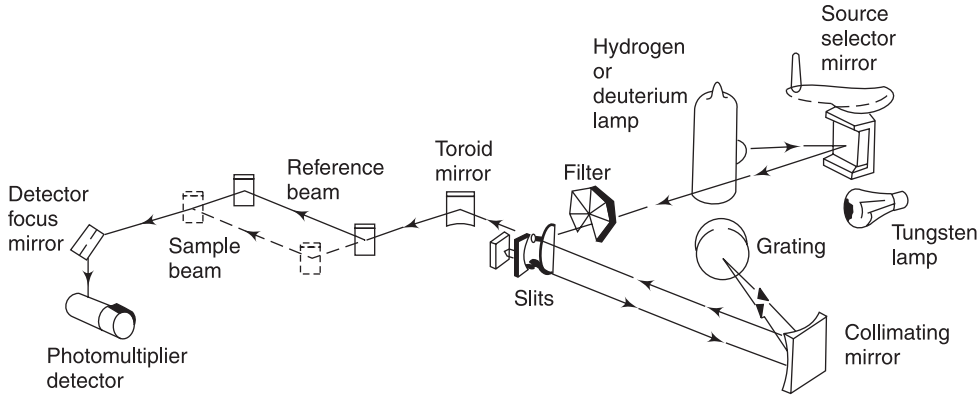


Figure 2.59 *Optical arrangement of Model 24–25 Beckman double-beam spectrophotometer*

The filter wheel is driven by DC motor, which is synchronised with the wavelength cam. As the wavelength cam moves, it causes the filter motor to drive till the correct filter comes in position. The wavelength cam drives the wavelength arm, which in turn causes the grating to pivot on its own axis, thereby causing the wavelength of light coming out of the monochromator to change. The monochromatic light is then directed to the sample and reference via a vibrating mirror bridge, which vibrates horizontally at a certain frequency. This bridge allows light to pass into the sample and reference cell holders alternately, with a frequency equal to the displacement frequency of the bridge. The vibrating bridge is controlled by the bridge drive circuitry. The reference and sample pulse train is then passed to the PMT, which converts the monochromatic light pulses to current pulses.

Split-Beam Design: The split-beam spectrophotometer (Figure 2.60) resembles the double-beam spectrophotometer but uses a beam splitter instead of a chopper to send light along the blank and sample paths simultaneously to two separate but identical detectors. This configuration enables the blank and the sample to be measured at the same time. Although the split-beam design is mechanically simpler than the true double-beam instrument and requires fewer optical elements, the use of two independent detectors introduces another potential source of drift. This design

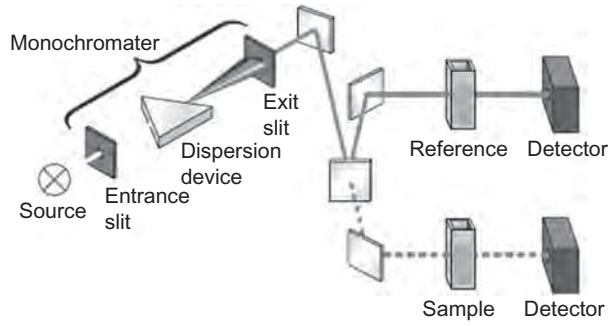


Figure 2.60 *Optical system of a split-beam spectrophotometer (Courtesy: M/s Agilent Technologies)*

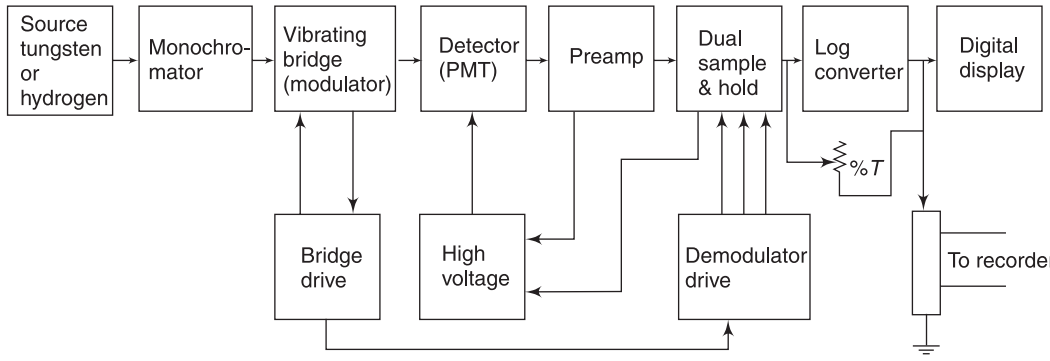


Figure 2.61 *Block diagram of electronic part of Model 24-25 Beckman double-beam spectrophotometer*

provides high stability, although not as high as a double-beam instrument since two detectors can drift independently.

Figure 2.61 is a block diagram of the electronic part of the instrument. As the vibrating bridge chops light energy coming from the monochromator at 35 Hz, the output of the PMT will be an AC signal. This signal is passed to the preamplifier where it is amplified. The input of the preamplifier is a FET, which offers a high input impedance to the signal. The amplified signal is then given to a demodulator, which separates reference pulses from sample pulses and converts sample and reference pulses to a DC potential. However, the demodulation process requires synchronisation, so that when the bridge is directing light through the sample path, the electronics is demodulating the sample pulse and the same will hold true for tie reference pulse. To achieve synchronisation, the same signal that is used to drive the coil for the vibrating bridge is tapped *off* and used as the input to the demodulator.

The DC output of the reference side is also fed back to the high voltage power supply. If the reference signal should decrease, the high voltage would increase, thus restoring the reference channel to a constant potential. This would ensure constant energy through the reference channel during the scan.

For making absorbance measurements, the DC potential from the sample-and-hold amplifier is passed to the log convenor. Differential absorbance can be measured between – 0.3 A to + 0.7 A by switching in bucking potential at the output stage. The analog DC potential is converted into binary coded decimal and this information is then displayed as an absorbance or concentration value on the digital display. The output of the log converter is applied to a divider network, which in turn drives an external recorder.

2.6.3 Microprocessor-Based Spectrophotometers

Computers have long been used in spectrophotometry, especially for on-line or off-line data processing. Since the advent of the microprocessor, their application has not been limited to processing of data from analytical instruments, but has been extended to control of instrument functions and digital signals processing, which had been performed conventionally by analog circuits. This has resulted in improved performance, operability and reliability over purely analog instruments.

A microprocessor, in a spectrophotometer, could be used for the following functions:

- *Control functions*: Wavelength scanning, automatic light source selection, control of slit width and detector Sensitivity, etc.
- *Signal processing functions*: Baseline correction, signal smoothing, calculation of % *T*, absorbance and concentration, derivative, etc.
- *Communication functions*: Keyboard entry, menu-driven operation, data presentation, warning display, communication with external systems. etc.

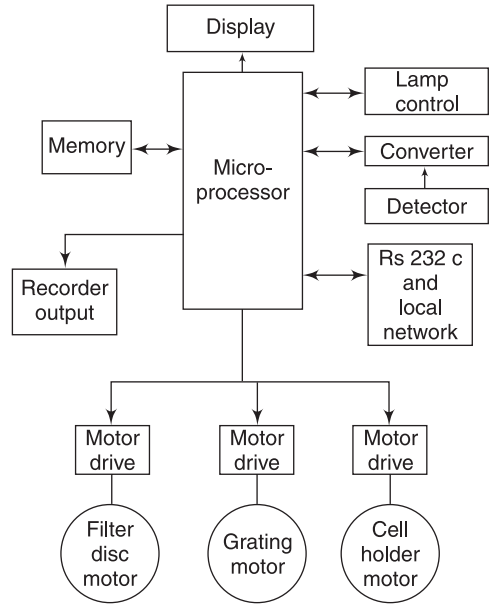


Figure 2.62 *Block diagram of a microprocessor controlled spectrophotometer*

Figure 2.62 shows a block diagram of a microprocessor controlled spectrophotometer. The diagram shows only the post-detector electronic handling and drive systems, all controlled via a single microprocessor. Once the operator defines such parameters as wavelength, output mode and relevant computing factors, the system automatically ensures the correct and optimum combination of all the system variables. Selection of source and detector are automatically determined; any filters introduced at appropriate points and sample and reference cells are correctly managed in the sample area. Output in the desired form (transmittance, absorbance, concentration, etc.) is presented along with the sample identification. Secondary routines such as wavelength calibration and self-tests become available on demand.

For wavelength scanning, a stepper motor is used, which ensures accurate and fast scanning. The automatic selection of samples is also made with a motor driven system under the control of a microprocessor.

The signal from the photodetector is amplified in a preamplifier and convened into digital form in an A-D converter. The signals are differentiated into sample signal S, reference signal R and zero signal *Z* and stored in the memory. From these values, the microprocessor calculates the transmittance $T = (S-Z)/(R-Z)$ and absorbance = $-\log T$. *In* order to obtain *R* or *S* values within specified range, the microprocessor provides control signals for slit width and high voltage for photomultiplier.

Baseline compensation due to solvent and optical unmatching of cells, which has been difficult with conventional instruments is conveniently possible in microprocessor-based systems. Improvements have also been achieved in such functions as auto-zero, expanding and contracting of the photometric scale, automatic setting of wavelength as well as in ensuring repeatable and more accurate results.

The digital output from the microprocessor is converted into analog form with D-A converter and given to an X-Y recorder as Y-axis signal, whereas the wavelength forms the X-axis, to obtain absorption or reflected spectral. Microprocessors have also enabled making such measurements as higher order derivative spectra and high speed sampling and storage of fast reaction processes and

for presenting processed data during and after the completion of the reaction. Figure 2.63 shows a DU series UV visible spectrophotometer from M/s Beckman Coulter Instruments.

Modern spectrophotometers invariably provide for electronic storage of method parameters. Ideally, all relevant parameters are stored in a single method file under a unique name. When the operator enters the name of the method, all parameters get set automatically, thus eliminating potential error.

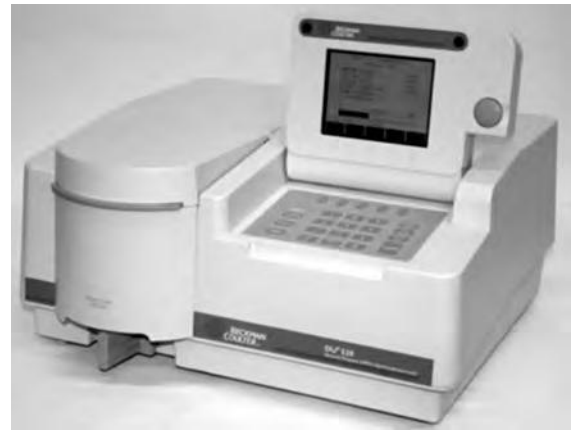


Figure 2.63 *Typical microprocessor-based spectrophotometer model-520 (Courtesy: M/s Beckman Coulter Instruments)*

Perkin -Elmer LAMBDA 9 Double-Beam Spectrophotometer: Perkin-Elmer LAMBDA 9 is a micro-computer-based double-beam UV-Vis-NIR (near infrared) spectrophotometer. It incorporates a video display, a keyboard and a printer/plotter for recording of spectra and data. This instrument covers the wavelength range from 3,200 to 185 nm. A photomultiplier is used as a detector in the UV-Vis range, whereas a PbS (lead-Sulphide) cell takes over in the NIR range. The detector changes at 860.8 nm. An aligned deuterium lamp for the UV range and a halogen lamp for the visible and the NIR range serves as light source. The source change takes place automatically at 319.2 nm. The double monochromators in Littrow arrangement are each provided with one holographic grating with 1,440 lines for the UV-Vis range and one holographic grating with 360 lines for the NIR range. In the UV-vis range, the spectral bandwidth can be adjusted from 0.05 to 5nm and on the NIR range from 0.1 to 20 nm.

Figure 2.64 shows the optical arrangement of the instrument. For operation in the near infrared and visible ranges, source mirror M_1 reflects the radiation from the halogen lamp onto mirror M_2. At the same time, it blocks radiation from the deuterium lamp. For operation in the ultraviolet range, source mirror M_1 is raised to permit radiation from deuterium lamp to strike mirror M_2. Source change is automatic during monochromator scanning.

From mirror M_4, the radiation is reflected through the entrance slit of monochromator 1. The radiation is collimated at mirror M_5 and reflected to one of the gratings depending on the wavelength range. After appropriate segment of the spectrum is selected, it is reflected to Mirror M_5 and from there to the exit slit. The exit slit of monochromator 1 serves as the entrance slit of monochromator 2. The radiation is reflected via mirror M_6 to the appropriate grating and then back via mirror M_6 through the exit slit to mirror M_7.

A choice is provided between a fixed slit width and a servo slit programme in the UV-Vis range. During scanning, the slit widths change automatically to maintain constant energy to the detector. A servo slit program is provided for the NIR range.

From mirror M_7, the radiation beam is reflected via toroid mirror M_8 to the chopper assembly. When the chopper rotates, a mirror segment, a window segment and dark segments are brought alternately into the radiation beam. The resulting beams emerge as follows:

- When a mirror segment enters the beam, radiation is reflected via mirror M_9, creating the sample beam *S*.

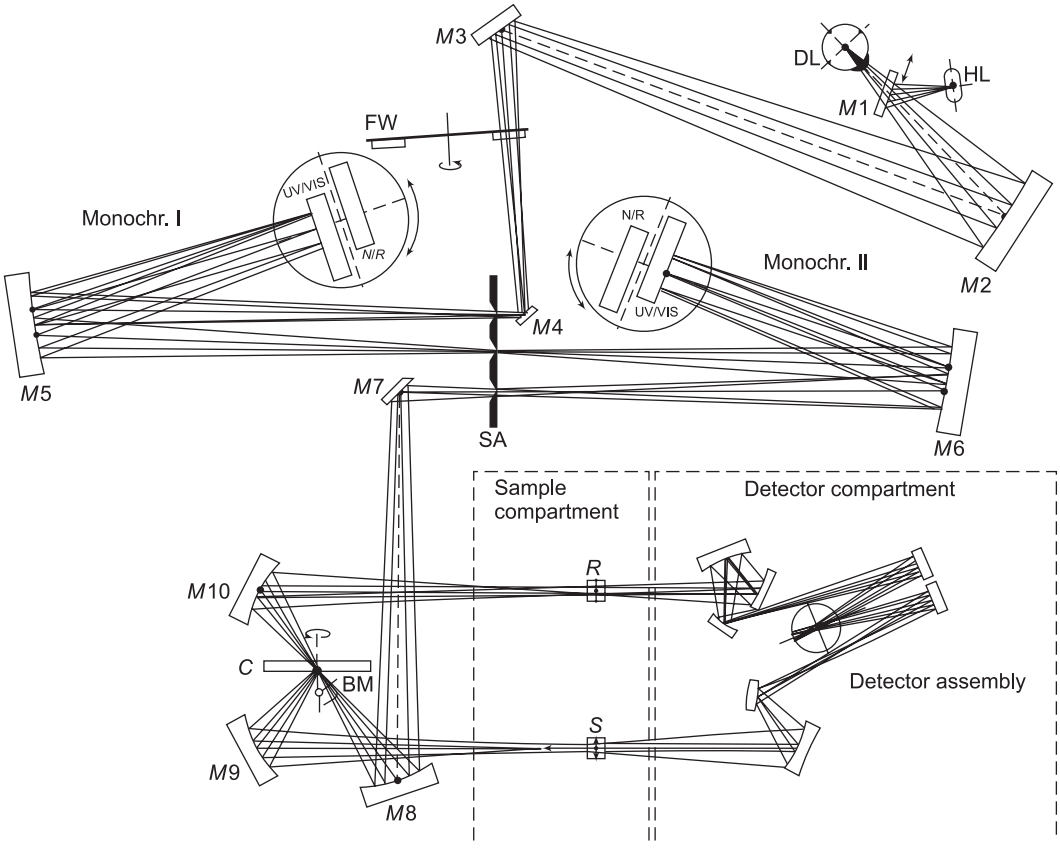


Figure 2.64 *Optical diagram of LAMBDA 9 Perkin-Elmer Spectrophotometer*

- When a window segment enters the beam, radiation is reflected via mirror M_{10}. Creating the reference beam *R*.
- With a dark beam no radiation reaches the detector, thereby creating the dark signal.

The radiation passing alternately through the sample and reference beams falls on the appropriate detector; a photomultiplier for the UV-Vis range while Pbs detector for the NIR range. Detector change is automatic during monochromator scanning. During all scanning operations, the monochromators stop slewing and plotter/printer chart advance is stopped until the respective filter, source or detector change is complete.

The electronic part of the instrument is based around Motorola MC 6808 microprocessor which provides control and data processing functions. The processor has 8-bit data and 16-bit address buses. The programme is stored in 3 EPROMS type 2764. 4 RAMs with 2k × 8 bit each are used for data storage. In ease of power failure, the RAMs and their control obtain their operating voltage from a battery.

The photomultiplier which is used as a detector in the UV-Vis range converts the light received into current. The supplied current of approximately 0.1 μA is converted to approximately 2 V in a preamplifier. Similarly, the resistance changes detected in the Pbs detector are converted into a

voltage signal of approximately 2 V for 100% transmittance. Following amplification of the signal is an A-D converter which converts the analog signal to digital information for processing by the micro-computer. The conversions carried out continuously one after the other is sample signal, reference signal and calibration signal.

Wavelength drive, slit drive, filter wheel drive, source selector mirror drive, detector change and grating change are all carried out by stepper motors. Stepping control is effected by the micro-computer, with the pulse frequency depending on the individual scan speed.

A computer unit processes the data supplied by the microprocessor and transmits them with the suitable format to the video display screen and to the keyboard. It also supplies the data to the printer/plotter.

2.6.4 High Performance Spectrophotometers

The model 8450A spectrophotometer from Hewlett Packard is based on a different concept, in which the sequence of optical components is reversed compared to the more traditional design approach. The principle is shown in Figure 2.65. The source is now focused on the sample instead of the monochromator. After passing through the sample, the remaining light enters a spectrograph, rather than a monochromator. This permits access to all wavelength information simultaneously, rather than serially as with a monochromatic system. Thus, we can acquire the same information on all resolution elements simultaneously offering tremendous speed advantage. Also, since there are no moving parts in the system, the uncertainty caused by wavelength reproducibility due to mechanical linkages is avoided (Knud et al., 1980).

Baseline stability in a reversed optical system can be a serious problem, which is taken care of by the adaptive optical system shown in Figure 2.66. The system is basically divided into three principal sections, which are detailed below:

The source section contains two light sources which are combined and focused on a source slit. The first element *is a 20* W tungsten-halogen lamp. Its filament is imaged through a spectral flattening filter onto *the aperture* of a see-through deuterium lamp. The source slit *is* 0.120 mm by 0.600 mm. A replicated ellipsoid with the lamp at one focus and *the* slit at the other focus serves *as* the condenser mirror.

The sample section contains two flat mirrors on a common shaft, which move the light beam *to* different sample positions. The shaft is under control of the computer and a servo mechanism. The light diverging from the common ellipsoid focus at the sample cell position strikes a field lens, which is located as close as mechanically feasible to the sample position. This field lens images the first sample section ellipsoid onto the second sample section

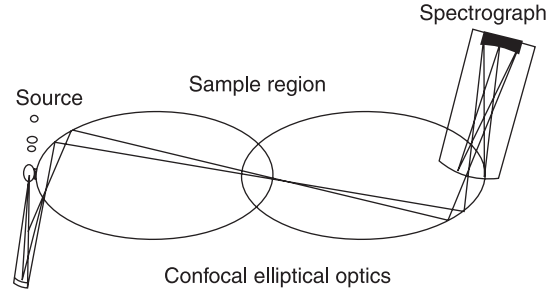


Figure 2.65 *Principle of HP spectrophotometer Model 8450A. Here, the traditional optics principles are reversed. The light passing through the sample is wideband instead of a single wavelength. The detector is a spectrograph that measures all wavelengths simultaneously, instead of one at a time (Courtesy Hewlett Packard, USA)*

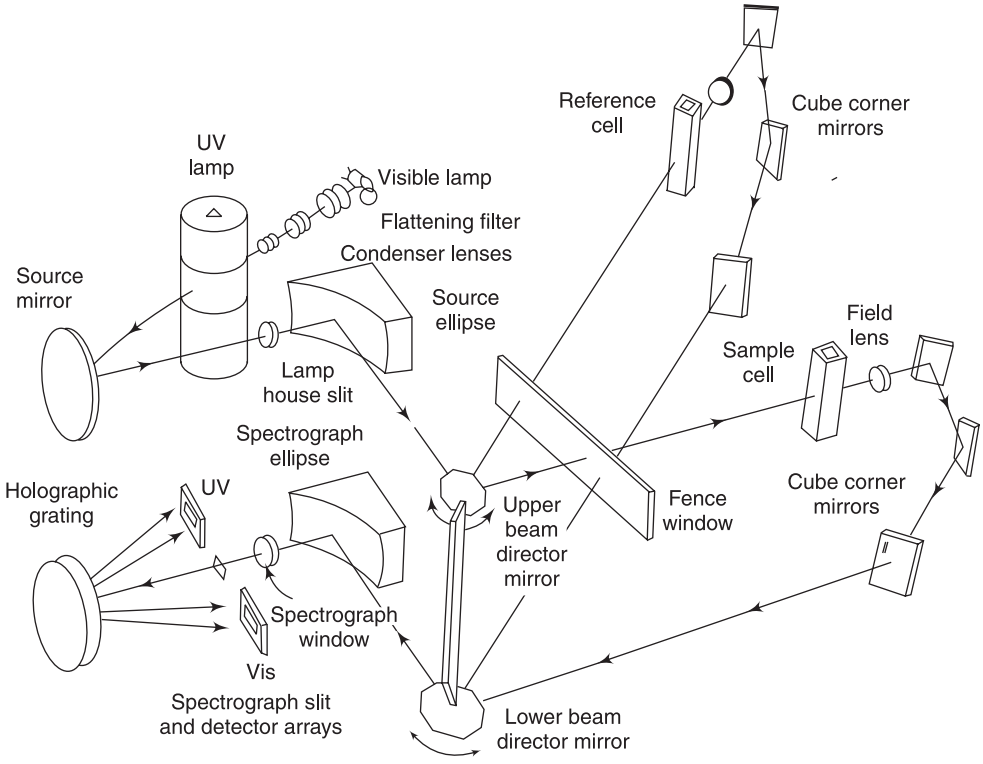


Figure 2.66 *The folded optical system of the H.P 8450A spectrophotometer (Courtesy Hewlett Packard, USA)*

ellipsoid, to reduce clear aperture requirements at the second ellipsoid and to increase flux through the system.

Next, there are three flat mirrors. The mirrors form a cube corner, whose diagonal intersects the beam director shaft midway between the centres of the two-beam director mirrors. The cube corner returns the entering beam back, along the direction it entered. After reflection from the lower beam director mirror, light is focused onto a 0.05 mm by 0.50-mm slit, by the second sample section ellipsoid. The image of the 0.120 mm by 0.600-mm source slit is formed on top of the spectrograph slit. The slit jaws are chisel mirrors tilted at l5° to direct light from the sides of the image on to photodiodes, on each side of the entering beam. These diodes provide a signal for the servomechanism, which controls the beam director shaft location, by balancing signals from the two diodes.

The spectrograph section contains two holographically recorded diffraction gratings, which receive light passing through the slit. The gratings are formed on a common substrate and the grating lines are tilted at 3.5° to the vertical to separate their spectra. A photodiode array is positioned at the first order spectrum of each grating (Hopkins and Schwartz, 1980). Each of these arrays uses 200 elements. One grating (UV) covers 200–400 nm, while the other grating (the Vis) overs 400–800 nm. Second order spectra signals are eliminated by using absorption filters placed over appropriate portions of the diode arrays.

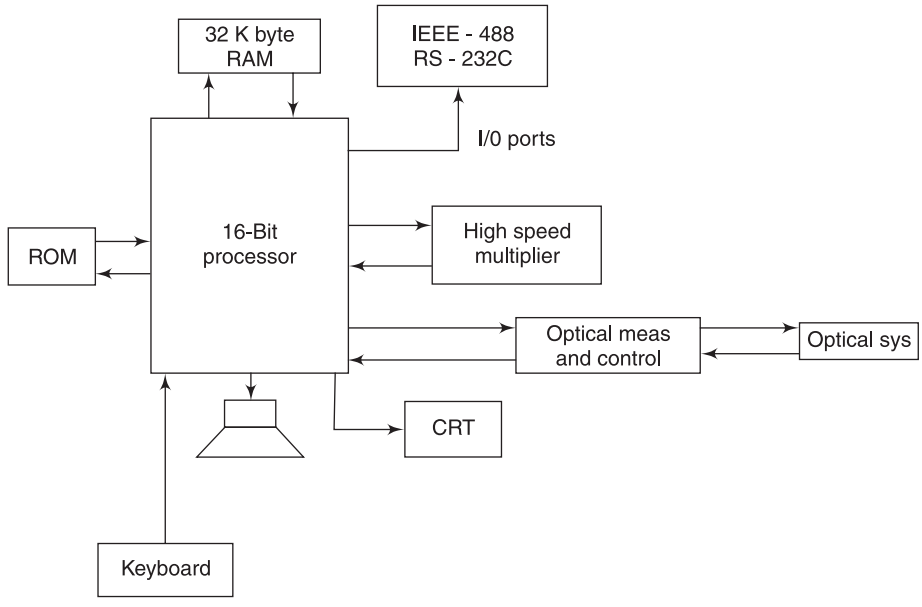


Figure 2.67 *Block diagram of the microprocessor control of the Model 8450A Spectrophotometer, (Courtesy Hewlett Packard, USA)*

Figure 2.67 gives a block diagram of the overall control and communication system of the spectrophotometer Model 8450A. It incorporates HP MC-5805 (Silicon-on-Sapphire) 16-bit microprocessor. The interactive control program occupies 57,344 bytes of ROM, which enables control of the instrument highly user-oriented. Besides the ROM control memory, the processor makes use of 32 KB of RAM, which is expandable to 64 KB. The signal from the photodiode arrays is connected to the input of the first amplifier, which is configured as a low-noise charge integrator. This results in a voltage proportional to light intensity, at the output of the integrator stage. This voltage is stored by the sample-and-hold circuit and the integrator is reset, ready to access the next diode.

The output of the integrator is fed to the input of a programmable-gain amplifier, which normalises the level of signal from each diode to the analog-to-digital converter (ADC) input voltage range. This is necessary because the photocurrent generated in the diodes varies considerably over the spectrum, primarily because of variations in the output of the lamps. The ADC converts the analog signals at the sample-and-hold stage into 14-bit digital words that are read by the microprocessor system. The processor enables the system to select the appropriate service routines and carries out necessary data processing and calculations.

The operation of the spectrophotometer and the measurement sequence is controlled from a keyboard. For a one-second measurement, the beam director starts from the resting dark position. It is commanded to move to the sample position, and when the system verifies that it is there, the sample integration begins when complete, the beam moves to the reference position and then the integrator cycle for the reference path is initiated. After the sample and reference measurements, the beam is returned to the dark position and one additional dark measurement is made for the running average.

When all the measurements have been taken, the final calculation of absorbance is initiated. A fast table-look-up algorithm for the calculation of logarithms with interpolation for the necessary decision is used to complete the calculation for all 401 wavelength values and then statistics within 1 s.

Like all modern microprocessor-based instruments, the display of results and information is on the LCD display, which can show simultaneously alphanumeric and graphic plots simultaneously. The user can communicate with a printer for hard copy of results and also with a remote computer. Communication is established by connecting these devices to either IEEE-488 or the RS-232C connectors.

2.6.5 Dual-Wavelength Spectrophotometer

The dual-wavelength spectrophotometer permits the recording of absorbance changes in the same sample to be made at two different wavelengths alternately, and virtually simultaneously. This function is performed by means of a control and reversible motor, which automatically adjusts the monochromator wavelength control to alternate between the two wavelengths selected. Following completion of the measuring cycle of the cuvette positioned at the first wavelength, the direction of the drive motor is reversed and the monochromator adjusted to the second wavelength. The two absorbance measurements are registered on the chart almost simultaneously.

There are three modes of operation for dual-wavelength spectrophotometers, which all depend upon chopping the light source in such a way as to time-share two signal sources on the detector output. First is the dual-wavelength mode. In this arrangement, two wavelengths of light are alternately passed through a single sample. The difference between transmittance or absorbance at the two wavelengths is measured as a function of time. This mode is used primarily to monitor the kinetics of reactions in the sample. In the second method, the two wavelengths of light are alternately passed through a single sample. In this case, one wavelength is scanned over some small range, while the other wavelength is held fixed at some reference point. The third mode is called the split-beam mode. Here a single wavelength is alternately passed through two separate samples. The wavelength is scanned over any region and a transmittance or absorbance difference between spectra of the two samples is the desired output.

Figure 2.68 illustrates the optical system of a dual-wavelength spectrophotometer. Light from the source is focused on the entrance slit of duochromator by quartz condensing system L_1 and L_2. The lens L_3 causes an image of the mask in back of L_2 to be projected on the gratings G_1 and G_2. This mask has two adjacent rectangular windows. Light going through one window illuminates grating G_1 and light going through the other illuminates grating G_2. The rotating shutter blade mounted on the motor allows alternate opening and closing of the two windows in 60 Hz sequence, and the two monochromatic beams leaving the exit slit S_2 are time-shared and bear a fixed frequency and phase relationship with the driving line voltage.

In the split-beam mode of operation, since both beams must be of the same wavelength, the gratings are driven angularly in synchronism by the scanning motor. This is achieved by setting the reference grating at any wavelength below the region to be scanned, for example 300 nm. When this is done the measure grating drives the reference grating at equal angle in a captive fashion. The wavelength driving motor scan is continuously indicated by the measure side. The voltage developed across a potentiometer, which is coupled to the lead screw that determines the grating position, is used to drive the X-axis of the x-y recorder. Upon leaving the duochromator, both beams are merged through reference and measure cuvettes by mirrors M_4 and M_5.

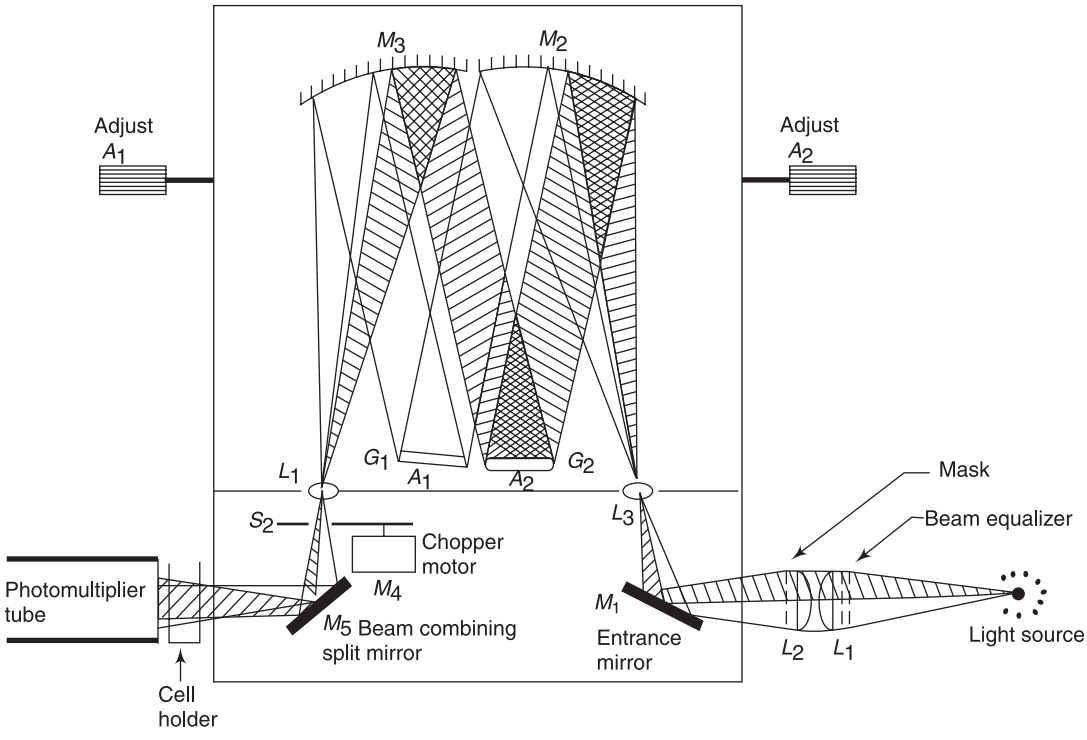


Figure 2.68 *Optical system of dual-wavelength spectrophotometer*

In the dual-wavelength mode, each of the gratings is set by dialling the appropriate wavelengths by means of the two controls on the panel of the instrument. Thus, radiant energy of these selected wavelengths passes alternately through the sample, where it is absorbed by or transmitted through the material under study. When a difference of transmittance along the two optical paths occurs, there is an alternating error signal, which is amplified, demodulated and read out as the difference between the absorbance readings at the two wavelengths.

Monitoring at alternate wavelengths can result in considerable time savings on lengthy reactions. In addition, the comparison of absorbance measurements at two different wavelengths can result in more effective analysis of the effluent from chromatographic columns.

Figure 2.69 shows the schematic of the electronic system. It consists of two basic circuits: the measure circuit and the reference circuit. The measure circuit provides a signal to the recorder, which is the difference of the intensities of the measure beam from the reference beam in optical density units. In dual-wavelength operation, the difference in intensity of the reference-monochromatic wavelength beam from the measure-monochromatic wavelength beam is plotted on the recorder in terms of an optical density difference. In split-beam operation, a difference or a ratio spectrum can be obtained. The reference circuit serves to internally standardise the instrument electronically at the frequency of the mains supply. It does this by dynode feedback control technique. In this method, the signal from the photomultiplier is electronically sampled during the time the reference cell or reference wavelength is being seen. It is then compared to a reference

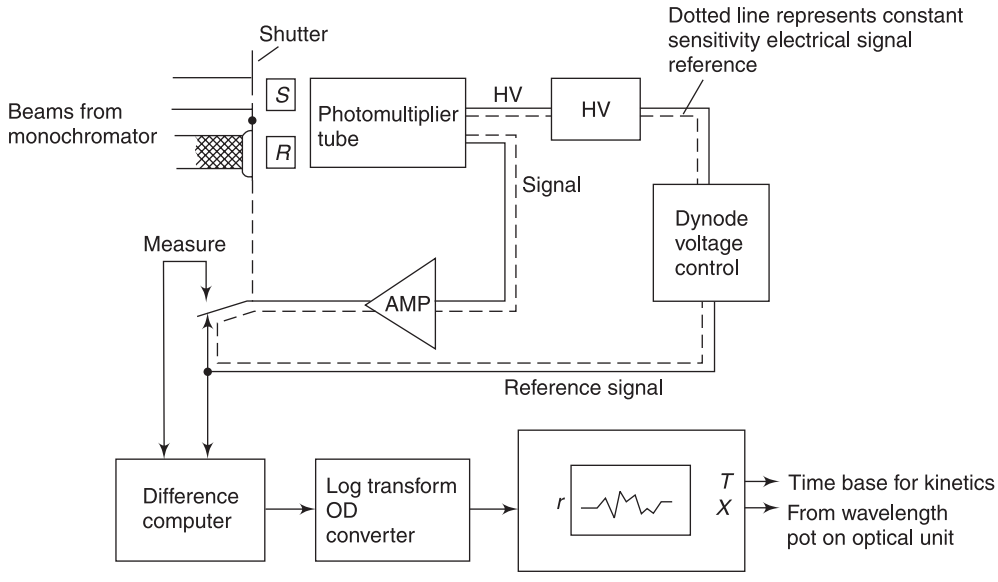


Figure 2.69 *Signal processor for dual-wavelength spectrophotometer*

voltage and an error signal is generated, which is fed back to raise or lower the high voltage applied to the photomultiplier, so that the signal coming from it, during the reference portion of operation is exactly equal to the reference voltage. In dual-wavelength operation, the reference circuit assures that the basic sensitivity of the instrument does not change with time, since there is continuous re-standardisation. This is important because changes in lamp intensity and photomultiplier instabilities would cause the instrument to drift. The signals are processed in a computer and given to the recorder.

2.6.6 Scanning Spectrophotometers

Scanning spectrophotometers are dispersive devices that normally utilise diffraction gratings to scan across a spectral region. Scanning devices can be used for multiple-component analysis applications in the entire UV, visible, and NIR regions. An important development has been the interfacing of fibre optics wavelengths with conventional scanning spectrophotometers as illustrated in Figure 2.70.

The optical waveguide is usually a single fibre optic cable. An advantage of using fibre optics is the elimination of the sample handling system. In the fibre optic design, the polychromatic light from the source passes through lenses and filters and onto the fibre optic cable. The light is transferred along the cable to the sample probe. The sample modified light is then collected by a second fibre cable and transferred to the monochromator, where it is diffracted into individual wavelengths and measured by the detectors.

The data from the analyser is processed by a personal computer (PC). The PC allows for the use of multi-variate calibration techniques. The measurement of multi-component solvent mixtures and the determination of octane number for gasoline samples are two typical applications performed on fibre optic scanning spectrometers.

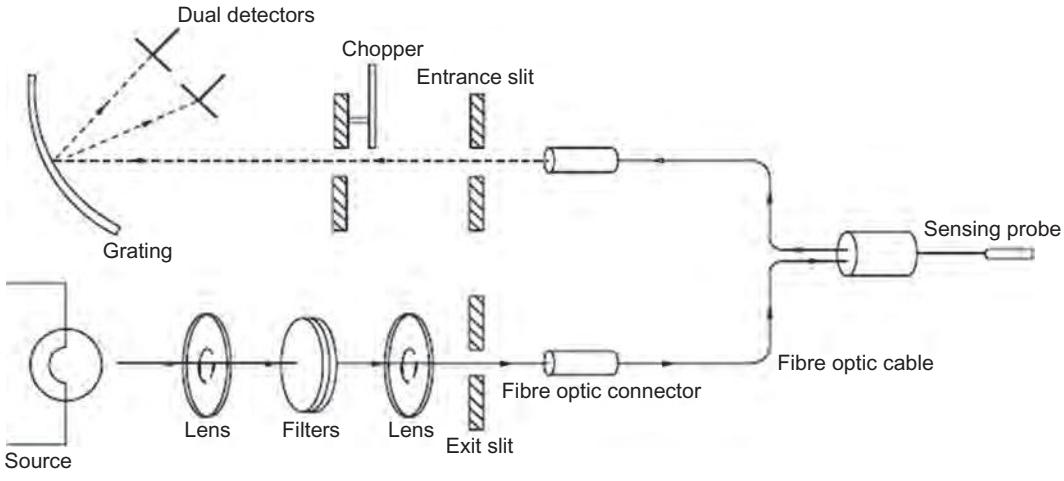


Figure 2.70 *Scanning spectrophotometer with fibre optic interfacing sensing probe (after Zetter, 1987)*

2.6.7 The Derivative Technique

The absorption spectrum of solids can consist of a large number of overlapping absorption bands. In order to isolate the bands, it is necessary to use a system having high spectral resolution. However, in most of the cases, it is only possible to resolve the first few absorption bands, and the higher quantum number bands appear as points of inflection on the sloping recorder trace of transmitted light intensity due to overlap. In such cases, $dT/d\lambda$ can give a clearer indication of small spectral details (Chopra, 1986).

A derivative attachment for a recording prism spectrophotometer, which gives a direct measurement of the first or second wavelength derivative of the light intensity transmitted by a sample is shown in Figure 2.71. The arrangement essentially employs a torsional scanner, which consists of a small mirror mounted on one prong of a tuning-fork, which can be set into torsional oscillations at its resonant frequency. The amplitude of oscillation can be varied by use of an electronic drive circuit, which provides a pure sine wave reference signal. The oscillating mirror moves a selected portion of the spectrum sinusoidally across the photomultiplier entrance slit. The original paper shows mathematically that the signal transferred to the recorder would be proportional to either the first or second wavelength derivative of transmittance. Calibration method is also illustrated.

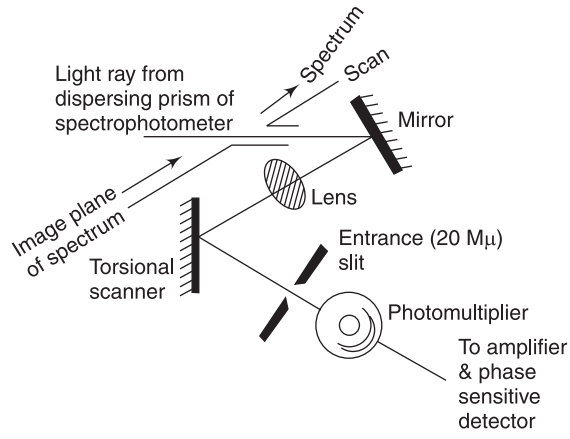


Figure 2.71 *Schematic of derivative attachment*

Some spectrophotometers are provided with plug-in derivative mode module. This module enables the spectrophotometer to measure the

instantaneous slope of the absorbance curve at any wavelength. In other words, the spectrophotometer can measure $dA/d\lambda$. The module utilises a passive resistor-capacitor electrical network to create the value $dA/d\lambda$, at each wavelength for the corresponding sample absorption curve. Due to a slight lag in the signal response with the electrical network, an absolute value of $dA/d\lambda$ is obtained only if scan speed is very low. The derivative curve can be used as a qualitative analysis tool to ascertain impurities, whose absorption peaks occur near or under the absorption peaks of the sample of interest.

The wavelength scanning speed influences the amplitude of the derivative spectrum and the back-ground noise. Increasing the scanning speed will generally improve the signal-to-noise ratio.

Modern spectrophotometers and associated software are frequently configured to operate in the first to fourth derivative modes. The use of even higher derivative orders is possible and they could be expected to produce even greater improvements in resolution by further banding. However, the apparent usefulness of high derivative orders may be compromised by the increasingly complex effect of side band satellites on the overall profile.

2.7 SOURCES OF ERROR IN SPECTROPHOTOMETRIC MEASUREMENTS

The sources of error in spectrophotometric measurements can be divided into two categories instrument-related errors and non-instrumental errors.

2.7.1 Instrument-related Errors

The major source of instrument-related errors is the stray light, which is the unwanted component of radiant energy outside the spectral bandwidth. Stray light causes serious measurement errors, particularly its primary effect is to reduce the observed peak height, where absorbance is high (at an absorption peak), or where instrument sensitivity is low (at the wavelength limits or near 190 nm), the errors introduced by stray light are relatively larger.

The main source of stray light in most spectrophotometers is usually the dispersing element in the monochromator, either a prism or a diffraction grating. Scattering of light and unwanted reflections from other optical elements can also add significantly to the stray light. It is also possible for stray light to arise outside the monochromator, such as from light leaks in the instrument, allowing some light directly to the sample or detector from outside the instrument or directly from the light source. However, in a well-designed and well-constructed instrument, the latter source of stray light would be negligible.

Stray light causes negative deviations from Beer's law and a level of 0.1% stray light at any wavelength will prevent accurate absorption measurements of greater than 3A.

Electronic noise in the detector and the noise element associated with the random fluctuations of the photon beam reaching the detector are also sources of problem in spectrophotometers. The noise is usually apparent in the amplifier output, especially where beam energy is low. Noise problems may be reduced by integration with respect to time, or by digital signal processing using microprocessors.

2.7.2 Non-Instrumental Errors

These errors may originate from the nature of the solution to be analysed. Multi-component mixtures usually create difficulty as more than one constituent absorbs light at a wavelength of interest. Absorbance in these conditions is additive and a Beer's law plot for one component may no longer be valid. However, it is normally possible to take readings at several wavelengths, to construct a set of simultaneous equations and solve them with a computer interfaced to a spectrophotometer.

The total attenuation of beam radiation may be due to the following factors:

- Reflection of air/cell and solution/cell interfaces
- Scattering by any suspended particles
- Absorption by the solution

In practice the effects of reflection and scattering are restricted to less than significant levels by the use of quality sample cells, matched where possible and by careful sample handling practice. There may be errors due to additional fluorescence component, as a result of absorbed energy being re-emitted at a longer wavelength from that of the incident radiation. Fluorescence effects may be reduced by chemical inhibition, or by appropriate cut-off filters.

2.8 CALIBRATION

Wavelength calibration of a spectrophotometer can be checked by using a holmium oxide filter, as a wavelength standard. Holmium oxide glass has a number of sharp absorption bands, which occur at precisely known wavelengths in the visible and ultraviolet regions of the spectrum. Holmium oxide filter wavelength peaks are given below:

Ultraviolet range with deuterium lamp: 279.3 nm, 287.6 nm
Visible range with tungsten lamp: 360.8 nm, 418. 5 nm, 453.4 nm, 536.4 nm, 637.5 nm

In double-beam spectrophotometers, zero is adjusted with sample and reference beam. Then holmium oxide filter is placed in sample beam. The wavelength control is manually scanned through each wavelength, until the absorption peak is found, always approaching each point from the longer to the shorter wavelength. The spectrum is then recorded.

The wavelength calibration can also be checked in the visible region by plotting the absorption spectrum of a didymium glass, which has been in turn, calibrated at National Bureau of Standards, USA.

3

INFRARED SPECTROPHOTOMETERS

3.1 IR SPECTROSCOPY

The infrared (IR) spectrophotometer has become almost indispensable in the chemistry laboratory, as it is ideally suited for carrying out qualitative and quantitative analysis, particularly of organic compounds. Its use in the applications to inorganic compounds is limited, because of the strong absorption of IR radiation by water. This constitutes a serious limitation in practical applications, since it necessitates the study of inorganic materials in the solid state.

IR region extends from 0.8 to 200 μ in the electromagnetic spectrum. However, most of the commercial instruments are available in the region from 0.8 to 50 μ. The position of absorption bands in the IR spectrum is expressed both in wavelength as well as in wave numbers. The wavelength λ is generally measured in microns (μ). The wavenumber (υ) is the number of wavelengths per centimetre and is given by

$$\upsilon \text{ (in cm}^{-1}) = 10^4/\lambda \text{ (in } \mu)$$

IR spectrophotometers using prisms produce spectra which are spread linearly with wavelength, whereas instruments fitted with gratings generally deliver spectra spread linearly with wavenumber. However, the results are preferably reported in wavenumbers in either case, since these are proportional to molecular properties like frequency and energy, whereas the wavelength is a property of the radiation only.

Each of the vibrational motions of a molecule occurs with a certain frequency, which is characteristic of the molecule and of the particular vibration. The energy involved in a particular vibration is characterised by the amplitude of the vibration, so that the higher the vibrational energy, the larger the amplitude of the motion. Associated with each of the vibrational motions of the molecule, there is a series of energy levels or states. The molecule may be made to go from one energy level to a higher one by absorption of a quantum of electromagnetic radiation. In undergoing such a transition, the molecule gains vibrational energy, and this is manifested in an increase in the amplitude of the vibration. The frequency of light required to cause a transition for a particular vibration is equal to the frequency of that vibration, so that we may measure the vibrational frequencies by measuring the frequencies of light which are absorbed by the molecule.

Since most vibrational motions in molecules occur at frequencies of about 10^{14} s^{-1}, then light of wavelength $\lambda = c/f = 3 \times 10^{10}$ cm/s/10^{14} s^{-1} = 3×10^{-4} cm = 3 μ will be required to cause

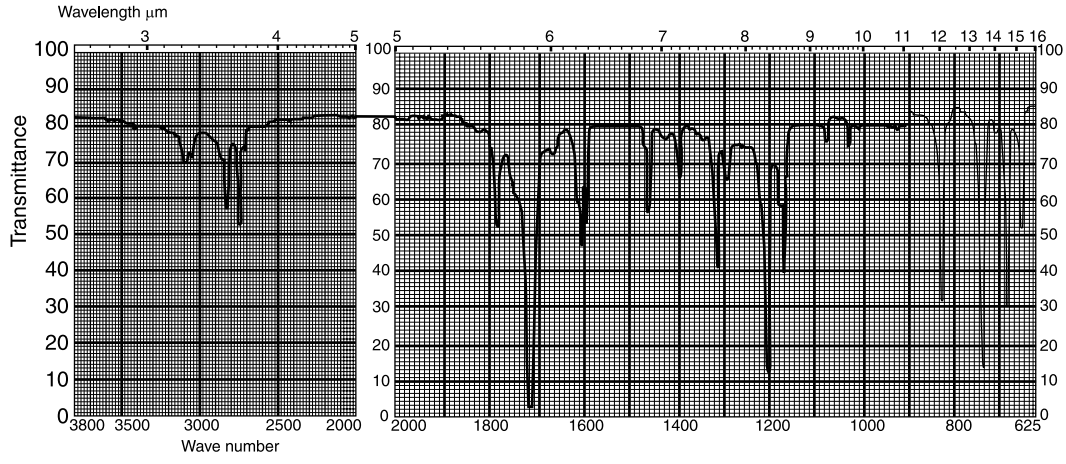


Figure 3.1 *Typical infrared spectrum*

transitions. As it happens, light of this wavelength lies in the so-called IR region of the spectrum. IR spectroscopy, then, deals with transitions between vibrational energy levels in molecules, and is therefore also called vibrational spectroscopy. An IR spectrum is generally displayed as a plot of the energy of the IR radiation (expressed either in microns or wavenumbers) versus the percent of light transmitted by the compound. This is indicated schematically in Figure 3.1. Here, sample transmittance is usually presented linearly on the vertical axis in an IR spectrum and absorption bands are generally presented pointing downwards. Transmittance in this case is defined as the radiant power of the radiation, which is incident on the sample, divided by the radiant power transmitted by the sample.

The energy acquired by a molecule can be utilised in three ways, namely electronic excitation, vibrational change and rotational change and the various molecular energy transformations that may take place are quantised. The amount of energy necessary to cause the various types of transitions generally correspond to definite regions of the electromagnetic spectrum. For a molecule, the average energy involved in electronic excitation is 5 eV. For molecules in a particular electronic state, the average energy involved in a vibrational excitation is 0.1 eV and a rotational excitation involves about 0.005 eV.

By definition

$1 \text{ eV} = 1.602 \times 10^{-19} \text{ J}$
$h = 6.626 \times 10^{-34} \text{ JS}$
and $E = h\upsilon$

(i) 5 eV corresponds to $\upsilon = 1.2 \times 10^{15}$ Hz
so $\upsilon = 40{,}000 \text{ cm}^{-1}$
or $\lambda = 2500$ Å$= 250$ nm

(ii) 0.1 eV corresponds to $\upsilon = 2.4 \times 10^{13}$ Hz
so $\upsilon = 833 \text{ cm}^{-1}$
or $\lambda = 12$ μm

(iii) 0.005 eV corresponds to $\upsilon = 1.2 \times 10^{12}$ Hz
so $\upsilon = 40$ cm^{-1}
or $\lambda = 250$ μm

This shows that light absorption at 250 nm Å (ultraviolet range) produces electronic change. Light absorption at 12 μm (IR) produces vibrational change. Light absorption at 250 μm (far IR) produces rotational change.

In IR spectroscopy, we are interested mainly in the vibrations and rotations induced in a molecule by absorption of radiation. The IR spectrum based on these absorption properties provides a powerful tool for the study of molecular structures and identification. Chemical identification is based on the empirical correlations of vibrating groups with specific absorption bands, and the quantitative estimations are dependent on the intensity measurements.

Normally, the data obtained from an IR spectrum should be used in conjunction with all other available information, like physical properties, elemental analysis, NMR, ultraviolet, etc., as IR data taken in isolation can at times be grossly misleading.

IR spectrophotometry is applicable to solids, liquids and gases. It is fast and requires small sample sizes. It can differentiate between subtle structural differences. On the other hand, interpretation of IR spectra is highly empirical and requires huge libraries of reference spectra. Interpretation of spectra is a skilled art; nevertheless, it is now a part of the repertoire of many chemists. IR spectrophotometry provides means for monitoring many common atmospheric pollutants such as ozone, oxides of nitrogen, carbon monoxide, sulphur dioxide and others.

The region of the IR spectrum which is of greatest interest to organic chemists is the wavelength range 2.5 to ≈ 15 micro-meters (μ). In practice, units proportional to frequency, (wave number in units of cm^{-1}) rather than wavelength, are commonly used and the region 2.5 to ≈15 μ corresponds to approximately 4000 to 600 cm^{-1}.

Modern IR instruments employ microprocessors for control of spectrometer functions. This provides better control or the spectrometer and in many cases additional features such as peak sensing or quantitative analytical capability. The internal representation of data in such systems is of course digital. Therefore, it would be quite feasible to produce data, which can be accepted by more powerful computer systems.

Table 3.1 *Various regions of the IR range of the spectrum*

Region	Wavelength range (λm)	Wavenumber range (cm^{-1})
Near	0.78–2.5	12800–4000
Middle	2.5–50	4000–200
Far	50–1000	200–10

It is useful to divide the infrared region into three sections: near, mid and far IR (FIR) (Table 3.1).
The most useful IR region lies between 4000 and 670 cm^{-1}.

Most commercial IR spectroscopy instruments now offer data handling facilities for a variety of purposes: signal-to-noise ratio improvement by computer averaging, difference spectroscopy by subtraction of spectra to reveal minor differences in related samples, spectral deconvolution to

provide resolution enhancement and spectral searching to identify unknown samples and mixtures. However, the most significant change that has resulted from the decrease in cost and increase in sophistication of small computer systems is the availability of Fourier transform IR (FTIR) spectrometers for routine applications at a price competitive with grating instruments (Morrisson, 1984).

3.2 BASIC COMPONENTS OF IR SPECTROPHOTOMETERS

Spectrophotometers for the IR range are composed of the same basic elements, as instruments in the visible and ultraviolet range, namely:

- a source of radiation
- a monochromator for dispersing the radiation
- a detector which registers the residual intensity after selective absorption by the sample

However, the materials used in the construction of the optical parts are quite different and they are suitably described in the sections to follow.

3.2.1 Radiation Sources

The source of radiation in IR spectrophotometers is ideally a black body radiator. All practical sources fall short of this to a lesser or greater extent. The energy emitted by a black body radiator varies with wavelength and with temperature. In particular, increasing the temperature of the source raises the energy of emission enormously in the short wavelength region, but has relatively small effect at long wavelengths.

The optimum IR source is an inert solid heated electrically to temperatures between 1500 and 2000 K. The maximum radiant intensity at these temperatures occurs at 1.7–2 μ (5000–6000 cm^{-1}). At larger wavelengths, the intensity reduces continuously, until it is about 1% of the maximum at 15 μ (667 cm^{-1}). On the short wavelength side also, the decrease is much more rapid and a similar reduction is noticed at about 1 μ (10,000 cm^{-1}).

There are three common practical IR sources: the *Globar rod*, the *Nernst filament* and the *nichrome wire*. Spectral characteristics of these IR sources are shown in Figure 3.2.

The Globar is a silicon carbide rod, which has a positive temperature coefficient of resistance. The rod is about 5 cm in length and 0.5 cm in diameter. It is electrically heated and run at a current to produce a temperature of about 1300 K. The heat dissipation at the ends of the rod is high, as they are the coolest parts. This sometimes leads to arcing and burnout. Therefore, water cooling of electrical parts is required to prevent arcing. The Globar finds applications for work at wavelengths longer than 15 μ (650 cm^{-1}), because its radiant energy output decreases less rapidly. Since the resistance of the rod increases with length of time, provision must be made for increasing the voltage across the element. It can be conveniently done with a variable transformer.

The Nernst filament is a small rod composed of fused rare earth oxides of zirconium and yttrium. The filament is of cylindrical shape having a diameter of about 1–2 mm and a length of 20–30 mm. Platinum leads are sealed to the ends of the cylinder to allow passage of current through it. The filament is heated to temperatures between 1500 and 2000°C and produces maximum radiation at about 7100 cm^{-1}. The device has a negative temperature coefficient of electrical resistance and is, therefore, operated in series with ballast resistance in constant voltage circuit. It must be externally

heated to a dull-red hot, because it is non-conducting when cold and has a tendency to crack or separate from its connections on cooling. Therefore, it should be run continuously whenever possible.

The nichrome strip, though, gives less energy than the Globar or Nernst, and is extremely simple and reliable in operation. In construction, it could be a tightly wound spiral of nichrome wire, heated by passage of current. The temperature is about 800–900°C. Some IR spectrophotometers employ a ceramic rod source, in which is embedded a platinum-rhodium filament producing temperature up to 1200°C. This radiator consumes only 45 W power.

Several workers have reported the use of tuneable lasers as energy sources. The major advantages are high energy concentrated in a narrow spectral band, a highly directional beam and a very short pulse time (5 ns). Light from any one of these sources is concentrated by a mirror and is focused onto the entrance slit of the monochromator.

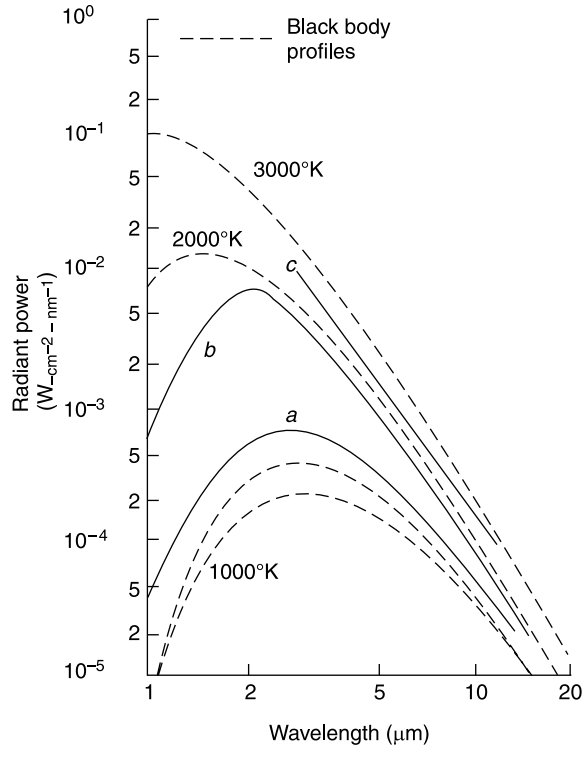


Figure 3.2 *Typical spectral characteristics of some IR sources (a) Globar, (b) Nernst glower, (c) Tungsten glower*

Gagliardi et al. (2002) have demonstrated the generation of continuous wave tuneable FIR radiation by mixing a quantum cascade laser and a CO_2 laser in a metal-insulator-metal (MIM) diode. The system provides full spectral coverage of the FIR radiation region up to 6 THz (Tera Cycles), making a fundamental contribution to coherent spectroscopy.

3.2.2 Monochromators

Light from the entrance slit is rendered parallel after reflection from a collimating mirror and falls on the dispersing element. The dispersed light is subsequently focused on to the exit slit of the monochromator and passes into the detector section. The sample is usually placed at the focus of the beam, just before the entrance slit to the monochromator. IR monochromators generally employ several mirrors for reflecting and focusing the beam of radiation in preference over lenses to avoid problems with chromatic aberrations.

Both prisms and gratings are used for dispersing IR radiation. However, the use of gratings is relatively a recent development. As a general rule, instruments operating below 25–40 μ have prism monochromators, whereas reflection gratings are utilised above 40 μ, because transparent materials are not easily available in that range. Materials for prism construction, which are found most suitable in the IR region, are listed in Table 3.2.

Table 3.2 *Materials for prism with their range of operation*

Material	Optimum range
Glass ($Si0_2$)	300 mμ to 2 μ (5000 cm^{-1})
Quartz	800 mμ to 3 μ (3300 cm^{-1})
Lithium fluoride	600 mμ to 6.0 μ (1670 cm^{-1})
Calcium fluoride	200 mμ to 9.0μ (1100 cm^{-1})
Sodium chloride	200 mμ to 14.5 μ (625 cm^{-1})
Potassium bromide	10 to 25 μ (400 cm^{-1})
Caesium iodide	10 to 38 μ (260 cm^{-1})

An ideal prism instrument would contain a large number of prisms made from different optical materials, so that each could be used in sequence in its most effective region. Such an instrument would of course be extremely expensive. High resolution prism instruments contain combination of SiO_2, NaCl and KBr prisms. Low-cost instruments use an NaCl prism over the full range. They give highest resolution in the vital fingerprint region. Some of these materials are very hygroscopic. Therefore, a good control of the humidity in the room, in which an IR monochromator is installed, is essential. A prism monochromator is sensitive to temperature changes and must be thermostated to maintain constant wavelength calibration.

Prism monochromators employ the Littrow mount, which reflects the beam from a plane mirror behind the prism and return it through the prism a second time (Figure 3.3), thereby doubling the dispersion produced. In a double-pass system, the beam returns through the prism again, producing a total of four passes through the prism, which results in improvement in resolution.

Figure 3.4 shows the optical arrangement of IR spectrophotometer from Carl Zeiss. Emanating from the radiation source (1), the reference beam is reflected by spherical mirrors (2) and (3) through the attenuator diaphragm (4) and passes the reference cell (5). A plane mirror (6) and a concave mirror (7) project it on to the rotating sector mirror (8). The sample beam takes an almost analogous course via mirrors (10) and (9), through the sample cell (11), the 100% adjusting diaphragm (12) and via a concave mirror (13). The rotating sector mirror (8) alternatingly allows one of the two light beams to reach the entrance slit of the monochromator, via a toroidal mirror (14). The monochromator used is according to the Ebert principle and is equipped with a prism in Littrow arrangement with 67° prisms either NaCl or KBr.

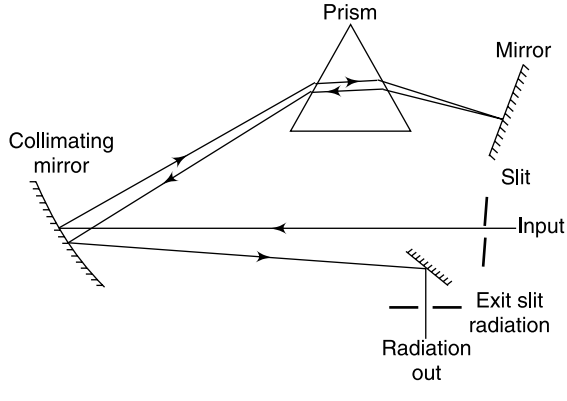


Figure 3.3 *Littrow mounting IR monochromator*

The instrument provides high spectral resolution achieved by long, curved entrance and exit slits. The scanning conditions within the entire wavenumber region are improved by flushing desiccated air through the path of rays. All hygroscopic elements are protected from the ambient air. In order to obtain wave number stability,

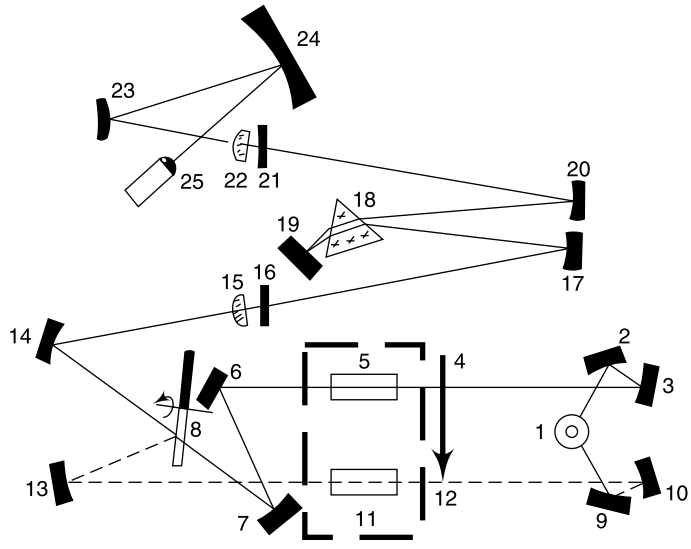


Figure 3.4 *Optical arrangement of SPECORD IR spectrophotometer (Courtesy: M/s Carl Zeiss, Jena)*

the NaCl prism is temperature regulated to ± 1°C. An optical imaging system consisting of a field lens (22), two mirrors (23 and 24) and the lens cemented on the detector (25) projects the beam, leaving through the monochromator's exit slit on to the receiver surface. The detector is a thermocouple of high sensitivity and short time constant.

Efficient balancing of the intensities of the two beams within the entire wavenumber region is achieved by the 100% adjusting diaphragm (12). It is operated by means of a cam, which allows to balance the 100% line at any part of the wavenumber scale.

The use of gratings as dispersing elements in the monochromators offers a number of advantages for the IR region. Better resolution is possible, because there is less loss of radiant energy than in a prism system. This facilitates employing of narrower slits. Also, gratings offer nearly linear dispersion. With these advantages, gratings have almost replaced prisms.

The standard grating used in IR spectrophotometry is an echelon reflection grating. They are usually constructed from glass or plastic that is coated with aluminium. Grating instruments incorporate a sine-bar mechanism to drive the grating mount, when a wavelength readout is desired and a cosecant-bar drive when wave numbers are desired.

One disadvantage of using gratings is that, they disperse radiant energy into more than one order. The higher order reflected rays would emerge from the monochromator at the same angle and act as unwanted radiation passing through the sample giving rise to high transmission values. It necessitates the use of an additional separation method. There are two methods of removing these higher unwanted orders of wavelength, using either optical interference filters or a low-dispersion prism. Some instruments make use of two gratings, each covering a part of the range, along with filters for removing unwanted orders. The two gratings have different construction, one having 60 lines/mm for the range 400–133 cm^{-1} and 180 lines/mm for 1200–1400 cm^{-1}. It is possible to maintain comparatively high resolution at a much lower cost by using an Ebert monochromator. It employs a single grating in its first and second orders. Figure 3.5 shows the optical diagram of IR

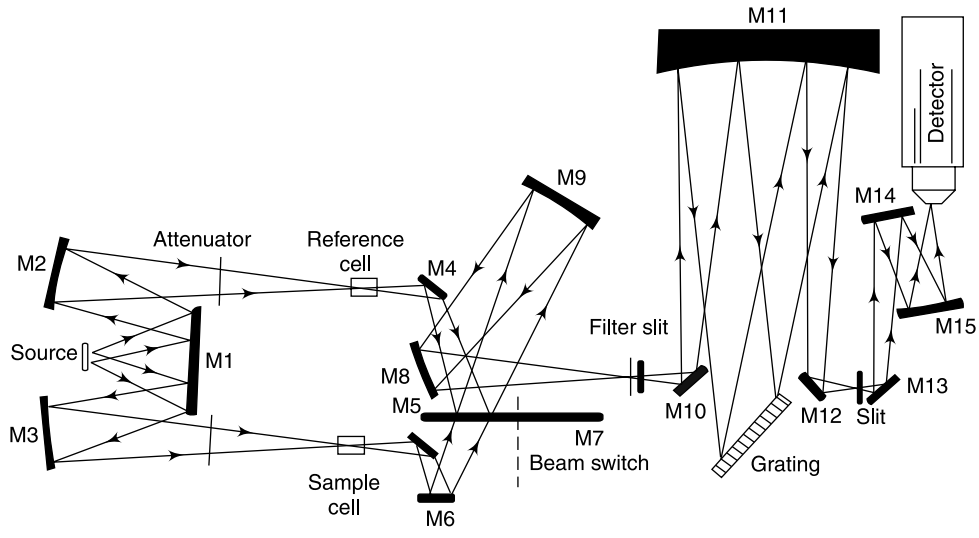


Figure 3.5 *Ebert Monochromator used in IR spectrophotometer*

spectrophotometer SP 1000 of Pye Unicam, making use of Ebert monochromator. The spectrum is scanned by rotating the grating about a vertical axis.

In the Beckman 4200 series IR spectrophotometers, monochromaticity is provided by a grating and set of circular variable filters (Figure 3.6). Two annular filter segments are mounted on a rotating wheel to select the desired wavelengths from the continuous output of a nichrome wire energy

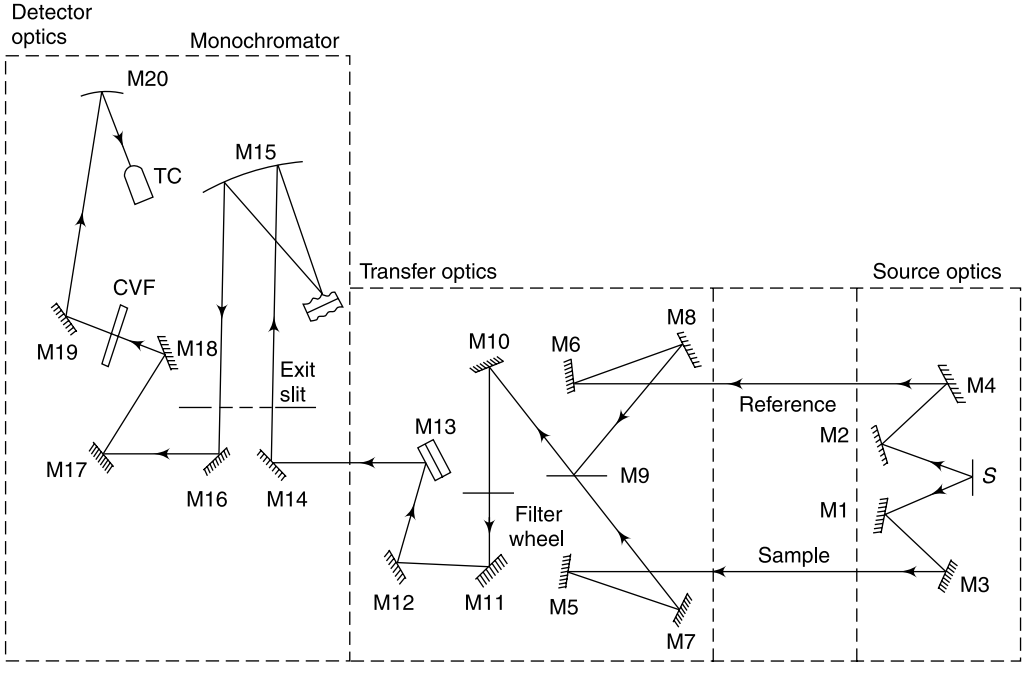


Figure 3.6 *Optical diagram of Beckman 4200 series IR spectrophotometer*

source. This is designated as a wedge filter system and is coupled to a grating wavelength selector. The annular filter segments consist of IR-transparent material, coated to transmit a continuously varying band of wavelengths when rotated in front of a V-shaped slit. The slit is positioned on the optical centre line of the filter system.

A single element thermocouple receives energy transmitted by the wedge filter and generates voltage for amplification by the electronics system. In double-beam operation, amplified thermocouple output, which is proportional to the difference of reference and sample beam intensities, drives an optical attenuator into the reference beam until beam intensities become equal (i.e. until optical null is achieved). Attenuator position in the reference beam is thus directly proportional to sample transmission at any given wavelength. Since the recorder pen is coupled mechanically to the attenuator, the pen records sample transmission as a function of wavelength. In single-beam operation, with the reference beam physically blocked by the operator, sample beam transmission produces thermocouple voltage that is proportional to sample beam intensity. The recorder functions potentiometrically. Amplified thermocouple voltage is compared with the output of the pen potentiometer, which receives reference voltage from a regulated supply. The difference between the two voltages is then directed to the pen servomotor, where it results in linear representation of energy in the sample beam.

The optical arrangement incorporates a number of mirrors and entrance and exit slits in the optical system. The slit width is adjustable manually from 0.005 to 7.0 mm. It also permits slit selection in programmed condition and keeps energy within dynamic range of servo system.

Diffraction gratings should not be touched and unless absolutely essential, only blowing with air should be resorted to for cleaning. In no case should these be washed with corrosive solvents. Prisms and lenses are generally hygroscopic and can get easily scratched. In case they become foggy, the only remedy lies in getting them polished, but in most cases they are beyond repair and hence extreme care should be taken to prevent exhalation of vapours near instruments that are opened.

3.2.3 Entrance and Exit Slits

In all continuous comparison techniques in spectrophotometry, part of the optical radiation emerging from the monochromator is directed on to the reference detector and the rest is allowed to fall into the sample. As the wavelength of the monochromator is scanned, it is arranged in such a way that the signal from the reference detector is maintained at an approximately constant level. It is possible to adjust the intensity of a tungsten lamp to obtain this condition, but when wide range of wavelengths in the IR are to be covered, the only practicable method is to control the monochromator slits. Most of the monochromators use a specially shaped cam to control the slit width, as the wavelength is scanned. Besides this, a servo system can also be employed to achieve the same purpose of the slit servo system, which maintains the output of an optical monochromator at a constant level, as the wavelength is scanned. In this system, after amplification, the signal from the detector is compared with a reference voltage and the error signal is converted to a frequency. The series of pulses drive a DC stepper motor coupled to the monochromator slits, and as balance is reached the motor slows down, thus preventing overshoot.

The slit width control has the same importance as it has in ultraviolet and visible spectrophotometers, and a compromise of different factors is to be made while selecting the slit width to be used. Narrow slits produce smaller bandwidth which consequently results in better spectra definition, whereas wider slits permit larger amount of radiant energy reaching the detector and consequently greater photometric accuracy.

3.2.4 Mirrors

As the materials that are used for lenses are not transparent to IR radiation over the entire wavelength range, lenses are generally not preferred. Front surfaced mirrors are usually used in the IR instruments, of which plane, spherical, parabolic and toroidal types are the most common. Although highly reflecting aluminium-coated mirrors with a protective coating are usually employed, after long periods of operation, the mirrors are bound to become somewhat dusty. Under these conditions, the recording accuracy may not be affected very much; the reflective power will be reduced. As fingerprints or other contaminations may give their own absorption bands, mirrors should be cleaned, if required, only by blowing hot air over them. In case the mirrors are too dirty, these can be cleaned by washing with detergents and rinsing with distilled water. Under no conditions should corrosive solvents be used.

3.2.5 Detectors

Detectors used in IR spectrometers usually convert the thermal radiant energy into electrical energy, which can subsequently be plotted on a chart recorder. The detector ranges in format from single element, uncooled detectors to specialised multi-spectral array detectors. Selection of a specific detector depends on the waveband of interest, the sensitivity required and the cost constraints. The detectors used may be divided into thermal and quantum types. The quantum detectors are useful in the near IR region, whereas thermal detectors can be used very much beyond this range.

The main features of thermal-type detectors include responsively with little dependence on wavelength and operation at room temperature. However, the response speed and detectivity are lower than the quantum type. The commonly used thermal detectors are thermopiles, bolometers, pneumatic detectors and pyroelectric detectors.

Quantum-type detectors feature high detectivity and fast response speed. Responsitivity is wavelength dependent and except for detectors in the near IR range, cooling is normally used with these detectors. Quantum-type detectors are classified into intrinsic types and extrinsic types.

The wavelength limits of intrinsic-type detectors are determined by their inherent energy gap, and responsitivity drops drastically when the wavelength limit is exceeded. Typical examples of this type of detectors are photoconductive and photovoltaic detectors such as Hg CdTe or PbSnTe.

Extrinsic-types of detectors are photoconductive detectors whose wavelength limits are determined by the level of impurities doped in high concentrations to the Ge or Si semiconductors.

The major difference between intrinsic-type detectors and extrinsic-type detectors is the operating temperature. Extrinsic-type detectors must be cooled down to the temperature of liquid helium.

3.2.5.1 Quantum-type detector

Photoconductive cells: These are essentially electrical resistors, which decrease in resistance in relation to the intensity of light striking their surface and are characterised by greater sensitivity and rapidity of response. They are constructed from a thin layer (0.1 μ) of semiconductor, like lead sulphide or lead telluride supported on a backing medium like glass and sealed into an evacuated glass envelope. These detectors are sensitive up to 3.5 and 6 μ for the lead sulphide and lead telluride cells, respectively. Typical response time of these cells is 0.5 ms.

Solid-State Photodetectors: Certain semiconductor materials exhibit photoelectric effect, which can be used for detection of IR radiation. Detectors of PbS were the earliest example of this type

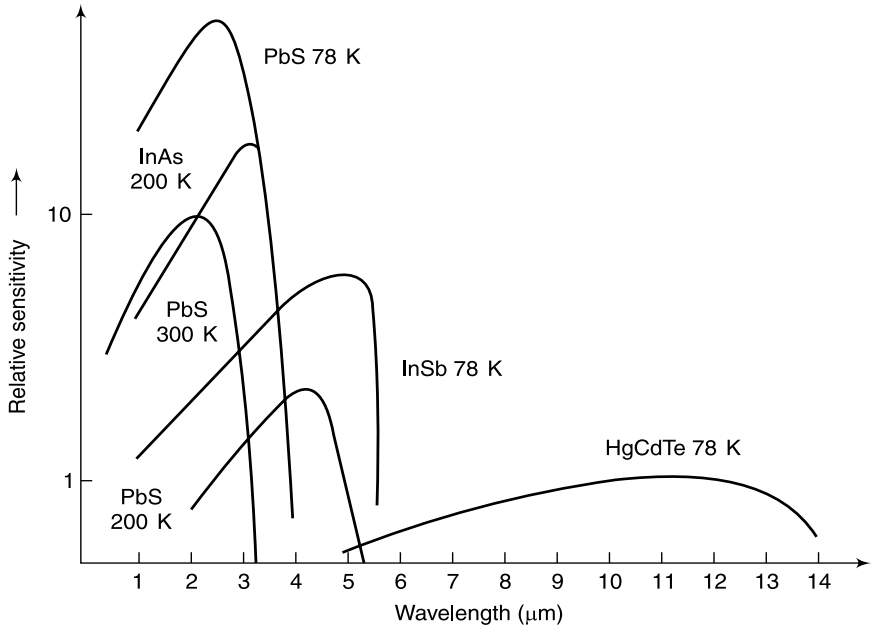


Figure 3.7 *Spectral response of typical intrinsic detectors*

of detectors. However, they do not respond to radiation at wavelength greater than 3 μm. Indium antimonide (In Sb) is another detector which is useful in the 2–6 μm range. But they are highly sensitive and are capable of detecting small temperature variations as compared to thermistors. Another detector making use of an alloy of cadmium, mercury and telluride (CMT) and cooled with liquid nitrogen, though has a peak response at 10–12 μm, has a moderate sensitivity. The spectral response of various photodetectors is shown in Figure 3.7.

It may be noted that the excitation energy required is very small in the long wavelength devices. The detectors must be cooled to prevent background thermal excitation, obscuring measurement of the incident IR radiation. Figure 3.7 shows the highest sensitivity at peak response and the temperature required for proper operation of the detectors.

InGaAs photodiode arrays are specifically suited for near IR spectroscopy using reflection measurements and for line-scan imaging applications. The linear image sensor is a hybrid assembly of an array of InGaAs photodiodes connected to a charge amplifying multiplexer.

The sensors come in vacuum-sealed packages with anti-reflection-coated windows. Proper cooling arrangement reduces the dark current and enables longer exposure times, ranging from 1 μs up to 20 ms. The sensors are delivered with a flat bottom package with fixation holes for optimal thermal coupling. Typical operating temperature is between 210 K and 225 K when the base plate temperature is kept at 293 K. The array is useful between 1.1 and 2.5 μm wavelength range and can be organised with 128, 256 and 512 pixels linear array format. The sensor from M/s XenIcs (www.XenIcs.com) is shown in Figure 3.8 along with its typical spectral response.

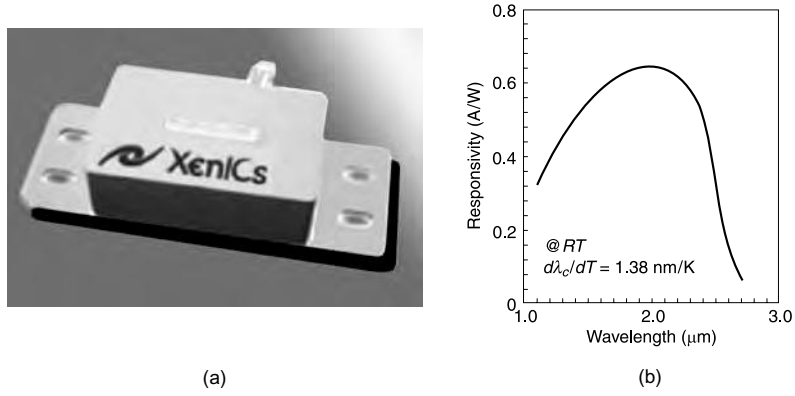


Figure 3.8 *(A) Photodiode (INGaAS) Array For IR Spectroscopy (B) Typical Spectral Response*

3.2.5.2 Thermal detectors

These detectors depend on their response on the heating effect of the radiation. They are useful for detection of all but the shorter IR wavelengths. The IR radiations are absorbed by a small black body and the resultant temperature increase is measured. Even under the best of circumstances, the temperature changes are extremely small and are confined to a few thousandths of a degree centigrade. Thermocouples, bolometers, pneumatic and pyroelectric detectors are the three commonly used thermal detectors.

Thermocouples are the most widely used detectors employed in IR spectrophotometers. In these detectors, the signal originates from a potential difference caused by heating a junction of unlike metals. They are made by welding together two wires of metals in such a manner that they form two junctions. One junction between the two metals is heated by the IR beam and the other junction is kept at constant temperature. Due to difference in work functions of the metals with temperature, a small voltage develops across the thermocouple. The receiver element is generally blackened gold or platinum foil, to which are welded the fine wires comprising the thermoelectric junction. The other junction is shielded from the incident radiation. Changes in temperature of the order of 10^{-6} °C can be detected. It is possible to increase the output voltage by connecting several thermocouples in series. This arrangement is called the thermopile. Thermopiles are made from both metals and semiconductors.

The average electrical output is about 1 μV. Amplification of such a low signal is difficult because of their low resistance (10–20 Ω) and the slow response of the average thermocouple. The thermocouple is enclosed in a magnetically shielded housing to reject spurious signals. Stray light can often be troublesome and should be avoided. Some of the combinations of metals for thermocouples that have been used are Ag-Pd, Sb-Bi and Bi-Te.

Bolometers give an electrical signal as a result of the variation in resistance of a conductor with temperature. It consists of a thin platinum strip in an evacuated glass vessel, with a window transparent in the IR range. Irradiation by the IR beam produces an increase in resistance of the metal strip, which is measured with a Wheatstone bridge. Usually, two identical elements are used in the opposite arms of the bridge. One of the elements is placed in the path of the IR beam and the other is used to compensate for the changes in the ambient temperature. Alternatively, the platinum strips may be replaced with thermistors, which show a negative thermal coefficient of electrical resistance. Bolometer arrays have become the focus of most uncooled detector development.

The pneumatic detector described by Golay (1947) essentially measured the intensity of IR radiation by following the expansion of a gas upon heating. The Golay cell (Figure 3.9) comprises a chamber containing xenon, a gas of low thermal conductivity. It is sealed at its front end by a blackened receiver. The rear wall is a flexible membrane with a mirrored surface on its rear side. IR energy falling on the receiver warms up the gas in the chamber. A rise in temperature of the gas in the chamber produces a corresponding rise in pressure and therefore, a distortion of the mirror diaphragm. Light from a lamp inside the detector housing can be focused on the diaphragm, which reflects the light on to a photocell. Movements of the diaphragm corresponding to the amount of incident IR energy changes the incident light energy on the photocell surface and causes a change in the photocell output. By periodically interrupting the incident radiation with a chopper, an AC signal is produced by the photocell which can be amplified.

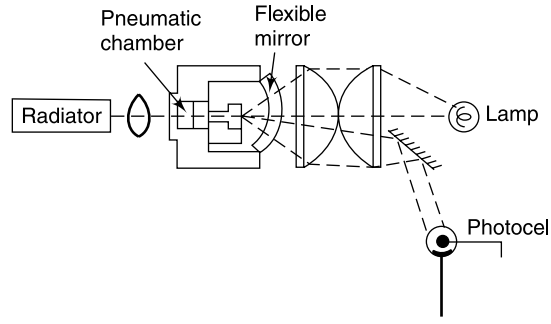


Figure 3.9 *Golay pneumatic cell*

Another form of pneumatic detector is capacitor microphone type in which the varying expansion of the gas affects the capacitor film, which in turn produces the variation in the electrostatic capacity.

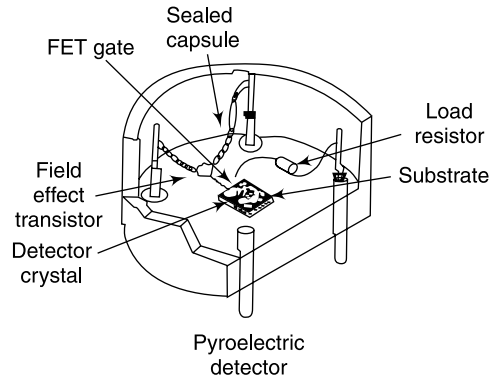


Figure 3.10 *Pyroelectric detector*

Pneumatic detectors with large receiver area are suitable for instruments in which wide slits are necessary. They function at all wavelengths throughout the IR region (as far as 400 μ using a diamond window). They have a low response time. The whole receiver is sensitive to the radiations, thereby eliminating the need for optical alignment.

In the case of pyroelectric detectors, certain types of crystals (ferroelectric) get polarised in a well-defined direction, known as the polar axis. Because the degree of polarisation is temperature dependent, heating or cooling a slice of such a crystal will create an accumulation of charge (on the faces normal to the polar axis), that is, proportional to the variation in polarisation caused by the temperature change. This is the pyroelectric effect. A pair of electrodes normal to the polar axis of the crystal may be used to measure the voltage generated within the crystal due to temperature changes.

Pyroelectric detectors are mostly made from single crystal triglycine sulphate (TGS). The detector construction is similar to a capacitor. Two electrodes, one of them transparent, are formed on opposite sides of a TGS slice. The transparent electrode allows the radiation to fall on the slice. The voltage generated is usually applied to a field effect transistor, which is an integral part of the detector package (Figure 3.10). Pyroelectric detectors are current sources with an output that is proportional to the rate of change of their temperatures.

Figure 3.11 shows a circuit arrangement to process the electrical signal received by the FET. The FET source terminal pin 2 connects through a pull down resistor of 100 K to ground and feeds into a two-stage amplifier having signal conditioning circuits. The amplifier is typically bandwidth

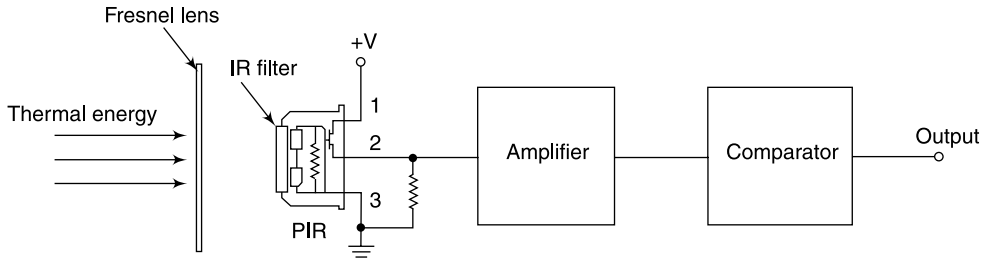


Figure 3.11 *Block diagram of signal processing circuit using pyroelectric detector*

limited to below 10 Hz to reject high frequency noise and is followed by a window comparator that responds to both the positive and negative transitions of the sensor output signal. A well-filtered power source of 3 to 15 volts is connected to the FET drain terminal pin 1.

The sensor has two sensing elements connected in a voltage bucking configuration. This arrangement cancels signals caused by vibration, temperature changes and sunlight. A body passing in front of the sensor will activate first one and then the other element, whereas other sources will affect both elements simultaneously and be cancelled. The radiation source must pass across the sensor in a horizontal direction when sensor pins 1 and 2 are on a horizontal plane so that the elements are sequentially exposed to the IR source. A focusing device is usually used in front of the sensor.

The device used for focusing is a Fresnel lens which is a Plano convex lens that has been collapsed on it to form a flat lens that retains its optical characteristics but is much smaller in thickness and therefore has less absorption losses.

The FL65 Fresnel lens from M/s Glolab (www.glolab.com) is made of an IR transmitting material that has an IR transmission range of 8–14 μm which is most sensitive to human body radiation. It is designed to have its grooves facing the IR sensing element so that a smooth surface is presented to the subject side of the lens which is usually the outside of an enclosure that houses the sensor.

The lens element is round with a diameter of 1 inch and has a flange that is 1.5 inches square. This flange is used for mounting the lens in a suitable frame or enclosure. The lens has a focal length of 0.65 inches from the lens to the sensing element. It has been determined by experiment to have a field of view of approximately 10° when used with a pyroelectric sensor.

Pyroelectric materials absorb quite strongly in the FIR and have essentially flat wavelength response from the near IR through the FIR. Though the pyroelectric detectors have low sensitivity as compared to Golay detector, they are preferred in the FIR range due to faster response.

3.2.5.3 General considerations regarding detectors

Due to the low intensity of available sources and the low energy of the IR photon, the task of measurement of IR radiation is particularly difficult. The electrical signal produced by the various detectors is quite small and requires large amplification before it can be put to a recorder. To prevent the very small signals being lost in the noise signals, which might be picked up by the connecting wires, the pre-amplifier is located as close to the detector as possible.

In addition to this, the radiation beam is chopped with a low frequency light interrupter. By using narrow bandwidth electronic amplifiers, the alternating signal corresponding only to the chopping frequency is measured. This arrangement minimises stray light signals.

The response times of different types of detectors are typically as follows:

Photoconductive cells	0.5 ms
Golay cell	4 ms
Bolometer	4 ms
Thermocouple	15–60 ms

The sensitivity of photoconductive detectors is generally higher than thermal detectors. Nevertheless, there is no strong choice among thermal detectors on sensitivity considerations.

When using thermal detectors, it is essential to shield the detector to reduce its heating by nearby extraneous objects. Therefore, the absorbing element is placed in a vacuum and is carefully shielded from thermal radiation emitted by other bodies in the area.

3.2.5.4 Pre-amplifier for use with photoconductive IR detectors

When photoconductive IR detectors such as cadmium mercury telluride are used, it is necessary to employ a low-noise pre-amplifier, such as shown in Figure 3.12. Basically, the circuit consists of a FET input stage, which has sufficient gain to make the noise contribution of the next stage negligible. This is followed by a low-noise integrated amplifier. A common source stage Q_1 is used with a bootstrapped drain load Q_2. To obtain the low noise, the FETs are used with drain currents near to I_{DSS} (drain current of FET with a zero source gate voltage). The feedback resistor is made as small as possible, so that the minimum bias is applied to Q_1. The current in Q_2 is determined by the current in Q_1. Therefore, to ensure that the gate junction of Q_2 is not forward biased, the FET with the higher I_{DSS} is used in this position. The amplifier at room temperature, with a 51 Ω source impedance and a bandwidth from 8 Hz to 10 kHz, has a noise level of 2 μV peak-to-peak (Gore and Smith, 1974).

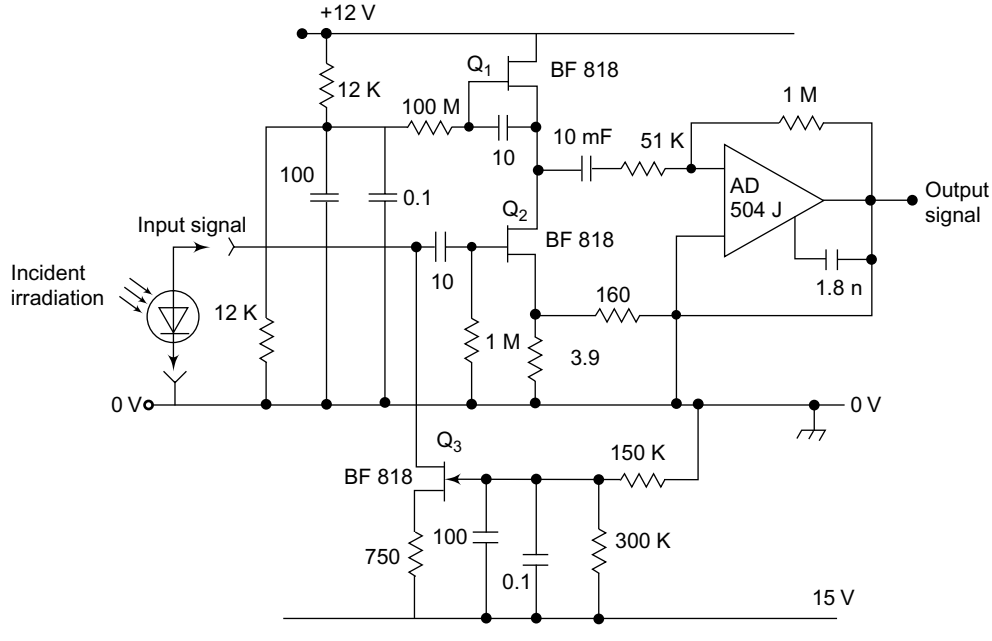


Figure 3.12 *Pre-amplifier for use with photoconductive IR detectors*

The detector requires a 10 mA bias current supply, which must have a low noise level, if the system performance is not to be degraded. This current is obtained from Q_3, which is also a low-noise FET. The noise voltage across the detector is about 19 mV rms, which is small compared to the theoretical amplifier noise.

3.3 TYPES OF IR SPECTROPHOTOMETERS

Infrared spectrophotometers are produced by several instrument manufacturers. Almost all the commercial designs employ a double-beam system, where the radiant energy passes alternately through the sample and then a reference to a single detector. They incorporate a low-frequency chopper (5–13 Hz) to modulate the output radiation from the source. Various sources and detectors described above are incorporated in one or more of the commercial instruments.

The double-beam system offers the advantage that scans are not disturbed by absorption bands produced by atmospheric water vapour and carbon dioxide. Also, fluctuations in source output detector sensitivity and gain have no influence on the record.

There are two types of arrangements for recording the IR spectra. These are the ratio recording and optical null method.

3.3.1 Optical Null Method

Infrared this method, the IR radiation is passed simultaneously through two separate channels, one containing the sample, the other the reference. The two beams are re-combined into a common axis and are alternately focused on the detector. If the intensities of the sample and reference beams are exactly equal, then no alternating intensity radiation goes through the slit. If the sample absorbs some radiation, an alternating intensity radiation is observed by the detector, which produces an AC signal. This AC signal can be selectively amplified in a tuned amplifier. As the amplifier is tuned to the chopping frequency of one light beam, signals of frequency different from the chopping frequency are not amplified. The alternating signal from the detector is used to drive a servomotor, which is mechanically coupled to an optical wedge or a fine-toothed comb attenuator. Movement of the comb occurs when a difference in power of the two beams, is sensed by the detector. The motor will stop when the reference and the sample beam intensities are exactly equal. The movement of the motor is synchronised with the recorder pen, so that its position gives a measure of the relative power of the two beams and thus the transmittance of the sample. The record obtained, therefore, is of the sample absorption as a function of spectral frequency. The teeth of the attenuator comb are accurately cut, so that a linear relationship exists between the lateral movement of the comb and the decrease in power of the beam.

Figure 3.13 shows the diagram of a typical recording-type double-beam optical-null principle IR spectrophotometer. Emanating from the source, the two light beams pass through the sample and reference cells. With a switching frequency of 12.5 Hz, the rotary sector mirror alternately conducts the radiation of the sample and reference channels into the monochromator. Here, the light of the respective wave number is reflected and passed on to the thermocouple, which delivers a 12.5 Hz AC voltage signal, when the intensities of the two optical paths are not equal. After amplification in the pre-amplifier and main amplifier and after phase-sensitive rectification in the demodulator, this AC voltage signal controls the servomotor, which adjusts the attenuator

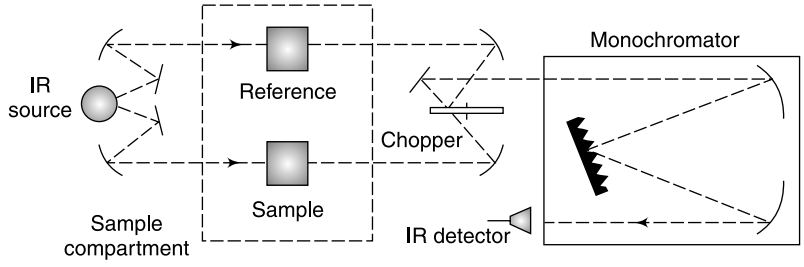


Figure 3.13 *Block diagram of a double-beam IR spectrophotometer*

diaphragm in the reference channel until the intensities of both beams are equal and the signal disappears. The servomotor also drives the recording pen, which records the sample's transmittance corresponding to the position of the attenuator diaphragm.

The Beckman Model IR 4200 series IR spectrophotometers are automatic recording double-beam optical null instruments, which cover a range of 4000–200 cm^{-1}. They employ nichrome/Nernst source and a thermocouple detector, and are available with scanning speeds from 2 cm^{-1}/mm to 1000 cm^{-1}/mm. The monochromator is filter/grating combination and provides a resolution of 0.5 cm^{-1} at 900 cmn^{-1} and 1.4 cm^{-1} at 3000 cm^{-1} in the better models.

3.3.2 Ratio Recording Method

In the direct ratio recording system, radiation from the source is passed alternately through two separate channels, one passing through the sample cell and the other through the reference cell. The two signals from the detector are amplified, rectified and recorded separately. The sample signal is displayed as a proportion of the reference signal. In this method, attempts are not made to have a physical equalisation of the sample and reference beam intensities. The reference signal is used to drive the slit width control, so that the energy level reaching the detector is constant. This system is, however, not favoured in IR instruments, as it requires a very stable amplifier system and the chopping system.

3.3.2.1 Perkin-Elmer 780 series IR spectrophotometers

Modern IR spectrophotometers are microprocessor-based and provide a continuous record of the IR transmittance or absorbance of a sample as a function of frequency, expressed in wave number units. The chart is driven in synchronism with the monochromator so that a pen moving laterally across the chart records the sample transmittance or absorbance as a function of wave number.

In the Perkin-Elmer 780 series IR spectrophotometers, two microprocessors have been used (Figure 3.14). The wave number scan motor drives the grating monochromator scan mechanism which is synchronised with the recorder drive by the abscissa microprocessor, to accurately reproduce the wave number settings on the chart. The instrument is a double-beam system (Figure 3.15) in which the energy radiated by the source is split into the sample and reference beams. In the photometer section, the two beams are combined by a rotating sector mirror to form a single beam containing pulses of radiation from the sample and reference beams. This combined pulsed beam passes into the monochromator where it is dispersed by the grating into its spectral components.

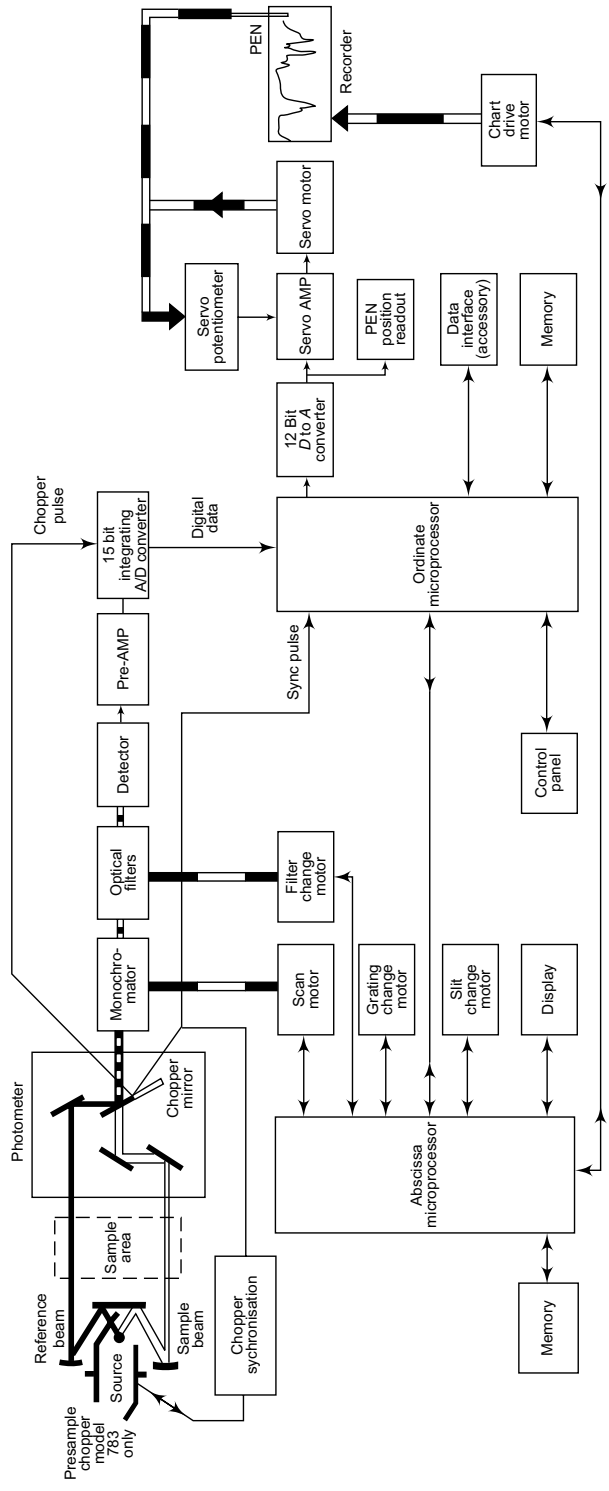


Figure 3.14 *Block diagram of a scanning IR ratio recording IR spectrophotometer*

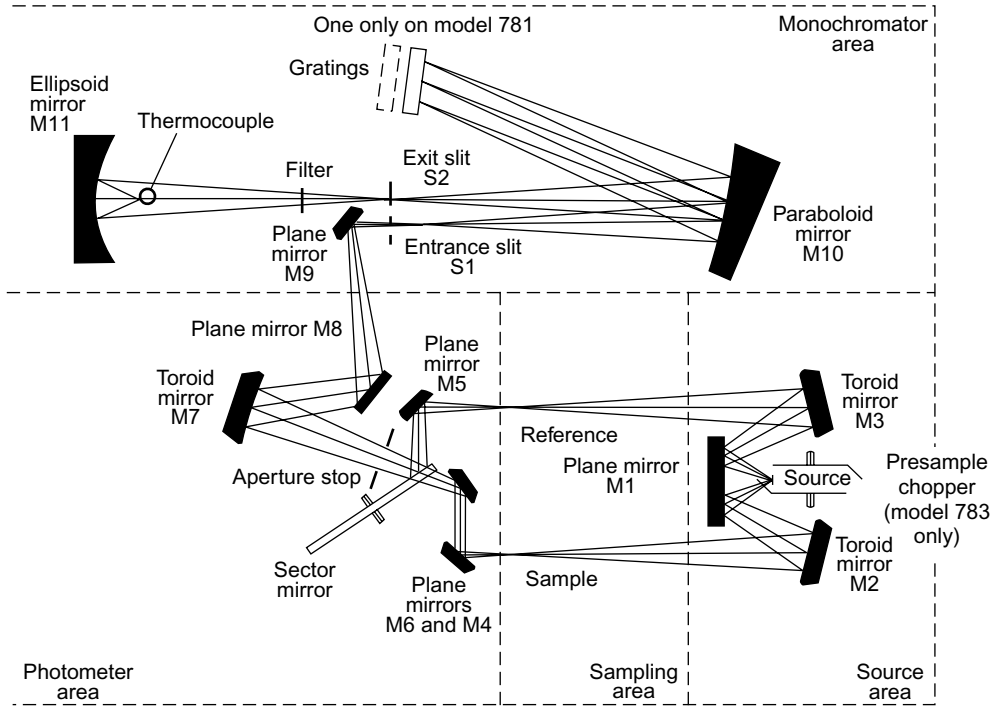


Figure 3.15 *Optical diagram of Perkin-Elmer IR spectrophotometer*

As the grating is rotated, the dispersed spectrum is scanned across the monochromator exit slit. The mechanical width of the monochromator slit determines the width of the wave number band emerging from the monochromator.

After leaving the monochromator, the radiation passes through one of a set of optical filters. The appropriate filter is automatically selected for the spectral region being scanned. The optical filter rejects unwanted radiation diffracted from the grating. Finally, the transmitted radiation is focused onto a thermocouple detector.

The electrical signal from the thermocouple detector is applied via a pre-amplifier to an analog-to-digital converter. The digital data produced is applied to the ordinate microprocessor which controls the ordinate function of the instrument. The microprocessor output is converted back to an analogue form, which is applied to the servo system.

Although a smaller slit width would decrease the bandwidth and provides improved resolution, it results in decreased intensity of the emerging radiation, thereby decreasing the signal-to-noise ratio. To maintain a uniform signal-to-noise response, the detector output is generally maintained at an approximately constant level over the range of the instrument by programming the widths of the monochromator slits by means of a cam drive.

A ceramic tube heated by an internal metallic element acts as a source of radiant energy. The ceramic tube when heated to about 1100°C produces a continuous spectrum of electromagnetic energy, most of which is in the IR region. The incandescent portion of the ceramic tubing is approximately 15 mm long and 3 mm in diameter.

The radiant energy is split into the reference and sample beams by the plane mirror M1 and toroidal mirrors M2 and M3. The toroidal mirror M2 focuses the sample beam onto the sample aperture in the sample panel and M3 focuses the reference beam onto the reference aperture in the sample panel. The use of toroidal mirrors eliminates astigmatism in the source images.

The sample and reference beams are combined by the sector mirror which rotates at 10 revolutions per second and gives a modulation frequency of 20 Hz. Plane mirrors M4, M5 and M6 are so oriented that the sector mirror will alternately pass the sample beam and reflect the reference beam through the aperture stop which is imaged on the grating in the monochromator and ensures that both beams are the same size. The toroidal mirror M7 then focuses this combined beam on the monochromator entrance slit SI, the beam being directed by plane mirrors M8 and M9. The beam diverges from S1 until the 19° off-axis parabolic mirror M10 reflects it as collimated radiation onto the grating. The radiation, after being diffracted by the grating, is focused in the plane of the exit slit S2 by M10.

In order to make measurements over a wide spectral range, two gratings are used, which are mounted back to back. One grating has 100 while the other has 25 lines per millimetre. The grating is selected automatically depending on the wavenumber setting of the instrument. To cover the full range of the instrument, the gratings are selected as follows:

Range	Grating	Order
4000–2000 cm^{-1}	100 lines/mm.	2nd
2000–600 cm^{-1}	100 lines/mm.	1st
600–200 cm^{-1}	25 lines/mm.	1st

The radiation of different wavenumbers corresponding to different orders of diffraction from the grating emerges from the exit slit and impinges on the optical filter assembly. The optical filter rejects radiation from all but the desired order of diffraction. The optical filters are mounted on an eight-position wheel. The first three positions of the wheel correspond to the second order of the 100 lines/mm grating. To obtain second order diffraction radiation to pass while blocking first order radiation, two transmission filters are used in each of the three positions. In the other five filter positions, the gratings are operated in their first orders only for which single long wavelength pass filters are employed. Filter changes occur at 3120, 2500, 2000, 1150, 700, 400 and 250 cm^{-1}. Variation of the slit width over the range of the instrument is achieved by the action of a cam, whose radius is sensed to control the separation of the slit jaws with the help of the slit motor.

The detector (thermocouple) and the monochromator exit slit are located at the foci of the ellipsoid M11, the ratio of its focal lengths being 1:8. The radiant energy leaving the exit slit is therefore, in linear dimensions by a factor of eight. A caesium iodide lens on the thermocouple assembly further reduces the linear dimensions of the slit, image falling on the thermocouple target by a factor of 1.4.

The signal from the thermocouple is approximately 0.5 μV with the medium slit width. The signal is amplified in a low noise-high gain pre-amplifier before it is given to an A-D converter. The digital data from the A-D converter is applied to the ordinate microprocessor. The function of the instrument is controlled through two stepper motors the monochromator scan and chart drive motors. The speeds of these motors are determined by the repetition rate of the pulses applied to their stator coils. These pulses are derived from a voltage-controlled oscillator which is phase locked to the mains frequency of 50 Hz.

The Perkin-Elmer instrument is a double-beam ratio recording instrument which covers a range of 4000 to 200 cm^{-1}, has three scan speeds and provides for a slit selection from four ranges to give resolution from 1.2 cm^{-1} to 5.5 cm^{-1}. The recommended environmental conditions for the instrument are 15°C to 35°C temperature and 75% maximum relative humidity.

3.4 SAMPLE HANDLING TECHNIQUES

A wide variety of samples can be analysed using IR spectroscopy. Solids, liquids or gases can all be handled. However, sample handling presents a number of problems, since no rugged window material for cells exists, which is transparent over the entire IR range and is also inert. The most commonly used window material is NaCl, which transmits down to about 650 cm^{-1}. KBr transmits down to about 400 cm^{-1}, CaBr to about 250 cm^{-1} and CsI to about 200 cm^{-1}. These materials being all water soluble, the surfaces of the windows made from them are easily fogged by exposure to atmospheric water vapour or moist samples. Therefore, they require frequent polishing when used under such conditions.

3.4.1 Gas Cells

Gas cells usually have a path length of 100 mm, since this thickness gives a reasonable absorbance level for the majority of gases and vapours at the normally encountered partial pressures. The inner cell diameter is typically 40 mm and the volume about 125 cm^3. The ends of the cells are ground square, to which IR-transparent windows are glued with sealing gaskets. Two tubes with a stopcock are attached to the cell for connecting the cell to the gas handling system. A typical glass cell may have a body of uniform cross section, but it is preferable to use a tapered construction to conform closely to the section of the radiation beam and requires less sample volume for a given thickness, when weak bands are to be studied, it is necessary to use very long cell paths, which are inconvenient to fit into the instrument. In such cases, multiple reflections using a combination of a mirror system, with a beam condenser, enables the spectra of minor components to be recorded.

3.4.2 Liquid Cells

The extinction coefficient of most liquid hydrocarbons in the IR region are such that a pure sample of thickness between 0.01 and 0.05 mm gives an absorption spectrum quite suitable for analysis. The transmittance lies between 15 and 70%. Other liquid materials are not markedly different.

Two types of cells are generally available for the examination of liquid samples; sealed cells for liquids of high vapour pressure and demountable ones for all other liquids. Both types are with path lengths of 0.02, 0.04, 0.06, 0.10, 0.16, 0.25, 0.4, 0.6 and 1.0 mm and with the earlier stated window materials. The external cell diameter is 30 mm. About 100 µl sample volume per 0.1 mm path length is required.

Liquid cells of this thickness consist of IR-transparent windows separated by thin gaskets of copper and lead, which have been amalgamated with mercury before assembly is securely clamped together. As the mercury penetrates the metal, the gasket expands providing a tight seal. The filling is done with hypodermic syringe. The demountable-type cells can be assembled for different path lengths, in which case circular spacing foils are placed between the windows.

3.4.3 Variable Path Length Cells

These are employed for determining the absorption coefficient and concentration of the test substance, as well as for establishing calibration curves. When using the cell in the reference beam, the solvent's absorption bands may be completely compensated by continuously changing the path length. The cell path length is usually kept adjustable, in the range 0–5 mm via a differential thread with an accuracy of ±1. The path length can be read off the setting drum to an accuracy of 1 μ. In precisely fitted piston guideways, the cell windows are displaced against each other, thus achieving an extreme tightness of the cell. This permits long-time scans to be carried out with highly volatile solvents. This type of cell is normally supplied with KBr windows. CaF_2 or NaCl windows are also available. Length of the cell is typically 120 mm and diameter 75 mm.

3.4.4 Sampling of Solids

Different methods are available for sampling solids for examination in the IR spectrometer. These methods are described below:

3.4.4.1 Solids dissolved in solutions

In case it is possible to obtain suitable solutions of the solids, the solids are dissolved and examined as dilute solutions by running in one of the cells for liquids. However, there is no single solvent which is transparent through the entire IR range. To cover the main spectral range between 4000 and 650 cm^{-1} (2.5–15.4 μ), the combination of carbon tetrachloride and carbon disulphide are employed.

3.4.4.2 Pressed pellet technique

In the pressed pellet technique, the solid sample is finely ground and mixed with an alkali halide like KBr and then pressed into the form of a disc for examination in the instrument. Pressing is done in an evacuable die, under high pressure. The resulting disc or pellet is transparent to be directly run. Other alkali halides like CsI and CsBr are used for measurement at longer wavelengths.

3.4.4.3 Mull technique

The IR spectrum of finely powdered solids may be obtained by dispersing the powder within a liquid medium, so that a thick slurry is produced. The thick slurry is spread between IR transmitting windows. The liquid is so chosen that it has the same refractive index *as* the sample, so that energy losses due to scattering of light are minimised. The most commonly used liquid mulling agent is a mixture of liquid paraffins, known as Nujol (mineral oil). Although Nujol is transparent throughout the IR spectrum, it cannot be used if C-H stretching and bending frequencies are to be observed In this case, a second mull like perfluorokerosene of hexchlorobutadiene may be used.

3.4.5 Micro-sampling

When the quantity of sample available for IR spectrophotometric examination is very small, standard sampling techniques cannot generally be used. This is, because, the absorbance of a given sample is proportional to the number of grams of absorbing material and inversely proportional to the area of the sample. Thus, in order to increase the absorbance for a given weight of sample, it is necessary

to decrease the area of the sample. The identification of gas chromatographic fractions, new compounds and materials isolated from natural products frequently involve micro-gram quantities of samples and require the use of micro-sampling techniques for the measurement of their IR spectra.

In cases where the quantity of available sample is very small, or where the spectrophotometer design does not permit small sample areas to be used, it is necessary to employ a beam condenser. Basically, a beam condenser consists of an appropriate combination of lenses and mirrors[1] which reduce the size of the spectrophotometer beam, focus it on the sample, and then return it to its normal size before entering the monochromator. Beam condensers are usually designed to fit directly into the sample compartment and to hold a variety of micro-liquid, solid, and gas cells. Typically, beam condensers have condensing factors of about four- or six-to-one and are available either with all-reflecting optics or with refracting optics, employing alkali halide lenses. Ultra-micro-cells for use in beam condensers have volumes of around 2 µl, with a 0.1 mm spacer.

3.5 FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier transform infrared spectroscopy (FTIR) has become a common feature in modem spectroscopy laboratories. A wide range of commercial FTIR spectrometers with very different specifications are now available. This has become possible with the availability of inexpensive micro-computers.

The Fourier transform technique depends upon the basic principle, that any wave function could be represented as a series of sine and cosine functions with different frequencies (Davies et al., 1985). This is illustrated in Figure 3.16a, which shows the simple ease of adding a series of sine waves. Figure 3.16b shows two sine waves, one having half the amplitude and the other having double the frequency. The sum of these two sine waves is shown in Figure 3.16c. Curves of higher and higher frequency can be added and in fact Figure 3.16d shows a series of 15 sine waves and their addition. It can be seen that in the limit, the curve will become a square wave. Logically, in order to fit more complicated waves, it becomes necessary to consider both sine and cosine series. The determination of the sine and cosine components of a given wave function is known as Fourier transformation (FT).

Fourier transformation facilitates two different presentations of the same experimental data, known as a do*mains*. These are most commonly the *time domain*, in which the data are recorded as a series of measurements at successive time intervals and the *frequency*

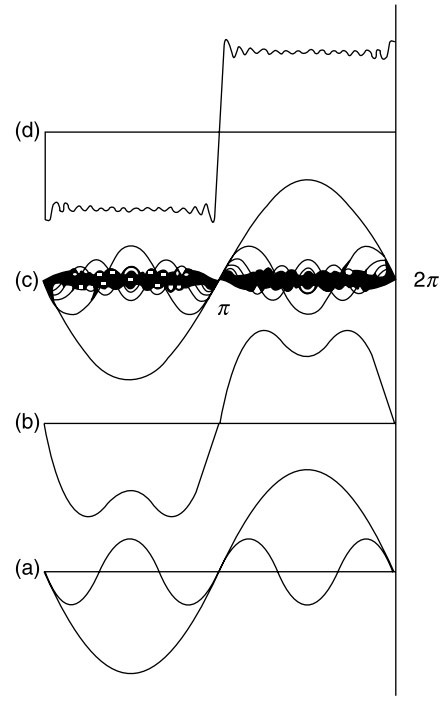


Figure 3.16 *Summation of sine waves to illustrate the idea of the Fourier series*

domain, in which the data are represented by the amplitudes of its sine and cosine components at different frequencies. Using the FT into practice can become tedious and time consuming even when using computers, and especially when a large number of points have to be considered. The situation was considerably eased by the invention of fast Fourier transformation (FFT) algorithm by Cooley and Tukey (1965), which made it possible to carry out FT of complex data in a matter of few seconds. Most of the present day applications of FT in analytical techniques are dependent on Cooley-Tukey FFT.

There are many advantages of using FT spectroscopic technique over using dispersive or continuous wave (CW) instruments, to record signal intensities directly as a function of frequency. If we measure a signal as a function of time, we obtain information on all frequencies in the spectrum simultaneously, whereas a CW instrument is confined to measuring only one frequency, or a very narrow band of frequencies at a time. Further, a typical spectrum consists of a few sharp peaks, with long stretches of noisy base line (i.e. CW scan wastes most of the measuring time by recording this base line). A considerable gain in signal-to-noise for a given total measurement time is achieved by repeatedly measuring an interferogram and then Fourier transforming the data, rather than by scanning through the frequencies directly.

3.5.1 FTIR spectrometers

In order to obtain spectra of the samples, a spectrometer must be able to separate the optical frequencies of the IR light after it passes through the sample. Dispersive spectrometers separate the optical frequencies spatially using a prism or a diffraction grating before the light passes through the sample.

FTIR spectrometers, however, modulate the IR beam before passing through the sample. This is done by the interferometer which causes each IR frequency to be modulated with a unique frequency of modulation. After the IR beam passes through the sample, the radiation intensity is detected and the frequencies are demodulated via a FT (ABB, 2003).

In essence, the heart of a FTIR spectrometer is a two-beam interferometer, most commonly of the Michelson type (Chalmers, 1983). The basic optical components of the interferometer are shown in Figure 3.17. The radiation entering the interferometer is split into two beams by a beam splitter. Beam *A* follows the straight path before returning to the beam splitter, whereas the distance travelled by beam *B* can be varied, before it re-combines with beam *A*. When the beams *A* and *B* recombine, an interference pattern is produced, which is incident on a detector. When the two beams are in phase at the beam splitter, maximum intensity will reach the detector. The intensity will be minimum in case the beams are out of phase. If provision is made in such a way that mirror M_2 is displaced uniformly, the detector output will be a sine wave, whose frequency is determined by the translation velocity of M_2 and the wavelength of the monochromatic radiation, and the amplitude of the signal will depend upon the intensity of incoming radiation.

If the incoming radiation is polychromatic, the detector output wave of unique frequency is produced for each component. The overall detector output as a function of time, will be the sum of the waves for each frequency component. The resulting signal is called an *interferogram* which has the unique property that every data point (a function of the moving mirror position) which makes up the signal has information about every IR frequency which comes from the source. This means that as the interferogram is measured, all frequencies are being measured simultaneously.

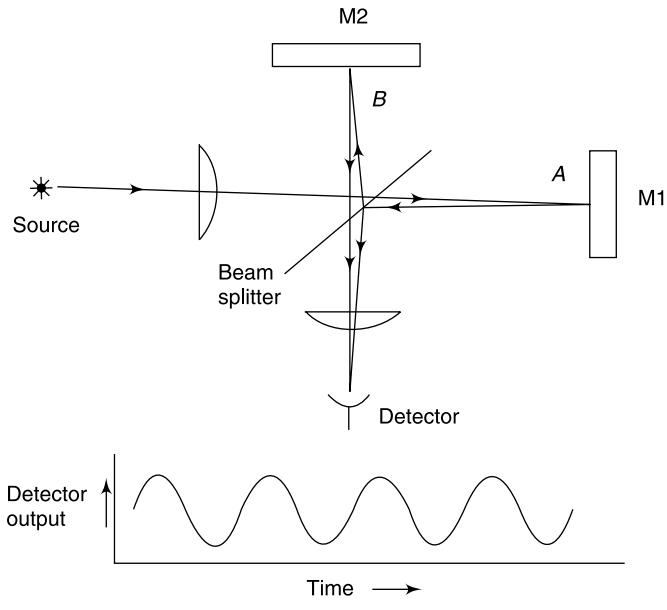


Figure 3.17 *Principle of Michelson interferometer*

Because the analyst requires a frequency spectrum (a plot of the intensity at each individual frequency) in order to make an identification, the measured interferogram signal cannot be interpreted directly. A means of 'decoding' the individual frequencies is required. This can be accomplished via a well-known mathematical technique called the Fourier transformation. This transformation is performed by the computer which then presents the user with the desired spectral information for analysis.

3.5.2 Major Components of FTIR Spectrophotometer

The major components and principle of operation of FTIR Spectrometer are shown in Figure 3.18.

- *The Source*: IR energy is emitted from a glowing black body source. This beam passes through an aperture which controls the amount of energy presented to the sample and, ultimately, to the detector.
- *The Interferometer*: The beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exits the interferometer.
- *The Sample Compartment* : The beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.
- *The Detector*: The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.
- *The Computer*: The measured signal is digitised and sent to the computer where FT takes place. The IR spectrum is then displayed user for interpretation and any further manipulation.

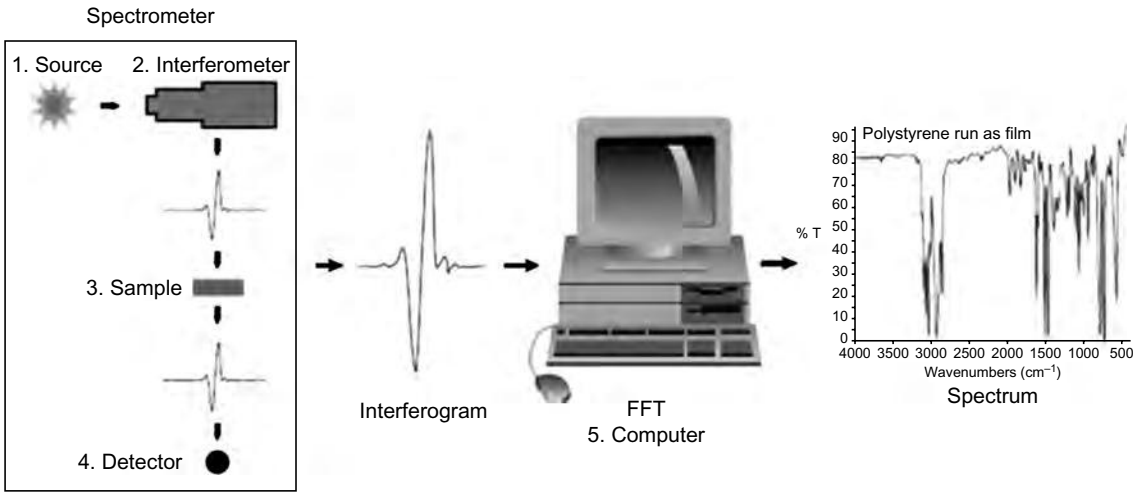


Figure 3.18 *Principle of FTIR Spectrometer (Courtesy: M/s Thermo Nicolet Corp., 2001)*

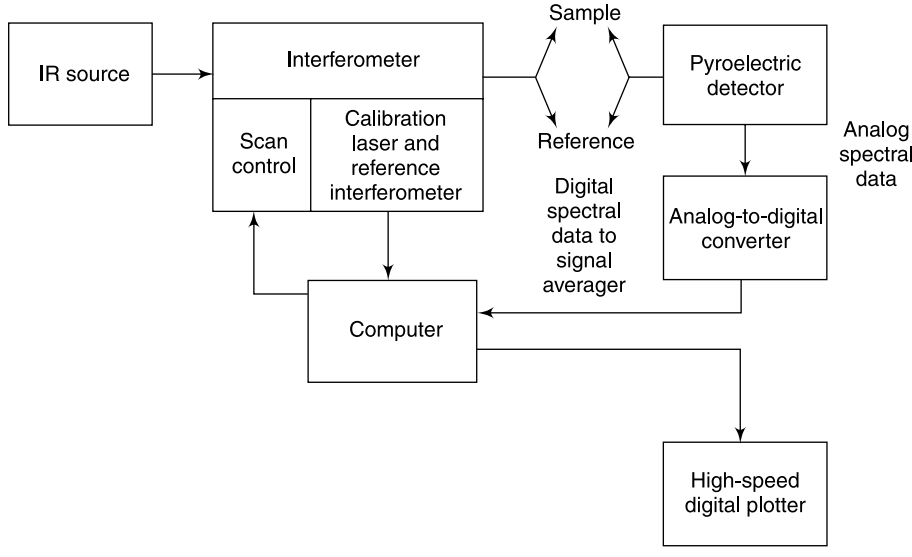


Figure 3.19 *Block diagram of a typical FT spectrometer*

Figure 3.19 shows the detailed schematic of a FT spectrometer. The source and detector are the conventional ones that are used in IR spectrometry. A small computer is employed to control the scan system and carry out the mathematical transformation. The heart of the system is the mirror scan mechanism. For faithful reproduction of the spectrum from the interferogram, the detector output must be known as a function of mirror displacement. The measurement of mirror displacement becomes increasingly difficult, as the wavelength decreases. At shorter wavelengths, a special technique known as fringe referencing technique is used. Here, one transducer is used to

drive simultaneously the movable mirrors of two interferometers through identical displacements. Sample radiation passes through one interferometer and produces the sample interferogram. Reference interferogram is recorded using a He-Ne laser. This reference laser ensures that sampling of the detector signal occurs reproduceably from scan to scan. Since the interferogram of a monochromatic source (laser) is a sine wave, its zero crossings provide an accurate trigger for digitised data collection of the main interferogram. Further, use of He-Ne laser reference interferogram provides an internal frequency calibration, important to high resolution studies.

Figure 3.20 shows the optical layout of double-beam Model 1800 FTIR spectrophotometer from Perkin-Elmer, which is based on scanning Michelson interferometer. The instrument gives a resolution of better than 0.2 cm^{-1} in tilt wavelength range of 4500–450 cm^{-1}. The instrument uses TGS detector and Ge/KBr beam splitter. The instrument employs three different microprocessor systems. The first is the system controller, a Perkin-Elmer PE 7700 professional computer. The PE 7700 is a 16-bit micro-computer, which comes equipped with 1 Mb RAM, two double-sided, double density floppy disk drives, a 20 or 40 M byte Winchester disk drive, colour CRT with eight soft keys, and four RS-232C and IEEE-488 communications ports. The PE 7700 processor performs all the data acquisition and subsequent calculations. A number of software packages are available for different data processing routines. The other two microprocessor systems are coupled to the optical unit. The instrument supervisor is based on the Motorola MC 68000. Actual control of the optical hardware is performed by a coprocessor based upon the Motorola 6802. The instrument processor seems to acquire and perform a wide range of calculations using 32-bit precision.

Recording spectrum by the FT technique requires more effort than conventional dispersion technique. Therefore, at present, the chief areas of application of FTIR are in the FIR absorption studies with remote sources, in IR emission studies of weak emitters and in rapid-scanning measurements.

The application of FT spectrometry has been extended to uv/visible region in the entire spectral range from 160 nm–700 nm (Snook, 1986). The resolution achieved is much greater than the instruments based on grating monochromators.

3.5.3 Advantages of FTIR

Some of the major advantages of FTIR over the dispersive technique include:

- *Speed*: Because all of the frequencies are measured simultaneously, most measurements by FTIR are made in a matter of seconds rather than several minutes.
- *Sensitivity*: Sensitivity is dramatically improved with FTIR for many reasons. The detectors employed are much more sensitive, the optical throughput is much higher which results in much lower noise levels, and the fast scans enable the co-addition of several scans in order to reduce the random measurement noise to any desired level.
- *Mechanical Simplicity*: The moving mirror in the interferometer is the only continuously moving part in the instrument. Thus, there is very little possibility of mechanical breakdown.
- *Internally Calibrated*: These instruments employ a HeNe laser as an internal wavelength calibration standard. These instruments are self-calibrating and never need to be calibrated by the user.

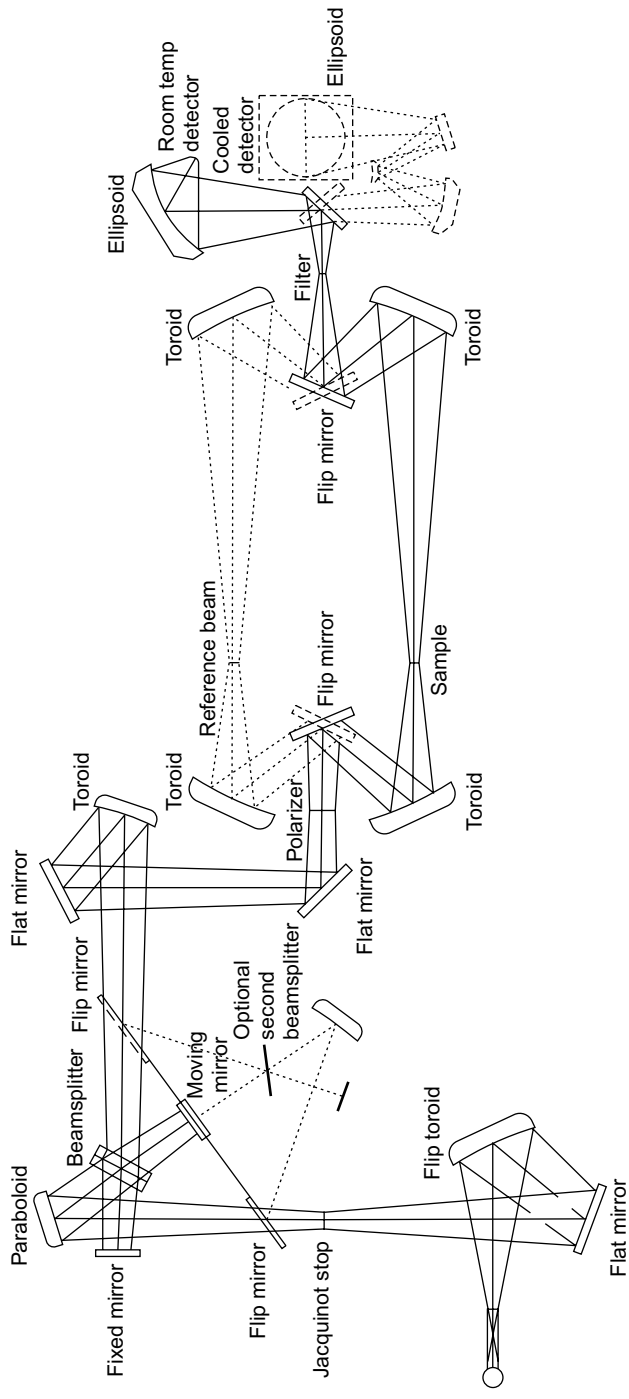


Figure 3.20 *The optical layout of the Model 1800 Perkin-Elmer FTIR spectrophotometer*

3.6 CALIBRATION

In order to check IR spectrophotometer, spectrum of a polystyrene film is run and the absorption spectrum is compared with that provided by the company. In case any difference in the absorption spectrum is detected, the instrument has to be thoroughly checked and recalibrated. To facilitate speedy recalibration of low resolution FIR instruments, special broad-band wavelength calibrators are often used.

The transmittance of the filter obtained between 800 and 350 cm^{-1} on a Perkin-Elmer 457 spectrophotometer shows that it provides three accurate calibration points: one due to the polythene matrix itself (at 722 ± 1 cm^{-1}) and the other two (at 590 ± 2 cm^{-1} and 500 ± 1 cm^{-1}) due to mercuric oxide.

3.7 ATTENUATED TOTAL REFLECTANCE TECHNIQUE

IR methods can be used to study materials which are light scattering or opaque, or in the form of coatings on opaque materials. The technique usually employed is based on obtaining reflectance spectrum. However, a spectrum obtained by reflection of the radiation from the surface of a chemical material is generally very poor. To overcome this difficulty, a technique known as Attenuated Total Reflectance is used which enables one to obtain reflection spectra of satisfactory quality.

An attenuated total reflection technique operates by measuring the changes that occur in a totally internally reflected IR beam when the beam comes into contact with a sample.

Figure 3.21 shows the optical arrangement of a simple ATR system. In this technique, energy from the source enters into a prism and is reflected almost entirely from face *C* when the beam strikes it at less than critical angle. The prism consists of a crystal of material of high refractive index and transparent to IR radiation.

If the sample is placed in contact with the face *C*, the internal reflection will be attenuated or reduced at the sample-crystal surface. The attenuation will take place at those wavelengths, where the material absorbs IR energy. This enables to achieve absorption spectra of coatings and liquids even if they are opaque. The information conveyed by the ATR spectra is essentially the same as that conveyed by transmission spectra.

Single reflection ATR is not always adequate because of the low sensitivity observed. Improvement in sensitivity can be obtained by the use of multiple internal reflection system, in which internal reflections take place 25–50 times before emerging out of the crystal. This is illustrated in Figure 3.22. This strengthens the absorption pattern of a material placed on one surface of the optical flat. This is best achieved by reducing the width of the entrance face of the crystal and increasing its length. However, there is a practical limitation, as the entrance face cannot be made smaller than the monochromator slit, otherwise it would result in serious loss of energy.

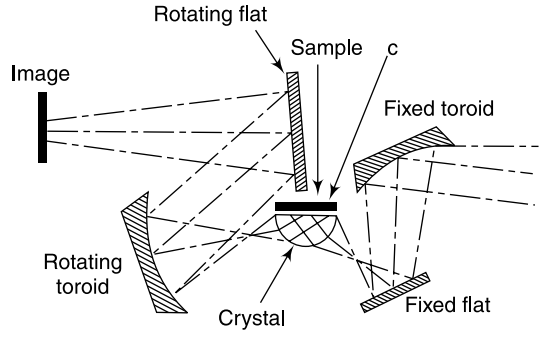


Figure 3.21 *Optical arrangement of a simple attenuated total reflectance system*

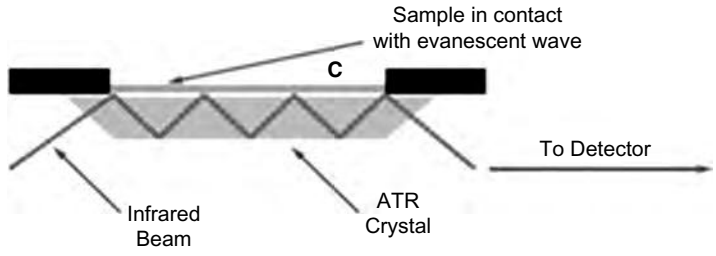


Figure 3.22 *Multiple internal reflections*

There are a number of crystal materials available for ATR such as silver chloride and KRS-5. Zinc Selenide (ZnSe) and Germanium are by far the most commonly used material for ATR sampling. Zinc Selenide is a relatively low cost ATR crystal material and is ideal for analysing liquids and non-abrasive pastes and gels but it is not particularly robust with a working pH range of 5–9. ZnSe scratches quite easily and so care must be taken when cleaning the crystal. Germanium has a much better working pH range and can be used to analyse weak acids and alkalis. Diamond is by far the best ATR crystal material because of its robustness and durability.

One of the main advantages of the ATR technique is that the spectrum obtained is independent of the sample thickness. Typically, the reflected radiation penetrates the sample to a depth of only a few microns. Consequently, the method has been found to be particularly useful for establishing the surface characteristics of many materials.

4

FLAME PHOTOMETERS

4.1 PRINCIPLE OF FLAME PHOTOMETRY

According to the quantum theory, the energy states of an atom or molecule are sharply defined and any change from one state to another therefore, requires a sharply defined quantity of energy. When radiation falls on a material, or the material is supplied with extra energy in some form, some part of the energy is taken up by the material and results in altering the state of the atoms or molecules of which it is composed. The atoms or molecules of the material are promoted to higher energy states. However, the higher energy states are rather unstable. The particles at the higher energy levels tend to lose the extra energy and return to the original level or ground state, either by undergoing a chemical reaction or by dissipating the energy as heat, or by emitting the energy as radiation. If it loses all or part of the energy as radiation, it will emit photons of an energy corresponding to difference between two energy levels. Since the levels are clearly defined for a given atom, the radiation will be emitted at clearly defined frequencies only. The frequencies are shown up as bright lines if the emitted light is dispersed as a spectrum. By measuring the wavelength of the emission, one can find out what atoms are present. Also, by measuring the intensity of the emission, one could compute the concentration of the element.

In short, the principle of flame photometry is based on the fact that if an atom is excited in a flame to a high energy level, it will emit light as it returns to its former energy level. By measuring the amount of light emitted, we can measure the number of atoms excited by the flame.

It is common knowledge that when sodium is introduced into a flame it emits a radiation in the yellow region of the visible spectrum. Table 4.1 gives details of the measurable atomic flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the colours produced.

Table 4.1 *Emission wavelength of various metals*

Element	Emission Wavelength (nm)	Flame Colour
Barium (Ba)	554	Lime Green
Calcium (Ca)	622*	Orange
Lithium (Li)	670	Red (Carmine)
Potassium (K)	766	Violet
Sodium (Na)	589	Yellow

*Calcium is measured by using the calcium hydroxide band emission at 622 nm. However, the main atomic emission occurs at 423 nm.

Flame photometry offers the following advantages:

- The technique is very rapid. It does not require any chemical preparation except preparing a solution of suitable concentration.
- The method is most useful for analysis of some elements, which are difficult to measure by other methods.
- The technique is most suited to analytical problems, in which a large number of samples of similar types are to be measured.
- The method is quite cheap as it does not require any other expensive reagents.

In addition, the analysis of alkali and alkaline earth metals by flame photometry has the following two major advantages:

- Their atoms reach the excited state at a temperature lower than that at which most other elements are excited.
- Their characteristic wavelengths are easily isolated from those of most other elements due to wide spectral separation.

4.2 BASIC FLAME PHOTOMETER

The equipment for flame photometric determinations is simple. A solution of the sample to be analysed is prepared. A special sprayer operated by compressed air or oxygen is used to introduce this solution in the form of a fine spray (aerosol) into the flame of a burner operating on some fuel gas, like acetylene or hydrogen. Conversion of sample solution into an aerosol by atomiser does not bring about any chemical change in the sample. However, the heat of the flame which vaporises sample constituents, molecules and ions of the sample species are decomposed and reduced to give atoms. The heat of the flame causes excitation of some atoms into higher electronic states. Excited atoms revert to the ground state by emission of high energy of characteristic wavelength.

The radiation of the element produced in the flame is separated from the emission of other elements by means of light filters or a monochromator. The intensity of the isolated radiation is measured from the current it produces when it falls on a photocell. The measurement of current is done with the help of a galvanometer, whose readings are proportional to the concentration of the element. After carefully calibrating the galvanometer with solutions of known composition and concentration, it is possible to correlate the intensity of a given spectral line of the unknown sample, with the amount of the same element present in a standard solution.

Flame photometry is characterised by a high degree of constancy and reproducibility. The spectrum of an element as produced in a flame is relatively simple, consisting normally of only a few lines. Identification of the line is simple and spectral interference is less frequent. The most usual application of flame photometry is for the analysis of sodium and potassium in body fluids and these analyses constitute the bulk of the determinations generally performed. However, the method is slowly replacing more troublesome methods for other elements also.

Flame photometry is concerned mostly with atoms. Molecules cannot normally survive the high temperatures employed in flame photometry.

The numbers of elements to which flame photometric methods can be applied depend mainly on the source temperature developed by the fuel-mixture employed. This is because not all atoms are easily excited in the flame. The atoms of some elements have their energy levels so spaced that a large number

of different lines are emitted, while for some others, almost all the light they emit is concentrated in one spectra line. The upper energy levels or atoms of some elements are so high above the ground state that they are very difficult to excite. Also, their emission lines could be of a wavelength, which may not be directly usable. All these factors determine the lowest concentration of atoms which can be detected in the sample and thus varies very widely for different atoms.

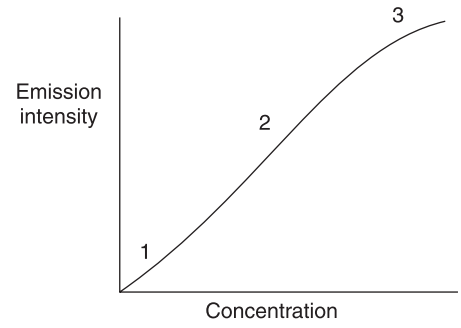


Figure 4.1 *Emission intensity Vs concentration*

A typical plot of emission intensity vs. concentration of ionic species in the solution being measured is linear over a wide range, but with a deviation at both *low* and *high* concentrations. This is shown in Figure 4.1. The deviation from linearity at very low concentrations is because the emission falls below expected due to ionisation where some atoms get converted back to ions ($K \rightarrow K^+ + e^-$). The ionisation is insignificant at higher concentrations. On the other hand, the negative deviation observed at high concentration is due to *self-absorption*. This involves partial absorption of photons emitted by excited atoms by the ground state atoms in flame.

4.3 CONSTRUCTIONAL DETAILS OF FLAME PHOTOMETERS

A flame photometer has three essential parts (Figure 4.2).
Emission System: It consists of the following:

(i) Fuel gases and their regulation: comprising the fuel reservoir, compressors, pressure regulators and pressure gauges.
(ii) Atomiser: consisting, in turn, of the sprayer and the atomisation chamber, where the aerosol is produced and fed into the flame.
(iii) Burner: receives the mixture of the combustion gases.
(iv) Flame: the true source of emission.

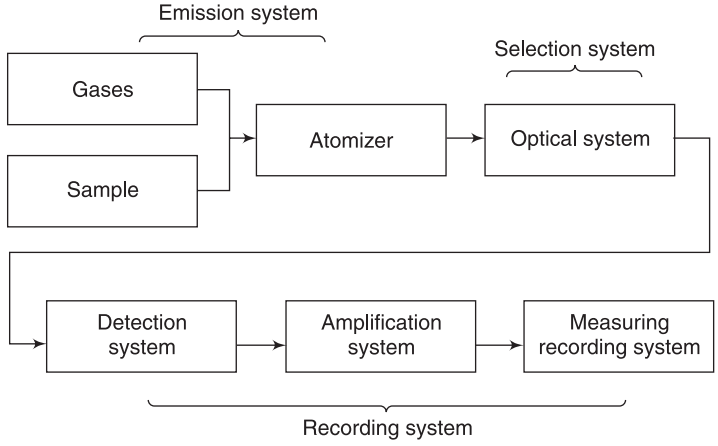


Figure 4.2 *Essential parts of a flame photometer*

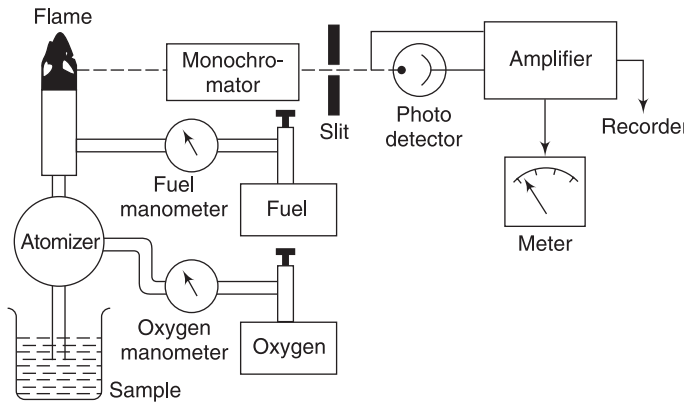


Figure 4.3 *Block diagram of a flame photometer*

Optical System: It consists of the optical system for wavelength selection (filters or monochromators), lenses, diaphragms, slits, etc.
Recording System: It includes detectors like photocells, phototubes, photomultipliers, etc., and the electronic means of amplification, measuring and recording. Figure 4.3 shows a typical block diagram of a flame photometer.

4.3.1 Emission System

4.3.1.1 Fuel gases and their regulation

Pressure Regulators: In order to obtain a steady-emission reading, it is imperative to have a flame that is perfectly steady and free from flickers. To achieve this, the air or oxygen and fuel pressure is maintained constant during the operation of the instrument. Suitable pressure gauges are therefore, provided in the instrument to indicate the pressure actually present in the line. A 25-lb gauge for the oxygen or air supply and a 10-lb gauge for the fuel are generally used. Pressure regulators are usually followed by needle valves for control of flow. Gauges provided with the regulators are often not sensitive enough to detect small changes of pressure, which have profound effect on the flame photometer operation. Therefore, narrow range pressure regulators and manometers are installed in the line, in order to observe small changes in pressure or gas flow.

Flowmetres: A flow metre may be inserted in the line from the gas reservoir to the atomiser in order to detect any clogging of the orifice. For the same flow rate, an appreciable change in the gauge pressure indicates partially clogged orifice. By controlling the individual flow rates of the fuel and oxygen, the operator can choose various fuel-oxygen mixtures ranging from lean flame mixtures to fuel-rich types of flames. The flow rates usually vary from 2 to 10 Cu. ft/h.

Fuel Supply: The fuel gas normally used in flame photometry is the acetylene gas, which is commercially available in cylinders of various sizes. Cylinder acetylene consists of acetylene gas dissolved in acetone, which in turn is absorbed on a porous filling material. Consequently, after the flame is lit, it should be allowed to burn several minutes before adjustments are attempted in order to vent the excess acetone initially present in the vapour phase. Consumption of acetylene ranges from 1 to 5 s Cu. ft/h. The other fuels used in flame photometry are propane, butane and hydrogen. When the available gas

pressure is less or is variable, a booster pump is necessary. The pump generally used is a motor-driven diaphragm pump, which delivers the gas to the burner at the required volume and pressure.

Oxygen Supply: Oxygen from cylinders should be supplied to the burner through a regulator capable of delivering approximately 12 Cu. ft/h. at a pressure of 12–15 lb per sq. inch.

Air Supply: The air supply can be supplied from a cylinder of compressed air or from an air compressor through a tank held at about 10 lb/sq. inch. A pressure compensation valve is placed between the reservoir and the burner. When compressors are used, the air should be filtered through glass wool. Consumption of air is approximately 10 Cu. ft/h for an acetylene-air flame.

4.3.1.2 Atomiser

One of the most exacting problems in the flame photometer design is the manner in which the sample is fed to the flame at a uniform rate. The usual method is to prepare solutions of known concentrations and to spray these into the flame, using some form of aerosol production. Use of an aerosol permits the distribution of the same throughout the body of the flame, rather than its introduction at a single point. In flame photometry, the name atomiser is given to a system which is used to form aerosol by breaking a mass of liquid into small drops. This little device is responsible for introducing the liquid sample into the flame at a stable and reproducible rate. The atomiser must not be attacked by corrosive solutions.

Two types of atomisers which are in common use are: (i) Those which introduce the spray into a condensing chamber and into the flame by the air of the combustible gas air mixture. Large droplets are removed in the condensing chamber. (ii) Those in which the sample is introduced directly into the flame (i.e. the atomiser and the burner are an integral unit).

Figure 4.4 shows the construction of the first type of atomiser. This is called the discharge-type atomiser and consists of two capillary tubes sealed into the walls of a glass chamber in such a manner that their bores are perpendicular to each other. The sample solution is poured into a funnel or drawn up from a container and is atomised by the blast of air from the tip of the other capillary. However, the atomised stream is composed of coarse spray with large droplets, which condense on the walls of the chamber and helical tube leading to the burner. The condensate flows down to the waste drain. The smaller droplets, in the form of a virtual fog are carried by the air stream into the burner, where it is mixed with the burner gases and carried into the region of active combination. Two removable hypodermic needles of stainless steel or glass are commonly used. With this type of atomiser, the consumption tilt sample is comparatively high and ranges between 4 and 25 ml of solution per minute. Of this amount only 5% actually reaches the flame. The sensitivity of this type of atomiser can be markedly increased by using a chamber which is heated by an electric heater placed around its

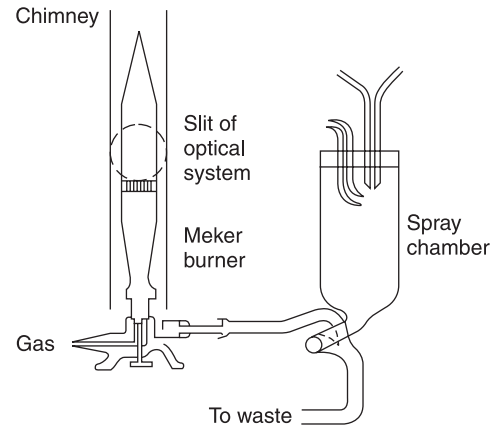


Figure 4.4 *Discharge-type atomiser with condensing chamber and burner*

walls. This hastens the process of vaporisation of the solvent and produces an aerosol of very fine particles, all of which are swept into the burner. In this case, sample consumption is only 0.2–0.6 ml/min and a substantial portion is carried directly into the burner to yield a much higher sensitivity.

The second type of atomiser is the integral burner-atomiser. In the sample solution is introduced directly into the flame. This is shown in Figure 4.5. The unit made of glass or metal is constructed of two concentric tubes. The sample solution is drawn through the innermost tube by the passage of oxygen through the orifice of the middle annulus. At the tip of the inner sample capillary, the liquid is sheared off and dispersed into droplets. The outer annulus supplies the combustible gas to the flame. The body of the unit is machined from brass and the capillary for solution intake is of palladium. All droplets, both large and small, are introduced directly into the flame. Sample consumption is between 0.8 and 2 mi/min. Each atomiser requires separate calibration and is not strictly interchangeable with another of the same type.

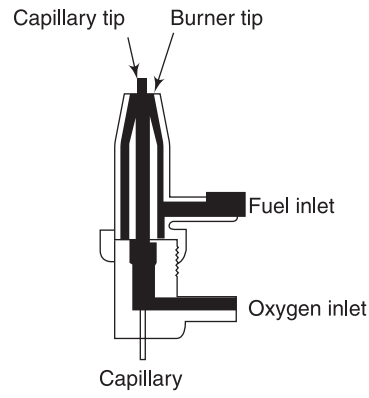


Figure 4.5 *Integral burner-atomiser*

Pneumatic nebulisation, though one of the most common methods of aerosol generation, produces a relatively wide droplet size distribution and limited aerosol density. Other techniques, such as the spinning disk aerosol generator, atomiser impactor and isolated droplet generation techniques have also been used, but they produce only very limited concentrations of in place of *d* aerosol. Ultrasonic techniques, when used for aerosol generation, offer unique combination of high solution to aerosol conversion rates, relatively independent of carrier flow gas and have the ability to generate varying-sized droplet populations. These workers describe the design of an ultrasonic nebuliser system for the generation of high density aerosol dispersions.

Periodic cleaning of the atomiser and capillary tube is necessary as blocked atomiser will give unstable reading, intermittent reading and low sensitivity. Cleaning is usually done by flushing with copious amount of distilled water. If blockage occurs, the atomiser is removed from its reading and flushed with dry air or cleaned it by using thin wire.

4.3.1.3 Burner

The burner brings the fuel, oxidant and sample aerosol together, so that they may react safely and produce a good flame. Burners used in flame photometry must fulfil the following conditions:

(i) Supply fuel and oxygen at constant pressure to enable the shape, size and temperature of the flame will remain constant.
(ii) Assist in a perfect distribution of the mixture of gases and the aerosol, which carries the atomised solution under analysis.
(iii) Have a tip of suitable shape to produce a symmetrical flame and ensure a homogeneous flow and distribution of the gases, avoiding a strike back in the burner from accidental fluctuations in the feed system.
(iv) Prevent condensation of the aerosol in the stem of the burner, which would reduce the effective quantity of the sample brought into the flame.

The most commonly used burner for low temperature flames is Meker type. Here, the fuel-gas issues from a small orifice and passes through a venture throat, where considerable air is entrained. The mixture of gas and the entrained air passes up the burner tube and burns at the top of the burner, where the combustion is assisted by the surrounding air.

A deep grid of metal across the mouth of the burner prevents the flame from striking back down the tube. To screen the flame from air drafts, it is surrounded by a glass chimney, which also protects the operator.

4.3.1.4 Flame

The flame is the most important part of the flame photometer, since it forms the source in which the light radiations, characteristic of the elements under analysis, are produced. The flame performs the following functions:

(i) It converts the constituents of the sample to be analysed from the liquid or solid state into gaseous state.
(ii) Decomposes these constituents into atoms or simpler molecules.
(iii) Excites the resulting atomic or molecular species to emit light radiations.

In order to produce accurate results, the flame must be very stable. If its temperature or structure shows a change over a period of time, the emission produced by a given sample will also change.

The flame temperature must be high enough to excite the atoms to higher energy levels, so that emission may take place. In general, the flames are produced by burning the gases as given in Table 4.2.

All the fuels employed in flame emission spectroscopy produce both continuous background as well as certain band spectra. The nature and intensity of the spectrum for a given fuel is strongly dependent upon fuel-to-oxidant ratio and the flame temperature.

Table 4.2 *Temperature achieved by a variety of gas mixtures*

Mixture	Temperature (°C)
Acetylene/oxygen	3100–3200
Acetylene/nitrous oxide	2900–3000
Natural gas/oxygen	2700–2800
Hydrogen/oxygen	2500–2700
Acetylene/air	2100–2400
Hydrogen/air	2000–2100
Propane/air	1900–2000

4.3.2 Optical System

The complex light emission produced in the flame by a solution containing one or more elements, makes it necessary to select the radiations of different wavelengths for each of the elements present in the solution. This job is accomplished by the optical system, which collects the light from the steadiest part of the flame, renders it monochromatic and then focuses it on to the surface of the photodetector. A concave mirror is often placed behind the flame, with its centre of curvature in the flame. In this way, the intensity of the emitted light is nearly doubled.

4.3.2.1 Filters

Atoms in the vapour state give Line Spectra and not band spectra because no covalent bonds and hence no vibrational sub-levels to cause broadening. Therefore, wavelength selection may be done by optical filters or monochromators. Less expensive instruments make use of filters, which may be absorption type or interference type. The usual glass or gelatine absorption filters have wide spectral bandwidths of 35–45 nm, in width at one-half the maximum transmittance. Their overall transmittance is only 5–25%, decreasing with improved spectral isolation. Absorption filters do not give the degree of monochromaticity required for analysing complex systems and hence, the flame photometers so equipped are restricted in use to the determination of only sodium, potassium, calcium and lithium.

To improve the resolution, interference filters are often used. They are the most suitable for flame photometry as compared with absorption filters, because they allow a much narrower band of wavelengths to pass. They are similar to monochromators in selectivity, but are less expensive. Standard interference filters having spectral bandwidths from 10 to 17 nm at one-half the maximum transmittance are commercially available. The same characteristics are shown by continuous wedge filters, which allow a continuous selection of different wavelengths. These filters usually have a working interval of 400–700 nm. They provide a dispersion of 5.5 nm/mm, with a transmittance which is usually not higher than 35%. The filters must be kept clean as a dirty filter can result in unstable reading.

4.3.2.2 Monochromators

Monochromators are incorporated in the instruments, which are expected to provide better isolation of spectral energy. This requirement emanates when the spectrum lines are very close or very weak or both. By using narrow slit widths and sensitive detecting circuits, quite narrow bands of radiant energy can be isolated. In flame photometry prism monochromators have been most widely accepted. They may be of glass or quartz. The glass prisms are only suitable for emissions essentially in the visible region, whereas the quartz prisms enable many atomic lines to be studied that occur in the far ultraviolet region of the spectrum.

Dispersal of light may also be obtained by means of diffraction in a grating. A grating is produced by ruling grooves at extremely close intervals, of the order of 15,000 grooves per inch, on a highly polished surface. The dispersion is linear, which permits a constant bandwidth to be used throughout the spectrum. The wavelength scale in this case is linear.

For the analysis of complex spectra, the resolving power should he very high. Resolving power is a measure of the ability of the monochromator to resolve two closely spaced lines of about equal intensity. Perhaps, the most straight test of an instrument's resolving power concerns the separation of the manganese line at 403.3 nm, the potassium line at 404.4 nm and the lead line at 405.8 nm from each other.

4.3.2.3 Other optical components

Reflectors and condensing lenses can be placed in the optical path to concentrate the light. A concave metal mirror with its focus in the flame itself is placed immediately behind the flame. Condensing lenses are used to render parallel beams of light before it falls on the interference filters. Some apparatus are fitted with an iris diaphragm in the optical path of the rays, which serves to reduce the sensitivity when using very concentrated solutions. Similarly, uniform metal grids with different size meshes may be used. The slit is of special importance in apparatus fitted with monochromators.

Its width is more important than its length, because the sensitivity, the accuracy, the ratio of total emission-flame background and the resolving power, depend on the width of the slit.

4.3.3 Photosensitive Detectors

After the isolation of the lines or bands emitted by the chemical elements under analysis in the optical system, the light intensities of these radiations have to be measured quantitatively. This is done by causing the radiation selected to fall on a photosensitive element. The electric current produced is then measured, either directly or after amplification. The intensity of electric current produced is a function of the concentration of the solution under analysis. The photodetectors are either of photovoltaic or photo-emissive type or any one of the photodetector types explained in Chapter 2.

The preferred detector for weak light intensities is the photomultiplier tube. This is many times more sensitive than the simple phototube, which in turn, permits greater spectral resolution without loss of signal. They are available with envelopes of ordinary glass or with quartz window inserts for use in the ultraviolet region below 350 nm.

4.3.4 Recording System

The final step in recording the light radiations produced in the source of emission consists in transforming the electrical signal produced by the selected radiation in the photosensitive element into a reading which is related to the concentration of the solution under analysis.

4.3.4.1 Amplifiers for phototube circuits

The current from the phototube is generally so small that it is necessary to amplify it, quite often by a factor of 10^5 to 10^6. The amplification of a direct current can be achieved with the help of electronic circuits with high input impedance.

In order to reduce the rapid and random fluctuations, a long enough time constant is incorporated in the amplifier circuit to aid in the integrating process, and thus the metre is rendered unresponsive to sudden flashes of light in the flame. Usually, the time constant is chosen to be approximately 1 s. To overcome the problem of drift of DC amplifiers, the light from the flame can be interrupted, and the photoelectric current can be amplified by an AC amplifier. A synchronously rotating disc is placed before the flame and the photoelectric current is thus convened to a chopped DC signal, which can then be amplified by an AC amplifier.

4.3.4.2 Amplifiers for use with photomultiplier tubes

The output current from the last amplifying stage in a photomultiplier tube for many solutions and concentration ranges normally employed, often lie between 10 and 100 μA. This requires to be appreciably increased by an amplifying circuit. Figure 4.6 shows a circuit diagram, which can be used to amplify current obtained in a photomultiplier. The output of the PMT is applied to the gate of FET Q_1. Q_1 inverts this negative going signal to positive and applies this to the non-inverting side of operational amplifier Z_1. Due to divider network R_9 and R_8, the gain of the operational amplifier Z_1 is held at 10. The output of the circuit can be directly connected to a moving coil metre.

Digital display type flame photometers are now available in which the analog voltage is converted to digital form and then given to LEDs for numerical display.

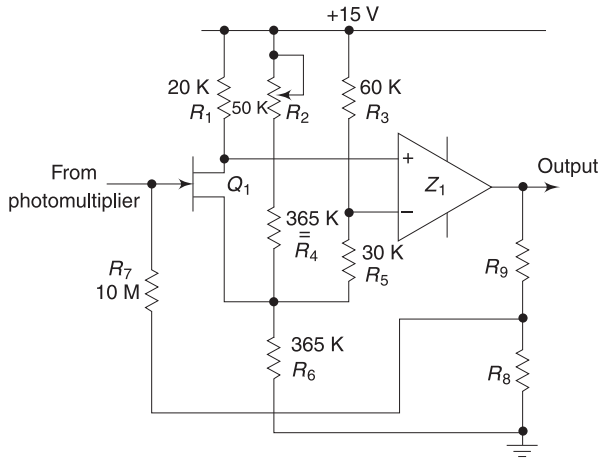


Figure 4.6 *Typical amplifier circuit used in flame photometers*

4.4 CLINICAL FLAME PHOTOMETERS

The flame photometer is one of the most useful instruments in clinical analyses. This is due to the suitability of the flame photometer for determining sodium, potassium and calcium, which are of immense importance in the development of the living being and indispensable to its physiological functions. In the clinical analysis of sodium and potassium, the flame photometer gives, rapidly and accurately, numerous differential data for normal and pathological values.

The normal plasma sodium concentration is about 140 mmol/l. A high plasma sodium concentration of more than 145 mmol/l is referred to as *hypernatremia*. This can occur due to simple dehydration, excess sodium intake, steroid therapy as well as in diabetic insipidus. *Hyponatremia*, with plasma sodium concentration less than 130 mmol/l, can occur due to diuretic medication, kidney disease, excessive sweating, congestive heart failure or gastrointestinal disorder.

Potassium is the major intracellular cation and influences muscular activity, cardiac function and nerve conduction process. Acute hyperkalaemia condition in which the plasma potassium concentration exceeds 5.5 mmol/l is a medical emergency. In hypokalaemia, the potassium level will be less than 3.5 mmol/l, which can occur due to excessive loss in gastrointestinal secretions and urine.

Many companies manufacture flame photometers KLiNa Flame System of M/S Beckman is a dedicated instrument for simultaneous analysis of sodium and potassium. In this instrument, sample handling is automatic, as the system has a turntable, which will hold up to 20 samples in cups and an automatic positive piston displacement dilutor that dilutes the sample prior to entering the spray chamber (Figure 4.7).

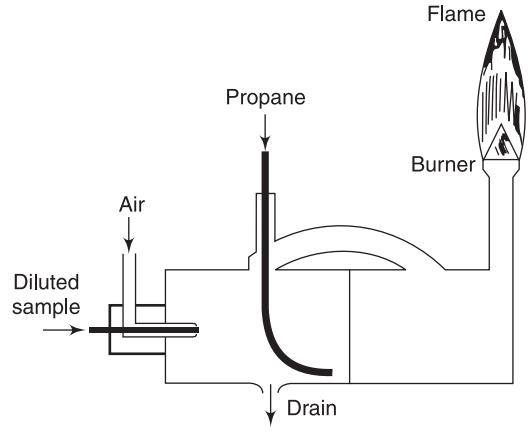


Figure 4.7 *Flame spray chamber and burner of KLiNa flame photometer (Courtesy M/S Beckman Instruments, USA)*

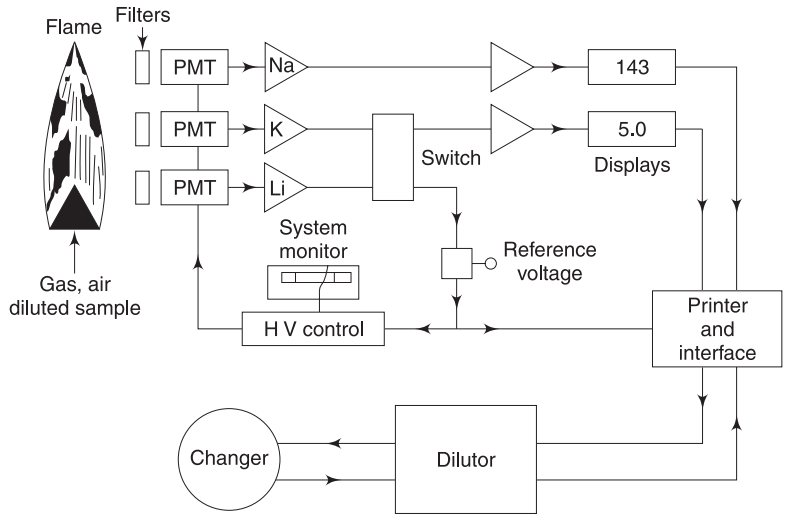


Figure 4.8 *Schematic diagram of Beckman KLiNa flame photometer*

The ignition and shutdown of the flame are automatic. When calibrate button is depressed, the flame is ignited and the circuits are energised. Passing a standby button extinguishes the flame, but maintains thermal equilibrium. Twenty-four micro-litres of samples are aspirated for simultaneous sodiums and potassiums, providing micro-sample analysis suitable for paediatric or geriatric work. After analysis, the results are displayed directly in millimoles per litre or milliequivalents per litre.

For the precise and accurate determination of sodium (Na) and potassium (K) concentrations, use is made of the fact that lithium normally not present in significant concentrations in serum, exhibits about the same flame emission characteristics as Na and K. Lithium ions are added to the diluent used for samples, standards and controls. The lithium in the diluent is referred to as the internal standard.

The diluted sample containing the fixed known amount of lithium, in the form of a dissolved salt, is nebulised and carried by the air supply into the first of two compartments in the spray chamber (Figure 4.8). Heavier droplets fall out of the stream on to the chamber walls or separate from the stream upon striking a partition in the chamber and flow to a drain from the compartment. Propane enters the first chamber to mix with the air and sample stream, and carry it through a tubular glass bridge into a second compartment; the aerosol and propane mixture travels up from the chamber to the burner head, where the mixture is burned. Exhaust gases are vented to room air from a cover located on top of the instrument.

In order to provide internal standardisation, the response of the sodium and potassium detector is a ratio function of the response by the lithium detector. Thus, any change in air-flow rate or fuel pressure that may affect the sample would proportionately affect the lithium detector.

The flame is monitored continuously by three photomultiplier detectors. Each detector views the flame through an optical filter that passes only the wavelength band of interest to the particular detector. The sodium detector therefore responds only to wavelengths in a narrow band centred at 589 nm; the potassium detector responds only to wavelengths in a narrow band centred at 766 nm; the lithium detector responds only to wavelengths in a narrow band centred at 671 nm.

The specifications for the KLiNa Flame System are +0.2 mmol/l for potassium and lithium, and ±2.0 mmol/l for sodium. Potassium and lithium both show linearity to 20 mmol/l, while sodium is linear to 200. In addition to the 0–20 scale, potassium may be re-scaled to read out to 200 mmol/l for convenient analysis of urine samples.

The fully automatic ignition and flame optimisation sequences reduce set up and calibration time. An automatic gas shut off mechanism activates if the flame is accidentally extinguished. If no flame is detected within a fixed time, about 10 seconds, the system automatically shut down the flow of fuel and air (Alpret, 1974). Warning lights indicate whether the problem is either low air or low fuel pressure. The lithium internal standard signal reduces fluctuation in flame conditions, drift and dilution errors and ensures reproducible results. In the microprocessor based instruments monitoring and control software allow measurements only after blanking and calibration.

By observing simple precautions and maintenance procedures, flame photometers have been found to give a long working life. The following procedures may be followed to correct any malfunctioning of the instrument:

(i) If the instrument shows low sensitivity, it may be due to blocked atomiser, low gas pressure, or faulty photocell. Necessary corrective steps should be taken to clean the blockage of the atomiser, restoring the gas pressure or changing the photocell.

(ii) The reason for varying or intermittent readings is usually a contaminated burner, faulty photocell, low gas pressure, blocked atomiser, improper air supply and excessive vibration. The instrument must be placed on a shockproof base. Appropriate checks must be made for the above faults.

The capability of flame photometers has been extended by including measurement of Sodium, Potassium, Lithium, Calcium, Barium, Strontium, Rubidium and Caesium; the most extensive range available from any Flame Photometer manufacturer by using suitable filters. This facility is available in the instrument Model 410 from M/s Sherwood Scientific Ltd., UK which is shown in Figure 4.9.

The instrument comes with a preloaded filter stick which houses a maximum of three element filters. The filter stick is easily removed and replaced within the chimney housing and as such it is a relatively simple operation to introduce alternative element specific filters. Flame photometers are available from simple single channel to standalone or software-driven multichannel instruments.

Figure 4.9 *Flame photometer (Courtesy: M/s Sherwood Scientific Ltd., UK)*

4.5 ACCESSORIES FOR FLAME PHOTOMETER

Modern flame photometers come with many useful accessories. For example, a diluter, sample changer and a printer.

The diluter is a motor-driven cam-programmed system that functions through a cycle of operations. These operations involve sample pick-up and transport to an internal mixing cup, the addition of a measured volume of diluent, mixing to ensure a properly prepared sample aliquot, coupling of the mixing cup to spray chamber, so that sample aspiration can occur, and finally the

washing and draining of the mixing cup. The diluter uses positive displacement pumps to assure exact sample dilutions in the operator selectable ratios of 50:1, 100:1 or 200:1. The solutions are generally diluted to fall within linear part of emission curve.

The dilutor based on peristaltic pump provides accurate dilute ratios independent of sample viscosity. Variation in dilution ratios is achieved by changing the bore size of the pump tubing.

The automatic changer enables automatic presentation to the diluter sample probe up to 20 successive samples. It is a turntable, which rotates stepwise to locate each sample cup under the extended and down position of the diluter sample probe. The probe is extended from the diluter, once for each sample determination probe tip enters the sample and a measured volume is taken for transport to the diluter mixing cup. Individual sample trays are placed on die chamber turntable. Each tray can hold 20 sample cups. The sample cup could be 0.25, 0.5 or 2.00 ml size. The 2.0 ml size is generally recommended.

The Printer provides a paper tape print-out to record the result of each sample determination. Each print-out is a data line that from left to right, reads the sample position number in the Changer sample tray, the type of determination performed and the results expressed in milliequivalents per litre.

4.6 EXPRESSION OF CONCENTRATION

The concentration of solutions is usually expressed in. parts per million (ppm) in flame photometry. This type of expression enables to make easy calculations on dilute solutions and the concentrations can be expressed in weight/weight, weight/volume and volume/volume ratios. The ratios can be expressed in volume, if the density of the liquid is near unity and hence 1 ml = 1 g. This is true especially in dilute solutions, whose density is approximately that of distilled water. The equivalents are as follows:

1 ppm = 1 mg/l = 1 mg/kg
1 ppm = 1×10^{-3} g/l = $1. 10^{-3}$ mg/ml
1 ppm = 1×10^{-6} g/ml

4.7 INTERFERENCES IN FLAME PHOTOMETRY

Flame photometry is subject to several types of interferences, which arise during the course of analysis through variations in any of the parts of the instrumental system or in the composition of the samples. These interferences give rise to experimental errors. Some of these interferences are given below.

4.7.1 Flame Background Emission

A flame is an extremely complex mixture of reacting gases. Since these gases are hot, they emit a certain amount of light, even when no sample atoms have been injected into the flame. This light is the flame background. The lines produced by a sample are superimposed on this background. Failure to correct properly for the background reading can be a source of serious error. It is desirable to eliminate, as far as possible, the flame background and other emission lines from the measurements. The narrower the bandwidth of the monochromator used, the more efficient is this elimination.

4.7.2 Direct Spectral Interference

This interference occurs when the emission lines are so close together that the monochromator cannot separate them. For example, the emission of the orange band of CaOH interferes with the sodium line at 589 nm. Interference is more serious when absorption filters are used in place of monochromators. In this case, the two lines overlap partially or completely, and will be read together in proportion to the degree of overlap. If the interference cannot be obviated by increased resolution, the difficulty must be overcome by prior removal of one element, perhaps by selective solvent extraction.

4.7.3 Self-Absorption

Interference due to self-absorption occurs due to the absorption of radiant energy through collision with atoms of its own kind present in the ground energy level. If some of the radiant energy is self-absorbed, the strength of the spectral line is weakened. Self-absorption primarily depends upon the number of atoms present in the ground state, the concentration of atomised solution and by the probability that these atoms will be excited by the incident radiation from excited atoms of its own kind. Self-absorption is insignificant at very low concentrations of a test element.

4.7.4 Effect of Anions

Anionic disturbances result from a radiation in the number of free metallic ions by stable combination in the flame with the anions. There is usually no interference with concentrations less than 0.1 M. Above this concentration, sulphuric, nitric and phosphoric acids, in particular, show a marked effect in lowering metallic emission. For example, the strong depression of the calcium emission in the presence of phosphate, aluminate and other similar anions has been fairly well known. In practice, over limited intervals of concentration, the depression is linear and this forms the basis for indirect determination of the depressant in the presence of a standard amount of calcium.

4.7.5 Effect of Ionisation

The easily excitable alkali metals, such as potassium show the phenomenon of ionisation through excessive dissociation and consequently result in the depletion of the number of available neutral atoms that can be excited. This in turn, weakens the intensity of the atomic spectrum, whereas any ionic spectrum is strengthened. When small quantities of easily ionised metals are added to a flame, the number of neutral atoms tend to increase mote rapidly in comparison to the ionised atoms, in proportion to a concentration of metal sprayed into the flame. As a result, the curve of intensity versus concentration may initially be concave upward.

4.7.6 Solution Characteristics

The physical properties of the solutions strongly influence the atomisation rate, which in turn influences the luminescence of the flame. The main factors responsible for this are the differences in viscosity, tension, density, volatility and temperature between standards and samples. For example, an increase in viscosity lowers the atomisation rate, with a consequent weakening of the emission, causing errors and loss of sensitivity. Similarly, variation in surface tension is considered as another source of error. They affect the emission in a similar manner to viscosity, through their effect on atomising conditions. Added salts and acids hinder the evaporation of solvent, large droplets result

in a diminished quantity of aerosol reaching the burner, because the larger droplets are more likely to settle upon the walls of the condensing chamber.

There are several standard ways of eliminating or avoiding the influence of the above mentioned factors. The techniques of overcoming interferences demand high skill and experience. With the development of atomic absorption spectrophotometry, in which the problems of interference and flame stability are not as severe, flame emission analysis has been largely limited to the determination of sodium potassium and less frequently lithium, calcium, manganese and copper in relatively simple mixtures.

4.8 PROCEDURE FOR DETERMINATIONS

4.8.1 Calibration Curve Method

In order to determine the concentration of an unknown element X in a given sample, or a series of samples, the instrument is first standardised by making a calibration curve. For this, it is desirable to know the probable range of concentration of X in the sample, so that a series of calibration standards can be made up; having a suitable spread over the range expected. The instrument is first adjusted to give a zero-metre reading, when pure distilled water is aspirated into the flame. The instrument settings are then added to give the full-scale reading, with the most concentrated standard sample. Without altering this adjustment, emission intensities for the remaining standards and the unknown are measured. Calibration curves are thus plotted and the concentrations read off against this calibration, concentration of the unknown is obtained from the calibrated curve.

Since it is difficult to obtain a flame of high long-term stability, it is usual to check the calibration at frequent intervals by spraying a standard. This ensures that drift can be measured and taken into account. In fact calibration curves are difficult to reproduce on the same day; virtually impossible on different days.

4.8.2 Internal Standard Method

The flame system is a critical part of the flame photometer and its long-term stability is dependent upon (i) fuel flow, (ii) air pressure, (iii) nebulisation rate and (iv) burner/atomising chamber temperature. While the first two factors can be controlled effectively to within acceptable limits by suitable instrument design, the other two are less easily controlled in this way. To overcome variation from these sources, internal standard procedure has been developed.

In this method a fixed quantity of the internal standard element is added to the blank sample and standard solution. The radiation of the element is measured together with those of the elements under analysis by dual detectors, or by scanning successively the two emission lines. The ratio of the emission intensity of the analysis line to that of the internal standard line is plotted against the concentration of the analysis element on log-log paper, to prepare the calibration curve for a number of standards. As a ratio rather than an absolute light intensity that is being measured, because of which fluctuations due to variable operation of the nebuliser-burner system, are greatly minimised. This procedure also reduces errors due to differences in the composition of the test and standard solutions.

For analysis of alkali metals and even of other elements, lithium has been preferred as an internal standard, as it satisfies most of the above mentioned requirements.

5

ATOMIC ABSORPTION AND EMISSION SPECTROPHOTOMETERS

5.1 ATOMIC SPECTROSCOPY

The science of atomic spectroscopy involves three techniques for analytical use: atomic emission, atomic absorption and atomic fluorescence. The process of excitation and decay to the ground state of an atom is involved in all three fields of atomic spectroscopy. Either the energy absorbed in the excitation process or the energy emitted in the decay process is measured and used for analytical purposes.

Atomic Emission Spectroscopy: In atomic emission, a sample is subjected to a high energy, thermal environment in order to produce excited state atoms, capable of emitting light. The energy source can be an electrical arc, a flame or more recently, a plasma. The emission spectrum of an element exposed to such an energy source consists of a collection of allowable emission wavelengths, commonly called emissions lines, because of the discrete nature of the emitted wavelengths. This emission spectrum can be used as unique characteristic for qualitative identification of the element. Atomic emission using electrical arcs has been widely used in qualitative analysis.

Atomic Absorption Spectroscopy: Atomic absorption spectroscopy consists in measuring the amount of light at the resonant wavelength which is absorbed as the light passes through a cloud of atoms. As the number of atoms in the light path increases, the amount of light absorbed increases in a predictable way. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte element present can be made. The use of special light source and careful selection of wavelength allow the specific quantitative determination of individual elements in the presence of others.

Atomic Fluorescence Spectroscopy: Atomic fluorescence spectroscopy technique incorporates aspects of both atomic absorption and atomic emission. Like atomic absorption, ground state atoms created in a flame are excited by focusing a beam of light into the atomic vapour. Instead of looking at the amount of light absorbed in the process, the emission resulting from the decay of the atoms excited by the source light is measured. The intensity of this 'fluorescence' increases with increasing atom concentration, providing the basis for quantitative determination.

While this chapter covers atomic absorption and atomic emission spectrophotometers, the next chapter details out instrumentation for atomic fluorescence spectroscopy.

5.2 ATOMIC ABSORPTION SPECTROSCOPY

Atomic absorption spectroscopy is an analytical technique based on the absorption of radiant energy by atoms. It was explained in the chapter on flame photometry, that when a dispersion of the atoms of a sample is produced in a flame, some of these atoms get thermally excited and emit characteristic radiation, as they return to the ground level. Most of them, however, remain in the ground state. When a beam of light is made to pass through the flame, a portion of it will be absorbed by dispersed atoms, in the same manner that a beam of light passing through a solution will be absorbed by the dispersed molecules of a solute. It is possible to find a series of absorption bands corresponding to the energy levels of the atoms sprayed into the flame. The wavelength of the bands is characteristic of the atoms of the element concerned and the absorbance of the band is proportional to the concentration of the atoms in the flame.

The potential of atomic absorption spectroscopy for the determination of metallic elements in chemical analysis was first realised by Alan Walsh, who, during the mid-1950's developed it into its modern form. Standard commercial equipment became available by about 1960. Since that time, the use of this technique in routine analysis has become wide spread, where it replaces many traditional wet methods for the estimation of metals in solution.

The versatility of atomic absorption spectroscopy is demonstrated by the fact that it permits the estimation of between 60 and 70 elements at concentrations that range from trace to macro quantities. It is applicable to the estimations of metals in organic and mixed organic-aqueous solvents, as well as to those in aqueous solution.

In atomic absorption spectrophotometry, the absorption lines are very narrow, approximately 0.02 Å wide. This is because, being atomic bands, they do not have broadening due to rotational or vibrational structure, as is the case in molecular absorption bands. However, the lines are very weak, because there would be relatively few atoms in the light path. Also, a narrow absorption band of the atoms in the flame necessitates the use of a very narrow source line, and therefore, a continuous radiation source, like tungsten or hydrogen lamp cannot be used in atomic absorption spectrophotometers. Since a different hollow cathode lamp is required for each element to be determined, atomic absorption is practically useless for qualitative analysis. Quantitative analysis is easily performed by a measurement of the radiation absorbed by the sample. As little as 0.01–0.1 ppm of many elements can be determined.

In technique, atomic absorption spectrophotometry is quite similar to flame photometry. In principle, the two methods are complementary and therefore, in combination provide a powerful analytical tool. Whereas, the flame emission technique measures energy that is emitted by atoms, atomic absorption measures the energy that is absorbed by the atoms. Atomic absorption is able to make use of a much larger number of metal atoms in the flame that do not acquire sufficient energy to emit light. These are the atoms in the ground state.

Each element has its own unique absorption wavelengths. A *source* is chosen for the element to be tested, which emits the characteristic line spectrum of that element. The sample, normally in liquid solution for fine suspension, is sprayed into a flame. The flame vaporises the sample and puts the atoms in a condition where they can absorb energy. The atoms must be chemically uncombined and in their minimum energy or ground state. A monochromator selects a characteristic sample line and illuminates adjacent lines. A photodetector measures the light passing through the flame, both before and after introduction of the sample into the flame.

The amount of light absorbed by the atoms in the flame is proportional to the concentration of the element in the sample solution. The unknown concentration of the element in the sample may

be determined by comparing the absorbance reading on a metre with a calibrated chart showing the relationship of absorbance *versus* concentration made from data of known concentrations of the element.

5.3 ATOMIC ABSORPTION INSTRUMENTATION

Performing atomic absorption spectroscopy requires a primary light source, an atom source, a monochromator to isolate the specific wavelength of light to be measured, a detector to measure the light accurately, electronics to process the data signal and a data display or reporting system to show the results. Figure 5.1 shows a block diagram of the atomic absorption spectrophotometer.

The light source normally used is a hollow cathode lamp (HCL) or an electrodeless discharge lamp (EDL). In general, a different lamp is used for each element to be determined, although in some cases, a few elements may be combined in a multi-element lamp. A hollow cathode lamp is inserted into the AAS machine which emits a distinctive spectrum for that particular metal element. This spectrum passes through an air-acetylene flame into which a solution of the metal being tested for is sprayed using a nebuliser to obtain a fine mist. The metal ions being tested absorb some of the wavelengths passing through the flame. The light beam then passes through a monochromator which acts as a filter to separate light of the chosen wavelength from other light. In the past, photomultiplier tubes have been used as the detector. However, in most modern instruments, solid-state detectors are now used.

The atom source used must produce free analyte atoms from the sample. The source of energy for free-atom production is heat, most commonly in the form of an air/acetylene or nitrous oxide/acetylene flame. The sample is introduced as an aerosol into the flame by the sample introduction

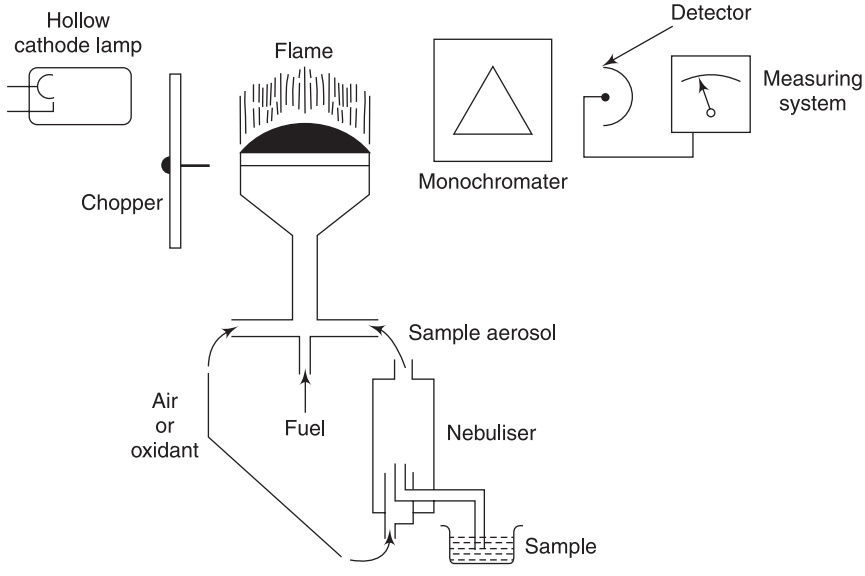


Figure 5.1 *Block Diagram of an atomic absorption spectrophotometer (Baudean et al., 1978)*

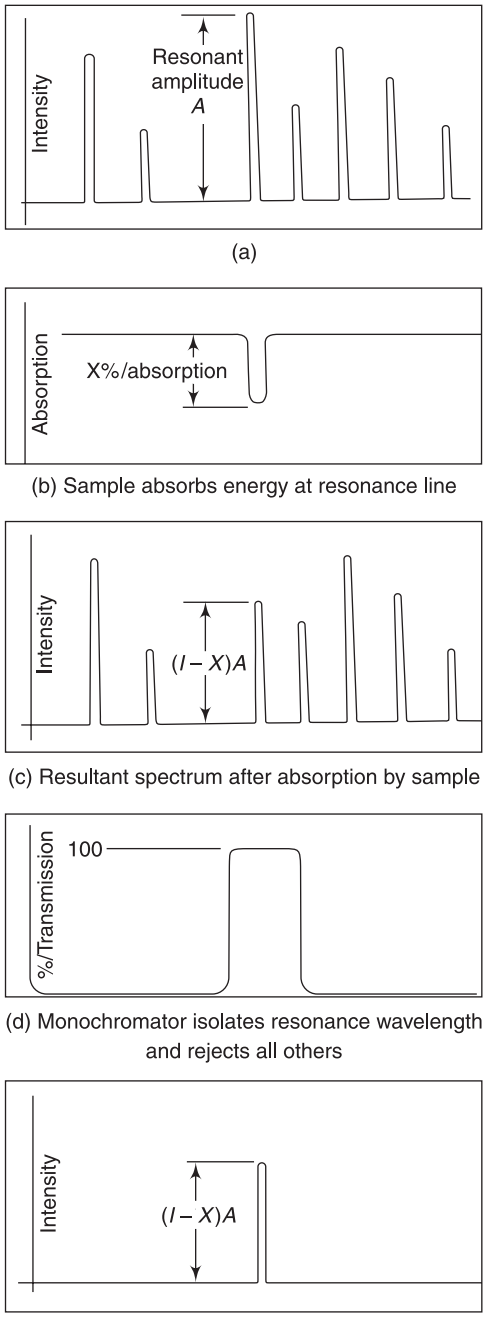


Figure 5.2 *Principle of an atomic absorption spectrophotometer*

system consisting of a nebuliser and spray chamber. The burner head is aligned so that the light beam passes through the flame, where the light is absorbed. The major limitation of Flame AA is that the burner-nebuliser system is a relatively inefficient sampling device. Only a small fraction of the sample reaches the flame, and the atomised sample passes quickly through the light path. An improved sampling device would atomise the entire sample and retain the atomised sample in the light path for an extended period of time, enhancing the sensitivity of the technique. Which leads us to the next option – electrothermal vaporisation using a graphite furnace.

The principle of an atomic absorption spectrophotometer is illustrated in Figure 5.2, in which the source of radiation is a HCL, whose cathode is made of the element to be investigated. This lamp emits the line spectrum of that element Figure 5.2a.

The sample is sprayed into the flame and the atoms of the element are dispersed in the gaseous phase. The atoms absorb the radiation only at a particular line called the resonance line (Figure 5.2b), giving the result as shown in Figure 5.2c. The resonance line gets diminished after passing through the flame containing the sample vapour, while all other lines remain unaffected. The other lines are removed by a monochromator, which selects a band of wavelengths around the resonance line and rejects all other lines (Figure 5.2d). The photodetector measures the diminished resonance line (Figure. 5.2e) and displays it on suitably calibrated scale. Atomic absorption obeys Beer's Law and calculations for concentration can be performed accordingly.

Many elements emit strongly in the flame at the same wavelength at which they absorb. This creates a serious error in measurement of the absorption. This error can be eliminated by modulating the light from the lamp, either by using an AC discharge or by using a mechanical or electronic chopper between the lamp and the flame. The detector circuit is so designed that only the modulated light is recorded and the unmodulated flame emission gets eliminated.

5.3.1 Radiation Sources

5.3.1.1 Hollow cathode lamps

The HCL is the most commonly used source of radiation for atomic absorption spectroscopy. It is a discharge lamp which emits the characteristic light of the element to be analysed.

The lamp consists of a cylindrical thick walled glass envelop, which has a transparent window of glass or silica affixed to one end (Figure 5.3). It contains one anode and a cup shaped cathode, which are both connected to tungsten wires, for taking out the electrical connections. The cathode is a hollow metal cylinder 10–20 mm in diameter, and is constructed of the metal whose spectrum is desired, or it may simply serve as a support for depositing a layer of the element. The tube is filled with a highly pure inert gas at low pressures of 1 or 2 mm. The gases generally used are helium, neon or argon. Mica sheets are placed inside the lamp to limit the radiation to within the cathode.

Choice of the window material depends upon the wavelengths of the resonance lines of the element concerned. For wavelength shorter than 250 nm, a silica window is chosen, whereas for wavelengths longer than 250 nm, either silica or ultraviolet, glass be employed.

HCLs have been constructed for almost every naturally available element. However, for atomic absorption spectroscopy, only lamps of the metallic elements are used. They usually have a long operating life, of the order of 1,000 h. The operating current requirements of a particular lamp would depend on the element concerned, and it is usually between 5 and 25 mA. The warm-up period of such type of lamps is normally between 5 and 30 min. A lamp which requires longer than 30 min may be regarded as unsatisfactory.

When a potential is applied between the electrodes, a discharge is struck and ionisation of the gas takes place. Current would flow as a result of movement of the ions to the electrodes. If the potential is sufficiently large, the gaseous cations acquire sufficient kinetic energy to dislodge some of the metal atoms from the cathode surface and produce an atomic cloud. This process is known as sputtering. Some of the sputtered metal elements in the excited state give out light characteristic of the excited atoms. If we excite an atom of element X by an electrical discharge, into a higher energy state, and if it returns to the ground state and emits a photon, this photon would have exactly the correct energy to be absorbed by another atom of X and to raise it to the same excited state.

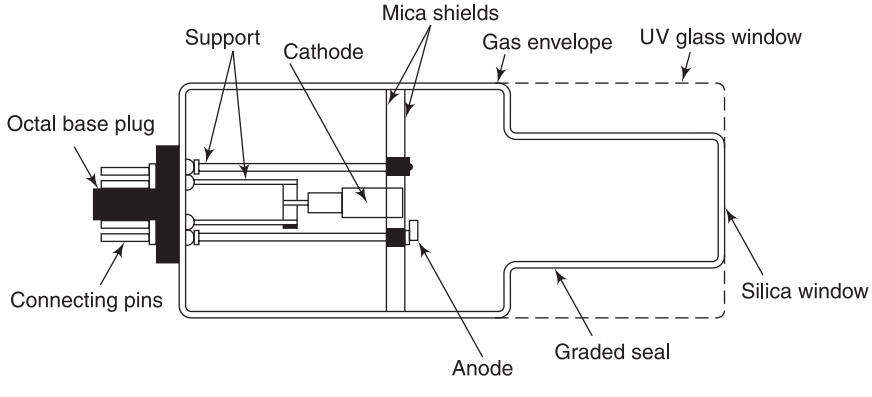


Figure 5.3 *Constructional details of a hollow cathode lamp*

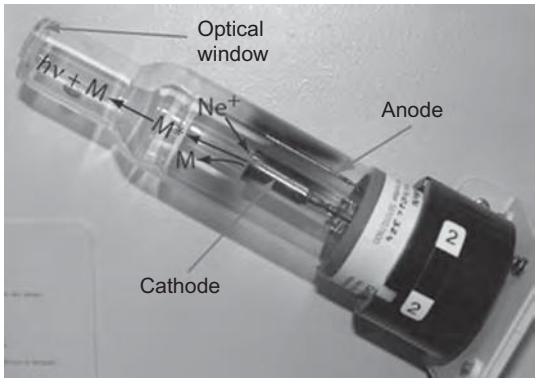


Figure 5.4 *Typical multi-elemental hollow cathode lamp*

In atomic absorption spectrophotometers, delay is encountered while changing from one element to another, due to the warm-up time for the HCLs. It may be possible to overcome this lag by warming up several lamps at once, but lamp life gets wasted. It is found possible to combine certain elements in the same hollow cathode in such a way, as to provide very nearly equal performance to single element lamps. Sintered cathodes are used for some of these lamps. Highly pure powders of the elements are mixed in the correct proportions and press-formed. These lamps are called multi-element lamps. The instruments are provided with a switching system, which puts each lamp into its approximate current range. Multi-element lamps emit very complex spectra. Therefore, only dual or triple element lamps, especially for related elements such as calcium-magnesium, sodium-potassium and copper-nickel-cobalt, are generally constructed. Figure 5.4 shows a typical multi-elemental HCL. The cathode in this lamp is fashioned from an alloy containing Co, Cr, Cu, Fe, Mn and Ni, and is surrounded by a glass shield to isolate it from the anode. The lamp is filled with Ne gas. However, the multi-elements lamps have the disadvantage that with continued operation, the resonance line intensity of one or more elements may fall off more rapidly than others.

The HCLs are operated from a regulated power supply, which provides chopped or AC power to ensure that the emission from the flame which is unchopped and, therefore results in DC signal, is ignored by the electronic system.

Several researchers have been using a *continuum source* for atomic absorption (AA) to avoid the need for element-specific light sources. An important advantage of such a technique is that, it provides the opportunity for multi-element analysis rather quickly. The instrument makes use of a high-resolution monochromator having a smaller spectral bandpass than the conventional AA instruments. Such a monochromator is *echelle* monochromator and the system provides separate detector channels for each of the elements to be determined. By modulating the wavelength at relatively high frequency over a narrow spectral range, a signal is generated that permits background measurements to be made. The chief limitation of this technique is that signal-to-noise ratio is diminished by the requirements of the continuum sources, especially if the system is to be used for very fast analytical signals. In practice, this is quite serious for the elements that are determined in the far UV, where the very bright continuum sources are not very useful (Galan, 1986).

5.3.1.2 Electrodeless discharge lamp

For most elements, the HCLs are completely satisfactory source for atomic absorption. In a few cases, however, the quality of the analysis is impaired by limitations of the HCL. These are primarily the volatile elements where low intensity and short lamp life are a problem. The atomic absorption determination of these elements can often be dramatically improved with the use of the 'electrodeless discharge lamp' (Beaty, 1978).

The EDL design is shown in Figure 5.5. A small amount of the element or salt of the element for which the source is to be used is sealed inside a quartz bulb. This bulb is placed inside a ceramic cylinder on which the antenna from a radio frequency generator is coiled. When an RF field of sufficient power is applied, the coupled energy will vaporise and excite the atoms inside the bulb into emitting their characteristic spectrum. An accessory power supply is required to operate an EDL.

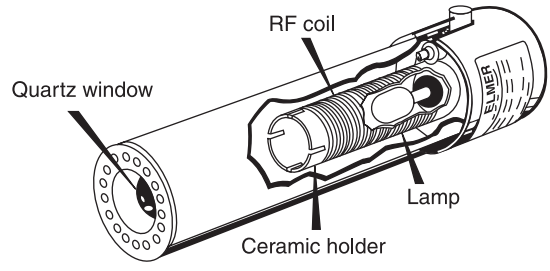


Figure 5.5 *Electrodeless discharge Lamp (Beaty, 1978)*

EDLs are typically much more intense and in a few cases more sensitive than comparable HCLs. They therefore offer the analytical advantages of better precision and lower detection limits. IN addition to providing superior performance, the useful lifetime of an EDL will exceed that of a HCL for the same element. IT should be noted, however, that the source of light in the EDL is considerably larger than that in the HCL. As a result, the performance benefits of the EDL can only be observed in instruments with optical systems designed to take advantage of these benefits.

EDLs are available for the 17 elements listed in Table 5.1. HCLs also exist for these elements. The EDLs, however, offer definite performance and lifetime benefits for instruments which are optically compatible with the EDL source.

Table 5.1 *Electrodeless discharge lamps – elements (Courtesy M/s PerkinElmer)*

Antimony	Arsenic	Bismuth
Cadmium	Caesium	Germanium
Lead	Mercury	Phosphorus
Potassium	Rubidium	Selenium
Tellurium	Thallium	Tin
Titanium	Zinc	

5.3.2 Burners and Flames

The essential requirements of a burner for atomic absorption are unique. The flame must extend over a reasonable length to improve sensitivity. This is directly analogous to the use of longer path cells in normal spectrophotometry. This follows from the fact that if the flame can be spread out into a long, narrow band, more of the atoms it contains may be brought into the light beam. In this way, absorption bands become strong and improve the sensitivity. Also, in order to avoid problems due to scattering of light by unburned droplets and large sample droplets, which cannot be burned, they must be removed before reaching the flame. In addition to this, the burner must be able to take up solutions with a high concentration of dissolved solids, limit clogging problems and reduce requirements for sample dilution.

The burners employed for the flame emission instruments are of the total consumption type. In these burners, the sample solution, the fuel and the oxidising gas are carried through separate

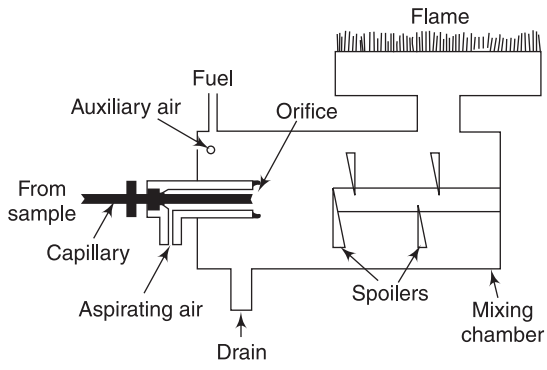


Figure 5.6 *Constructional details of a premix burner*

passages and meet at an opening at the base of the flame. However, for atomic absorption studies, a premix type of burner (Figure 5.6) is mostly preferred. In this type of burner, the sample is aspirated through a thin capillary tube by the air flowing into the atomiser section. The air-sample mixture emerges from the atomiser as a fine spray of droplets, which is then mixed with the fuel, usually acetylene. The mixture is rendered turbulent by the flow spoilers and is then forced up into the burner head. Larger drops of the sample collect at the bottom of the chamber and are drained off.

In another design, gas mixing and large drop removal is achieved by passing the aerosol through a constriction, which functions as a turbulent jet. This geometry lead to improved detection limits in atomic absorption spectroscopy. Because of long path available, a slot burner is used in atomic absorption spectrometry. The slot dimensions are dependent upon the fuel and oxidant gases. Usually, a 10 cm slot is used for air-acetylene flame and a 5 cm slot for a nitrous oxide-acetylene flame. The port is usually 0.4–0.6 mm in width. Very thin flames arc used, so as to obtain high analyte concentrations in the flame for longer slot. The burner head is fabricated from aluminium, stainless steel or titanium.

Another burner system, denoted as Autolam-burner consists of a nebuliser, spray chamber and burner head. It differs from the pre-mix burners in that the nebuliser and burner head are 180° out of the alignment; the analyte droplets must complete a semicircle from the inlet to the flame. The design enables uniform mist to reach the flame and prevents clogging of the slot.

The desirability of avoiding flashback has led to the development of capillary burners. They are more stable and for a number of elements the emission signal is greater than that from a conventional burner. With a suitable adaptor, they can be made to fit any premix-burner assembly.

The mechanism of atom production is quite complex. Apparently, it is not the same for all solvents and flames. One of the important factors that need consideration is the possible formation of oxides. Many metal oxides are very stable and once formed are very difficult to reduce, even in a high temperature flame. Therefore, the reducing nature of the flame (amount of fuel to oxidant) assumes critical importance.

For flame atomic absorption spectrophotometers, the design of nebulisers and spray chambers appears to have been empirically optimised to provide the best aerosol drop size in the flame for interference-free analyte vaporisation. No surprise that nebulisers and spray chambers have undergone a steady progression during the past 20 years. However, pneumatic nebulisation approach is used in the vast majority of atomic spectroscopy determinations in case of liquid samples.

The main practical requirements (Browner and Boorrn, 1984) for pneumatic nebulisers are the following:

- A high velocity gas stream
- A reasonable pressure drop of the liquid injection capillary for Venturi effect natural aspiration

- Maximum interaction between the gas and liquid streams for fine aerosol production
- Freedom from blockage resulting from either particles suspended in the solution or from salt build-up at the nebuliser top.

All these requirements mean that the construction of pneumatic nebulisers is a demanding engineering challenge, as tolerances must be very precisely kept on annular spaces. These spaces may be as small as 10–20 μm for ICP nebulisers, compared to 150–250 μm for flame atomic absorption nebulisers.

A substantial control over the gas-liquid interaction can be obtained by varying the position of liquid uptake tube in a conically or parabolically converging gas tube (Figure 5.7). This need is avoided in cross-flow nebulisers (Figure 5.8). However, it requires very precise and rigid positioning of the gas-liquid tubes. Cross-flow nebulisers are much less fragile than concentric nebulisers, which are usually made of glass.

The major limitation of air pneumatic nebulisers of conventional design is that, they produce aerosols with wide drop range. This means that high transport efficiency can be achieved only at the expense of allowing large drops to reach the atomiser. On device that produces a much finer aerosol is the ultrasonic nebuliser. In this device, up to 30% efficient production of droplets in the size range from 1.5 to 2.5 μm has been found at a 0.3 ml/min solution flow rate.

Figure 5.9 shows the constructional details of ultrasonic nebuliser. With this device, the principle of aerosol production is significantly different from pneumatic nebulisation. In the ultrasonic nebuliser, instead of drops being stripped from a liquid cylinder by a high velocity gas jet, surface instability is generated in a pool of liquid by an ultrasound beam, which is generated by a piezoelectric transducer.

Ultrasonic nebulisers produce aerosols with mean drop diameters that appear to be a function of exciting frequency. At frequencies below 50 kHz, cavitation is the main mode of

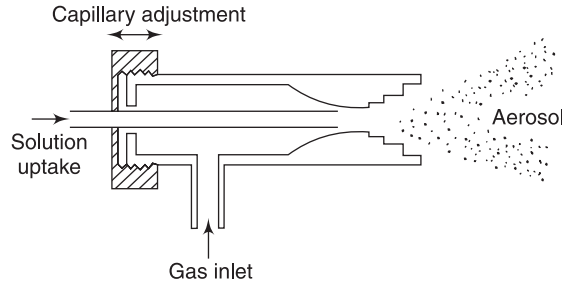


Figure 5.7 *Adjustable concentric nebuliser (re-drawn after Browner and Boorn, 1984)*

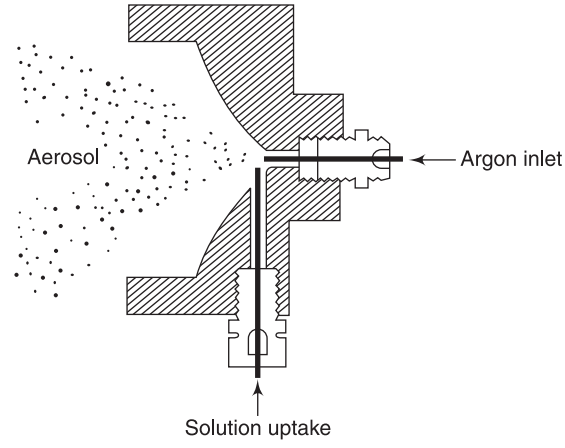


Figure 5.8 *Cross-flow nebuliser*

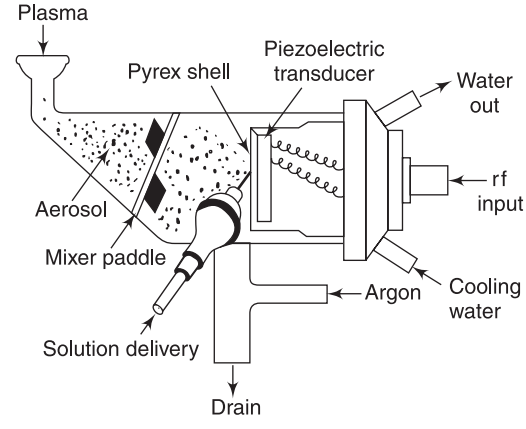


Figure 5.9 *Ultrasonic nebuliser (re-drawn after Browner and Boorn, 1984)*

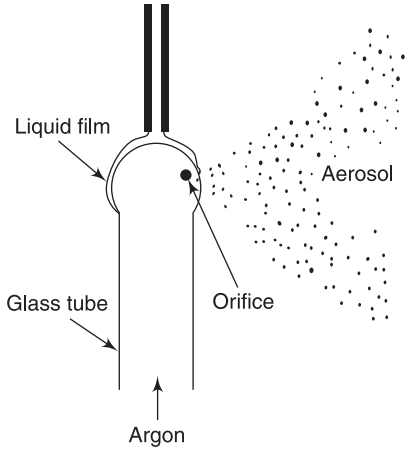


Figure 5.10 *Babington nebuliser*

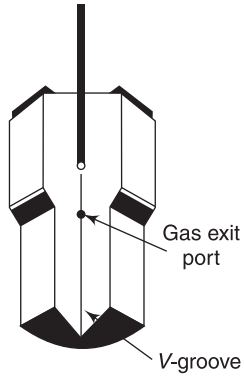


Figure 5.11 *V-groove nebuliser (re-drawn after Browner and Boorn, 1984)*

droplet production. At the high frequencies commonly used in modern ultrasonic nebulisers, typically 1 MHz or greater, the mechanism of aerosol production changes from cavitation to geyser formation. With the geyser formation mechanism, it is the power density and not the operating frequency which determines the drop size.

Although ultrasonic nebulisers have advantage of producing small-sized droplets, many unanswered questions remain about the general reliability and freedom from interference of these devices.

The problems of nebuliser blockage inherent in pneumatic nebulisers are effectively overcome with Babington type pneumatic nebuliser. The principle of this device is that of concept used for paint spraying. It involves a spherical surface with an array of holes around a circumference. The gas supply comes from within the sphere, and as the liquid flows over the outside of the sphere and passed over the gas stream, it is nebulised (Figure 5.10). Babington type nebulisers are ideal when solutions with suspended particles must be analysed and when prior acid or other dissolution to dissolve the particles is not convenient. These nebulisers can be made either entirely of glass, metal or Teflon, or by embedding a sapphire orifice in a Teflon block.

Another design of a nebuliser is the *V-groove* type (Figure 5.11) in which a liquid stream is passed down a V-groove, with a small hole drilled in its centre for the gas stream. This type of nebuliser is quite popular and is available commercially.

Nebulisers and spray chambers operate interactively. They must be optimised as a unit rather than individually. However the spray chamber must meet the following specific requirements:

- Effective removal of aerosol droplets larger than the cut-off diameter. This is necessary for interference-free movement.
- Rapid wash-out characteristics, both to increase the possible rate of analysis and to avoid cross-contamination problems.
- Smooth drainage of waste aerosol from the chamber, to avoid pressure pulses in the atomiser.

Since the thermal energy from the flame is responsible for producing the absorbing species, flame temperature is an important parameter in atomic absorption spectrometry. The temperatures for some flames used in atomic absorption are given in Table 5.2.

It may be noted that cooler flames are subject to more interference problems resulting from insufficient energy for complete atomisation. While the air-acetylene flame is satisfactory for the majority of elements determined by atomic absorption, the hotter nitrous oxide-acetylene flame is required for many refractory-forming elements. Moreover, nitrous oxide-acetylene flame is

Table 5.2 *Temperature of premix flames*

Gas	Temperature
Air-Methane	1875
Air-Hydrogen	2000
Air-Acetylene	2300
N_2O-Acetylene	2955
Air-Propane	1925

Table 5.3 *Elements which may be determined by atomic absorption spectroscopy with different flames*

Air/propane or Air/acetylene	Air/acetylene	Air/acetylene or N_2O/acetylme	N_2O/acetylene
Arsenic	Antimony	Barium	Aluminium
Bismuth	Chromium	Calcium	Beryllium
Cadmium	Cobalt	Molybdenum	Germanium
Copper	Iron	Strontium	Silicon
Gold	Lithium	Tin	Tantalum
Lead	Magnesium		
Manganese	Nickel		Titanium
Mercury	Platinum		Tungsten
Potassium			
Silver			
Sodium			
Zinc			Zirconium

effective in interference control in other situations. Table 5.3 gives a list of elements which may be determined by atomic absorption spectroscopy with different flames.

If a high standard of analytical performance is to be expected from the instrument, the burner must be kept in a clean condition. The burner head can usually be disassembled for cleaning purposes. Inorganic deposits on the surface of the burners can be removed by soaking it in water. Layers of organic material may be removed with the help of a fine emery paper. Soaking in acid solutions is generally not recommended. The burner should be cleaned after every day of operation.

5.3.3 Graphite Furnace for Atomisation

In recent years, there has also been an increasing interest in the use of non-flame techniques, like electrothermal and cold vapour atom formation devices in the recent years. They primarily include carbon filament and furnaces, metal filament atomisers, cold vapour devices, etc.

The furnace AA system is probably the most sensitive analytical technique for the determination of trace metals. In this method, a very small sample is taken and converted completely to an

atomic vapour. The subsequent integration of the absorbance pulse yields useful signal about the element of interest. The instrument manufacturers have modified the earlier designs to carry out the furnace work due to the usefulness of the graphite furnace.

There are different methods by which the sample can be placed in the furnace (Slavin, 1982). The simplest technique is the one in which the sample is applied on the wall of the furnace. When the wall reaches a temperature at which the analyte will vaporise, the metal is driven from the surface into the gas phase. This temperature will vary depending upon the matrix constituents. Also, the rate at which the metal comes off will depend upon the quantity and the specific nature of the matrix constituents.

An improved technique is to add a graphite platform (plate) at the bottom of the graphite tube, on which the sample is deposited. Here, the graphite plate is heated by radiation from the walls, so that the temperature of the sample on the plate is delayed relative to the wall of the tube, and therefore to the gaseous vapour within the tube. In this situation, the atomic vapour is generated at a constant temperature and the absorbance signal is directly proportional to the number of atoms present in the sample and independent of the rate at which the atoms are generated.

In order to operate stabilised temperature platform furnace, the tube must be heated very rapidly to ensure that the platform will have lagged the heating cycle sufficiently for the tube and the gas to come to steady-state temperature, prior to the evolution of the analyte of interest. A heating rate in excess of 1,000°C seems necessary to achieve the necessary degree of interference freedom. Also, the electronic system to record the integrated absorbance signal must be very fast, since these peaks are generated quite rapidly and appear quite rapidly. The older analog electronic circuits developed for flame AA, which have relatively long time constants, will yield analytical errors when used for the graphite furnace.

Another variation of AA furnace is called a *probe furnace*, which involves putting the sample on a surface outside the furnace. The sample is dried and charred just outside the furnace, thus avoiding contamination of the analytical furnace itself, by the char products. The furnace is then brought to the desired atomisation temperature. When that temperature has been achieved, the probe with the charred sample on it is inserted rapidly into the furnace. The technique is far from practical due to some difficult engineering problems, but the physical considerations are correct. Figure 5.12 shows a cross-sectional view of the graphite furnace.

The graphite tube, which is aligned in the spectrophotometer optical path, is an open-ended cylinder of graphite with a small hole in the centre through which the sample is introduced. The arrangement is shown in Figure 5.13. The tube is held between two large graphite rings, which provide electrical contact. The graphite tube acts as a resistor in an electrical circuit. When a voltage is applied to the tube, current flow will cause an increase in temperature of the tube. By controlling the amount of current flow, the temperature of the tube can be adjusted to any desired level up to a maximum of about 3,000°C. The graphite system is held in place

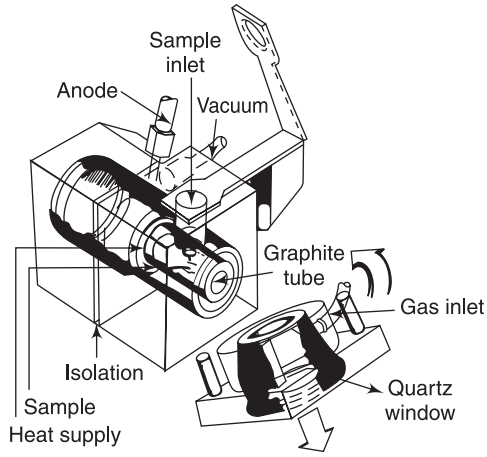


Figure 5.12 *Cross-sectional view of the graphite furnace*

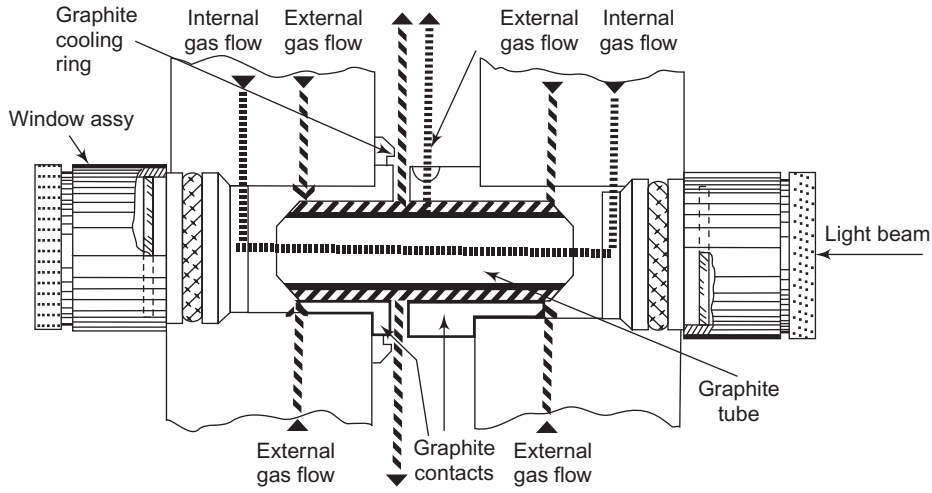


Figure 5.13 *Longitudinally heated graphite furnace atomiser (After Beaty and Kerber, 1993)*

by a water-cooled housing. By circulating water through the housing, the outside temperature of the furnace is kept at a safe level, and after atomisation the graphite tube is rapidly cooled to room temperature where it is ready to accept the next sample.

In order to prevent tube destruction at high temperatures from air oxidation, an inert gas purge of the graphite tube is provided. An external purge around the outside of the tube shields the tube from the oxidising atmosphere. A separately controllable internal gas is extremely valuable in controlling matrix effects from major constituents of the sample. The internal gas is introduced directly into the inside of the tube from the open ends and escapes out the centre hole of the tube. The internal gas flows only during furnace operation and interrupts after the completion of each measurement to allow the next sample to be introduced.

The graphite tube may be constructed entirely of high density graphite or it may be covered with a relatively thin coating of pyrolytic graphite. The sensitivity for some elements is enhanced with the pyrolytic graphite tube. The refractory-forming elements (elements which form compounds of a very high thermal stability) usually benefit most from pyrolytic graphite. Figure 5.14 shows Varian Model Spectra 10/20 atomic absorption spectrophotometer with a graphite furnace attachment for flameless analysis.

Figure 5.14 *Atomic Absorption Spectrophotometer Varian Spectra 10/20 for analysis of metals in the ppm range with flame, and ppb range with graphite furnace (Courtesy: M/s Varian)*

The prospect of release from the tedious and time-consuming sample dissolution step

used in an analysis has prompted further studies into the possibility of the direct introduction of solid samples. The fundamental advantages of vapour introduction, compared to liquid sample introduction are as follows:

- It allows pre-concentration of the sample from a relatively large volume of solution, into a relatively small volume of vapour. Therefore, sample transport can be achieved with an efficiency approaching 100%, compared to the 0–10% typical of liquid sample introduction.
- The greater transport efficiency gives adequate detection limits, which can be critically important for several elements like arsenic, selenium and tellurium.

The disadvantage of electrothermal atomisers is that sample inhomogneity and dispensing problems account for poor precision (Faithfull, 1983).

5.3.4 Optical System

The optical system of an atomic absorption spectrophotometer is typically as shown in Figure 5.15. The system employs a photometer which is of the single-beam type. The source lamp is a HCL, which is AC-operated and produces a modulated light beam. The light beam is passed through a limiting stop and focused at the mid-point of a slot burner. After travelling through the flame, the beam is re-focused on the entrance slit of the Littrow grating monochromator. The slit width is kept continuously variable and may be controlled by a cam and cam-follower mechanism.

Littrow mounting uses only one piece of quartz or silica with the back surface of the prism metallised. Since the light passes back and forth through the same prism and lens, polarisation effects are eliminated. This type of monochromator provides adequate resolution to separate the resonance line in the lamp from the other lines. Also with its high dispersion, it is possible to open the slits wide and admit high energy. Conventional prism systems do not meet these specifications. Figure 5.16 shows the optical diagram using a silica prism as a monochromator.

A Littrow grating monochromator with a dispersion of 16 Å/mm provides a bandpass as low as 2 nm at the standard slit width of 1 mm. Instruments using grating monochromators not only provide good uniform dispersion over the whole spectrum, but also high resolving power. They have a superior dispersion to a prism instrument at high wavelengths, and are at least as good at low wavelengths.

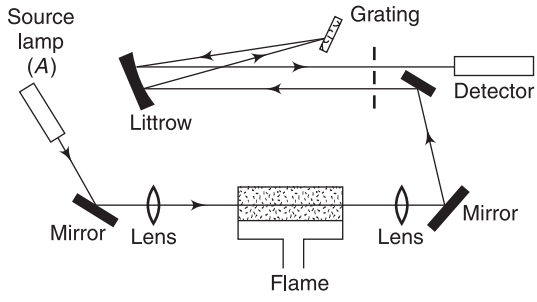


Figure 5.15 *Optical system of an atomic absorption spectrophotometer*

For atomic absorption determination of most of the common metals, a very moderate monochromator is quite suitable. For determinations of metals for which the lamps possess poor output and complex spectra, a better monochromator is necessary. Floyd et al. (1980) illustrate a computer-controlled scanning monochromator system which can be combined with an inductively coupled plasma excitation source.

The radiation emerging from the exit slit falls on the cathode of a photomultiplier, sensitive over the range 210–800 nm. The lenses used in the system are of fused silica, whereas the mirrors are coated with MgF_2 to preserve reflectivity in the UV region.

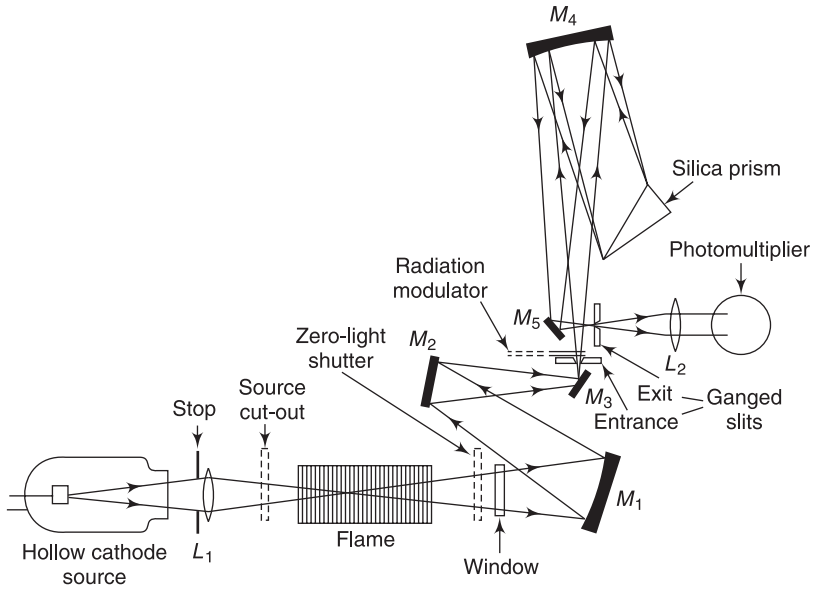


Figure 5.16 *Optical diagram of the SP 90 Pye Unicam atomic absorption spectrophotometer*

5.3.4.1 Double-beam atomic absorption spectrophotometer

In this design, the beam from the HCL is split through a mirrored chopper, one half passing by the atomiser and the other half around it, as schematically displayed in Figure 5.17. The two beams are then recombined through a half-silvered mirror and passed through the monochromator. The

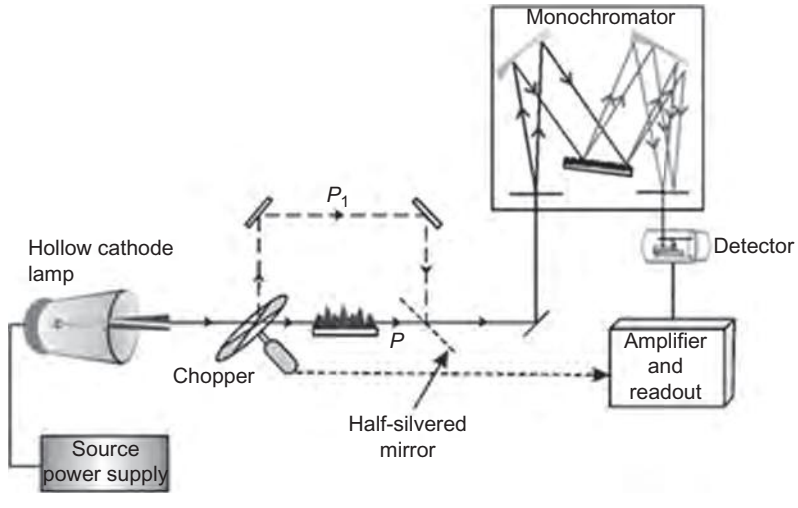


Figure 5.17 *Schematic diagram of a double-beam atomic absorption spectrophotometer (Courtesy: M/s ExpertsMind.com)*

ratio among the reference and sample signal is then amplified and fed to the readout device and recorder. These instruments correct the fluctuations within the intensity of radiation coming from the radiation source and for changes within the sensitivity of the detector. It must be remembered that reference beam within double-beam instruments does not pass by the flame and thus corrects for the loss of radiant power because of absorption or scattering through the flame itself. Changes in peak shape or position could be indicative of interference problems. Most instruments are equipped with a device for background correction.

5.3.5 Electronic System

The emission system not only produces light of a particular wavelength, originating from the lamp, falling upon the photodetector, but also light of the same wavelength arising from the flame. It is necessary to distinguish between these two sources of radiation, since it is the measurement of that from the lamp only, which is required. This requirement is attained by modulating the light from the lamp, with either a mechanical chopper or by using an AC power supply and tuning the electronics of the detector to this particular frequency. With a DC system, it would become very difficult to determine calcium in the presence of sodium, because the presence of sodium or potassium in a flame contributes a variable amount of continuous background radiation, which adds to the measured intensity of all spectral lines. Sodium also interferes with magnesium when the DC system is employed, because there is a sodium emission line at 285.2 nm, only 0.07 nm away from the magnesium line at 285.28 nm. On the other hand, the AC system is entirely free from spectral interference and the DC signals from the flame and other extraneous sources are eliminated.

The main advantage of electronic modulation over mechanical chopper is that no moving parts are involved. However, in a double-beam system, in which the light from the measuring and reference beams is passed on to the same photomultiplier, the use of a mechanical chopper becomes essential.

The random background noise that originates from the flame is generally noticeable at low frequencies. Also the high frequency photomultiplier noise increases above at about 1,000 Hz. The optimum modulation frequency of the chopper is therefore, selected around 350–400 Hz.

The most innovative method for background correction developed is the one involving the Zeeman effect. In this method, a magnetic field is applied to either source or generated atoms to split the Zeeman components of each spectral line. Since these components are polarised, it becomes possible to isolate them using stationary or rotating optical polarisers. This method, known as Zeeman modulation, can thus be employed for high precision background correction. Accurate instrumental subtraction of flame background can also be accomplished by directly measuring the background in a reference flame and subtracting it from the signal produced by an analytical flame. This dual flame approach has been employed in some systems.

The Zeeman background correction technique has been incorporated into several new AA spectrophotometers which utilise, in addition, a considerably improved form of the graphite furnace atomiser. The simple insertion of a small platform into the graphite tube furnace, rather than using a cup built into the furnace wall, ensures volatilisation and atomisation under nearly constant temperature conditions, giving a far more reliable and reproducible transient.

Another form of background correction is the Hieftje Method, in which HCL depends on the so-called self-reversal of HCL emission at high current. Operation of an HCL at high current not

only broadens the atomic emission line (because of the higher operating temperature), but because of the high concentration of unexcited atoms produced, leads to absorption within the HCL so that an apparent doublet, centred about the true emission line, is emitted.

Thus, by measuring absorption when the lamp is operating at low current and then repeating the operation while the lamp is briefly operated at high current, the true atomic absorption will be given by the difference between the two. The absorbance under the high current conditions is, of course, assumed to be due to a broad molecular band and could be an overestimate of the background if this is not so.

Figure 5.18 shows a block diagram of the electronic system of an atomic absorption spectrophotometer.

The low-level signal developed across a PMT anode resistor is either chopped by mechanical interruption of the light beam directed on to the PMT or is electronically chopped by a transistor switch. In most of the spectrophotometers, where transistor switch is used, it is operated by the waveform supplied from the synchronising circuits to ensure that the chopped amplified signals are correctly phased before being applied to the synchronised decoder circuits. In either mode of operation, the signal is usually chopped at the frequency used in the synchronising system, say 50 Hz, and is amplified in a low-noise amplifier. To provide high input impedance, the input stage contains a bipolar transistor. The preamplifier circuit also contains signal limiting diodes, circuit for eliminating the spikes produced by electronic chopping and suitable filters in the power supply line.

The circuit shown in Figure 5.19 has a gain of approximately 4. The signal applied via C_1 to the gate of field effect transistor T_1 originates at the anode of the photomultiplier, R_1 being the anode resistor. The zener diode protects the gate emitter junction of T_1 by limiting the signal amplitude. The stage gain of the amplifying stage is linearised by DC feedback from the junction of R_3 and R_4 to T_1 source. Capacitor C_2 provides AC feedback between the collector and base of T_2. R_5 and C_3 act as filter in the power supply line.

The EHT oscillator is a high frequency oscillator, operating in the range of 15–20 kHz. It is an a stable multi-vibrator, whose output is connected to the primary winding of the EHT transformer, through an output stage comprising a Darlington stage.

The EHT requirements for PMT are of the order of 2 kV at a maximum current of 200 μA. The transformer's primary windings may be bifilar wound to reduce losses and are connected in series to permit a push-pull signal. The secondary winding is connected to four EHT rectifying diodes

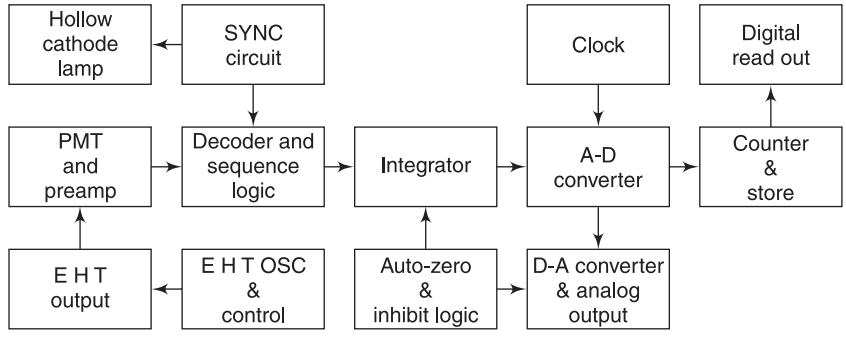


Figure 5.18 *Block diagram of the electronic circuit of an atomic absorption spectrophotometer*

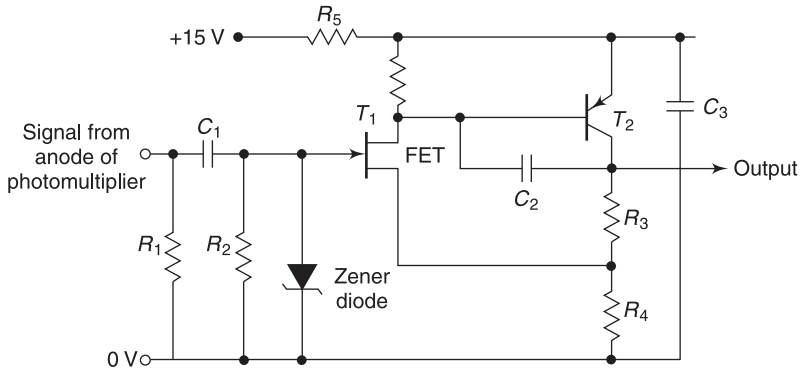


Figure 5.19 *Preamplifier circuit used with photomultiplier detector*

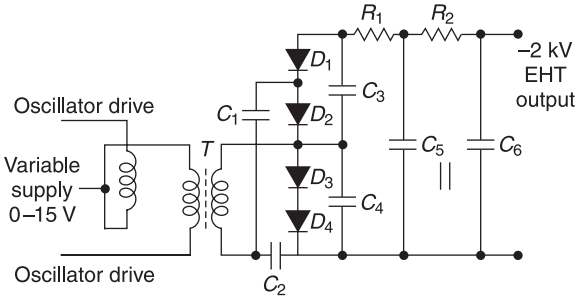


Figure 5.20 *EHT supply circuit for a photomultiplier*

(Figure 5.20) arranged in quadrupler rectifier circuit, which is followed by smoothing filters. In most of the spectrophotometers, the positive EHT line is earthed at the photomultiplier preamplifier and the polarity of the output is, therefore, negative.

The main synchronising circuit derives its input from the mains supply sine wave signal, or 50 Hz frequency. The signal is squared in Schmidt trigger circuit, to get lamp modulation waveform at 50 Hz pulse frequency. The same circuit also provides for sample decoder drive at 50 Hz and reference decoder voltage, which are connected to PMT signal processing circuit in decoder and sequence logic circuit

The amplified signal from the PMT preamplifier is given to the decoder circuit, where it is amplified, encoded and given to the integrator. The sequence logic circuit controls various timings of the cycle. The operation can be manual or it could be remote controlled.

The integrator provides DC voltage corresponding to signal and reference signals. The integrator could be conventional op-amplifier connected in integrator configuration, having a capacitor in the feedback circuit. Auto-zero potentiometer is connected in feedback of the amplifier following the integrator.

Basically, the analog-to-digital converter (ADC) employs single ramp principle. It comprises of a comparator circuit, which evaluates the difference between the reference and sample signals. It produces a pulse of width proportional to the signal difference. The conversion is carried out according to linear or logarithmic law, the latter is required in the absorbance mode.

The comparator is fed with a ramp generator and a switch circuit. The ramp waveform is determined by the voltage charge on the ramp capacitor. When a linear ramp is required, the ramp voltage decreases at a constant rate, the capacitor getting discharged at a constant current.

A logarithmic ramp is produced when the capacitor discharges towards a pre-set voltage and the discharge current is passed via a resister.

The output of the comparator is given to a sign circuit of logic gates, which produces two output signals in anti-phase. The output signals indicate the polarity of the signal differences evaluated in the comparator.

An additional circuit provides for timing pulses like 10-Hz reset pulse output, a 5-Hz set zero pulse output and a 5-Hz transfer data pulse output. These pulses are obtained by dividing the mains frequency in a divider.

The basic clock frequency for the ADC is obtained from an astable multi-vibrator. The clock pulses are responsible for causing operation in digital display. The clock frequency can be varied for scale expansion. The logic circuit provides gating of the clock output with the comparator output pulse. The resulting bursts of oscillator pulses provide the digital equivalent to the voltage difference of the DC analog signals.

The bursts of clock pulses are counted in decade counters and given to BCD to seven-segment decoder/drivers, which contain the decoding logic and drive transistors for each of the seven segments of each numeral of the display.

Modern instruments are computer-controlled in which one significant development is the use of a multi-lamp turrets controlled by the same computer which tunes the desired wavelength. This makes the technique multi-element in nature. The computer also controls the flame composition and burner height. The instruments are also self-optimising with the gases controlled via a binary flow system with feedback of absorbance signal to automatically set the optimum fuel flow (Ebdon, 1984).

5.3.6 Sampling System

A Sampling system is used to supply a constant feed-rate of sample into the burner. The most common method employed makes use of solution which is introduced into the flame by an atomiser. The design and construction of the atomiser are similar to that used in emission spectroscopy. A complete sampling system comprises of a nebuliser and a spray chamber is shown in Figure 5.21.

The nebuliser reduces the sample solution to a spray of droplets of various diameters by a pneumatic action. The spray is directed into the spray chamber, where the larger droplets are precipitated and the air stream carries the remaining spray into the burner and thence to the flame. Normally, sample uptake rate of the system is 3–4 ml/min. It has been experimentally established that an improvement in analytical sensitivity, when using non-aqueous solvents, occurs by employing a more efficient nebulisation of the sample solution. Organic solvents are often used in place of water, when the sample may be an organic liquid or a solid, soluble only in organic solvents. The choice of a suitable organic solvent is very important, because the solvent can affect the physical properties of the flame and may influence the decision, and sensitivity of the analysis.

The sampling system may be coupled to an automatic sample changer, which may have a number of sample holding polythene cups. Each cup passes the sampling point in turn, and contents are sampled by an automatic head.

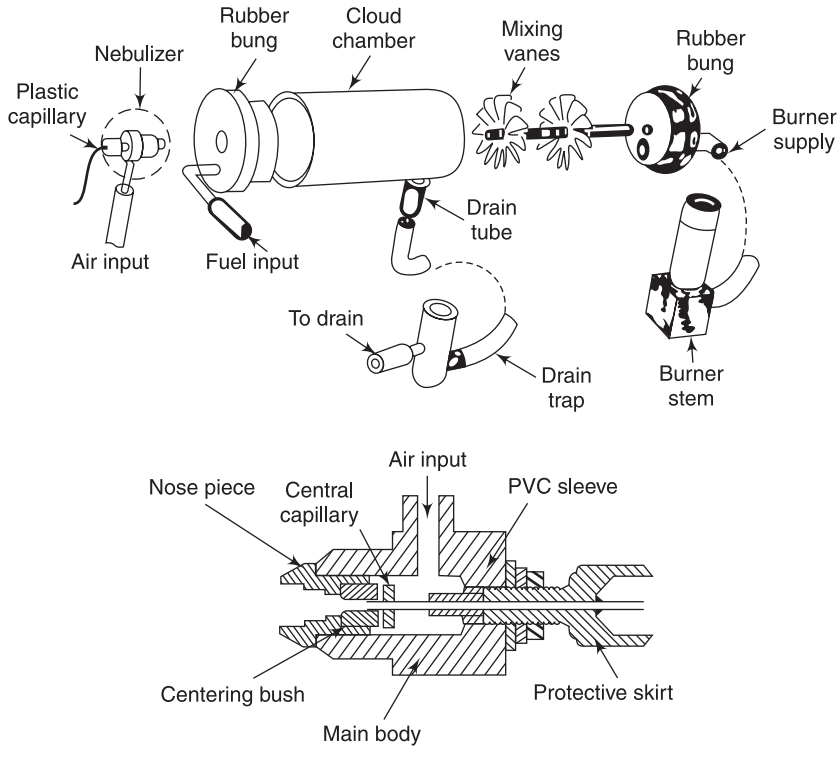


Figure 5.21 *Nebuliser and spray chamber used in atomic absorption spectrophotometric measurements*

One way to reduce wash-out time significantly is to use flow-injection technique. The pump used is peristaltic and the system injects the sample in pulsed-form, similar to the one used in liquid chromatography. This technique offers the following advantages:

- Only a relatively small volume of sample is necessary to achieve a signal comparable to continuous nebulisation.
- Because of the transient nature of the signal, exponential decay in the wash-out process starts much sooner than with continuous sample introduction. Consequently, the signal decays to baseline more quickly than with continuous sample introduction. It is, therefore, possible to inject samples at the rate of approximately 4/min, as against 1. 5/min, with conventional sample introduction.

The sample introduction procedure selection depends upon the following:

- Type of sample; liquid, solid or gas
- Levels and also the range of levels for the elements to be determined
- Accuracy required
- Precision required
- Amount of material available
- Number of determinations required per hour

In addition, all the measurement techniques available, regardless of whether they are flame atomic absorption spectroscopy, inductively coupled plasma-atomic emission spectroscopy or DC plasma atomic emission spectroscopy, have a major effect on the choice of the procedure selected.

5.4 ATOMIC EMISSION SPECTROSCOPY

Atomic emission spectroscopy (AES) is a method of chemical analysis that uses the intensity of light emitted from a flame, plasma, arc or spark at a particular wavelength to determine the quantity of an element in a sample. The wavelength of the atomic spectral line gives the identity of the element, while the intensity of the emitted light is proportional to the number of atoms of the element.

Historically, emission techniques have centred around flame excitation for easily excited elements (flame photometry) and electric arc and spark excitation when higher energies are required. In the last decade, instruments using a high temperature gas plasma (Ar) emission have become common-place. Flame excitation is limited in scope due to the fact that the excitation conditions vary widely from element to element and the energy in a flame is low. Arc and spark (and now plasma) excitation sources are much higher energy allowing the breakdown of refractory materials into the component atoms and good emission spectra for many elements. Under the same set of excitation conditions this allows for a simultaneous determination of multiple analytes.

Methods based on plasma sources normally have linear calibration ranges over a much wider range than atomic absorption technique, requiring less need for dilution or concentration. Because of the higher energy sources, number of lines in an emission spectrum is very large; higher resolution is a necessity for quantitative analysis (i.e. high quality optics and monochromators). Plasma instruments have the advantage of speed but are not likely to totally replace flame atomic absorption instruments with their lower requirements – initial purchase, operating costs, operator skill – for very similar sensitivities.

AES is ideally suited for multi-elemental analysis because all analytes in a sample are excited simultaneously. If the instrument includes a scanning monochromator, it can be programmed to move rapidly to an analyte's desired wavelength, pause to record its emission intensity and then move to the next analyte's wavelength. This sequential analysis allows for a sampling rate of 3–4 analytes per minute.

Another approach to a multi-elemental analysis is to use a multi-channel instrument that allows us to simultaneously monitor many analytes. A simple design for a multi-channel spectrometer couples a monochromator, with multiple detectors that can be positioned in a semicircular array around the monochromator at positions corresponding to the wavelengths for the analytes.

5.5 ATOMIC EMISSION SPECTROPHOTOMETER

An atomic emission spectrometer is similar in design to the instrumentation for atomic absorption. In fact, it is easy to adapt most flame atomic absorption spectrometers for atomic emission by turning off the HCL and monitoring the difference in the emission intensity when aspirating the sample and when aspirating a blank. Many atomic emission spectrometers, however, are dedicated instruments designed to take advantage of features unique to atomic emission, including the use of plasmas, arcs, sparks, and lasers as atomisation and excitation sources, and an enhanced capability for multi-elemental analysis.

Atomic emission requires a means for converting a solid, liquid or solution analyte into a free gaseous atom. The same source of thermal energy usually serves as the excitation source. The most common methods are flames and plasmas, both of which are useful for liquid or solution samples. Solid samples may be analysed by dissolving in a solvent and using a flame or plasma atomiser.

Atomisation and excitation in flame atomic emission is accomplished using the same nebulisation and spray chamber assembly used in atomic absorption. The burner head consists of single or multiple slots, or a Meker-style burner. Older atomic emission instruments often used a total consumption burner in which the sample is drawn through a capillary tube and injected directly into the flame.

In the inductively coupled plasma-atomic emission spectrophotometer (ICP-AES), the sample is exposed to the extremely high temperature of an argon plasma (up to 10,000 K) that breaks the sample into atoms, ionises these atoms and electronically excites the resulting ions. When the excited electrons in these ions fall back to lower energy levels, they emit light. The wavelengths of light emitted by a particular element serve as a 'fingerprint' for that element. Therefore by measuring the wavelengths of light emitted by our sample, we can identify the elements in the sample; and by measuring the amount of light emitted by a particular element in the sample, we can determine the concentration of that element.

Figure 5.22 shows the basic design of the ICP-AES instrument. The sample solution is pumped by a peristaltic pump into the nebuliser where it is broken into an aerosol of fine droplets by a fast stream of argon gas. From the nebuliser it passes through the spray chamber (which eliminates the larger droplets)

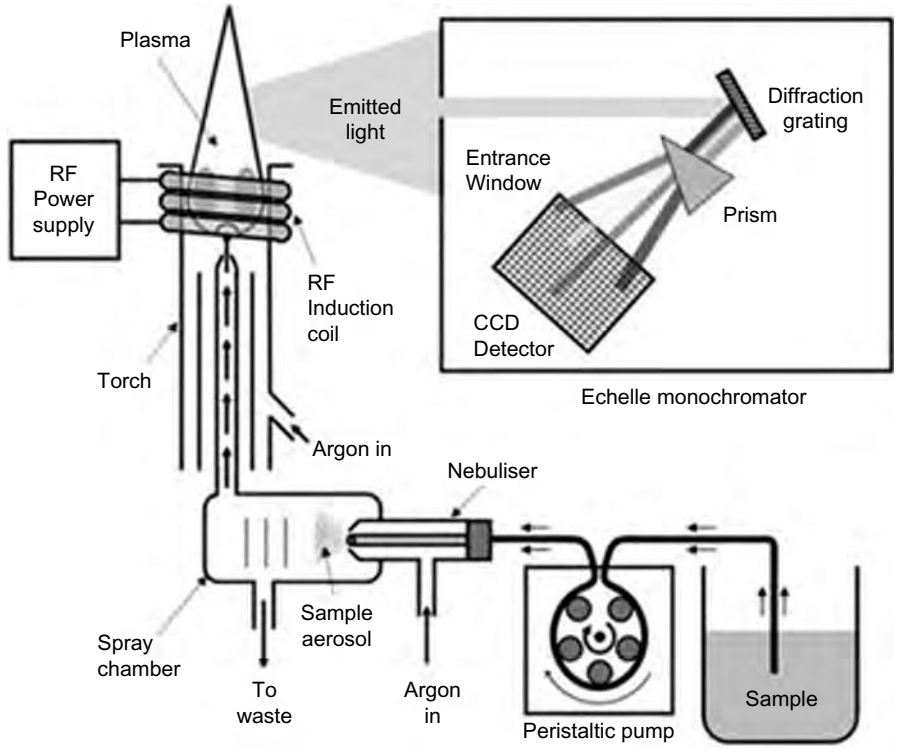


Figure 5.22 *Block diagram of ICP-AES instrument*

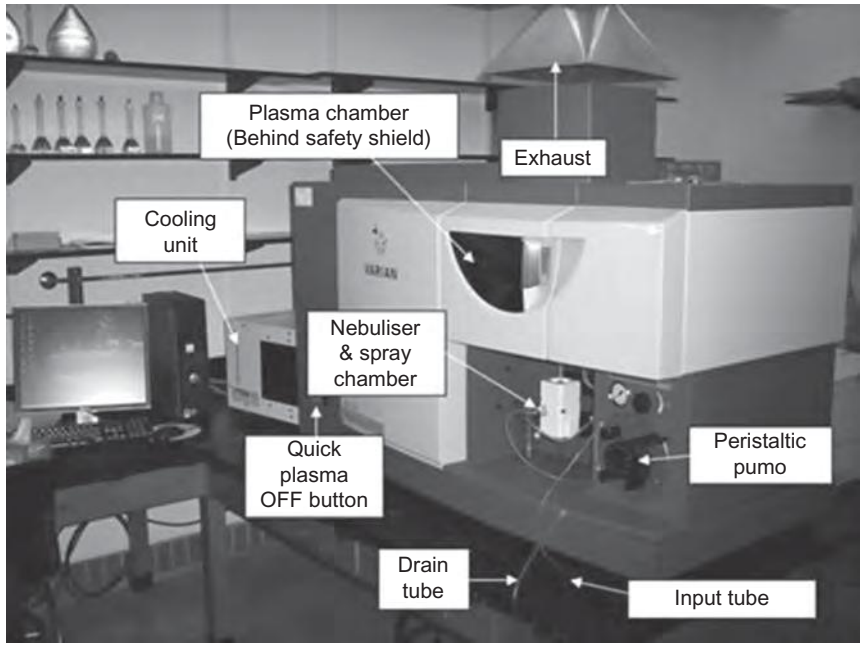


Figure 5.23 *Inductively coupled atomic emission*

and on to the quartz plasma torch. The plasma ionises and excites the atoms of the sample. Emitted light from the ions in the plasma then passes through the entrance window to the monochromator where it is separated into its various wavelengths (colours). The monochromator is a high-resolution 'Echelle' design that makes use of both a diffraction grating and a prism to generate a two-dimensional pattern of individual wavelengths of light. This light hits the charge-coupled device (CCD) detector, similar to what you find in a digital camera, where thousands of individual picture elements (pixels) capture the light and turn it into a digital signal that we can measure. Figure 5.23 illustrates the Varian Inductively coupled atomic emission spectrophotometer, showing placement of its various parts.

The commonly used excitation sources are flame, arc and spark and plasma. However, plasma excitation sources are the ones which are mostly used in the present day instruments.

5.6 PLASMA EXCITATION SOURCES

In order to produce strong atomic emission from all chemical elements, it is necessary to attain temperatures considerably above those of simple flames. A convenient means of obtaining temperatures in the range 7000 K–14000 K is to generate an inert-gas plasma. Plasma is a hot gaseous fourth state of matter which is electrically conductive because of the major concentrations of essentially free electrons and highly charged cations present. Plasma is very effective medium for volatilisation, atomisation and ionisation.

A plasma is a hot, partially ionised gas that contains an abundant concentration of cations and electrons. The plasmas used in atomic emission are formed by ionising a flowing stream of argon gas, producing argon ions and electrons. A plasma's high temperature results from resistive heating

as the electrons and argon ions move through the gas. Because plasmas operate at much higher temperatures than flames, they provide better atomisation and a higher population of excited states.

A significant development in atomic spectrochemical equipment has been the development of the inert gas, electrical discharge plasma excitation source. Unlike other electrical discharges that have been optimised principally for solid or powdered samples, the inert-gas plasma has been developed specifically for solution sample types, as a replacement for the flame excitation source.

The inert-gas electrical discharge plasma, commonly using argon, comes in the following principal versions:

- Direct current plasma (DCP)
- Inductivity coupled plasma (ICP)
- Microwave-induced plasma (MIP)

The DCP and ICP have been specifically developed for solution analysis. The MIP does not perform as well for solution analysis. This is because of the sample introduction being improperly matched to the material input limitations of the MIP. Organic liquids are handled well by DCP and ICP systems. All the plasmas do poorly with solid sample introduction. They are, however, not as robust as DC arcs and graphite atomisers.

5.6.1 Direct Current Plasma (DCP)

The direct current plasma (DCP) operates at DC and uses a multiple electrode system as shown in Figure 5.24. Typically, it is a three-electrode system having two pyrolytic graphite anodes and one thoriated tungsten cathode. It has four electrical poles; two anodes and two cathodes and uses two power supplies. The single cathode connects to the negative pole of both power supplies. Each electrode is sufficiently cooled by flowing argon. The electrode set keeps the generated plasma volume in position, which provides much of the stability of the generated signal.

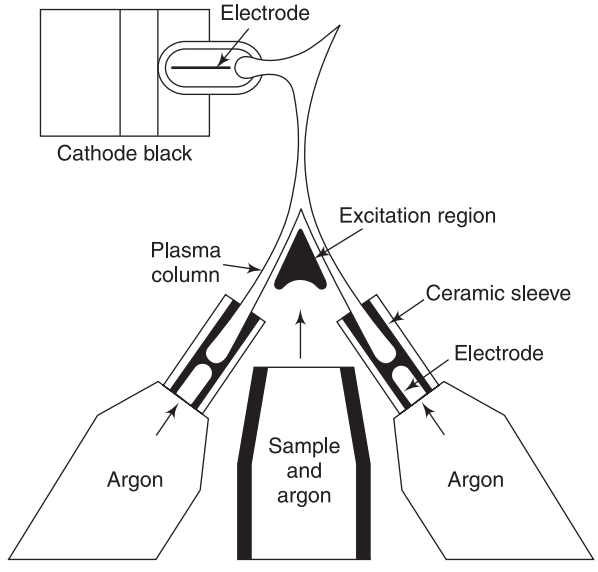


Figure 5.24 *Multiple-electrode direct current plasma system (re-drawn after Zander, 1986)*

The DCP uses nearly 10 l/min of argon, with the bulk of that going to the single type of nebuliser developed for it, the argon carrier gas for the DCP should be at least 99.995% pure. The generation of a DCP within a three-electrode set requires two 7A arcs at 40–50 V. Consequently, about 0.75 kW (max.) can be applied to the discharge.

A result of the size of the generated plasma volume is the size of the viewing zone for spectroanalytical observation. The DCP viewing zone is a volume confined between two pinched arcs. The cross-sectional area imaged on the spectrometer slit block containing the most useful emission is about 8 mm^2. The gas temperature is about 4000–6000 K.

5.6.2 Inductively Coupled Plasma (ICP)

The operating principle of inductively coupled plasma (ICP) torch is simple in that an electric discharge (e.g. from a Tesla Coil) is used to cause partial ionisation in argon gas and this gas is then passed through an oscillating magnetic field from an induction coil operating at radiofrequencies. The ions initially created are accelerated and through collision with atoms, cause further ionisation until a plasma results. Power consumption in the RF generator for the induction coil is typically around 10 kW.

ICP systems typically operate at 27.12 or 40.68 MHz. The torch arrangement shown in Figure 5.25 uses a radio frequency coil antenna surrounding a ceramic, flow controlling torch. Usually, the coil is made of copper, is protected from oxidation and corrosion and is water cooled. The torch body generally a concentric arrangement of quartz tubes, is usually made of silica and must be cooled by an argon flow. A separate argon flow is used in plasma generation and a third is necessary as the sample aerosol carrier, as sample introduction is that used in conventional AAS. The pneumatic arrangement tends to hold the generated plasma volume in position. The normal observation of the emission from the plasma is either on the side (A) or axial (in line B) to the torch. Figure 5.26 shows the various parts of a commercially available inductively coupled atomic emission spectrophotometer.

Most ICPs use between 10–20 l/min of argon, with the bulk of that cools to the gas that cools the quartz torch. The ICP requires more power capacity, since it generally uses more power at 220 V, 20–30 A, single-phase. The ICP is generated from about 2.5 kW radiated power at a coil antenna. The viewing zone in ICP is 20–60 mm^2

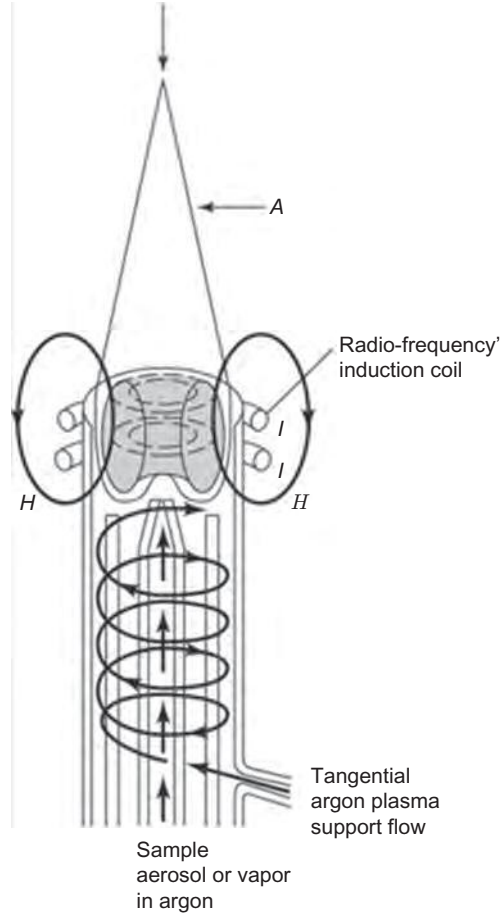


Figure 5.25 *Schematic diagram of inductively coupled plasma torch (Courtesy: M/s Thomson Higher Education)*

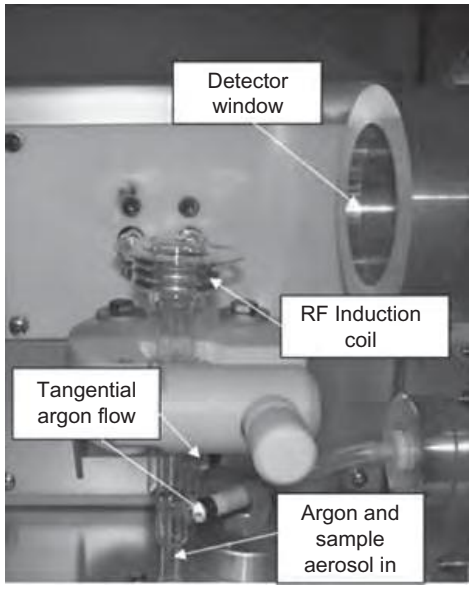


Figure 5.26 *Shows picture of the plasma torch compartment in the ICP-AES. (Courtesy: Concordia College, Minnesota)*

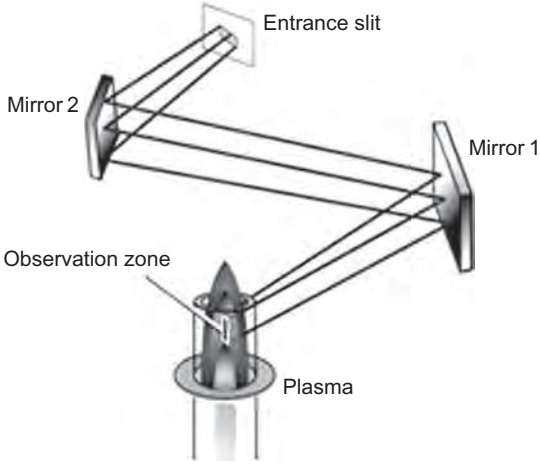


Figure 5.27 *Radially viewed plasma with a vertical slit image in the plasma (Courtesy: M/s PerkinElmer)*

and the temperature 4,000–6,000 K. The ICP is available commercially as a complete atomic emission analysis system from several companies. The wash-out-times necessary for a drop to 1% and 0.1% of peak for a typical ICP spray chamber are 25 and 40 s compared to AA values of 1 and 3 s. Therefore, larger wash-out times is a particular problem in ICP systems.

A major development in the area of atomic absorption spectroscopy has been the introduction of commercial ICP torches in place of chemical flames. The ICP torch provides extremely high sensitivity, low matrix interferences and multi-element analysis capability for samples in solution. However, the ICP systems are extremely expensive and less convenient to operate than typical flame spectrometers. Nevertheless, their preference cannot be overlooked in laboratories, where high sample throughput is imperative and where determination of at least five elements in each sample is required. In most other cases, the chemical flame will remain the choice for the majority of determinations. The attractive features of ICP are:

- The high efficiency of atomisation and excitation gives high sensitivity for most elements. This facilitates working at low detection limits.
- The high temperature, relatively uniform structure and chemical mildness of a plasma minimises both chemical and spectral interferences. This leads to greater dynamic range.
- Only one optical system can be used for all elements. There is no need for a separate component for every element as with HCL-based AAs.
- Simultaneous determination of all elements in a sample is possible, leading to a very high throughput in analysis.

The main disadvantage of IPC AEs analysis is due to the extreme complexity of atomic emission spectra (i.e. presence of lines due to different elements close to each other). This requires very high-resolution and expensive monochromator.

There are two ways of viewing the light emitted from an ICP. In the classical ICP-OES configuration, the light across the plasma is viewed radially (Figure 5.27), resulting in the highest upper linear ranges. By viewing the light emitted by the sample looking down the centre of the torch (Figure 5.28) or axially, the continuum background from the ICP itself is reduced and the sample path is maximised. Axial viewing provides better detection limits than those obtained via radial viewing by as much as a factor of 10. The most effective systems allow the plasma to be viewed in either orientation in a single analysis, providing the best detection capabilities and widest working ranges.

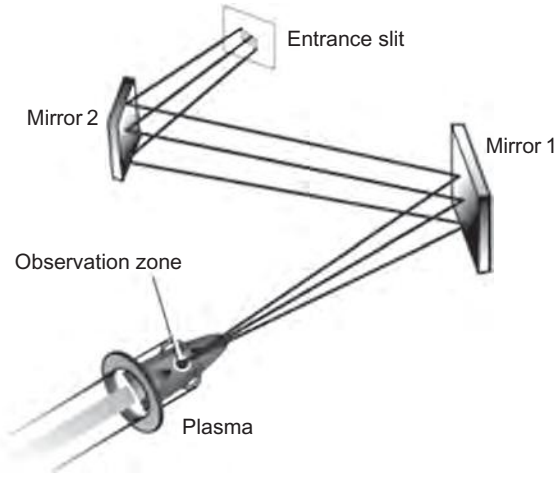


Figure 5.28 *Axially viewed plasma with an axial slit image in the plasma (Courtesy: M/s PerkinElmer)*

In ICP systems, three important considerations need to be followed in sample introductions:

- It is necessary to introduce sample to the atomiser with drops no larger than a certain maximum size.
- The solvent introduction rate must fall within a certain permissible band of values.
- To maintain good system reproducibility, the temperature must be maintained constant in the plasma box, as a means to reduce the baseline drift caused by variable solvent vapour loading.

Loon (1980) reviews the methods used for direct trace elemental analysis of solids by atomic spectrometry.

5.6.3 Microwave-Induced Plasma (MIP)

The microwave-induced plasma (MIP) uses 2,450 MHz resonant cavity in a quartz or ceramic tube, which is cooled by the flowing carrier gas. The resonant cavity can be made of pure copper. Brass or aluminium can also be used if they are silver-coated. Figure 5.29 shows a schematic diagram of the resonant MIP cavity. The MIP typically uses argon as the carrier gas, but newer versions permit atmospheric pressure operation with helium. It uses very little carrier gas, 50 ml/min to a few litres per minute. The popular versions of MIP use more expensive helium, which greatly offsets its reduced gas consumption. The MIP is generated from a few watts to a few hundred watts of radiated power.

Sample introduction in the MIP may usually cause an alteration of the power transfer efficiency to the plasma and consequent power density fluctuations. As a result, sample introduction into an MIP with retention of power suitability and thereby signal stability is quite difficult. Even if the MIP system is operated at relatively high power, the small size of the plasma and the viewing of the plasma itself make sample introduction a complex problem. The details of various types of plasma sources have been given by Zander (1986).

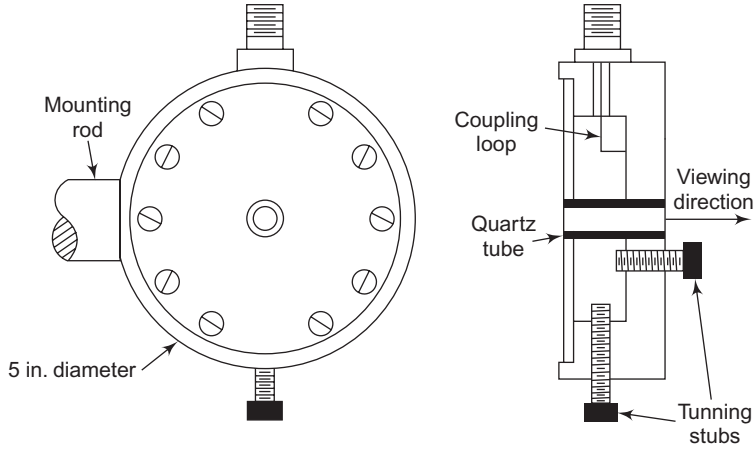


Figure 5.29 *Schematic diagram of a 2,450 MHz microwave-induced plasma (re-drawn after Zander, 1986)*

5.7 PERFORMANCE ASPECTS

The performance of an atomic absorption spectrophotometer is usually described by sensitivity and detection limit.

By definition, sensitivity is the concentration of an element in solution, which absorbs 1% of the incident light. Its value depends upon atomiser efficiency, the design of the burner and optical system. It is independent of amplifier gain and noise from flame or lamp.

Detection limit is defined as that concentration of an element, in ppm, which gives a reading equal to twice the standard deviation of the background signal, the standard deviation being computed from at least 10 readings. In practice, the detection limit is the minimum concentration of the element in solution that can just be detected. This parameter depends both on sensitivity and noise generated by the flame and lamp. It is, therefore, more meaningful and a better criterion of spectrophotometric performance.

5.8 SOURCES OF INTERFERENCES

5.8.1 Anionic Interference

The atomic absorption spectroscopic technique is generally recognised to be free from spectral interference, because the bandwidth or the monochromator is essentially the width or the source emission line. Thus, closely spaced lines such as Mg 285.21 and Na 285.28, would present no problem in absorption work. The wavelengths at which individual elements absorb are well defined and the possibility of two elements absorbing at exactly the same wavelength is quite remote.

However, the response of an clement at its resonance wavelength may sometimes depend on another component in the same solution. This effect is known as chemical interference. The major source of this effect is due to the presence of anions with the metal ions in the sample,

which affect the stability of the metal compounds formed during atomisation and hence the efficiency of metal atom production. The effect is important when determining calcium and magnesium in biological fluids and aluminium alloys, etc. Sulphate, phosphate, silicate, aluminate and a number of other radicals all interfere with these metals, by tending to bind the calcium into a compound that is not associated in the flame. Generally, all these interferences may be completely removed by the addition of an excess of lanthanum, which competes with the calcium for the interfering anions. Lanthanum is also useful in removing similar interferences from strontium and barium.

5.8.2 Viscosity Interference

Viscosity interference is caused by the fact that solutions of widely differing viscosities enter the burner at different rates. Fortunately, this effect is negligible in most of the analyses. However, if it is desirable to overcome this interference, it may be necessary to match the standards to the samples. The method usually adopted is that of additions. In this method, a crude determination of the concentration of the desired element is made, after which the sample is divided into three aliquots. Different additions are made to two of the aliquots, while the third is left blank. All three are then determined by atomic absorption, and a graph is plotted for absorbance versus ppm added. The intercept of the curve on the ppm axis gives the concentration in the unknown. With the method of additions, atomic absorption measurements are independent of sample characteristics, and the making up of standards is not necessary.

5.8.3 Ionisation Interference

Ionisation interference is usually observed at high flame temperature and manifests itself as an enhancement in the response of the element under determination. At any temperature in the flame, there is an equilibrium between neutral atoms and ions. For elements having relatively low ionisation potentials, the proportion of ions will be high. If another easily ionisable element is added into the flame, the equilibrium for both elements is shifted towards the atomic state, producing an enhancement in analytical sensitivity. The interference is generally overcome by addition of the same quantity of the interference to the standard solutions, as is present in the samples, or by addition of an easily ionisable metal like sodium or potassium to both standard and sample solutions.

Since the concentration of the analyte element is considered to be proportional to the ground state atom population in the flame, any factor that affects the ground state population of the analyte element can be classified as an interference. Factors that are likely to affect the ability of the instrument to read this parameter can also be classified as an interference.

5.8.4 Broadening of Spectral Line

There are several factors which read to broadening of the spectral line. These are:

Doppler Effect: This effect arises because atoms will have different components of velocity along the line of observation.

Lorentz Effect: This effect occurs as a result of the presence of foreign atoms in the environment of the emitting or absorbing atoms. The magnitude of broadening varies with the pressure the foreign gases and their physical properties.

Quenching Effect: In a low-pressure spectral source, quenching collision can occur in flames as the result of the pressure of the foreign gas molecules with vibrational levels very close to the excited state of the resonance line.

Self-absorption Effect: The atoms of the same kind as that emitting radiation will absorb maximum radiation at the centre of the line than at the wings, resulting in the change of shape of the line as well as its intensity. The effect is more predominant if the vapour which is absorbing radiation is considerably cooler than that which is emitting radiation.

6

FLUORIMETERS AND PHOSPHORIMETERS

6.1 PHOTOLUMINESCENCE SPECTROSCOPY

Photoluminescence relates to the emission of light through absorption of ultraviolet or visible energy.

Fluorescence, which is the immediate release (10^{-8} s) of absorbed light energy as opposed to *phosphorescence*, which is the delayed release (10^{-6} to 10^{2} s) of absorbed light energy.

Chemiluminescence is the emission of light energy caused by chemical reactions.

Bioluminescence refers to the light emission through the use of enzymes in living systems.

Photoluminescence spectroscopy is divided into two categories: Fluorescence spectroscopy and Phosphorescence spectroscopy.

6.2 FLUORESCENCE SPECTROSCOPY

The analytical technique based on the absorption of infrared, visible and ultraviolet light has found extensive applications in chemistry. However, a limitation of this technique is the difficulty of differentiating between different substances with similar absorption spectra. Many solutions when irradiated with visible or ultraviolet light, not only absorb this light, but reemit light of a different wavelength. This effect is known as fluorescence and its exploitation opens up possibilities in the discrimination and accurate determination of many substances in very dilute solutions. The difference in wavelength between incident and re-emitted light enables efficient discrimination to be made between substances with similar absorption characteristics.

Fluorescence spectroscopy, thus, is a form of analysis that utilises the emission properties of specific molecules rather than tendency to absorb certain wavelength of light. This inherently makes it more selective than absorption spectroscopy due to the fact that all molecules that fluoresce must absorb, but not all molecules that absorb necessarily fluoresce. Therefore, only specific types of molecules can be studied using fluorescence spectroscopy.

The main advantage of fluorescence detection compared to absorption measurements in the greater sensitivity achievable because the fluorescence signal has in principle a zero background. Major analytical applications include quantitative measurements of molecules in solution and fluorescence detection in liquid chromatography.

Fluorescent analysis makes valuable contributions to many branches of research and industry. However, it has found widest application in biomedical laboratories. Clinical laboratories employ it for and such studies as adrenalin and noradrenalin in urine and blood and in screening tests for tumours of adrenal glands. Tests for transminase, lactic dehydrogenase, DPN (diphosphoneucleotide), steroids, DNA (deoxyribonucleic acid), histamine and many others are routinely made with fluorimeters. The value of the fluorimetric technique, for such studies as air pollution and analysis of alkaloids, has tremendously increased. Industrial health laboratories use fluorimeters for routine determination of beryllium. Agriculture and food chemists use this technique for vitamin and insecticide residue studies. Water, air pollution and sanitation engineers find it effective and convenient for studying flow and diffusion of air and water.

6.3 PRINCIPLE OF FLUORESCENCE

Normally, in the ground state of molecules, molecular energies are constant and at minimum values. When a quanta of light impinges on a molecule, it is absorbed and an electronic transition to a higher electronic state takes place. This absorption of radiation is highly specific, and radiation of a particular wavelength and energy is absorbed only by a characteristic structure. In the ground state of most molecules, each electron in the lower energy levels is paired with another electron, whose spin is opposites its own spin. This state is called a singlet state. But when the molecules absorb energy, they are raised to an energy level of an upper excited singlet state or S_1 or S_2 or S_3 (Figure 6.1), represented by the transition G-S_1 or G-S_2. These singlet transitions are unstable and the molecular energy tends to revert almost immediately to a lower level. In going to a lower energy level, the absorbed energy is lost by steps from the upper singlet state to the lowest single-state through the transition S_3-S_1 or S_2-S_1. These singlet transitions are responsible for the visible and ultraviolet absorption spectra.

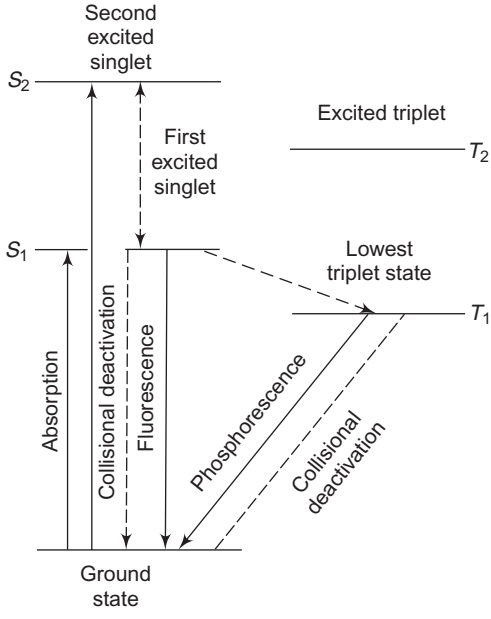


Figure 6.1 *Principle of fluorescence and phosphorescence*

The excited singlet state persists for a time, which is of the order of 10^{-8} to 10^{-4} s. During this time interval, some energy in excess of the lowest vibrational energy level is rapidly dissipated. In case all the excess energy is not further dissipated by collisions with other molecules, the electron would return to the ground state with the emission of energy. This phenomenon is called fluorescence. Since this transition involves less energy than in the original absorption process, the fluorescence is emitted at wavelengths longer than those of the exciting source. This is shown in Figure 6.2. Many vibrational levels are actually involved, so molecular fluorescence spectra are generally observed as continuous bands, with one or more maxima. Filters are used to filter out the exciting wavelengths, so that only fluorescent energy reaches the detector.

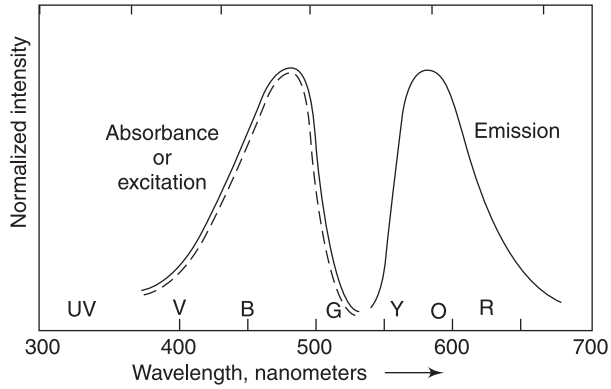


Figure 6.2 *Representation of excitation (absorption) and emission spectrum (www.turnerdesigns.com)*

Any fluorescent molecule has two characteristic spectra, the excitation spectrum and the emission spectrum. The excitation spectrum shows the relative efficiency of different wavelengths of exciting radiation to cause fluorescence, whereas the emission spectrum would indicate the relative intensity of radiation emitted at various wavelengths. Normally, the shape of the excitation spectrum should be identical to that of the absorption spectrum of the molecule, and independent of the wavelength at which fluorescence is measured. However, due to instrumental errors, this is seldom the case. Also, the shape of the emission spectrum is independent of the wavelength of exciting radiation. If the exciting radiation is at a wavelength different from the wavelength of the absorption peak, there will be less absorption of radiant energy, and consequently less energy will be emitted.

6.3.1 Relationship Between Concentration and Fluorescence Intensity

The total fluorescence intensity is equal to the rate of light absorption multiplied by the quantum efficiency of fluorescence (η), that is

$$F = \eta\,(P_o - P) \tag{6.1}$$

where P_o is the power of the beam incident upon the solution and P is its power after traversing a length b of the medium.

In order to relate F to the concentration c of the fluorescing particle, we can apply Beer's Law. In such a case, the fraction of light transmitted is

$$P/P_o = e^{-\tau bc} \tag{6.2}$$

where τ is the molar absorptivity of the fluorescent molecules and τbc is its absorbance A.

$$\begin{aligned} 1 - P/P_o &= 1 - e^{-\tau bc}\,P \\ (P_o - P) &= P_o\,(1 - e^{-\tau bc}) \end{aligned} \tag{6.3}$$

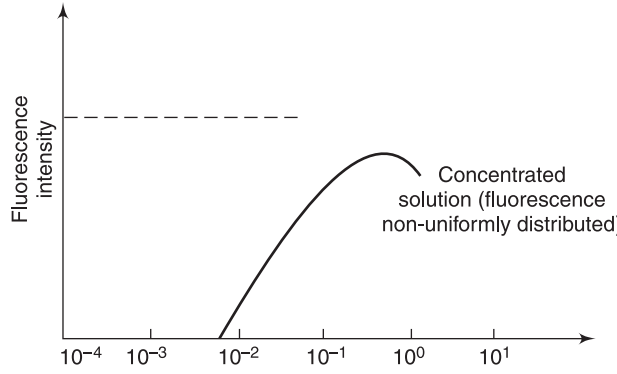


Figure 6.3 *Plot between concentration and fluorescence intensity*

substituting Eq. (6.3) in Eq. (6.1), we get

$$F = \eta P_o (1 - e^{-\tau bc}) \tag{6.4}$$

For very dilute solutions in which A (τbc) <0.05 and only a small fraction of light is absorbed, the exponential term can be expanded, and Eq. (6.4) reduces to

$$F = K'.\ \eta.\ P_o (2.3\ \tau bc)$$

or

$$F = K.\ C_M$$

This equation shows that for very dilute solutions, a plot of the fluorescent power of a solution versus concentration of the emitting particles would be linear. However, at higher concentrations (Figure 6.3) linearity is lost and the curve bends downwards as the concentration increases.

The linearity of a sample depends upon many factors, including the chemical composition of the sample and the path length, the light must travel. Also, concentration has effect on linearity. When concentration is too high, light cannot pass through the sample to cause excitation; this very high concentration can have very low fluorescence. Similarly, at intermediate concentrations, the surface portion of sample nearest the light absorbs so much light that little is available for the rest of the sample. So, the readings will not be linear, though they will be within the range of a calibration curve.

While making fluorescent measurements, it is always advantageous to make the exciting energy as large as possible. However, photodecomposition should be avoided.

6.3.2 Advantages of Fluorescence Technique

The inherent advantages of fluorescence technique are:

- *Sensitivity*: Sensitivity of a fluorometer refers to the minimum detectable quantity of a compound of interest under specified instrument condition. High sensitivity of fluorometry allows the reliable detection of fluorescent materials using small sample sizes. Fluorometers achieve 1,000–5,00,000 times better limits of detection as compared to spectrophotometers detectability to parts per billion or even parts per trillion is common for most analytes. Therefore, picogram quantities of luminescent materials are frequently studied.

- *Selectivity*: Fluorometers are highly specific and less susceptible to interferences because fewer materials absorb and also emit high (Fluorosce). This is because two characteristic wavelengths are involved in these measurements. In contrast, spectrophotometric techniques are prone to interference problems because many materials absorb high, making difficult to isolate the targeted analyte in a complex matrix.
- *Variety of sampling* methods like dilute and concentrated samples, suspensions, solids, surfaces and combination with chromatographic systems.
- *Wide concentration Range*: Since the fluorescence output is linear to sample concentration over a broad range, the technique can be used over three to six decades of concentration without sample dilution or modification of the sample cell.
- *Simplicity and speed*: Fluorometry is a relatively simple analytical technique. Its sensitivity and specificity reduce or eliminate the sample preparation procedures and thereby expedite analysis.

6.4 MEASUREMENT OF FLUORESCENCE

Fluorescence measurements are carried out in instruments called fluorometers or fluorimeters. The various components of these instruments are similar to those in photometers. These are analogous to photometers as they too make use of filters to restrict the wavelengths of the excitation and emission beams. Fluorescence measuring instruments making use of monochromators like spectrophotometers are called spectrofluorimeters. These are of two types: in one type, a filter is employed to limit the excitation radiation and grating or prism monochromator to isolate a peak of the fluorescent emission spectrum. The other type of instruments are equipped with two monochromators. One of the monochromators restricts the excitation radiation to a narrow hand, whereas the other permits the isolation of a particular fluorescent wavelength. The latter types are the true spectrofluorimeters.

The basic principle of measurement of fluorescence is shown in Figure 6.4. The arrangement contains an excitation source, sample cell and fluorescence detector. Molecules in solution are usually excited by UV light and the excitation source is a deuterium or xenon lamp. Broad band excitation light from a lamp passes through a monochromator, which passes only a selected wavelength. The fluorescence is dispersed by another monochromator and detected by a photomultiplier tube while the excitation monochromator gives excitation spectrum, scanning the fluorescence monochromator gives the fluorescence spectrum. Simple instruments sometimes use only a bandpass filter to select the excitation wavelength.

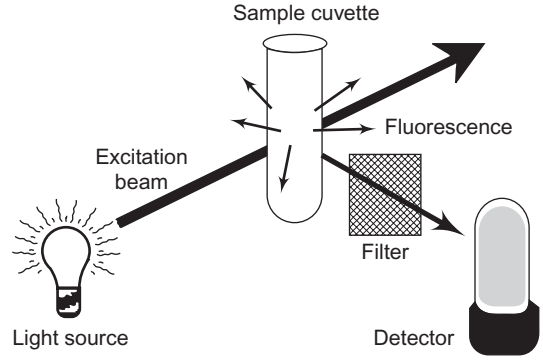


Figure 6.4 *The basic principle of measurement of fluorescence (Tissue, 2004b)*

6.4.1 Single-Beam Filter Fluorimeter

Figure 6.5 shows a block diagram of a single-beam filter fluorimeter. The source of light is a high pressure mercury discharge lamp, with

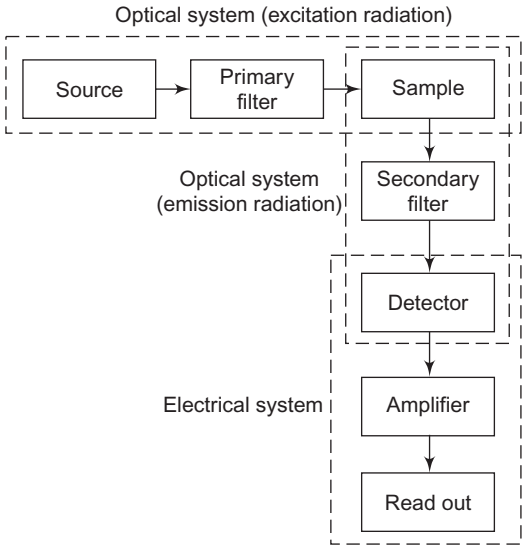


Figure 6.5 *Schematic diagram of the components of typical fluorimeter*

glass or fused silica envelope. Selection of the exciting wavelength is made by inserting a primary filter in the incident beam. This enables to isolate one of the principal lines at 365, 405, 436, 546 or 579 nm and to have intense line spectrum with good spectral purity. Low pressure lamps, equipped with a silica envelope can produce intense radiation at 254 nm. The key components of a signal-beam filter-based fluorometer are shown in Figure 6.6. The lamp or light source which provides the energy that excites the compound of interest include xenon lamps, high pressure mercury vapour lamps, xenon-mercury arc lamps, lasers and LED's. Lamps emit a broad range of light (i.e. more wavelength than those required to excite the compound). LEDs and lasers emit more specific wavelengths.

Both cylindrical and rectangular cells constructed of glass or silica are employed in fluorescence measurements. The cuvette material must allow the compound's absorption and emission light energy to pass through. Also, the size of the sample cell affects the measurement. The greater the path length (or diameter) of the cell, the lower the concentration that can be read. Fluorometers commonly use 10 mm sq. cuvettes and/or

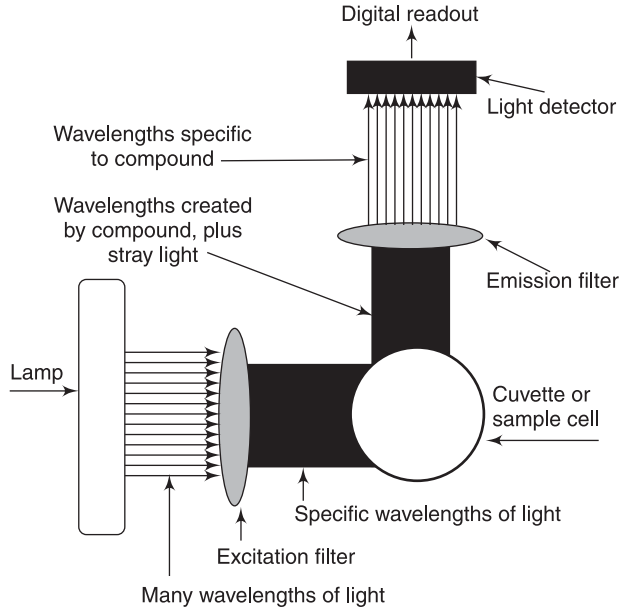


Figure 6.6 *Key components of a filter fluorometer (www.turnerdesigns.com)*

13 or 25 mm test tubes. For small volumes of solutions, adaptors are available for capillary tubes (9ml) and mini-cells (100 ml). Cuvettes are made from borosilicate or quartz glass that can pass the selected wavelengths. Adequate care is taken in the design of the cell compartment to reduce the amount of scattered radiation reaching the detector. For continuous, on-line monitoring of samples for fluorescence studies, flow-cells are used in the instruments sample chamber, through which samples are pumped. Flow cells too, are available in various diameters. The fluorescence emitted by the sample is measured by selecting the wavelength of fluorescence radiant energy by a second optical filter, called the secondary filter. This is placed between the sample and a photodetector located at a 90° angle from the incident optical path. The fluorescent signal is generally of low intensity and requires large amplification for carrying out necessary measurements. The detector used in fluorescence measurements are the sensitive photomultiplier tubes. After signal amplification, the signal is displayed on a duly calibrated micro-ammeter or recorder.

It is always difficult to measure absolute fluorescence intensity. Therefore, measurements are made with reference to some standard substance, which may be chosen arbitrarily. The circuit is balanced at any chosen setting by placing the standard solution in the instrument. Without making any adjustment, the standard solution is replaced by additional standard solutions of lesser concentration. Lastly, fluorescence of the solvent and cuvette alone is measured to establish a true zero concentration. With the help of these settings, a curve is plotted between the concentration against scale readings, which gives the fluorescence-concentration curve. Unknown concentrations can be estimated from this curve, if the scale reading is known.

The detection system used in the commercially available in fluorimeters is almost exclusively based on the use of the photomultiplier tube in a single-channel configuration. Yet the parallel multi-channel optoelectronic image detectors should be clearly superior, because of its ability to record the entire spectrum simultaneously. Parallel detection can result in either a significant increase in signal-to-noise ratio, or a corresponding reduction in analysis time. The detector is a silicon intensified target vidicon, whose control is achieved with a computer.

It is known that the dark current of a photomultiplier is primarily due to the emission of thermionic electrons from the cathode and it is generally reduced by cooling the photomultiplier. In the case of very weak light measurements, the reduction of the dark current is an important factor in improving the signal-to-noise ratio. Special arrangements are used to cool photomultipliers to a temperature, where the thermal component of the dark current is virtually eliminated.

In order to study the dynamic properties of excited molecules, one must know not only the decay time of the luminescence, but also its quantum yield, that is the number of emitted photons divided by the number of absorbed photons. The number of absorbed photons can be determined easily by any absorption measurements, but the number of emitted quanta cannot be found in a straightforward manner. The recorded-emission spectra for the spectral response of the detection system thus need to be corrected. The spectral response of the detection system is not always known, and also it is considered too much of a trouble to re-draw the spectra point by point. The latter can be conveniently done by interfacing the detection system to a computer. A simple and cheap solution to the problem is possible by the use of programmable read-only memories.

For studying the decay of fluorescence on the nanosecond time scale, impulse technique is generally preferred. In this method, the sample is excited by a short pulse of light of suitable wavelength and the emitted fluorescence is detected and recorded as a function of time. The excitation and detection of the fluorescence are repeated many times and the results are averaged in order to

obtain acceptable signal-to-noise ratios. However, if any drift occurs in the excitation light source, or in the detection electronics, the measured profile of the light source will not truly represent the correct profile and may introduce serious errors. Automated luminescence spectrometers using a microprocessor for correcting and recording absorption, excitation and emission spectra are greatly extending the qualitative and quantitative analytical capabilities of the technique.

A single-beam fluorometer is a good choice when sensitive quantitative measurements are desired for specific compounds. The comparative ease of handling and low cost make filter fluorometers ideal for dedicated and routine measurements.

Filter fluorometers are calibrated by compensating for blank (solution containing zero concentration of the substance to be read) and adjusting the instrument to reflect a known concentration of sample (standard). Calibration is required for each instrument from the same manufacturer and certainly from manufacturers to manufacturer. As fluorescence is subject to temperature and environmental effects, the fluorometer must be calibrated in conditions as close as possible to the actual conditions of study. Due to extremely sensitive nature of measurement in fluorometry, it is important to be rigorous in laboratory procedures such as carefully preparing standards and cleaning labware.

6.4.2 Double-Beam Filter Fluorimeter

Double-beam instruments are direct reading type and are generally preferred because of this obvious advantage. In these instruments, the circuit is so arranged that the current from the reference detector opposes that generated by the measuring photocell. The reading on the calibrated scale, at which the two currents neutralise each other directly, gives the fluorescence reading.

Figure 6.7 shows a schematic diagram of a double-beam fluorimeter. A mercury lamp is used as a source of radiation. A collimated beam is passed through the primary filter, which then falls on a cuvette containing the sample. The measuring photocell is mounted at right angles to the excitation beam. Fluorescent radiation passes through secondary filters, which remove scattered ultraviolet radiation, but pass visible light.

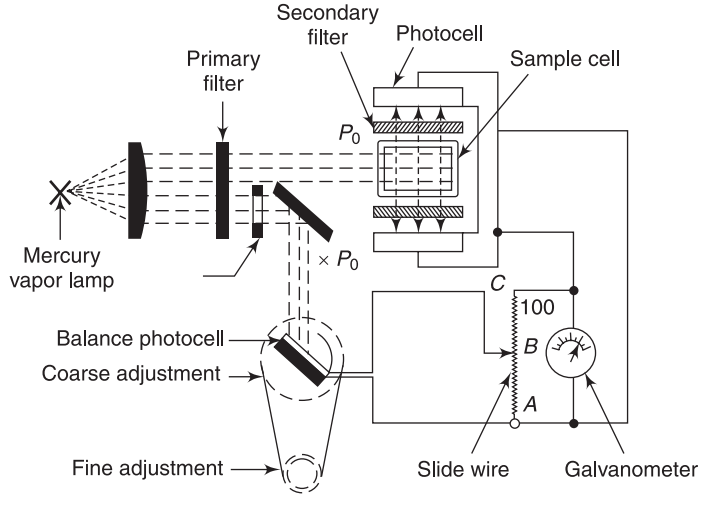


Figure 6.7 *Schematic diagram of a double-beam fluorimeter*

A part of the excitation beam is passed through a reduction plate for reading the intensity of the reference beam, in order that its power be of the same order of magnitude as the weak fluorescent beam. The attenuated reference beam is reflected on to the surface of a reference photocell, mounted on a rotating table. By adjusting the angle of the photocell, the amount of radiation falling on the photocell and thus the current output, can be adjusted.

The outputs of the reference and the measuring photocells is compared and a sensitive galvanometer is used to check the null point. The circuit is arranged, so that the current from the reference cell opposes that generated by the measuring photocell, which respond to fluorescent radiation. In this arrangement, the concentration *C* is given by

$$C = K.\, R_{AB}/R_{AC}$$

where *AC* is the total resistance. The constant *K* is independent of P_o.

Quite often, two photocells, connected in parallel, are used to measure the weak fluorescent radiation. This arrangement increases the sensitivity of the instrument.

6.4.3 Ratio Fluorimeters

A ratio fluorimeter which enables direct readings for rapid analysis of multiple samples is shown in Figure 6.8.

In this instrument, the exciting energy is matched to the sample by the two primary filters located on both sides of a specially designed source lamp. The lamp cycles in-phase with the AC line voltage and has a dark time, as the lamp anodes alternately reach zero potential during the change in phase of the line voltage.

During one phase, light reaches the reference test tubes and produces fluorescence, which strikes the photomultiplier and produces the reference signal. During the other phase, light falls on the sample and causes it to fluoresce. This fluorescent energy when strikes the photomultiplier, produces the sample signal. The reference and sample beams pass through the secondary filter. This filter isolates the fluorescence radiation from spurious radiation. By setting the reference signal equal to 100% on the metre, the signal at the metre indicates the ratio of the sample to the reference. The ratio can also be recorded by using a high impedance recorder.

The source of excitation used in a typical instrument is divided into two sections by a light shield, with electrons flowing from a common cathode alternately to two anodes, one on the reference side and the other on the sample side, as the lamp cycles in-phase with AC line voltage. When one beam is on, the other is completely off. Thus, the lamp supplies excitation radiation alternately to the reference and sample photocells. The lamp is surrounded by phosphor coated glass sleeve, which has a

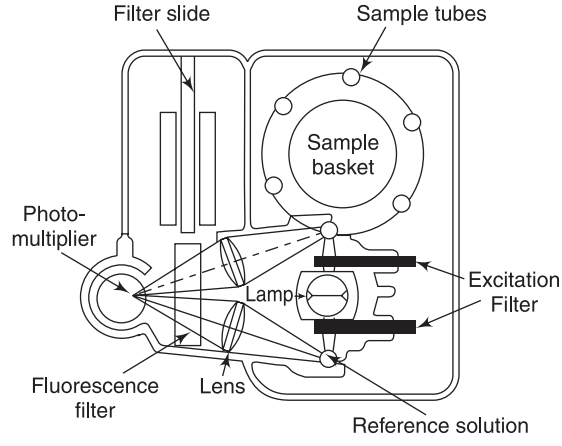


Figure 6.8 *Optical diagram of the ratio fluorimeter*

clear portion plus coatings with three different phosphors. By rotating the sleeve, mercury wavelengths about 237 nm through the clear portion, plus peaks at 310, 360 and 450 nm through the phosphor coated portions are achieved. This dual source lamp provides double-beam operation without light choppers, beam splitters or other similar devices. The sample beam and the reference beam consist of fluorescent energy of the same wavelength, thus making readings independent of lamp temperature variations. Two types of filters are employed: two primary (excitation) filters and one secondary (fluorescence) filter. Both types of filters can be easily changed for analysis of different samples. Glass test tubes can be used as sample containers for most of fluorescence that the photomultiplier receives. Since fluorescence is greater at lower temperatures, provision is made for water cooling of the sample, so that sample fluorescence is as strong as possible. The sample tube area may be cooled by a continuously flowing supply of water.

Reference bars are supplied with fluorimeters for establishing upscale metre values. These are glass rods fused with varying concentration of uranium salts. Each bar emits about three times more fluorescence intensity, successively from numbers 1 through 6. They cover an approximate range of 5–1,000 ppb of quinine sulphate equivalent. In some cases, they may be more useful than standard solutions, because their fluorescence values are stable indefinitely, if not scratched or chipped.

Double-beam fluorimeters using two photomultipliers to compensate for fluctuations in light level from the exciting source are generally used. Nevertheless, the stability of these instruments is not particularly good, and is generally not better than ±1% over a 10 min period. This poor performance has been found to be due to gain changes and drifts in dark current of the two photomultipliers, caused by variation in high voltage ambient temperature and fatigue effects. A simple solution to this problem is to use a single photomultiplier for both reference and sample beams.

Another version of the double-beam type fluorimeter makes use of an optical bridge that is analogous to the Wheatstone bridge, used in measuring electrical resistance. The bridge detects and measures the difference between the light emitted by a sample and that of the calibrated rear light path. This is the principle of the Turner Model 110 Fluorimeter (Figure 6.9).

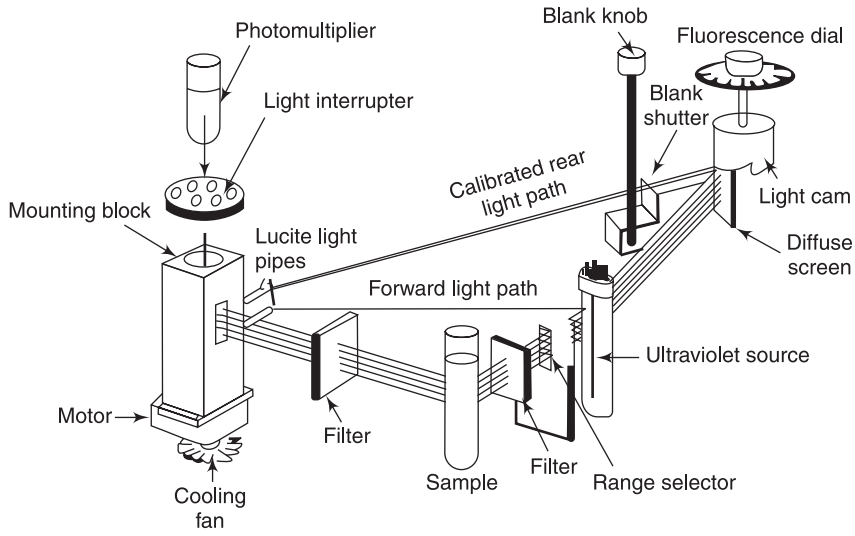


Figure 6.9 *Double-beam fluorimeter based on the optical bridge principle (Principle of Turner fluorimeter)*

The instrument employs a single photomultiplier (PMT) tube as a detector. A rotating mechanical light interrupter surrounds the PMT and causes the reference beam and the fluorescent beam to strike the PMT, alternately. The output of the PMT is thus an alternating current, which can be amplified in an AC amplifier. The next stage is a phase-sensitive detector, whose output will either be positive or negative depending upon whether the fluorescent beam or the reference beam is stronger. The power of the reference beam can be adjusted manually or automatically, by the rotation of the light cam which increases or decreases the intensity of reference beam reaching the detector. This is done until the amount of light reaching the PMT from the rear light path equals the amount from the sample. The light cam is attached to a linear dial, each small division of which corresponds to an equal fraction of light. For a totally non-fluorescent sample, no light would reach the detector in one of the phases and the null point could then be obtained from one direction only. For accurately adjusting the null point from both directions, a third (forward) light path of constant intensity is made to fall on the detector, so that under all conditions some radiation strikes the PMT. This permits a correct operation, both above and below zero with non-fluorescent blanks. Two lucite light pipes are employed in the optical system, for the rear light path and forward light path.

The PMT is mounted inside a rotating cylindrical interrupter, with two sets of light slots. The upper slots allow light to pass alternately to the PMT from the rear calibrated light path and the combination of the sample and forward light path. The lower slots interrupt the light path from the reference beam, which in turn generates the reference signal. This circuit arrangement cancels out variations in line voltage, light source and PMT sensitivity.

The PMT produces an AC signal at about 400 Hz that is proportional to the light unbalance. This signal is amplified and fed as signal to the phase-sensitive detector.

The reference signal is generated by a lamp with special glass filter around it, which passes only infrared light and directs it on to a red-sensitive phototube. The reference lamp and phototube generates an AC signal. This signal is amplified and is given as reference to the phase detector, its phase depending upon the position of the light interrupter. The phase detector generates a dc signal proportional to the signal received, which is applied to the chopper, where the dc signal is converted to AC. The AC signal is amplified. The AC signal is used to drive the servomotor, which in turn drives the light cam, so that a null balance is obtained.

6.5 SPECTROFLUORIMETERS

There are a large number of instrument manufacturers that provide spectrofluorometers. Spectrofluorimeters normally make use of two monochromators, one to supply selectively the excitation radiation and the other to isolate and analyse the fluorescence emission. The dual monochromator system thus permits to determine and selectively utilise the peaks of excitation and fluorescence wavelength. The spectrum of the fluorescence emitted by the sample can be studied at any frequency of exciting light. Also, the variation of the intensity of fluorescence with the frequency of the exciting light used can be determined. They are widely employed in analytical chemistry as they provide very high sensitivity. Although the number of naturally fluorescent substances is limited, with the use of suitable fluorescent reagents many others can be made fluorescent.

Figure 6.10 shows a schematic of the optical system used in a spectrofluorimeter. The source of light is a 150 W high pressure xenon arc lamp. The ellipsoidal mirror (ME) collects radiation from

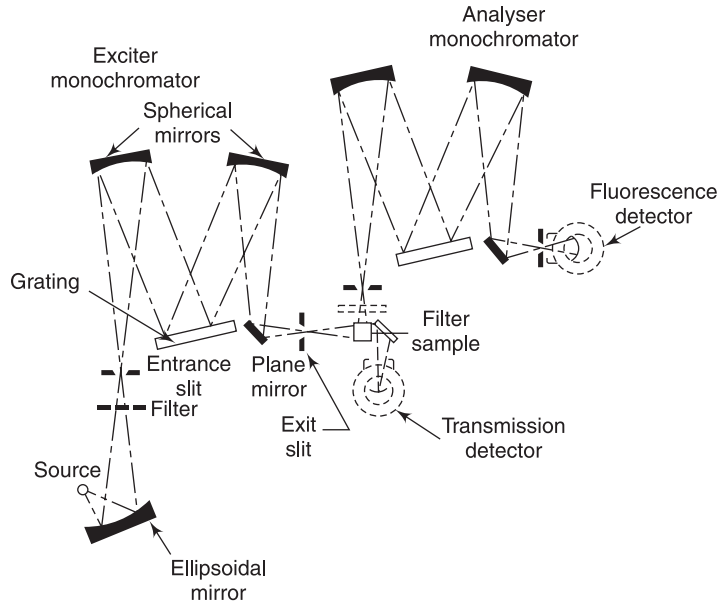


Figure 6.10 *Optical system of a typical spectrofluorimeter*

this lamp and focuses an enlarged image of the arc upon the entrance slit of the excitation monochromator, completely filling the $f/3.5$ mirrors.

Monochromatic radiation of specific wavelength is selected by angular positioning of the grating. A desired bandpass is achieved by the selection of appropriate interchangeable slits for the entrance and exit positions of the monochromator. The radiant energy emerging from the exit slit of the excitation monochromator activates specific molecules within the sample, resulting in fluorescent emission. The emitted radiation is viewed by the analysing monochromator, which permits only characteristic sample emission to reach the detector, blocking all undesired spectral regions.

When fluorescent energy falls on the PMT, the registered photocurrent can be indicated on the metre, recorder or oscilloscope. The spectrum can be manually or automatically scanned.

Many compounds exhibit far greater fluorescence at reduced temperatures than at normal, ambient or higher temperatures. Therefore, spectrofluorimeters are usually accompanied by cryogenic accessories to cool the sample by means of an immersion probe, which is cooled by easily replenished reservoir refrigerant. Using liquid nitrogen, for example, provides a sample temperature very close to 77 K.

The optical layout of a typical spectrofluorometer using diode array as a detector is shown in Figure 6.11. A xenon lamp is used as the light source which is focused by an elliptical mirror onto a diffraction grating which can either select light of a specific wavelength or scan a range of wavelengths. By means of two plane mirrors and an elliptical mirror, the excitation light is passed through the sample contained in the cuvette (sample cell) and then reflected back along its own path to increase excitation efficiency. The fluorescent light emitted at right angles is focused onto the second diffraction grating via two plane mirrors and three elliptical mirrors. The resulting fluorescent spectrum generated by the grating is then focused by means another elliptical and plane mirror onto a diode array sensor. Thus, the whole fluorescent spectrum can be recorded or, if a

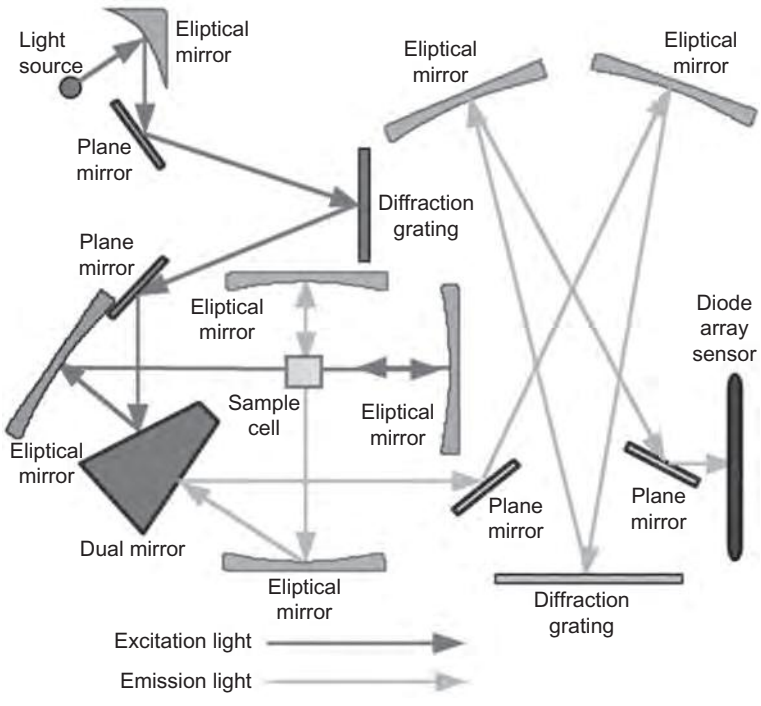


Figure 6.11 *Optical layout of a typical of spectrofluorometer using diode array as a detector*

specific diode is selected representing fluorescent light of a specific wavelength, then by programming the first grating an excitation spectrum can be recorded.

Baird Atomic Model SF 1OOE spectrofluorimeter (Figure 6.12) employs a unique optical system comprised of four grating monochromators; two are used in tandem for dispersing excitation energy and two used for observation of fluorescent spectra. This double-grating system in conjunction with folded optics offers a significant advantage in the reduction of physical dimensions. Each monochromator incorporates a 600 line/mm plane reflection grating. Wavelength control is provided by means of a cam, which may be operated manually or automatically. The calibrated

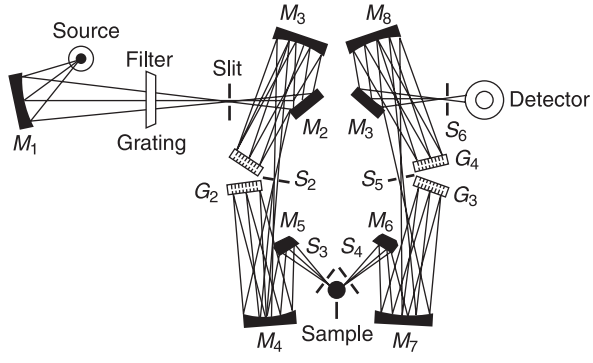


Figure 6.12 *Optical diagram of a spectrofluorimeter (Courtesy: M/S Baired Atomic, USA)*

operating range of each pair of monochromators is 220–700 nm. Wavelength settings are indicated by a scale, which is calibrated in 2 nm increment, The spectral widths of the entrance and exit slits to each pair of monochromators are each independently variable, in steps of 2, 8 and 32 nm.

The excitation source is a specially selected 150 W dc xenon arc lamp, which gives a very stable, high intensity light output in the UV, visible and infrared regions. An off-axis ME forms an enlarged image of the source arc at the entrance slit of the excitation monochromator. The lamp can be very simply focused and laterally adjusted.

The detector used is a PMT, which is supplied with a stabilised power supply. The detector signal is amplified in an operational amplifier.

Miller (1984) has reported successful adoption of pulsed xenon lamps, in place of the continuous arc lamps used previously. The pulsed sources have much longer lifetime, and do not require bulky power supplies. They can be conveniently used with electronic gating systems, to distinguish between the virtually instantaneous emission of fluorescence and the delayed emission of phosphorescence.

6.6 MICROPROCESSOR-BASED SPECTROFLUOROMETER

Iwata et al. (1986) illustrate the details of a microprocessor-based spectrofluorometer (Figure 6.13). The instrument takes advantage of the microprocessor in both instrument control and data manipulation originating in the data station unit. The microprocessor enables to have a variable spectral bandwidth in steps from 1.5 to 20 nm, wavelength scanning steps selectable in steps from 1 to 1,000 mm/min, a softwave-controlled shutter that closes the aperture of the excitation monochromator and an auto-zero function.

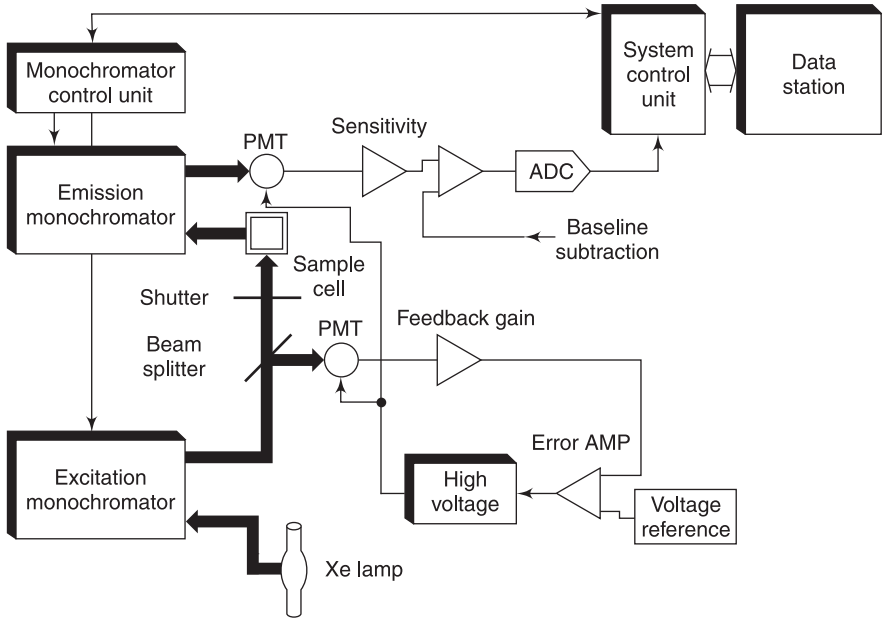


Figure 6.13 *Block diagram of a microprocessor-based spectrofluorimeter (After lwata et al., 1986)*

Sensitivity improvement in the instrument is achieved by: (i) Using holographic gratings of 1,800 grooves/mm. The stray light of this optical system is less than 3.5% at 220 nm excitation wavelength and 5-nm spectral bandwidth. (ii) Rotating the slit image of the monochromator formed on the sample cell by 90°, in which the observed volume of the sample illumination is enlarged by the slit's length-to-width ratio, resulting in higher emission intensity and an increase in sensitivity.

A dynode-feedback technique is employed to compensate for characteristics of the light source and excitation monochromator, as well as light source fluctuation. The feedback electronically controls the PMT high voltage. By changing the gain of the feedback loop in steps, the feedback technique can effectively cover a wide dynamic range of incident radiation.

The data acquisition hardware comprises three modules: (i) a high resolution, 12" cathode ray tube for display, (ii) a thermal graphic plotter/printer and (iii) a processor module that houses microprocessors, memory, bus and associated electronics. To maximise the data throughput, two slave processors are employed to help the master processor by sharing relatively low priority jobs. The spectra produced by fluorescence spectrometers are affected by the solvent background and the optical characteristics of the instrument. Appropriate software permits to obtain corrected emission spectra. This can be achieved by scanning both excitation and emission monochromators synchronously. The inverse of the corrected data stored in the memory of the spectrofluorometer. The software program multiplies the inverse of the corrected data with the raw spectral data, so that the resultant corrected emission is in real time.

The microprocessor-based instruments usually provide menu-driven operation, enhancing operating ease. Each set of programs for a particular application is labelled with a specific method number, so the operator can easily select the desired program. The operator can determine instrument conditions by means of interactive communication, with the instrument through the screen. The facilities available include real-time display and record of emission spectrum, excitation spectrum, concentration correction of spectra, data processing (e.g. smoothing, derivatives, peak selection and arithmetic manipulation), PMT tube voltage control and self-diagnostic features.

A typical example of a microprocessor-based fluorescence spectrometer is the PerkinElmer LS-5B. In, this instrument, the excitation monochrometer covers the wavelength range 230–720 nm, while the emission monochromator covers the wavelength range 250–800 nm. A red-sensitive PMT tube is used for the 650-800 nm region. The instrument provides a choice of five scan speeds ranging from 30 to 480 nm/min. A pre-scan mode combines both the abscissa and ordinate controls, and is designed to search automatically for and display the maximum excitation and emission wavelengths of an unknown fluorescing or phosphorescing material. On completion of the search, the excitation and emission monochromators display the peak wavelengths. The spectra are recorded on a printer-plotter connected to the instrument, Through the use of an RS 232 C interface, the instrument can be connected to a computer for instrument control and external data manipulation.

Linking a micro-computer to a fluorescence spectrometer confers several advantages. As we know, conventional single-beam fluorescence spectrometers produce excitation and emission spectra that are uncorrected (i.e. they reflect the wavelength dependence of the optical components of the instrument as well as the properties of the sample). Correction of the spectra requires the acquisition and storage of arrays of correction factors, which can be conveniently done by a

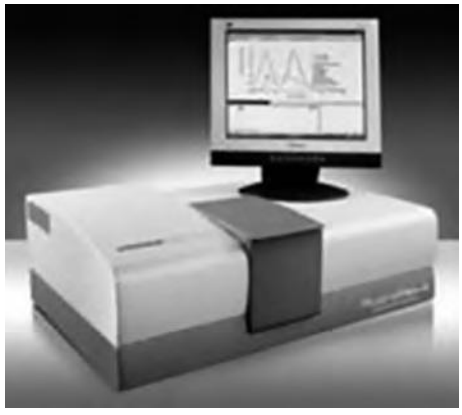


Figure 6.14 *Typical Spectrofluorometer model FluoroMax (Courtesy: M/s Horiba scientific)*

micro-computer. Micro-computers are also useful to expedite the acquisition of derivative fluorescence spectra, fluorescence polarisation values and difference spectra.

Each fluorophore is characterised by two spectra, excitation and emission. A three dimensional presentation of excitation wavelength, emission wavelength and intensity is required for its complete description. Such 3-D pictures can be conveniently obtained as isometric projections with a micro-computer. The advent of 16- and 32-bit computers ensures that the required high speed and large memory capacity are available in modem micro-computers.

Moder spectroflurometers are generally PC-based. Figure 6.14 shows a typical commercial instrument from M/s Horiba Scientific.

6.6.1 PerkinElmer Fluorescence Spectrometer Model LS-3

The optical system of the model LS-3 PerkinElmer Fluorescence spectrometer is shown in Figure 6.15. The source is a pulsed xenon flash tube which provides a continuum of energy over the spectral range of interest. Energy from the source is selected by ME and reflected by the toroidal mirror (MT) onto the entrance slit 2, from where it goes to the 1,200 lines per mm grating, a spherical mirror (MS) and exit slit 1. The energy diffracted by the grating emerging from slit 1 is of narrow wavelength. The wavelength of the excitation beam is determined by the setting of the grating, the angle of which is controlled by means of a stepper motor. The energy emerging from the slit 1 is directed onto the sample through the toroidal and plane mirrors.

Energy emitted by the sample is focused by the toroidal and plane mirrors onto the entrance slit of the emission monochromator. The monochromator consists of the entrance slit 1, a spherical mirror, a 960 lines per mm grating and exit slit 2. A narrow wavelength band of energy diffracted by the grating emerges from slit 2. The wavelength of the light falling on the PMT is determined by the setting of the grating, the angle of which is controlled by a stepper motor.

A microprocessor controls the stepper motors that move the excitation and emission monochromator gratings. The monochromators can be scanned over their ranges independently or synchronously. Also, they can be driven to selected points in their ranges. The normal range of the excitation monochromator is 230–720 nm, and that of emission monochromator as 250–800 nm. A red-sensitive PMT is necessary for measurements in the emission range of 650–800 nm.

The xenon source lamp is made of quartz and is very susceptible to contamination. It is therefore recommended that glass envelope of the source should not be at any time, touched by hand. If the solace becomes contaminated accidentally, it can be cleaned with ethanol or methanol using a piece of soft non-fluffy material.

The replacement of the source lamp becomes necessary if it shows deterioration characteristics. Symptoms of source deterioration are a noticeable drop in intensity (caused by blackening of the tube), low sensitivity, audible misfiring of the trigger supply and consequently resulting in an erratic display.

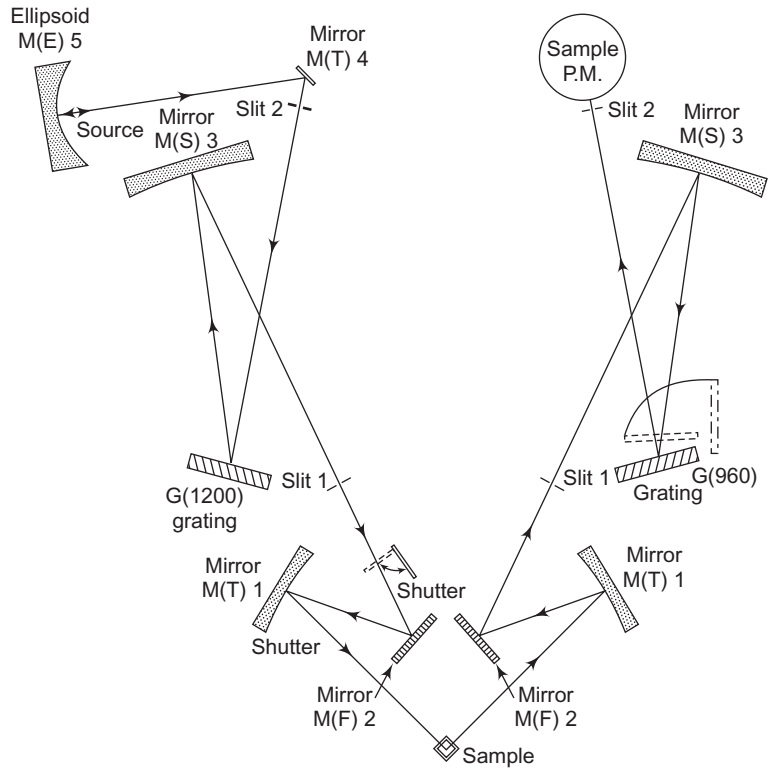


Figure 6.15 *Optical diagram of Perklin Elmer Fluorescence spectrometer Model LS-3*

6.7 MEASUREMENT OF PHOSPHORESCENCE

In the phosphorescence process, two transients are involved between the lowest excited singlet state and the ground state. This involves an inter-system crossing or transition from the singlet to the triplet state. A triplet state results when the spin of one electron changes, so that the spins are the same or unpaired. The first is a non-radiative transition, as the molecular energy level lowers to the vibrational level of a metastable triplet state (S to T_1). From the triplet state, the molecular energy level drops to the ground state; the radiation emitted during the transition T_1–G is called phosphorescence (Figure 6.1). The time involved in the transition is of the order of 10^{-3}s, which gives relatively long periods of phosphorescence emission. A characteristic feature of phosphorescence is an afterglow (i.e. emission which continues after the exciting source is removed). Since the energy level of the triplet state lies below the excited singlet levels, the phosphorescence is emitted at wavelengths longer than those of fluorescence. Because of the relatively long lifetime of the triplet state, molecules in this state are much more susceptible to deactivation processes, and only when the substance is dissolved in a rigid medium can phosphorescence emission usually be observed.

Phosphorescence emission is characterised by its frequency, lifetime, quantum yield and vibrational pattern. These properties form the basis for qualitative analysis, whereas the correlation of intensity with concentration gives the bases for quantitative measurements.

6.7.1 Phosphorescence Spectrometer

Phosphorescence is measured in a similar manner as that of fluorescence, except for employing a mechanical method of distinguishing between them by their time delay as shown in Figure 6.16. Light from the xenon lamp is passed through an excitation monochromator. It falls on the sample via a fixed slit system, and through a slotted-cam type shutter, which surrounds the sample cell. The slotted cam is driven by a variable speed motor, having the sample cuvette at the axis of rotation. The slots are so arranged that the sample is first illuminated and then darkened. While it is dark, its phosphorescence is allowed to pass and go to the phosphorescence monochromator, placed at right angles to the excitation radiation. The mechanical device accomplishing the modulation of the radiation from the light source incident on the sample and simultaneously modulating the luminescence radiation from the sample, which is incident on the photodetector, is known as phosphoriscope. The modulation is periodic and out of phase, so that no incident exciting or luminescent radiation reaches the photodetector during one phase, whereas only long decaying phosphorescence radiation reaches the photodetector during the other phase. Hence, the phosphoriscope allows measurement of phosphorescence in the presence of fluorescence and other scattered radiation. The phosphorescence spectrum is obtained by setting the excitation monochromator at the wavelength corresponding to a phosphorescence maximum and allowing be emission monochromator to sweep throughout its wavelength range.

Phosphorescence is not observed at room temperature, as the energy of the triplet state is readily lost by a collisional deactivation process involving the solvent. Therefore, phosphorescence is normally observed at reduced temperatures in solidified samples. The sample is generally placed in small quartz tube, which is then placed in liquid nitrogen (77 K), and held in a quartz Dewar flask. The incident radiation passes through the unsilvered part of the Dewar flask, and phosphorescence is observed through the sample part of the flask at right angles to the incident beam. The sample cell is immersed directly into the coolant, which is usually liquid nitrogen. Several commercial fluorimeters are provided with phosphorescence attachments.

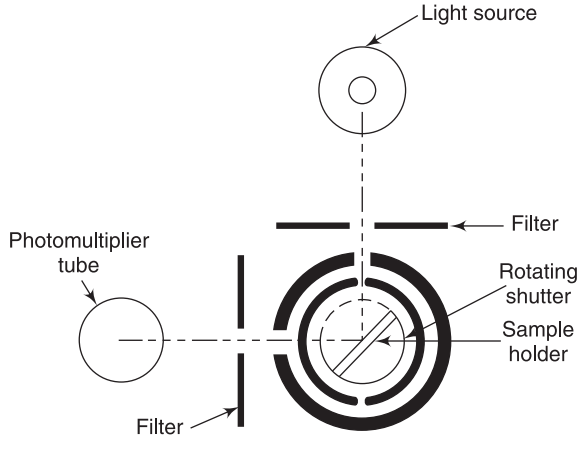


Figure 6.16 *Phosphorescence measurement method*

Until 1970, it was widely believed that solution phosphorescence could only be observed at cryogenic temperatures. More recently, several studies have demonstrated practicable ways of observing room temperature phosphorescence, and the method now holds a great promise in high performance liquid chromatography (HPLC). As in other photoluminescence methods, the main areas of application are likely to be in biochemical and environmental analysis.

Pulsed source-time resolved phosphorimetry which has been commercialised by PerkinElmer in their Model LS-5 Luminescence spectrometer gives the following advantages over the conventional mechanical chopper method:

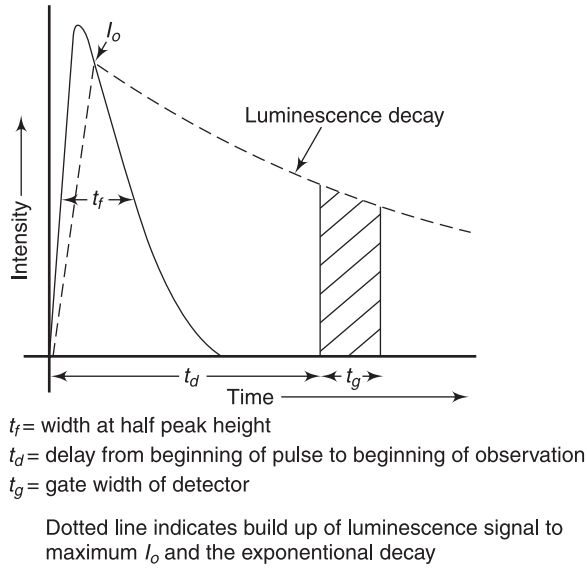


Figure 6.17 *Schematic diagram of events occurring during the excitation of a sample with a pulsed xenon source in phosphorescence mode (After Williams, 1981)*

- A pulsed source produces higher peak intensities than a continuously operated xenon lamp, resulting in greater phosphorescence emission intensity.
- Pulsed source phosphorimetry has the advantage of time resolution, compared with a mechanically modulated system, permitting the analysis of organic phosphors with short lifetimes in the range of 0.1–50 ms.

Figure 6.17 illustrates the events occurring during the excitation of a sample with pulsed xenon source in the phosphorescence mode. The xenon lamp produces a burst of energy with a width at half-peak intensity of less than 10 µs. During this period, the phosphorescence intensity rises to the peak value (I_o) and then theoretically decays exponentially. The signal from the detector (photomultiplier) are gated and both, the delay of the start of the gate after the start of the flash (t_d) and the duration of the gate (t_g) can be varied in multiples of 10 µs. During the cycle, a quantum-corrected reference PMT is used to measure the flash intensity and the signals from the sample and reference PMTs are ratioed to compensate for any source instability, and to provide corrected excitation spectra.

7

RAMAN SPECTROMETER

The Raman effect was experimentally discovered in 1928 by C.V. Raman. It is a powerful analytical tool for the quantitative analysis of complex mixtures, for locating various functional groups or chemical bonds in molecules and for the elucidation of molecular structure. The fields of application of Raman spectroscopy are quite diverse and include polymers, paints, semiconductors, corrosion, medicine, biology, ceramics, catalysts and catalysis and photochemistry, etc. It has often been observed that Raman spectroscopy is most effective when used in conjunction with IR data. While this is true in some cases, it is also an undeniable fact that Raman spectroscopy is complimentary to IR and can be used very effectively even alone.

Raman spectrometers basically employ one of the two technologies for the collection of spectra: dispersive Raman and Fourier transform Raman. Each technique has unique advantages and each is ideally suited to specific analyses.

7.1 THE RAMAN EFFECT

It was shown by Lord Rayleigh in 1871 that when a sample is irradiated with monochromatic light in the visible region, the majority of the light simply passes through the sample in the direction of the incident beam. However, a small amount (about 1 part in 10^5) is scattered by the sample in all directions, which can be observed by viewing the sample at right angles to the incident beam. The scattering of light at the same frequency as the incident radiation is called Rayleigh scattering. It can be shown that scattering from small particles is proportional to the inverse fourth power of the wavelength. This beautifully explains the blue colour of the sky and the redness of the setting sun, where scattering is predominantly due to particles of molecular dimensions. The sky is illuminated by sunlight, which is scattered by the atmosphere; the blue light intensity predominates, because of wavelength dependence of Rayleigh scattering.

It has been observed from spectroscopic investigations of light scattered from a sample illuminated with a monochromatic beam that about 1% of the total scattered intensity occurs at frequencies that are different from the incident frequency. This is called Raman scattering. This phenomenon results from an interaction between the incident photons and the vibrational energy levels of the molecules. The interaction is not simply absorption, but rather a transfer of a part of the energy of the photon to the molecule or vice versa.

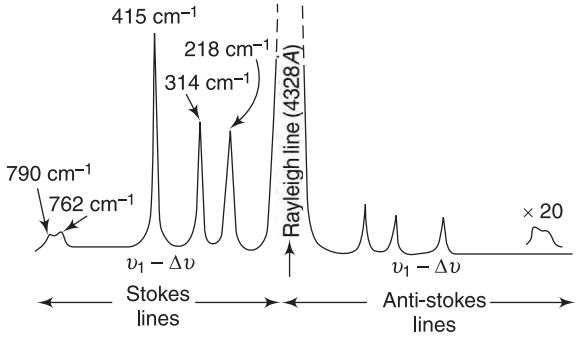


Figure 7.1 *Raman spectrum of CCL_4. This is only a schematic showing the stokes and anti-stokes lines. The wave number values on the diagram are Δr, that is the Raman shift, and correspond to vibrations of the molecule*

The equation for the energy interchange is

$$h\upsilon_1 + E_1 = h\upsilon_2 + E_2$$

$$\Delta E = h\upsilon_1 - h\upsilon_2 = \pm h\Delta\upsilon$$

where υ_1 and υ_2 are the frequencies of the incident photon and the scattered radiation respectively; E_1 and E_2 are the initial and final energy states of the scatterer and $\Delta\upsilon$ corresponds to energy transitions (rotational, vibrational or electronic), within the sample itself. The sample may be solid, liquid or gaseous. A plot of $\upsilon \pm \Delta\upsilon$ against photon intensity yields a spectrum of vibrational transition, which is analogous to, but not identical with, an IR spectrum this is shown in Figure 7.1. The scattered lines are called Raman lines and are characteristics of the vibrational modes of the substance irradiated and represent a sort of fingerprint of that substance.

It may be noted that the shift in the frequency of the scattered Raman lines is proportional to the vibrational energy involved in the transition. The shift is in fact a measure of the separation of the two vibrational energy states of the molecules. It is independent of the frequency of the incident radiation.

Absorption spectroscopy such as UV, visible and IR, etc., are resonance processes, in that they utilise photons which have energies equal to differences in energy-level spacings of the sample. In general, the Raman process is non-resonant, since the incident photon has energy much greater than the transition of interest. This is shown in Figure 7.2, in which the molecule is in virtual energy state. This is not a stationary state of the molecule and one can regard the absorbed photon as recognising the unstable state and being re-emitted by the molecule which then returns to one of its stationary states. If the final and initial IR states are identical, then this is Rayleigh scattering, and if they are different, we have Raman scattering.

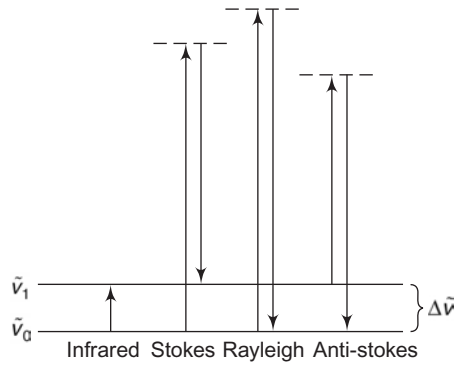


Figure 7.2 *Energy levels in Raman scattering. The* dashed lines *show non-existent states at levels determined only by the energy of the incoming photons*

The transition occurring below the Rayleigh or exciting line are termed Stokes lines ($\upsilon_1 + \Delta\upsilon$), while those above it are called anti-Stokes ($\upsilon_1 + \Delta\upsilon$). It is usual to scan the stokes side, because these bands are much more intense than the anti-stokes lines. Figure 7.3 shows the principle of Raman spectroscopy.

It may be observed that in Raman scattering, the absorption and emission of the photon

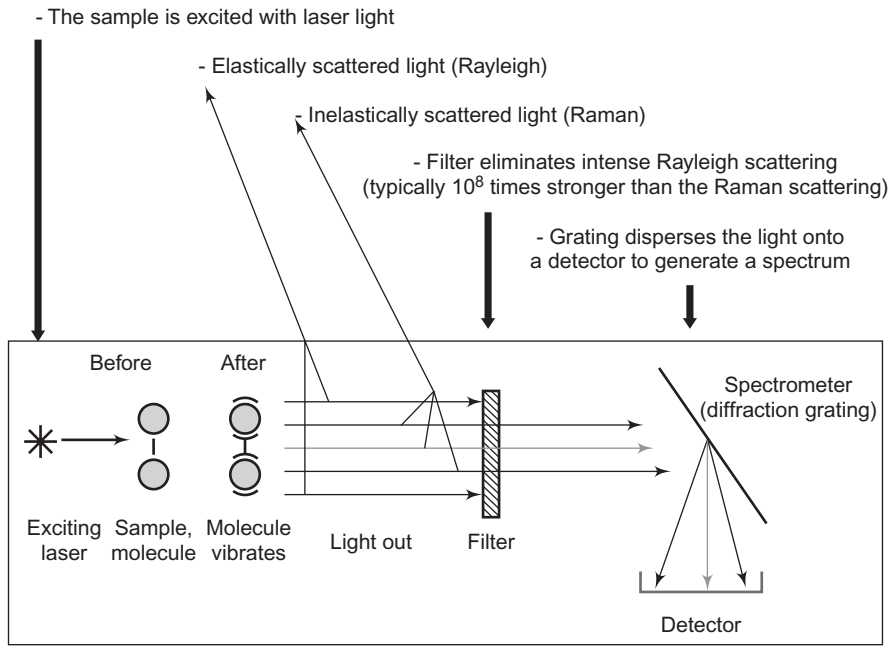


Figure 7.3 *The principle of Raman Spectroscopy (Windisch, 2003)*

are actually simultaneous events. The Raman effect differs from fluorescence in exactly this respect. In fluorescence, the incident photon is absorbed and the molecule attains an excited stationary state, which has a well-defined lifetime. Thus, the absorption and emission steps are actually sequential.

Raman spectroscopy has certain intrinsic advantages over IR spectroscopy. In Raman spectroscopy both the incident and scattered photons are typically in the visible region, while their difference ($\Delta\upsilon$) is in the IR. This enables the use of excellent detectors, like photomultiplier tubes, which can have single photon counting capability and are more sensitive than the IR counterparts.

7.2 RAMAN SPECTROMETER

The Raman spectrum can be observed by illuminating the sample with monochromatic light and observing the light scattered at right angles to the incident radiation. Raman intensities are approximately 0.01% of the incident radiation and therefore, the Raman spectrometer must employ intense source of radiation, sensitive detector, high light-gathering capability coupled with freedom from extraneous scattered incident light.

Raman spectrum can be recorded in the following two different ways:

- By focusing the spectrum from the prism or grating onto a photographic plate and measuring the line frequencies and intensities using external equipment after the plate is developed; and
- By focusing the spectrum produced by the monochromator on to a photomultiplier tube amplifying the detected signal and recording it. This method has the advantage, that the response to the Raman line intensity is linear, which greatly simplifies quantitative analysis.

The earlier Raman instruments made use of a cylindrical sample cell (Raman tube) placed inside a helical mercury-arc lamp, which radiates energy at 435.8 or 546.1 nm. Between the mercury lamp and the sample is a filter jacket (organic dye), which isolates monochromatic light of either 435.8 or 546.1 nm. This monochromatic radiation irradiates the sample tube from all sides and the light which is scattered out of the end of the tube is introduced into the monochromator. The mercury-arc source is quite inefficient, as a 2.5 kW arc lamp radiates about 50W at 4358 A, of which only about 1 W is actually effective in exciting the sample. The mercury-arc lamp also generates considerable heat, which must be dissipated by circulating the filter solution, that also serves as a coolant Glass absorption filters are not suitable, as too much heat is generated.

Modern Raman instruments employ laser as an excitation source and use computers for data acquisition and control. The major components in a Raman system are:

- A source of monochromatic radiation
- Sample compartment and associated optics
- Spectrometer or monochromator
- Detection system
- Computer

Figure 7.4 shows the arrangement of these components in a Raman spectrometer. The minimum system would consist of a laser, a double monochromator and a photomultiplier tube detector with a recorder. Today, the most modern Raman instruments are completely integrated into a single unit and computer controlled, are interlocked for laser safety, have automated protocols for calibration and offer large spectral libraries. These advances make the collection and utilisation of Raman spectra a routine exercise.

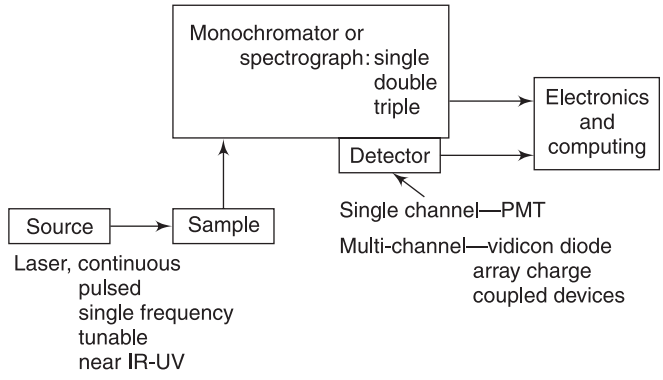


Figure 7.4 *Block diagram of a laser-based Raman spectrometer. The diagram also shows some choices associated with the selection of a Raman system*

7.2.1 The Source

In modern Raman spectrometers (Figure 7.5), lasers are used as a photon source due to their highly monochromatic nature and high beam fluxes. This is necessary as the Raman effect is weak, typically the Stokes lines are ~10^5 times weaker than the Rayleigh scattered component.

Figure 7.5 *Raman Spectrometer (Courtesy: M/s Evans Analytical Group)*

The helium-neon laser which emits highly monochromatic light at 632.8 nm is a commonly used excitation source in the modern Raman spectrometers. This wavelength being in the red region of the spectrum, there is a loss of scattering intensity associated with the use of this longer wavelength and the photoelectric detectors are less sensitive. These problems can be overcome, using lasers with power outputs in the range of 5–30 mW. They produce as good intensity Raman spectra as the older arc sources. The longer wavelength, on the other hand, offers very significant advantages. Usually the Raman spectrum cannot be obtained, if the sample absorbs the exciting wavelength. At 632.8 nm, fewer samples have interfering absorption bands. Another difficulty in Raman spectrometry is that of the sample fluoresced when irradiated; the spectrum would be observed by the fluorescence spectrum. Because red radiation is frequently less effective in generating fluorescence, this problem is automatically minimised.

Howard (1986) explains that the argon-ion (visible) laser is the most commonly used source in Raman spectrometers. However, tuneable dye lasers are increasingly being used and the present range of interest includes the visible and UV. The type of laser required depends on the sample and the Raman technique to be used. Specialised Raman methods may sometimes involve the simultaneous use of several lasers of different types.

Raman Spectrometer usually employs visible laser radiation. Typical laser wavelengths are 780, 633, 532 and 473 nm, although others can also be used. One advantage of using shorter wavelength lasers is the enhancement in the Raman signal that occurs at these wavelengths. The efficiency of Raman scatter is proportional to $1/\lambda^4$, so there is a strong enhancement as the excitation laser wavelength becomes shorter.

7.2.2 Sample Chamber

In Raman spectrometers, samples may be examined as solids, liquids or solutions, or in the gas phase. However, a Raman spectrum is most easily obtained using liquid samples. The narrow and readily collimated laser beam can be simply focused into a capillary tube containing the liquid. Water is an excellent solvent for Raman spectroscopy, because it has few interference bands and dissolves readily large numbers of inorganic materials.

For study of Raman spectrum of gases, the gas sample can be placed inside the laser cavity. In case of solid samples, the laser beam is focused into a capillary tube containing the powdered solid. Only a few milligrams of a powdered solid sample are adequate to give good spectra.

Most Raman spectrometers for material characterisation use a microscope to focus the laser beam to a small spot (<1 – 100 μm diameter). Light from the sample passes back through the microscope optics into the spectrometer.

7.2.3 The Spectrometer

It was explained that the intensity of Raman lines is much weaker than the exciting line. Thus, an excellent optical system would be needed to ensure stray light rejection. This is most important if it is required to measure close to the Rayleigh line. This is usually achieved by using two or three monochromators. The use of holographic gratings and multiple slits ensures proper levels of luminosity.

The *grating* has a strong influence on spectral resolution and instrument throughput. The higher the number of grooves on the grating, the wider the dispersion angle of the exiting rays and higher the resolution. Grating response is also wavelength dependent, so the dispersion (resolution) across the wave number axis is not linear, but instead, the dispersion becomes greater at higher wave numbers. For this reason, spectral resolution must be stated for a specific wave number and will vary across the spectrum.

7.2.4 The Detector

The most commonly used detector is the photomultiplier tube, which provides excellent sensitivity, low noise and large dynamic range. This is however a single-channel detector and requires point-by-point spectral data acquisition and consequently long scan times. Multi-channel detectors are presently assuming more popularity, with the obvious advantages for time resolved measurements. Multi-channel detector may be a one-dimensional diode-array, with 512 or 1024 pixels (each 2.5 mm high by 25 μm wide) or two-dimensional type like vidicon or charge coupled device with arrays of 512 × 512 pixels. Multi-channel detectors offer certain advantages over photomultiplier tubes for many applications, as they permit the collection of large portions of a vibrational spectrum in seconds.

The CCDs are also commonly used for dispersive Raman spectrometers. They are silicon devices with very high sensitivity. The detecting surface of the CCD is a two-dimensional array of light-sensitive elements, called pixels (usually each pixel is <30 μm). Each pixel acts as an individual detector, so each dispersed wavelength is detected by a different pixel or closely spaced group of pixels.

CCD detectors have a large wavelength response region, routinely extending from approximately 1,100 nm and down into the UV range. Many common CCDs have very weak responses for the higher wavenumber response of the NIR laser, and going any higher in laser wavelength rapidly disqualifies the CCD as a viable detector.

7.2.5 Computer

The computers incorporated in the modern instruments are essential for spectral manipulation (addition, subtraction, self-deconvolution, etc.). They are indispensable when using a multi-channel detector and offer a major advantage with a single-channel system. Good software and graphics facilities for Raman spectroscopy are constantly under intensive development.

Raman spectroscopy is a rapidly developing analytical tool. Several new advanced techniques are under development, with a view to enhance its utility. There has recently been substantial interest in using Fourier transform techniques, to collect Raman data. This technique would appear to have a bright future, particularly as it can be add-on to IR spectrometer.

7.3 PC-BASED RAMAN SPECTROMETER

De Graff et al. (2002) illustrate the details of PC-based laser Raman Spectrometer using CCD detection. The layout is shown in Figure 7.6. The beam from a 532-nm linearly polarised laser is passed directly into the sample without focusing. To observe polarisation effects, a half-wave plate is placed between the laser and the sample to rotate the polarisation of the beam. In order to measure depolarisation ratios quantitatively, a polarisation analyser is placed between the sample and the fibre optic. Samples are held in a standard 1-cm glass fluorimeter cell and scattered light is collected at a 90° angle from the laser beam path. A notch filter is placed between the sample cell and the fibre optic coupling to reduce the Rayleigh scattered 532-nm excitation light. The notch filter provides a 2-nm bandpass width for the notch filters. This corresponds to ~70 cm^{-1} at the 532-nm Nd/YAG line; however, the wavelength of the notch is rather angle-dependent. A collecting lens focuses the scattered light into the fibre optic patch. The fibre optic patch to the spectrometer has a 400 µm diameter. A box surrounds the sample, filter, lens and fibre optic cable end in order to prevent room light from reaching the detector. The other end of the fibre optic cable is connected to the spectrometer which contains a 50 µm slit and a 600-groove/mm grating with a blaze wavelength of 400 nm. The multi-pin electronics cable from the spectrometer leads to an A/D converter card in a Pentium PC. The software is used to collect and process the data.

The spectrometer software allows integration times to be varied from 1 ms to 65 s. Because of the relative weakness of the Raman signal in comparison to pixel noise, a dark spectrum (with laser light blocked) must be subtracted from the overall signal. The software explicitly allows for dark spectrum subtraction.

Like IR spectrometry, Raman spectrometry is a method of determining modes of molecular motion, especially the vibrations, and their use in analysis is based on the specificity of these

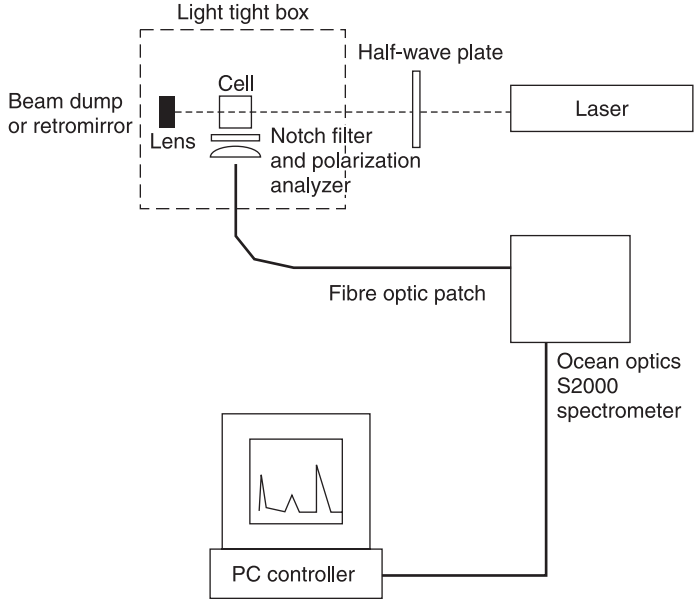


Figure 7.6 *Block diagram of Raman Spectrometer (De Graff et al, 2002)*

vibrations. The methods are predominantly applicable to the qualitative and quantitative analysis of covalently bonded molecules rather than to ionic structures.

7.4 FT RAMAN SPECTROMETER

FT-Raman was developed to overcome some of the problems occasionally encountered in dispersive Raman spectroscopy. An FT-Raman instrument typically employs a 1 μm excitation laser, an interferometer and a high-sensitivity near-infrared detector. By using the longer wavelength excitation laser, there is less energy supplied, so the virtual state is lower and less likely to overlap an upper electronic state. This greatly reduces fluorescence interferences.

Indium gallium arsenide (InGaAs) or liquid nitrogen-cooled germanium (Ge) detectors are typically used for FT-Raman spectroscopy. These detectors are very sensitive, but are still less sensitive for near-infrared radiation than the silicon CCD is for visible radiation. The advantages of the Fourier transform technique are necessary to provide the sensitivity to extract functional spectral information from this lower intensity signal.

By virtue of the Fourier transform technique, FT-Raman offers:

- High resolution with minimal throughput loss
- Measurement of all wavelengths at once
- Increased signal-to-noise by signal averaging
- Superior wavelength accuracy due to the internal calibration inherent to an interferometer

The interferometer employs a beam splitter (Figure 7.7) optimised for near-infrared radiation, which divides the incoming Raman scatter into two optical beams, one transmitted and one reflected. The reflected beam travels to and reflects off a flat mirror that is fixed in place. The transmitted beam travels to and reflects off a flat mirror attached to a mechanism that allows the mirror to move a short distance (typically a few millimetres) away from the beam splitter.

The two beams recombine at the beam splitter where, because they travelled different distances to and from the mirrors, they constructively and destructively interfere with each other. The moving mirror has a constant frequency and fixed motion, so this interference is modulated. The resulting interferogram has the unique property that every data point has information about every frequency of the Raman scatter collected from the sample.

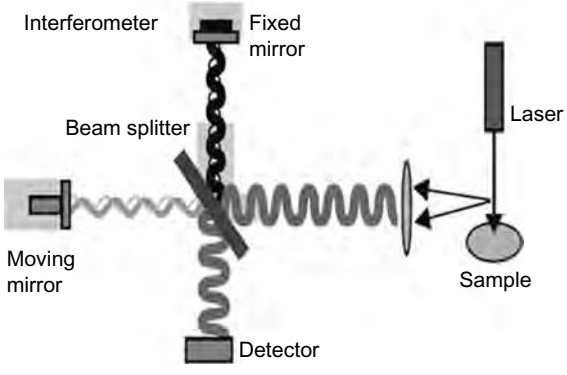


Figure 7.7 *Interferometer used in FT Raman spectroscopy*

Vibrational spectra are typically presented as frequency spectra (a plot of intensity at each individual frequency) because the measured interferogram signal is not readily interpreted. The individual frequencies are decoded using the well-known mathematical technique called Fourier transformation. The computer performs this transformation and the desired spectral information is presented as shown in Figure 7.8.

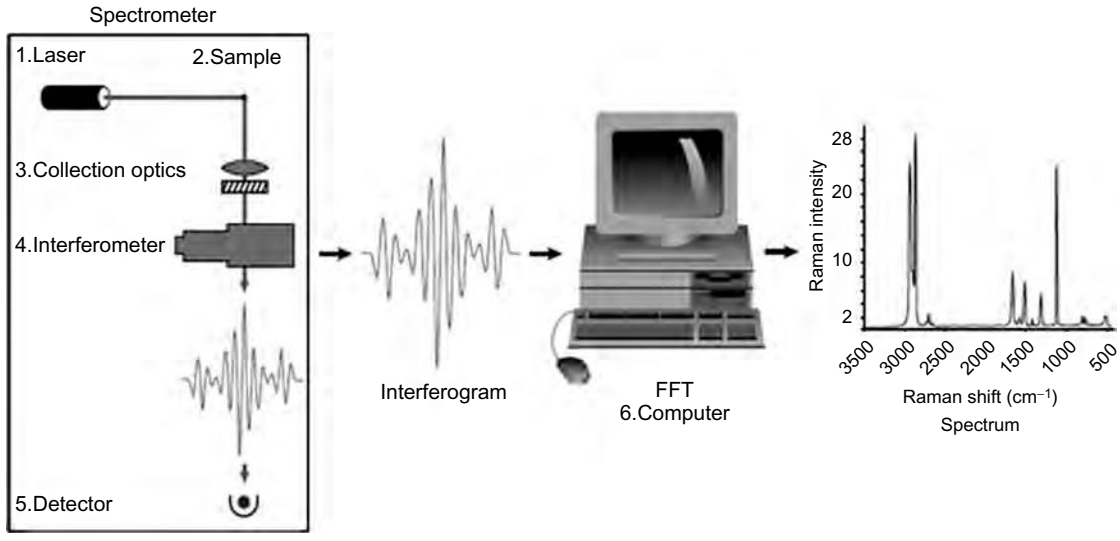


Figure 7.8 *FT Raman Spectrometer (Courtesy: M/s Thermo Electron Corporation)*

Figure 7.9 shows the optical diagram of the FT Raman Spectrometer. Light from a near-infrared laser source is focused onto the solid sample that is held on a support. The support also acts as a screen to prevent laser light directly entering the optical system of the FTIR spectrometer. The scattered light is then focused by means of a large ellipsoidal mirror and a collimating lens, so that it enters the FTIR optical system and strikes the beam splitter. From there on the optical system is very similar to that of the FTIR spectrometer.

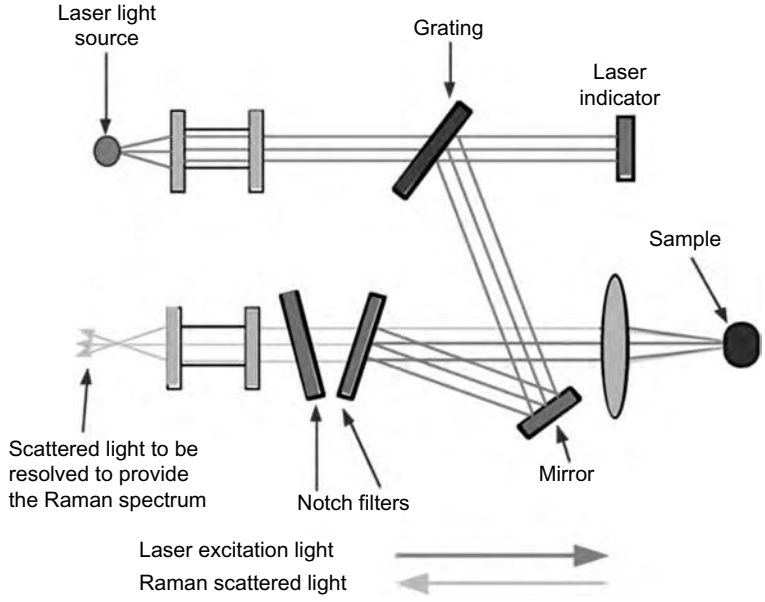


Figure 7.9 *Optical System of FT Raman spectrometer*

Light from a suitable laser source is collimated onto a diffraction grating that selects the light of the wavelength to be used to produce Raman scattering. Some of the light is focused onto a secondary photosensor, the output of which is used to indicate the activity of the laser.

Light of the selected wavelength is directed by means of a mirror onto a reflecting system that also acts as a notch filter to remove any light of extraneous wavelength. The reflected light is focused onto the sample that generates the scattered light. The scattered light is directed back through the optical system and through a second notch filter that removes any traces of the incident light. The scattered light is collimated and is then ready to be resolved on a suitable diffraction grating to produce the required Raman spectrum.

7.5 INFRARED AND RAMAN MICROSPECTROMETRY

Both the Raman and the IR spectrum yield a partial description of the internal, vibrational motion of the molecule in terms of the normal vibrations of the constituent atoms. Neither types of spectrum alone give a complete description of the pattern of molecular vibration, and, by analysis of the difference between the Raman and the IR spectrum, can help procure additional information about the molecular structure.

The coupling of optical microscopy to vibrational spectroscopy (IR and Raman) enables the chemical characterisation of samples or domains as small as 10 μm (IR) or 1 μm (Raman). These techniques are applied to analyse polymers, rubbers, papers, and organic and inorganic materials. Results are obtained by spectral interpretation or imaging the functional groups. This has opened up the possibility of Raman microstructural investigations on the μm scale. A schematic diagram of a typical system (ISA Jobin Yvon LABRAM micro-Raman) is shown in Figure 7.10.

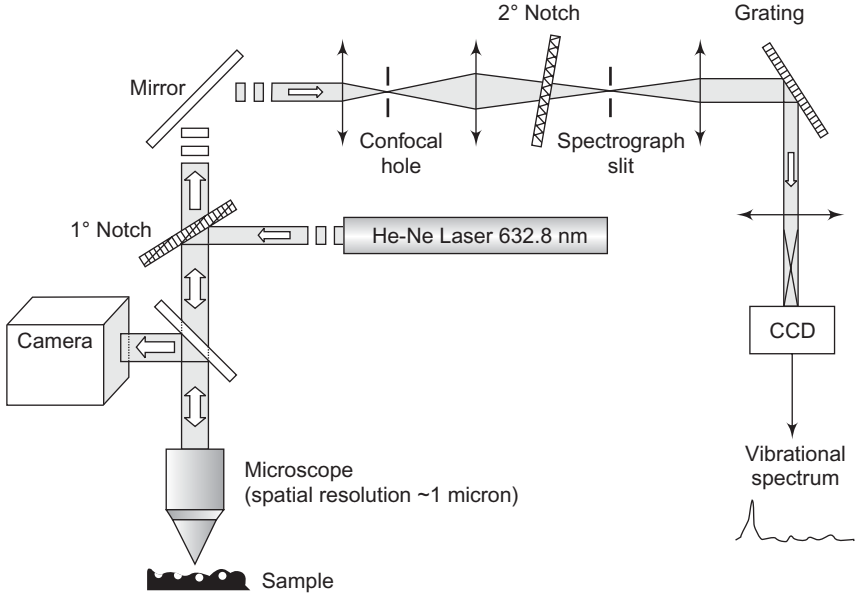


Figure 7.10 *Micro-Raman apparatus*

The system uses two excitation lines 632.8 nm (He-Ne) and 784.8 (diode). It is completed with an XY motorised stage with spatial resolution of about 1 μ and with an auto focus system which allows to obtain Raman mappings on micrometric scale in three dimensions.

Although there are many different molecular spectroscopes, the most versatile ones for identification are IR and Raman spectroscopies. The basic differences in the two methods are worth noting (Katon et al. 1986) Raman Spectrometry is normally carried out in the visible region of the spectrum, and thus normal microscopes, with the usual glass optics are quite satisfactory for coupling to the spectrometer. Because the source for Raman Spectrometry must be monochromatic, an argon-ion laser is typically used. On the other hand, glass is not transparent in the IR region. Therefore, all IR microscopes must use reflecting optics and cassegrainian objectives or mirrors of the proper configuration, for focusing and collecting the incident and transmitted beams, respectively.

8

PHOTOACOUSTIC AND PHOTOTHERMAL SPECTROMETERS

8.1 PHOTOACOUSTIC SPECTROSCOPY

Photoacoustic (PA) spectroscopy is the measurement of the effect of absorbed electromagnetic energy, particularly in the visible region, on matter by means of acoustic detection. The discovery of the PA effect dates back to 1880 when Alexander Graham Bell showed that thin discs emitted sound when exposed to a beam of sunlight that was rapidly interrupted with a rotating slotted disk. The absorbed energy from the light causes local heating and through thermal expansion, a pressure wave or sound is generated. Later, Bell showed that materials exposed to the non-visible portions of the solar spectrum (i.e. IR and UV) can also produce sounds.

A PA spectrum of a sample can be recorded by measuring the sound at different wavelengths. This spectrum can be used to identify the absorbing components of the sample. The PA effect can be used to study solids, liquids and gases (Kreuzer, 1978).

The principle of PA spectroscopy is explained by Rosencwaig and Gersho (1976) and is illustrated in Figure 8.1. When a light absorbing substance is subjected to modulated incident radiation, a fraction of the radiation falling upon the sample is absorbed and results in excitation. When a molecule absorbs energy, it must lose an equivalent quantity of energy via photodecomposition, photoluminescence or thermal relaxation. The last process is usually predominant and therefore, non-radiative de-excitation processes, which normally occurs, gives rise to the generation of thermal energy within the sample.

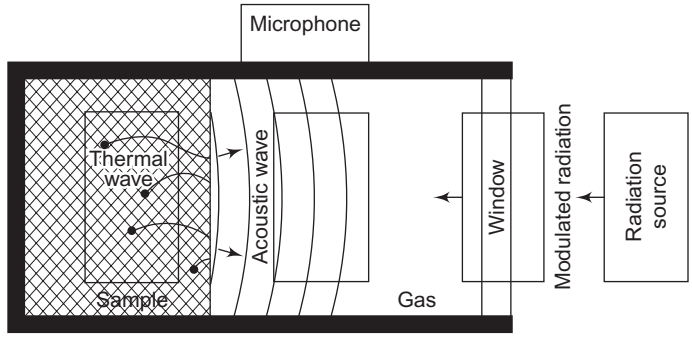


Figure 8.1 *Principle of photoacoustic spectroscopy*

If the incident radiation flux is periodically interrupted (modulated), the energy released by the sample will also be periodic, and if contained in a cell having a constant volume, will give rise to acoustic pressure pulses. This is called PA signal. Usually, a condenser microphone is used for the detection of and measurement of the PA signal. In effect, the sample is enclosed with a microphone inside a sealed cell containing a gas and fitted with an acoustically isolated window for the illumination of the sample. The modulated variations in the temperature of the surface of the sample result in the generation of an acoustic wave in the gas. This wave propagates through the volume of the gas to the microphone, where signal is produced.

It may be observed that the PA signal is a function of the following two processes occurring in the sample:

1. The absorption of electromagnetic radiation (specified by the absorption coefficient β)
2. Thermal propagation in the sample (specified by the thermal diffusivity X).

It is possible to define a characteristic thermal diffusion length μs. The thermal wave produced in the sample is heavily damped and may be fully damped out within a distance $2\pi\mu s$. Thus, only those thermal waves originating from a depth less than or equal to μs will give an appreciable contribution to the PA signal intensity.

8.1.1 System Components

The PA signal is normally of rather small magnitude and it is essential to maximise the signal-to-noise ratio. As the magnitude of the PA signal is proportional to the incident power of the radiation falling upon the sample, high intensity sources are generally employed in PA systems. Lasers operating in the near-UV, visible and mid-IR region of the spectrum have been used. However, in majority of the applications, a relatively wide spectral range is required and continuum sources are essential. Usually, 300 W xenon arc lamps are used for studies in the UV-visible-near IR regions. The PA signal is produced when the incident radiation is modulated. Usually, amplitude modulation is employed, which is implemented either electronically or by a mechanical chopper. A grating monochromator is used to achieve wavelength dispersion of the incident radiation. Although condenser microphones are widely used as detectors in PA spectrometers, piezoelectric transducers are becoming more popular (Wetsel, 1980).

The PA signal is a vector which has magnitude and phase components. Therefore, a lock-in amplifier is commonly used for signal recovery from the PA cell, which has the same frequency and a particular phase relationship to the modulation waveform.

The output power of the source in PA spectrometers is not constant with the variation in the wavelength. Therefore, any PA spectrum obtained will be observed as the product of the true PA spectrum, and the power spectrum of the source. The following methods are employed to obtain a corrected PA spectrum:

- Using a double-beam spectrometer with two matched PA cells, one normally containing finely produced carbon as a reference absorber. The ratio of sample and the reference signals provide corrected spectrum.
- Using a double-beam system with a pyroelectric detector in the reference channel. The greater sensitivity of the pyroelectric detector results in only about 10–20% of the source power to be

used in the reference channel. This has the advantage of giving corrected PA spectrum in real time, together with automatic correction for any fluctuation in source intensity.

- Having a single-beam system and producing digital storage for the uncorrected sample spectrum and a spectrum of carbon black. The corrected PA spectrum is obtained by calculation on a point-to-point basis. The technique, however, does not correct for source drift.

8.1.2 Typical Photoacoustic Spectrometers

Figure 8.2 shows the experimental setup for a typical PA spectrometer. In this setup, white light (Halogen or Xe arc lamp, 150–1,000 W) passes through a monochromator (180–3,800 nm). A mechanical chopper modulates the light and the beam goes to the PA cell. Cell must have an optical window to allow radiation to reach the testing sample placed inside. A chopper driver (1 Hz–3 kHz) gives the pulse reference that synchronises the PA signal (1 μV–2 mV) collected by the electret microphone (20–50 mV/Pa), which is monitored by a lock-in amplifier. The PA experiment runs varying two main variables: wavelength or frequency.

Blank and Wakefield (1979) described another arrangement of double-beam PA spectrometer for use in the UV, visible and near-infrared spectral regions. A functional diagram of the system is shown in Figure 8.3. The spectral range is 240–250 nm, and it is determined by the output of the lamp at both ends of the spectrum. The lamp used is 300 W xenon arc lamp, with an integral parabolic reflector and a sapphire window. All transmitting optical components except filters are fabricated in UV-grade quartz and UV-grade sapphire, including lenses. The UV sapphire and

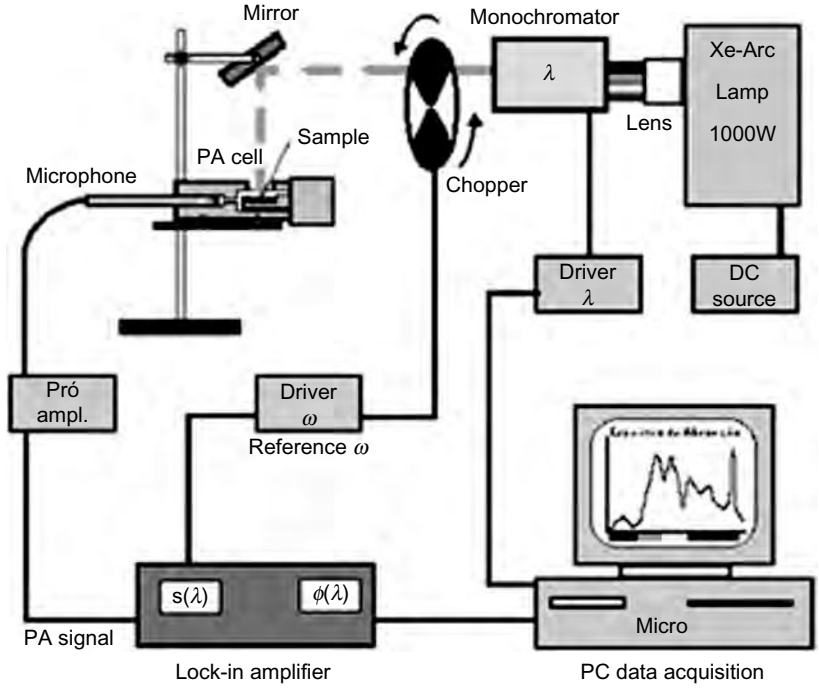


Figure 8.2 *Block diagram of photoacoustic spectrometer (Bento, 2002)*

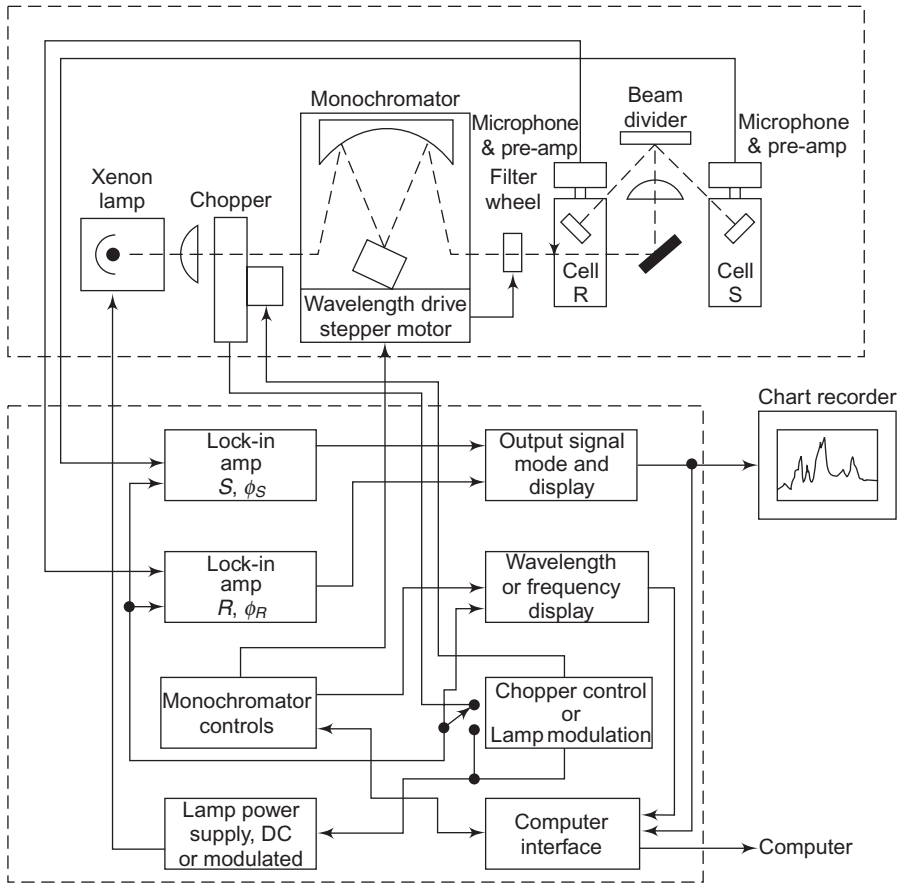


Figure 8.3 *Block diagram of a double-beam photoacoustic spectrometer (Redrawn after Blank and Wakefield, 1979)*

quartz are stable and transmit well in the entire spectral region, except that UV quartz exhibits some absorbing bands in the near-infrared.

Either the radiation beam can be modulated with an electromechanical chopper or the arc lamp can be current-modulated. Although, the latter method enables to have higher modulating frequencies (from 100 to 10,000 Hz), desired for control of μs, most designs employ electromechanical modulation at frequencies from 10 to 1,000 Hz, due to better signal-to-noise ratio capability. Hereafter, the radiation enters Ebert monochromator, which carries four gratings mounted in a turret. The gratings have 2,360, 1,180, 590 and 295 grooves/mm and are blazed at 300, 500, 1,200 and 2,100 mm, respectively. Wavelength scanning is done by a stepper motor drive, and in a scan, the grating interchange is automatic. Near the exit slit, is a wheel containing absorption and interference order sorting filters. The filters are automatically selected as wavelength is changed.

After emerging from the monochromator and filter, the beam is converged and divided into two parts by a beam divider. After division, each of the two beams is directed into its respective PA cell, reference cell and the sample cell. The pressure fluctuations in each of the cells are fed to the

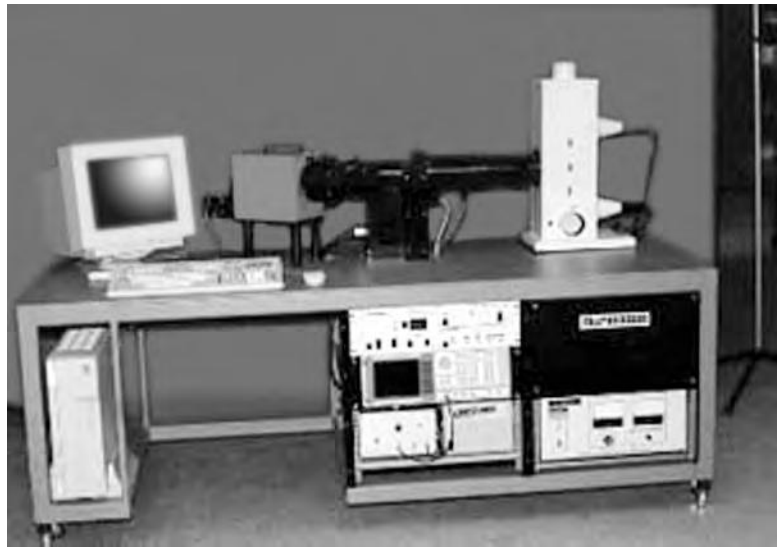


Figure 8.4 *Photoacoustic spectrometer (Courtesy: M/s McPherson Inc., USA)*

microphone and adjacent pre-amplifier signal conditioners, before it is sent to the lock-in amplifier. The lock-in amplifier can output the signal phase or magnitude at adjustable fixed phase. The phase is measured relative to that of the modulator. The signals from the sample and reference cells are called S&R respectively. Although several modes of operation are possible, the most commonly used mode till now is S/R, wherein R is derived from a saturated reference material such as carbon black. The resulting signal from each cell consists of the sample spectrum, with a superimposed system power spectrum, due to the lamp output and optics. The reference spectrum from carbon black consists of the system power spectrum. The ratio S/R divides out the unwanted power spectrum leaving only the true sample spectrum. The chopping frequency is continuously variable and either wavelength or frequency can be displayed. Figure 8.4 shows a PA spectrometer from M/s McPherson Inc., USA.

Cox et al. (1980) illustrated a double-beam PA spectrometer, which employed a linear scanner to alternately illuminate S&R materials contained in a cell, which employs a single microphone. Data acquisition requires only one pre-amplifier and one lock-in amplifier. The linear scanner is an analog device, for which the angle of deflection of the shaft is proportional to the power input. The basic function of the scanner is to move the monochromatic radiation, which is in fact an image of the source from sample to reference and back.

8.1.3 FTIR Photoacoustic Spectroscopy

It has been found that there is a poor signal-to-noise ratio of the PA response, and therefore, repeated experiments and ensemble averaging of the results would be required. Kirkbright (1984a, b) reports advances in Fourier transform IR PA spectrometry and correlation techniques as a possible method of overcoming the limitations of the conventional PA spectroscopy.

Figure 8.5 shows the measurement principle of FTIR PA spectroscopy. The solid sample to be measured is placed in a sealed vessel to which small microphone is attached. When a modulated IR light beam is absorbed by the sample, heat is generated due to the incident light. This heat causes

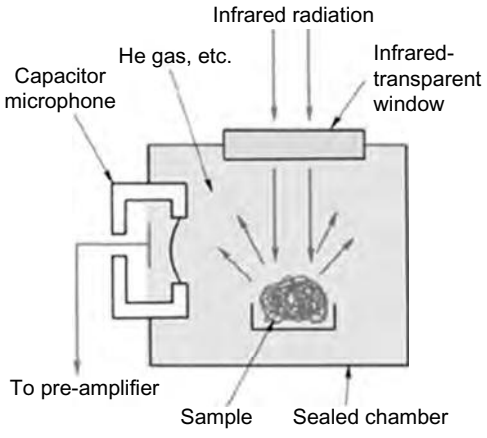


Figure 8.5 *Shows the measurement principle of FTIR photoacoustic spectroscopy*

pressure changes in the surrounding gaseous layer, which can be detected by the high-sensitivity microphone. The signals from the microphone are acoustic interference waves. Applying Fourier transformation to these signals produces a spectrum similar to an absorption spectrum.

For an optically transparent sample, the PA signal reflects the sample optical properties, regardless of the sample thermal properties. However, only when the sample is optically opaque and thermally thick, the PA signal is proportional to the sample absorbance coefficient and the thermal diffusion length.

In case of solid samples although the sample is optically opaque, it is said to be photoacoustically transparent. Since the thermal diffusion length is a function of the modulation frequency of the incident light, increasing the modulation frequency and reducing the thermal diffusion length, makes an optically opaque sample photoacoustically transparent. Also, as the PA signal is proportional to the thermal diffusion length for a thermally thick sample, the depth from which the PA signal is emitted can be changed by changing the modulation frequency. In other words, increasing the modulation frequency reduces the depth and the signal intensity. For a normal Michelson interferometer, the modulation frequency f is expressed as $f = 2V\nu$. It is dependent on the velocity of the movable mirror V (cm/s) and the wavenumber ν (cm^{-1}). Therefore, f decreases as the movable mirror speed decreases. Conversely, as the thermal diffusion length increases, information is obtained from a deeper depth from the surface.

A schematic view of the FFT PA spectrometer (Arnott et al. 1999) which measures in situ light absorption by aerosol is shown in Figure 8.6. The laser beam power is modulated at the acoustic

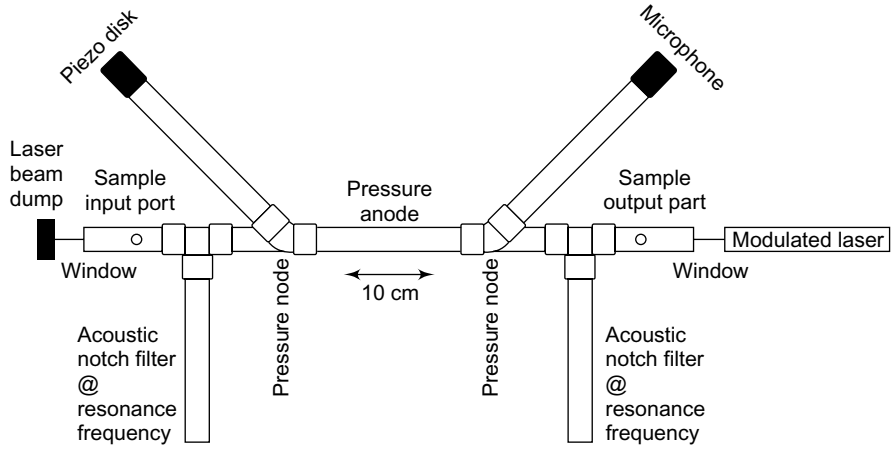


Figure 8.6 *Schematic view of the photoacoustic spectrometer (Redrawn after Arnott et al., 1999)*

resonance frequency of the PA spectrometer. Light absorbing components (gas or aerosol) convert laser beam power to an acoustic pressure wave through absorption-induced gas expansion. A microphone detects the acoustic signal, and hence a measure of light absorption is produced. The piezoelectric disk is used to determine the acoustic resonance frequency of the spectrometer and the resonator quality factor (gain) to calibrate the system. Acoustic notch filter block most of the air-flow pump noise and spurious sound produced by absorption of light on the windows from entering the spectrometer.

Light absorption in dimensions of inverse distance, is determined from the acoustic pressure measured with (calibrated) microphone and corrected for pre-amplifier gain, resonator quality factor Q; resonance frequency f_0; the Fourier component of laser beam power at f_0; and resonator cross-sectional area.

A block diagram of the prototype PA spectrometer and detection electronics is shown in Figure 8.7. A pump was used to draw outside air through the spectrometer with a volume flow rate of 3 l min^{-1}. Considering the volume of the spectrometer, this gives a flow rate time constant of about 8 s. Acoustic notch filters were placed between the inlet and the spectrometer, and between the pump and the spectrometer to reduce the ambient and pump generated sound spectral energy at and near the resonance frequency of the spectrometer. These are in addition to the filters on the spectrometer. The laser power is modulated at the spectrometer acoustic resonance frequency using the square wave output of the waveform generator. The waveform generator provides timing for phase sensitive detection of the microphone signal by the lock-in amplifier and the Fast Fourier Transform (FFT) analyser. The microphone signal is amplified with a low noise pre-amplifier (typically by a factor of 100) and is band pass filtered (50 Hz band width centred at 500 Hz). Pre-amplification is necessary to provide adequate signal level for the FFT analyser. The FFT analyser determines the magnitude of the Fourier component of acoustic pressure at frequency f_0. The FFT analyser is also used to calibrate the spectrometer by issuing a single cycle of a sine wave to the piezoelectric disk and obtaining the ratio (transfer function) of the spectral resonator response to

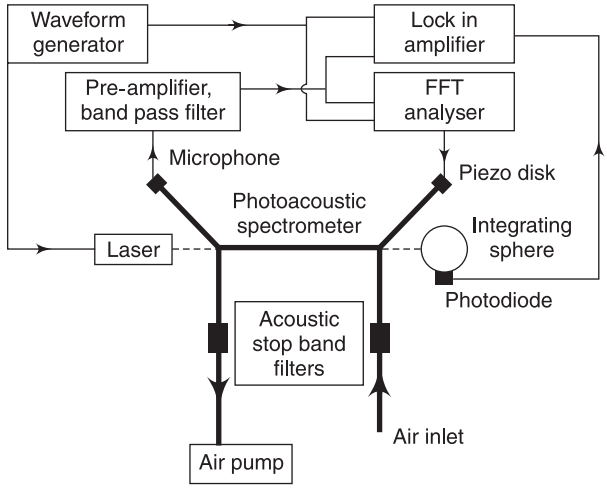


Figure 8.7 *Block diagram of the photoacoustic spectrometer and detection electronics (Adapted from Arnott et al., 1999)*

the issued spectrum. The computer receives the transfer function, and fit it to a standard resonance response function as a function of Q, f_0, and the peak of the transfer function. The lock-in amplifier is used to determine the phase of the microphone signal relative to the waveform generator timing signal, and also determines the photodiode response at f_0. The photodiode is calibrated with a laser power metre.

The entire measurement procedure is automated with use of the computer. The pre-amplifier communicates to the computer via a serial port. All other instruments are equipped with IEEE 488 communication capability. National Instruments Lab View software is used to provide a convenient user interface. Each measurement of light absorption is preceded with a resonator calibration using the FFT analyser. The lock-in time constant was set to 10 s for light absorption measurements, and 128 averages lasting 4 s each are used with the FFT analyser.

Muller et al. (2003) present an all solid states, PA spectrometer for highly sensitive mid-IR trace gas detection. A complete spectral coverage between 3.1 and 3.9 μm is obtained using a PPLN-based continuous-wave optical parametric oscillator pumped by a Nd: YAG laser at 1,064 nm. A low threshold is achieved by resonating the pump, and spectral agility by employing a dual-cavity setup. Output idler power is 2 × 100 mW. The frequency tuning qualities of the OPO (optical parametric oscillators) allow reliable scan over gas absorption structures.

The PA spectrometer (Figure 8.8) consists of the OPO, chopper photoacoustic cell (PAC), pyroelectric detector and the wavemeter and is installed on a 120 cm × 75 bread-board. The amount of radiation absorbed by the molecules is measured by its conversion into heat. The 3 μm beam is modulated in amplitude at the resonance frequency of the PAC, generating a standing acoustic wave.

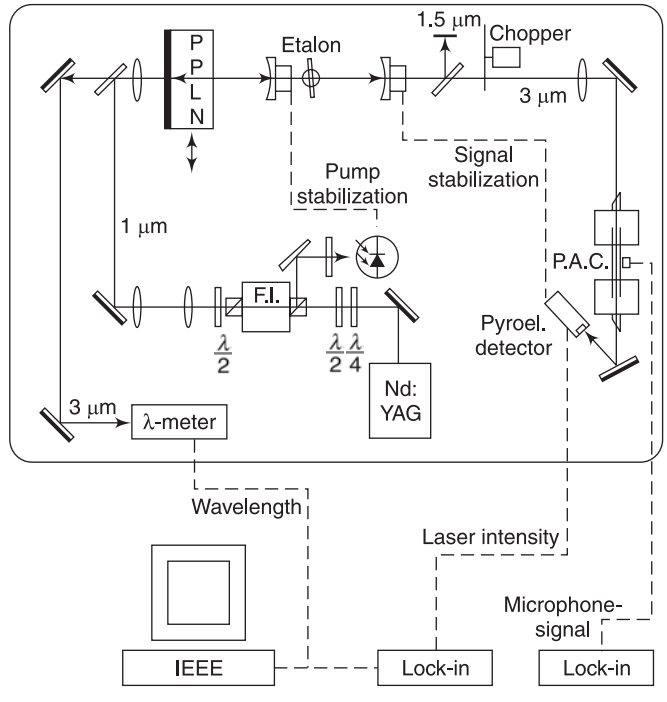


Figure 8.8 *Schematic of the OPO-based photoacoustic spectrometer (Muller et al., 2003)*

The signals from the microphone and the pyroelectric detector behind the cell are processed with two lock-in amplifiers. About 70 mW of idler power are available at the front-side of the PAC. For measurements two different PAC are used. The small PAC (resonator length of 7 cm and diameter of 6 mm), which allows a fast gas exchange, has a *Q*-factor of 17.4. The large PAC (16 cm resonator length and diameter of 14 mm) has a Q-factor of 51.1. To avoid memory effects during gas exchange the inner surfaces of both cells are passivated against molecular adsorptions.

The power supply operates in the range from 0–50 mA and maximum output voltage of 12 kV. Two separate power supplies are used to obtain equal current on both arms of laser tube.

The PAC consists of a polished brass resonator (l = 100 mm, ϕ = 15 mm) inside an aluminium housing. The first longitudinal mode frequency is around 1,600 Hz. The cell has acoustic internal insulators ($L = N4$) close its extremities that are closed with ZnSe Brewster windows. Two electret microphones (sensitivity 22 mV/Pa at 1,600 Hz) are glued in the centre of the resonator.

The best PA detection limits are usually achieved in instruments utilising resonant PAC. Resonant cells allow build-up of the acoustic wave over many cycles of the resonant frequency. These resonances, which can be due to radial, azimuthal, or longitudinal modes of the cell, are typically at a few kHz and the amount of build-up (the quality factor, or Q, of the resonator) typically exceeds the expected loss in PA signal that would be predicted in shifting to higher frequencies.

8.2 PHOTOTHERMAL SPECTROSCOPY

Photothermal spectroscopy is a high-sensitivity method used to measure optical absorption characteristics of a sample. It is a more direct measure of optical absorption in terms of sample heating than optical transmission-based spectroscopies. The basis of photothermal spectroscopy is a photo-induced change in the thermal state of the sample. Light energy absorbed and not lost by subsequent emission results in sample heating. This heating results in a temperature change as well as changes in thermodynamic parameters of the sample which are related to temperature. Measurement of the temperature, pressure, or density changes that occur due to optical absorption are ultimately the basis for the photothermal spectroscopic methods.

The photothermal absorption techniques can be categorised by their spatial temperature distribution and corresponding refractive-index change produced by radiation absorption. Laser light, rather than other types of sources, is the usual energy source. In liquids or gases or weakly absorbing solids, the refractive-index changes are probed in the absorbing medium itself. Opaque or highly scattering solids, such as powders are probed by the refractive-index gradient generated in a coupling fluid in contact with the solid.

8.2.1 Excitation Sources

Photothermal spectroscopy is usually performed using laser light sources. There are two main reasons for this. The first is the high spectral purity and power. For an excitation of a sample with a given absorption coefficient, the temperature change will be proportional to the optical power, in the case of continuous excitation, or energy, in the case of pulsed excitation. The photothermal spectroscopy signal is generally proportional to the temperature change. Thus the greater the power or energy, the greater the resulting signal. Lasers can deliver high powers or pulse energies over very narrow optical bandwidths thereby enhancing the photothermal signals.

Table 8.1 *Main sample excitation schemes used in photothermal spectroscopy*

Excitation	Signal	Detection
Pulsed	Short-lived transient, magnitude decreases with time	Peak magnitude estimation and transient waveform analysis
Continuous	Long-lived transient, magnitude increases with time	Steady-state magnitude estimation and transient waveform analysis
Modulated	Periodic modulation, magnitude and phase are functions of frequency	Periodic wave magnitude and phase analysis using frequency selective filters or lock-in amplifiers

The second reason is spatial coherence. The temperature change is not only proportional to the optical power or energy, but also is inversely proportional to the volume over which the light is absorbed since heat capacity scales with the amount of substance. The spatial coherence properties of laser sources allow the light to be focused to small, diffraction limited, volumes. The small volumes used in photothermal spectroscopy enhance signal magnitudes, allow photothermal spectroscopy to be used in small volume sample analysis and allow for microscopic analysis of heterogeneous materials.

There are three main types of time dependent excitation and detection schemes for photothermal spectroscopy. These are given in Table 8.1.

8.2.2 Basic Processes in Photothermal Spectroscopy

The basic processes responsible for photothermal spectroscopy signal generation are shown in Figure 8.9. Optical radiation, usually from a laser, is used to excite a sample. The sample absorbs some of this radiation resulting in an increase in the internal energy. The increased internal energy results in a temperature change in the sample or the coupling fluid placed next to the sample. This temperature change results in a change in sample or coupling fluid density.

If the photothermal-induced temperature change occurs faster than the time required for the fluid to expand, then the rapid temperature change will result in a pressure change. The pressure perturbation will disperse in an acoustic wave. Once the pressure has relaxed to the equilibrium pressure, a density change proportional to the temperature will result.

In either case, there will be a change in temperature induced by the absorption of optical energy. This temperature change will in turn result in a density change in the sample. In combination, temperature and density changes affect other properties of the sample. Photothermal spectroscopy is based on a measurement of these properties. In particular, the sensitive photothermal methods are based on measurement of the refractive-index change that occurs with changes in temperature and density of the sample.

Photothermal spectroscopy signals are directly related to light absorption whereas scattering and reflection losses do not produce photothermal signals. Therefore, photothermal spectroscopy more accurately measures optical absorption in scattering solutions, in solids and in interfaces.

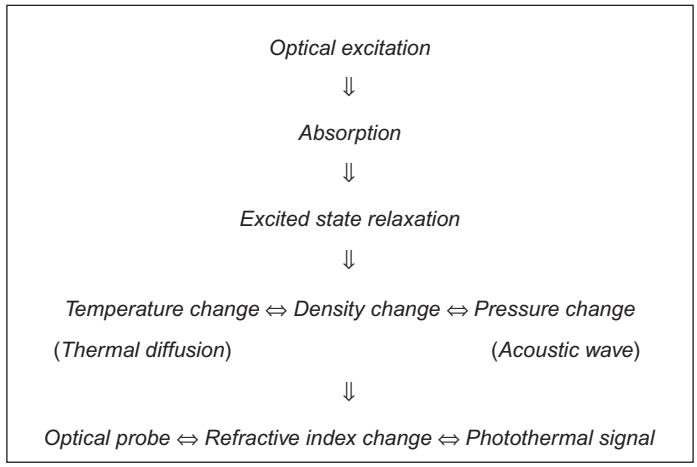


Figure 8.9 *Basic processes involved in photothermal spectroscopy*

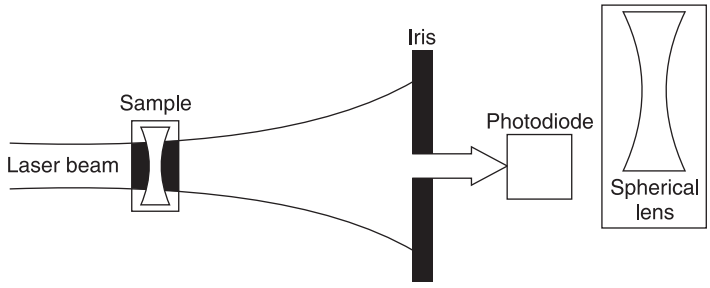


Figure 8.10 *Photothermal Lens effect*

The four widely used photothermal spectroscopic techniques are illustrated as follows:

Thermal Lens: When a focused laser beam is used for excitation, the resulting temperature gradient produces a lens-like optical element, which can be measured by its effect on the divergence of the laser beam. This is shown in Figure 8.10.

Transverse Photothermal Deflection: By creating a thermal gradient in a sample with optical excitation at an interface, the resulting thermal prism which can be detected by its deflection of a laser beam Figure 8.11.

Photothermal Refraction: It involves probing the thermal lens in a sample at right angles to the laser beam that caused it, as shown in Figure 8.12, the thermal lens will appear as a cylindrical lens to the probe beam.

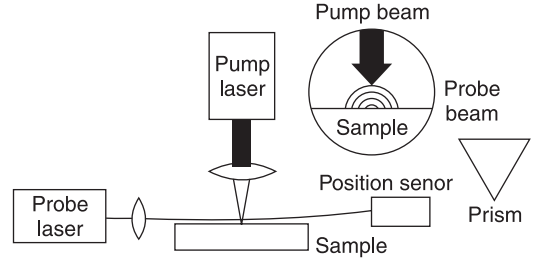


Figure 8.11 *Transverse photothermal deflection (prism effect)*

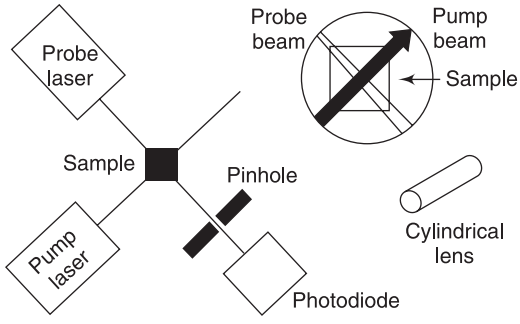


Figure 8.12 *Photothermal refraction effect (cylindrical lens effect)*

Photothermal Diffraction: A periodic temperature distribution can be generated by an excitation interference pattern and probed *as* a thermal transmission grating by diffraction of a laser beam into a detector. Thus, diffraction photothermal effects can be generated with two crossed laser beams and probed with a third (Figure 8.13).

It may be observed from the above that in each case, a different discrete optical element approximates the behaviour of the refractive-index distribution as generated and measured. Since a laser beam can be defocused or deflected by a refractive-index distribution, no matter how it is generated, lasers are invariably used in photothermal instrumentation.

Unlike conventional transmission or reflection measurements, the sensitivity of photothermal techniques depends on the power of the radiation used for excitation and the thermo-physical properties of the sample. Solvents which exhibit a large change in refractive index with temperature (dn/dt), are more suitable for study with this method, since a given increase in temperature produces a large change in optical path.

Photothermal effects are closely related to the PA effect. As we observed in the last section, in PA measurements, the heat generated by light absorption is detected as a pressure change, using a microphone or other pressure transducer in contact with the sample, whereas it is refractive-index changes which are detected in photothermal spectroscopy. Both techniques are capable of measuring absorbances down to 10^{-9} units, although this level is rarely reached. By that standard, nominally transparent materials which may have absorbances of 10^{-6}–10^{-3} /cm, are strongly absorbing (Morris, 1986).

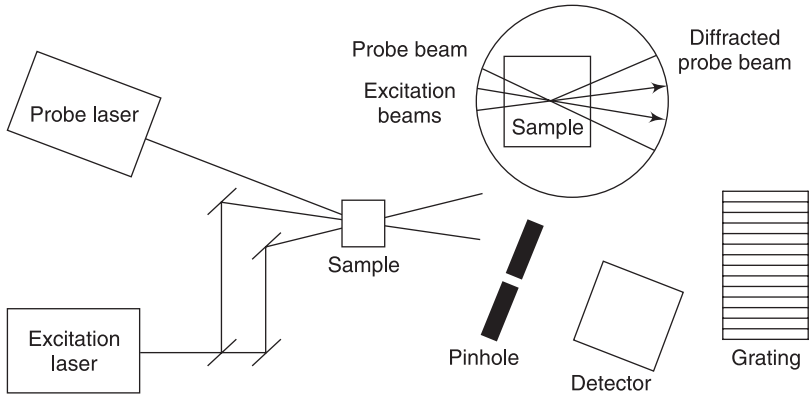


Figure 8.13 *Photothermal diffraction effect (grating effect)*

8.2.3 Photothermal Instrumentation

Laser light sources are usually used to perform photothermal spectroscopy. The reason for this is the high spectral purity and power. For an excitation of a sample with a given absorption coefficient, the temperature change will be proportional to the optical power in the case of continuous excitation, or energy in the case of pulsed excitation. The photothermal spectroscopy signal is generally proportional to the temperature change. Thus the greater the power or energy, the greater the resulting signal. Lasers can deliver high powers or pulse energies over very narrow optical bandwidths thereby enhancing the photothermal signals. Another reason is spatial coherence of the laser beam. The temperature change is not only proportional to the optical power or energy, but also is inversely proportional to the volume over which the light is absorbed since heat capacity scales with the amount of substance. The spatial coherence properties of laser sources allow the light to be focused to small volumes. The small volumes used in photothermal spectroscopy enhance signal magnitudes, allow photothermal spectroscopy to be used in small volume sample analysis and allow for microscopic analysis of heterogeneous materials.

A schematic diagram illustrating the main components of the instrument used for photothermal spectroscopy is shown in Figure 8.14. Most instruments consist of the following six components:

- Sample
- Light used for sample excitation
- Light used to monitor refractive-index perturbations
- A mask, aperture or other form of spatial filter for the probe light
- An optical detector used to detect the optically filtered probe light
- Electronic signal processing equipment

The excitation light heats the sample. The probe light monitors changes in the refractive index of the sample resulting from heating. The spatial and propagation characteristics of the probe light will be altered by the refractive index. The spatial filter selects those components of the altered probe light that change with the samples' refractive index. The optical detector monitors changes in the probe light power past the spatial filter. In some apparatuses, a spatial filter and a single channel detector are combined using an image detector. Signals generated by the photodetector are processed to enhance the signal-to-noise ratio.

In addition, an apparatus may also be equipped with detectors to monitor the excitation and probe light power, a thermostatic sample holder, and optical spatial filters to control the spatial profiles of the excitation and probe light. This additional equipment is used to control the experiment environment and to measure the optical power required to accurately quantify changes that occur in the sample. These components are necessary when the data must be used to determine absolute absorption of the sample.

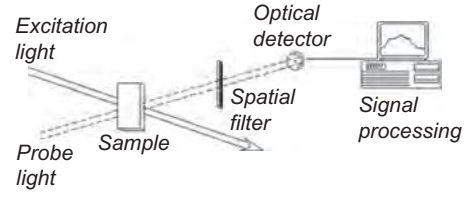


Figure 8.14 *A generic photothermal spectrometer showing essential features (Adapted from Bialkowski, 1996)*

The most popular technique in photothermal instrumentation is the transverse photothermal deflection system. A functional block diagram is shown in Figure 8.15. The heating

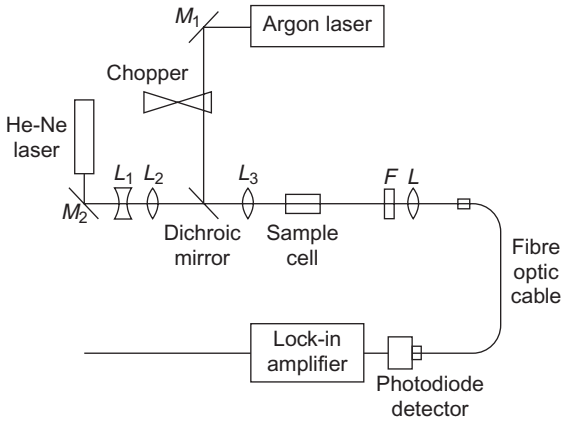


Figure 8.15 *Thermal lens measurement system—basic building blocks*

beam is a low-power laser beam, which is modulated at 5–20 Hz. The modulation is achieved by a mechanical chopper. The typical power output of the argon laser (heating laser) is 20–100 mW. The heating beam is combined with a helium-neon probe beam. The power output of this probe beam is 0.5–2 mW. The combined beams are focused into the sample with a short (30–100 mm) focal length lens. A simple glass filter is used to isolate the probe beam beyond the sample. An optical fibre serves as the limiting aperture and also to relay the probe beam to a photodiode detector. This is followed by a lock-in amplifier for demodulation, before it is fed to a read-out system.

For quantitative analysis, the concentrations of samples can be obtained simply by calibration at a fixed wavelength with standards. If the wavelength of the pump beam is varied, the deflection of the probe beam is then a measure of the absorption spectrum of the sample investigated.

Several factors determine the signal-to-noise ratio in photothermal measurements. In general, the intensity of photothermal effect increases linearly with power density. Also, in the refractive-index distribution generated in any photothermal technique, there is a sample region with maximum refractive-index change. Probing this region yields maximum sensitivity. However, in many analytical applications, photochemical reactions are potentially serious sources of error in transverse photothermal deflection measurements or any other measurements of solids.

The key to the success of sensitive photothermal apparatuses lies in measurement of a thermal change and not the thermal state itself. Although apparatuses could directly or indirectly measure the thermodynamic parameters such as temperature, pressure, density and energy state, the limiting absorption that could be measured would be imposed by thermodynamic fluctuations.

Sensitive photothermal spectroscopy methods circumvent direct measurements by measuring refractive-index changes due to a non-equilibrium change in the energy of the sample. The change occurs in both space and time. Photothermal spectroscopy methods measure some effect that the spatially or temporally dependent refractive-index change has on the propagation characteristics of light used to monitor the refractive index.

Photothermal effects are useful in characterising physical and chemical properties of a wide variety of solid, liquid and gaseous materials. Wider availability of compact and inexpensive lasers that are suitable for photothermal measurements, are likely to bring a rapid increase in the number of problems readily solved by photothermal spectroscopy.

9

MASS SPECTROMETERS

9.1 BASIC MASS SPECTROMETER

In a mass spectrometer, the sample to be analysed is first bombarded with an electron beam to produce ionic fragments of the original molecule. These ions are then sorted out by accelerating them through electric and magnetic fields, according to their mass-to-charge (m/e) ratio. A record of the numbers of different kinds of ions is called the mass spectrum. The uniqueness of the molecular fragmentation is the basis for identification of different molecules in a complex mixture, as no two molecules will be fragmented and ionised in exactly the same manner. Very small sample sizes, which may be of the order of a few tenths of a milligram, are generally sufficient as long as the material is able to exist in the gaseous state at the temperature and pressure existing in the ion source. Several other useful applications of mass spectrometry include the direct determination of the molecular weight, the placement of functional groups into certain areas of the molecules and their interconnection and investigation of reaction mechanism.

One of the main advantages of mass spectrometry over other spectroscopic techniques is its sensitivity. It is possible on some instruments to obtain full mass spectra with 1 ng of material in about a second. Compounds now amenable to mass spectrometry vary from low molecular weight gas mixtures to high molecular weight natural products. Various ionisation techniques are used to provide complementary structural information and molecular weight and elemental composition data are readily obtainable.

A significant advantage of mass spectra is its suitability for data storage and library retrieval, since the positions (masses) of the peaks in the spectrum of a given compound are fixed. Another asset of mass spectrometry is its capability in handling complex mixtures, currently via gas chromatograph-mass spectrometer.

The procedure for analysing a substance by mass spectrometry starts by converting the substance into a gaseous state by chemical means. The gas is introduced into the highly evacuated spectrometer tube, where it is ionised by means of an electron beam. The positive ions thus formed are deflected and focused by means of suitable magnetic and electric fields. For a given accelerating voltage, only positive ions of a specific mass pass through a slit and reach the collecting plate. The ion currents thus produced are measured by using a sensitive electrometer tube. By varying the accelerating voltage, ions from other mass species may be collected, and the ion currents measured in such cases would be proportional to the amount of the given mass species present. A great

advantage of mass spectrometry over other direct methods is that the impurities of masses different than the one being analysed do not interfere with the results.

Mass spectrometers also generate three-dimensional data. In addition to signal strength, they generate mass spectral data that can provide valuable information about the molecular weight, structure, identity, quantity and purity of a sample. Mass spectral data add specificity that increases confidence in the results of both qualitative and quantitative analyses.

Some mass spectrometers have the ability to perform multiple steps of mass spectrometry on a single sample. They can generate a mass spectrum, select a specific ion from that spectrum, fragment the ion and generate another mass spectrum; repeating the entire cycle many times. Such mass spectrometers can literally deconstruct a complex molecule piece by piece until its structure is determined (Agilent Technologies, 2001).

9.2 PRINCIPLE OF OPERATION

Figure 9.1 shows the principle of operation of a mass spectrometer. The molecules in the gas sample (*A*) to be analysed are bombarded with electrons to produce ions (*B*). These ions are accelerated in a high vacuum into a magnetic field (*C*), which deflects them into circular paths (*D*). Since the deflection for light ions is greater than that for heavy ions, the ion stream separates into beams of different molecular weight. A suitably placed slit (*F*) allows a beam of a selected molecular weight to pass through to a collection electrode (*E*). As the accelerating voltage on the ion source is gradually reduced, ion beams of successively greater mass pass through the slit. When these ions fall on the collector electrodes, they produce minute electric currents, which after suitable amplification, may be measured. Their amplitude will indicate the number of ions in each beam. The proportion of molecules of different masses in the gas sample may, thus, be found and a complete analysis of the gas sample may be made; provided all the constituent gases have a different molecular weight. This is usually the case in respiratory gas analysis work.

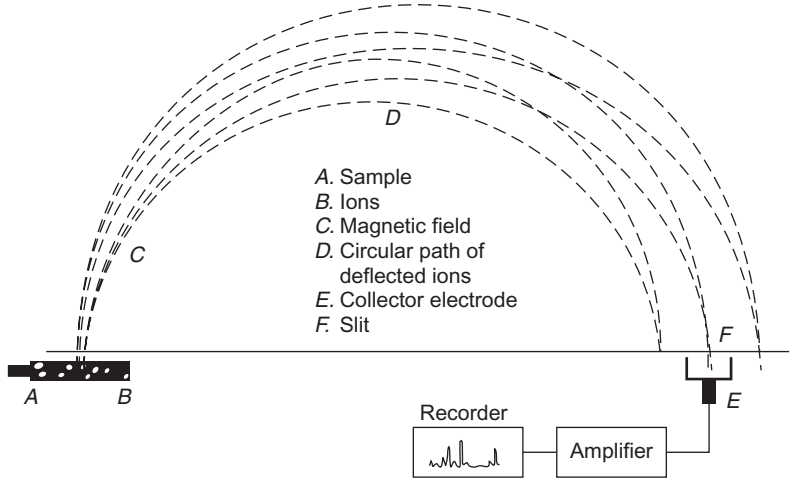


Figure 9.1 *Principle of operation of a mass spectrometer*

It is apparent that there is a close analogy between scanning mass spectrometry and scanning optical spectroscopy. This is shown in Figure 9.2.

In optical spectroscopy, the source is a visible light which is composed of individual colours (different wavelengths of light) that are present at different intensities. A prism (or grating) is used to separate the light into its different wavelengths followed by a slit which selects the wavelength reaching the detector. The different wavelengths are then scanned across the detector slit and the light intensity is measured as a function of wavelength.

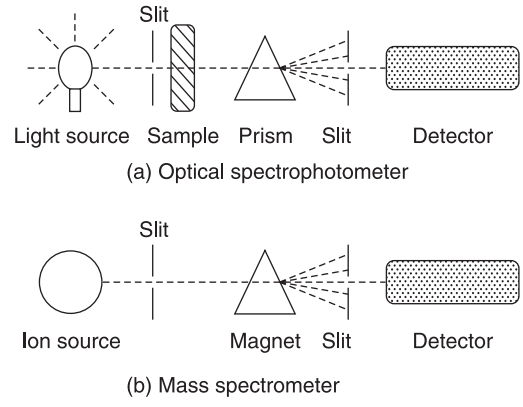


Figure 9.2 *Analogy between optical spectroscopy and mass spectrometry*

In scanning mass spectrometry, the starting point is a mixture of ions having different (*m/e*) ratios. A slit is used to select which *m/e* ratio reaches the detector. The different *m/e* ratios are then scanned across the detector slit and the ion current is recorded as a function of time (mass).

Mass spectrometers are used for all kinds of chemical analyses, ranging from environmental analysis (e.g. detection of poisons such as dioxin) to the analysis of petroleum products, trace metals and biological materials (including the products of genetic engineering).

Let us take water (H_2O) as an example to illustrate the principle of mass spectrometry. A water molecule consists of two hydrogens (H) and one oxygen (O). The total mass of a water molecule is the sum of the mass of two hydrogens (approximately 1 atomic mass unit per hydrogen) and one oxygen (approximately 16 atomic mass units per oxygen):

H:	1 u (atomic mass units)
+H:	1 u
+O:	16 u
$=H_2O$:	18 u

Let us suppose that we put some water vapour into the mass spectrometer. A very small amount of water is all that is needed – the water is introduced into a vacuum chamber (the 'ion source') of the mass spectrometer. If we shoot a beam of electrons through the water vapour, some of the electrons will hit water molecules and knock off an electron. If is lost an electron (negatively charged) from the neutral water molecule, the water will be left with a net positive charge. In other words, we have produced charged particles, or 'ions' from the water.

$$H_2O + 1 \text{ (fast) electron} \rightarrow [H_2O]^+ + 2 \text{ electrons}$$

Some of the collisions between the water molecules and the electrons will be so hard that the water molecules will be broken into smaller pieces, or 'fragments'. For water, the only possible fragments will be $[OH]^+$, O^+ and H^+.

The mass spectrum of water is shown in Figure 9.3. It shows peaks that can be assigned to masses of 1, 16, 17 and 18.

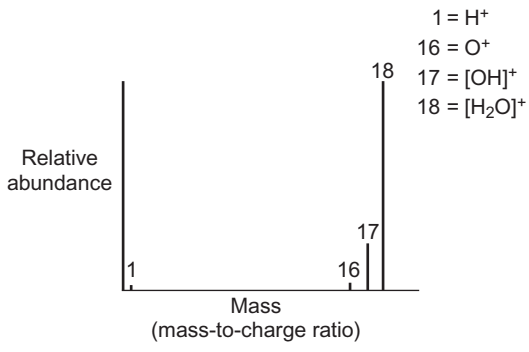


Figure 9.3 *Mass spectrum of water*

$1 = H^+$
$16 = O^+$
$17 = [OH]^+$
$18 = [H_2O]$

It is obvious that only certain combinations of elements can produce ions that have these masses. For example, the ammonium ion $[NH_4]^+$ also has an approximate mass of 18 atomic mass units, but there would be peaks at mass 14 and 15 in the mass spectrum of ammonia corresponding to a N^+ and $[NH]^+$ (nitrogen is atomic mass 14)

A trained mass spectrometrist can interpret the masses and relative abundances of the ions in a mass spectrum and determine the structure and elemental composition of the molecule. Computer programs, such as those that search libraries of mass spectra for the best match, can also be used to interpret a mass spectrum.

Mass spectra can provide other information as well. For example, a high-resolution mass spectrometer can determine the mass of an ion very precisely. If we knew that the mass of our hypothetical ion at mass 18 was actually mass 18.010, we could easily distinguish it from an ammonium ion, which would have an exact mass of 18.035. Given an accurate mass and an estimated error tolerance, a computer can easily calculate the elemental composition of the molecule.

9.3 TYPES OF MASS SPECTROMETERS

Several methods of producing mass spectra have been devised. However, the principal difference between the various types of spectrometers lies in the means for separating the ions according to their *m/e* ratio. The important types are described below.

9.3.1 Magnetic Deflection Mass Spectrometer

Figure 9.4 shows the essential parts of a typical magnetic deflection mass spectrometer tube due to Nier (1940). The heated tungsten filament produces an electron beam, which passes between plates *A* and *B*. A difference in electrical potential between *A* and *B* pulls ions out of the beam, so that they pass through the slit in *B* into the region between *B* and *C*. The potential difference between *B* and *C* is adjustable from 0 up to several thousand volts. The ion beam then enters the space between two trapezoid shaped magnet poles, where it is deflected through an angle of 60, 90, 120 or 180.

When ions of mass *m* and charge *e* pass through an accelerating electric field, they would attain a velocity *V* which can be expressed in terms of the accelerating voltage *V* as follows:

$$1/2\, mv^2 = eV \tag{9.1}$$

where $1/2\, mv^2$ is the kinetic energy of the ion as it leaves the electric field.

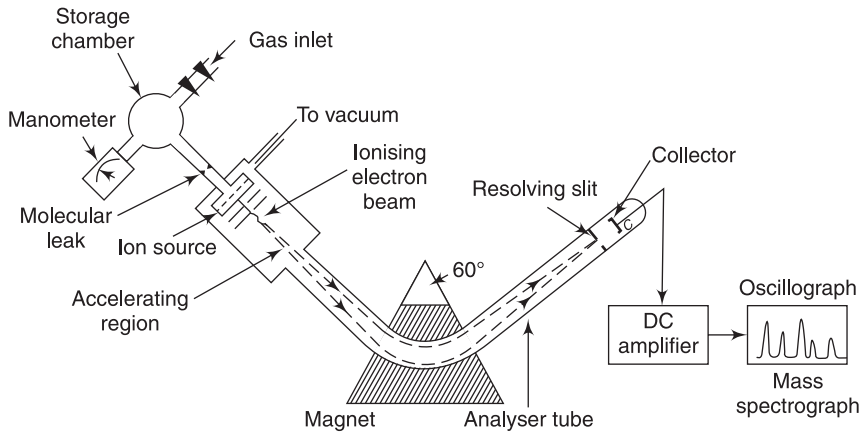


Figure 9.4 *Schematic diagram of a Nier 60° sector mass spectrometer*

From Eq. (9.1), v can be written as follows:

$$v = [2\ eV/m]^{1/2} \tag{9.2}$$

If the ions next enter a magnetic field of constant intensity h, which is applied at right angles to their direction of motion, the ions would be diverted into circular orbits. From physics, it is known that when acceleration is applied perpendicular to the direction of ion motion, the objects velocity remains constant, but the object travels in a circular path. Therefore, the magnetic sector follows an arc. The radius and angle of the arc vary with different in optical designs. Equating the centripetal and the centrifugal forces results in the following formula:

$$mv^2/r = hev \tag{9.3}$$

The radius of curvature of the trajectory is given as follows:

$$r = mv/eh \tag{9.4}$$

Substituting for v from Eq. (9.2), we get

$$\begin{aligned} r &= [2\ eV/m]^{1/2} \times m/eh \\ &= [2\ eV/m \times m^2/e^2h^2]^{1/2} \\ &= [2V/h^2 \times m/e]^{1/2} \end{aligned} \tag{9.5}$$

This equation shows that the radius of the orbit is a function of the m/e ratio of the particles.

In practice, all the quantities of Eq. (9.5) are kept constant with the exception of m and V. By varying the accelerating voltage V, it is possible to cause an ion of any mass to follow the path which may coincide with the arc of the analyser tube in the magnetic field. Ions of different will ratio m/e strike the tube at some point and would get grounded.

Under specified conditions, the ions which will be collected would follow the expression:

$$m/e = h^2 r^2 /2V \tag{9.6}$$

The relationship (9.6) shows that for obtaining a mass spectrum, the accelerating voltage or the magnetic field strength can be varied. Usually, it is the magnetic field which is kept constant and the voltage is adjusted to bring to a focus specific *m/e*. The magnetic field must be very uniform over a large area. If electromagnets are employed, they would require large power supplies capable of providing several kilowatts of power and must be highly regulated. For a particular instrument, the angle of deflection (radius) is fixed for a given analyser tube (180°, 90°, 60°). Therefore, Eq. (9.6) can be written as

$$mV = constant$$

If this constant is, say 2000, then as V is set at 100 V, particles of mass 20 can reach the collector plate, while to direct particles of mass 25 to it, V must be set at 80 V. Direction focusing is achieved by deflecting the ion beam along a 180° trajectory through the magnetic field. A large magnet is required for a 180° mass spectrometer. When the ion source is in the uniform magnetic field, the gap between the poles must be large enough to contain the ion source. To overcome this difficulty, Nier (1940) employed the direction focusing properties of a 60° sector magnetic field. In this arrangement, the magnetic field does not envelop the ion source; a separate source magnet is required. With Sector type instruments, a mass resolution of 200–600 mass units can be obtained.

The use of simultaneous direction and velocity focusing results in a high-resolution mass spectrograph. This is done by placing an electrostatic analyser between the ion-accelerating slits and the magnetic field. After the usual acceleration of the beams in an electrostatic field, the ions are deflected through a tandem arrangement of an electrostatic analyser and then a magnetic analyser. The arrangement enables focusing of ions having the same *m/e* but different initial velocities and directions. Resolving power of 8500 with a 0.05 mm entry aperture has been achieved by utilising the focusing properties of electric and magnetic sector fields in a parabolic configuration.

Commercial mass spectrometers appeared in the USA in the early 1940s. In these instruments, ions were separated according to the quotient *m/e* and the separated ion beams were recorded directly on a photographic plate. Later, the design of a mass spectrometer for precision mass measurements was developed. The instrument incorporated the arrangement, known as Nier-Johnson geometry, which involves a deflection of $\pi/2$ radians in a radial electrostatic field analyser, followed by a magnetic deflection of $\pi/3$ radians. One ion beam at a time is brought to a focus on an exit slit and measured electrically. These types of instruments became commercially available in the early 1960s.

About the same time, commercial mass spectrographs using Mattauch-Herzog geometry also became readily available. In this arrangement, a deflection of $\pi/4\sqrt{2}$ radians in a radial electrostatic field is followed by magnetic deflection $\pi/2$ radians, and all ions of different mass can be simultaneously focused on a photographic plate. The spectrographs with photographic recording are used for analysis of solids and for the recording of organic mass spectra. An accuracy of 1 part in 10^9 has been obtained in precision mass measurements.

A magnetic sector alone will separate ions according to their *m/e*. However, the resolution will be limited by the fact that ions leaving the ion source do not all have exactly the same energy, and therefore do not have exactly the same velocity. To achieve better resolution, it is necessary to add an electric sector that focuses ions according to their kinetic energy. Like the magnetic sector, the electric sector applies a force perpendicular to the direction of ion motion, and therefore has the form of an arc. Figure 9.5 shows the schematic diagram of the JEOL M station double focusing mass spectrometer. This arrangement is called 'Reverse Geometry' magnetic sector mass spectrometer, which means that the magnetic sector precedes the electric sector.

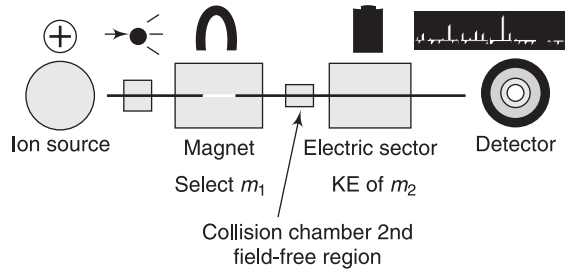


Figure 9.5 *Reverse geometry mass spectrometers (Hold magnetic fold strength constant and scan electric field)*

The electric sector is usually held constant at a value which passes only ions having the specific kinetic energy. Therefore, it is the magnetic fold strength '*B*' which is most commonly varies. The magnetic fold is usually scanned exponentially or linearly to obtain the mass spectrum. An alternative is to hold '*B*' constant and scan '*V*'. The electric sector field is not subject to hysteresis, so the relationship between *m/e* ratios and accelerating voltage is a simple linear relationship.

9.3.2 The Time-of-Flight Mass Spectrometer

In a time-of-flight mass spectrometer, ions of different *m/e* ratio are separated by the difference in time they take to travel over an identical path from the ion source to the collector. This requires that the starting time (the time at which the ions leave the ion source) is well defined. Therefore, ions are either formed by a pulsed ionisation method (which is usually matrix assisted laser desorption ionisation) or various kinds of rapid electric field switching are used as a 'gate' to release the ions from the ion source in a very short time. In the pulsed mass spectrometer, in which ion packets of a few micro-seconds duration are emitted at intervals of a few milliseconds from a voltage source. The ions traverse an evacuated tube called the drift tube to reach the detector. The detector is sensitised for a brief instant to register their arrival. Since ions of different masses arrive at the detector at different times, the accurate measurement of the time between activating the source and sensitising the detector gives information concerning the mass of the ions. The signal from the ions reaching the detector is amplified and applied to the vertical deflection plates of an oscilloscope. The horizontal axis deflection of the oscilloscope commences as the ion packets start out. This produces a mass spectrum on the screen of the oscilloscope. The device, thus, gives a mass spectrum in a very short time. The essential parts of a time-of-flight instrument are shown in Figure 9.6. It consists of the following parts:

- An electron gun for the production of ions.
- A grid system for accelerating ions to uniform velocities in a pulsed mode.
- An evacuated tube, called the drift tube.
- An ion detector and suitable electronic circuitry for translating the time-dependent arrival of ions of different velocities into a time base that is related to mass number.

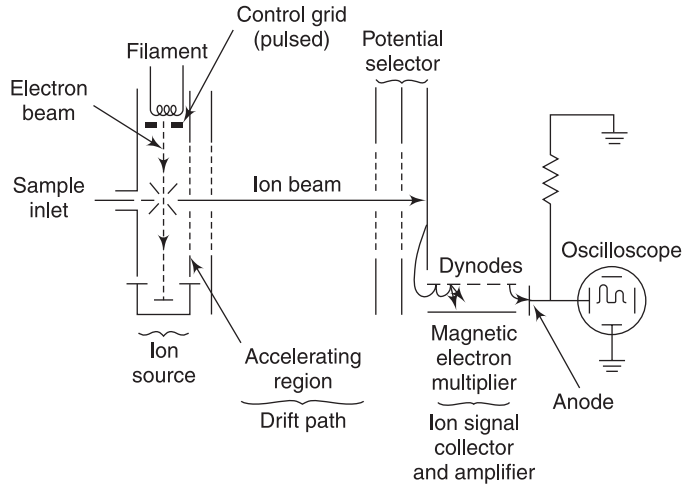


Figure 9.6 *Schematic diagram of a time-of-flight mass spectrometer*

If L is the length of the drift tube in centimetres and t is transit time in micro-seconds, for singly charged ions of mass m and constant energy Ve, then

$$\begin{aligned} t &= L\,[m/2\ Ve]^{1/2} \\ &= L\,[m/e \times 1/2V]^{1/2} \\ m/e &= 2V/L^2 \times t^2 \end{aligned} \tag{9.7}$$

If the detector is sensitised for a period Δt at time t, the resolution $\Delta m/m$, for constant energy, is given by

$$\text{Resolution} = \Delta m/m = 2\ \Delta t/t$$

Equation (9.7) shows that the time resolution will increase with increased drift tube length and will decrease with increasing accelerating voltage.

The current produced by the ions arriving at the collector may have a very short duration, which necessitates the use of a wide-band amplifier. Specially designed magnetic electron multiplier is used for this purpose. This multiplier uses a strip of semiconducting material for the multiplying surface instead of dynodes. A gain of 10^7 is attained with a dark current of only 3×10^{-21}A.

The ions leaving the ion source of a time-of-flight mass spectrometer have neither exactly the same starting times nor exactly the same kinetic energies. Various time-of-flight mass spectrometer designs have been developed to compensate for these differences. One such design makes use of a device known as *Reflectron*. The 'Reflectron' is an ion optic device in which ions in a time-of-flight mass spectrometer pass through a 'mirror' (reflection) and their flight is reversed.

The linear reflection allows ions with greater kinetic energies to penetrate deeper into the Reflectron than ions with smaller kinetic energies. The ions that penetrate deeper will take longer to return to the detector. If a packet of ions of a green m/e ratio contains ions with varying kinetic energies, then the Reflectron will decrease the spread in the ion flight times, and therefore improve the resolution of the time-of-flight mass spectrometer. The arrangement is shown in Figure 9.7.

The main advantages of the time-of-flight spectrometers include its speed and ability to record entire mass spectrum at one time. A conventional spectrometer detects only one peak at a time. Its accuracy depends on electronic circuits, rather than on extremely critical mechanical alignment and on the production of highly stable and uniform magnetic fields. The main disadvantage of the TOF spectrometers is their poor resolution due to display on an oscilloscope screen.

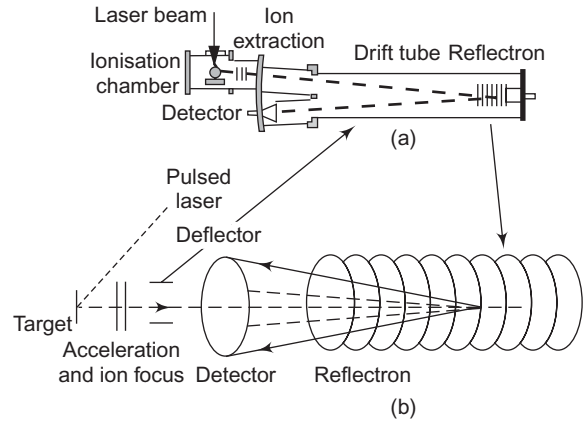


Figure 9.7 *(a) Ablation of ions by use of a laser from a solid sample (b) Principle of working of a reflectron (Adapted from www.jeol.com/ms/docs/ms-analysers.html)*

9.3.3 Radio Frequency Mass Spectrometer

The most popular radio frequency (rf) mass spectrometers make use of Bennett (1950) tube. The arrangement in the instrument is such that the charged particles emerging from the ion source are all accelerated to the same energy in an electrostatic field, and then they pass through a system of rf electrodes. The energy acquired by the ions in this process is a function of their specific *m/e* ratio. Maximum energy increment would be acquired by these ions, only if the ions start with the correct velocity at the optimum phase of the rf field. A potential energy selector is placed before the detector, which balances out the energy of the ion beam and the mass spectrum is recorded by detection of the ions with the highest energies as the frequency of the alternating rf voltage is varied.

The rf field is applied in one or more rf stages. Each stage is a series of three equally spaced parallel grids. An alternating rf voltage is applied to the central grid, with respect to the outer grids, which are kept at ground potential.

If v is the velocity attained by the ions in phase with the rf field, then

$$v = df$$

where f is the frequency of the rf field in MHz and d is the spacing between adjacent grids in centimetres. The *m/e* ratio of the ion beam reaching the detector is given by

$$m/e = 0.266\ \text{V}/d^2f^2 \text{ (in cgs units)}$$

The rf mass spectrometer does not require a magnet and therefore is comparatively light weight and simple in construction. In the Bennett spectrometer, the rf voltage has a fixed frequency and is modulated at 10% at 1 kHz. The current received at the detector is amplified with an AC amplifier tuned to the modulation frequency. The DC ion-accelerating voltage is swept from 50 to 250 V, twice per second. The spectrum in the range $M = 10–50$ is reproduced twice per second.

It has been shown through investigations that the resolving power of a Bennett tube is primarily determined by the distances between the individual rf accelerating stages. A two-stage tube

would be the most favourable, but the instrument tends to provide spurious lines. Separation of the principal line from the spurious lines with a retarding field would greatly affect the sensitivity of the spectrometer. The spurious lines may be suppressed in a three-stage tube, although resolving power is slightly reduced.

The resolution of the Bennett tube can be improved by using a square-wave rf signal, in place of sinusoidal rf voltage. Several improvements have been incorporated over the original design and mass spectrometers for special applications have been built up based on this principle.

9.3.4 Quadrupole Mass Spectrometer

Quadrupole type mass spectrometers feature simple construction, light weight compared to sector-type mass spectrometers, high speed electronic scanning and low cost. A quadrupole mass spectrometer consists of an ion source, a quadrupole mass filter and a lens system to focus the ions into the quadrupole filter. Figure 9.8 shows the principle of quadrupole mass filter. The arrangement consisting of four cylindrical rod-shaped electrodes which provide a potential field distribution, periodic in time and symmetric with respect to the axis which will transmit a select mass group and cause ions of improper mass to be deflected away from the axis. This mass selection scheme uses a combination of DC potential plus a rf potential. By proper selection of potentials and frequency, an ion of desired mass can be made to pass through the system, while unwanted masses will be collected on one of the electrodes.

In practice, opposite electrodes of the filters are connected together, and to one pair a potential $\phi(t) = u + v \cos(2\pi ft)$ is applied, where u is a DC voltage and v is the peak amplitude of the rf voltage at a frequency f. To the other pair of electrodes, the same potential but of the opposite sign is applied. Ions emitted out of the ion source and focused into the quadrupole filter are made to undergo transverse motion by the rf and DC field perpendicular to the Z-axis.

The motion of the ions in the X-Y plane can be described by the differential equations, and their solutions contain either exponential or oscillatory factors depending on the value m/e of the ions. With proper selection of μ and U, ions of a given m/e will have stable trajectories; these ions will oscillate about the Z-axis and ultimately emerge from the exit of the mass filter assembly. Ions with

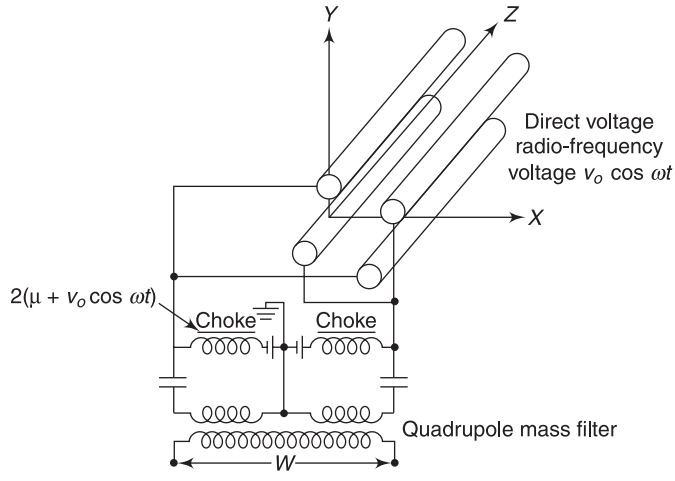


Figure 9.8 *Quadrupole mass filter*

other values of *m/e* will have unstable solutions; they will move away from the Z-axis and ultimately strike the electrodes thus being removed.

Conditions for stable trajectories are shown in Figure 9.9. Stable conditions are obtained if the values *a* and *q*, expressed by the following equations are within the hatched area.

$$a = 8\, eu/mr_o^2\, \omega^2$$

$$q = 4\, ev/mr_o^2\, \omega^2\ (\omega = 2\pi f)$$

where e = electric charge
v = maximum value of rf voltage
m = mass number
r = radius of electric filed
f = frequency
u = DC voltage

When the frequency f and the ratio *u/v are* kept constant, the value *a* is linearly proportional *to* the value *q*, *a/q* is constant. Because the ratio *a/q (= 2u/v)* is not mass dependent, all ions of different masses in the stability diagram lie on a straight line through zero, its gradient only depending on the ratio of *u* and *v*. Only ions of the *q* intervals inside the hatched region of the figure are stable.

By increasing the ratio *u/v*, the stable interval, which corresponds to a stable mass interval is reduced, so that only ions of one mass can pass the quadrupole filter. In other words, the resolution of a quadrupole mass spectrometer is maximised when the straight line of *a/q* cuts the apex of hatched area. The lower the gradient, the lower is the resolution.

When the value of q is kept constant, the value of *m/e* is proportional to the value of *v*. Therefore

$$m/e = k.\ v/r_o^2 f^2$$

where *k* is a constant

By variation of the rf amplitude, *m/e* varies. In short, in a quadrupole mass spectrometer, masses are scanned by changing the value *v*, with the *u/v* ratio kept constant.

Changing the slope of the scan line will change the resolution. Increasing the resolution decreases the number of ions that reach the detector. Good resolution also depends on the quality of the machining for the quadrupole rods.

The performance of a mass filter depends on the quality of the quadrupole rods, the stability of the applied voltage and the field characteristics at the two ends of the rods. Especially, it is essential that the quadrupole rod assembly is produced with super-high precision.

Where a differentiation is required between very similar substances, another technique, making use of quadrupole principle, is the *Pyrolysis* mass spectrometry. In this arrangement, the sample to be analysed, usually a solid or involatile liquid, is rapidly heated in a

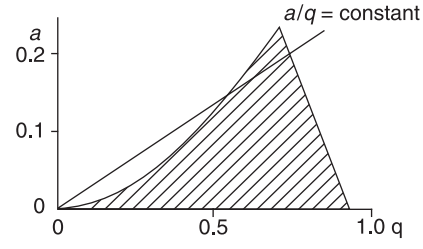


Figure 9.9 *Stability diagram of quadrupole mass spectrometer*

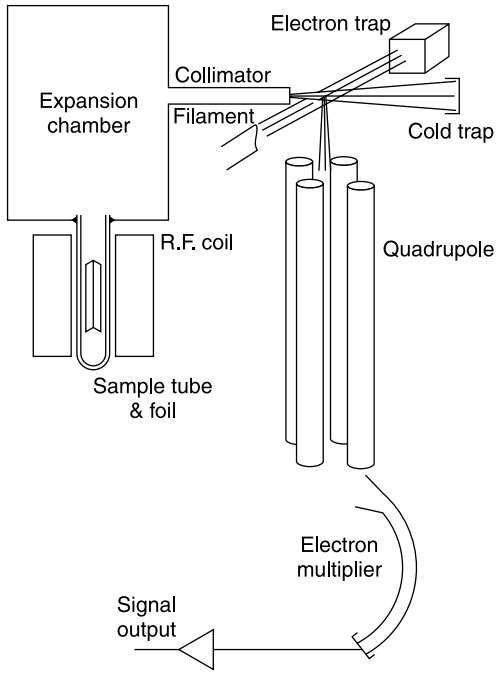


Figure 9.10 *Schematic diagram of the pyrolysis spectrometer (Ottley, 1986)*

vacuum to a precise temperature. This causes thermal breakdown and subsequent gas-phase reactions. The gas or pyrolysate is then analysed directly in a mass spectrometer.

The block diagram of pyrolysis mass spectrometer is shown in Figure 9.10 (Ottley, 1986). The sample is held on a metal substrate, usually a wire of foil, made of a magnetic alloy. This is heated by induction from an external coil operating in the 1 MHz range. This causes rapid heating to a specific temperature, known as Curie point at which the magnetic permeability drops abruptly. The gas produced during pyrolysis is then held in a small buffer volume before being formed into a molecular beam. A liquid nitrogen cooled shield surrounds the ion source and reduces source contamination.

Once the gas has entered the ion source of the mass spectrometer, electron impact ionisation creates an ion beam which is then analysed in a quadrupole mass analyser. High speed pulse counting using an electron multiplier is employed to permit fast scanning over a mass range typically between 12–200 amu.

Spectra from several samples of the same substance are averaged to enable a library of known materials to be built up. Unknown samples may then be matched against the library and hence identified.

9.4 COMPONENTS OF A MASS SPECTROMETER

Common to most mass spectrometer instruments, following are the five units shown in Figure 9.11:

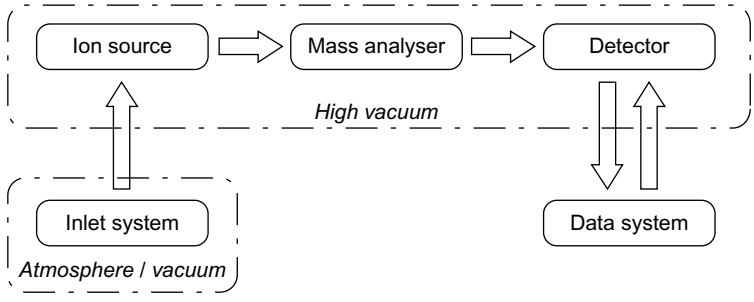


Figure 9.11 *Diagram of the major components common to all typical modern mass spectrometers (Paulo et al., 2012)*

- the inlet sample system
- the ion source
- the mass analyser
- the amplifier and the detector
- the data system and display system
- vacuum system.

9.4.1 The Inlet Sample System

Gaseous Samples: Introduction of gases involves merely transfer of the sample from a gas bulb into the metering volume. The arrangement is a small glass manifold of known volume attached to a mercury manometer. The pressure range is generally from 30 to 50 mm of Hg.

The gas sample is introduced into the mass spectrometer ion source through a leak of some kind. Generally, the leak is a pin-hole in metal foil. Hogg (1969) explains the construction of a variable leak inlet system used in high-resolution mass measurement.

Liquid Samples: Liquid samples may be introduced by hypodermic needle and injected through a silicon rubber dam, or by a break-off device which consists in touching a micro-pipette to a sintered glass disc under mercury. The low pressure in the reservoir draws in the liquid and vaporises it instantly.

Solid Samples: Solid samples can be vaporised to gaseous ions by instantaneous discharges with a power up to 100 kW by using a rf (1 MHz) spark. Under these conditions, all constituents of the sample are converted to gaseous form at an equal rate without regard to their vapour pressure, thereby eliminating the possibility of preferential vaporisation.

9.4.2 Ion Sources

Following the leak is the ionisation chamber, which is maintained at a low pressure (10^{-4} to 10^{-7} mm Hg) and at a temperature of 200°C. The electron gun is located perpendicular to the incoming gas stream. Electrons are emitted from a filament (Figure 9.12) normally of carbonised tungsten, but for special purposes, tantalum or oxide coated filaments may be used. They are drawn off by a pair of positively charged slits, through which they pass into the body of the chamber. The potential present in the slits control the electron emission and the energy of the electrons. An electric field applied between these slits accelerates the electrons, which on subsequent collisions with molecules of the passing gas stream produce ionisation and fragmentation. To obtain a mass spectrum, the electric field is kept between 50 and 70 V. The electron beam is usually collimated by a magnetic field, which is confined to the ionisation region.

9.4.2.1 *Inductively Coupled Plasma (ICP) as ion source*

Inductively coupled plasma (ICP) has been shown to be a potentially effective ion source (Houk, 1986). ICP hardware is designed to generate plasma, which is a gas in which atoms are present in an ionised state. The basic

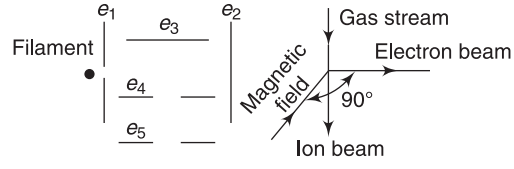


Figure 9.12 *Schematic diagram of electron gun and ionisation chamber*

set up of an ICP consists of three concentric tubes, most often made of silica. These tubes, termed outer loop, intermediate loop and inner loop, collectively make up the torch of the ICP. The torch is situated within a water-cooled coil of an rf generator. As flowing gases are introduced into the torch, the rf filed is activated and the gas in the coil region is made electrically conductive. This sequence of events forms the plasma.

The formation of the plasma is dependent upon an adequate magnetic field strength and the pattern of the gas streams follows a particular rotationally symmetrically pattern. The plasma is maintained by inductive heating of the flowing gases. The induction of a magnetic field generates high frequency annular electric current within the conductor. The conductor, in turn, is heated as the result of its ohmic resistance.

In order to prevent possible short-circuiting as well as meltdown, the plasma must be insulated from the rest of the instrument. Insulation is achieved by the concurrent flow of gasses through the system. Three gases flow through the system – the outer gas, intermediate gas and inner or carrier gas. The outer gas is typically Argon or Nitrogen. The outer gas has been demonstrated to serve several purposes including maintaining the plasma, stabilising the position of the plasma and thermally isolating the plasma from the outer tube. Argon is commonly used for both the intermediate gas and inner or carrier gas. The purpose of the carrier gas is to convey the sample to the plasma. However, the ICP operates at atmospheric pressure, whereas mass spectrometer (MS) requires an operating pressure $\leq 10^{-5}$ torr for resolution and detection of ions. The solution acts as an interface between the two.

The conventional method of sample introduction for ICP mass spectroscopy is by aspiration, via a nebuliser, into a spray chamber. A small fraction of the resulting aerosol is swept by argon into the torch. Approximately 1 ml of sample is required per analytical run, about 99% of which is wasted.

Recently, low cost, low uptake rate, high efficiency nebulisers have been employed to combat this problem. The high efficiency nebuliser operates more efficiently at 10–200 l/min. The detection limits and precision obtained with the high efficiency nebuliser are superior to conventional nebulisers.

Figure 9.13 shows the arrangement presently used for extracting ions from the ICP. The plasma flows around the tip of a water-cooled metal cone called the sampler. This cone has a circular orifice of 0.5–1.00 mm diameter drilled into its tip. Gas from the ICP enters this orifice from a cross-sectional area of diameter about three times the orifice diameter, so that a 1.5–3 mm wide section of the axial channel is sampled. Most of the gas flow is evacuated by a mechanical pump that maintains a pressure of the order of 1 torr.

The central orifice of a conical skimmer is located behind the sampler in such a way that the sampled beam is transmitted into a second vacuum chamber. The pressure here is low enough, and the mean free path long enough for ion lenses to collect, focus and transmit the ions to the mass analyser.

The formation of singly charged ions is very efficient in the ICP. Some 54 elements are expected to be ionised with an efficiency of 90% or more. Even metalloid or non-metallic elements such as P and As, for which ionic emission lines are either absent or not prominent, are ionised with reasonable efficiency. Therefore, combination of the ICP with MS shows promise of extending the sensitivity and selectivity of MS to elemental analysis of solutions.

9.4.2.2 Glow discharge ion source

In recent years, the glow discharge has been developed as a low-energy alternative ion source, which, coupled with a quadrupole mass filter, serves as a simple and inexpensive mass spectrometer.

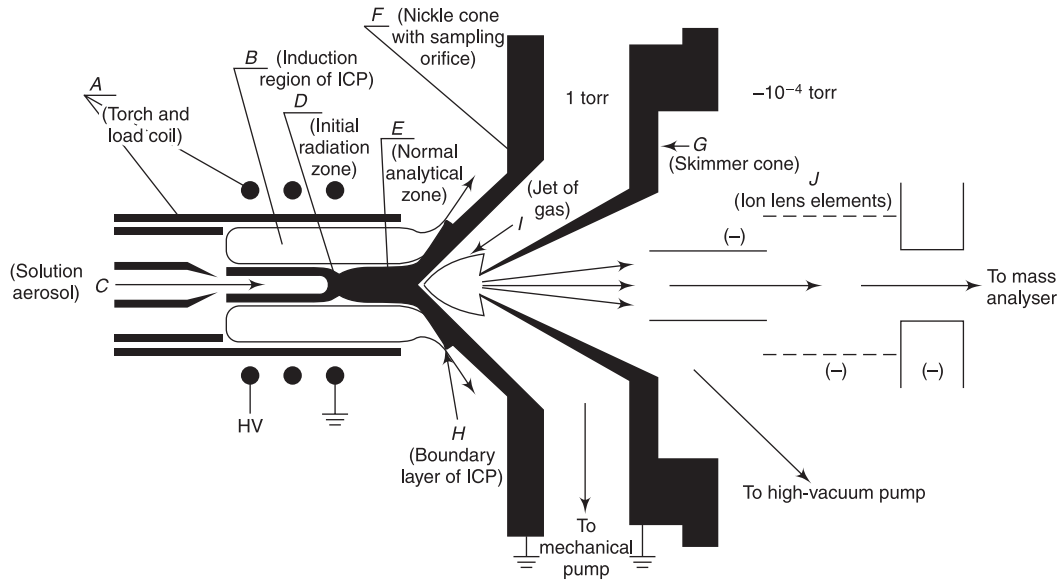


Figure 9.13 *Inductively coupled plasma as an ion source (redrawn after Houk, 1986)*

Basically, the glow discharge is a simple two electrode device, filled with a rare gas to about 0.1–10.0 torr. A few hundred volts applied across the electrodes cause breakdown of the gas and formation of the ions. Figure 9.14 shows the basic components and discharge regions. The sample to be analysed serves as the cathode, whereas the anode material is not particularly critical (Harrison et al., 1986).

The sample is atomised into the discharge by a process known as sputtering. The glow discharge not only atomises the solid sample, but also provides the means by which these atoms are ionised. Sputtered atoms diffuse into the negative glow, which contains energetic electrons,

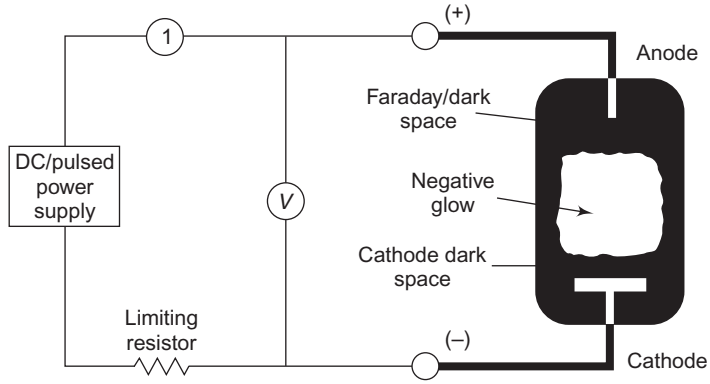


Figure 9.14 *Schematic representation of a glow-discharge ion source (Redrawn after Harrison et al., 1986)*

ions and metastable atoms. We are basically concerned with the ionisation of the sputtered atoms, rather than the bulk ionisation process, by which the discharge sustains itself.

The glow discharge is adaptable to many discharge types and ion source configurations. Sometimes, the sample type itself dictates a certain cathode geometry, whereas other source models arise from more fundamental considerations. Glow discharge ion sources have been operated in the DC, pulsed-DC and rf modes.

The glow-discharge source presents a flowing gas load to the mass spectrometer, requiring differential pumping to interface the 1-torr source with the 10^{-6} to 10^{-7} torr analyser chamber. Most of the reported work in glow-discharge mass spectrometer has been carried out using quadrupole analyser. This analyser makes it possible to design instruments with low sampling voltages and high ion transmission, particularly at low mass ranges.

The glow discharge possibly ionises only a small percentage of the sputtered sample atoms. Coupling the output of a tuneable laser to the glow-discharge plasma permits very selective ionisation of a given element. The focused laser beam is capable of close to 100% ionisation efficiency. In this arrangement, the glow discharge functions primarily as an atomisation source and the laser as the means of ionisation, Harrison et al. (1986) have reviewed the developments in glow-discharge mass spectrometry.

9.4.2.3 Other ionisation methods

Plasma discharge: A plasma is a hot, partially ionised gas that effectively excites and ionises atoms. The most common plasma source is an ICP.

Electron impact (EI): An Electron Impact source uses an electron beam, usually generated from a tungsten filament, to ionise gas-phase atoms or molecules. An electron from the beam knocks an electron off of analyte atoms or molecules to create ions.

Electrospray ionisation (ESI): This source consists of a very fine needle and a series of skimmers. A sample solution is sprayed into the source chamber to form droplets. The droplets carry charge when they exit the capillary. As the solvent evaporates, the droplets disappear leaving highly charged analyte molecules. The method is particularly useful for large biological molecules that are difficult to vaporise or ionise.

Fast-atom bombardment (FAB): In this method, a high-energy beam of natural atoms, typically Xe or Ar, strikes a solid or low-vapour-pressure liquid sample causing desorption and ionisation. It is used for large biological molecules that are difficult to get into the gas phase. The sample is usually dispersed in a matrix such as glycerol. FAB causes little fragmentation and usually gives a large molecular ion peak, making it useful for molecular weight determination.

The atomic beam is produced by accelerating ions from an ion source though a charge-exchange cell. The ions pick up an electron in collisions with neutral atoms to form a beam of high-energy atoms.

Field ionisation: Molecules can lose an electron when placed in a very high electric field. High fields can be created in an ion source by applying a high voltage between a cathode and an anode called a field emitter. A field emitter consists of a wire covered with microscopic carbon dendrites, which greatly amplify the effective field at the carbon points.

Laser Ionisation (LIMS)B: A laser pulse ablates material from the surface of a sample, and creates a micro-plasma that ionises some of the sample constituents. The laser pulse accomplishes both vaporisation and ionisation of the sample.

Matrix-assisted laser desorption ionisation (MALDI): This is a laser-based method of vaporising and ionising large biological molecules such as proteins or DNA fragments (Cotter, 1999). The biological molecules are dispersed in a solid matrix such as nicotinic acid or dihydroxybenzoic acid. A UV laser pulse ablates the matrix which carries some of the large molecules into the gas phase in an ionised form so they can be extracted into a mass spectrometer.

Thermal ionisation (TIMS): Thermal ionisation is used for elemental or refractory materials. *S* ample is deposited on a metal ribbon, such as Pt or Re, and an electric current heats the metal to a high temperature. The ribbon is often coated with graphite to provide a reducing effect.

9.4.3 Electrostatic Accelerating System

Positive ions, which are separated from electrons by a weak electric field, are accelerated in a strong electrostatic field between the first and second accelerating slit. Voltages of the order of 400–4,000 V accelerate the ions to their final velocities of up to 1,50,000 miles/s. and they acquire a kinetic energy of a few thousand electron volts. Such a relatively high kinetic energy is imparted to the ions to produce an almost mono-energetic beam, when it finally emerges out of the final accelerating slit, which is approximately 0.076 mm in width. The electrostatic voltages are highly stabilised to an accuracy of better than 0.01%.

9.4.4 Ion Detectors and Recording of Mass Spectrograph

Faraday Cup: The ion beam passing through the exit slit of the analyser tube is normally collected in a cylinder (Faraday cage), which is connected to the grid of an electrometer tube, whose output is in turn amplified. The use of an electrometer tube is necessitated because of an extremely low magnitude of the ion current (10^{-6} to 10^{-10} A). Vibrating electrometers have also been used in order to convert the DC current output to an AC signal. The amplified ion current is recorded as a function of the ratio m/e on an oscillograph or a pen-and-ink strip chart recorder. Since a Faraday cup can only be used in an analog mode it is less sensitive than other detectors that are capable of operating in pulse-counting mode.

Channeltron: A channeltron is a horn-shaped continuous dynode structure that is coated on the inside with an electron-emissive material. An ion striking the channeltron creates secondary electrons that have an avalanche effect to create more secondary electrons and finally a current pulse.

Electron multiplier tube (EMT): Electron multiplier tubes are similar in design to photomultiplier tubes. They consist of a series of biased dynodes that eject secondary electrons when they are struck by an ion. They therefore multiply the ion current and can be used in analog or digital mode.

Micro-channel plate: A micro-channel plate (MCP) consists of an array of glass capillaries (10–25 μm inner diameter) that are coated on the inside with a electron-emissive material. The capillaries are biased at a high voltage and like the channeltron, an ion that strikes the inside wall, one of the capillaries creates an avalanche of secondary electrons. This cascading effect creates a gain of 10^3 to 10^4 and produces a current pulse at the output. The schematic of a MCP is shown in Figure 9.15.

The recorder must have a provision for automatically recording peaks of widely varying amplitudes. This is achieved by using five separate recording channels with relative sensitivities of

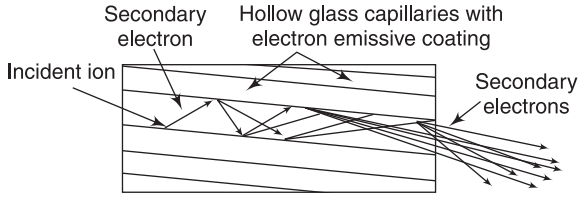


Figure 9.15 *Schematic of a micro-channel plate*

1,3,10.30 and 100. This arrangement enables the height of any peak to be recorded within better than 1% accuracy, over a range of magnitude of 1 to nearly 1,000.

9.4.5 Vacuum System

To prevent undue scattering by collision of ions with residual gas molecules, the mass spectrometer requires a good vacuum system. Generally, separate mercury or oil diffusion pumps are employed in the source and analysing regions of the spectrometers.

The two important parameters of a vacuum pump are its lowest attainable pressure and its pumping speed, typically listed as litres per minute (lpm) or cubic feet per minute (cfm). The lowest attainable pressure depends on the design of the pump. The pumping speed of the different types of pumps depends on the physical size of the pump. Table 9.1 gives the types of vacuum pumps along with the lowest attainable pressure

Mechanical pumps consist of an inlet and exhaust with a one-easy valve, and an off-centre rotating piston in a cylindrical cavity. As the piston rotates, gas is pulled into the cavity and forced out through the exhaust port. The rotating piston has spring-loaded vanes to create a seal with the cavity walls. This seal and the exhaust port valve are lubricated with allow-vapour-pressure oil. A two-stage mechanical pump consists of two pumping cavities in series to achieve a lower vacuum pressure. Accessories needed when using mechanical pumps are a mist filter or vent to trap oil mist in the pump exhaust and a trap to prevent oil vapour from backstreaming into the volume being evacuated.

A diffusion pump consists of a bath of boiling oil that streams through a jet-shaped volume. The oil entrains gas molecules and transports them in the direction of the oil flow. A mechanical pump can then pump away the exhaust from the diffusion pump.

A turbomolecular pump or just turbo pump contains a turbine that is spinning at a very high rate of revolution, typically tens of thousands revolutions per second. The turbine blades are spinning faster than the average speed of gas atoms or molecules, so that any gas-phase species that enter the turbo pump are physically forced out of the pump by the turbine blades. A mechanical pump is required to maintain a low pressure and pump away the exhaust from a turbo pump.

The mechanical pumps allow a vacuum of about 10^{-3} torr to be obtained. Once this vacuum is achieved the operation of the remainder of the vacuum system allows a vacuum as high as 10^{-10} torr to be reached.

Many analytical instruments including mass spectrometry, X-ray photoelectron and Auger electron spectroscopies, and electron microscopy require a low pressure to operate. Special gadgets or instruments are required to measure such low level pressures.

Table 9.1 *Common vacuum pumps*

Pump	Lowest Attainable pressure	Typical Use
Mechanical pump	10^{-3}–10^{-4} torr	Rouging or backing pump
Diffusion pump	10^{-6} torr	Vacuum lines
Turbomolecular pump	10^{-9} torr	High-vacuum systems

Table 9.2 *Common pressure gauges*

Gauge	Pressure Range	Typical Use
Manometer	760–1 torr	Systems near atmospheric pressure
Thermocouple gauge	$1–10^{-3}$ torr	Monitoring mechanical pumps
Ionisation gauge	$10^{-3}–10^{-9}$ torr	High-vacuum systems

By definition, pressure is the force per unit area, with units of pounds per square inch (PSI), or newtons per square metre ($N*m^{-2}$). In practice, pressure is usually reported in atmospheres (atm), which is the atmospheric pressure at sea level, or torr, which is 1.0 mm of mercury in an Hg manometer. One atm = 760 torr. Table 9.2 shows various types of pressure gauges and then range of measurement.

A *manometer* consists of a U-shaped tube that is closed and evacuated at one end, and filled with Hg or oil. One atmosphere of pressure at the open side of the tube pushes mercury in the tube to a height of 760 mm in the evacuated side of the tube. Lower pressure on the open side of the tube pushes the *H* mercury to less than 760 mm, and provides a measure of the pressure.

Thermocouple gauges operate on the dependence of thermal-conductivity on gas pressure. In these gauges, a constant current is applied to a metal filament to heat the filament. The temperature of the filament depends on the heat transfer to gas molecules, which depends on the pressure. The temperature of the filament is measured by making a thermocouple junction with the filament. The pressure reading is based on a calibration, which depends on the gas present in the vacuum system.

The most common type of ionisation gauge is a thermionic or hot-cathode gauge. It consists of an electrically heated filament and two electrodes. The filament (at ground voltage) emits electrons that are accelerated to the positively electrode. If the electrons collide with gas atoms or molecules they produce positive ions. Positive ions are collected at the negative electrode, creating an ion current which can be measured. The measured current depends on the density and ionisation cross section of the gas-phase species. Thermocouple gauges are used for calibration of different gases in the vacuum system.

Extreme cleanliness must be maintained on the surfaces in all regions of the evacuated system. The hands should not touch any interior surface, or any volatile lubricant used.

9.5 INDUCTIVELY COUPLED PLASMA MASS SPECTROMETER

Inductively coupled plasma mass spectroscopy (ICPMS) was developed in the late 1980s to combine the easy sample introduction and quick analysis of ICP technology with the accurate and low detection limits of a mass spectrometer. The resulting instrument is capable of trace multi-element analysis, often at the part per trillion level (Worley and Kvech, 2005).

ICP technology uses the same principles as used in atomic emission spectrometry. Samples are decomposed to neutral elements in high temperature argon plasma and analysed based on their *m/e* ratios. An ICP-MS consists of four main processes, (i) sample introduction and aerosol generation, (ii) ionisation by an argon plasma source, (iii) mass discrimination and (iv) the detection system. The schematic (Figure 9.16) illustrates this sequence of processes.

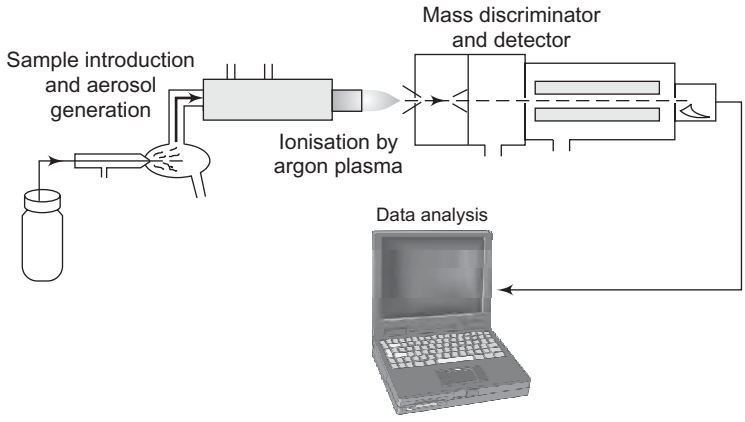


Figure 9.16 *Schematic of ICP-MS main process*

Unlike the atomic emission spectrometer, ICP-MS spectrometers can be used to analyse both solid as well as liquid samples. Solid samples are introduced into the ICP by way of a laser abalation system. Aqueous samples are introduced by way of a nebuliser which aspirates the sample with high velocity argon, forming a fine mist. The aerosol then passes into a spray chamber where larger droplets are removed via a drain (Jarvis et al., 1992). Typically, only 2% of the original mist passes through the spray chamber (Olesik, 1996). This process helps to produce droplets small enough to be vaporised in the plasma torch.

After the sample passes through the nebuliser and is partially desolvated, the aerosol moves into the torch body where it is mixed with more argon gas. A coupling coil is used to transmit rf to the heated argon gas, producing an argon plasma 'flame' located at the torch. The hot plasma removes any remaining solvent and causes sample atomisation followed by ionisation. In addition to being ionised, sample atoms are excited in the hot plasma. Next stage is an ICP torch (Figure 9.17). The aerosol moves into the bottom of the torch body. The ports on the right side of the torch body are where more argon is introduced to the flow. At the top are two high quality quartz tubes and an inner alumina injector tube.

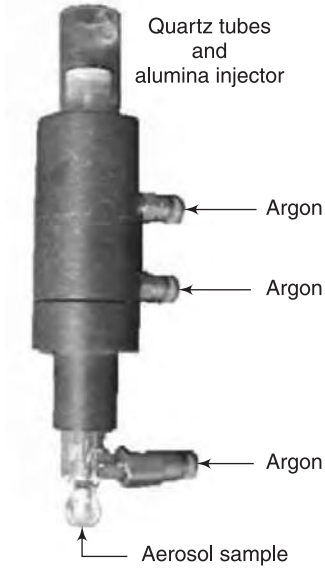


Figure 9.17 *ICP torch*

Atomisation/ionisation occurs at atmospheric pressure. Therefore, the interface between the ICP and MS components becomes crucial in creating a vacuum environment for the MS system. Ions flow through a small orifice, approximately 1 mm in diameter, into a pumped vacuum system. Here a supersonic jet forms and the sample ions are passed into

the MS system at high speeds, expanding in the vacuum system. The entire mass spectrometer is kept in a vacuum so that the ions are free to move without collisions with air molecules. Since the ICP is maintained at atmospheric pressure, a pumping system is needed to continuously pull a vacuum inside the spectrometer. In order to most efficiently reduce the pressure, several pumps are typically used to gradually reduce pressure to 10^{-5} mbar before the ion stream reaches the quadrupole. If only one pump were used, its size would be excessive to reduce the pressure immediately upon entering the mass.

In the first stage of the mass spectrometer, ions are removed from the plasma by a pumped extraction system. An ion beam is produced and focused further into the actual unit. Different types of mass analysers can be employed to separate isotopes based on their *m/e* ratio. Quadrupole analysers are compact and easy to use but offer lower resolution when dealing with ions of the same m/e ratio. A series of ion lenses, maintained at appropriate voltages, are used to direct the ions into quadrupole mass analyser. The ions are transmitted through the quadrupole on the basis of their *m/e* ratios and then detected by an electron multiplier.

The use of a quadrupole mass analyser gives better than unit mass resolution over a mass range up to $m/z = 300$. The ICPMS system is considered a sequential multi-element analyser that has scan times less than 20 ms for one sweep. The signal intensity is a function of the number of analyte ions in the plasma and the mass-dependent transport through the mass spectrometer. Double focusing section analysers offer better resolution but are larger and have higher capital cost.

The most common type of ion detector found in an ICP-MS system is the channeltron electron multiplier. This cone or horn-shaped tube has a high voltage applied to it opposite in charge to that of the ions being detected. Ions leaving the quadrupole are attracted to the interior cone surface. When they strike the surface, secondary electrons are emitted which move farther into the tube emitting additional secondary electrons. As the process continues, more electrons are formed, resulting in as many as 10^8 electrons at the other end of the tube after one ion strikes at the entrance of the cone. This results in an avalanche effect to create a current pulse.

The efficiency of the ICP in producing singly charged positive ions for most elements makes it an effective ionisation source for mass spectrometry. ICPMS is unique among the flame and plasma spectroscopy techniques in the ability to discriminate between the mass of the various isotopes of an element where more than one stable isotope occurs. Isotope dilution, in which the change in isotope ratio for two selected isotopes of an element of interest is measured in a solution after the addition of a known quantity of a spike that contains enrichment of one of the isotopes, permits calculation of the concentration of the element. Isotope dilution is the most reliable method of accurate determination of elemental concentration.

ICP-MS has been used widely over the years, finding applications in a number of different fields including drinking water, wastewater, natural water systems/hydrogeology, geology and soil science, mining/metallurgy, food sciences, and medicine (Olesik, 1996).

The most important advantages of ICP-MS include multi-element capability, high sensitivity and the possibility to obtain isotopic information on the elements determined. Disadvantages inherent to the ICP-MS system include the isobaric interferences produced by polyatomic species arising from the plasma gas and the atmosphere. The isotopes of argon, oxygen, nitrogen and hydrogen can combine with themselves or with other elements to produce isobaric interferences. ICP-MS is not useful in the detection of non-metals.

9.6 TRAPPED ION MASS ANALYSERS

The ion-trap mass spectrometer uses three electrodes to trap ions in a small volume. The advantages of the ion-trap mass spectrometer include compact size and the ability to trap and accumulate ions to increase the signal-to-noise ratio of a measurement.

The mass analyser consists of a ring electrode separating two hemispherical electrodes. A mass spectrum is obtained by changing the electrode voltages to eject the ions from the trap.

This class of instruments operate by storing ions in the trap and manipulating the ions by using DC and RF electric fields in a series of programmed timed events. This arrangement provides very high resolution and high sensitivity. There are two types of trapped-ion mass analysers:

- Static Traps: Ion cyclotron Resonance mass spectrometer
- Dynamic Traps: Three-dimensional quadrupole in trap mass spectrometer

9.7 QUADRUPOLE ION TRAP MASS SPECTROMETER

An ion-trap analyser consists of a central doughnut-shaped ring electrode and a pair of end cap electrodes. A variable rf is applied to the ring electrode while the end cap electrodes are grounded. Fragment ions circulate in a stable orbit within the cavity surrounded by the ring. As the rf energy is increased, the orbits of heavier ions become stabilised, while those with lighter ions become destabilised, causing them to collide with the walls of the ring electrode. When rf is scanned, the destabilised ions leave the cavity of the ring electrode via openings in the lower end cap and pass into a transducer. The arrangement is shown in Figure 9.18.

9.8 FOURIER TRANSFORM MASS SPECTROMETRY (FT-MS)

In FTMS, masses are represented by frequencies, and because frequencies can be measured very accurately, FTMS can offer potentially very high mass measurement accuracy. There are two types of mass analysers that employ Fourier transforms (FTs) to determine *m/z* ratios. These are

- Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICRMS) and
- The orbitrap

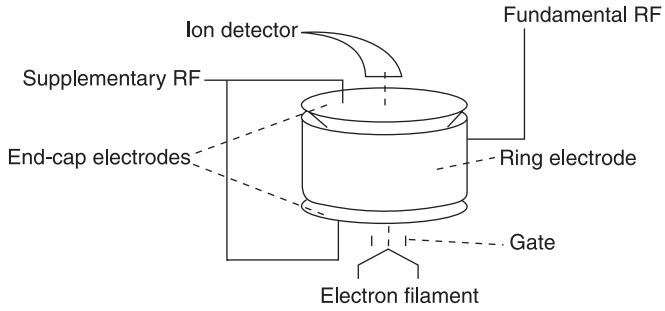


Figure 9.18 *Ion trap mass spectrometer*

9.8.1 Ion Cyclotron Resonance (ICR) Mass Spectrometery

It takes advantage of ion cyclotron resonance (ICR) to select and detect ions. Ions move in a circular path in a magnetic field. The cyclotron frequency of the ions circular motion is mass dependent. By measuring the cyclotron frequency, the mass of the ion can be determined (Tissue, 2000a).

By equating the centripetal force mv^2/r and the Lorentz force evB experienced by an ion in a magnetic field, the working equation can be derived as follows:

$$mv^2/r = e\ v\ B$$

solving for the angular frequency (ω), which is equal v/r then

$$\omega = v/r = e\ B/m$$

Ions of the same *m/e* ratio have the same cyclotron frequency, but they move independently and out of phase. If an *excitation pulse* is applied at the cyclotron frequency, the resonant ions will absorb energy and are brought into phase with the excitation pulse.

If several different masses are present, an excitation pulse that contains components at all the cyclotron frequencies must be applied. The ions re-emit the radiation, which is picked up by the receiver plates as *image currents*, amplified and digitalised. The signal induced in the receiver plates depends on the number of ions and their distance from the receiver plates.

The image currents induced in the receiver plates will contain frequency components from all of the *m/e* ratios. The various frequencies and their relative abundances can be worked out mathematically by using an FT which converts a time-domain signal (the image currents) to a frequency-domain spectrum (the mass spectrum).

9.8.1.1 Components of FT ICR-MS

All FTICRMS systems have in common the following five main components, as shown in Figure 9.19:

- A magnet (usually a superconducting magnet)
- Analyser cell (placed in the strong magnetic field created by the magnet)
- Ultra-high vacuum system
- Ion source
- Data system (many of the components in the data system are similar to those used in NMR).

In this section, we shall only discuss the ICR cell, which is the heart of the FTICRMS instrument. It is here that the ions are stored, mass analysed and detected.

Ion cyclotron frequency, radius, velocity and energy, as a function of ion mass, ion charge and magnetic field strength, follow directly from the motion of an ion in a spatially uniform static magnetic field.

Ion cyclotron motion may be rendered coherent (and thus observable) by the application of a spatially uniform RF electric field (excitation) at the same frequency as the ion-cyclotron frequency. The ICR signal results from induction of an oscillating 'image' charge on two conductive infinitely extended and opposed parallel electrodes. A frequency-domain spectrum is obtained by Fourier transformation of the digitised ICR signal.

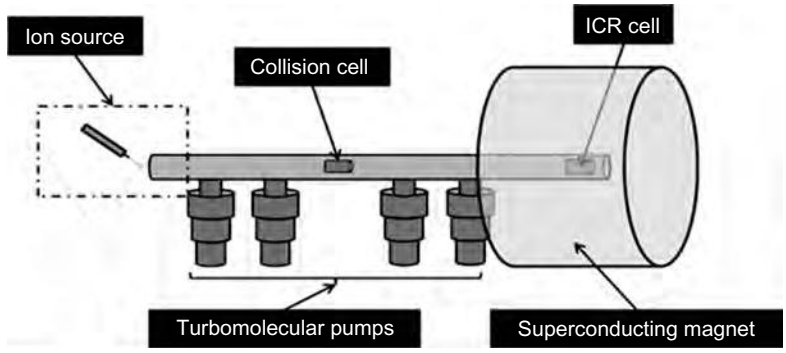


Figure 9.19 *Schematic representation of an FTICR mass spectrometer. Note that not all components are present in this scheme, for example, the ion optics is not presented, nor the rotary vacuum pumps that are needed for the proper functioning of the turbo molecular pumps (Paulo et al., 2012)*

Confinement of ions by application of a three-dimensional axial quadrupolar DC electric field shifts the ion-cyclotron frequency, whereas excitation and detection remain essentially linear, but with a reduced proportionality constant.

The detection of the ions occurs as the ion packets pass two detector plates. As the ion packets have past these plates, charge moves within the detection circuit to counteract the proximity of the ions. The potential change (voltage) between the detection plates can be measured as a function of time and it is from here that the raw data is obtained. It should be noted that the ions repeatedly pass the detector plates for the duration of the acquisition, as non-destructive detection is employed. The magnitude of the signal is proportional to the total charge and to the proximity of the ions to the detection plates (orbital radius), and is independent of magnetic field strength.

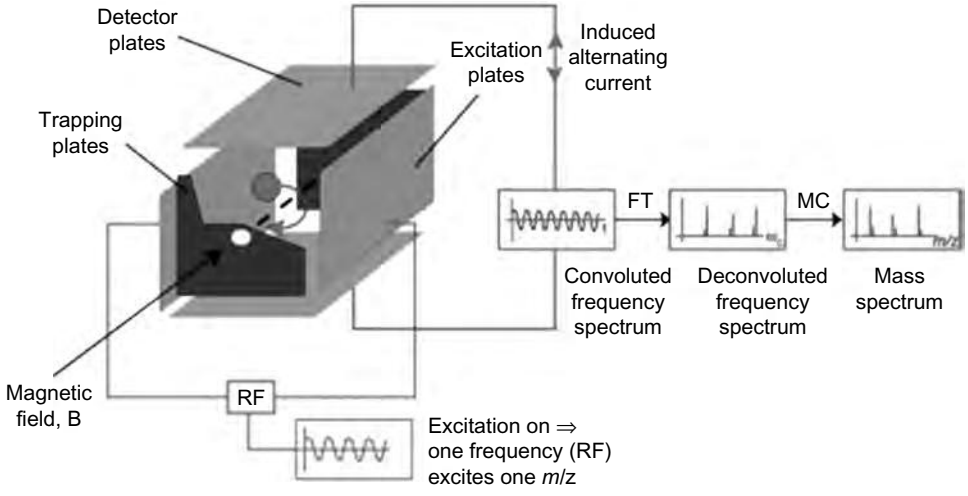


Figure 9.20 *Process of obtaining a mass spectrum from the time-domain data through Fourier transform. (Courtesy: University of Bristol)*

The raw data will represent the detections of all the ions at the same time, with their different cyclotron frequencies. It is therefore necessary to extract data concerning the different ion packets. This is done through the usage of FT, where frequency information is obtained from time-domain data. Figure 9.20 illustrates the process of obtaining a mass spectrum from the time-domain data through FT, conversion to *m/z* and calibration. Unlike other mass spectrometers (e.g. sector instruments, time-of-flight, quadrupole) where mass analysis and ion detection are spatially separated events, in FTICR all analytical steps are made on the same spatial place but separated in time. Figure 9.21 shows a typical FT ICR-MS system.

Figure 9.21 *A typical FT-ICR mass spectrometer (Courtesy: M/s Thermo Fisher)*

9.8.2 Orbitrap Mass Spectrometry

In the orbitrap (Figure 9.22) mass analyser, ions are injected tangentially into an electric field between specially shaped electrodes and trapped because their electrostatic attraction to the inner electrode is balanced by centrifugal forces. Thus, ions cycle around the central electrode in rings – rotational motion (elliptical orbiting). In addition, the ions also move back and forth along the axis of the central electrode – axial oscillation. Therefore, ions of a specific *m/e* ratio move in rings which oscillate along the central spindle-like electrode. The frequency of these harmonic oscillations is independent of the ion velocity and is inversely proportional to the square root of the *m/e* ratio (*m/z*). By sensing the ion oscillation in a manner similar to that used in FT-ICR (ion image current detection and FFT), the trap can be used as a mass analyser.

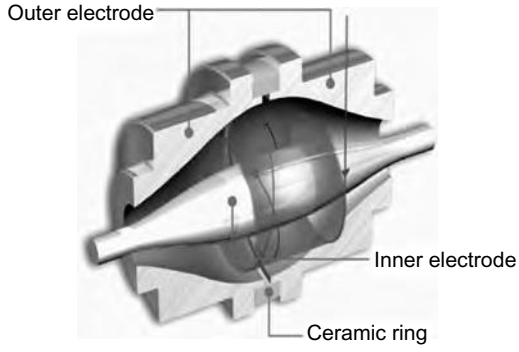


Figure 9.22 *Orbitrap mass analyser. Ions are captured in a quadro-logarithmic electrostatic field. An outer electrode enclosing a central spindle electrode consists of two halves separated by a dielectric material. The image current of ions moving as concentric rings along the central electrode is picked up by the outer electrode sections. (Courtesy: M/s Thermo Fisher Scientific)*

Features of the orbitrap at its present stage of development include high mass resolution (up to 1,50,000), large space charge capacity, high mass accuracy (2–5 ppm), a *m/e* range of at least 6000 and dynamic range greater than 103. The current commercially available orbitrap systems are equipped with several features that increase the range of applications. Figure 9.22 depicts a hybrid ion-trap orbitrap

mass spectrometer equipped with an Electron Transfer Dissociation (ETD) module. ETD is the ion trap equivalent to Electron Capture Dissociation (a very popular fragmentation technique used in FTICRMS) that enables, for example, the probing of post-translational modifications of proteins (Jung et al., 2010).

The final element of the mass spectrometer is the detector. The detector records either the charge induced or the current produced when an ion passes by or hits a surface. In a scanning instrument, the signal produced in the detector during the course of the scan versus where the instrument is in the scan (at what m/z) will produce a mass spectrum, a record of ions as a function of m/z.

Typically, some type of electron multiplier is used, though other detectors including Faraday cups and ion-to-photon detectors are also used. Because the number of ions leaving the mass analyser at a particular instant is typically quite small, considerable amplification is often necessary to get a signal. MCP detectors are commonly used in modern commercial instruments. In FTMS and orbitraps, the detector consists of a pair of metal surfaces within the mass analyser/ion-trap region which the ions only pass near as they oscillate. No DC current is produced, only a weak AC current is produced in a circuit between the electrodes.

9.9 TANDEM MASS SPECTROMETRY (MS/MS)

Mass spectrometers are commonly combined with separation devices such as gas chromatographs (GC) and liquid chromatographs (LC). The GC or LC separates the components in a mixture and the components are introduced, one by one, into the mass spectrometer. MS/MS is an analogous technique where the first-stage separation device is another mass spectrometer. A tandem mass spectrometer is one capable of multiple rounds of mass spectrometry, usually separated by some form of molecule fragmentation.

Let us assume that we want to analyse a mixture of components. Each component of the mixture produces characteristic ionic species and has a unique molecular weight. The mass spectrum of the mixture contains peaks for each compound present in the mixture. However, the entire mass spectrum tells us the molecular weight, but we would really like to see fragment ions that provide structural information for the component of interest.

The simplest form of tandem mass spectrometry is shown in Figure 9.23. It combines two mass spectrometers. The first mass spectrometer is used to select a single (precursor) mass that is characteristic of a given analyte in a mixture. The mass-selected ions pass through a region where they are activated in some way that causes them to fall apart to produce fragment (product) ions. This is usually done by colliding the ions with a neutral gas in a process called collisional activation (CA) or collision-induced dissociation (CID). The second mass spectrometer is used to separate the fragment ions according to mass. The resulting 'MS/MS' spectrum consists only of product ions from the selected precursor. Chemical background and other mixture components are absent.

Early work on MS/MS was done with reverse-geometry double focusing mass spectrometers. A reverse-geometry mass spectrometer is one in which the magnetic sector precedes the electric sector. A magnetic sector alone can be used as a mass spectrometer, with roughly unit resolution. Therefore, the magnetic field strength can be adjusted to select a given precursor mass. The precursor mass is then activated by collisions in the second field-free region, just between the magnetic and electric sectors and then the electric sector is used to analyse the kinetic energies of the product ions. This is referred to as 'mass-analysed ion kinetic energy spectrometer' (MIKES).

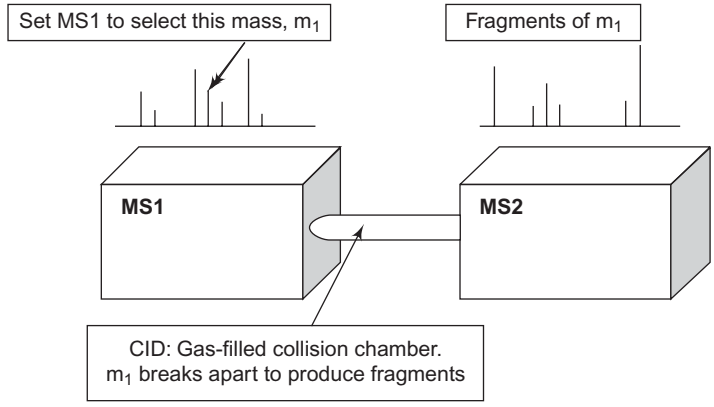


Figure 9.23 *Tandem mass spectrometer (MS/MS)*

MIKES experiments, we are measuring product-ion kinetic energies instead of product-ion *m/e* ratios. MIKES spectra provide a 'fingerprint' that can be used to identify a given analyte, and they are useful for ion chemistry studies.

Most magnetic sector analyses are done by setting the accelerating voltage and electric sector to a fixed accelerating voltage and electric sector. MS/MS can also be done by scanning the electric and magnetic sector together according to certain scan laws.

Consider what happens to ions during a product-ion linked scan where collisions occur in the first field-free region. Ions leaving the ion source are accelerated to a kinetic energy that depends only on the accelerating potential and the number of charges on the ion:

$$T = eV = \frac{mv^2}{2}$$

All ions with the same number of charges will have the same kinetic energy. Assuming that the ions have only a single charge, it is apparent from the above expression that ions with different masses must have different velocities if their kinetic energies are the same.

Let us assume that precursor ions with mass m_1 fall apart in the first field-free region to for product ions with mass m_2. Let us also assume that the velocity does not change when the ions fall apart. This is a safe assumption because we will only be observing ions that undergo grazing collisions, and any change in velocity will be small compared to the total velocity of ions accelerated to say, 10 kilovolt kinetic energies. The product ions will still have the same velocity as the precursor ions. If we can select ions according to their velocities, we can tell which product ions were formed from precursor ions with known velocities.

Recall that the magnetic sector separates ions according to their momentum (mv) and the electric sector selects ions according to their kinetic energy ($mv^2/2$). The ratio of B to E is related to the velocity, which is as follows:

$$\frac{B}{E} = \frac{mv}{mv^2/2} = \frac{2}{v}$$

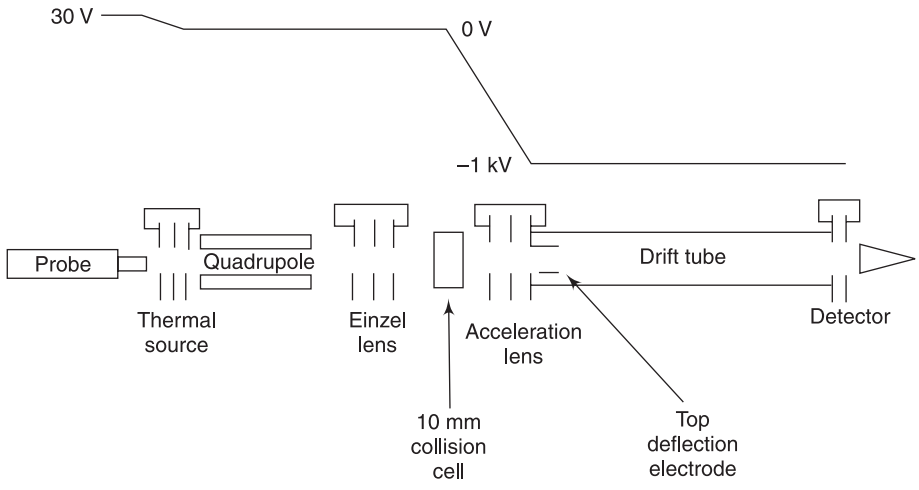


Figure 9.24 *Schematic of the tandem quadrupole/time-of-flight instrument*

Thus, a B/E ratio can be chosen to select ions with a given velocity. If we scan B and E together, always keeping a constant B/E ratio, we will detect product ions from the precursor ion with the specified velocity. This means that a B/E scan is a product-ion scan.

A tandem mass spectrometer for MS/MS studies consists of a quadrupole mass filter followed by a time-of-flight mass analyser (Glish and Goeringer, 1984). This is shown in Figure 9.24. A collision cell is located in the region between the two analysers. Low energy parent ions (≤100 eV is mass analysed by the quadrupole and injected into the collision cell and is accelerated by 1,000 eV upon exiting the collision cell. These accelerated ions are their gated into the time-of-flight for mass analysis.

Ionisation or solvent molecules are produced by a corona discharge at the discharge needle. The solvent ions formed produce analyte ions by atmospheric pressure chemical ionisation source. These ions are focused and declustered through a dry nitrogen region, and then through a 100 pm orifice into the high vacuum analyser region of the MS, where they are mass analysed. Typically, a nebuliser vapour temperature of 125–150°C is maintained, which is suitable for a variety of applications.

9.10 RESOLUTION IN MASS SPECTROMETRY

Several different definitions of resolution are used in mass spectrometry. Some of the commonly referred to definitions are:

The difference between each mass from the next integer is defined a unit resolution the capability of the equipment to distinguish mass say 500 from mass 501. This definition is commonly used when describing resolution on quadrupole and ion-trap mass spectrometers peak shapes in quadrupole mass spectrometers are usually 'flat shaped' (Figure 9.25).

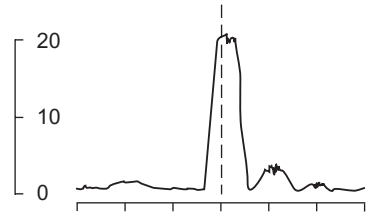


Figure 9.25 *Flat shaped peaks in ion-trap mass spectrometer*

In magnetic sector mass spectrometers, resolution is defined as the mass divided by the difference in mass number between two distinguishable neighbouring lines of equal height in the mass spectrum. If two ions of mass M_1 and M_2 differing in mass by ΔM give adjacent peaks in the mass spectrum as shown in Figure 9.26 and the height of peak is H above the base line, and height of the valley h is less than or equal to 10% of the peak H (i.e. $h \leq 10$), the resolution is then $M_1/\Delta M$. For example, if the peaks representing two masses 100.000 and 100.005 are separated by a 10% valley, the resolution is 100.000/0.005 (i.e. 20,000).

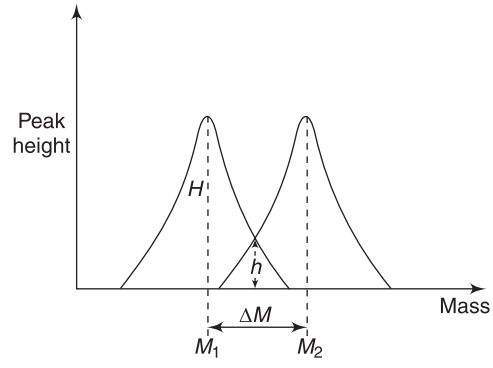


Figure 9.26 *Resolving power calculation for mass spectrometer*

Time-of-flight mass spectrometers usually use the 50% peak-height (h) definition. Similarly, Fourier Transform ion cyclotron resonance (FTICR) mass spectrometers also usually use the 50% valley definition for resolution because of the broadening near the base lime of the peak shape. Resolving power is inversely proportional to mass in FTICR. So, it is important to know the mass at which a given resolving power was obtained in order to determine what the resolution should be at another mass.

9.11 APPLICATIONS OF MASS SPECTROMETRY

In addition to general inorganic analysis and trace analysis in inorganic chemistry, rocket-borne mass spectrometers now analyse the upper atmosphere. Perhaps the most impressive experimental achievement in mass spectroscopy is the successful mass spectrometric analysis carried out on the surface of Mars. Process monitor mass spectrometers, with the idea of direct control of industrial process have been constructed.

In the early 1950s, mass spectrometers were used for the quantitative analysis of mixtures of the lighter hydrocarbons containing molecules with up to six or seven carbon atoms. The mass spectra of the various components in a mixture were taken to be linearly additive, and the analysis was worked out by setting up a set of simultaneous linear equations and solving them, usually by matrix inversion, using desk calculators. The advances in digital computers have considerably transformed the situation concerning calculations of such equations. Mass spectrometry is presently used to make analysis of mixtures into different hydrocarbon types, even when many hydrocarbons are present with 20–30 carbon atoms in the molecule. Therefore, they are used in refineries for trace element investigations, analysis of lubricating oils and quantifying the substances in mixtures of organic compounds.

In addition, they are used in detecting and measuring the concentrations of pollutants in air and water.

Each mass analyser has its own special considerations and applications and its own benefits and limitations. The choice of mass analyser is usually based upon the application, cost and performance desired. There is no ideal mass analyser that is goof for all applications.

10

NUCLEAR MAGNETIC RESONANCE SPECTROMETER

10.1 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive technique for mapping molecular structures and learning how molecules function and relate to each other. It is recognised as one of the most powerful techniques for chemical analysis. The importance of this technique is reflected in the efforts that have been made to extend its applicability to smaller and smaller sample sizes. NMR spectrometer provides an accurate and non-destructive method of determining structure in liquids and soluble chemical compounds.

The study of absorption of radio frequency (rf) radiation by nuclei in a magnetic field is called nuclear magnetic resonance. For a particular nucleus an NMR absorption spectrum may consist of one to several groups of absorption lines in the rf region of the electromagnetic spectrum. They indicate the chemical nature of the nucleus and the spatial positions of neighbouring nuclei (Grant, 1985).

NMR spectroscopy uses rf radiation to induce transitions between different nuclear spin states of samples in a magnetic field. NMR spectroscopy can be used for quantitative measurements, but it is most useful for determining the structure of molecules along with IR spectroscopy and mass spectrometry. The utility of NMR spectroscopy for structural characterisation arises because different atoms in a molecule experience slightly different magnetic fields and therefore, transitions at slightly different resonance frequencies in an NMR spectrum. Furthermore, splittings of the spectra lines arise due to interactions between different nuclei, which provide information about the proximity of different atoms in a molecule.

10.2 PRINCIPLE OF NMR

10.2.1 Nuclear Spin

Elementary particles such as electrons or a nucleus are known to behave as if they rotate about an axis and thus have the property of spin. The angular momentum associated with the spin of the particle would be an integral or a half-integral multiple of $h/2\pi$, where h is Planck's constant. The maximum spin component for a particular particle is its spin quantum number I. Subatomic particles (electrons, protons and neutrons) can be imagined as spinning on their axes. In many atoms (such as ^{12}C) these spins are paired against each other, such that the nucleus of the atom has

no overall spin. However, in some atoms (such as ^{1}H and ^{13}C) the nucleus does possess an overall spin. The rules for determining the net spin of a nucleus are as follows:

(i) If the number of neutrons and the number of protons are even, the spin would be zero. Nuclei of this type do not give rise to an NMR signal. Neither do they interfere with an NMR signal from other nuclei. Examples are C^{12}, 0^{16}.
(ii) Nuclei having either the number of protons or the number of neutrons as odd have half-integral spin. Examples are H^{1} B^{11}, p^{31}, etc.
(iii) Nuclei which have both the number of neutrons and the number of protons as odd would have integral spin. For example, H^{2} and N^{14}.

10.2.2 Nuclear Energy Levels

Since a nucleus possesses a charge, its spin gives rise to a magnetic field that is analogous to the field produced when an electric current is passed through a coil of wire. The resulting magnetic dipole or nuclear magnetic moment μ is oriented along the axis of spin and has a value that is characteristic for each kind of particle.

When spinning nucleus is placed in a strong uniform magnetic field *(H)* (Figure 10.1), the field exerts a torque upon the nuclear magnet. This would make the nucleus to assume a definite orientation with respect to the external field. The torque is a vector with its direction at right angles to the plane of μ and H. This results in a rotation of the nuclear axis around the direction of the external field. This is called precessional motion.

Each orientation of the nucleus corresponds to a different energy level or state. The interrelation between particle spin and magnetic moment leads to a set of observable magnetic quantum states given by the following formula:

$$m = I, I-1, I-2, \ldots, -(I-1), -1$$

The overall spin, I, is important. Quantum mechanics tell us that a nucleus of spin I will have $2I + 1$ possible orientations. A nucleus with spin 1/2 will have 2 possible orientations. In the absence of an external magnetic field, these orientations are of equal energy. If a magnetic field is applied, then the energy levels split. Each level is given a magnetic quantum number, m.

The spin number for both the electron and proton is 1/2. Thus each has two spin states, corresponding to $I = 1/2$ and $I = -1/2$. The spin number of a nucleus is related to the relative number of protons and neutrons it contains. Therefore, for heavier nuclei, the spin number

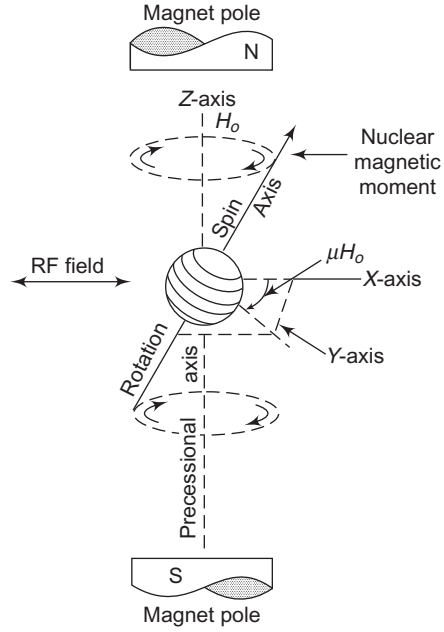


Figure 10.1 *Spinning nucleus in a magnetic field*

range from zero to at least 9/2. When the nucleus is in a magnetic field, the initial populations of the energy levels are determined by thermodynamics, as described by the Boltzmann distribution. This is very important, and it means that the lower energy level will contain slightly more nuclei than the higher level. It is possible to excite these nuclei into the higher level with electromagnetic radiation. The frequency of radiation needed is determined by the difference in energy between the energy levels.

When brought into the influence of an external magnetic field, a particle that possesses a magnetic moment, tends to become oriented such that its magnetic dipole and hence its spin axis is parallel to the field. The energy levels are a function of the magnitude of the nuclear magnetic moment and the strength of the applied magnetic field.

10.2.3 Resonance Conditions

When an alternating rf field, superimposed over the stationary magnetic field, rotates at exactly the frequency of an energy level, the nuclei will be provided enough energy to undergo a transition from a lower energy level to a higher-energy level. In general, the energy difference between states is given by

$$\Delta E = \mu\beta\, H_o / I$$

where H_o is the strength of the external magnetic field in gauss, β is a constant called the nuclear magnetron. 5.049×10^{-24} ergs – gauss^{-1}, μ is the magnetic moment of the particle expressed in units of nuclear magnetrons. The value of μ for the proton is 2.797 nuclear magnetrons.

The frequency υ of the radiation that will effect transitions between energy levels can be determined from the Planck's equation as given below:

$$\Delta E = h\upsilon = \mu\beta\, H_o / I$$

The frequency of the resonance absorption can be adjusted by varying the value of the applied magnetic field. Difficulties in construction of large magnets limit the field to approximately 23,000 gauss. In that case

$$\upsilon = \mu / h\, \beta\, H_o / I \qquad h = 6.626 \times 10^{-27} \text{ erg.s}$$

$$= \frac{2.797 \times 5.05 \times 10^{-24} \times 23{,}000}{(6.6256 \times 10^{-27})\,(1/2)}$$

$$= 95 \times 10^{6} \text{ Hz} = 95 \text{ MHz}$$

Therefore, proton will precess 95 million times per second in a fixed field of 23,000 gauss. The frequency 95 MHz lies in the rf range of the electro-magnetic spectrum.

With a field strength of 14,092 gauss, the frequency would be 60 MHz. Similarly, for a fixed field strength of 10,000 gauss, the frequency is 40 MHz.

10.2.4 NMR Absorption Spectra

For understanding NMR absorption spectra, the behaviour of a charged particle in a magnetic field needs to be understood.

Imagine a nucleus of spin 1/2 in a magnetic field. This nucleus is in the lower energy level (i.e. its magnetic moment does not oppose the applied field). The nucleus is spinning on its axis. In the presence of a magnetic field, this axis of rotation will precess around the magnetic field.

The frequency of precession is termed the Larmor frequency, which is identical to the transition frequency.

The potential energy of the precessing nucleus is given as follows:

$$E = -\mu B \cos \theta$$

where θ is the angle between the direction of the applied field and the axis of nuclear rotation.

If energy is absorbed by the nucleus, then the angle of precession, θ, will change. For a nucleus of spin 1/2, absorption of radiation 'flips' the magnetic moment so that it opposes the applied field (the higher-energy state).

It is important to realise that only a small proportion of 'target' nuclei are in the lower energy state and can absorb radiation. There is the possibility that by exciting these nuclei, the populations of the higher and lower energy levels will become equal. If this occurs, then there will be no further absorption of radiation. The spin system is saturated. The possibility of saturation means that we must be aware of the relaxation processes which return nuclei to the lower energy state.

NMR absorption spectra can be obtained either by changing the frequency of the rf oscillator or by changing the spacing of the energy levels by varying the magnetic field. Constructing a highly stable oscillator, whose frequency can be varied continuously, is a difficult job. Also, there are no dispersing elements analogous to a prism and a grating for rf radiation. Therefore, it is more practical to hold the oscillator frequency constant and vary the magnetic field continuously. Since for a given nucleus, frequency and field strength are directly proportional, the magnetic field *(H)* can be used equally well as abscissa for recording an NMR absorption spectrum.

10.2.5 Relaxation Process

When a nuclei in the higher-energy state return to the lower state, emission of radiation takes place. However, the emission is insignificant because the probability of re-emission of photons varies with the cube of the frequency. At radio frequencies, re-emission is negligible. Therefore, non-radiative relaxation processes will give more useful information.

The following are the two major relaxation processes:

- Spin-lattice (longitudinal) relaxation
- Spin-spin (transverse) relaxation

10.2.5.1 Spin lattice relaxation

Nuclei in an NMR experiment are in a sample. The sample in which the nuclei are held is called the lattice. Nuclei in lattice are in vibrational and rotational motion, which creates a complex magnetic field. The magnetic field caused by motion of nuclei within the lattice is called the lattice field. This lattice field has many components. Some of these components will be equal in frequency and phase to the Larmor frequency of the nuclei of interest. These components of the lattice field can interact with nuclei in the higher-energy state, and cause them to lose energy thereby returning to the lower

state. The energy that a nucleus loses increases the amount of vibration and rotation within the lattice resulting in a tiny rise in the temperature of the sample.

10.2.5.2 Spin-spin relaxation

Spin-spin relaxation describes the interaction between neighbouring nuclei with identical precessional frequencies but differing magnetic quantum states. In this situation, the nuclei can exchange quantum states; a nucleus in the lower energy level will be excited, while the excited nucleus relaxes to the lower energy state. There is no net change in the populations of the energy states, but the average lifetime of a nucleus in the excited state will decrease. This can result in line-broadening.

10.2.6 The Chemical Shift

It is the phenomenon that occurs in which a specification, that is, a carbon or hydrogen atom, in a given molecule resonates at a slightly different frequency based on its local chemical environment. In other words, the difference between the field necessary for resonance in the sample and in some arbitrarily chosen reference compound is called the chemical shift. For protons, it is usual to refer spectra to tetramethyl silane (TMS_i) with extrapolation to infinite dilution in an inert solvent such as CCI_4. TMS_i gives sharp resonance line at the high field end of the range of observed proton shifts and therefore, it does not obscure any other proton lines arising from the sample.

The chemical shift is expressed as follows:

$$\delta = \frac{H_{\text{sample}} - H_{\text{TMSi}}}{H_1} \times 10^6$$

where H_{sample} and H_{TMSi} are the positions of the absorption peaks for the sample and reference material respectively in Hz and H_1 is the rf of the signal used. The chemical shift 'δ units' is expressed in parts per million.

NMR takes advantage of the phenomenon of the chemical shift so it allows a chemist to obtain a picture of not only how many particular atoms are in a molecule, but also an idea of how the atoms are joined together in the molecule.

10.3 TYPES OF NMR SPECTROMETERS

There are two NMR spectrometer designs: continuous-wave (CW) and pulsed or Fourier transform (FT-NMR).

10.3.1 Continuous-Wave NMR Spectroscopy

A CW-NMR spectrometer consists of a control console, magnet, and two orthogonal coils of wire that serve as antennas for rf radiation. One coil is attached to an rf generator and serves as a transmitter. The other coil is the rf pick-up coil and is attached to the detection electronics.

Since the two coils are orthogonal, the pick-up coil cannot directly receive any radiation from the generator coil. When a nucleus absorbs rf radiation, it can become reoriented due to its normal movement in solution and re-emit the rf radiation is a direction that can be received by the pick-up

coil. This orthogonal coil arrangement greatly increases the sensitivity of NMR spectroscopy, similar to optical fluorescence.

Spectra are obtained by scanning the magnet and recording the pick-up coil signal on paper at the control console.

CW NMR spectrometers have largely been replaced with pulsed FT-NMR instruments. However due to the lower maintenance and operating cost of CW instruments, they are still commonly used for routine ^{1}H NMR spectroscopy at 60 MHz. Low resolution CW instruments require only water-cooled electromagnets instead of the liquid-He-cooled super-conducting magnets found in higher-field FT-NMR spectrometers.

10.3.2 Fourier Transform NMR Spectroscopy

FT NMR spectrometers use a pulse of rf radiation causes nuclei in a magnetic field to flip into the higher-energy alignment. Due to the Heisenberg uncertainty principle, the frequency width of the rf pulse (typically 1–10 μs) is wide enough to simultaneously excite nuclei in all local environments. All of the nuclei will re-emit rf radiation at their respective resonance frequencies, creating an interference pattern in the resulting rf emission versus time, known as a free-induction decay (FID). The frequencies are extracted from the FID by a Fourier transform of the time-based data.

Because it allows NMR transition to be observed simultaneously rather than serially, it has increased the sensitivity of NMR by more than a factor of 10 and has reduced the time required to obtain NMR date by a factor greater than 100.

An FT-NMR spectrometer consists of a control console, magnet, and a coil of wire that serves as the antenna for transmitting and receiving the rf radiation. Only one coil is necessary because signal reception does not begin until after the end of the excitation pulse. Because the FID results from the emission due to nuclei in all environments; each pulse contains an interference pattern from which the complete spectrum can be obtained. Because of this multiplex advantage, repetitive signals can be summed and averaged to greatly improve the signal-to-noise ratio of the resulting FID.

10.4 CONSTRUCTIONAL DETAILS OF NMR SPECTROMETER

Figure 10.2 shows the block diagram of an NMR spectrometer. It is a complex system integrating several technologies into an analytically powerful, information rich system. The Key parts of the system are:

- A magnet, which produces magnetic field in the range 10,000–25,000 gauss
- Radio frequency transmitting system
- The signal amplifier and detector
- A display device, which may be a recorder or an oscilloscope
- A non-magnetic sample holder, which holds the sample
- Computer workstation which stores and processes the NMR data using complex software to generate spectrum for the same

The various sub-systems in an NMR spectrometer are shown in Figure 10.3.

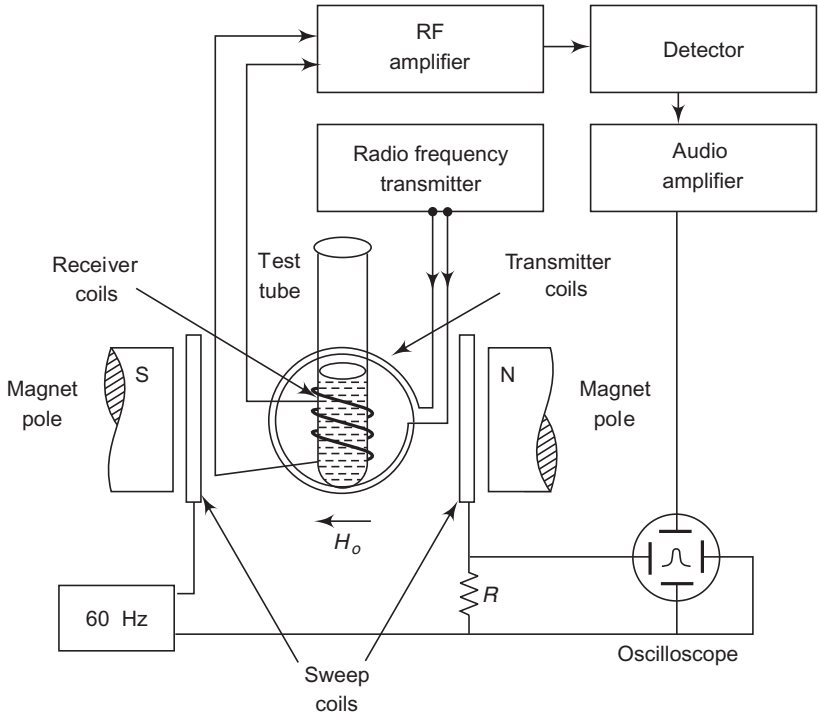


Figure 10.2 *Block diagram of a nuclear magnetic resonance spectrometer*

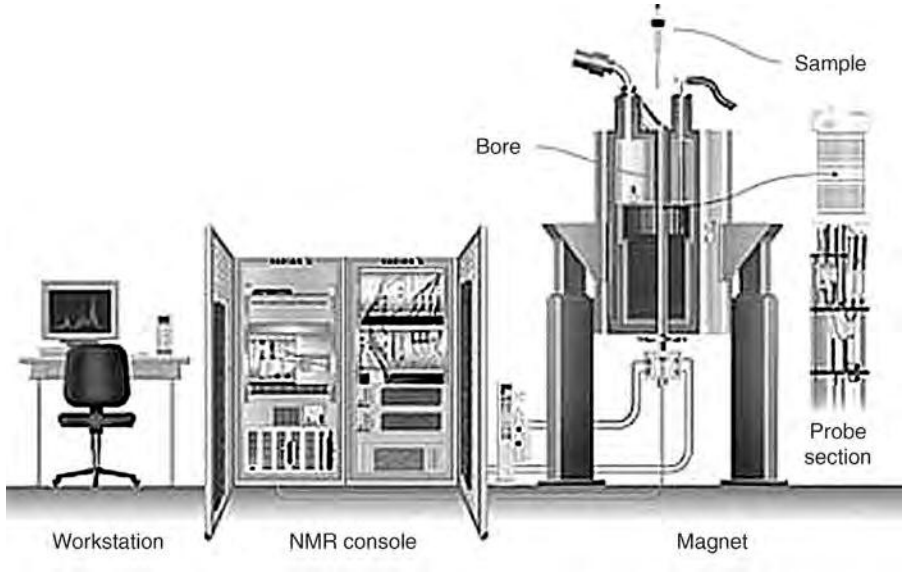


Figure 10.3 *Various sub-systems of an NMR spectrometer (Courtesy: M/s Varian, USA)*

10.4.1 Magnetic Field

The magnet used in these instruments may be permanent magnet or electromagnet. Alternately, the magnetic field may be produced from super-conducting solenoids. Decidedly, a permanent magnet is less expensive, but it does not allow the observation of the resonance of different nuclei and of a given nucleus, at two different field strengths. Therefore, either electromagnet or super-conducting magnets are generally used.

Important requirement of the magnet is that, it should be stable and homogeneous. In electromagnets, stability of the magnetic field is achieved by continuously compensating for small rapid fluctuations in magnetic field with coils wound around the pole faces and by controlling the temperature of the magnet to minimise thermal fluctuations. Inhomogeneity is compensated also with small magnetic fields, produced by passing DC current through small electrical coils located on the faces of the magnet, and spinning the sample to effectively average out field gradients in the direction perpendicular to the spin axis. This, however, produces spinning side bands, due to modulation of the magnetic field. They can be identified from the main signal by changing the spinning rate. The magnetic field must be highly homogeneous in the sample area and the order of homogeneity is 1 part in 10^8. In high-resolution instruments, to ensure the field stability of the magnet, it is temperature compensated. The magnet is placed in a thermostated oven and is surrounded by heavy thermal insulation. Special magnetic and rf shielding minimises the effects of environmental perturbations, which can disrupt NMR operation. In case of electromagnets, the pole pieces are about 12 inches in diameter and are spaced about 1.75 in apart.

For the study of NMR, there are great advantages in working at the highest possible applied magnetic field strength, especially for the resonances of nuclei other than hydrogen and fluorine. Also, the expected signal-to-noise ratio increases rapidly, as the strength of the magnet is increased. Super-conducting magnets are operated at the temperature of liquid helium which is 4.2 K at standard pressure and for the highest available fields even at 2 K. The magnet coils are constructed using niobium alloy wires embedded in a copper wire which allows the winding of a solenoid. The niobium alloys used become super-conducting at low temperatures unlike copper.

In addition to the main coil, a super-conducting magnet contains additional super-conducting coils, shim coils, which produce specific magnetic field gradients that can be used to improve the basic homogeneity of the main coil. Still the homogeneity required for high-resolution NMR experiments can only be reached by additional non-super-conducting shim coils which are mounted in the room temperature bore of the super-conducting magnet.

Advances in magnet technology have resulted in the availability of commercial high-resolution spectrometers with super-conducting solenoids producing fields of 12 tesla (500 MHz ^{1}H resonance) with the probability of 14.4 T just around the corner. The principal advantages of these high fields are in the greater dispersion of complex ^{1}H spectra and the higher sensitivity of the basic NMR technique. However, the higher-field strength magnets are of comparatively high cost.

The strength of a super-conducting magnet is typically specified in terms of the resonance frequency for the hydrogen atom expressed in megahertz (MHz). Depending on the magnet, the field strength can range from 200 to 900 MHz. The 900 MHz NMR magnet is 10 times stronger than the most powerful magnetic resonance imaging (MRI) system used in hospitals and 2,00,000 times stronger than the earth's magnetic field.

A typical super-conducting solenoid is 26 cm long, with a 3 cm bore and outside diameter 8.36 cm. It is wound with Nb-Zr 25% wire and produces a field of 5T (1 T = 10,000 gauss) with a current

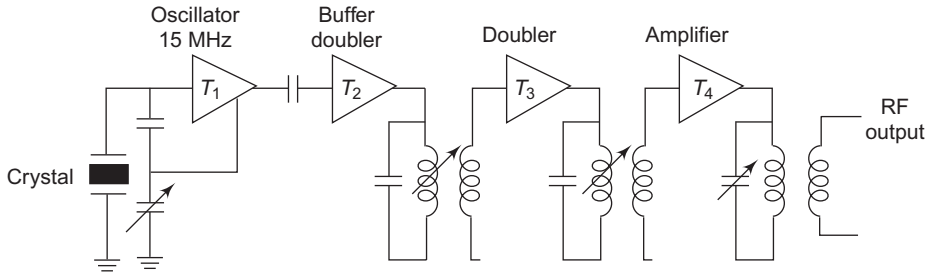


Figure 10.4 *Transmitter circuit*

of about 20 Å. Permanent magnets can yield maximum magnetic fields, approximately 14,000 gauss, whereas electromagnets can produce fields up to about 24,000 gauss. Super-conducting solenoids have been used to give approximately 70,000 gauss.

10.4.2 The Radio-Frequency Transmitter

The rf transmitter is a 60 MHz crystal controlled oscillator. The rf signal is fed into a pair of coils mounted at right angles to the path of the field. The coil that transmits the rf field is made in two halves, to allow insertion of the sample holder. The two halves are placed in the magnetic gap. For high-resolution work, the transmitted frequency must be highly constant to about 1 part in 10^8. The oscillator is of low power, generally of less than 1 W. The basic oscillator (Figure 10.4) is usually crystal controlled, at a fundamental frequency of 15 MHz. It is followed by a buffer doubler, the frequency being doubled by tuning the variable inductance to the second (30 MHz). It is further connected to another buffer doubler, with the connector inductance, tuned to 60 MHz. A buffer amplifier T_4 is provided to avoid circuit loading on the tuned doubler. Precision resistors are used in the transmitter for their low noise characteristics.

10.4.3 The Signal Amplifier and Detector

The rf signal produced by the resonating nuclei is detected by means of a coil that surrounds the sample holder. This coil consists of a few turns of wire and is placed at right angles to the source coil and the stationary field to minimise pick up from these fields. Even so, the coupling between transmitting and receiving coils cannot be completely eliminated and some leakage is always known to be present. The problem of this coupling is solved to some extent by using devices called paddles, which act as inductors mutually coupled to both receiver and transmitter.

The signal results from the absorption of energy from the receiver coil, when nuclear transitions are induced and the voltage across the receiver coil drops. This voltage change is quite small and must be amplified in an rf amplifier before it can be displayed.

Referring to Figure 10.5, after impedance matching in a transformer T_1, the input rf signal is amplified in a three-stage low noise cascade amplifier. The output of the last rf stage is applied to the AGC (automatic gain control) and spectrum detectors. The AGC voltage is fed back to the previous stage through an AGC amplifier, which is kept cut-off due to a delaying bias, until a pre-determined signal level is reached. This delayed AGC allows maximum gain on weak signals and prevents overdriving on strong signals.

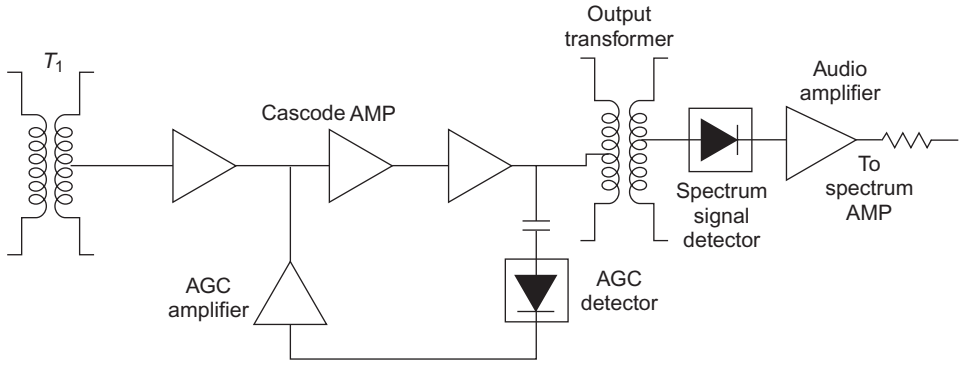


Figure 10.5 *Receiver and detector circuit*

10.4.4 The Display System

The detected signal is applied to the vertical plates of an oscilloscope to produce the NMR spectrum. The total amplification required is usually of the order of 10^5. The spectrum can also be recorded on a chart recorder. NMR spectrometers have built-in electronic integrators for measuring the relative areas under the peaks. The integrated spectrum is a step function, with the height of each step being directly proportional to the area under the peak corresponding to the step.

10.4.5 Data Display and Record

Modern NMR machines are computer controlled. General purpose digital computers are used for data acquisition and data display. It is possible to place under computer control such functions as pulse timing, delay and acquisition timing, digitisation rate, filter bandwidth, transmitter and decoupler offsets, receiver gain, noise bandwidth, plotter and pulse sequence. This leaves only a few essential manual adjustments, such as establishing the NMR lock for field-frequency ratio stabilisation and trimming up the field homogeneity controls.

In modern NMR spectrometers, the computer workstation and complex software directs the NMR experiment from start to finish. NMR signals are subjected to complex digital signal processing algorithm, including the Fourier Transform, to convert the NMR information into a form that is easily interpreted by the end user. The signals are displayed as a series of peaks or spectrum, on the workstations monitor (Hawkes, 1984). The data are usually stored on a disc and the processed data are plotted on a paper or displayed on a graphics display terminal.

10.4.6 The Sample Holder

The sample holder for NMR studies consists of a glass tube, generally of 0.5 cm outer diameter. Micro-tubes for smaller sample volumes are also available. The sample is invariably in the liquid form. In case studies are to be made on solid or gaseous samples, the solids may be studied above their melting point, and gaseous samples below their liquification point. Samples can be less than 1/1,000 cubic inch of the gas, liquid or solid.

The sample holder is placed in a sample probe, which also contains the sweep source and detector coils. This ensures reproducible positioning of the sample with respect to these components.

The sample probe is also provided with an air-driven turbine for rotating the sample tube along its longitudinal axis at several hundred rpm. This rotation averages out the effects of inhomogeneities in the field and provides better resolution.

It is frequently necessary in NMR studies to determine the behaviour of the measured parameters of a sample as a function of temperature. Often the range from room temperature down to the boiling point of liquid nitrogen is appropriate for these studies. This necessitates sample temperatures to be kept stable for several hours, while allowing rapid changes to be made when passing a cooled gas over the sample or using a controlled heat leak from the sample to a liquid nitrogen reservoir. For temperature regulation air flows around the sample tube after passing an electrical heating element, and a feedback system regulates the temperature of the air by controlling the power fed to the heater.

In the Varian T60A NMR spectrometer, the sample tube, with spinner attached, is simply dropped into a chimney-like outlet and floats down on to a cushion of compressed air, eliminating the possibility of damage to inner components of the probe. Spinning speed is adjustable and the speed can be optically monitored and displayed on a directly calibrated metre.

NMR Probe: In practice, the antenna that provides the rf link between the sample and the instrument electronics is called the 'NMR' probe. Inserted in the magnet, the probe holds the sample at the centre of the magnetic field. It bombards the sample with rf energy and then receives the very weak rf responses from the sample, which it sends to the receiver.

The probe contains resonance circuitry with a coil that acts as antenna which transmits the rf pulses to the sample and subsequently receives the response of the spins *via* the precessing magnetisation which induces a voltage across the coil. The use of two different coils for transmitting and receiving would have some exceptional advantages but designs with two coils at the same frequency suffer from interference effects between the coils and result in inferior performance. The coil produces a linearly polarised oscillating magnetic field perpendicular to the main static magnetic field. The coil is mounted in the probe with some solid material and possibly glue.

10.5 COMPUTER CONTROLLED NMR SPECTROMETER

Figure 10.6 displays a block diagram which shows the major components of a high resolution NMR spectrometer. The whole system is controlled by a dedicated spectrometer computer which is directed by the operator *via* a general purpose host computer work station (Wider, 1998).

The main parts of the spectrometer are drawn with heavy lines; the arrows indicate the pathway from the spectrometer computer to the probe and back to the computer where the NMR signal is stored. The parts drawn and connected with thin lines provide auxiliary functions of the spectrometer which are essential for a spectrometer used for applications with biological macromolecules.

Functionally, first the spectrometer computer instructs the rf control processor to set the necessary frequencies in the synthesisers and to send rf pulses from the transmitter to the different amplifiers according to the requirements of the experiment. Based on digitally stored data, the transmitter generates the pulses with the required shapes, phases, durations and power settings before sending them to the linear amplifiers. Through the linear amplifiers, the rf pulses reach the probe which contains a coil that delivers the rf frequency to the sample located in the top of the probe.

The receiving path starts in the rf coil in the probe and ends with the digitised signal in the computer memory. After being picked up by the receiver coil, the signal is routed to the pre-amplifier.

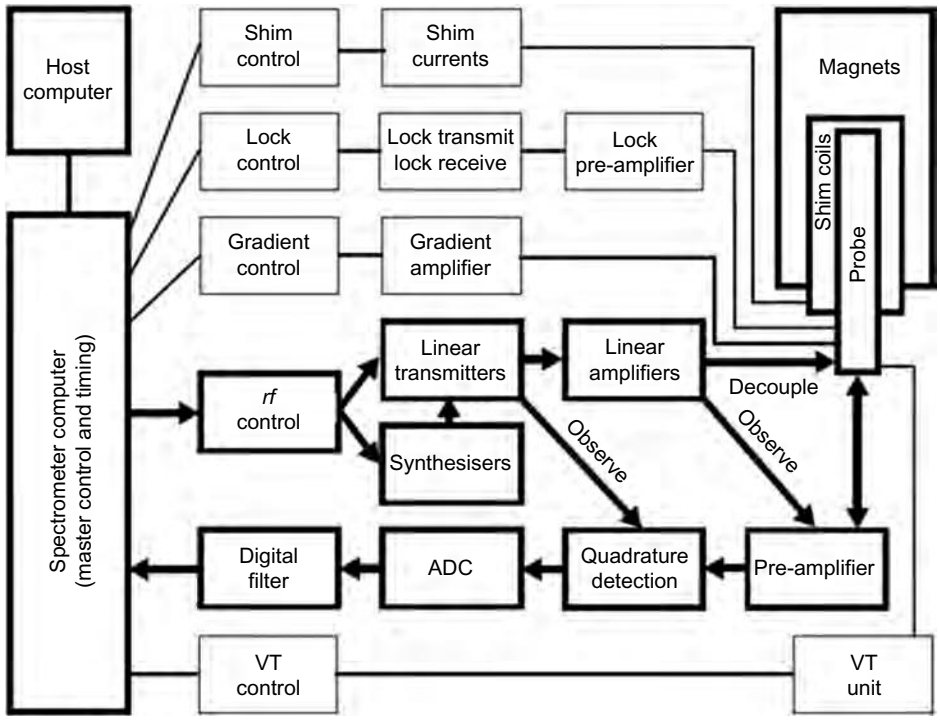


Figure 10.6 *Block diagram of a typical high resolution NMR spectrometer (Adapted from Wider, 1998)*

The typical voltage induced in the coil by the nuclear magnetisation of interest is in the range of μV or smaller. Transmission losses have to be minimised by placing the pre-amplifier as close as possible to the detection coil. A special safety circuitry protects the pre-amplifier from the very high voltage present during an rf pulse and directs the pulse only towards the coil.

The signal cannot be digitised at the NMR frequency and must be transformed into a frequency range of a few tens of kHz where digitisers with a high dynamic range exist. Reducing the frequency does not happen in one step. First all NMR frequencies are transformed to the same intermediate frequency and the two quadrature channels are created. A further mixing step reduces the signal frequencies of the quadrature channels to lower frequencies which can be digitised in the analog-to-digital converter (ADC). The final spectral range is selected using digital filters which can be designed to have a much better performance than corresponding analog filters. After digitising and filtering, the signal is sent to the computer memory where different scans are summed up and the data can be processed.

Very stable adjustable shim currents are supplied to the shim coils which create a correcting magnetic field to obtain the required homogeneity of the main magnetic field. The homogeneity thus obtainable by far outperforms the long-term stability of the frequency sources and the magnetic field. To obtain sufficient stability the ratio of the frequencies and the magnetic field is kept constant by permanently monitoring a reference NMR signal at a specific frequency.

Probes are built for a specific sample tube diameter. The most commonly used diameter is 5 mm. Probes for 3, 8 or even 10 mm are available but not so universally applicable. In addition, these

probes suffer from more severe problems with the H_2O resonance because shimming tends to be more difficult and adverse effects like radiation damping are more pronounced.

Precise and stable control of the sample temperature in the probe is very important for optimal performance of the spectrometer. More specifically the variable temperature (VT) control system must fulfil the following requirements. Firstly, it must be very stable and keep the temperature within 0.05 K of the set temperature. This is because the field-frequency lock is based on the deuterium resonance of water which is very temperature sensitive with a resonance shift of 0.01 ppm/K. Even small temperature changes will degrade the performance of the spectrometer by causing field corrections which are not based on a field-frequency drift.

In recent years pulsed magnetic field gradients (PFGs) have found widespread applications in experiments used for studies of biological macromolecules. To allow such experiments to be carried out, a *z* gradient channel which can deliver gradients up to 0.3 T/m (30 G/cm) or more are available. A gradient channel includes the gradient control hardware and the gradient amplifier as well as a special actively shielded gradient coil in each probe. Presently available triple axis gradient coils seem to reduce the performance of the *z* gradient due to interactions of the three different gradient coils. Currently, most applications require only a *z* gradient and the necessity of installing triple axis gradients on a particular spectrometer must be carefully evaluated.

Modern spectrometers use a general purpose work station as host computer which does not really need a special configuration except for a fast link with the spectrometer computer. For optimal performance large memory and disk capacities are an advantage.

10.6 SENSITIVITY ENHANCEMENT FOR ANALYTICAL NMR SPECTROSCOPY

The sensitivity of an instrument is a measure of the ability to differentiate signal from the surrounding noise. It is usually measured as the ratio of peak signal amplitude to rms noise.

$$\text{Sensitivity} = \text{S/N} = \text{Peak signal height/rms noise} \tag{10.1}$$

where rms noise = peak-to-peak noise/2.5

Equation (10.1) slows that the sensitivity may be improved either by increasing the signal amplitude or by reducing the observed noise. Both these are considered in the initial design of a NMR spectrometer. However, special operating techniques allow the NMR operator to optimise them for a particular sample and spectrometer system.

These are three operating techniques for sensitivity enhancement: (i) optimisation of sample volume, (ii) optimisation of instrumental parameters and (iii) time averaging. The first two enhancement techniques (optimisation of sample volume and spectral parameters) can often enhance the sensitivity by a factor of 10 over the normal operating conditions. Further use of signal averaging with a computer can achieve a further increase of 10 within reasonable time limits. This can significantly increase the applicability of analytical NMR to the investigation of small samples to give the NMR operator the best possible use of spectrometer.

The signal averaging technique is based on the principle that the signal, if added coherently, will increase linearly with the number of scans *N*, while the noise being random, will add as the square root of the number of the scans. Thus, the sensitivity increases by the square root of the number of

scans ($N^{1/2}$). To have a long measuring time for increasing sensitivity, it is better to have several fast scans than a slow single scan.

Signal averaging basically involves:

- A system, which must provide to repetitively scan the spectral region of interest.
- Some storage device for storing the spectral information.
- A system to coherently add the individual spectra.

These are usually accomplished by a small computer (multi-channel pulse height analyser), suitably modified to provide an NMR sweep and coherent spectral addition. These special purpose computers are commercially available from manufacturers of NMR spectrometers.

10.7 SPIN DECOUPLER

Spin decoupler permits spin-coupled interactions between nuclei to be eliminated through the use of a technique called nuclear magnetic double-resonance. In this technique, specific regions of the spectrum are irradiated by a strong component of rf power or rf side-band power, causing the multiplet patterns from spin-coupled resonances in other regions to coalesce into less complex patterns more easily assignable. In principle, double-irradiation helps to solve problem introduced by complicated proton couplings. The second rf field is adjusted to the resonant condition for the group, whose coupling is to be eliminated. Under these circumstances, the proton being split sees only one equivalent state and single peak results.

In addition to the usual H_1 rf field used to examine the NMR response to the sample, a second rf field H_2 is introduced simultaneously into another region of the proton spectrum. The spectrum is recorded by sweeping the main magnetic field H_o and the H_2 field, while the H_1 field is held constant H_1 modulation of the H_0 field generates the sidebands for the observing field. For frequency sweep, a voltage proportional to the magnetic field is applied to a voltage controlled oscillator, which supplies the decoupling frequency. The magnitude and frequency of the H_2 are controllable.

Figure 10.7 shows a block diagram of spin decoupler arrangement. The decoupling frequency to the field modulator is supplied by 5 kHz voltage controlled oscillator.

The oscillator is a Wien Bridge Oscillator, whose gain stability is provided by a thermistor in the feedback loop. This frequency is amplified and applied to the AC sweep coils to generate the H_2 or decoupling field. The decoupling power level is adjusted at the oscillator output. In frequency sweep operation, tracking of the oscillator with the sweep is accomplished through the frequency control circuit. As the DC component of the field is swept, a differential voltage is developed across the resistor R placed in series with the sweep coils. The differential voltage is amplified in the differential amplifier and applied to the error amplifier. The error amplifier receives input from three different sources; the frequency control setting the differential voltage from the DC sweep coils, and the feedback voltage from the frequency-to-voltage converter.

The frequency control sets the DC operating level that produces the desired frequency from the voltage controlled oscillator. The DC sweep voltage from the differential amplifiers adds to the operating level and the oscillator follows the sweep. The frequency of the oscillator is sensed by the frequency-to-voltage converter, which applies an error signal to the error amplifier when the oscillator drifts or overshoots. Frequency-to-voltage converter provides a direct conversion of a sinusoidal signal to a DC signal. In this circuit, the input signal is squared by a Schmidt trigger

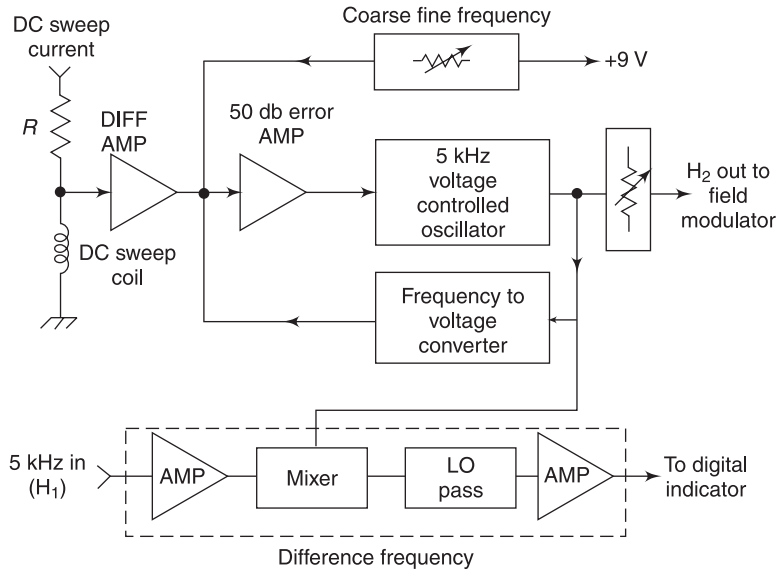


Figure 10.7 *Block diagram of spin de-coupler (Courtesy: M/s Varian, USA)*

circuit and differentiated to trigger a monostable multi-vibrator. The pulses from the monostable multi-vibrator are applied to a low-pass *RC* filter, which smooth the pulses to a DC voltage.

A difference frequency circuit is included to provide a readout frequency, for the digital indicator. This circuit provides a means of mixing two audio signals and a filter to recover the low-frequency component. The readout frequency is the difference frequency between the 5 kHz spectrometer control oscillator *(H_1)* and the spin decoupler oscillator frequency *(H_2)*. The Spin Decoupler module temperature is maintained to ± 1°C by temperature controller circuit. This circuit is a DC amplifier, which compares a DC reference voltage to the voltage change produced by a thermistor mounted in the Spin Decoupler oven. The output of the DC amplifier is applied to a power transistor, which controls the power supplied to the oven heaters.

10.8 FOURIER TRANSFORM NMR SPECTROSCOPY

As mentioned in section 10.3, 'Continuous Wave' or CW type of NMR spectrometer are now obsolete The limitations of CW spectrometers are low sensitivity and long analysis time. In pulsed NMR spectrometers, a single pulse of rf energy is used to simultaneously activate all nuclei. The excited nuclei returning to the lower energy level generate an FID signal that contains in a time domain all the information obtained in a frequency domain with CW spectrometer. Time domain and the frequency domain responses form a pair of Fourier Transforms: the mathematical operation is performed by a computer after ADC conversion. After a delay allowing for relaxation of the excited nuclei, the pulse experiment (transient) may be repeated and the response coherently added in the computer memory, with random noise being averaged out. Modern NMR spectrometers operate in the 'pulsed Fourier Transform' (FT) mode, permitting the entire spectrum to be recorded in 2–3 s rather than 5 min.

The block diagram of a typical high-resolution pulsed FT-NMR spectrometer is shown in Figure 10.8. It uses a super-conducting (cryogenic) solenoid as the source of the magnetic field. Introduction of FT-pulsed NMR spectrometer has made the acquisition of spectra routine and has also allowed proton spectra to be obtained in much less time, and with smaller amounts of specimen as compared to CW techniques. Figure 10.9 shows a typical FT NMR installation.

This data is digitised and a computer performs a Fast Fourier Transform to convert it from an FID signal as a function of time (time domain) to a plot of intensity as a function of frequency (frequency domain). This 'spectrum' has one peak for each resonant frequency in the sample. The real advantage of the pulsed- FT method is that, because the data is recorded so rapidly, the process of pulse excitation and recording the FID can be repeated many times, each time adding the FID data to a sum stored in the computer. The signal intensity increases in direct proportion to the number of repeats or 'transients', but the random noise tends to cancel because it can be either negative or positive, resulting in a noise level proportional to the square root of the number of transients. Thus

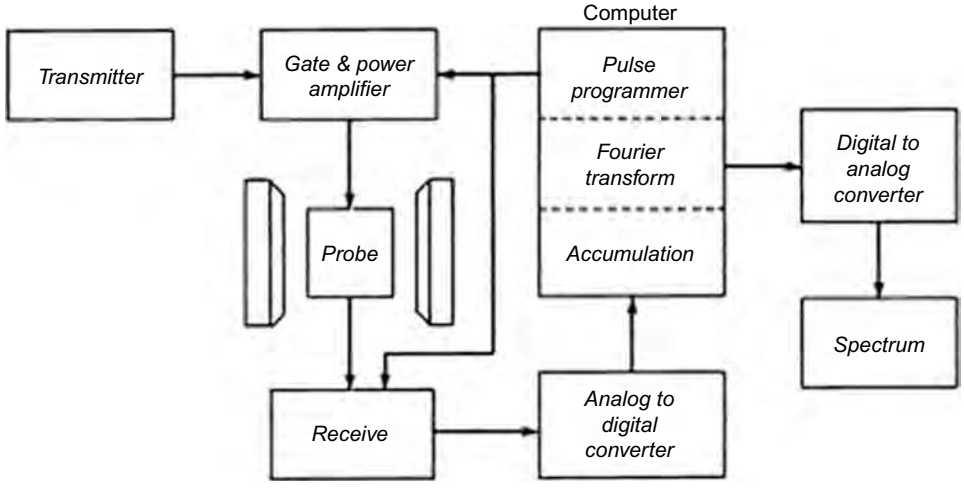


Figure 10.8 *Block diagram of a typical pulsed FT-NMR spectrometer (Courtesy: US Pharamacopeia)*

Figure 10.9 *Typical 500 MHz FT-NMR spectrometer (Courtesy: M/s Bruker)*

the signal-to-noise ratio increases with the square root of the number of transients. This signal averaging process results in vastly improved sensitivity over the old frequency sweep method.

An electronic integrator is a feature of most NMR spectrometers. On a CW instrument (^{1}H and ^{19}F) the integrator, connected to the spectrometer output stage, determines the relative areas of the resonance peaks and presents these areas as a series of stepped horizontal lines when a sweep is made in the integration mode. On FT-MNR spectrometers, an integration algorithm is included in the spectrometer software, and the resonance peak areas may be presented graphically as stepped lines or tabulated as numeric values. The use of computer-generated tabulated/numeric integration data should not be accepted without a specific demonstration of precision and accuracy on the spectrometer in question.

Sensitivity enhancement is the major attraction of the Fourier transform technique. Thus for proton NMR, it is possible to consider samples that are an order of magnitude less concentrated than the previous limit, and this has great importance in biochemical field. XL-100 Pulsed-Fourier Transform NMR spectrometer from Varian is a high resolution 23.5 k gauss spectrometer, which can be operated in the frequency range from 6 to 100 MHz. This instrument is designed around a fundamental clock frequently. A 15.4 MHz crystal oscillator generates the deuterium resonance frequency at 23.5 k gauss. The deuterium resonance signal is then used to lock the magnetic field to the clock frequency. The choice of a 15 inch or 12 inch magnet system permits to accommodate a wide size range of sample tubes. This permits to optimise sensitivity versus availability of samples. For Fourier transform, the spectrometer is provided with programmable rf pulse hardware and digital computer programmed to control, acquire and transform spectral data.

11

ELECTRON SPIN RESONANCE SPECTROMETERS

11.1 ELECTRON SPIN RESONANCE

Electron spin resonance involves detecting a physical phenomenon of absorption of electromagnetic radiation in the microwave region by paramagnetic species that are subjected to an external magnetic field. It is the study of magnetic dipoles of electronic origin by applying, usually, fixed microwave frequencies to a sample residing in a varying magnetic field. Also known as electron paramagnetic resonance (EPR) spectroscopy, it is a valuable research and analytical tool in Chemistry, Physics, Biology and Medicine. It is used in the study of molecular structures, reaction kinetics, molecular motion, crystal structure, electron transport and relaxation properties.

The phenomenon of electron spin resonance is based on the fact that an electron possesses a spin, and associated with the spin, there is a magnetic moment, the value of which is called the Bohr magneton. The atoms, ions or molecules having an odd number of electrons exhibit characteristic magnetic properties, which arise from the spinning or orbiting action of the unpaired electrons about the nucleus. When a strong magnetic field is applied to the unpaired spins of an electron, the electrons will be split into two groups. In one group, the electron dipoles or magnetic moments of the electrons are aligned either parallel or anti-parallel to the direction of the external magnetic field. The electrons will process about the axis of the magnetic field at a frequency proportional to both the applied magnetic field and the electron magnetic moment.

If a second, weaker radiofrequency alternating magnetic field, having the frequency of precession of the electron is applied at right angles to the fixed magnetic field, resonance occurs. At resonance, the absorption of energy from the rotating field causes the spin of the electrons to flip from the lower energy level to the higher level. The two levels are separated by

$$E = h\upsilon = 2\,\mu H$$

when h is Planck's constant and υ is the frequency. In comparison to NMR, the electron has a much smaller mass and larger magnetic moment than a proton. For a given magnetic field, the precession frequency is, therefore, much higher. For a free electron, the frequency of absorption is given by

$$\begin{aligned}\upsilon &= 2\,\mu H/h \\ &= (2\mu/h) \times H = (2.8026 \times 10^6) \times H\end{aligned}$$

In a field of 3,400 gauss, the precession frequency is approximately 9,500 MHz.

In actual practice, the radio frequency (rf) is in the microwave region and is held at a certain constant value, and the magnetic field strength is varied to obtain conditions where resonance occurs.

The incident radiation is absorbed by the electrons in the lower energy level and they jump into higher energy state.

In general, if n_1, the population of the ground state, exceeds n_2 the population of the excited state, a net absorption of microwave radiation takes place. The signal would be proportional to the population difference (n_1–n_2).

By the Boltzmann distribution law, the population ratio in the two states is given by

$$n_1/n_2 = e^{-2\,\mu H/kt}$$

where k is the Boltzmann constant. The sensitivity of measurement is greatly enhanced by using a high magnetic field.

The expression tells us that ESR sensitivity (net absorption) increases with decreasing temperature and with increasing magnetic field strength. Since field is proportional to microwave frequency, in principle sensitivity should be greater for K-band or Q-band or W-band spectrometers than for X-band. However, since the K-, Q- or W-band waveguides are smaller, samples are also necessarily smaller, usually more than cancelling the advantage of a more favourable Boltzmann factor.

In most of the substances, chemical bonding produces paired electrons, as they would be either transferred from one atom to another to form an ionic bond or electrons are shared between different atoms to form covalent bonds. The magnetic moments and spins of paired electrons point in opposing directions, with the result that there is no external spin paramagnetism. However, in a paramagnetic substance having an unpaired electron, resonance occurs at definite values of the applied magnetic field and incoming microwave radiation. The deviation from the standard behaviour of the unpaired electron due to the presence of magnetic fields in its surroundings, gives knowledge about the structure of the substance under study.

The ESR measurements are made by placing the sample under study in a resonant cavity positioned between the pole pieces of an electromagnet. The microwave frequency is set to a matched condition with the help of a tuning device. The magnetic field is varied to bring about resonant conditions and the microwave energy absorbed by the sample is plotted on a recorder. The spectrum is then analysed to determine the behaviour and mechanisms associated with the unpaired electrons interaction with the external magnetic field and its environment.

For routine investigations with the ESR spectrometer, it is a general practice to employ an X-band frequency (8.5–10 GHz). This cannot be generated by using ordinary vacuum tubes and special tubes capable of operating at microwave frequencies are employed. Microwaves are normally handled using waveguides designed to transmit over a relatively narrow frequency range. Waveguides look like rectangular cross-section pipes with dimensions on the order of the wavelength to be transmitted. As a practical consideration, waveguides cannot be too big or too small –1 cm is a bit small and 10 cm a bit large, the most common choice, called X-band microwaves, has wavelength (λ) in the range 3.0–3.3 cm (frequency 9–10 GHz). Although x-band is by far the most common, ESR spectrometers are available commercially in several frequency ranges as given Table 11.1.

Table 11.1 *Frequency bands in ESR spectrometers*

Band	Frequency GHz	Wavelength λ, cm	B Magnetic field Tesla
S	3.0	10.0	0.107
X	9.5	3.15	0.339
K	23	1.30	0.82
Q	35	0.86	1.25
W	95	0.315	3.3

Under ideal conditions, a commercial X-band spectrometer can detect the order of 10^{12} spins (10^{-12} moles) at room temperature. By ideal conditions, we mean a single line, on the order of 0.1 G wide; sensitivity goes down roughly as the reciprocal square of the line width. When the resonance is split into two or more hyperfine lines, sensitivity goes down still further. Nonetheless, ESR is a remarkably sensitive technique, especially compared with NMR.

The limitations of triode, pentode and similar tubes that arise at very high frequencies as a result of transit time effects are avoided by employing types of tubes which make the use of transit time in achieving their normal operation. One such tube is the reflex klystron or reflex oscillator, as it is sometimes called. It requires only a single resonant cavity. Since it has an efficiency of only a few per cent, the reflex klystron is essentially a low-power device, typically being used to generate 10–500 mW. The reflex klystron is particularly satisfactory for use in the frequency range 1,000–25,000 MHz.

11.2 BASIC ESR SPECTROMETER

Although many spectrometer designs have been produced over the years, the vast majority of laboratory instruments are based on the simplified block diagram shown in Figure 11.1 Microwaves are generated by the klystron tube and the power level adjusted with the attenuator. The circulator routes the microwaves entering from the klystron towards the Cavity where the sample is mounted.

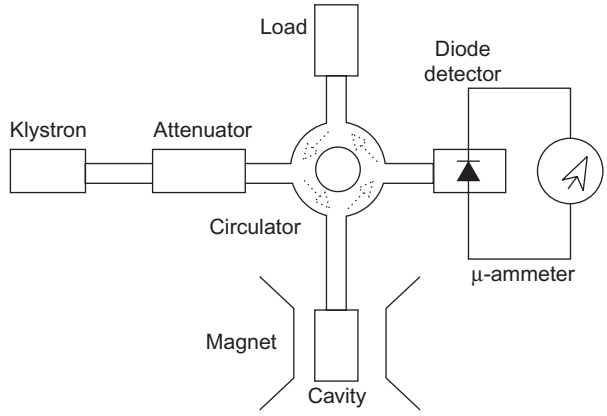


Figure 11.1 *Block diagram of a basic ESR spectrometer*

A majority of EPR spectrometers operate at approximately 9.5 GHz. The radiation may be incident on the sample continuously or pulsed. The sample is placed in a resonant cavity which admits microwaves through an iris. The cavity is located in the middle of an electromagnet and helps to amplify the weak signals from the sample. Numerous types of solid-state diodes are sensitive to microwave energy and absorption lines can be detected when the separation of the energy levels is equal or very close to the frequency of the incident microwave photons. In practice, most of the external components, such as the source and detector, are contained within a microwave bridge control. Additionally, other components, such as an attenuator, field modulator and amplifier, are also included to enhance the performance of the instrument.

A wave metre is put in between the oscillator and attenuator to know the frequency of microwaves produced by klystron oscillator. The wave metre is usually calibrated in frequency unit (megahertz) instead of wavelength. Waveguide is a hollow, rectangular brass tube. It is used to convey the wave radiation to the sample and crystal.

The power propagated down the waveguide may be continuously decreased by inserting a piece of resistive material into the waveguide. The piece is called variable attenuator and used in varying the power of the sample from the full power of klystron to one attenuated by a force 100 or more.

Isolators are used to prevent the reflection of microwave power back into the radiation source. It is a strip of ferrite material which allows microwaves in one direction only. It also stabilises the frequency of the klystron.

Microwaves reflected back from the cavity are routed to the diode detector, and any power reflected from the diode is absorbed completely by the load. The diode thus produces a current proportional to the microwave power reflected from the cavity. Thus, in principle, the absorption of microwaves by the sample is detected by noting a decrease in current in the micro-ammeter. In practice, such a DC measurement is too noisy to be useful.

The signal-to-noise ratio can be improved by introducing small amplitude field modulation. An oscillating magnetic field is super-imposed on the DC field by means of small coils, usually built into the cavity walls. When the field is in the vicinity of a resonance line, it is swept back and forth through part of the line, leading to an AC component in the diode current. This AC component is amplified using a frequency selective amplifier, thus eliminating a great deal of noise. Thus the detected AC signal is proportional to the change in sample absorption.

A schematic drawing of the microwave-generating klystron tube is shown Figure 11.2. There are three electrodes: (i) a heated cathode from which electrons are emitted, (ii) an anode to collect the electrons and (iii) a highly negative reflector electrode which sends those electrons which pass through a hole in the anode back to the anode.

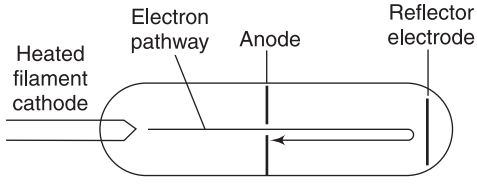


Figure 11.2 *Schematic diagram of a microwave-generating klystron tube*

The motion of the charged electrons from the hole in the anode to the reflector and back to the anode generates an oscillating electric field and thus electromagnetic radiation. The transit time from the hole to the reflector and back again corresponds to the period of oscillation. Thus the microwave frequency can be tuned, over a small range by adjusting the physical distance between the anode and the

reflector or by adjusting the reflector voltage. In practice, both the methods are used; that is, the metal tube is distorted mechanically to adjust the distance as a coarse frequency adjustment and the reflector voltage is adjusted as a fine control.

The sample is mounted in the microwave cavity as shown in Figure 11.3. The cavity is a rectangular metal box, exactly one wavelength in length. An X-band cavity has dimensions of about 1 × 2 × 3 cm. The electric and magnetic fields of the standing wave are also shown in the figure. Note that the sample is mounted in the electric field nodal plane, but at a maximum in the magnetic field.

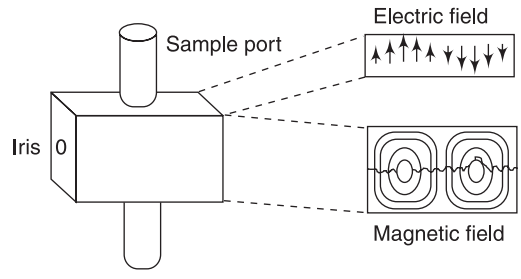


Figure 11.3 *Microwave cavity*

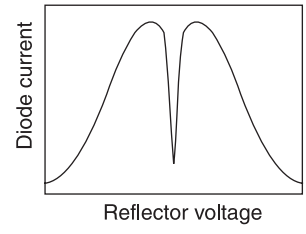


Figure 11.4 *Klystron mode and cavity dip*

Since the cavity length is not adjustable but it must be exactly one wavelength, the spectrometer must be tuned such that the klystron frequency is equal to the cavity resonant frequency. The tune-up procedure usually includes observing the klystron power mode. That is, the klystron reflector voltage is swept, and the diode current is plotted on an oscilloscope or other device. When the klystron frequency is close to the cavity resonant frequency much less power is reflected from the cavity to the diode, resulting in a dip in the power mode as shown in Figure 11.4. The cavity dip is centred on the power mode using the coarse mechanical frequency adjustment with the reflector voltage used to fine tune the frequency.

Most EPR experimental setups do not operate in the 'continuous wave' (CW) format only. Instead, advanced EPR experiments utilise pulsed microwave energy to selectively excite different transitions of a paramagnetic sample. These experiments have the same basic components as previously described, but require different amplifying and detecting equipment specifically designed for pulse creation and detection.

11.3 COMPONENTS OF AN ESR SPECTROMETER

Figure 11.5 shows the block diagram of an ESR spectrometer. The sample is irradiated by microwave energy from the klystron in the microwave bridge. A klystron normally operated at 9.5 GHz, generates a microwave field. The magnetic field at the sample is modulated at 100 kHz. The klystron output passes through an isolator, a power leveller and a directional coupler. The field is applied to the resonant cavity, which is connected to one arm of the microwave bridge. During the magnetic field scan, when field intensity reaches the value required to induce electron spin resonance in the sample, a change occurs in the amount of microwave energy absorbed by the sample. This causes a change in the microwave energy reflected from the cavity. The reflected microwave energy, which is modulated at the field modulation frequency, is directed to the detector crystal.

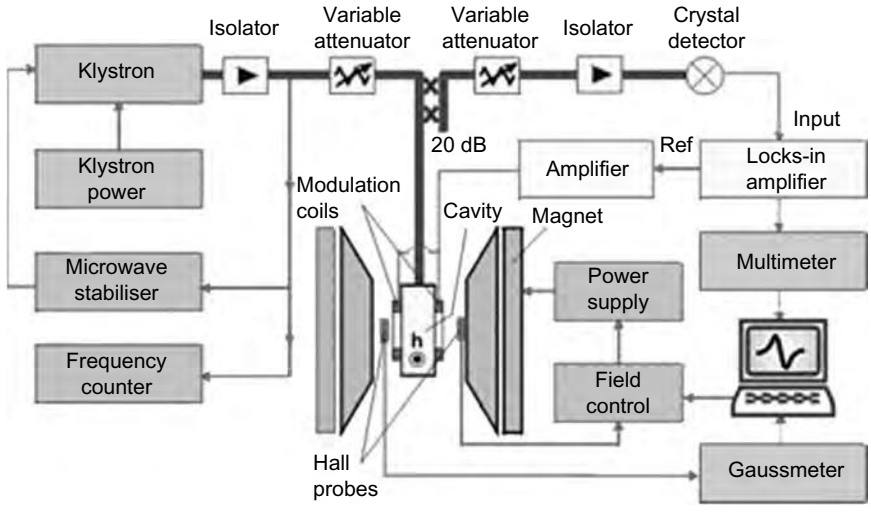


Figure 11.5 *Block diagram of ESR Spectrometer (Courtesy: M/s Varian, USA)*

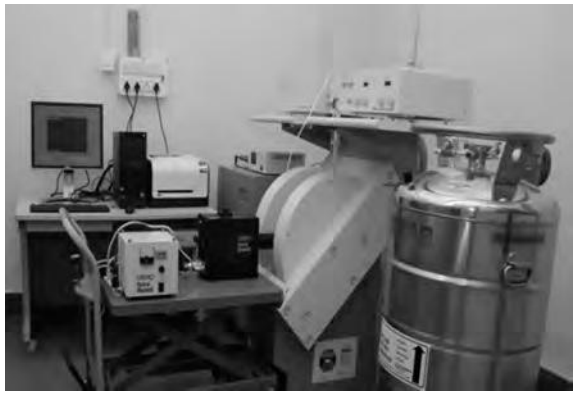


Figure 11.6 *ESR Spectrometer (Courtesy: M/s Jeol, Japan)*

The detector is usually a silicon tungsten crystal rectifier. After detection at the crystal, the resulting 100 kHz, which contains the ESR information, is amplified in the pre-amplifier circuit and applied to the receiver section of the 100 kHz modulation unit. The amplified signal is phase detected to obtain the spectrum, which appears as a deflection on the Y-axis of the recorder. The field scan potentiometer is linked mechanically to the recorder X-axis. The X-axis is calibrated in gauss. The ESR signal may also be applied to the oscilloscope for visual display. The field controller accurately controls the magnetic field to the desired set value. A typical EPR Spectrometer is shown in Figure 11.6

11.3.1 The Magnet and the Magnetic Field Controller

The magnet used in the ESR spectrometers is usually of the electromagnet type. It provides a homogeneous magnetic field, which can be varied from 200 m gauss to 20 k gauss is calibrated in steps. Stability of 1 part in 10^6 is satisfactory for adequate resolution of ESR spectra.

The magnetic field controller provides direct control and regulation of the magnetic field in the air gap. It is an AC carrier type servo system that accurately controls the magnetic field. Figure 11.7 shows the schematic diagram of the magnetic field controller in a spectrometer.

The driver amplifier supplies a 30 mA rms, 1,230 Hz exciting current through the field set reference resistor (R), the Hall effect magnetic field sensor element and the primary of the scan voltage

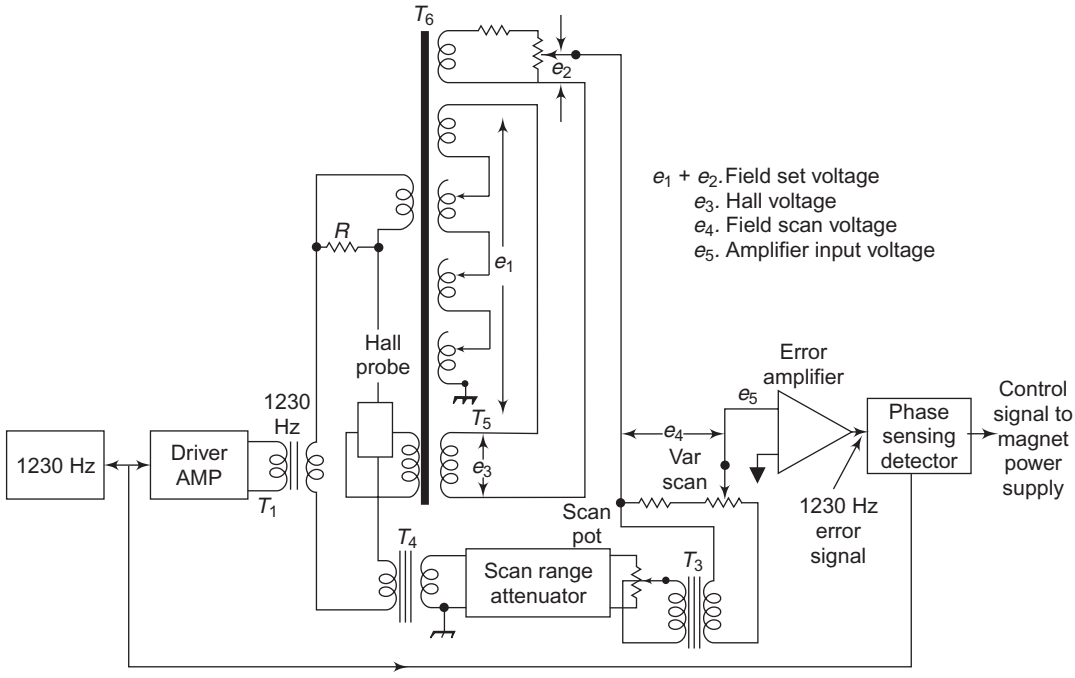


Figure 11.7 *Schematic diagram of magnetic field controller*

transformer (T_4). A field reference voltage is developed across R. Similarly, a field scan reference voltage is developed across the constant input impedance of scan range attenuator by the transformed reference current in the secondary winding of T_4.

The output of the summation circuit (i.e. input to the error amplifier) is the algebraic sum of the output of the Hall effect field sensor (transformed by T_5) added to the *field set* and *field scan voltage*. The sum of these three voltages is amplified by the error amplifier and applied to the input of the phase-sensitive detector. When the output of the Hall effect field sensor is less than the sum of the field set and field scan voltages, the output of the phase-sensitive detector is of a polarity which turns on the magnet power supply, to increase the magnet current and thus the magnetic field. In a similar manner, when the output of the Hall effect field sensor exceeds the sum of the field set and field scan voltages, the current through the magnet winding is decreased and the magnetic field is decreased.

The field scan potentiometer is centre-tapped and the wiper is driven by the recorder's horizontal axis-drive mechanism. The voltage output of the scan potentiometer acts to oppose the field set voltage, when the recorder pen is in the left half of its operating range, but adds to the field set voltage, when the recorder pen is moved into the right half of the recorder chart.

- The 1,230 Hz oscillator is a stable Wein bridge oscillator, whose output level is internally regulated.
- The error amplifier is a high gain stable amplifier with a passband centred near 1,230 Hz. It has a phase shifting potentiometer, which permits the phase to be adjusted, so that the signal component of the amplified error signal is exactly in-phase and the spurious quadrature

component of the amplified error signal is exactly out-of-phase, so that the latter is completely rejected by the phase-sensitive detector.
- The phase-sensitive detector converts the amplified 1,230 Hz output signal of the summation circuit into DC control voltages to drive the magnet power supply.
- The Hall probe is maintained at a constant temperature. The temperature control is maintained by controlling current through a heater, sensed by a thermistor.
- The magnet power supply provides controlled DC current to the low impedance electromagnet. The magnet current is supplied by the main transformer and rectifiers.

The magnets used could be 9, 12 or 15 inch. They are generally floor mounted and require 2.5 to 22.5 kW power supplies to drive them. The magnets are equipped with ring-shim pole caps designed for maximum field homogeneity. A 3-inch clearance (air gap) provides ease of access. The pole caps are made compatible with tapered ring-shim tips, which reduce the air gap to 1.75 inch to achieve optimum field performance when operating at 35 GHz.

In order to improve magnet performance, the heat generated must be dissipated. To do so coolant lines are run around the magnet, magnet power supply and microwave bridge. If the temperature of the untreated water is below the dew point, condensation may occur in the magnet power supply. In this event, the untreated water can be run through the magnet first and then through the power supply. Also, the coolant water flow should be correct for magnet system in use. This is ensured by checking the difference between inlet and outlet pressure if the difference is more than that specified for the magnet system, the cooling system may be clogged.

If the ambient room-air temperature or magnet cooling water temperature changes, the setting of the Hall probe temperature should be examined to ensure that the temperature control system is regulating (i.e. it is not saturated or cut off). This is particularly important in those installations where large annual variations in room or water temperatures take place. Noise and jitter can be induced into the field control system by transients on the power lines that are coupled into the Hall probe circuit. As much as possible, the Hall probe cable should be isolated from power lines connecting the console and the magnet power supply.

It is often difficult to detect or troubleshoot instabilities of the magnetic field, as normally no convenient external field measuring device is available to monitor the magnetic field with sufficient stability or accuracy. However, the manufacturers of instruments suggest several test techniques that may be employed with easily obtainable auxiliary test equipment. If the magnet current rises beyond normal, the Hall sensor probe may be improperly phased. This is corrected by removing power from the system and reversing power leads to magnet coils.

11.3.2 Microwave Bridge

A microwave radiation source is necessary for any EPR spectrometer, because this is the energy that corresponds with a detectable splitting of the electronic spin states. Microwave radiation is typically produced and amplified by a klystron, which is capable of tuning waves to a precise frequency, amplitude and phase. The microwaves are then channelled into the resonant cavity by use of either waveguides or coaxial cables. Waveguides are the most common method of microwave propagation. Waveguides are essentially open air, brass rectangular channels with dimensions that correspond to the wavelength of the radiation that is to be propagated. However, for low power, low frequency microwave radiation, like that often used in EPR, special coaxial cables have been found to be just as effective.

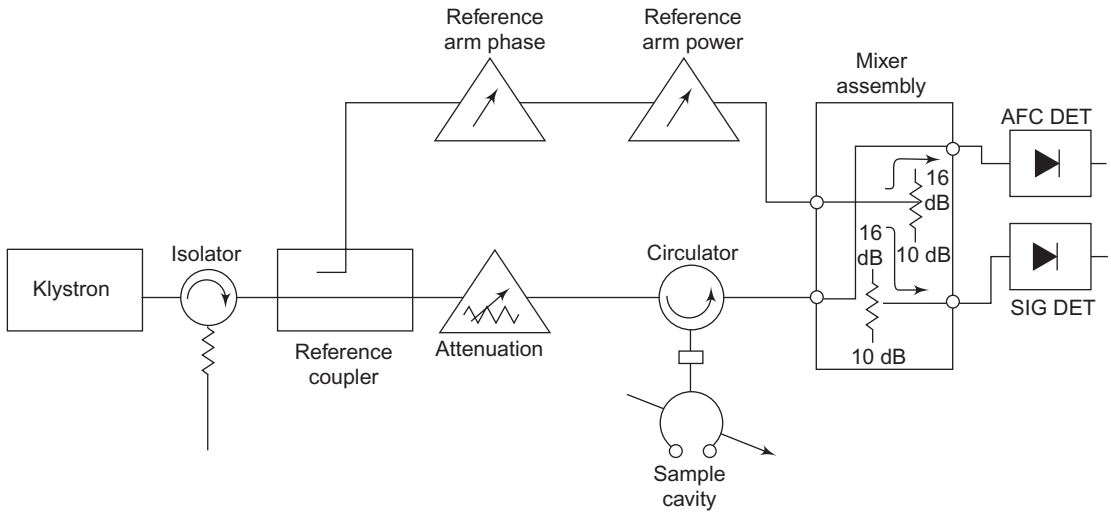


Figure 11.8 *Block diagram of the microwave bridge*

The microwave bridge detects the ESR signal reflected from the sample cavity. The bridge contains microwave circuitry, a klystron power supply, a pre-amplifier and an automatic frequency control circuit (Figure 11.8).

The klystron generates microwave energy at 9.5 GHz, which is used to irradiate the sample. The klystron output is applied to an isolator which allows signal flow only in one direction. Klystron tube acts as the source of radiation which is stabilised against temperature fluctuation by immersion in an oil bath or by forced air cooling. The frequency of the monochromatic radiation is determined by the voltage applied to klystron. It is kept a fixed frequency by an automatic control circuit and provides a power output of about 300 milliwatts. The klystron can also be water-cooled through its mounting flange.

The output of the isolator is given to four port circulator, where the microwave power is directed to the sample cavity. The circulator then directs reflected power from the sample cavity back to the microwave detector. The crystal DC bias current is indicated by the detector level metre.

When all the power is absorbed and none reflected, the detector receives no signal. At resonance, the sample absorbs microwave energy and the unbalances the impedance of the cavity. The absorption in the sample is detected as a dip in the output of the crystal detector. The crystal output containing the ESR signal and the 70 kHz AFC (automatic frequency control) signal is amplified by the pre-amplifier. It is then coupled to the receiver and to the AFC amplifier.

The automatic frequency control circuit contains 70 kHz crystal controlled oscillator, which generates AFC carrier and the AFC phase detector reference voltage. The AFC carrier is superimposed on the klystron reflector voltage, which results in a 70 kHz frequency modulation (fm) of the klystron output The frequency modulated rf is applied to the sample cavity, resulting in an fm to amplitude modulation (am) conversion. The resultant am modulated microwave power is detected and amplified in a pre-amplifier, followed by a 70 kHz tuned amplifier. The output of this amplifier is phase detected, amplified, filtered and applied to the klystron reflector tracking network. Another parallel output is given to an integrator, which quickly returns the phase detector DC output to zero. This arrangement permits a very accurate lock over a range of at least ±15 V reflector voltage correction.

Noise elimination is an important factor in enhancing the sensitivity of plotting ESR spectrum. Most of the noise is eliminated in the phase-sensitive detector, because only that pad of the noise which is at the same frequency and in-phase with the reference signal, is allowed to pass through it. The noise is further eliminated by using a long time constant filter, which averages out noise at frequencies greater than the reciprocal of its time constant. A better method of noise elimination is through continuous averaging by a computer, which employs a multi-channel pulse-height analyser. This technique of using computer for average transients efficiently removes both low and high frequency noise.

In order to achieve advantages of higher frequency operation such as, increased sensitivity for small samples, minimisation of second order shifts, increased resolution for powder samples with different g-values, the klystron frequency is set at 35 GHz instead of 9.5 GHz. The higher frequency also permits the observation of transitions that require higher energy.

The klystron requires +650 V for the klystron beam and up to −400 V for the klystron reflector. The supply voltages are obtained from the 20 V DC inputs in inverter circuits, working at a frequency of 35 kHz. This frequency is obtained by dividing the 70 kHz AFC modulation frequency by 2. The klystron body or tuning shaft should not be touched when the bridge is in tune or operate position because the klystron body is at +650 V when operating. It is insulated from ground by an insulating gasket placed between the klystron flange and the water cooling flange.

11.3.3 Modulation Unit

The 100 kHz modulation unit acts as a transmitter and receiver. The transmitter provides the power to drive the cavity modulation coils. The receiver processes the ESR signal from the bridge preamplifier and converts it to a DC voltage for application to the recorder Y-axis. Figure11.9 shows block diagram of the 100 kHz modulation unit.

- The 100 kHz oscillator is crystal controlled. Its output is attenuated by the modulation amplitude control and is applied to the output modulation amplifier. The gain of the amplifier is controllable. The 100 kHz modulation signal is transmitted to the cavity modulation coils.

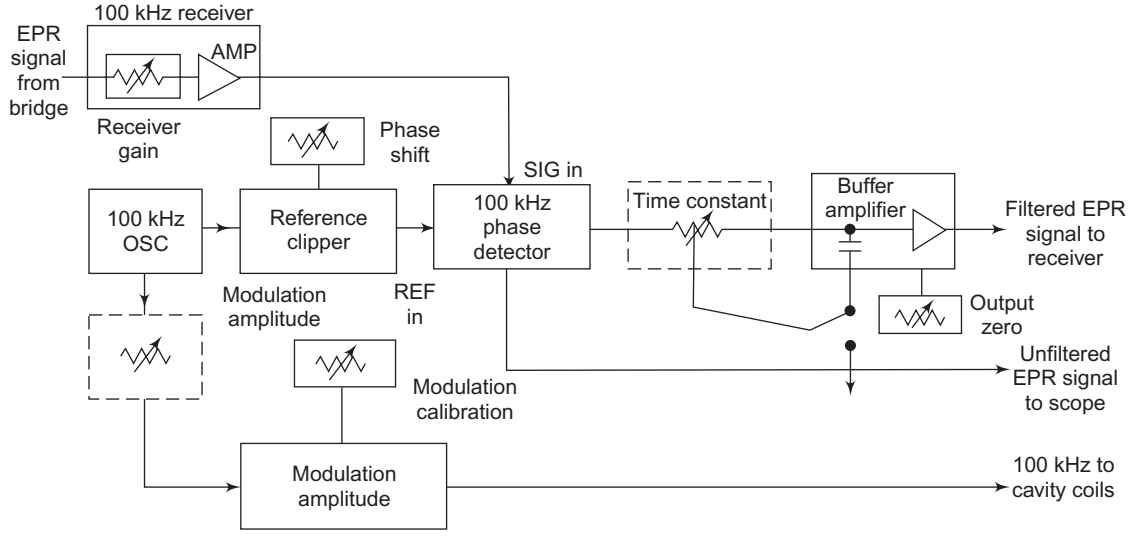


Figure 11.9 *Block diagram of 100 kHz modulation Unit*

- The 100 kHz ESR signal from the detector crystal in the bridge is filtered and amplified in the 100 kHz receiver. The receiver gain control can be set in 1 dB steps.
- The amplified 100 kHz ESR signal is applied to the input of the phase detector. The amplitude of the DC phase detector output is proportional to the amplitude of the 100 kHz signal input. A low-pass filter removes any residual 100 kHz or harmonics from the phase-detected signal. Additional noise filtering is provided by an *RC* network, which provides variable time constant. This is followed by a buffer amplifier, which provides low output impedance stage connection to the recorder.

Modulation may also be carried out at low frequencies like 10 kHz, 1 kHz, 270 Hz or 35 Hz. Low frequency module shown on the block diagram serves to accomplish this function.

11.3.4 Detection Methods

Various detection methods exist for EPR spectroscopy. The majority of EPR spectrometers are reflection spectrometers, meaning they measure the amount of radiation that is reflected back out of the resonator. Changes in the level of reflected microwave energy at various field strengths allow the observation of spectroscopic transitions. A special channelling device called a 'circulator' is used to insure that radiation from the microwave source is sent only into the cavity and that reflected radiation is sent only to the detector. A diode is used to measure the power of reflected energy. To achieve an accurate quantitative measurement of absorbed microwave energy, the diode must sometimes be 'biased,' or supplied with a steady level of current which insures that the diode is reading in the correct linear range.

CW spectrometers make use of a 'field modulator,' which creates an oscillating magnetic field around the sample, in addition to the applied magnetic field created by the electromagnet. This serves to fix the range of electromagnetic energy that reaches the detector, increasing the resolution.

11.3.5 Recorder

The recorder is used to produce a hard copy of the ESR spectrum, as a function of time on a 30 × 40 cm flat chart. By selecting the synchronised frequency to the stepper motor, the recording time can be selected from a few seconds to several hours.

Horizontal travel (X-axis) of the pen is achieved by a pulsed stepper motor and bolt driven capstan, which moves the pen carriage horizontally. Vertical (Y-axis) travel of the pen is controlled through a second system of pulleys and wire cable from a servomotor Y-axis movement results when the servo loop, of which motor is a part, is unbalanced by a signal applied to the unbalanced by a signal applied to the recorder amplifier. The recorder amplifier is turned on by the servomotor to balance the servo loop.

11.3.6 Oscilloscope

The oscilloscope permits direct visual observation of rapidly changing or decaying signals as well as optimisation of instrument parameters. The oscilloscope supplies 34 Hz sawtooth modulation to the sample cavity modulation coils and displays the resulting ESR spectrum. The amplitude of the field modulation is kept adjustable and may be selected by sweep width control. Noise on the displayed spectrum may be reduced by the addition of an input filter. The oscilloscope is also used to display the klystron mode. It is accomplished by applying the same 34 Hz sawtooth modulation

to the klystron reflector and displaying the output of the microwave detector on the oscilloscope. Modern PC-based equipment use LCD for display of EPR spectrum.

11.3.7 Sample Cavities

The heart of the ESR spectrometer is the resonant cavity containing the sample. The sample is contained in a resonance cavity. Rectangular TE120 cavity and cylindrical TE011 cavity have widely been used. In most of the ESR spectrometers, dual sample cavities are generally used. This is done for simultaneous observation of a sample and a reference material. Since magnetic field interacts with the sample to cause spin resonance, the sample is placed where the intensity of magnetic field is greatest.

The resonant cavity of an EPR spectrometer is designed with dimensions corresponding to the specific wavelength of microwave radiation that is to be used. For example, at X-band frequencies (~9.5 GHz), the wavelength of the electromagnetic wave is ~3 cm. In Figure 11.10, the resonant cavity has dimensions of ~3 cm. This allows the microwaves to resonate in the cavity. In a resonant cavity, the level of microwave energy is thousands of times greater than that in the waveguide. The sample is located in the cavity at a location that allows maximum absorption of magnetic energy from the microwaves, and minimum absorption of electric energy. This is because it is the magnetic field component of the microwave energy that excites and EPR transition, while the electric field component will be absorbed in a non-resonant manner. Typically, the sample is placed in a central location in the cavity, where it will absorb the greatest level of energy from the magnetic field of the microwaves.

Signal intensity from a particular sample can vary greatly, depending on the microwave cavity configuration. This is important when signal-to-noise is a serious problem. The two main effects arise from, (i) the difference between cylindrical (TE_{011}) and rectangular cavities (TE_{012}), (ii) the effect of quartz such as Dewar inserts, on the microwave field distribution. The use of a cylindrical cavity results in a net improvement over the rectangular cavity. Both types of cavities are however transverse electric (TE) modes, which means that the electric field lines are confined to the plane perpendicular to the longitudinal axis. No such restriction exists for the magnetic field lines. The sample cavity should be so designed that it can be held in the volume of maximum homogeneity of the magnetic field. The cavities are available for a wide variety of ESR signals, ranging from solids to liquids. They are designed to be compatible with other accessories like aqueous solution sample cell, flow mixing chamber, electrolytic cell, liquid nitrogen Dewar and the variable temperature accessory.

Figure 11.10 *An X-band resonant cavity, with waveguide attached. Note the dimensions of ~3 cm (Courtesy: Alan Wilder, UC Davis ChemWiki)*

ESR studies involving comparative measurements are made by using dual cavity. This cavity does this by allowing a reference sample of known magnetic resonance characteristics to be exposed to microwave energy simultaneously with and in the same cavity as, the sample being studied.

A rotating cavity is designed to facilitate ESR studies of crystal anisotropy. The cylindrical rotating cavity has exterior modulation coils, which are free to turn with the rotating electromagnet and thus provide a modulation field, which is always parallel to the DC magnetic field. The sample tube remains fixed in the cavity, which keeps the Q constant and eliminates the need for retuning after rotation.

A cryogenic cooling device is used to cool samples down to ultra-cold temperatures. This leads to a greater population difference in the split spin states, increasing the signal level. Many transitions can only be seen at ultra-cold temperatures, especially in biological samples. A temperature as low as 4 Kelvin is often necessary to gain a resolved spectrum. Liquid helium must be used to maintain these ultra-cold temperatures. The following two types of cryogenic cooling setups are common:

- A reservoir dewar, which maintain a constant level of liquid helium and/or liquid nitrogen surrounding the sample, or
- A continuously flowing liquid helium pump, which continuously pumps liquid helium into the space surrounding the sample cavity.

The latter is the setup commonly utilised on commercially available spectrometers. Naturally, these setups require the use of high-powered vacuum pumps to evacuate the areas insulating the lines and reservoirs that transfer and contain the cryogen.

11.3.8 Sample Cells

Aqueous solution sample cells are specially designed to carry out ESR studies in lossy or aqueous solutions. Further, as optical irradiation experiments are often performed with these cells, the quartz is selected to give a maximum optical transmission in the UV-visible region of the spectrum. Special types of flow cells, tissue cells and electrolytic sample cells are also used when required. Generally, the standard sample tube is made of high purity quartz and measures 3 mm ID and 4 mm OD. Glass is not used because it contains traces of FE^{3+}. For maximum sensitivity, the tube may be filled to a height of 2.5 cm for a rectangular cavity and 5 cm for a cylindrical cavity.

All sample tubes should be cleaned before inserting them into the cavity. Only standard ESR sample tubes should be used to prevent breakage and to minimise contamination signals. Sources of contamination, such as cigarette ashes or smoke should be kept away from the immediate vicinity of the cavity, whether the cavity is installed in the air gap or not. Loose magnetic materials must be kept away from the gap of the magnet to prevent damage to the cavity, when it is in the air gap. Watches should be kept away from the magnet gap. Sample materials or solvents (water-acetone) with high dielectric loss require flat cells. For materials with low dielectric, the regular 3-mm sample tube is used.

12

ELECTRON AND ION SPECTROSCOPY

12.1 SURFACE SPECTROSCOPIC TECHNIQUES

Electron and ion spectroscopic techniques find applications for surface[1] analysis. They can provide chemical information, which the classical methods like microscopy, reflectivity and adsorption isotherms cannot. One can obtain, with electron and ion spectroscopy, elemental analyses, information about oxidation states and organic functional groups; quantitative analyses either as elemental ratios or oxidation state ratios and distributions of materials either across the surface or from the surface inward, towards the bulk (Hercules and Hercules, 1984a).

The surface spectroscopic techniques can be best explained by Figure 12.1. When a beam is incident on the surface it penetrates to some depth within the surface layer. A second beam exits from the surface, which can be analysed by a spectrometer. The beams shown on the diagram may be photons, electrons or ions. Obviously, by varying the nature of the beams in and out of the surface, a large number of surface analytical techniques can be generated. Table 12.1 summarises the various methods which emanate from the electron and ion spectroscopy. In this chapter, only the following techniques are covered:

(a) Electron spectroscopy
  (i) Electron spectroscopy for chemical analysis (ESCA)
  (ii) Auger electron spectroscopy (AES)
(b) Ion spectroscopy
  (i) Secondary-ion mass spectrometry (SIMS)
  (ii) Ion scattering spectroscopy (ISS)

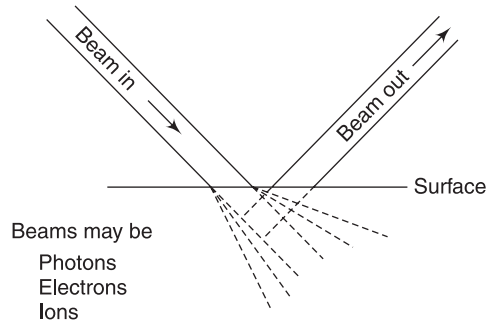


Figure 12.1 *Principle of electron and ion-beam spectroscopy*

[1] A surface is normally considered as the boundary layer of me phase at its interface with another. The surface is usually more than one atomic layer deep and is a region of non-uniform atomic potentials. Some workers also refer to the outermost layer of atoms as a surface, the transition layer as the selvedge and the remainder as the hulk

Table 12.1 *Techniques developed by combination of electron and ion beams*

Beam in	Beam out		
	X-rays	**Electrons**	**Ions**
X-rays	X-ray fluorescence X-ray diffraction	• photoelectrons • Auger electrons • Electrons spectroscopy for chemical analysis (ESCA) • X-ray photoelectron spectroscopy (XPS) • Induced electron emission (IEE)	Ionisation
Electrons	X-ray emission	• secondary emission • Auger electrons • Auger electron spectroscopy (AES), • Electron impact spectroscopy (EIS) • Low energy electron diffraction (LEED)	Electron-induced ion desorption (EIID)
Ions		Ion induced Auger electrons	• Secondary-ion mass spectrometry (SIMS) • Ion scattering spectroscopy (ISS)

12.2 ELECTRON SPECTROSCOPY

12.2.1 Electron Spectroscopy for Chemical Analysis (ESCA)

Electron spectroscopy for chemical analysis (ESCA), also known as X-ray Photoelectron Spectroscopy (XPS), is an effective technique to detect the elements and their bonding states on the surface of solid. The methods use soft X-rays to eject electrons from inner-shell orbitals. The kinetic energy of these photoelectron energies are dependent on the chemical environment of the atom, it makes XPS useful to identify the oxide state and ligands of an atom. On the other hand, however, the binding energy may shift (up to several eV) reflecting the chemical state of the atom; it is also possible to get information on the valence number and oxidation number, and the type of functional group present. Moreover, the ion etching technique provides the depth profiling from the surface.

Electron spectroscopy is based on the ionisation phenomenon brought about by either a photon (X-ray) or an electron (Hercules and Hercules, 1984a). An X-ray photon ionises an atom, producing

an ejected free election. The kinetic energy of the ejected photoelectron is dependent on the energy of the impinging photon expressed as

$$E_K = h\upsilon - E_b - \Phi$$

where E_K is the kinetic energy of the photon ejected, $h\upsilon$ is the X-ray energy, E_b is the binding energy of the parent atom relative to the ejected electron and Φ is the work function, typically <2 eV and often ignored.

The binding energy is specific for a given electron in a given element and can serve for the identification of that element. Measuring the E_K and calculating the E_b yields the 'fingerprint' of the parent atom. This forms the basis of ESCA – the electron spectroscopy for chemical analysis.

ESCA gives sufficient chemical information up to a depth of about 5–20 Å in metals, 15–40 Å in oxide and 40–100 Å in polymers. Thus sensitivity is sufficient enough to identify and detect fraction of a monolayer. It can identify elements in the periodic table above helium and adjacent elements are clearly distinguished.

In case of electron ionisation of an atom, both an excited ion and a second electron are produced. However, because of electron-electron interactions, discrete electron energies are not observed. Therefore, ESCA is not observed when using electron ionisation.

In ESCA, the goal is to catch the electrons in order to find out from which atom they are coming. The information we are interested in is the so-called binding energy which they had before leaving the atom. All what we do is counting these electrons versus their binding energy and we obtain a spectrum looking like the one shown in Figure 12.2.

In this spectrum, two main peaks at 284.6 and 532.5 energy counts are observed. The unit used for counting energy is electronvolt, abbreviated as eV. Accordingly, peaks are designated as 284.6 and 532.5 eV.

Each energy matches a specific atom type e.g. 284.6 eV matches carbon and 532.5 matches oxygen. From this we can conclude that this specimen contain carbon and oxygen. Each peak area is proportional to the number of atoms being present in the studied element. By calculating the respective contribution of each area, we obtain the specimen chemical composition (e.g. 25% oxygen and 75% carbon). In other words, among 100 atoms present at the surface of the material, 75 are carbon atoms. By studying the energy of this carbon peak, it is possible to find out if the surface of this material corresponds to C-O or C=O chemical form.

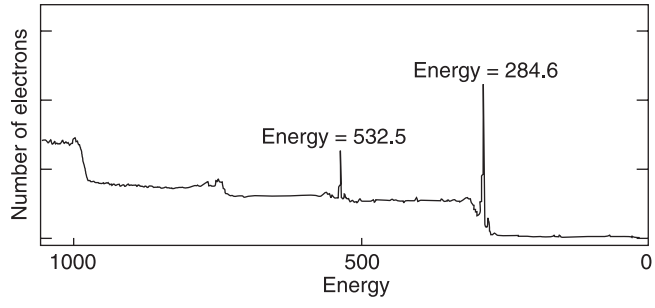


Figure 12.2 *Electron count plot versus energy (energy spectrum) (adapted from www.lasurface.com)*

12.2.2 Auger Electron Spectroscopy (AES)

Auger Electron Spectroscopy (Auger spectroscopy or AES) was developed in the late 1960s, deriving its name from the effect first observed by Pierre Auger, a French Physicist. It is a surface-sensitive analytical technique that utilises a high energy electron beam as an excitation source and utilising the emission of low energy electrons in the Auger process. It is one of the most commonly employed surface analytical techniques for determining the composition of the surface layers of a sample. Auger spectroscopy facilitates determination of the chemical composition of a surface. The smallest surface that can be characterised is a few nm wide for the best instruments.

In this technique, the electron beam is scanned over a variably sized area, or it can be directly focused on a small surface feature of interest. This ability to focus the electron beam to diameters of 10–20 nm makes Auger an extremely useful tool for elemental analysis of small surface features. When used in combination with ion sputter sources, Auger can perform compositional depth profiling.

AES can be used for surface chemical analysis in a way very similar to XPS. Since core levels are involved, the energy of the Auger is also very characteristic for the various elements. AES is, however, rather limited when it comes to very high resolution studies. On the other hand, a qualitative chemical analysis of the surface is still very often performed using AES. Figure 12.3 shows a typical equipment for AES for chemical analysis.

The Auger nomenclature follows the old X-ray notations. The Auger transitions are labelled ABC for the initial state hole (A) and the two final state holes (B) and (C). For ABC on sets the letter denoting the shell. A KLL Auger transition would be a transition starting from a hole in the 1*s* levels which would be filled up from the 2p level. A 2p electron would also be emitted. The more complicated nature of the upper levels causes a multiplet splitting in the Auger spectra.

An important point in AES is that it does not make any difference, how the initial core hole is created. In most practical cases this is achieved by bombarding the sample with electrons of 2–3 keV kinetic energy. The Auger electrons are detected with the electron analysers. For a quantitative analysis, the energy of the exciting electrons does, however, come into play because of the energy- and element-dependent ionisation.

It may be noted that photoionisation can produce either an ESCA electron or an Auger electron, while electron ionisation can produce only Auger electrons. Further, the kinetic energy of the Auger electron does not depend upon photon energy, whereas the ESCA photoelectron does. It may be noted that Auger phenomenon characterises a de-excitation process leading to the loss of one electron. This electron is called an Auger electron.

Figure 12.4 illustrates the relationship between ESCA, X-ray and Auger processes for photoionisation of a IS electron. In the ESCA process, the photon ejects a IS electron from the atom. In case of X-ray, an electron drops

Figure 12.3 *Auger electron spectrometer (Courtesy: M/s Evans Analytical Group)*

from the 2 p orbit to fill the IS hole and a photon is emitted, resulting in $K\alpha$ X-ray emission. In the Auger process, a 2s electron drops to fill the IS hole, simultaneously expelling a 2 p electron.

It is obvious from this diagram that Auger and X-ray emission are competitive processes. In general, for low energy (1,000 eV) processes, the Auger effect is predominant, while for higher energy (10,000 eV) processes, X-ray emission will be dominant.

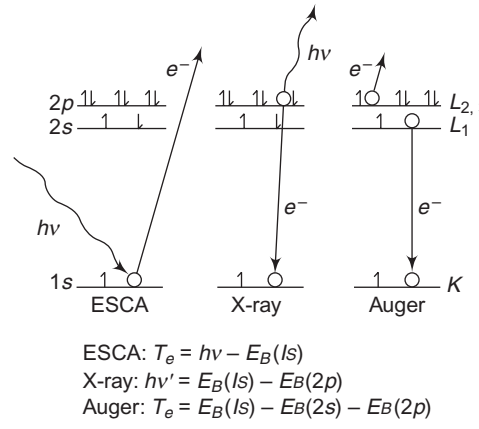


Figure 12.4 *ESCA, X-ray and Auger processes*

AES involves the irradiation of the surface to be analysed with a beam of electrons of energy in the 1–2 KeV range. Beam currents are typically 5–50 μA in a beam of diameter 0.5 mm. Since an electron with primary energy 1–2 keV can penetrate only a few atomic layers, AES is basically a surface chemical analysis technique providing chemical information from 4 to 10 Å depth. The technique is very sensitive and generally allows detection and identification of less than 0.1% of a monolayer of atoms. Table 12.2 gives analytical characteristics of ESCA and AES.

Table 12.2 *Analytical characteristics of ESCA and AES (after Hercules and Hercules, 1984b)*

Parameter	ESCA	Auger (ABS)
Energy range		
Kinetic energy	100–1,500 eV	50–2,500 eV
Escape depth	20 A	20 A
Peak locations	± 0.1 eV	±1 eV
Elemental sensitivity		
Elements	Z > 2	Z > 2
Specificity	Very good	Good
Sensitivity variations	50 X	50 X
Quantitative analyser		
Absolute	±30%	±30%
Relative	±5%	±5%
Detection limit	0.1% monolayer	0.05% monolayer
Other aspects		
Vacuum	10^{-5}–10^{-10} torr	10^{-8}–10^{-19} torr
x-y resolution	None	0.5 μ
Speed	Slow, typical run	Fast, takes minutes is 30 min
Sample destruction	None in 95% of the sample	Frequent, bad for organics

The basic technique of AES, primarily a surface-sensitive technique used for elemental analysis of surface, has also been adapted for the following use:

- *Auger Depth Profiling*: Providing quantitative compositional information as a function of depth below the surface. Depth profiles are obtained by employing a controlled sputtering process which enables elemental concentration to be plotted as a function of depth. 'Sputtering' is a process in which an ion gun is used to remove a few angstroms of the top most surface of a sample. Sputtering and analysis are alternated until the desired depth is reached.
- *Scanning Auger Microscopy (SAM)*: Providing spatially resolved compositional information on heterogeneous samples. Therefore, the Auger multi-probe is capable of producing elemental composition spectra, surface images, selective elemental line scans and maps, and depth profiles.

AES is a technique often described as more sensitive than XPS. The difference in sensitivity is primarily due to the difference in electron kinetic energies. For examples in the case of carbon, in XPS the kinetic energy is close to 1,000 eV as opposed to 250 eV in AES. Because the mean free path changes with the electron kinetic energy, the depth of analysis will be smaller in AES than in XPS in the case of a carbon containing specimen. As a matter-of-fact, the surface contamination (primarily carbon and oxygen) is a much more sensitive factor in AES. An ion etching is sometimes necessary to study surfaces with this spectroscopy.

12.3 INSTRUMENTATION FOR ELECTRON SPECTROSCOPY

Figure 12.5 shows a block diagram of an electron spectrometer for either ESCA or Auger measurements. It contains the following components:

(a) A source of radiation with which to excite the sample
(b) An electron energy analyser
(c) An electron detector
(d) Readout system
(e) A high vacuum system

In addition, the entire system must be shielded from the earth's magnetic field.

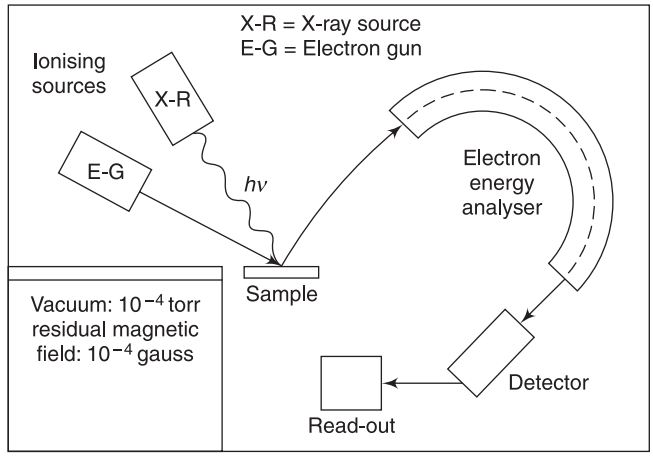


Figure 12.5 *Block diagram of an electron spectrometer*

12.3.1 Radiation Sources

12.3.1.1 X-ray sources

Traditionally, ESCA instruments have used X-rays as radiation sources and Auger spectrometers as electron guns. It is desirable that the radiation be homogeneous in energy.

The basic X-ray source includes a heated filament and a large target anode. Electrons from the filament are accelerated towards the anode to produce radiation consisting of continuum of bremsstrahlung radiation with characteristic X-ray lines superimposed upon it. The anode is held at a high positive potential, while the filament is held near ground potential. The positive potential on the anode ensures that scattered electrons do not enter the sample chamber but are retracted to the anode. The anode materials most commonly used, which give nearly monochromatic radiation sources, are magnesium or aluminium. These elements give high intensity and narrow wavelength bands of the $K\alpha$ lines. The most intense lines are called $K\alpha_1$ and $K\alpha_2$, according the old X-ray nomenclature (Figure 12.6**)** Often the doublet is viewed as one line and are called $K\alpha_{12}$. It has an energy of 1,253.6 and 1,486.6 eV, for Al and Mg, respectively (Hofmann, 2003).

The $K\alpha_{12}$ is the most intense line in the X-ray spectrum. The $K\alpha_1$ and $K\alpha_2$ lines do also have a certain width themselves which, together with their separation, determines the ultimate resolution achievable with an X-ray source. It is determined by life time of the core hole. The total width for the Al and Mg $K\alpha_{12}$ line is of the order of 1 eV.

As illustrated for a detailed chemical analysis, it is very desirable to have a higher energy resolution. In order to achieve this, the X-ray source can be equipped with a monochromator. This will increase the energy resolution and at the same time remove the 'satellite' lines which are due to photoemission induced by, for example, the $K\alpha_{34}$ line.

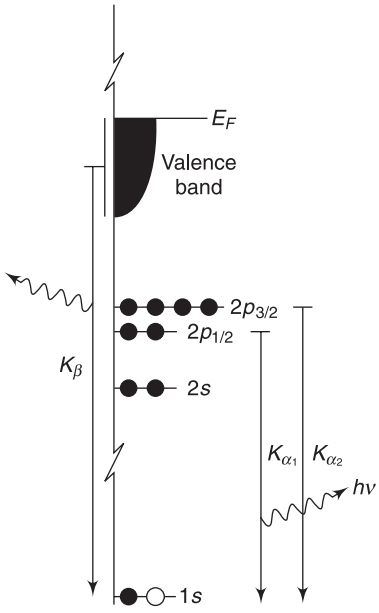


Figure 12.6 *Nomenclature in the X-ray decay in* Al *and* Mg

A thin X-ray transmitting window separates the excitation region from the specimen and prevents the entry of scattered electrons from the X-ray source into the sample chamber. The most commonly used window material is high purity aluminium or beryllium foil, which removes the K_β line and much of the background.

Another means of removing background produced by bremsstrahlung radiation and satellite peaks produced by the less intense characteristic X-ray transitions, is through the use of an X-ray monochromator. Most popularly used X-ray monochromator exploits Rowland's Theorem, which states that if a diffracting crystal with radius of curvature R is tangent to a circle of diameter R, and if an X-ray source and the sample replaced on this circle, so that they both make angle θ relative to the crystal surface, then the radiation striking the crystal surface which satisfies the Bragg diffraction law will be reflected and brought to a focus on the sample, and all the other radiation in the X-ray spectrum will not be reflected.

The Bragg diffraction law is

$$n\lambda = 2d \sin\theta$$

where n is any integer, λ is the X-ray wavelength and d is the spacing of the atomic planes within the crystal.

Figure 12.7 shows the arrangement of the monochromator. There are actually three crystals in the system, which helps to collect more X-ray intensity than is possible with a single crystal. Each crystal has a separate Rowland circle in a different plane from the others. These three planes intersect in the line passing through the anode and the sample. The reflection of the Al $K\alpha$ radiation from the crystal planes takes place at a Bragg angle θ of 78.5°. A Bragg angle close to 90° has the advantages of minimising the geometrical aberrations and maximising the Bragg diffraction intensity and the dispersion of the monochromator.

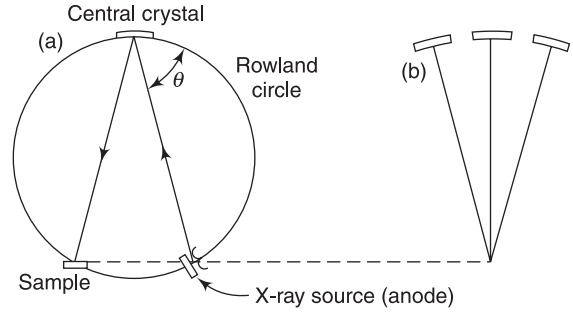


Figure 12.7 *X-ray monochromator. [It has three spherically bent crystals with three Rowland circles that intersect at the anode and sample]*

An electron beam for AES is produced in an electron gun, which basically consists of an emitting surface called cathode, an accelerating electrode (the anode) and one or several focusing electrodes, which control the characteristics of the electron beam. An electron beam can be used directly or with a monochromator. Generally the electrons from a heated cathode are fairly homogeneous, though there may be a small spread due to a range of kinetic energies of the emitted electrons. The beam can be made more homogeneous by employing an energy filter, of which there are several types.

Figure 12.8 shows typical arrangements used as electron energy filters. If the electrons are subject to a retarding field between two grids (Figure 12.8a), only those electrons which have sufficient energy to overcome the field will go to the right. This arrangement acts essentially as a high pass filter, in that there is a lower but not an upper limit to the energy of the emerging beam. If the electron beam enters the space at an angle of 45° into the space between two parallel plane conductors (Figure 12.8b), only those electrons within a specified narrow energy band will emerge through the exit slit. The energy band will depend upon the distance between the plates and the potential applied.

The use of cylindrical electrodes (Figure 12.8c) instead of plane electrodes results in good energy discrimination. Even those electrons which enter the filler at a slightly divergent angle will be focused on the exit slit. For double focusing effect, the angle $\pi/\sqrt{2}$ radians (127°17′) is required. Equally good focusing properties can be obtained by employing 180° spherical rather than 127° cylindrical segments as electrodes (Figure 12.8d).

12.3.1.2 Synchrotron radiation

Alternatively, one can use synchrotron radiation as an X-ray source. This radiation is caused by accelerating charged particles (mostly electrons), typically by forcing them to go around the corners of a storage ring (Figure 12.9).

Synchrotron radiation (SR) is emitted when charged particles moving with relativistic speeds are forced to follow curved trajectories in magnetic fields. In general, three kinds of magnets are

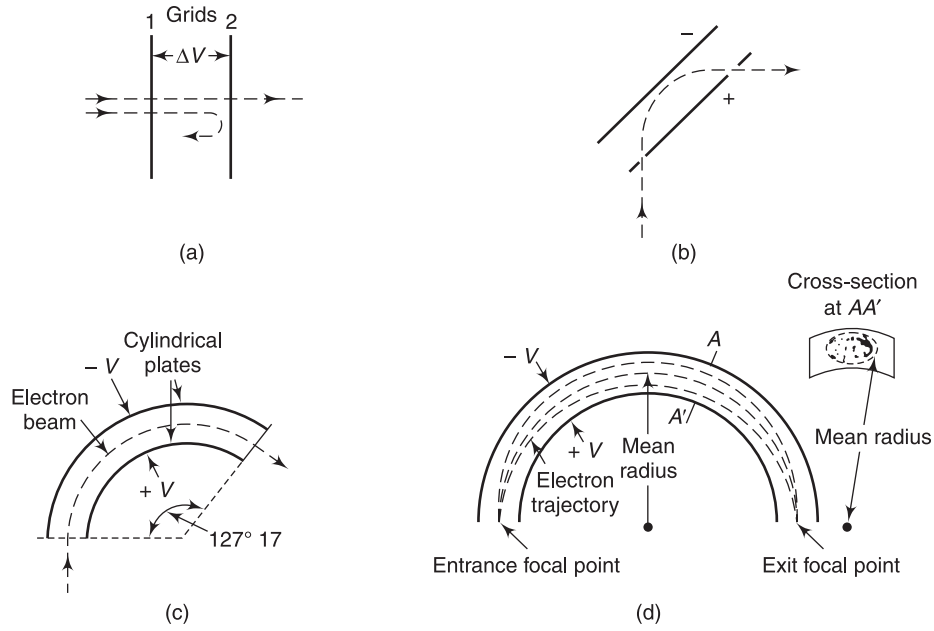


Figure 12.8 *Energy filters: (a) retarding field, (b) parallel plate arrangement, (c) cylindrical electrode arrangement, (d) spherical electrode arrangement*

used to make the necessary magnetic fields: bending magnets, wigglers and undulators.

For bending magnets, a sample dipole structure is used to constrain the electrons in a curved path. The radiation emitted is extremely intense and extends over a broad wavelength range from the infrared though the visible and ultraviolet, and into the soft and hard X-ray regions of the electromagnetic spectrum.

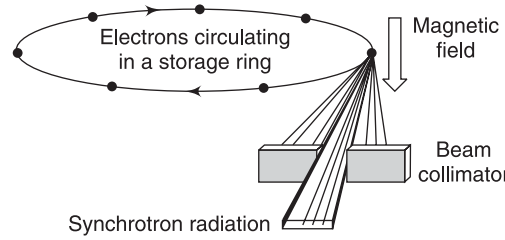


Figure 12.9 *Synchrotron radiation emission mechanism*

High-field wiggler magnets are often used as sources in order to increase the flux at shorter wavelengths. A wiggler can be considered as a sequence of binding magnets of alternating polarities which gives a $2N$ enhancement in the flux, where N is the number of poles. The properties of wiggler SR are very similar to that of dipole radiation with a reduction in the critical wavelength as a consequence of the higher field. For super-conducting wiggler magnets, a value of 6 tesla, as opposed to around 1.2 tesla for conventional dipoles, would be typical.

Undulators, consisting of periodic magnetic arrays, cause small electron deflections comparable in magnitude to the natural emission angle of the SR. The radiation emitted at the various poles interferes coherently resulting in the emission of a pencil-shaped beam peaked in narrow energy bands at the harmonics of the fundamental energy. For N poles the beam's opening angle is decreased by $N^{1/2}$ and thus the intensity per solid increases as N^2.

Synchrotron radiation has several advantages over conventional source: the resolution can be very high, the radiation is polarised and most important, the photon energy can be changed. This allows to shift the peaks in an X-ray spectrum to exactly the desired kinetic energy. In surface science, this is the energy where the mean free path of the electrons is shortest. The obvious disadvantage of synchrotron radiation is that you have to build a storage ring to get it.

12.3.2 Energy Analysers

The function of the energy analysers is to measure the number of photoelectrons as a function of their energy. This is done by using an electrostatic or magnetic analyser. Magnetic deflection analysers are effective, but less convenient to design and use than electrostatic types. The most widely used analyser in commercial instrumentation is double-pass cylindrical mirror (CMA) and 180° spherical sector analysers (SSA).

Figure 12.10 shows a schematic diagram of the double-pass CMA. Basically, the CMA consists of two conical cylinders with angular entrance and exit apertures cut in the inner cylinder. A negative potential is applied to the outer cylinder. The potential applied between the inner and outer cylinders produces α cylindrical retarding potential. From the theory of this analyser, it is found that optimum focus will be obtained with the angle a between the electron beam and the axis of symmetry within a few degrees of $42^{\circ}\,20'$. In the CMA, electrons which leave sample positioned at the focal point of the analyser pass through an annular slit then pass into the radial field between the cylinders to be focused back to the axis by the negative potential. The electrons pass into the second cylinder mirror analyser to be focused onto an electron multiplier.

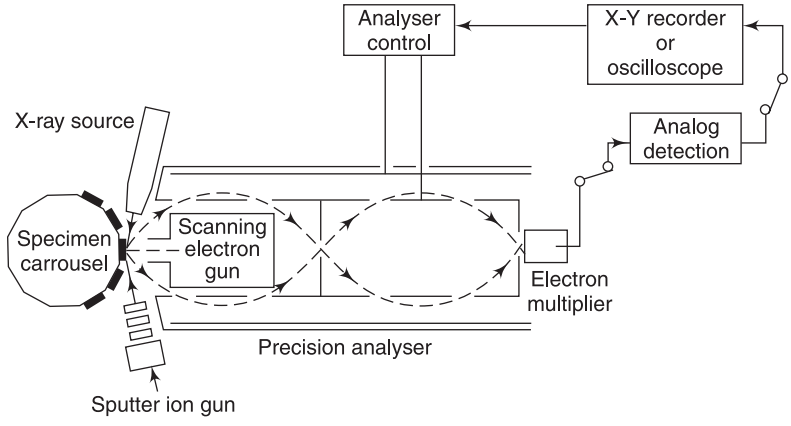


Figure 12.10 *The arrangement of a double-pass cylindrical mirror analyser used for X-ray photoelectron spectroscopy*

In SSA (Figure 12.11), electron energy is analysed by passing the electrons between two hemispherical domes that have a potential difference between them. Electrons of the desired energy follow a circular orbit between the domes and reach the detector, while electrons having higher or lower energies strike one of the domes and are not detected. The number of electrons striking the detector for a given potential difference is counted and plotted as a function of energy.

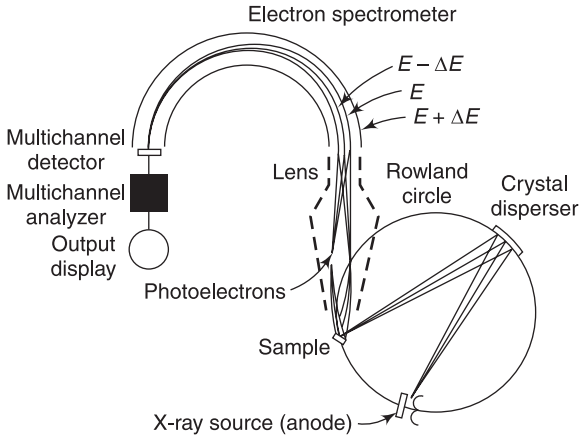


Figure 12.11 *Schematic diagram showing 180° hemispherical analyser with electrostatic lens and dispersion compensated electron optics*

The energy of the X-ray photon incident on the sample varies across the sample by about one electron volt (which is the width of the X-ray line). This results in variation of the kinetic energy of the photoelectrons from a given energy level by one electron volt. This inherent variation is cancelled by using an electron lens. The electron lens has four elements or three gaps, corresponding to three degrees of freedom in the electron optics. The first is used to retard the photoelectrons of interest to 115 eV kinetic energy, before they enter the electron spectrometer. The second forms a focused electron image of the target at the spectrometer entrance. The last degree of freedom is used to image; so the dispersion of the electron spectrometer at 115 eV kinetic energy precisely cancels the dispersion of the X-ray monochromator across the sample. This cancellation, called dispersion compensation, removes the inherent line-width limitation on instrument resolution.

The use of a retarding field formed by the lens improves the resolving power of the lens, if electrons leaving the sample with kinetic energy E_k are retarded to an energy E_R for transmission through the analyser, then the resolving power of the complete system will be improved by a factor equal to the retarding ratio E_K/E_R Retardation also gives rise to improvement in sensitivity at a fixed energy resolution, since a larger slit may be used at the lower transmission energies.

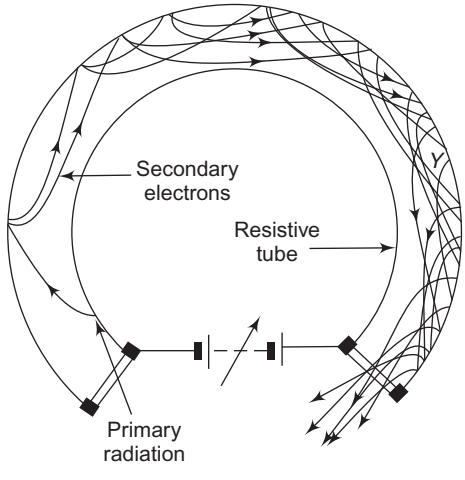


Figure 12.12 *Channel electron multiplier*

The fringe fields at the entrance and exit to the electrodes of both the CMA and SSA need to be suitably terminated fringing fields at the ends of CMA are usually terminated by a series of rings coupled by dividing resistors or by resistively coupled ceramic disks. In case of hemispherical analyser, the fringing fields are terminated by concentric wires or shaped electrodes biased at the electron pass energy.

12.3.3 Electron Detectors

The most commonly employed detectors in ESCA and AES instruments are *electron multipliers*. They are similar to photomultipliers, but accept electrons directly instead of from a photocathode. One type of such a multiplier is the channel electron multiplier described by Evans (1972) and shown in Figure 12.12. It consists of lead-doped glass tube with a secondary

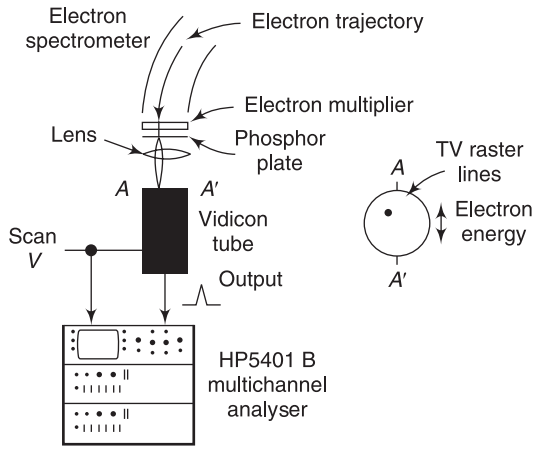


Figure 12.13 *Wide-area detections system. An incoming electron strikes a multi-channel electron multiplier, resulting in a flash of light from a phosphor plate. The flash is imaged onto a vidicon tube. As the vidicon scans, each flash produces a pulse that is counted by the multi-channel analyser and classified according to the incoming electron. The result is the ESCA spectrum, a plot of number of photoelectrons versus energy*

semiconducting coating possessing a high secondary electron yield. A voltage of 2–3 kV is applied between the ends of the multiplier to produce a gain of 10^6–10^8 due to the cascade of collisions, as electrons travel down the inside of the tube. The device accepts electrons at one end and emits more electrons at the other, hence acts as a current amplifier. The output of the multiplier is a series of pulses that are fed into a pulse amplifier-discriminator, then into a digital-to-analog converter, and stored in a multi-channel analyser or a computer.

The spectrometers in which electron energy can be related to position in the exit plane of the analyser are equipped with multi-array detectors. One such system is shown in Figure 12.13. The conventional exit slit at the output of the spectrometer is replaced with a 1.1 inch-square imaging electron multiplier. Whenever a single photoelectron strikes the input of this active surface, a corresponding pulse of 10^8 electrons exits from the same position on the output surface and strikes a phosphor plate. The resulting flash of light is imaged onto and stored within the target of a vidicon television tube. The event remains stored until erased by the conventional TV raster scan of the vidicon electron beam, which converts the event to an electrical pulse at the vidicon output. These pulses are transferred to multi-channel analyser (1,024 channels), which sort out the pulses according to the energies of the detected photoelectrons.

12.3.4 Read-Out System

Counts are accumulated in the various channels for an appropriate time and the resulting spectrum (a plot of counts per channel versus channel energy level) drawn by an x-y plotter can be displayed. The information can also be put into a computer for analysis or further processing. Since the atomic structure of each element in the Periodic Table is distinct from all the others, measurement of the electron binding energies enables identification of the presence of elements on the sample surface.

12.3.5 Vacuum Systems

Electron spectrometers must operate under a vacuum of 10^{-6} torr or lower, 10^{-10} torr is ideal. At pressures higher than 10^{-6} torr, the electrons would be scattered enroute from the sample to detector. Pressures of these orders can be achieved by a variety of techniques. However, the most common system is a getter-ion pump complemented by a sublimation pump, with a cryogenic shroud and sorption forepump. The vacuum system is of stainless steel construction, with crushed metal gaskets.

12.3.6 Magnetic Shielding

Electron spectrometers are high resolution instruments and therefore it is necessary to reduce the effect of stray magnetic fields within the volume of the analyser. This is required, since the path of the electron is disturbed by stray magnetic fields, including that of the earth. Magnetic shielding can be provided in several ways. Ferromagnetic shielding is usually preferred in commercial instruments, as it is simple and less sensitive to magnetic field variations. An alternative method is to use Helmholtz coils, which are adjusted to produce a field exactly equal and opposite to the stray field present at any time. The system is made automatic and includes a magnetometer probe placed on the vicinity of the spectrometer and connected electrically to adjust the current in the Helmholtz coils as required to maintain a constant field.

12.3.7 Sample Handling

Surface techniques usually require that the sample to be analysed be placed in a special environment, which in the case of ESCA should be a good vacuum. This is because electrons escaping from a solid at atmospheric conditions would be able to travel only a negligible distance before being stopped by air molecules, so analysis of their energy would be impossible. Moreover, if the sample is composed of a reactive material (e.g. aluminium), the pressure on the sample chamber must be very low, to prevent the surface being oxidised or otherwise contaminated. Figure 12.14 shows

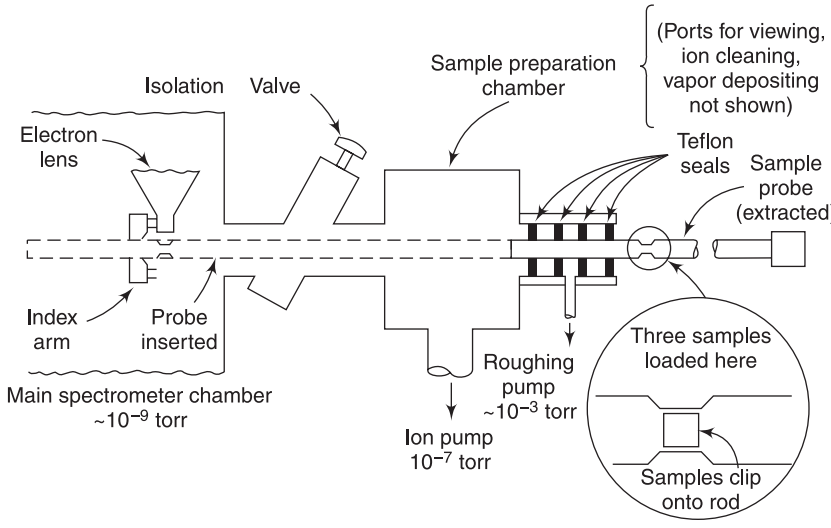


Figure 12.14 *Sample inlet system*

a technique to insert a sample originally at atmospheric pressure in a vacuum chamber. Three samples are attached to a rod, which slides through four seals. As the samples pass the second of these seals, they are evacuated to about 10^{-2} torr in a few seconds. They are then transported into the sample preparation chamber, where the pressure is approximately 10^{-7} torr. Here they can be cleaned by a beam of ions to remove layers of oxidation, or be otherwise prepared if desired.

The rod is then inserted farther into the main spectrometer chamber, and one of the three samples is placed in the proper position for analysis. The rod automatically positions the sample in the X-ray beam, so the emitted electrons can enter the electron lens.

The sample can be analysed at any desired temperature between −150 and 300°C by using a special sample rod. A controller is supplied with a variable temperature probe that automatically regulates the sample temperature at the desired value. Another special probe that permits samples to be vapour deposited on a substrate is also provided with some instruments. Any material volatile enough to be vaporised below 1700°C can be evaporated. This probe is useful for extremely reactive samples. Samples can be cleaned to remove layers of oxide or other surface impurities with an ion gun attached to the sample preparation chamber.

A source of difficulty in ESCA and AES is the sample charging effect. The X-ray incidents on the sample cause electrons to be emitted, so the sample surface acquires a net positive charge. If the sample is electrically conductive and in contact with the metal parts of the spectrometer, the positive charge cannot accumulate. But if the sample is an insulator, the positive charge will quickly build up and this charging effect could be very serious in some situations.

The charging effect can be turned off by supplying a flood of electrons having a uniform low energy to the sample. The surface potential can thus be clamped to a potential determined by the energy of these electrons. This capability actually result in additional useful information about the sample.

Improvement in computer technology and X-ray optics has made XPS the technique of choice for surface chemical characterisation, achieving spatial resolution of the order of 3–5 micrometres, good enough for chemical imaging.

12.4 ION SPECTROSCOPY

Ion spectroscopy is another powerful technique applied to surface characterisation. The two techniques usually adopted are ISS and secondary-ion mass spectrometry (SIMS). The principle of ion spectroscopy is shown in Figure 12.15. When primary ion, usually an inert gas ion, having kinetic energy of 0.3–5 keV is incident on a surface, one of the following phenomena can occur (Hercules and Hercules, 1984b):

(i) The primary ion can be elastically scattered by a surface atom, resulting in a reflected primary ion. The kinetic energy of the reflected primary ion will depend on the mass of the

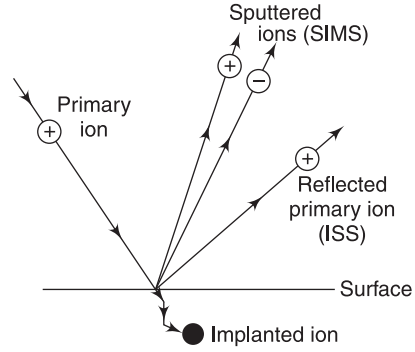


Figure 12.15 *Fundamental processes important for SIMS and ISS*

surface atom involved in the scattering process. It is the reflected primary ion which is measured in ISS.

(ii) The primary ion can penetrate the surface and become embedded in the solid. The penetration of the ion in the lattice results in considerable disruption by transfer of momentum to lattice atoms or molecules. This is called sputtering. For approximately 1 keV ions, this process takes place only several atomic layers deep, restating in expelling atomic and molecular fragments. These fragments can be either neutral atoms or ions, which could be both positive and negative ions. The ions are referred to as secondary ions, and thus the term secondary-ion mass spectrometry (SIMS).

Table 12.3 gives analytical characteristics of ISS and SIMS.

Table 12.3 *Analytical characteristics of ISS and SIMS (after Hercules and Hercules, 1984 c)*

Parameter	ISS	SIMS
Range	Energy range: 1 keV	Spectral range 0–500 amu
Analysis depth	Samples top atomic layer	40 Å (dynamic), monolayer (static)
Elemental sensitivity		
Elements	Li to U	All
Specificity	Variable	Good
Sensitivity variations	30 X	10^5
Quantitative analysis		
Absolute	±30%	Not possible
Relative	± 10%	±50%
Detection limit	10^{-3}% monolayer	10^{-4}% monolayer
Other aspects		
Matrix effects	Some	Severe
vacuum	10^{-5} torr of scattering gas	10^{-5} torr of ionising gas
Depth profiling capability	Yes, slow	Yes, rapid (dynamic SIMS)
X-Y resolution	Poor, 100 μ	1 μ with ion microprobe
Sample destruction	Some sputtering	Yes, sputtering of surface

12.4.1 Instrumentation for Ion Spectroscopy

A block diagram of a typical ISS/SIMS instrument is illustrated in Figure 12.16. Ions are formed by bombarding gas atoms with electrons. The positive ions are accelerated and focused on the sample at an angle of 45° Ions are scattered in all directions. However, only those electrons which are in a selected small solid angle are received in the 127° electrostatic analyser. The detector can be a channel electron multiplier or a solid-state (Si) device. To obtain ISS spectra, the backscattered primary ions are sampled by the CMA and their kinetic energies are measured.

The fundamental equation for single-event ion scattering is

$$E_1 = E_0 \left(\frac{\cos\theta \pm \sqrt{\alpha^2 - \sin^2\theta}}{1+\alpha} \right)^2 \qquad ...(12.1)$$

where E_0 = energy of the incident ion,
E_1 = energy after collision with a surface atom,
$\alpha = M_2/M_1$
where M_1 = the mass of the incident ion
and M_2 = mass of the target surface

Equation (12.1) is a complex function of the angle (θ) between the initial direction of the ion and the direction the ion takes after the scattering process. For a scattering angle of 90°

$$E_1 = E_0 \left(\frac{M_2 - M_1}{M_2 + M_1} \right)$$

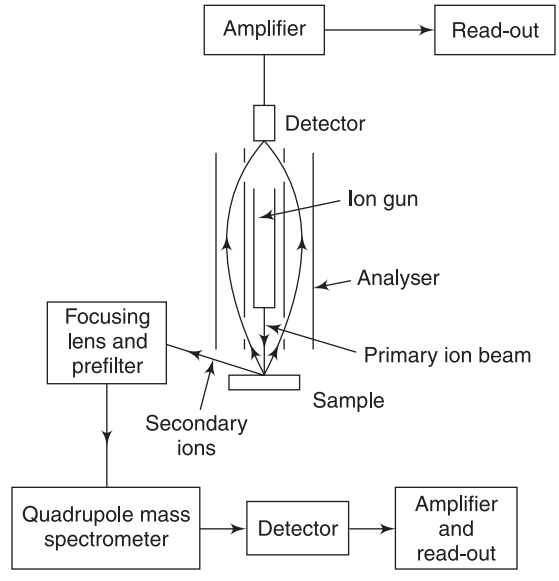


Figure 12.16 *Block diagram of ISS/IMS instrument*

This is valid only if $M_1 < M_2$. The energy E_1 is most sensitive to small differences in M_2 if M_1 is only slightly smaller than M_2. So, it is advantageous to use ions from a variety of gases. The noble gases, helium and Argon are most frequently selected to avoid side-effects.

ISS is sensitive *to* every element heavier than helium, since the lightest isotope used as a primary ion is ^{3}He and the scattering element must be heavier than the scattering gas. The specificity (ability to separate two particular elements) will vary depending on the scattering gas used. The detection limit of ISS is probably of the order of 10^{-3}% of a monolayer, i.e. less sensitive than SIMS, but more sensitive than either Auger or ESCA. ISS can be used effectively for depth profiling, particularly in the range 0–100 Å.

For recording of SIMS spectrum, the secondary ions are collected and their masses are determined in a small dedicated mass spectrometer. The secondary ions emitted from the sample are first focused through a lens and a pre-filter. The pre-filter effectively is a discriminator used to select ions having a particular kinetic energy range. The ions are then passed on to a quadruple mass spectrometer. The ions coming out of the mass spectrometer are detected by an electron multiplier, amplified and given to a read-out system. As with ESCA and Auger spectrometers, ISS/SIMS instrument must operate in high vacuum.

The quadruple mass spectrometer used in SIMS instruments operates in the range 500–1000 amu with a resolution of 1 amu. SIMS shows good specificity, although there is some overlap between peaks of different elements. Because of severe matrix effects, sensitivity variations between the most sensitive and least sensitive elements are about 10^5, therefore, performing absolute analyses with SIMS generally is not possible. Also, the relative standard deviations even for calibrated systems are poorer than for the other surface analysis techniques.

The analysis depth of SIMS varies depending on whether one is operating in the dynamic or static mode. In the dynamic mode, the primary ion current density is approximately 10^{-6} A/cm^2 and scrambling occurs in a layer at least about 40 Å at best. Thus, a dynamic SIMS has a depth resolution of 40 Å at best (Hercules and Hercules, 1984b). When operating in the static mode (10^{-9}A/cm^2), each sputtering event comes from a virgin surface, and thus, one can assume that the SIMS spectrum is characteristic of the top few atomic layers.

13

SCANNING ELECTRON MICROSCOPE

13.1 BACKGROUND

For obtaining information about the physical nature of surfaces, several different imaging and detection devices are used. The most basic image sensor, the eye, was the only means humans had of visually observing the world around them for thousands of years. Though excellent for viewing a wide variety of objects, the power of the eye has its limits, anything smaller than the width of a single human hair being able to pass unnoticed by the organ. Most objects, particularly biological samples, are far too small to be seen with the naked eye and require the use of high-power optical and electron microscopes for careful examination.

When optical microscope were developed in the late 1600s, a whole new world of tiny wonders was discovered. Electron microscopes, invented in the mid-twentieth century, made it possible to detect even tinier objects than optical microscopes, including smaller molecules, viruses and DNA. The detection power of most electron microscopes used today, however, stops just short of being able to visualise such incredibly small structures as the electron orbital systems of individual atoms.

13.1.1 Optical vs. Electron Microscope

The major difference in optical and electron microscopy lies in how much detail can be seen with each method. This difference comes from the very dissimilar sources of illumination that are used by the two methods. In optical microscopy, visible light (photons) is transmitted through or reflected from a sample and then passed through optical lenses to achieve magnification. The major limitation of standard optical microscopy lies in the diffraction limit of resolution. Diffraction is a phenomenon whereby a beam of light or other system of waves is spread out as a result of passing through a narrow aperture, typically accompanied by interference between the wave forms produced.

Figure 13.1 explains the concept of resolution in microscopes. As illustrated by the diagram, the minimum resolvable separation distance of a microscope depends on the wavelength of illumination and the numerical aperture of the lens, and is given by the following equation:

$$D = 0.61\,\lambda/NA$$

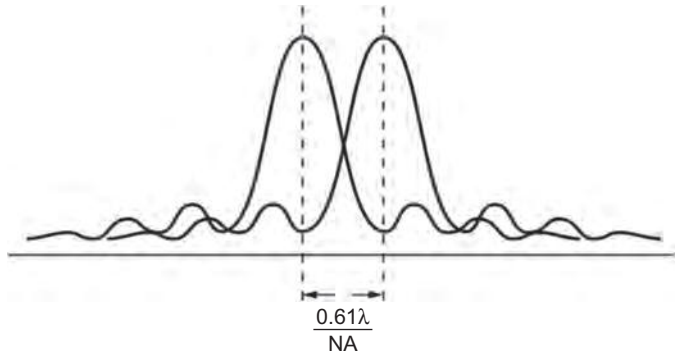


Figure 13.1 *The concept of resolution in microscopes*

where *NA* is the numerical aperture of the objective lens, and is typically inscribed on the lens by the manufacturer. This formula was derived by German physicist Ernst Abbe and is based on the Rayleigh criterion invented by English physicist John William Strutt.

The limit on what size can be resolved – irrespective of instrument type – is set by the wavelength. The wavelength of the visible light used in optical microscopes is between 400 and 700 nano-metres (nm). The maximum value of N.A. for optical microscope is approx. 1.4; it is obvious, therefore, that even the short blue light (= λ 436 nm) of the visible spectrum will yield a resolution of only 190 nm. The electron microscope however, utilises electrons for illumination. Electrons have the characteristics of both particles and waves. The wavelength of an electron beam is about 1,00,000 times less than that of visible light and hence the resolution of an electron microscope is far superior to that of the optical microscope. The current resolution of limit of the best electron microscope is approximately 0.05 nm.

The other feature of electron microscopy is its depth of focus. The depth of focus is the distance above and below the image plane over which the image appears in focus. As the magnification increases in the optical microscope, the depth of focus decreases.

The three-dimensional appearance of the specimen image is a direct result of the large depth of field of the electron microscopes. It is these large depths of fields that are the most attractive feature of these microscopes. This field arises because of the method in which the data is obtained with a fine electron beam scanned over the surface and with the detected secondary electrons forming an image on the 'TV'-like monitor.

The advantages of electron microscopes are:

- Remarkable depth of focus
- Imaging from millimetres to a sub-nano-metre
- Chemical composition with 0.1–1 μm resolution
- Optical properties on a micro-meter scale (via Cathodoluminesence)

Electrons microscopy is more powerful, as it can achieve better resolution. Resolution is the degree of detail visible in photographic image, or the smallest interval measureable by a scientific instrument such as a microscope.

13.2 SCANNING ELECTRON MICROSCOPE (SEM)

A scanning electron microscope (SEM) is an instrument for observing and analysing the surface micro-structure of a bulk sample using finely focused beam of energetic electrons. An electron-optical system is used to form the electron probe which may be scanned across the surface of the sample in a raster pattern.

Various signals are generated through the interaction of this beam with the sample. These signals may be collected or analysed with the application of appropriate detectors. For imaging, the signal amplitude obtained at each position in the raster pattern may be assembled to form an image.

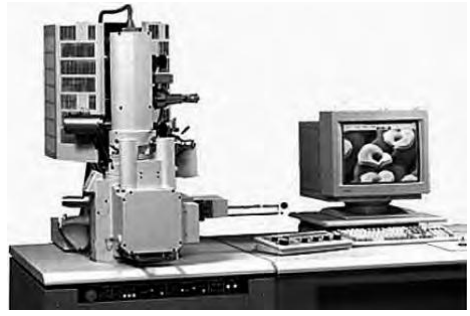


Figure 13.2 *Typical scanning electron microscope (Courtesy: M/s Hitachi)*

The scan of the electron beam and the digitisation of the image pixel value are synchronised with intensity proportional to the collected signal. Typically electrons emitted from the sample are detected to assemble the image.

Magnification is given by the ratio of the length of the line on display device to length scanned on the real sample.

Figure 13.2 shows a typical SEM – Hitachi Model S-4700. The microscope column, specimen chamber and vacuum system are on the left; the computer, monitor and many of the instrument controls are on the right.

Electrons are light-weight (1/1836 the mass of the proton) and are scattered or absorbed in air. The sample chamber in the SEM, therefore, must be in vacuum which limits the sample size to a few centimetres on edge. Electrons carry charge ($e = 1.6 \times 10^{-19}$ Coulomb) and therefore, the samples must be covered with a conducting coating.

13.3 TYPES OF SIGNALS IN SEM

Figure 13.3 shows the basic signals of SEM. The electrons interact with the atoms close to the sample surface and produce signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity. The types of signals produced by a SEM include secondary electrons (SEs), backscattered electrons (BSE), characteristic X-rays, light (cathode-luminescence), specimen current and transmitted electrons.

The signals come from an interaction volume in the specimen which differs in diameter according to different energies of the primary electrons (typically between 200 eV and 30 keV). The SE come from a small layer on the surface and yield the best resolution, which can be realised with an SEM. The well-known topographical contrast delivers micrographs which resemble with conventional light optical images.

Secondary electrons: SEs are low-energy electrons (<50 eV) ejected from the specimen atoms by the energetic primary beam. They are called 'secondary' because they are generated by other radiation

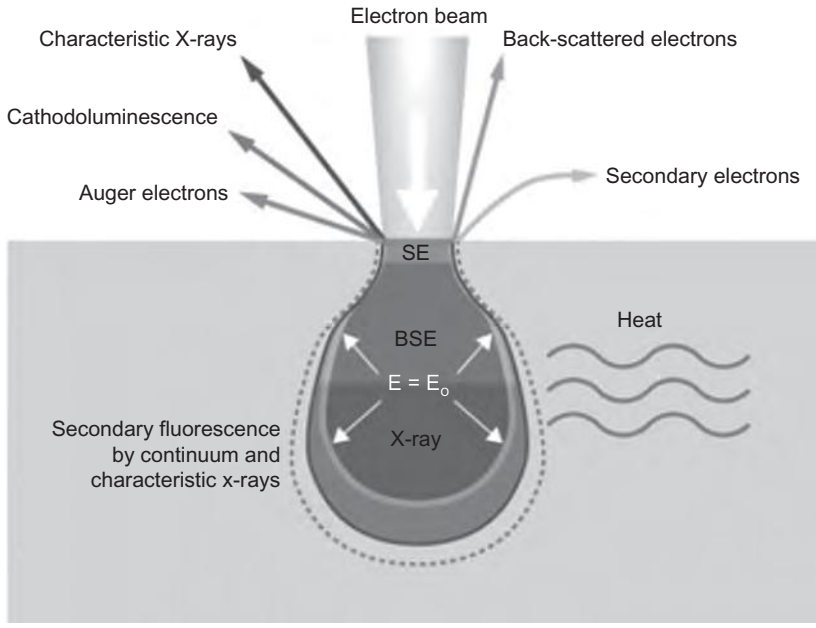


Figure 13.3 *Shows depth/lateral distribution of emitted electrons and other emissions (http://www.ammrf.org.au/myscope/pdfs/sem.pdf)*

(the primary radiation). This radiation can be in the form of ions, electrons or photons with sufficiently high energy (i.e. exceeding the ionisation potential). A SEM with secondary electron imaging (SEI) can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. Figure 13.4 shows energy distribution of emitted electrons when an electron beam (primary) strikes the sample surface.

Back-scattered electrons (BSE): They are beam electrons that are reflected from the sample by elastic scattering. BSE are often used in analytical SEM along with the spectra made from the characteristic

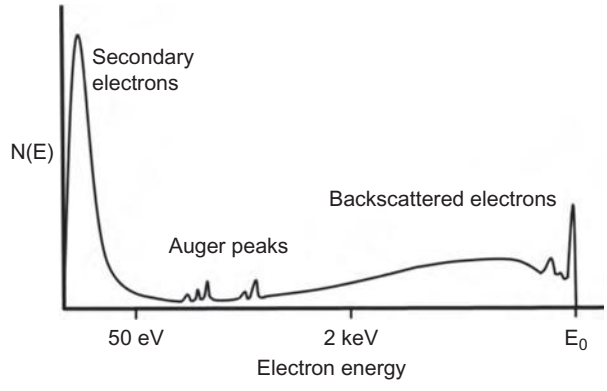


Figure 13.4 *Energy distribution of emitted electrons (Courtesy: Mabon and Sweich, 2012)*

X-rays. Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images can provide information about the distribution of different elements in the sample.

Characteristic X-rays: They are emitted when the electron beam removes an inner-shell electron from the sample, causing a higher energy electron to fill the shell and release energy. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample.

13.4 COMPONENTS OF SEM

Figure 13.5 shows the interconnection of various electronics sub-systems used in an SEM. If we take a look at the lower column and specimen chamber, we see the objective lens which focuses the electron beam on the specimen surface. A signal is generated from the specimen, acquired by the detector, and processed to produce an image or spectrum on the monitor display. The major components of a SEM are as follows:

- A source (*electron gun*) of the electron beam which is accelerated down the column.
- A series of *lenses (condenser and objective*) which act to control the diameter of the beam as well as to focus the beam on the specimen.
- A series of *apertures* (micron-scale holes in metal film) which the beam passes through and which affect properties of that beam.
- Controls for *specimen position* (*x, y, z* height) and orientation (tilt, rotation).
- An area of *beam/specimen interaction* that generates several types of signals that can be detected and processed to produce an image or spectra.
- All of the above maintained at high-vacuum levels.

A pair of deflector coils, controlled by the *Scan Generator*, is responsible for rastering that focus the electron beam across the specimen surface. The beam is rastered from left to right and top to bottom. There is a one-to-one correspondence between the rastering pattern on the specimen and the rastering pattern used to produce the image on the monitor. The resolution which is chosen to

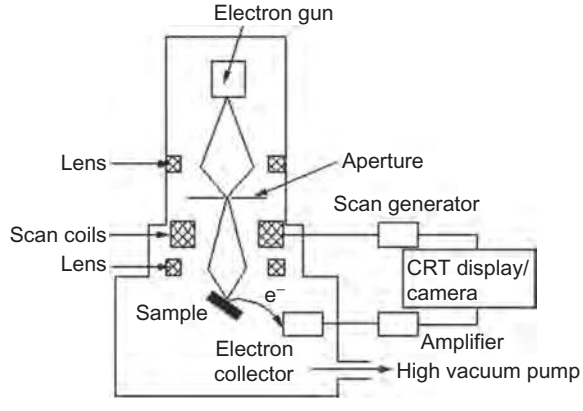


Figure 13.5 *Block diagram of scanning electron microscope (http://www.chm.bris.ac.uk/pt/diamond/stuthesis/chapter2.htm)*

image will obviously affect the number of pixels per row as well as the number of rows that constitute the scanned area.

The dot with in each pixel on the specimen represents an area of beam-specimen interaction from which the signal is derived. The signal is collected by the detector and subsequently processed to generate the image. That processing takes the intensity of the signal coming from a pixel on the specimen and converts it to a grey scale value of the corresponding monitor pixel. The monitor image is a two-dimensional rastered pattern of grey scale values.

With the beam focussed on the specimen surface, we all need to do to change magnification is to change the size of the rastered area on the specimen. The size of the monitor raster pattern is constant. Magnification will increase if we reduce the size of the area scanned on the specimen.

Magnification = area scanned on the monitor/area scanned on the specimen.

The following parameters control and define the major modes of imaging in the SEM:

- *Beam accelerating voltage (kV)*: The voltage with which the electrons are accelerated down the column.
- *Probe convergence angle*: The half-angle of the cone of electrons converging onto the specimen.
- *Probe current*: The current that impinges upon the specimen generates the various imaging signals
- *Probe diameter or spot size*: The diameter of the final beam at the surface of the specimen.

The detailed description of the various system blocks is given below.

13.4.1 Electron Beam Generator

The purpose of the electron gun is to provide a stable beam of electrons of adjustable energy. The following are the three main types of electron guns:

- Tungsten hairpin
- Lanthanhexaboride (LaB_6)
- Field emission gun (FEG)

The following are the two types of field emission guns (FEGs):

- Cold Emission
- Thermal-Field (Schottky) Emission

Figure 13.6 shows various types of electron beam generators. The schematic shows three filament types: (a) a tungsten, (W) wire, (b) a lanthanum hexaboride crystal assembly: LaB_6 and (c) a tungsten crystal (for FEGs). The tip of a tungsten wire hairpin filament is about 10 μm in diameter, whereas the tungsten crystal is sharpened to a much narrower tip.

13.4.1.1 Thermionic sources – electron gun

The electron beam is generated by the electron gun located at the top portion of the microscope column. The gun consists of a V-shaped tungsten filament. In the Figure 13.7, the filament (also called the emitter) is surrounded by the Wehnelt cylinder that closes over the filament assembly and has a small hole in the centre through which electrons exit. The electrode pins run to the filament through an insulator disc and carry the current flow to the filament. During operation, a high voltage is applied between the filament (–) and the anode (+), while an electric current is regulated

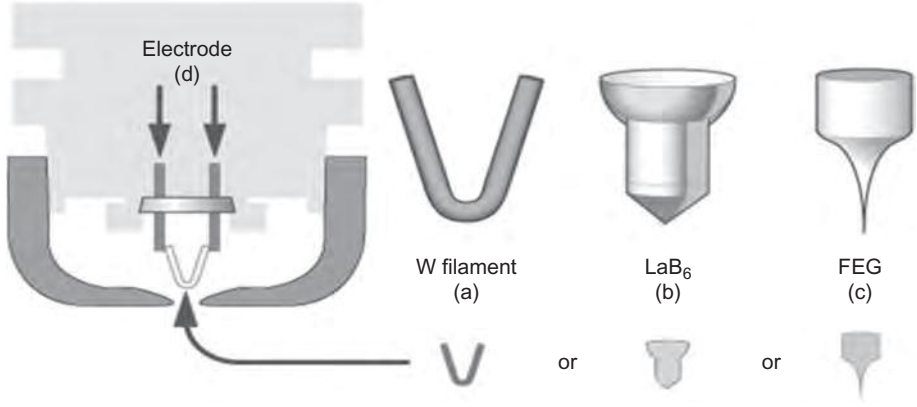


Figure 13.6 *Various types of Electron Guns (http://www.ammrf.org.au/myscope/pdfs/sem.pdf)*

through the filament causing it to emit electrons. These electrons are attracted by the + anode but are forced through the hole of the cathode shield. The negative charge around the hole forces electrons from the filament into a very narrow beam.

The electron beam, as seen below, is accelerated through a potential difference of voltage between the filament and the anode. The greater the voltage (V), the higher the speed of the electrons and the shorter the wavelength (l), as shown in below given formula:

$$\lambda = \sqrt{\frac{1.5}{V}} \text{ (nanometer)}$$

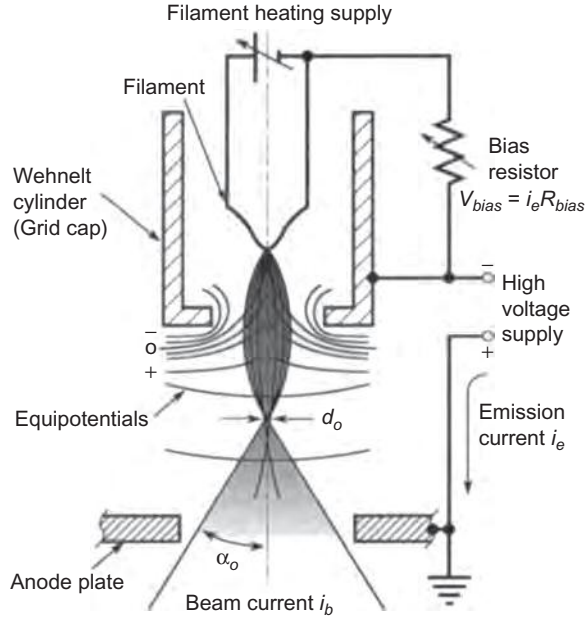


Figure 13.7 *Shows schematic of a generalised thermionic electron gun (Goldstein, 2003)*

The simplest and cheapest gun is the heated tungsten wire to produce electrons. The other expensive types make use of Lanthanum hexaboride gun which is made from a crystal of lanthanum hexaboride placed in a specialised housing. This material is a refractory ceramic material with a high melting point and is heated to generate electrons. It has the advantage of a longer usable lifetime than the thermionic tungsten filament.

13.4.1.2 Electron sources -cold field emission

Figure 13.8 illustrates the working principle of cold FEG. It uses a pointed single crystal tungsten wire filament that is not heated by a filament current. Instead, electrons are pulled off the cold filament by a strong electrostatic field called an extraction voltage. FEGs provide significant

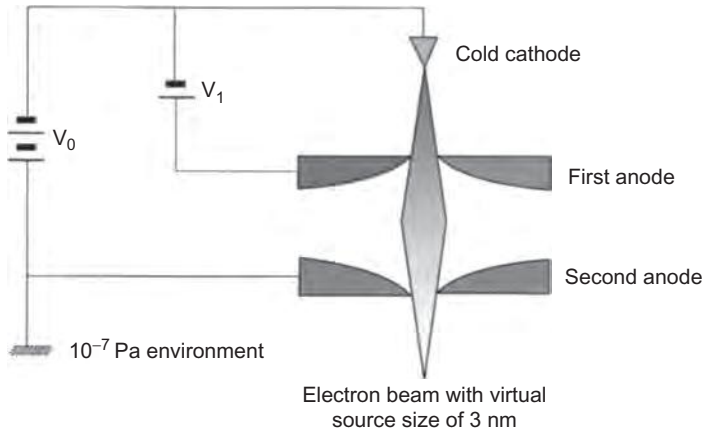


Figure 13.8 *Field emission gun–cold field emission (Courtesy: M/s Hitachi)*

advantages over thermionic filaments including a much smaller electron virtual source size, high current, high brightness (100×), low-energy spread and a long life. These advantages make the FEG SEM a high resolution machine for high magnification work.

However, while the cold FEG provides the most coherent source for high-resolution SEI, it is the least appropriate for energy dispersive X-ray analysis.

The major advantages of the cold FEG are that it is the highest brightness SEM source available and has a very long potential source lifetime – many years. The disadvantages are that is has poor short and long term probe current stability and requires ultra-high vacuum in gun area.

13.4.1.3 Electron sources – thermal-field (Schottky) emission

A Schottky field emission (hot-field emission) gun has some advantages compared to cold field emitters. The major advantages are better beam current stability, less stringent vacuum requirements and the fact that there is no need for periodic emitter flashing (heating the cold filament for a short time each day) to restore the emission current. The principle of operation of the thermal-field (Schottky) emission is shown in Figure 13.9.

Increasingly, the majority of high-resolution FEG SEMs use Schottky emitters. One significant advantage of the Schottky emitter is the high beam current (>100 nA) that can be achieved with little decrease in spatial resolution.

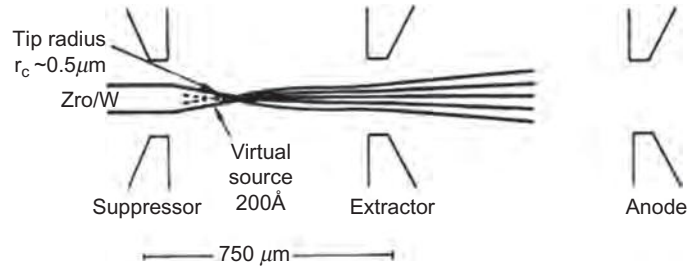


Figure 13.9 *Hot-field emisison- thermal-field (Schottky) emission (Courtesy: Mabon and Sweich, 2012)*

The ability to achieve a small probe diameter is directly related to the source size or the diameter of the electron beam exiting the gun. An electron beam emanating from a small source size is said to have high spatial coherency. Electron beams can also be characterised in terms of temporal coherency. Beam with high temporal coherency will have electrons of the same wavelength. In reality there is a certain 'Energy Spread' associated with the beam. As we will see, lower energy spreads result in be resolution and are particularly important in low accelerating voltage imaging.

Both types of field emission require that the tip remains free of contaminants and oxide and thus they require ultra-high vacuum conditions (10^{-10} to 10^{-11} Torr). The vacuum in the specimen chamber is in the 10^{-5} to 10^{-6} Torr range [1 Torr = 133 Pa = 1.33 mbar]. In the cold FEG, the electric field produced by the extraction voltage lowers the work function barrier and allows electrons to directly tunnel through it—thus facilitating emission. The cold FEGS must have their tip 'flashed' (briefly heated) periodically to free absorbed gas molecules. The thermally assisted FEG (Schottky field emitter) uses heat and chemistry (nitride coating) in addition to voltage to overcome the potential barrier level.

Although electron beam diameter is the determining factor of resolution in the ideal case, in practice there are other factors also, such as sample preparation and surface roughness, atomic mass and chemical composition of the sample, beam intensity, accelerating voltage, scanning speed, working distance, aberrations and hysteresis in the electron optics, and interaction volume of the electron beam with the sample. However, the electron beam diameter is the dominant factor.

In a microscope using a routine voltage of 50 kV, the wavelength will be approximately 0.0054 nm (1/100,000 wavelength of visible light). This value when applied to the resolution formula will yield a resolution limit of about 0.84 nm or 8.4 Angstroms.

13.4.2 Electron Lenses

A series of electromagnetic lenses and apertures are used to reduce the diameter of the source of electrons and to place a small, focused beam of electrons or spot onto the specimen.

The purpose of a lens is to change the path of the electron beam in a desired direction. Since electrons are charged particles and they can be bent in a magnetic field, lenses for electrons are constructed with ferromagnetic materials and windings of copper wire. A variable electric current through the lens will produce a magnetic field of variable strengths which will deflect or bend the electron beam passing through it. These produce a focal length which can be changed by varying the current through the coil. They are called electromagnetic lenses. The magnetic field bends electron paths in a similar way that solid glass lenses bend light rays. Under the influence of a magnetic field, electrons assume a helical path, spiralling down the column. This helical path can easily be demonstrated at low magnification by changing the focus up and down to cause image rotation.

There are two lens sets. The condenser lens is at the top and the objective lens at the bottom. Each does a specific and a different job. The condenser lens converges the cone of the electron beam to a spot below it, before the cone flares out again and is converged back again by the objective lens and down onto the sample. The condenser lens current controls the initial spot size and is referred to as the spot size control.

The objective lens also has some influence over the diameter of the spot size of the electron beam on the specimen surface. But its main role is in focusing the beam onto the sample. It may be noted that a focused beam produces a smaller spot on the surface than an under or over-focused beam.

In construction, an electromagnetic lens is a coil of wire through which current flows as shown in Figure 13.10. Because the current flow produces a magnetic field at right angles, the field pushes

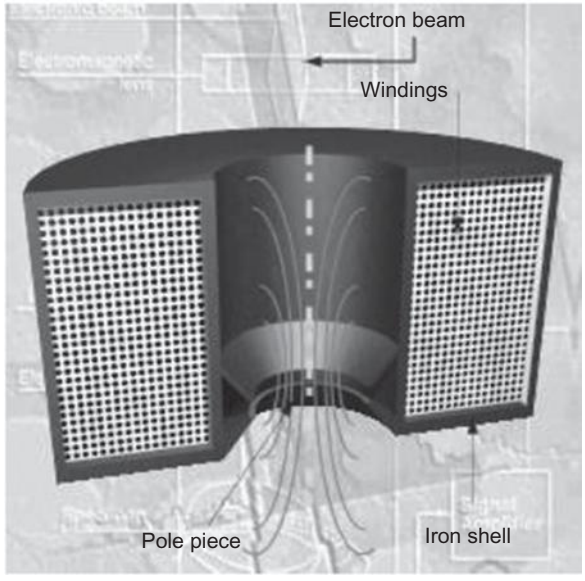


Figure 13.10 *Various components of electromagnetic lense (Courtesy: Mabon and Sweich, 2012)*

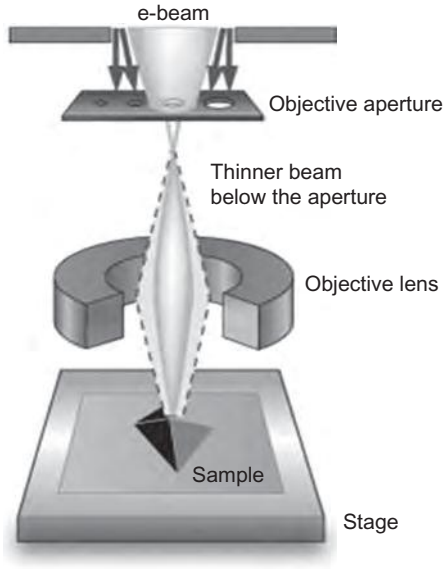


Figure 13.11 *Position of condenser aperture (http://www.ammrf.org.au/myscope/pdfs/sem.pdf)*

inwards into the hole in the centre. This acts to shape a beam of electrons travelling in their natural spiral path down the central hole.

When an electron passes through an electromagnetic lens it is subjected to two vector forces at any particular moment: a force parallel to the core (Z axis) of the lens; and a force parallel to the radius of the lens. These two forces are responsible for two different actions on the electrons, spiralling and focusing, as they pass through the lens. An electron passing through the lens parallel to the Z axis will experience the force causing it to spiral through the lens. This spiralling causes the electron to experience the force which causes the beam to be compressed towards the Z axis. The magnetic field is inhomogeneous in such a way that it is weak in the centre of the gap and becomes stronger close to the bore. Electrons close to the centre are less strongly deflected than those passing the lens far from the axis.

13.4.3 Focus and Alignment:

An important aspect of aligning the microscope is ensuring that the apertures are centred with respect to the beam and thus the optical axis of the microscope. If an objective aperture is not centred the image will move when you try to focus it. The way to correct this is to wobble the current to the objective lens and align the aperture to minimise movement in both the X and Y plane.

The objective aperture arm fits above the objective lens in the SEM. This is illustrated in Figure 13.11. It is a metal rod that holds a thin plate of metal containing four holes. Over this fits a much thinner rectangle of metal with holes (apertures) of different sizes. By moving the arm in and out different-sized holes can be put into the beam path.

The aperture stops electrons that are off-axis or off-energy from progressing down the column. It can also narrow the beam below the aperture, depending on the size of the hole selected.

13.4.4 Accelerating Voltage

The accelerating voltage in the SEM is kept variable from < 1 to 30 kV. The increasing accelerating voltage will

- decrease lens aberrations. The result is a smaller probe and thus better resolution.
- increase the probe current at the specimen. A minimum probe current is necessary to obtain an image with good contrast and a high signal to noise ratio.
- potentially increases charge-up and damage in specimens that are non-conductive.
- increase beam penetration into specimen and thus obscure surface detail.

In theory, an increase in accelerating voltage will result in a higher signal and lower noise in the final image (micrograph). Also, with a higher accelerating voltage, the electron beam penetration is greater and the interaction volume is larger. Therefore, the spatial resolution of micrographs created from those signals will be reduced. This is illustrated in Figure 13.12. So there will be a brighter image because the number of BSEs will increase but the resolution will be reduced. For SEI at typical voltages (say 15 keV), BSEs can enter the SE detector and degrade resolution because they come from deeper in the sample.

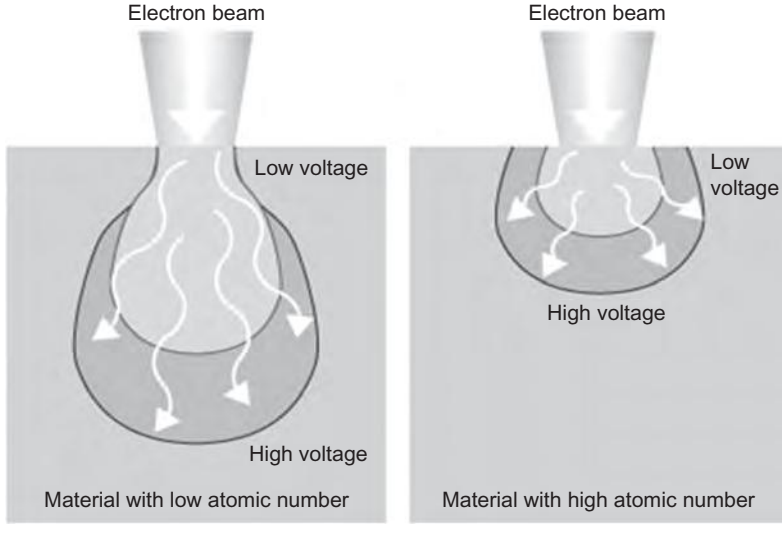


Figure 13.12 *Effect of accelerating voltage on resolution (http://www.ammrf.org.au/myscope/pdfs/sem.pdf)*

13.4.5 Detectors

13.4.5.1 Everhart-Thornley (ET) detector

The most common SE detector is the Everhart-Thornley (ET) detector shown in Figure 13.13. A Faraday cage over the detector can be variably biased from −50 to +250 V. A small negative bias will exclude low-energy SEs but will receive high-energy BSEs whose path is line of sight with the detector. A positive bias to the Faraday cage will allow the detector to attract the entire range of secondary signals as well as the line of sight BSE signal. A positive potential (10–12 kV) is applied

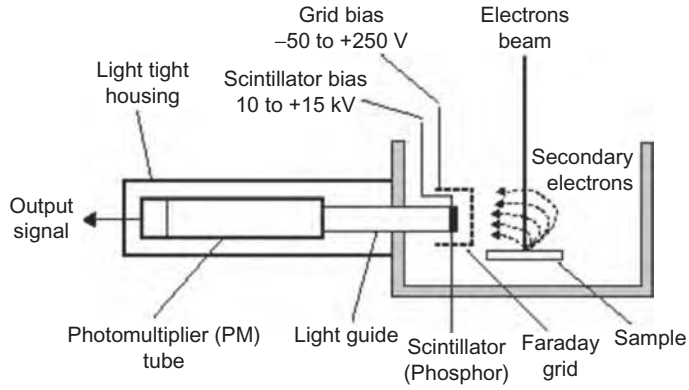


Figure 13.13 *Shows construction of Everhart-Thornley SE detector (Hafner, 2007)*

to the face of the phosphor coated scintillator to ensure that the electrons accepted through Faraday cage will be accelerated sufficiently to generate photons. The photons are conducted by a light guide to the photomultiplier where they are converted back to electrons and amplified up to a million-fold.

13.4.5.2 Through the lens detector

The SEMs usually have an additional detector, called 'through the lens' detector illustrated in Figure 13.14. In this detector, the magnetic field of the objective lens projects into the sample chamber and high-resolution SE are trapped by this field and follow spiral trajectories around the lines of magnetic flux up through the lens.

Shorter working distances are required when using these detectors. The image provided by the upper detector shows sharp edges and clearly defined details at high resolution/magnification.

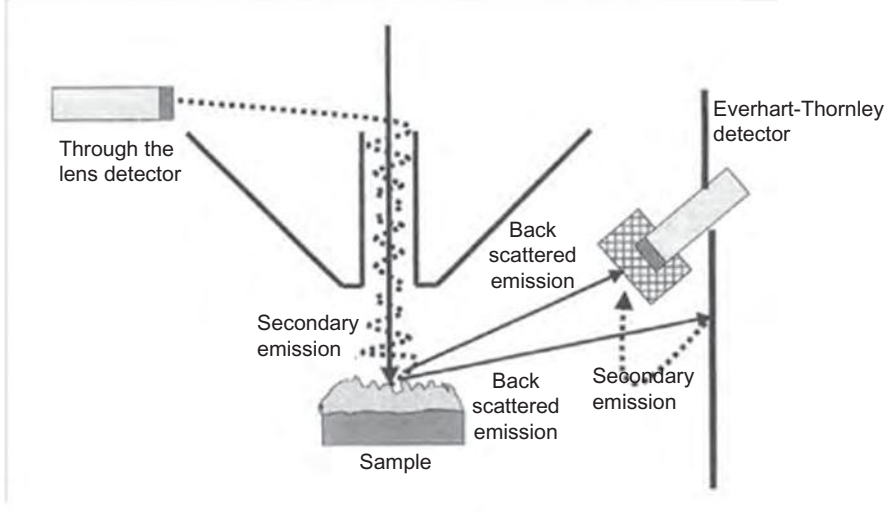


Figure 13.14 *Through the lens detector (Hafner, 2007)*

However, there is less topography observed using the upper detector. Specimens prone to charging may be more difficult to image with the upper detector given the reduced BSE signal. It is also possible to image with a mix of lower and upper detectors to achieve the desired effect.

Figure 13.15 *Shows Typical 4 quadrant solid state BSE detector (Courtesy: M/s Oxford Instruments Microanalysis Group: The Oxford Guide to X-Ray Microanalysis)*

13.4.5.3 Backscattered electron detector

Typically, BS detectors are solid state and comprise of a piece of silicon wafer. The detector is placed annularly to the bottom of the objective lens. The scheme of BSE detector is shown in Figure 13.15.

The incident beam passes through a hole in the detector before striking the sample. The silicon diode is divided into sectors or quadrants that can be summed or subtracted depending on the nature of image required. The normal BSE signal is referred to as *compositional* and provides information about the average atomic number of the sample. A *topographic* mode is also available which provides surface landscape information and includes no compositional contrast.

13.4.6 Display

Secondary electron (SE) images: For routineSEM images, SEs form the usual image of the surface. Secondary **electrons are low-energy electrons formed by inelastic scattering** and have energy of less than 50 eV. The low energy of these electrons allows them to be collected easily.

The major influence on SE signal-generation is the shape (topography) of the specimen surface. SEs provide particularly good edge detail. Edges look brighter than the rest of the image because they produce more electrons.

Because SEs have very low energies, only those produced at the surface of the sample are able to escape and be collected by the SE detector. Electrons emitted from a surface that faces away from the detector or which is blocked by the topography of the specimen, will appear darker than surfaces that face towards the detector. This topographical contrast due to the position of the SE detector is a major factor in the 'life-like' appearances of SE images.

Backscattered electron (BSE) images: BSEs are high-energy electrons (>50 eV) from the primary incident beam that are ejected back out from the sample. These BSE are used to produce a different kind of image. Such an image uses contrast to tell us about the average atomic number of the sample. The higher the average atomic number, the more primary electrons are scattered (bounced) back out of the sample. This leads to a brighter image for such materials.

The greater energy of BSE, compared with SE, means that BSE produced from deeper within the interaction volume are able to escape from the sample and be collected by the BSE detector, so BSE images have lower spatial resolution than SE images. In other words, the BSE can travel further in the sample before coming out again and so the information they carry is less restricted to the surface detail.

13.4.7 The Vacuum System

It is important to remember that the electron beam must be generated in and traverse through the microscope column under a high-vacuum condition. The presence of air molecules will result in the collision and scattering of the electrons from their path. In the electron microscope the vacuum is maintained by a series of highly efficient vacuum pumps.

A vacuum is obtain by removing as many gas molecules as possible from the column. The higher the vacuum the fewer molecules present. Atmospheric pressure at sea level is equal to 760 millimetres of mercury. A pressure of 1 millimetre of mercury is called a Torr. The vacuum of the SEM needs to be below 10^{-4} Torr to operate, although most microscopes operate at 10^{-6} Torr or greater vacuum. The higher the vacuum (the lower the pressure), the better the microscope will function. To pump from atmospheric pressure down to 10^{-6} Torr, the two classes of pumps that are used are a low-vacuum pump (atmosphere down to 10^{-3}) and a high-vacuum pump (10^{-3} down to 10^{-6} or greater depending on type of pump). There will be one or more of each class on a SEM.

Low-vacuum pumps used in a SEM are known as mechanical pumps or rotary pumps. This type of pump removes air by the mechanical rotation of an eccentric cam or rotor that is driven by an electric motor. These pumps will back stream, a process resulting in the condensation of oil out of the vacuum side of the pump.

Mechanical pumps are very efficient pumps while pumping at pressures near atmospheric. However, their efficiency decreases rapidly as they approach 10^{-3} Torr. Thus, to achieve the operating pressure of a SEM (10^{-4} Torr to 10^{-6} Torr) another type of pump must be used besides the mechanical pump. These types of pumps are classified as high-vacuum pumps.

Oil diffusion pumps represent one type of commonly used high-vacuum pump. The arrangement of the vacuum system is shown in Figure 13.16. This pump works by heating oil until it

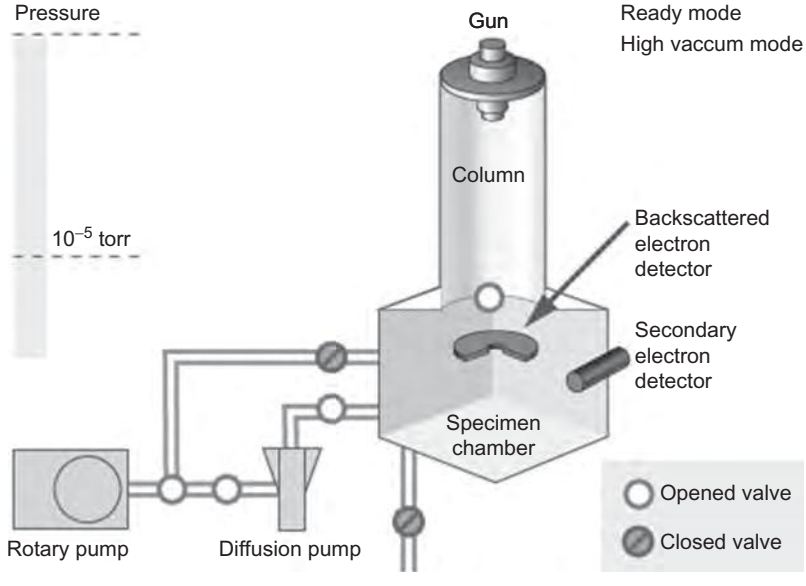


Figure 13.16 *Illustrates the vacuum system arrangement (www.ammrf.org.au/myscope/sem/practice/principles)*

vaporises. As the oil cools and condenses, the oil traps air lowering the pressure. Because there are no moving parts, a diffusion pump is a reliable as well as an inexpensive pump. Without proper cooling, hot oil will condense outside the pump contaminating the sample and the microscope, hence back streaming occurs.

Turbomolecular pumps (turbo pumps) are high precision units that simply blow the air out by means of a series of turbine fan blades revolving at speeds greater than 10,000 RPM. Turbo pumps are expensive pumps, since great precision is needed in manufacturing fan blades for high speeds and the stress associated with the speeds. Turbo pumps are known for their audio noise. A high pitched frequency is produced when the fan blades are at operating speed. Another disadvantage of the turbo pump is its moving parts and thus a possibility of vibrations may be introduced in the microscope. One major advantage is that turbo pumps do not back stream and therefore they are considered clean pumps.

Ion-Getter pumps (ion pumps) ionise air molecules in a high voltage field and absorb positively charged ions onto the negative cathode. Ion pumps are expensive but very clean as they pump by removing the contamination. These pumps are very slow in their pumping speed. It can take several hours to bring the vacuum down to operating pressures when changing samples.

Cryo pumps are the latest type of high-vacuum pumps. This type of pump lowers the pressure by cooling gases down to liquid nitrogen or liquid helium temperatures. Cryo pumps are expensive compared to other types of high-vacuum pumps and they are expensive to maintain due to the recurring nitrogen or helium charge. This type of pump is usually found on instruments with a freezing stage.

With two types of vacuum systems (high and low vacuum) and each not being able to handle the requirements of the vacuum system completely by itself, a valving system is used to change over between the two pumping systems whenever a change in vacuum is needed, e.g. changing samples. Accordingly, all modern microscopes have an automated valving system.

13.5 DIGITAL SEM

From its earliest inception, the SEM has been widely used as an imaging tool. It produces images by raster scanning an electron beam over a region of interest on a sample. Early SEM images were analog images which were preserved on 4 × 5 Polaroid or Kodak film.

For the analog SEM, the images were recorded on film, so the exposure and grain size of the film determined the smallest features which could be imaged. For the digital SEM pixel resolution determines the smallest features which could be imaged. Each pixel in a digital image contains just one element of information, i.e. a grey level from 0 (black) to 255 (white). The smallest feature which can be resolved is therefore linked to the pixel size.

The proliferation of high speed digital electronics has revolutionised SEM, whereby everything from digital scan control to digital data acquisition, to archival of digital images is not only common but expected by default on modern SEMs. Since this digital revolution, digital images are displayed on everything from desktop computer monitors to large projection screens and printed at various pixel densities on a wide variety of paper sizes.

With digital SEMs, like the Agilent 8500 FE-SEM, most of the signals are handled digitally with analog to digital converters (ADC), digital to analog converters (DAC) and field programmable gate arrays (FPGA) in conjunction with electrostatic optics. With this arrangement the scan

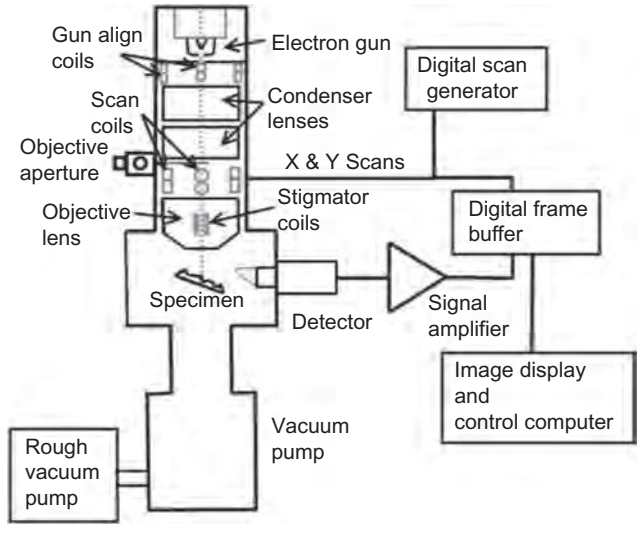


Figure 13.17 *Digital SEM (adapted from: Mabon and Sweich, 2012)*

waveform is generated digitally, that is, pixel by pixel in incremental steps, and the image is collected and displayed digitally in the same pixel by pixel fashion. A block diagram of the digital SEM is shown in Figure 13.17.

An advantage with digital image data is the intensity data that can easily be normalised. For example, with the typical 8-bit grey scale SEM image, the darkest pixel is set to 0 and the brightest is set to 255. Normalisation is done by auto brightness and contrast control and it allows for convenient storage and display of the data on the computer monitor without the need to worry about film speed as in analog SEM images. There are many sophisticated software programs for manipulating and analysing digital images. With digital images, analyses like particle size distribution, average fibre length, or area ratio of one phase to another in a multi-component system can be done easily.

Figure 13.18 *Scanning Electron Microscope Model 8500 FE-SEM (Courtesy: M/s Agilent)*

The new Agilent 8500 FE-SEM (Figure 13.18) offers a field emission SEM which is compact and innovative system optimised for low-voltage imaging, extremely high surface contrast, and resolution typically found only in much larger and more expensive field emission microscopes.

The system's continuously variable imaging voltage is tuneable from 500 to 2,000 volts as an operational parameter, rather than a setup choice. The system eliminates charging of non-conductive samples without the need to coat the samples, which can mask nanoscale features, or resort to increased pressure operation, which can degrade resolution.

In addition, the 8500 utilises a four-segment micro-channel plate (MCP) detector that provides topographic imaging along two orthogonal directions to enhance surface detail. This technique has been demonstrated to resolve sub-nano-metre atomic steps on the surface of crystalline substances.

The core technology inside a SEM is the electron beam column, which extracts, collimates, shapes, scans and focuses the electron beam. A conventional electron beam column relies on combination of precision machined electromagnetic and electrostatic elements to control the electron beam. The coils that form the critical elements are typically hand-wrapped by technicians to achieve uniform electromagnetic fields Moreover, closed-loop cooling and sophisticated vibration isolation are often required at the system level to manage the high currents in the lenses and other elements. The resultant high-resolution electron beam column is both large and expensive.

Silicon-based micro-fabrication techniques enabled Agilent Technologies to design and fabricate a miniature electrostatic electron beam column combined with a thermal-field emission electron source. The 8500 FE-SEM design utilises stacks of silicon on insulator to form all of the lenses, apertures and deflectors in the electron beam column. Patented technology allows these electron beam columns to be built wafer scale on 150 mm substrates. The columns are fabricated with the precise aperture diameters and repeatable alignment tolerances required to minimise aberrations that can degrade image quality.

The system's thermal-field emission electron source, meanwhile, provides high brightness, high stability, small virtual source size, low-energy spread and long-lasting consistent performance. A quad-segmented MCP detector is located just below the objective lens of the electron beam column directly and above the sample. This detector collects both backscattered and SEs. The MCP may be operated either in a standard mode, where all the channels are added together, or in a differential mode (topographic mode), in which opposite sides of the detector are dynamically subtracted.

For the Agilent 8500 FE-SEM the electron beam is nominally 10 nm and the pixel size on a standard computer monitor is 200 μm with 1920 × 1080 pixels yielding a 3μm scan size for a 1024 × 1024 image. So in terms of magnification, a 512 × 512 pixel image of a 3 μm scan size would be 32,500× instrument magnification, the corresponding magnification for a 1024 × 1024 pixel image would be 65,000×, and the corresponding magnification for a 2048 × 2048 pixel image would be 1,30,000×. If the 3 μm scan size is used to describe the digital image, it does not matter how the image is viewed or printed dimensionally; it represents the 3 × 3 μm area of the sample surface that was scanned by the electron beam (Agilent Technologies, 2011).

13.6 SCANNING TRANSMISSION ELECTRON MICROSCOPY (STEM)

The scanning transmission electron microscope makes use of a beam of high-energy electrons (typically 100–400 keV) which is collimated by magnetic lenses and allowed to pass through a specimen under high vacuum. The transmitted beam and a number of diffracted beams can form a resultant diffraction pattern, which is imaged on a fluorescent screen kept below the specimen. This is shown in Figure 13.19. The diffraction pattern gives the information regarding lattice spacing and symmetry of the structure under consideration. Alternatively, either the transmitted beam or one of the diffracted beams can be made to form a magnified image of the sample on the viewing screen as bright and dark field imaging modes respectively, which give information about the size

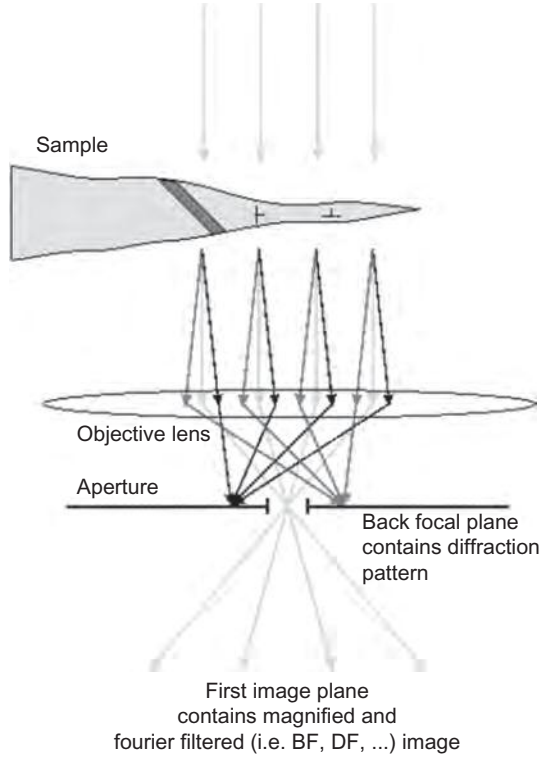


Figure 13.19 *Principle of scanning transmission microscope*

and shape of the micro-structural constituents of the material. High-resolution image, that contains information about the atomic structure of the material, can be obtained by recombining the transmitted beam and diffracted beams together.

The instrument comprises of a tungsten filament or LaB6 or an FEG as source of electron beam, objective lens, imaging lens, CCD camera, monitor, etc.

The beam of electrons scans the specimen, as it does in scanning electron microscopy. However, it is the transmitted electrons that are collected and amplified and form an image on a cathode ray tube. The small spot size of the beam allows different areas of the specimen to be discriminated and analysed.

14

SCANNING PROBE MICROSCOPES

14.1 SCANNING PROBE MICROSCOPY

Scanning probe microscopes (SPMs) are a family of instruments used for studying surface properties of materials from the atomic to the micron level. All SPMs contain the components shown in Figure 14.1.

Virtually in all SPMs, a piezoelectric scanner is used as an extremely fine positioning stage to move the probe over the sample or the sample under the probe. The probe is typically called the tip.

Briefly, scanning probe microscopy involves the following steps:

- Sharp probe tip is scanned across surface under study.
- Piezoelectric materials provide atomic-scale control of x, y, z motion of the tip.

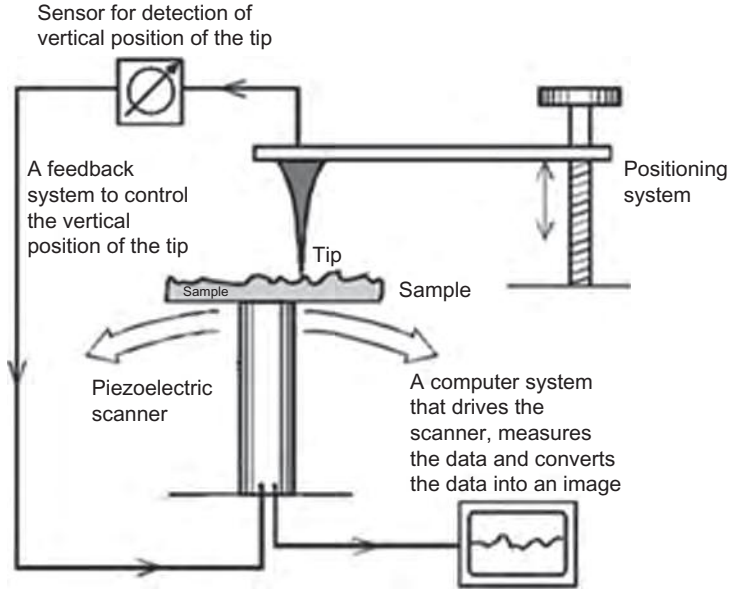


Figure 14.1 *Schematic of a generalised scanning probe microscope (Adapted from: Howland and Benatar, 2006)*

- Detect signal that reflects topography or other spatially varying property of interest.
- Good vibration isolation required.

Basically, the SPM is an imaging tool with a vast dynamic range, spanning the realms of optical and electron microscopes. It is also a profiler with a very high degree of three-dimensional resolution. In certain cases, SPMs can measure physical properties such as surface conductivity, static charge distribution, localised friction, magnetic fields and elastic moduli of the material under study. As a result, applications of SPMs are very diverse. In summary, the development of the various scanning probe microscopy techniques has revolutionised the study of surface structure – atomic resolution images have been obtained not only on single-crystal substrates in UHV but also on samples at atmospheric pressure and even under solution

The common unit of dimension used for making measurements at atomic scale is the nano-metre. A nano-metre is one billionth of a metre:

$$1 \text{ m} = 1{,}000{,}000{,}000 \text{ nm}$$
$$1 \text{ μm} = 1{,}000 \text{ nm}$$

Another common unit of measure is the Angstrom. There are ten angstroms (Å) in 1 nm:

$$1 \text{ nm} = 10 \text{ Å}$$

The scanning tunnelling microscope (STM) is the ancestor of all SPMs. It was invented in 1981 by Gerd Binnig and Heinrich Rohrer at IBM Zurich. Five years later they were awarded the Nobel prize in physics for its invention. The STM was the first instrument to generate real-space images of surfaces with atomic resolution. This invention was quickly followed by the development of a whole family of related techniques which, together with STM, are classified in the general category of Scanning Probe Microscopy (SPM) techniques. Of these later techniques, the most important is atomic force microscopy (AFM).

14.2 SCANNING TUNNELLING MICROSCOPE (STM)

14.2.1 Principle of STM

STM can image surfaces of conducting materials with atomic-scale resolution. It uses an atomically sharp metal tip that is brought very close to the surface. When the tip and sample are connected with a voltage source, a small tunnelling current flows between the tip and the conducting sample surface. This current can be measured, whose magnitude depends on the distance between the tip and the surface. As the tip is moved laterally across the surface, a feedback mechanism moves the tip up and down to maintain a constant tunnelling current. Rastering the tip across the surface therefore produces a topographic map of the surface. For tunnelling to take place, both the sample and the tip must be conductors or semiconductors. Unlike AFMs, which are discussed in the next section, STMs cannot image insulating materials (Brian and Tissue, 2004a).

Generally, a small bias voltage (mV to 3 V) is applied between an atomically sharp tip and the sample. If the distance between the tip and the sample is large, no current flows. However, when the tip is brought very close (10 Å) without physical contact, a current (pA to nA) flows across the gap between the tip and the sample.

Such current is called tunnelling current which is the result of the overlapping wave-functions between the tip atom and surface atom, electrons can tunnel across the vacuum barrier separating the tip and sample in the presence of small bias voltage.

The magnitude of tunnelling current is extremely sensitive to the gap distance between the tip and sample, the local density of electronic states of the sample and the local barrier height. The density of electronic states is the amount of electrons exit at specific energy. As we measure the current with the tip moving across the surface, atomic information of the surface can be mapped out.

The tunnelling current is an exponential function of distance; if the separation between the tip and the sample changes by 10% (on the order of 1 Å), the tunnelling current changes by an order of magnitude. This exponential dependence gives STMs their remarkable sensitivity. STMs can image the surface of the sample with sub-Angstrom precision vertically, and atomic resolution laterally.

The idea of tunnelling is applied to look at the surface profile of a material at the atomic scale. By moving the tip horizontally across the surface in close proximity (a few Å), it is found that the current varies exactly as the same pattern as the profile of the surface. In other words, the variation of current duplicates the profile of the surface and we can 'view' the surface simply by measuring the tunnelling current. The scanning tip of a STM is so 'sharp' that it only consists of a few atoms, and the tunnelling current is as small as a few nano-amperes. The lateral resolution is about 1 Å whereas a vertical resolution up to 0.01 Å can be achieved. The STM can be used in ultra-high vacuum, air or other environments.

14.2.2 Components of STM

Figure 14.2 shows its essential elements. A probe tip, usually made of W or Pt–Ir alloy, is attached to a piezodrive, which consists of three mutually perpendicular piezoelectric transducers: x piezo, y piezo, and z piezo. Upon applying a voltage, a piezoelectric transducer expands or contracts. By applying a sawtooth voltage on the x piezo and a voltage ramp on the y piezo, the tip scans on the xy plane. Using the coarse positioner and the z piezo, the tip and the sample are brought to within a fraction of a nano-metre each other. The electron wave-functions in the tip overlap electron wave-functions in the sample surface. A finite tunnelling conductance is generated. By applying a bias voltage between the tip and the sample, a tunnelling current is generated.

The components of a STM include scanning tip, piezoelectric controlled scanner, distance control and scanning unit, vibration isolation system, and computer.

- *Scanning tip*: Electrons tunnel from the scanning tip to the sample, creating the tunnelling current. Tips typically are made out of tungsten, platinum or a Pt-Ir wire. A sharp tip can be produced by: Cutting and grinding or Electrochemical etching
- *Piezoelectric controlled scanner*: Piezoelectric crystals expand and contract very slightly depending on the voltage applied to them and this principle is used to control the horizontal position x, y and the height z of the scanning tip.
- *Distance control and scanning unit*: Position control using piezoelectric means is extremely fine, so a coarse control is needed to position the tip close enough to the sample before the piezoelectric control can take over.
- *Vibration isolation system*: STM deals with extremely fine position measurements so the isolation of any vibrations is very important. The tip-sample distance must be kept constant within 0.01 Å to get good atomic resolution. Therefore, it is absolutely necessary to reduce

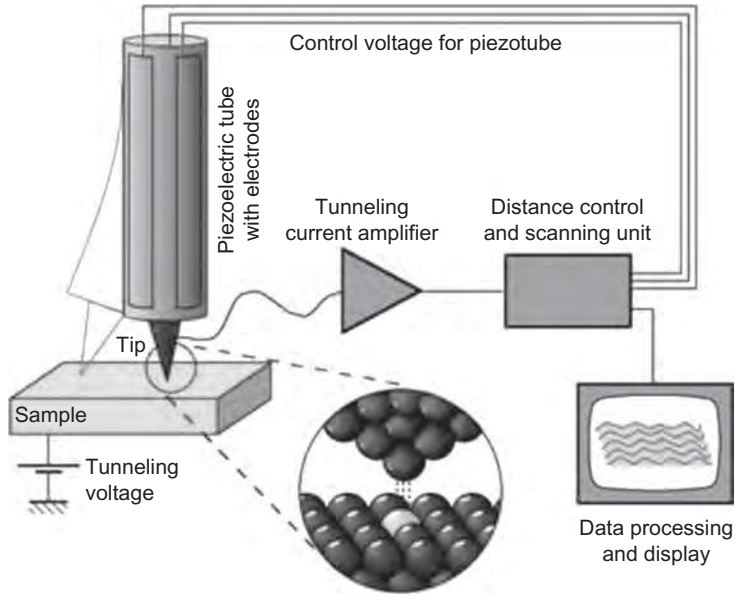


Figure 14.2 *Principle of scanning tunnelling microscope. The scanning waveforms, applying on the x and y piezos, make the tip raster scan on the sample surface. A bias voltage is applied between the sample and the tip to induce a tunnelling current. The z piezo is controlled by a feedback system to maintain the tunnelling current constant. The voltage on the z piezo represents the local height of the topography. To ensure stable operation, vibration isolation is essential (Mitchell, 2010)*

inner vibrations and to isolate the system from external vibrations. Environmental vibrations are caused due to the following reasons:

- Vibration of the building 15–20 Hz
- Running people 2–4 Hz
- Vacuum pumps
- Sound

Damping can be done by the following methods:

- Suspension with springs (including additional eddy current dampers)
- Stacked plate systems
- Pneumatic systems

- *Computer*: The computer records the tunnelling current and controls the voltage to the piezoelectric tubes to produce a three-dimensional map of the sample surface.

14.2.3 Requirements of Various Components

14.2.3.1 Operating parameters

- Voltage range ~ 0.1–2 V
- Current range ~ pA–nA
- Can operate in air, liquid or vacuum
- Sample and tip must be conducting

The requirements of various components are as follows:

- Controlling the tip-sample distance from a few mm down to 0.01 Å
- Exact lateral positioning
- Stabilised tip-sample distance
- Sharp tip
- Measuring a current in the range of 0.01—50 nA

The most widely used convention of the polarity of bias voltage is that the tip is virtually grounded. The bias voltage V is the sample voltage. If $V > 0$, the electrons are tunnelling from the occupied states of the tip into the empty states of the sample. If $V < 0$, the electrons are tunnelling from the occupied states of the sample into the empty states of the tip.

The tunnelling current is converted to a voltage by the current amplifier, which is then compared with a reference value. The difference is amplified to drive the z piezo. The phase of the amplifier is chosen to provide a negative feedback: if the absolute value of the tunnelling current is larger than the reference value, then the voltage applied to the z piezo tends to withdraw the tip from the sample surface, and vice versa. Therefore, an equilibrium z position is established. As the tip scans over the xy plane, a two-dimensional array of equilibrium z positions – representing a contour plot of the equal tunnelling-current surface – is obtained, displayed and stored in the computer memory.

The topography of the surface is displayed on a computer screen, typically as a grey-scale image. Usually, the bright spots represent high z values (protrusions), and the dark spots represent low z values (depressions).

The z values corresponding to the grey levels are indicated by a scale bar. The most convenient unit for x and y is nano-metre (nm, 10^{-9} m), and the most convenient unit for z is picometer (pm, 10^{-12} m).

To achieve atomic resolution, vibration isolation is essential. This is achieved by making the STM unit as rigid as possible, and by reducing the influence of environmental vibration to the STM unit.

14.2.3.2 Positioning

The large distance range the tip has to be controlled on, makes it necessary to use two positioners, namely a coarse positioner and a fine positioner. The fine positioner is also used as a scanner. Every fine positioner/scanner is made out of a piezocrystal or piezoceramic material.

Piezoelectric Effect: Applying stress on piezo materials causes electric polarisation.

Reverse Piezoelectric Effect: An electric field applied to a piezo material causes mechanical strain.

Piezocrystals: *Quartz, $BaTiO_3$ Piezoceramics: $Pb(Ti,Zr)O_3$ (PZT)*

14.2.4 Electronic Circuit

A typical electronic circuit used in STM is shown in Figure 14.3. When working with constant current mode, a feedback circuit is provided to control the z piezo.

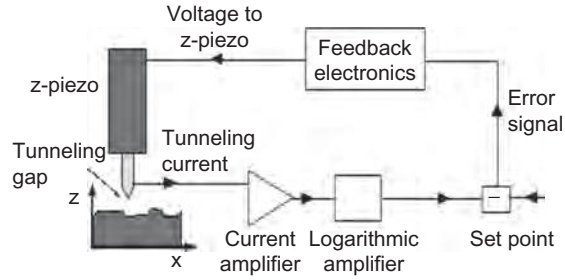


Figure 14.3 *Basic building blocks of electronic circuit of scanning tunnelling microscope (http://www2.fkf.mpg.de/ga/research/stmtutor/stmtech.html)*

The tunnelling current (0.01–50 mA) is converted into a voltage by a current amplifier. To get a linear response with respect to the tunnelling gap (the current is exponentially dependant on the tip-sample distance), the signal is processed by a logarithmic amplifier. The output of the logarithmic amplifier is compared with a pre-determined voltage which is used as a reference current. The error signal is passed to feedback electronics, which applies a voltage to the z piezo to keep the difference between the current set point and the tunnelling current small.

14.2.5 Modes of Operation

Imaging of the surface topology with STM can be carried out in one of the following two ways:

- Constant-height mode (in which the tunnelling current is monitored as the tip is scanned parallel to the surface)
- Constant-current mode (in which the tunnelling current is maintained constant as the tip is scanned across the surface)

14.2.5.1 Constant height mode

In this mode, the vertical position of the tip is not changed, which is equivalent to a slow or disabled feedback. The current as a function of lateral position represents the surface image. This mode is only appropriate for atomically flat surfaces as otherwise a tip crash would be inevitable. One of its advantages is that it can be used at high scanning frequencies (up to 10 kHz). In comparison, the scanning frequency in the constant current mode is about 1 image per second or even per several minutes.

If the tip is scanned at what is nominally a constant height above the surface, then there is actually a periodic variation in the separation distance between the tip and surface atoms as shown in Figure 14.4. At one point the tip will be directly above a surface atom and the tunnelling current will be large, while at other points the tip will be above hollow sites on the surface and the tunnelling current will be much smaller. The tunnelling current measured at each location on the sample surface constitutes the data set and the topographic image.

A plot of the tunnelling current v's tip position therefore shows a periodic variation which matches that of the surface structure - hence it provides a direct 'image' of the surface (and by the time the data has been processed, it may even look like a real picture of the surface!)

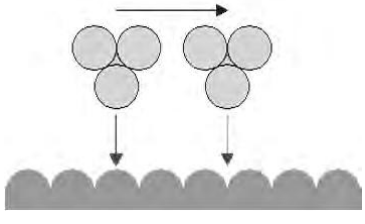


Figure 14.4 *Constant-height mode of STM (http://www.chem.qmul.ac.uk/surfaces/scc/scat76.htm)*

14.2.5.2 Constant current mode

In this mode of imaging, the surface is to maintain the tunnelling current constant while the tip is scanned across the surface. This is achieved by adjusting the tip's height above the surface so that the tunnelling current does not vary with the lateral tip position. In this mode, the tip will move slightly upwards as it passes over a surface atom, and conversely, slightly in towards the surface as it passes over a hollow. The concept is shown in Figure 14.5.

By using a feedback loop, the tip is vertically adjusted in such a way that the current always stays constant. As the current is proportional to the local density of states, the tip follows a contour of a constant density of states during scanning. A kind of a topographic image of the surface is generated by recording the vertical position of the tip.

Each mode has advantages and disadvantages. Constant-height mode is faster because the system doesn't have to move the scanner up and down, but it provides useful information only for relatively smooth surfaces. Constant-current mode can measure irregular surfaces with high precision, but the measurement takes more time.

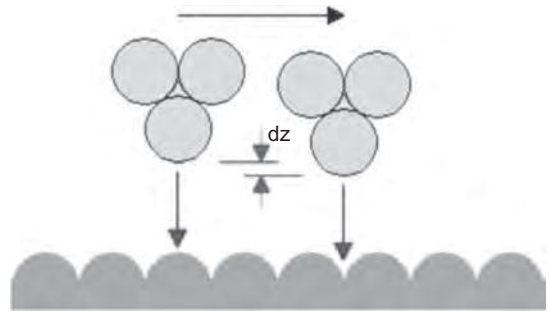


Figure 14.5 *Constant current mode of STM (http://www.chem.qmul.ac.uk/surfaces/scc/scat76.htm)*

14.2.6 Applications of Scanning Tunnelling Microscopy

STM has revolutionised the study of solid surfaces, and enabled for the first time tracking images and performing spectroscopy of such systems with atomic resolution. STM is the only device that allows scientists to study both topographical and electrical properties of materials, which are important for understanding the behaviour of microelectronic devices. STM is also the only instrument with the ability to rip atoms from the sample surface and relocate or otherwise manipulate them.

A key shortcoming of scanning probe procedures is the slow, serial method by which they operate. This characteristic has limited their use mainly to laboratory applications involving atom-at-a-time manoeuvres. Also, the STM is limited to operation with only the conducting surfaces. The surfaces of insulators, structures in liquids and biological samples can be imaged non-destructively with high resolution by the atomic force microscope (AFM).

14.3 ATOMIC FORCE MICROSCOPE

14.3.1 What Is Atomic Force Microscopy?

AFM is similar to STM in that it can image surfaces under study at atomic-scale resolution (Brian and Tissue, 2004b). The difference between AFM and STM is that AFM does not require that the sample be an electrically conducting material.

Figure 14.6 shows the principle of working of the AFM. Like STM it uses an atomically sharp tip that is brought very close to the surface. The tip will experience a chemical attraction or repulsion and will move up or down on its supporting cantilever. For example, when the cantilever tip is brought in contact with the surface to be imaged, an ionic repulsive force from the surface applied to the tip bends the cantilever upwards. The amount of bending, measured by a laser beam reflected on to a split photodetector, can be used to calculate the force. By keeping the force constant while scanning the tip across the surface, the vertical movement of the tip follows the surface profile and is recorded as the surface topography by the AFM.

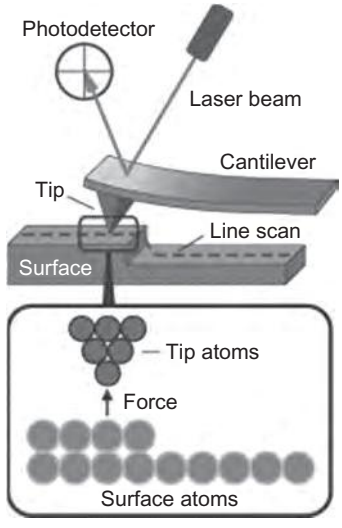


Figure 14.6 *Principle of operation of an AFM (Courtesy: M/s Keysight Technologies)*

The key to the sensitivity of AFM is in monitoring the movement of the tip. A common method of monitoring the tip movement is to use a laser beam that is reflected or diffracted by the tip or cantilever. Up or down movement of the tip is then detected by changes in the laser beam position. As in STM, rastering the tip across the surface produces a topographic map of the surface with atomic resolution.

For the techniques to provide information on the surface structure at the atomic level, the following steps have to be taken:

1. The position of the tip with respect to the surface must be very accurately controlled (to within about 0.1 Å) by moving either the surface or the tip.
2. The tip must be very sharp – ideally terminating in just a single atom at its closest point of approach to the surface.

In the modern AFM instruments, it is possible to accurately control the relative positions of the tip and the surface by ensuring good vibrational isolation of the microscope and using sensitive piezoelectric positioning devices.

The theory and operation of an AFM is similar to a stylus profiler. The primary difference is that in the AFM, the probe forces on the surface are much smaller than those in a stylus profiler. Because the forces in an AFM are much smaller, smaller probes are used, and the resolution is much higher than can be achieved with a stylus profiler.

In an AFM a constant force is maintained between the probe and sample while the probe is raster scanned across the surface. By monitoring the motion of the probe as it is scanned across the surface, a three-dimensional image of the surface is constructed. The constant force is maintained by measuring the force with the 'light lever' sensor and using a feedback control electronic circuit to control the position of the z piezoelectric ceramic.

14.3.2 Components of AFM

The main components of an AFM are shown in Figure 14.7.

Coarse Z motion translator (Z): This translator moves the AFM head towards the surface so that the force sensor can measure the force between the probe and sample. The motion of the translator is usually about 10 mm.

Coarse X-Y translation stage (T): The X-Y translation stage is used to place the section of the sample that is being imaged by the AFM directly under the probe.

X and Y piezoelectric transducer (X-P): With the X-P, the Y-P probe is moved over the surface in a raster motion when an AFM image is measured.

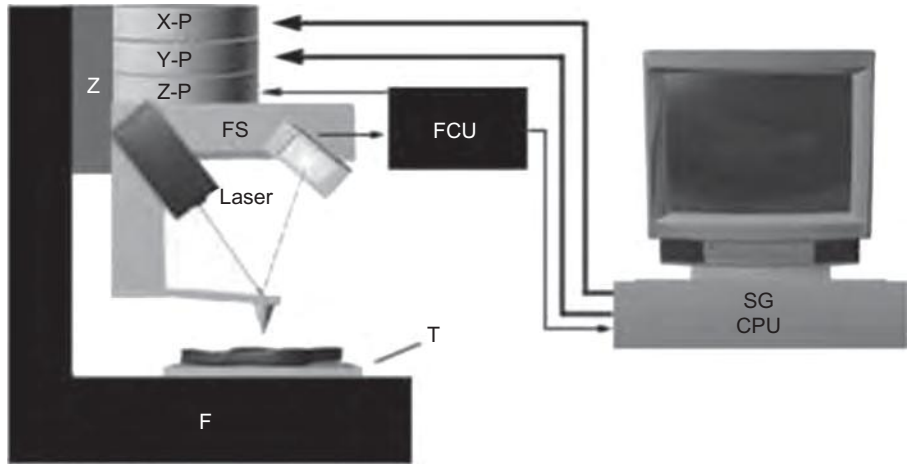


Figure 14.7 *Shows components and sub-systems of an atomic force microscope (Courtesy: M/s Pacific Nanotechnology, 2006)*

Force sensor (FS): The FS measures the force between the probe and the sample by monitoring the deflection of a cantilever.

Z piezoelectric ceramic (ZP): Moves the FS in the vertical direction to the surface as the probe is scanned with the X-Ps.

Feedback control unit (FCU): The FCU takes in the signal from the light lever FS and outputs the voltage that drives the ZP. This voltage refers to the voltage that is required to maintain a constant deflection of the cantilever while scanning.

X-Y signal generator (SG): The motion of the probe in the X-Y plane is controlled by the SG. A raster motion is used when an image is measured.

Computer (CPU): The computer is used for setting the scanning parameters such as scan size, scan speed, feedback control response and visualising images captured with the microscope.

Frame (F): A solid frame supports the entire AFM microscope. The frame must be very rigid so that it does not allow vibrations between the tip and the surface.

The functional parts of the AFM are illustrated in Figure 14.8

The Probe: AFM works by scanning a very sharp (end radius 10 nm) probe along the sample surface, carefully maintaining the force between the probe and surface at a set, low level. Usually, the probe is formed by a silicon or silicon nitride cantilever with a sharp integrated tip. The tip can be conical, tetrahedral or pyramidal, with the conical tips having diameters of 5 nm and the latter tips having diameters between 10 and 50 nm. Conical tips are generally sharper and so are better suited to feeling out steep features, but are also more liable to break than the other tip geometries which are used more frequently.

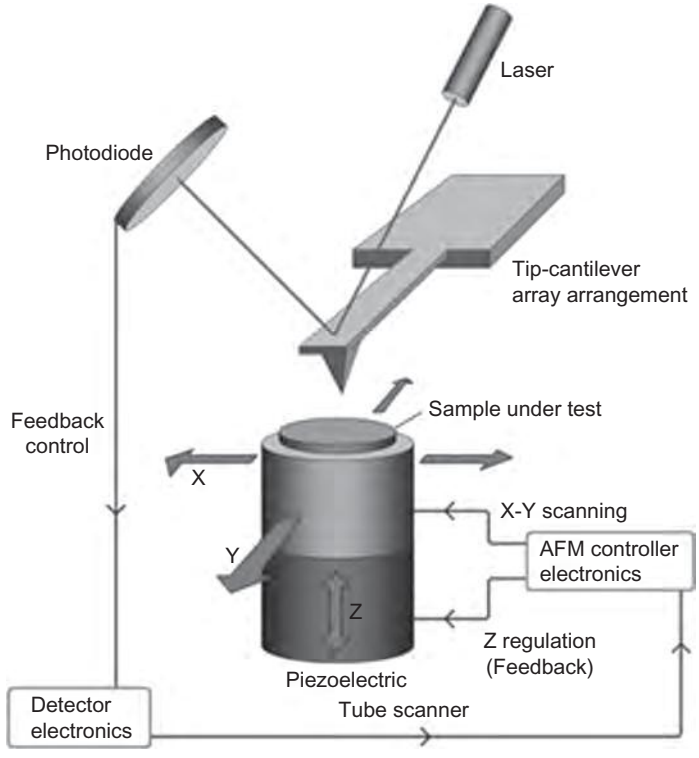


Figure 14.8 *Schematic of AFM operation (Adapted from: University of Virginia, http://pharm.virginia.edu/facilities/atomic-force-microscope-afm/)*

The tip is attached to the end of a cantilever), typically constructed from silicon oxynitride, a ceramic, and coated with gold or some other reflective material. AFM cantilevers are generally about 100 μm long. With the small deflections detected, the cantilever obeys Hooke's Law.

$$F = -k\,d$$

where F is force, k is the spring constant, and d is the deflection, so the forces at work can easily be calculated from the measured deflection. Cantilevers used in AFM vary greatly in flexibility, with spring constants starting from 0.1 N/m up to 100 N/m. Stiffer cantilevers are used in ambient conditions whereas more flexible cantilevers are used in aqueous environments. The spring constant is related to the resonant frequency of the cantilever by the following equation:

$$\Omega = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

where m is mass. m is very small since the cantilevers are on the micro-scale, so AFM cantilever resonant frequencies are fairly high, generally ranging from 100 to 400 kHz. High resonant frequency is important in order for the system to be quickly responsive to changes in the sample surface's topography.

Typically, probe radius varies from 5 to 20 nm. The bending of the cantilever normal to the sample surface is usually monitored by an optical lever, although other methods have been investigated. This system magnifies the normal bending of the cantilever greatly, and is sensitive to Angstrom-level movements.

A cantilever made of silicon has the following dimensions:

$$L = 100\ \mu m$$
$$W = 20\ \mu m$$
$$T = 1\ \mu m$$

The force constant, k, is approximately 1 N/m. Thus if the cantilever is moved by 1 nm, a force of 1 nano-newton is required. Measuring the motion of the cantilever is possible with the 'optical lever' method. In the optical lever method, light is reflected from the back side of the cantilever into a photodetector.

Scanner: A piezoelectric tube scanner is used to position the cantilever and the probe tip over the sample, since currently no mechanical motor provides adequate precision at the atomic scale for this purpose. (The tube scanner can alternatively be used to move the sample underneath the probe tip.) The scanner is generally made from the piezoelectric ceramic, lead zirconium titanate (PZT). A piezoelectric material undergoes a change in geometry when it is placed in an electric field. The amount of motion and direction of motion depends on the type of piezoelectric material, the shape of the material and the field strength.

The tube, as a piezoelectric transducer, expands when voltage is applied, with the piezoceramic's expansion coefficient ranging from 0.1 to 300 nm/V. A voltage is applied to the scanner in response to feedback, to maintain a constant cantilever deflection, applied force or probe height, depending on the mode used. The scanner can move with sub-Angstrom (<0.1 nm) resolution in the x, y and z (up and down) direction.

Optical lever: An optical lever system is used to measure the deflection of the cantilever, which in turn is used to provide the feedback needed to adjust the scanner according to the setting used (Baselt, 2006). The system is very sensitive and can detect sub-Angstrom movement in the cantilever. A laser beam hits the reflective cantilever, which produces a reflected beam that is magnified because of the relative angles at which the components are set up in the AFM. The reflected beam strikes a photodetector, made from two adjacent photodiodes. The difference in intensity between what is detected by each photodiode is translated into a voltage that is fed back to the piezoelectric scanner, which adjusts accordingly. The adjustment that is recorded or the deflection of the cantilever is used then to map out a surface topography in constant height mode. Motions as small as 1 nm are routinely measured with the 'optical lever' method in AFMs.

The vertical bending (deflection) of the cantilever due to forces acting on the tip is detected by a laser focussed on the back of the cantilever. The laser is reflected by the cantilever onto a distant photodetector. The movement of the laser spot on the photodetector gives a greatly exaggerated measurement of the movement of the probe. This set up is known as an optical lever. The probe is moved over the sample by a scanner, typically a piezoelectric element, which can make extremely precise movements. The combination of the sharp tip, the very sensitive optical lever, and the highly precise movements by the scanner, combined with the careful control of probe-sample forces result in the extremely high resolution of AFM.

The basic principle of AFM is that a probe is maintained in close contact with the sample surface by a feedback mechanism as it scans over the surface, and the movement of the probe to stay at the same probe-sample distance is taken to be the sample topography.

Feedback Control: Feedback control is used commonly for keeping the motion of an object in a fixed relationship to another object. In the AFM, feedback control is used to keep the probe in a 'fixed' relationship with the surface while a scan is measured.

For the realisation of a scanning force microscope, the force measurement must be supplemented by a feedback control, in analogy to the STM. The controller keeps the amplitude or the resonance frequency of the vibration of the cantilever (the tip), and thus also the distance, constant. During scanning, the feedback controller retracts the sample with the scanner of a piezoelectric ceramic or shifts towards the cantilever until the vibration amplitude or frequency has reached the set point value again. The scanning force micrographs thus show areas of constant effective force constant. If the surface is chemically homogeneous and if only the van der Waals forces act on the tip, the SFM image shows the topography of the surface.

The motion of the probe over the surface is generated by piezoelectric ceramics that move the probe and FS across the surface in the X and Y directions.

14.3.3 Modes of AFM

AFM can operate in contact, non-contact and tapping mode. Since AFM works essentially by measuring the forces between the tip and the surface (Horber and Miles, 2003), it is helpful to examine the force–distance curve (Figure 14.9) to determine the nature of the forces with the different modes.

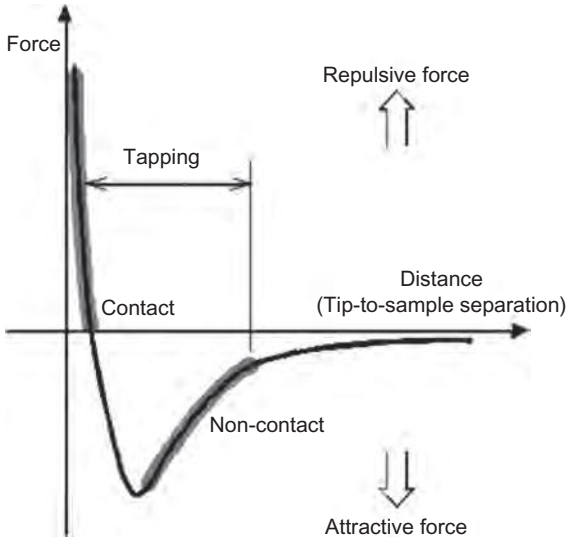


Figure 14.9 *Force versus distance curve with different modes of operation of STM (Howland and Benatar, 2006)*

When the tip is far away from the sample surface, the forces between the two are very small because the distance is too great; as the tip comes closer to the sample, attractive forces pull them together as dipoles and induced dipoles interact; and when the tip and sample are very close together, electron orbitals of the sample and tip come in contact and repulse each other.

When scanning a sample with an AFM, a constant force is applied to the surface by the probe at the end of a cantilever. Measuring the force with the cantilever in the AFM is achieved by two methods. In the first method, the deflection of the cantilever is directly measured. In the second method, the cantilever is vibrated and changes in the vibration properties are measured.

14.3.3.1 Static mode/contact mode

Using the feedback control in the AFM, it is possible to scan a sample with a fixed cantilever deflection. Because the deflection of the

Figure 14.10 *Different modes of operation of STM (a) Contact mode; (b) Non-contact mode; and (c) Tapping mode (Courtesy: M/s Pacific Nanotechnology, 2006)*

cantilever is directly proportional to the force on the surface, a constant force is applied to the surface during a scan. This scanning mode is often called 'contact' mode (Figure 14.10a). However, because the forces of the probe on the surface are often less than a nano-newton, the probe is minimally touching the surface.

AFM is most commonly operated in contact mode. As the name suggests, in contact mode, the probe tip is always in contact with the sample. The cantilever pushes down on the sample with forces ranging from 10^{-8} to 10^{-6}N (Howland and Benatar, 2000). The sample's topography is obtained in either constant height or constant force mode. With constant force mode, which could just as easily be called constant deflection mode, a force is set (of the order of 10^{-9} N) at which the scanner should hold the cantilever/probe. The topography is mapped out using the adjustments in the scanner's vertical movement made to maintain this set force. With constant height mode, the probe is held at a set height throughout scanning and the deflection of the cantilever is measured to map out the sample's topography.

Contact mode has a number of drawbacks. The constant contact with the sample can damage the sample surface as the probe is drawn across the surface. Furthermore, when the AFM is being operated in atmospheric conditions (i.e. in open air), the tip is probing a contaminant layer over the surface consisting of water vapour and nitrogen that pulls the probe in with capillary forces on the order of 10^{-8} N, producing distorted data.

14.3.3.2 Non-contact mode/dynamic modes

In an attempt to prevent the sample damage that sometimes occurs with contact mode, non-contact mode was developed (Li, 2006). In non-contact mode, the tip scans 50–150 Å over the sample, which is close enough to be in the weakly attractive regime of Figure 14.10b. Non-contact mode is not as effective as contact mode because the forces being measured are much weaker (on the order of 10–12 N); to compensate, the tip is oscillated from 100 to 400 kHz, around the cantilever's resonant frequency, so that the forces can be detected by quantifying changes in the cantilever response to the sample's 'force gradients'.

The cantilever in an AFM can be vibrated using a piezoelectric ceramic. Since in the non-contact regime the force between the tip and the surface is of the order of 10–12 N and therefore much weaker than in the contact regime the tip has to be driven in the dynamic mode, i.e. the tip is vibrated near the surface of the sample and changes in resonance frequency or amplitude are detected and used as input for the feedback circuit. In this case, the resonance frequency or the amplitude is held constant by moving the sample up and down and recording directly the topography of the sample.

In ambient conditions, the meniscus force draws the probe into the contaminant layer, which disrupts the oscillation and can distort the resulting images. This drawing in is referred to 'jump-to-contact' (Round, 2006). Consequently, non-contact mode is not frequently used.

14.3.3.3 Tapping mode

With tapping mode, like with non-contact mode, the probe tip is again oscillated around the resonant frequency of the cantilever, except that the tip is allowed to tap the surface on the downswing of its oscillation (Li, 2006). As Figure 14.10c illustrates, tapping mode goes between the strong repulsive forces experienced upon contact and the weakly attractive forces during lift-off.

When the probe contacts the surface, the oscillation is slowed due to the collision and the amplitude changes resulting are then quantified; the adjustment to maintain some set constant vibration amplitude is used to obtain a surface topography, as amplitude increases over dents in the sample and decrease over protrusions. Damage to the surface is negligible, since the frequency of the probe's contact makes the surface viscoelastic so the probe is not making extended contact with the surface. Furthermore, there is no shear force affecting the results since the force is always coming down vertically and then lifting off, unlike in contact mode where in scanning the tip is essentially pulled across the surface.

14.3.4 Magnification of AFM

An AFM is capable of imaging features as small as a carbon atom and as large as the cross section of a human hair. A carbon atom is approximately 0.25 nm in diameter and the diameter of a human hair is approximately 80 μm in diameter.

Magnification in an AFM is the ratio of the actual size of a feature to the size of the feature when viewed on a computer screen. Thus, when an entire cross section of a human hair is viewed on a 500 mm computer monitor (20 inch monitor), the magnification will be as follows:

$$\text{Magnification} = 500\ \text{mm}/0.08\ \text{mm} = 6{,}250\times$$

In the case of extremely high resolution imaging, the entire field of view of the image may be 100 nm. In this case, the magnification on a 500 mm computer screen is given below:

$$\text{Magnification} = 500\ \text{mm}/(100\ \text{nm}*1\ \text{mm}/1{,}000{,}000\ \text{nm}) = 5{,}000{,}000\times$$

14.3.5 Resolution in an AFM

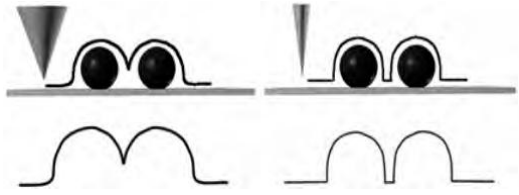


Figure 14.11 *Effect of tip size on the resolution in STM. The image on the right will have a higher resolution because the probe used for the measurement is much sharper (Courtesy: M/s Pacific Nanotechnology, 2006)*

Traditional microscopes have only one measure of resolution, namely the resolution in the plane of an image. An AFM has two measures of resolution: the plane of the measurement and in the direction perpendicular to the surface.

In Plane Resolution: The in-plane resolution depends on the geometry of the probe that is used for scanning. In general, the sharper the probe, higher the resolution of the AFM image. In Figure 14.11 the theoretical line scan of two spheres that are measured with a sharp probe and a dull probe.

Vertical Resolution: The vertical resolution in an AFM is established by relative vibrations of the probe above the surface. Sources for vibrations are acoustic noise, floor vibrations, and thermal vibrations. Getting the maximum vertical resolution requires minimising the vibrations of the instrument.

14.3.6 Applications of AFM

AFM has been a major breakthrough for the sciences. Used primarily as an imaging tool, AFM gives scientists the ability to visualise surfaces at an atomic scale, with up to 10 picometer resolution. AFM has been enormously helpful in the field of biology in particular, since the technique can be used with samples in vacuum, ambient air and aqueous environments. This flexibility allows imaging of samples under more physiological conditions than techniques like electron microscopy for instance, where imaging must be performed in vacuum. Furthermore, the sample does not need to be stained or undergo other forms of harmful treatment so that it isn't sacrificed in the process and can potentially be used again for further analysis.

AFM has been used to image live cells and DNA, as well as collagen and other proteins at the nano-scale. AFM's imaging capabilities have also helped scientists find new structures, like the cell membrane's fusion pores. AFM can provide three-dimensional images with resolution and provide quantitative information about the surface morphology. AFM has much broader potential and application because it can be used for imaging any conducting or non-conducting surface. These include life science, materials science, electrochemistry, polymer science, biophysics, nanotechnology and biotechnology.

15

RADIOCHEMICAL INSTRUMENTS

15.1 FUNDAMENTALS OF RADIOCHEMICAL METHODS

The use of radioactive isotopes has led to the development of radiochemical methods for analyses, and has made possible the examination of phenomena, the measurement of which was formerly complicated or even impossible. These operations are based on the fact that radio-isotopes (isotopes of elements with unstable atomic nuclei) emit radiation which can be detected by suitably located detectors. The proportion of radioactive atoms in the volume of material perceived by the detector can thus be determined by the measurement of the intensity of such radiation. Radiochemical methods offer the advantage of elimination of chemical preparation that usually precedes the measurement. These methods are both sensitive and specific and are often characterised by good accuracy.

In one method, a pure but radioactive form of the substance to be determined is mixed with the sample in known amount. After equilibrium, a fraction of the component of interest is isolated and the analysis is then based upon the activity of this isolated fraction. Alternatively, activity is induced in one or more elements of the sample by irradiation with suitable particles. The measurement of this activity gives information about the element of interest.

Before the advent of the cyclotron and more recently the chain-reacting pile, most of the work with radio-isotopes was done with naturally occurring radioactive elements. However, it is now possible to obtain artificially produced radio-isotopes of most of the elements. Also, it is possible to obtain these in large quantities and with extremely high activity.

15.1.1 Time Decay of Radioactive Isotopes

Each radioactive isotope is characterised not only by type and energy of radiations emitted, but also by the characteristic lifetime of the isotope. This is most conveniently designated by half-life or half-period of the isotope. The half-period of a radioactive isotope is the time required for half of the initial stock of atoms to decay. Thus, after one half-period has elapsed, the total activity of any single radioactive isotope will have fallen to half its initial value; after two periods, the activity will be one-quarter its initial value and so on. After 6.6 half-periods, the activity will be 1% of the initial activity.

Decay is a random process which follows an exponential curve. The half-life of a radioactive isotope is given here:

$$t^{1/2} = 0.693/\lambda$$

where λ is the decay constant for a particular radio-isotope. In practice, disintegration rates are determined by counting the number of disintegrations over a certain time t_m and finding the ratio of the number of disintegrations to the time t_m.

15.1.2 Units or Radioactivity

The unit of radioactivity is curie. This was originally defined to represent the disintegration rate of one gram of radium, but it is now used as the standard unit of measurement for the activity of any substance, regardless of whether the emission is alpha or beta particles, or X or gamma radiation. When used in this way, the curie is defined as an activity of 3.7×10^{10} disintegration. The curie represents a very high activity. Therefore, smaller units such as millicurie or micro-curie are generally used.

Energy: The basic unit used to describe the energy of a radiation particle or photon is the electron volt (eV). An electron volt is equal to the amount of energy gained by an electron passing through a potential difference of one volt. The energy of the radiation emitted is a characteristic of the radionuclide.

15.1.3 Types and Properties of Particles Emitted in Radioactive Decay

The theory of atomic structure proves that some elements are naturally unstable and exhibit natural radioactivity. On the other hand, elements can be made radioactive by bombarding them by high-energy charged particles on neutrons, which are produced by either a cyclotron or a nuclear reactor respectively. This process will alter the ratio of photons to neutrons in the atoms, thus creating a new unstable nucleus which could undergo radioactive decay. The extra neutron disintegrates and in the process, releases energy in the form of gamma radiation.

Radioactive emissions take place in three different forms, which are given below.

Alpha emissions: Alpha particles are composed of the protons and two neutrons. They are least penetrating and can be stopped or absorbed by air. They are most harmful to the human tissue.

Beta emissions: These are positively or negatively charged and are high-speed particles originating in the nucleus. They are not as harmful to tissue as alpha particles, because they are less ionising, but are much more harmful than gamma rays.

Gamma emissions: Emissions like X-rays constitute electromagnetic radiation that travels at the speed of light. They differ from X-rays only in their origin. X-ray originates in the orbital electrons of an atom, whereas gamma rays originate in the nucleus. They are due to an unstable nucleus. X-rays and gamma rays are also called 'photons' or packets of energy. As they have no mass, they have the greatest penetrating capability. Gamma rays are of primary interest in radiochemical methods of analysis.

Different types of particles are distinguished by their penetration power, particles with the greatest mass and charge being the least penetrating. The energies of alpha and beta particles and gamma radiations are expressed in terms of the electron volt. One electron volt is the energy that an electron would acquire, if it were accelerated through a potential difference of one volt. Radioactive emissions have energies of the order of thousands or millions of electron volts. Alpha emission is characteristic of the heavier radioactive elements such as thorium, uranium, etc. The energy of alpha particles is generally high and lies in the range 2–10 MeV (million electron volt). Their penetrating power is low and are completely stopped by foils and solid materials like aluminium. Due

to larger ionising power of alpha particles, they can be distinguished from beta and gamma radiations on the basis of pulse amplitude they produce on a detector.

Beta emission consists of a very energetic electron or positron (beta particles that carry a unit positive charge). Their penetration power is substantially greater than alpha particles, and have energy range 0–3 MeV.

Gamma rays are high-energy photons having high penetrating and low ionising power.

15.1.4 Interaction of Radiations with Matter

Beta particles interact primarily with the electrons in the material through which they pasts. The absorption depends mainly upon the number of electrons in their path. The molecules of the matter may be dissociated, excited or ionised. However, it is the ionisation which is of primary importance in the detection of beta particles.

Alpha particles have relatively large mass and higher charge, the specific ionisation produced by them is much larger than for beta particles.

Upon interaction with matter, gamma rays ionise energy by three modes. The *photoelectric effect* transfers all the energy of the gamma ray to an electron in an inner orbit of an atom of the absorber. This involves ejection of a single electron from the target atom. This effect predominates a low gamma energies and with target atoms having a high atomic number.

The *Compton Effect* occurs when a gamma ray and an electron make an elastic collision. The gamma energy is shared with the electron and another gamma ray of lower energy is produced which travels in a different direction. The *Compton effect* is responsible for the absorption of relatively energetic gamma rays. When a high-energy gamma ray is annihilated following interaction with the nucleus of a heavy atom, *pair production* of a positron and an electron results. *Pair production* becomes predominant at the higher gamma-ray energies and in absorbers with a high atomic number. The number of ion pairs per centimetre of travel is called specific ionisation.

15.2 RADIATION DETECTORS

Several methods are available for detection and measurement of radiation from radionuclides. The choice of a particular method depends upon the nature of the radiation and the energy of the particle involved.

If the radiation falls on a photographic plate, it would cause darkening when developed after exposure. The photographic method is useful for measuring the total exposure of workers to radiation, who are provided with film badges. Better methods are available for an exact measurement of the activity.

15.2.1 Ionisation Chamber

The fact that the interaction of radioactivity with matter gives rise to ionisation makes it possible to detect and measure the radiation. When an atom is ionised, it forms an ion pair. If the electrons are attracted towards a positively charged electrode and the positive ions to a negatively charged electrode, a current would flow in an external circuit. The magnitude of the current would be proportional to the amount of radioactivity present between the electrodes. This is the principle of the ionising chamber.

An ionisation chamber consists of a chamber which is gas filled and is provided with two electrodes. A material having a very high insulation resistance, such as polytetrafluoroethylene is used as the insulation between the inner and outer electrodes of the ion chamber. A potential difference of a few hundred volts is applied between the two electrodes. The radioactive source is placed inside or very near to the chamber. The charged particles moving through the gas undergo inelastic collisions to form ion pairs. The voltage placed across the electrodes is sufficiently high to collect all the ion pairs. The chamber current will then be proportional to the amount of radioactivity in the sample. lionisation chambers are operated either in the counting mode, in which they respond separately to each ionising current, or in an integrating mode involving collection of ionisation current over a relatively long period.

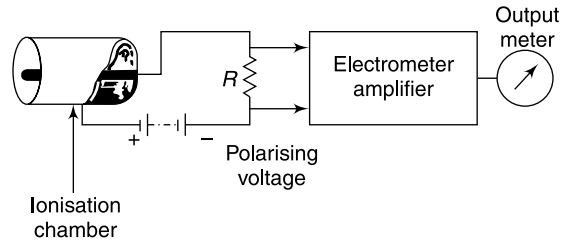


Figure 15.1 *Schematic diagram for measuring ionising current using dc ionising chamber*

Figure 15.1 shows an arrangement for measuring the ionising current. The current is usually of the order of 10^{-10}A or less. It is measured using a very high input impedance voltmeter, which has a MOSFET in the input stage. The current is indicated on a moving coil type ammeter. Alternatively, null method can also be used. In this method, the change in voltage produced across a capacitor by the ionising current is counterbalanced by an equal and opposite voltage supplied from a potentiometer. A potentiometric recorder of the self-balancing type can be used to record the signal.

The magnitude of the voltage signal produced can be estimated from the fact that the charge associated with the 100,000 ion pairs produced by a single alpha particle traversing approximately 1 cm in air would be around 3×10^{-14} coulomb. If this average charge is made to pass through a resistance of $3 \times 10^{10}\ \Omega$ in 1 s, a difference of potential of approximately 1 mV would develop across the high resistance. This voltage is a function of the rate of ionisation in the chamber.

Liquid samples are usually counted by putting them in ampoules and placing the ampoules inside the chamber. Gaseous compounds containing radioactive sources may be introduced directly into the chamber. Portable ionisation chambers are also used to monitor personnel radiation doses.

15.2.2 Geiger-Muller Counter

The Geiger counter is commonly called GM tube. This tube consists of a metal cylinder (Figure 15.2), which acts as a cathode and is about 1–2 cm in diameter. It has an axial insulated wire working as an anode and is capable of being maintained at a high positive potential of the order of 800–2,500 V. This assembly is placed in a tubular glass envelope containing a gas or mixture of gases, which is easily ionisable. The envelope is gas-tight and is typically filled to a pressure of 80 mm of argon gas and 20 mm of alcohol. Alcohol, butane or bromine acts as a quenching gas and argon as the ionising gas. The tube contains a window of thin mica or other suitable material, which permits effective passage of beta and gamma radiation, but not of alpha radiation.

As the gas is ionised in the counting tube, migration of ions takes place towards the appropriate electrodes under the voltage gradient. They soon acquire sufficient velocity, cause further ionisation and give rise to an avalanche of electrons travelling towards the central anode. As a

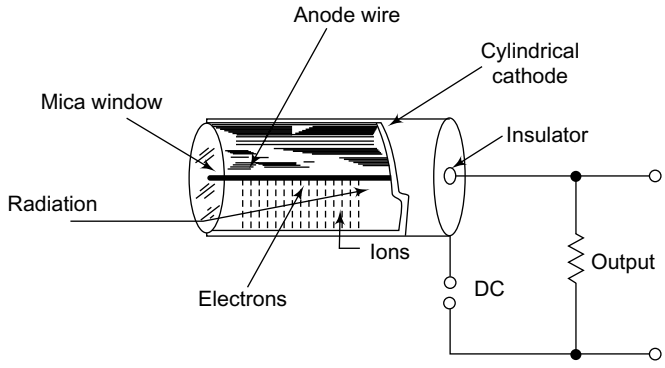


Figure 15.2 *Construction of a Geiger-Muller tube*

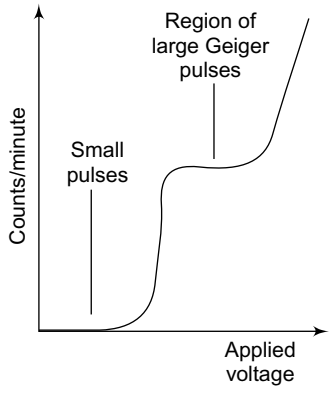


Figure 15.3 *Variation of the count rate with voltage using Geiger-Muller counter*

result, ion multiplication to a complete sheath around the anode, and the same pulse size is observed for each primary ionising event. The process, in fact, produces a continuous discharge, which fills the whole active volume of the counter in less than a micro-second. Each discharge builds up to a constant pulse of 1–10 V. This pulse amplitude is sufficient to operate a rate metre or scaler without using any amplifier.

Figure 15.3 shows the variation of the count rate recorded by a typical Geiger counter, when the polarising voltage is altered. The tube works in the voltage range exhibited by the plateau. Below the starting voltage, no counts are recorded. Between the starting voltage and beginning of the plateau, the voltage is too low to produce constant pulse size. Also, beyond the plateau the count rate increases, because of breakdown and spurious discharges through the tube. The plateau is observed between 800 and 1,400 V for commercial tubes. The slope of the plateau is generally expressed in terms of a percentage of the count rate per volt. It should not be more than 0.1% per volt for a counter in a satisfactory condition. In order to minimise the counts due to background, the counter is normally placed inside a lead shield.

Positive ions produced by ionisation, being much heavier, have much lower mobilities than electrons. Therefore, they move only a very short distance towards the cathode in the time interval required for the electrons to cross the space between electrodes. This travel time may be several hundred micro-seconds and can vary from counter to counter. During this period, the positive ions form a sheath around the anode wire, which effectively lowers the potential gradient to a point where the counter becomes insensitive to the entry of fresh ionising particles. This is called dead time of the counter. The dead time of self-quenched GM counter is usually reduced by rapidly dropping the counter voltage below the starting potential, several research workers. Use of quenching circuit is preferred because it offers two advantages: reduction of the counter dead time

and prolongation of lifetime of the counter by reducing the number of organic molecules dissociated in each discharge.

In a GM tube containing ethyl alcohols as a quenching agent, some of the vapours are dissociated each time a count is recorded, so that the counter has a limited life. The life of these tubes is limited to perhaps 10^8 to 10^{10} counts. The tubes containing halogens have much longer life, because halogens simply dissociate during ionisation and recombine afterwards. The counting tubes containing halogens (bromine) can be used at low temperatures. The halogens, however, may be consumed by reaction with electrodes and other metallic parts of the tube. Geiger counters are utilised more often than any other counter. They have also been made in the miniature form having dimensions less than one inch and diameter of 2 mm.

15.2.3 Proportional Counter

The proportional counter is an ionisation chamber that is operated at voltages beyond the ordinary ionisation chamber region, but below that of the Geiger region. These counters are called proportional counter, because the output pulse from the chamber stalls to increase with the increase in the electric field strength at the central electrode, but is still proportional to the initial ionisation. In these counters, the pulse produced is amplified by a factor of 1,000 or more. The design of the counter and the value of the applied voltages are so chosen, that a high voltage gradient exists close to the anode. The radius of cathode is about 1 cm and that of anode 0.001 cm, with a polarising voltage of the order of 1000 V. The output pulse is generally of a few millivolts and therefore, requires amplification before the signal can be given to a scaler for counting.

The radioactive source may be placed inside the counter or outside the counter. In the former case, it avoids window absorption. In the continuous gas-flow type counters, an argon-methane mixture flows at atmospheric pressure from compressed gas tank at a rate of 200 ml/min. Counter-life is, therefore, unlimited as the filling gas gets constantly replenished.

Figure 15.4 shows the schematic diagram of the counting equipment used with a flow type proportional counter. The pre-amplifier is a voltage follower, which provides high input impedance. This is followed by a low-noise linear amplifier having a very stable gain in the range of 500–1000. The amplifier is required to be of non-overloading type, since large pulses from gamma-ray background may overload a conventional amplifier for an appreciable time, causing counts to be missed. In order to avoid attenuation and distortion of pulses due to capacitance of the long connecting cables, the pre-amplifier should be placed very near the detector. Figure 15.5 shows range of operation of different types of counters.

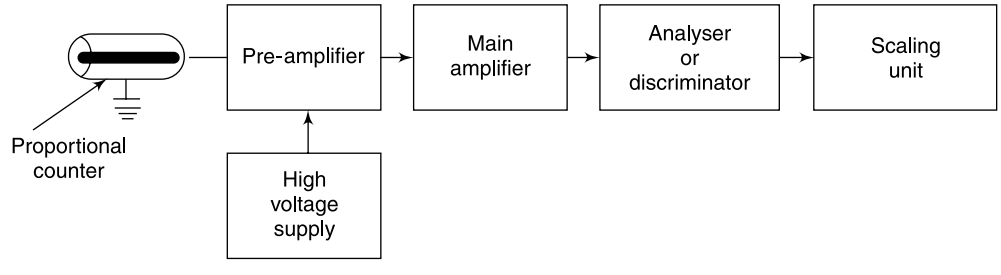


Figure 15.4 *Counting equipment used with a proportional counter*

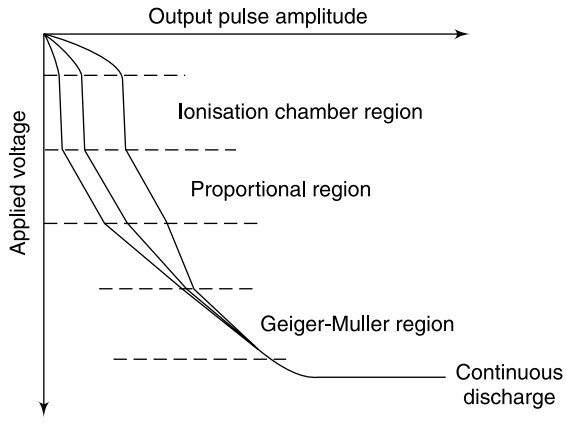


Figure 15.5 *Range of operation of different types of counts*

In proportional counter, different particles would yield output pulses of amplitude proportional to the isotope energy. By employing a pulse-height analyser, which counts a pulse only if its amplitude falls within certain specific limits, a proportional counter can be made to respond to beta rays or X-ray frequencies etc. In single-channel pulse-height analysers, provision is made for lower and upper energy discrimination, so that only pulses having amplitudes between the levels are passed. The voltage between the discriminating settings is called window, gate or width. The scaling unit counts down the pulses from the analyser, so that they are digitally displayed. A decade system of counting is employed, which displays units, tens, hundreds, thousands and ten thousands of counts. Most of the scalers incorporate a counter/timer, which displays time taken to record a definite number of counts, or number of counts which occur within a definite time interval. Scalers can often be replaced by rate metres, which continuously indicate or record the mean value of the rate, at which pulses from the analysers are applied to it.

15.3 PULSE HEIGHT ANALYSER

In radioactivity measurements, the individual particles are detected as single electrical impulses in the detectors. Also, various types of detectors can be set up to operate in a region in which the particular particle produces an electrical impulse having height proportional to the energy of the particle. The measurement of pulse height is thus a useful tool for energy determination. in order to sort out the pulses of different amplitudes and to count them, electronic circuits are employed. The instrument which accomplishes this is called pulse-height analyser. These analysers are either single or multiple-channel instruments.

Figure 15.6 shows a block diagram of a single-channel pulse-height analyser. The output pulses from the photomultiplier are amplified in a high input impedance low-noise pre-amplifier. Amplified pulses are fed into a linear amplifier of sufficient gain to produce output pulses in the amplitude range of 0–100 V. These pulses are then given to two discriminator circuits. A discriminator is nothing but a Schmitt trigger circuit, which can be set to reject my signal below a certain voltage. This is required for excluding scattered radiation and amplifier noise. The upper discriminator circuit rejects all but signal 3 and the lower discriminator circuit rejects signal 1 only and transmits signals 2 and 3. The two discriminator circuits give out pulses of constant amplitude. The pulses having amplitudes between the two triggering levels are counted. This difference in two levels is called the window width the channel width or the acceptance slit and is analogous to monochromators in optical spectrometry.

Schmitt trigger circuits are followed by anti-coincidence circuit. This circuit gives an output pulse when there is an impulse in only one of the input channels. It cancels all the pulses which trigger

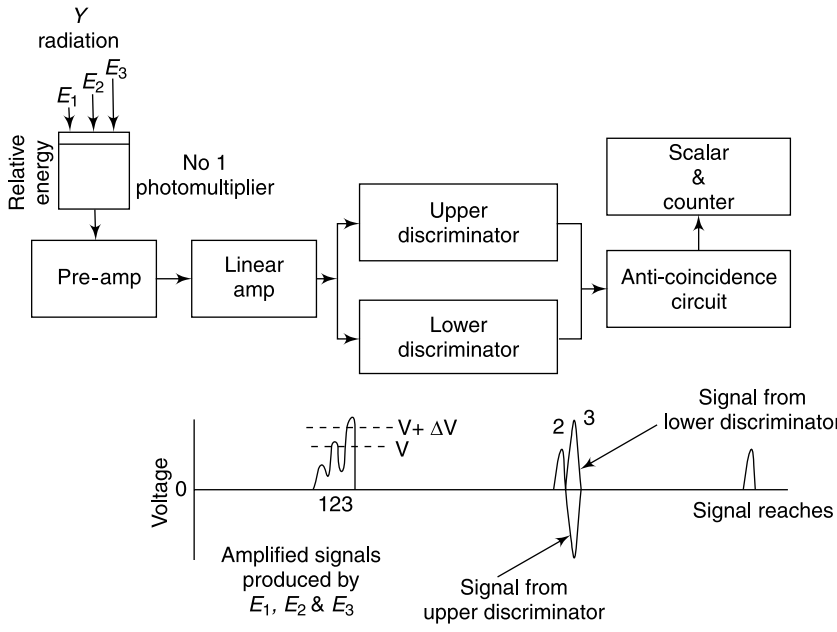


Figure 15.6 *Block diagram of a pulse-height analyser*

both the Schmitt triggers. This is accomplished by so arranging the upper discriminator circuit that its output signal is reversed in polarity and thus cancels out signal 3 in the anti-coincidence circuit. As a consequence, the only signal reaching the counter is one lying in the window of the pulse-height analyser. The window can be manually or automatically adjusted to cover the entire voltage range with width of 5–10 V. Scaler and counter follow the anti-coincidence circuit.

Multi-channel pulse-height analysers are often used to measure a spectrum of nuclear energies and may contain several separate channels, each of which acts as a single-channel instrument for a different voltage span or window width. The Schmitt trigger discriminators are adjusted to be triggered by pulses of successively longer amplitude. This arrangement permits simultaneous counting and recording of an entire spectrum.

A parallel array of discriminators is generally used, provided the number of channels is ten or less. If the number of channels is more than ten, problems of stability of discrimination voltages and adequate differential non-linearity arise.

15.4 SCINTILLATION COUNTER

Scintillation is the process of turning radioactive energy (e.g. the energy associated with a beta particle) into light using a 'scintillator'. A scintillator, thus, is a substance which produces minute flashes of light in the visible or near ultraviolet range, when it absorbs ionising radiation. In such cases, the number of fluorescent photons is proportional to the energy of the radioactive particle. The flashes occur due to the recombination and de-excitation of ions and excited atoms produced along the path of the radiation. The light flashes are of very short duration and are detected by using a photomultiplier

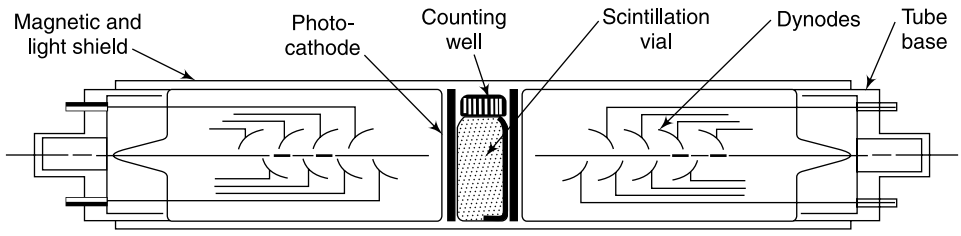


Figure 15.7 *Typical beta scintillation counter showing only the counting well and photomultiplier tube detectors*

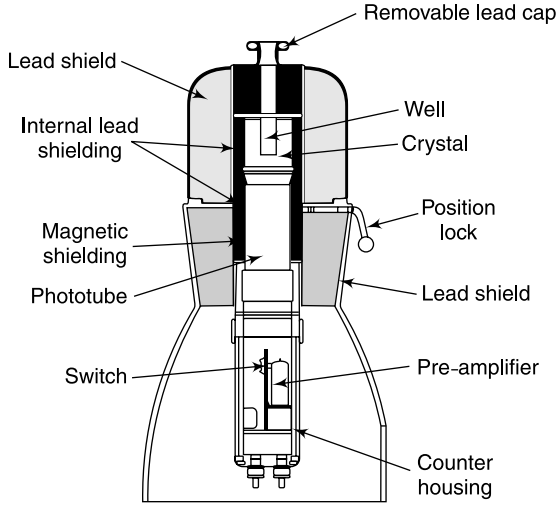


Figure 15.8 *A well-type scintillation counter (Courtesy: Texas Nuclear Dinsion, Ramsey Engg. Co. Austin)*

tube, which produces a pulse for each particle. A scintillator along with the photomultiplier tube is known as a scintillation counter.

The following are the two types of scintillators:

- Liquid Scintillators
- Solids Scintillators

Liquid Scintillators: These are used for low-energy β^- emitters such as ^{14}C, ^{35}S and ^{3}He. The radioactive material is mixed into a scintillation fluid referred to as cocktail. Liquid scintillation fluids are organic molecules dissolved in an organic solvent. The mixture is placed between two photomultipliers which record the light produced. Figure 15.7 shows the simple arrangement of a liquid scintillation counter. A count is recorded only when a pulse of light is simultaneously detected by both PMTs, thus acting as a coincidence counter. This helps to reduce back ground noise.

Solid Scintillators: These are used for high-energy β^- emitters such as 203_{Hg}, 59_{Fe}, 65_{Zn} and 109_{Cd}. The most commonly used solid scintillator is NaI (Sodium Iodide). Here, the radioactive sample is placed in a well (Figure 15.8) cut out of a crystal, whereas the photomultiplier tube is mounted on one face of the crystal. Each β^- particle produces several thousand photons of light (wavelength: 400 nm) as it passes through the crystal, which are counted.

15.5 GAMMA COUNTERS

Gamma radiations cannot be detected directly in a scintillating material, because gamma rays possess no charge or mass. The gamma-ray energy must be converted into kinetic energy of electrons present in the scintillating material. Thus the conversion power of the scintillating material will be proportional to the number of electrons (electron density) available for interaction with the gamma

rays. Because of its high electron density, high atomic number and high scintillating yield, the scintillating material which is generally used as gamma-ray detector is a crystal of sodium iodide activated with about 0.5% of thallium iodide. For counting beta particles, scintillator crystals of anthracene are employed. The crystal, being hygroscopic in nature, is usually mounted in a sealed aluminium container having a glass window on the side, which is in contact with the face of the photomultiplier.

For *in vitro* counting, the geometry of the scintillation crystal plays an important role. When it is convenient, the sample is placed in a well within the crystal and the crystal is coated on all sides with reflecting material, except for the side which is bonded to the face of the photomultiplier tube. In order to reduce the background counts, the crystal-photomultiplier tube assembly is mounted inside a cylindrical lead shield having a lead lid.

Instruments used for counting gamma particles are called gamma counting spectrometers. They may include an oscilloscopic display which is called energy scope. The energy scope provides a visual indication of isotope spectrum. The Beckman Biogamma counting system provides a means of selecting the counting window by adjusting the variable discriminators and aids in selecting the proper high voltage. The vertical gain can be set in two positions to determine display height of scope. The low position is used when measuring high-energy isotopes such as ^{59}Fe and ^{22}Na and the high position is used with low-energy isotopes such as ^{125}I and ^{57}Co. The activity indicator indicates the amount of activity in terms of flashes of light. If the light glows constantly, a highly active source is in the counting chamber. If the light flashes, a less active source is in the chamber. Two variable discriminators permit adjustment from 0 to 1,000 divisions to cover any part of an isotope spectrum. They are in fact two ten-turn potentiometers and are marked as upper and lower. The time selector switch is used to select one of the 10 time intervals (in minutes), that determines the length of time each sample is counted. Count selector switch selects minimum number of counts that must be accumulated in the first 0.1 min. If the minimum is not reached, counting is terminated and the system moves to the next sample. Gamma counting systems generally include automatic sample changers which may hold 20 vial trays with 10 vials each. The vials are moulded from polypropylene to reduce gamma absorption in the vial walls. A tele-typewriter can also be included to have a sample print out of the counts made.

Samples containing weak beta emitters, such as H^3 and C^{14} can be counted more efficiently by mixing the sample with a liquid scintillator, so that the scintillator is in intimate contact with the short-range beta rays. Counting is carried out with one inch diameter photomultiplier. The compound containing the radioactive source is dissolved in toluene or xylene, to which is added a primary scintillator and a secondary scintillator to increase the pulse height by acting as a wavelength shifter. The photomultiplier tube is dipped directly into the scintillator solvent and the counts are made.

Normally five adjustments are made in a scintillation counter for proper counting of the ionising particles. They are: high voltage setting, pulse-height-analyser threshold voltage, analyser channel-width, amplifier gain and time constant of the rate metre.

Figure 15.9 shows a block diagram of the automatic gamma counting system MR 1032 from M/s Roche Medical Electronics. The maximum capacity of the sample changer in this system is 86 racks, each holding 12 sample tubes. The bidirectional sample changer has facilities for advance and reverse travel and up-down movement for single measurements. The sample changing cycle takes approximately 7 s.

Sample and rack identification are sensed by on optical reader. Thus, each sample is identified and the rack number can be matched to a laboratory, or to a specific experiment. The detector is a

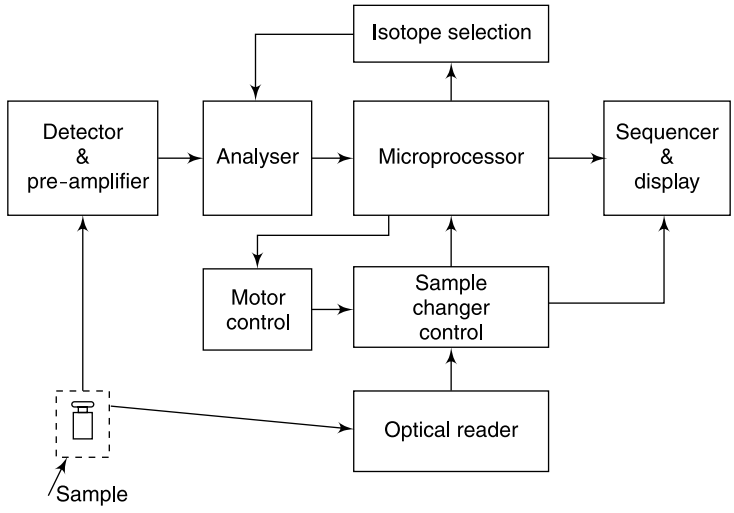


Figure 15.9 *Block diagram of the automatic gamma counting system (Courtesy: M/s Roche Medical Electronics, USA)*

well-type sodium iodide-crystal activated with a trace of Thallium and coupled to a photomultiplier tube. The crystal is hermetically sealed in a thin can of aluminium and optically coupled to the sensitive face of an end-on PMT. Crystal and tube are covered with lead shielding for low and constant background.

The low-energy models incorporate a pulse-height analyser with four pre-set windows, which discriminate between the following low-energy isotopes: ^{125}I ^{57}Co, ^{75}Se or ^{131}I. The high-energy models incorporate a 7-isotope pulse-height analyser. The capacity of the scalar is 1,000,000 counts. There is one scaler associated with each pulse-height analyser. An increase in the number of counts accumulated will lead to a reduction in the percentage of statistical error and in increase in measuring accuracy. The display has 7-digit display, which shows counts per minute, elapsed time of the count in progress or identification number and the number of the sample being counted.

The system is directed and controlled by a microcomputer set MCS-8080. This parallel 8-bit modular microcomputer has up to 65 KB memory capacity in PROM and RAM. The 8080 incorporates a central processor module, a RAM module, a PROM module and an input-output module, which is designed for interface with a keyboard. The results are printed out in numbered A-4 formats.

15.5.1 Semiconductor Detectors

A great deal of development work has been done on semiconductor radiation detectors. These detectors can be made very small arid robust, silicon and germanium crystals have been employed mainly for counting alpha and beta particles. They function in a manner similar to that of the gas ionisation chamber. On absorption of radiation in the crystal, electrons and positive holes are formed, which move towards opposite electrodes under the influence of applied potential. The resulting current is proportional to the energy of the ionising radiation.

15.6 LIQUID SCINTILLATION COUNTERS

Figure 15.10 shows a block diagram of a typical liquid scintillation counter which illustrates the manner in which the emitted radiation interacts with the cocktail (a mixture of a solvent and solute) leading to a count being recorded by the system.

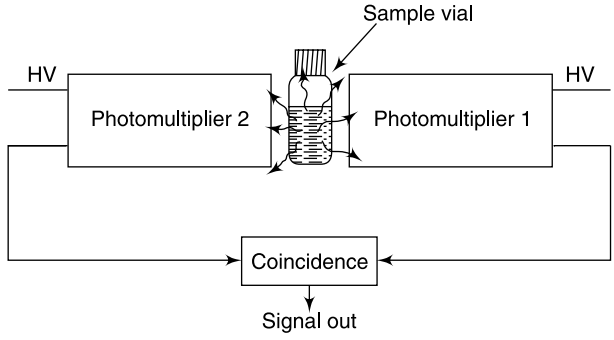


Figure 15.10 *A block diagram of a liquid scintillation counter*

Beta particles are emitted in a radioactive decay. In order to ensure efficient transfer of energy between the beta particle and the solution, the solution is a solvent for the solvent material. The kinetic energy of the beta particles is dissipated and gets absorbed by solvent molecules making them excited. Energy of the excited solvent is emitted as UV light and the solvent molecule returns to general state, and there by transfer energy to the solute.

The solute is a fluor. As the excited orbital electrons of the solute molecule return to the ground state, a radiation results, in this case a photon of UV light. The UV light is absorbed by fluor molecules which emit blue light flashes upon return to ground state.

Nuclear decay events produce approximately to photons per keV of energy. The energy is dissipated in a period of time on the order of 5 ns. The total number of photons from the excited fluor molecules constitutes the scintillation. The intensity of the light is proportional to the beta particles initial energy.

Blue light flashes fall on the photocathode of the photomultiplier tube (PMT) which get converted into electrical pulses. The counter normally has two photomultiplier tubes. The signal from each PMT is fed into a circuit which produces an output only if the 2 signals occur together, i.e. within the resolving time of the circuit, which are generally 20 ns. By summing the amplitude of the pulses from each PMT, an output is obtained which is proportional to the total intensity of the scintillation.

The amplitude of the PMT pulse depends on the location of the event within the vial. The pulse is large of it is closer to the PMT, than if the event is more remote.

The analog electrical pulses are converted into a digital value. The digital value represents the beta particle energy. The pulses are then passed to an analyser, where it is compared to digital values for each of the channels of the counter. Each channel is the address of a memory slot in a multi-channel analyser (MCA) which consists of many storage channels or storage slots covering the energy range from 0–2 keV. Instruments generally employ automatic data reduction including averaging of repeat sample counts, low count rejection and result normalisation.

Figure 15.11 *Scintillation counter Model LS 6500 (Courtesy: M/s Beckman Coulter Scintillation Counter)*

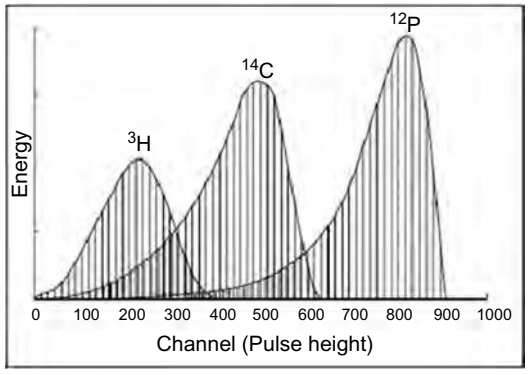


Figure 15.12 *Pulse-height spectra of three radioisotopes from a liquid scintillation counter*

Modern liquid scintillation counters, such as Beckman Coulter Model LS 6500 (Figure 15.11) are programmable instruments. Parameters which can be programmed include count time, sample repeat and count channel as well as radio-isotope which are to be observed. The instruments count samples in liquid form, either in the standard 20 ml glass or plastic vial or in a 6 ml miniature vial. The instrument has a 32,768 channel MCA which gives you an eight-fold improvement in effective resolution (0.06 keV per channel) over that of conventional liquid scintillation counters (0.5 keV per channel).

The ability to sort the pulses detected according to their amplitude allows discrimination between emissions of different energies. For example, as shown in Figure 15.12, three commonly used isotopes ^{3}H, ^{14}C and ^{32}P have beta energies of 18.3, 156 and 1,710 keV, respectively. By setting windows judiciously, it is possible to specify which energy range is to be measured.

The number of pulses in each channel is displayed on a CRT or printed as a hard copy. In this manner, the sample is analysed and the spectrum can be plotted to provide information about the energy of radiation or the amount of radioactive material dissolved in the cocktail.

Quenching: Anything added to a counting vial (colour, solvents, filters, swabs) can reduce the efficiency of the scintillation process. This reduction in counting efficiency is called *quenching*. There are three major forms of quench:

- Chemical quench: Some chemicals will affect the transfer of energy between the solvent and the fluors resulting in reduction in the amount of light and a subsequent reduced counting efficiency.
- Colour quench: Solid and liquid scintillators emit light in the blue region of the spectrum. Red, green and yellow colours in the counting vial may absorb the light, resulting in reduced efficiency.
- Self absorption: This occurs when radiation emitted by an isotope remains undetected due to absorption of the radiation by the sample itself e.g. in precipitates, cells.

In practice, all samples are quenched to some degree. The counts per minute observed (cpm) may differ substantially from the true radioactive decay rate, (disintegrations per minute, (dpm), depending on the efficiency of the counting process. The following is the counting efficiency by definition:

$$\text{Counting efficiency} = \text{cpm}/\text{dpm}$$

$$\text{Therefore, dpm} = \text{cpm}/\text{Counting efficiency}$$

In order to determine the counting efficiency, the amount of quenching has to be known. Several methods are used to characterise and quantify the quenching for a particular sample. A common method uses the so-called 'H-number' (*proprietary technique by Beckman Instruments) which is assigned by an on-line analysis of the Compton electron spectrum generated in the sample by shining briefly an external standard source on this sample.

A quench calibration curve can be constructed by plotting the counting efficiency versus the H-number using a set of samples of known constant activity but containing varying amounts of quenching agent. In modern instruments, the quench curve can be stored in the machine's electronic memory such that the quenching correction is made automatically to provide directly the output as dpm.

Sometimes, spurious, non-reproducible counts will be observed. The cause may be static electricity. Handling of plastic scintillation vials with surgical gloves can build up a charge on vial. It is advisable not to wear gloves when loading the scintillation counter. Modern instruments incorporate electrostatic controllers to minimise the effect of static electricity.

It is known that photomultipliers used in liquid scintillation counters are a source of instability. The sources of these instabilities are (i) variation of gain with temperature, (ii) variation of gain with tube current, (iii) variation of gain with sudden large changes in tube current and (iv) effects due to ageing. Stabilisation systems based on the use of radioisotopes are common in scintillation counters used in nuclear spectroscopy. However, such systems are not practicable in a liquid scintillation counter, intended for counting low activity beta radiation sources. Several workers have reported stabilisation systems which are not based on radioisotopes, and which, therefore, are potentially useful for liquid scintillation counters. These include systems based on the use of gas discharge tubes and those based on the use of modulated and other light sources. Light emitting diodes have also been employed for the stabilisation of photomultipliers. This is done by the use of LEDs for stabilisation of gain in photomultiplier, by means of adjustment of high voltage or pre-amplifier amplification.

15.7 GAMMA SPECTROMETRY

From an analytical standpoint a great majority of radioactive nuclei release gamma rays in their head-long tumble to a more stable existence, that is to say when they undergo radioactive decay. It is only a small minority that is not detectable by gamma spectrometry. Some of these are ^{3}H and ^{14}C and of course, the measurement of their beta decay by liquid scintillation is very well established.

Figure 15.13 shows decay scheme showing origin of gamma radiation. In case of ^{32}P the energies of the beta particles emitted range up to a maximum of 1710 keV. In case ^{60}Co, (Figure 15.13b), beta decay occurs again, but not to the ground state of ^{60}Ni but to an intermediate energy state. It is the dc-excitation of the state, which in this case occurs through an intermediate energy level, which is the source of gamma radiation. It may be observed that the gamma emission is not a decay process in the same sense as beta decay and alpha decay; but is one of the ways in which the excitation energy that the nucleus usually has, after one of those processes has occurred is dissipated (Hemingway, 1986).

The essence of gamma spectrometry is the fact that the energies and abundances of gamma rays emitted are specific to a radionuclide. For example, in the figure shown, the energies of the two gamma rays released are Y_2 = 1,173.2 keV (2,505.7–1,332.5) and Y_2 = 1,332.5 keV (1,332.5–0).

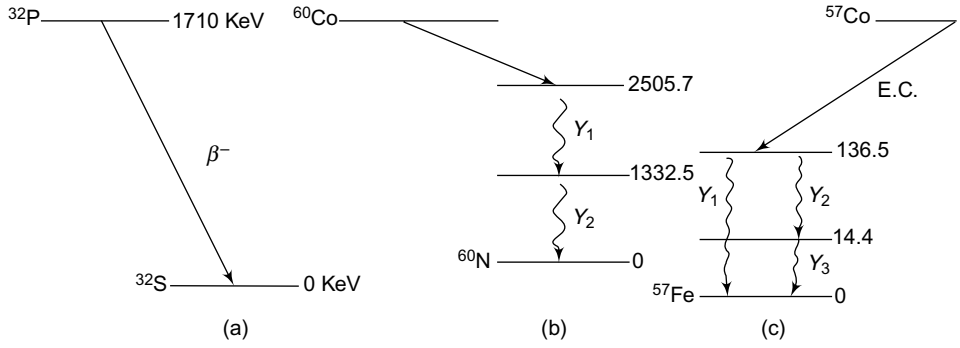


Figure 15.13 *Decay scheme showing origin of gamma radiation*

If a sample emits two gamma rays of these energies, then you can be confident that the sample contains ^{60}Co, as these are unique to the decay of this nuclide. In general, every gamma-emitting radionuclide would emit its own characteristic pattern of monoenergetic gammas, but there are often dozens of different energies, each energy with its own particular abundance

Gamma spectrometry is based on the use of semiconductor detectors, i.e. germanium and silicon. The germanium detector is the most common, which in the earlier days required lithium compensation for the residual charge carriers due to impurities. These were called jelly [Ge (Li)] or lithium drifted germanium. In these detectors, Li is incorporated into the semiconductor lattice by annealing the semiconductor with Li at a high temperature (~500°). A voltage of approximately 1,000 V is placed across the semiconductor material with two electrodes and the electron cascade produced by a photoelectron is detected as an electrical pulse at the anode. But in high purity germanium, lithium compensation is not required and the detectors are called intrinsic or high purity.

In addition to being more robust than gas-filled or scintillator detectors, these semiconductor detectors also provide a much higher resolution. However, they need cooling to decrease the dark noise of the detector and current-to-voltage pre-amplifier.

Both types of detectors are used at liquid nitrogen temperature (77 K), where the thermally induced electronic noise is reasonably low. Most manufacturers have developed portable detectors, which can use a small dewar or 0.5–21 volume of liquid nitrogen and have a start holding time of 24 h. While the Ge (Li) detectors must be maintained at 77 K all the lime, the intrinsic detectors can be allowed to warm up to room temperature without causing damage, and can thus be transported easily without depending on guaranteed supply of liquid nitrogen.

Figure 15.14 shows a block diagram of a gamma spectrometer, when gamma radiation is absorbed in the sensitive region of the detector, the energy deposited promotes electrons across the band-gap, with the number of electron-hole pairs thus produced being proportional to the absorbed energy. This charge is collected by a potential of a few thousand volts supplied by a high voltage power supply. A pre-amplifier covers the charge collected to a pulse, which is passed to the main amplifier. The pre-amplifier is necessarily mounted close to the detector. The main amplifier amplifies the pulse and suitably shapes it to be passed on to the MCA. MCA sorts the pulses by height to give a pulse-height spectrum, which is equivalent to an energy spectrum.

Most modern gamma spectrometers use personal computers (PC) to control and operate the MCA. The PC displays the gamma spectrum and the disk units to store the spectra. Packages

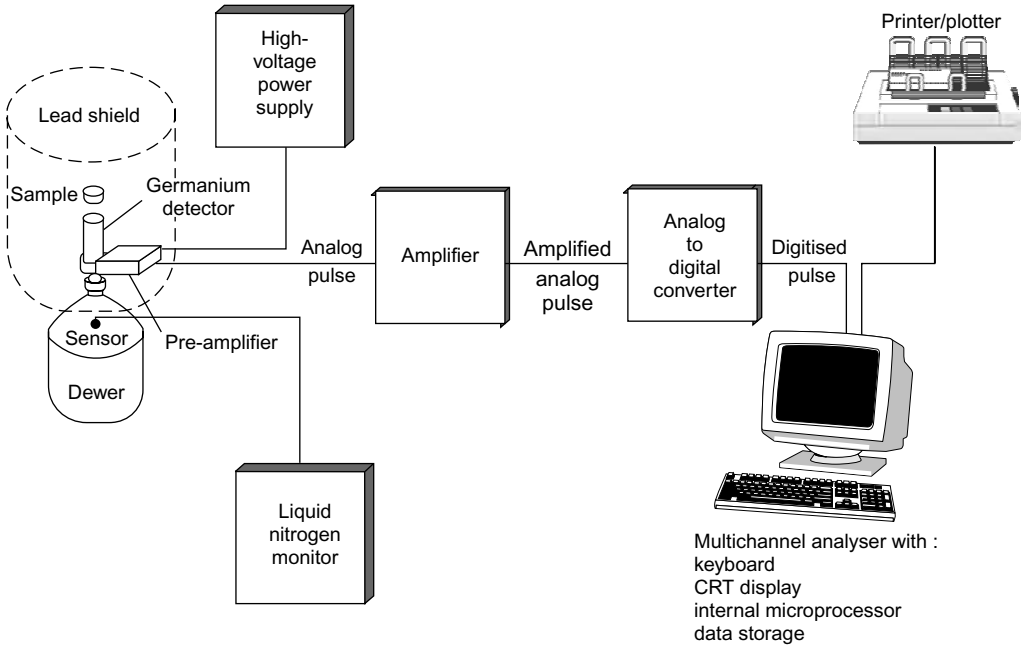


Figure 15.14 *General arrangement of gamma spectrometry system equipped with a high purity germanium detector (Courtesy: M/s Nuclear Data, IL)*

containing MCA hardware and spectrum analysis software that are contained on a plug-in card into a PC are available. The information obtainable from analysis would be the energy of peak maximum, which identifies the nuclide and the area of the peak, which when divided by the counting time to give a counting rate, quantifies the nuclide.

15.8 NEUTRON ACTIVATION ANALYSIS INSTRUMENTS

15.8.1 Neutron Activation Analysis

Neutron Activation Analysis (NAA) is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analysis of trace elements in samples from almost every conceivable field of scientific or technical interest. For many elements and applications, NAA offers sensitivities that are superior to those attainable by other methods, of the order of parts per billion or better. In addition, because of its accuracy and reliability, NAA is generally recognised as the 'Reference Method' of choice when new procedures are being developed or when other methods yield results that do not agree (Glascock, 2014).

In NAA, samples are activated by neutrons. During irradiation the naturally occurring stable isotopes of most elements that constitute the rock or mineral samples, biological materials are transformed into radioactive isotopes by neutron capture. Then the activated nucleus decays according to a characteristic half-life; some nuclides emit particles only, but most nuclides emit gamma-quanta, too, with specific energies. The quantity of radioactive nuclides is determined by

measuring the intensity of the characteristic gamma-ray lines in the spectra. For these measurements, a gamma-ray detector and special electronic equipment are necessary. As the irradiated samples contain radionuclides of different half-lives, different isotopes can be determined at various time intervals.

The basic essentials required to carry out an analysis of samples by NAA are a source of neutrons, instrumentation suitable for detecting gamma rays, and a detailed knowledge of the reactions that occur when neutrons interact with target nuclei.

15.8.2 Principle of Neutron Activation

The sequence of events occurring during the most common type of nuclear reaction used for NAA, namely the neutron capture reaction, is illustrated in Figure 15.15. When a neutron interacts with the target nucleus via a non-elastic collision, a compound nucleus forms in an excited state. The excitation energy of the compound nucleus is due to the binding energy of the neutron with the nucleus. The compound nucleus will almost instantaneously de-excite into a more stable configuration through emission of one or more characteristic prompt gamma rays. In many cases, this new configuration yields a radioactive nucleus which also de-excites (or decays) by emission of one or more characteristic delayed gamma rays, but at a much slower rate according to the unique half-life of the radioactive nucleus. Depending upon the particular radioactive species, half-lives can range from fractions of a second to several years.

In principle, therefore, with respect to the time of measurement, NAA falls into two categories: (1) prompt gamma-ray neutron activation analysis (PGNAA), where measurements take place during irradiation, or (2) delayed gamma-ray neutron activation analysis (DGNAA), where the measurements follow radioactive decay. The latter operational mode is more common; thus, when one mentions NAA it is generally assumed that measurement of the delayed gamma rays is intended.

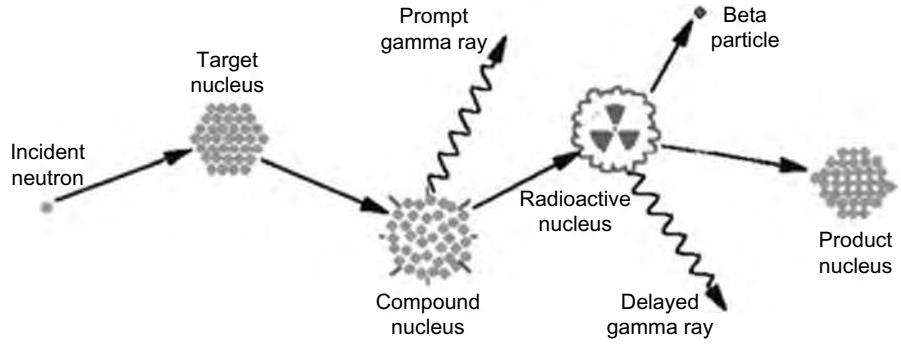


Figure 15.15 *Diagram illustrating the process of neutron capture by a target nucleus followed by the emission of gamma rays (Adapted from Glascock, 2014)*

15.8.3 Neutron Sources

Although there are several types of neutron sources such as reactors, accelerators, and radioisotopic neutron emitters one can use for NAA, nuclear reactors with their high fluxes of neutrons from uranium fission offer the highest available sensitivities for most elements. Different types of

reactors and different positions within a reactor can vary considerably with regard to their neutron energy distributions and fluxes due to the materials used to moderate or reduce the energies of the primary fission neutrons.

These neutron sources are accelerators where a convenient target material is bombarded by accelerated charged particles and the neutrons are produced in a nuclear reaction. In the most frequently used and commercially available neutron generators, deuterons are accelerated and the target material is tritium.

15.8.4 Instrumentation for Neutron Activation Analysis

The instrumentation used to measure gamma rays from radioactive samples generally consists of a semiconductor detector, associated electronics, and a computer-based, MCA. Most NAA labs operate one or more hyperpure or intrinsic germanium (HPGe) detectors which operate at liquid nitrogen temperatures (77K) by mounting the germanium crystal in a vacuum cryostat, thermally connected to a copper rod or 'cold finger'. Although HPGe detectors come in many different designs and sizes, the most common type of detector is the coaxial detector which in NAA is useful for measurement of gamma rays with energies over the range from about 60 keV to 3.0 MeV.

With the use of automated sample handling, gamma-ray measurement with solid-state detectors, and computerised data processing, it is generally possible to simultaneously measure more than thirty elements in most sample types without chemical processing. The application of purely instrumental procedures is commonly called instrumental neutron activation analysis (INAA) and is one of NAA's most important advantages over other analytical techniques, especially in the multi-element analysis. If chemical separations are done to samples after irradiation to remove interferences or to concentrate the radioisotope of interest, the technique is called radiochemical neutron activation analysis (RNAA). The latter technique is performed infrequently due to its high cost.

16

X-RAY SPECTROMETERS

X-rays are the short-wavelength, high-energy electromagnetic radiation associated with electronic transitions in atoms. The generation of X-rays and the interactions of X-rays with matter have numerous consequences of chemical importance.

X-radiation provides powerful tools for analytical purposes, which have advantages over other techniques. For example, in elemental analysis, it is, in principle, if not necessarily in practice, a non-destructive technique. Thus, a material may be analysed for its elemental constituents without the need for conversion (e.g. in atomic absorption spectroscopy) to some particular chemical form. Several distinct ways in which X-radiation can be employed for this purpose are based on their following characteristics:

Absorption of X-rays gives information about the absorbing material just as in other regions of the spectrum.

Fluorescence emission of X-rays enables to identify and measure heavy elements in the presence of each other and in any matrix.

Diffraction of X-rays enables analysis of crystalline materials with a high degree of specificity and accuracy.

16.1 X-RAY SPECTRUM

The most commonly used device for obtaining X-radiation is X-ray tubes. The conventional tube consists of a cathode (electron emitter) and an anode (target). Electrons from the cathode are accelerated by a high voltage (say 40 kV) applied to the target which is usually a heavy metal like molybdenum. The electrons, upon impact on the anode, produce a broad range X-ray spectrum. It is due to the deceleration of the impinging electrons by successive collisions with the atoms of the target material. It has been observed that the emitted quanta are generally of larger wavelength than the short-wavelength cut-off. The intensity of the continuum rises to a broad maximum and falls off gradually with increasing wavelength. This cut-off wavelength is given (in nanometres) by

$$\lambda_{\min} = \frac{hc}{Ve} = \frac{1240}{V}$$

where, h = Planck's constant
c = velocity of electromagnetic radiation
e = electronic charge
V = X-ray tube voltage in volts

When the potential across the tube is increased, a stage is reached where the energy is sufficient to eject an electron from one of the inner shells of the atoms constituting the target material. The place of the ejected electron is filled up promptly by an electron from an outer shell, releasing a photon of X-radiation, whose wavelength is dependent on the energy levels and hence characteristic of the material in the anode, or a specimen applied on the target. When originating in an X-ray tube, these lines will be superimposed on the continuum. As the inner electrons are not related to the state of chemical combination of the atoms (except for lighter elements), it follows that the X-ray properties of the elements are independent of chemical combination or physical state to a close degree of approximation.

X-ray wavelengths are normally specified as kX units, where 1 kX unit = 0.100202 nm. The commonly used wavelength unit in X-ray studies is the Angstrom (Å). However, in SI units, the X-ray wavelengths are expressed in nanometres (nm) or picometers (pm). For analytical purposes, the useful X-ray wavelength range is 70–200 pm.

16.2 INSTRUMENTATION FOR X-RAY SPECTROMETRY

Figure 16.1 highlights basic components of instrumentation associated with X-ray methods. The following are the important parts:

- X-ray generating equipment
- Collimator
- Monochromators
- Detectors

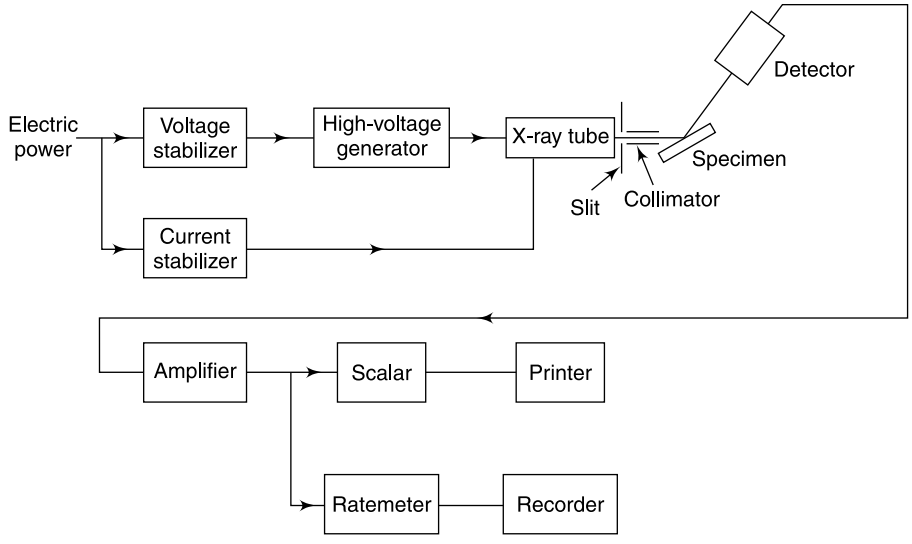


Figure 16.1 *Basic components of instrumentation for X-ray spectroscopy*

16.2.1 X-Ray Generating Equipment

X-ray generators used in analytical instruments are basically the same as the system first discovered to emit X-rays by Rontgen in 1996. They are generated in specially designed high-vacuum sealed-off tubes, which have heavy metals such as copper or molybdenum as target. Targets made of tungsten, iron, chromium, nickel are also sometimes used for special purposes.

Most conventional wavelength-dispersive X-ray spectrometers use a high-power (2–4 kW) X-ray bremsstrahlung source. Energy dispersive spectrometers use either a high-power or low-power (0.5–1.0 kW) primary source, depending on whether the spectrometer is used in the secondary or primary mode. In all cases, the primary source unit consists of a very stable high-voltage generator, capable of providing a potential of typically 40–100 kV. The current from the generator is fed to the filament of the X-ray tube, which is typically a coil of tungsten wire. The applied current causes the filament to glow, emitting electrons. A portion of this electron cloud is accelerated to the anode of the X-ray tube, which is typically a water-cooled block of copper with the required anode material plated or cemented to its surface. The impinging electrons produce X-radiation, a significant portion of which passes through a thin beryllium window to the specimen.

The target is usually ground to a slight angle, so that it gives the desired shape of the focal spot from the side. For fluorescence work, the focus is of larger size (about 5×10 mm) and is viewed at an angle of about 20°. However, for diffraction studies, wherein sharper definition is required, the focal spot is a narrow ribbon and the source appears to be very small when viewed from the end.

X-ray tubes are normally operated at 50–60 kV. Tubes operating at higher voltages, in the range of 100 kV, results in enhanced sensitivity, as the intensities of all lines increase with the increase in voltage. High voltages are generated in specially constructed transformers and employing full wave rectification.

16.2.2 Collimators

Collimation of X-ray beam is achieved by using a series of closely spaced, parallel metal plates, or by a bundle of tubes, 0.5 mm or less in diameter. Increased resolution is obtained by reducing the separation between the metal plates of the collimator, but it will be at the cost of intensity. Collimators may be used (i) between the specimen and the analyser crystal and (ii) between the analyser crystal and the detector.

16.2.3 Monochromators

An X-ray tube generates not only lines but also continuum radiation. This is because deceleration of high-energy electrons within the target leads to the emission of Bremsstrahlung (braking radiation) of all energies upon to a maximum determined by the voltage applied to the tube. True X-ray emission from the target atoms, of course, does not occur until the critical inner shell ionisation energy is achieved. Once this is exceeded, the intensity of line emission increases much more rapidly with increasing tube voltage than does the intensity of the background Bremsstrahlung or white radiation. Only the sharp features are determined by the nature of the target and, hence, they are termed the characteristic lines.

An X-ray tube however, can be used as a near-monochromatic source in a rather simple way which depends upon the special features of X-ray absorption by matter. Quantitatively, X-ray

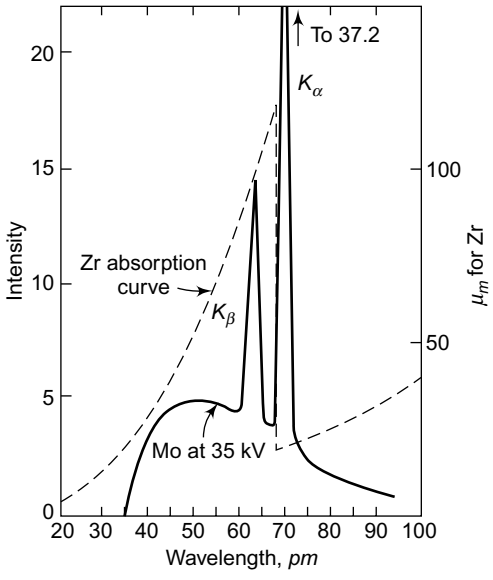


Figure 16.2 *The zirconium absorption curve and the molybdenum emission spectrum*

absorption at a given wavelength by a pure element is described by a law equivalent to the Lambert-Beer Law of UV and visible spectrophotometry, that is,

$$I_t = I_o e^{-\mu_m \rho x}$$

where

I_t = incident intensity of X-ray
I_o = transmitted X-ray intensity
μ_m = mass absorption coefficient (characteristic of the absorber)
ρ = density, and
x = thickness of absorber

Mass absorption coefficients are found to show a complex wavelength dependence showing sharp discontinuities, known as absorption edges, which correspond to the particular, allowed energy states of the absorbing atom.

It is, however, not so simple to construct a monochromator which can be varied as desired in the X-ray region, though narrow bands of wavelengths of various discrete points are simpler to obtain. Narrow bands can be achieved by different methods, which are discussed below:

(i) Using filters which consist of an element or its compound, which has a critical absorption edge (sharp discontinuities) at just the right wavelength to isolate a characteristic line from a source target. It is a common practice to insert a thin foil in the primary X-ray beam, to remove K_β-line from the spectrum, while transmitting the K_β-line with only a small loss of intensity. For example, 35-kV molybdenum emission spectrum results in *Mo-K*α (70.9 pm) and *Mo-*K_β (63.2 pm). Therefore, if a material could be found with a critical edge between these two wavelengths, it would absorb the β-line, while passing the α-line. Such a filter can be made of zirconium, which has its K-absorption edge at 68.9 pm. Figure 16.2 shows the zirconium absorption curve along with molybdenum emission spectrum. Characteristic wavelengths and filters for common targets of X-ray tubes are given in detail in Liebhafsky, (1972).

(ii) Using a monochromator, in which an analysing crystal of known spacing acts as a diffraction grating. This system could produce a narrow band of wavelengths at any point in the spectrum, if a continuous source of X-rays were available. The monochromator gives much better signal-to-noise ratio as compared to filters.

Since X-rays are fundamentally electromagnetic waves, the equation for diffraction by a grating which is $n\lambda = d \sin \theta$ is also applicable to X-rays. As the X-ray wavelengths are smaller by a factor of 1,000 or more, the grating space (d) should be smaller by about the same factor to obtain reasonable values of θ. This requirement is difficult to meet by a grating, as it is impracticable to rule it so finely. Fortunately, such orders of spacing exist between adjacent planes of atoms in crystals. The crystals suited for X-ray gratings are lithium fluoride, sodium chloride, topaz, calcium fluoride, gypsum, etc.

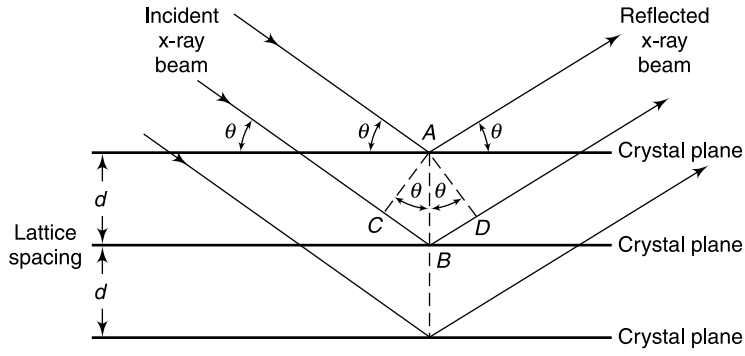


Figure 16.3 *Diffraction of X-rays from a set of crystal planes*

The monochromator arrangement consists in reflecting the X-ray beam from a plane crystal and selecting the wavelength by varying the angle. Referring to Figure 16.3, since the waves reflected at successive crystal planes must pass twice across the space between planes, the diffraction equation becomes

$$n\lambda = 2d \sin \theta$$

where d is the distance between adjacent planes in the crystal, and angle between the direction of the incident beam and that of the diffracted beam is 2θ. Obviously, the range of wavelengths obtainable from various analysing crystals is governed by the d-spacing of the crystal planes and by the geometric limits to which the goniometer (on which the crystal is mounted) can be rotated.

(iii) Using radioactive sources which emit radiations in the X-ray region, in which case X-ray tube with associated high-voltage power supply is not required. They are not preferred, because they cannot be turned off and radiation hazard is always present.

16.2.4 X-ray Detectors

The earliest method of detecting X-rays was the use of photographic film, which is no longer used for quantitative purposes, except for certain diffraction studies. Modem equipment use detectors, which are based on the properties of X-rays to produce flashes of light (scintillation) in certain materials or to cause ionisation in others. Details of their construction and working are given in Chapter 15. However, it may be noted that the spectral sensitivity of each detector varies with the wavelength of the X-radiation. This is illustrated in Figure 16.4., which shows the relative spectral response of the three most important

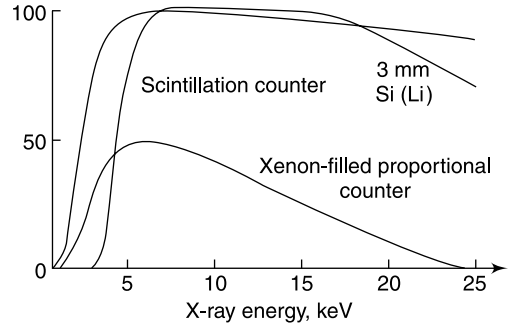


Figure 16.4 *Spectral response of solid-state [Si (Li)], scintillation and gas-filled proportional counters*

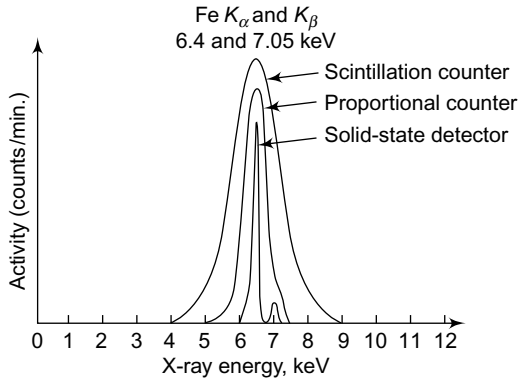


Figure 16.5 *Comparison of resolution between scintillation, proportional and Si (Li) detectors*

detectors. Each detector gives a somewhat broadened peak for a given monochromatic wavelength as shown in Figure 16.5. The abscissa scale is given in energy units (reciprocal of wavelength). It may be appreciated that the Fe-K_α and Fe-K_β lines at 6.40 and 7.05 keV (193.7 and 175.7 pm) are reproduced only by Si(Li) detector and not by other detectors.

Solid-state detectors are now the most widely used detectors. They employ silicon or germanium, sensitised to ionising radiation, such as X-radiation, by the addition of lithium. This diffusion of lithium into the crystalline material is called drifting. Figure 16.6 is a schematic illustration of typical lithium-drifted silicon detector. When an X-ray photon enters the crystal, it dislodges electrons from the lattice, causing vacancies or holes. When a potential is applied across the detector, a potential gradient is set up. When an X-ray photon hits the detector, electron-hole pairs are formed, which are collected at the electrodes. A pulse which is proportional to the energy of the X-ray is generated when the electron reaches the electrodes. The number of pulses indicates the intensity of the radiation.

The Si(Li) detector (Figure 16.7) consists of a small cylinder about 1 cm diameter and 3 mm thick of p-type silicon that has been compensated by lithium to increase its electrical resistivity. In order to inhibit the mobility of the lithium ions and to reduce electronic noise, the diode and its pre-amplifier are cooled to the temperature of liquid nitrogen. The pre-amplifier is located as close to the detector as possible to avoid amplification of electronic noise rather than signal. It incorporates a field-effect transistor (FET), which is used to reset the pulse amplification circuitry. The

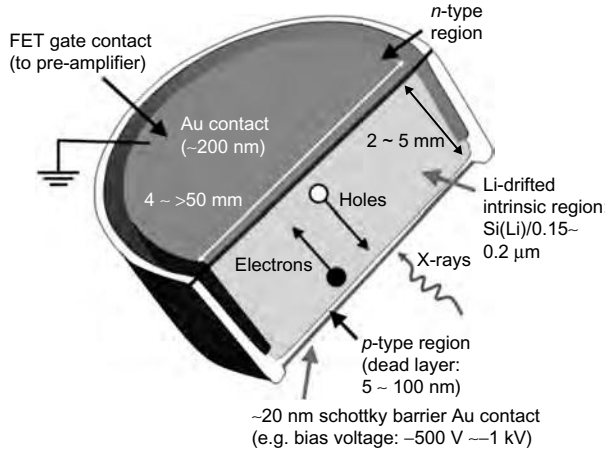


Figure 16.6 *Schematic illustration of typical lithium-drifted silicon detector (Courtesy: M/s Bio-Rad Laboratories)*

detector crystal and pre-amplifier are housed in a liquid N_2-cooled jacket separated from the electron column by a thin (8 mm) Be-foil window that is transparent to most X-rays of interest. The voltage pulses produced by the detector are fed into a linear pre-amplifier and then a multi-channel analyser, which is used to sort the arriving pulses at its input in the same fashion as to produce a histogram representation of the X-ray energy spectrum.

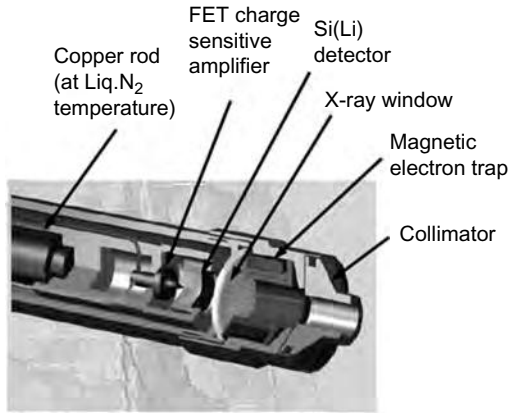


Figure 16.7 *SiLi X-ray detector (Courtesy: M/s Oxford Microanalytical Instruments)*

The electrical output from the solid-state detectors is much smaller than the corresponding signal from a scintillation detector, or a gaseous ionisation detector. Therefore, high gain, low noise and amplification is required when using these detectors. To diminish noise pick-up, the pre-amplifier is generally located close to the detector in the same housing. Using a pulse-height discriminator, it is possible to discriminate electronically unwanted signals of different wavelengths.

Gas-proportional counters and sodium-iodide scintillation counters suffer from poorer energy resolution. They are therefore, unable to differentiate between adjacent metals on the periodic table, which makes them unsuitable in such applications. Solid-state detectors prove useful in such a situation.

16.3 X-RAY DIFFRACTOMETERS

Diffraction is a wave property of electromagnetic radiation that causes the radiation to bend as it passes by an edge or through an aperture. Diffraction effects increase as the physical dimension of the aperture approaches the wavelength of the radiation. Diffraction of radiation results in interference that produces dark and bright rings, lines or spots, depending on the geometry of the object causing the diffraction.

These interference effects are useful for determining dimensions in solid materials, and therefore, crystal structures. Since the distances between atoms or ions is on the order of 10^{-10} m (1 Å), diffraction methods require radiation in the X-ray region of the electromagnetic spectrum.

The diffraction of X-rays is of great analytical significance, as it is applied to the study of the crystalline material producing the diffraction. No two chemical substances would form crystals, in which the spacing of planes is identical in all analogous directions. Thus, every crystalline substance would scatter the X-rays in its own unique diffraction pattern, giving a fingerprint or its atomic and molecular structure. X-ray diffraction is, therefore, adaptable to quantitative applications, because the intensities of the diffraction peaks of a given compound in a mixture are proportional to the fraction of the material in the mixture. This technique also furnishes a rapid, accurate method for the identification of the crystalline phases present in a material. In fact, sometimes it is the only method available for determining which of the possible polymorphic forms of a substance are present (e.g. carbon in graphite or in diamond).

16.3.1 Diffraction and Bragg's Law

Diffraction occurs as waves interact with a regular structure whose repeat distance is about the same as the wavelength. The phenomenon is common in the natural world, and occurs across a broad range of scales. For example, light can be diffracted by a grating having scribed lines spaced on the order of a few thousand Angstroms, about the wavelength of light (www.geosci.ipfw.edu).

X-rays have wavelengths on the order of a few Angstroms, the same as that of typical inter-atomic distances in crystalline solids. This means, X-rays can be diffracted from minerals which, by definition, are crystalline and have regularly repeating atomic structures.

When certain geometric requirements are met, X-rays scattered from a crystalline solid can constructively interfere with each other, there by producing a diffracted beam. In 1912, W.L. Bragg recognised a predictable relationship among the following factors:

- The distance between similar atomic planes in a mineral (the inter-atomic spacing) which is the d-spacing measured in Angstroms.
- The angle of diffraction theta measured in degrees. For practical reasons, the diffractometer measures an angle twice that of the theta angle. Not surprisingly, the measured angle is '2-theta'.
- The wavelength of the incident X-radiation, symbolised by the Greek letter lambda and which is equal to 1.54 Å.

These factors are combined in Braggs's Law:

$$n\lambda = 2d\sin\,\theta$$

where

n = an integer – 1, 2, 3,…, etc. (n = 1 for our calculations)
λ (lambda) = wavelength in Angstroms (1.54 Å for copper)
d (d-spacing) = inter-atomic spacing in Angstroms

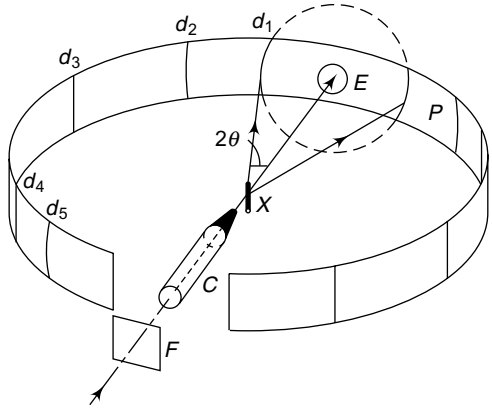


Figure 16.8 *Debye-Scherrer X-ray powder camera—geometrical features*

X-ray diffractometers are basically analogous to an optical grating spectrometer, with the difference that lenses and mirrors are not used with X-rays. Therefore, they appear quite different from their optical counterparts. The X-ray tubes, in some equipment, have several ports in different horizontal directions (when the tube is so mounted that the anode is vertical), permitting a number of experiments to be carried out simultaneously.

The diffracted beam of X-rays may be detected by one of the detectors described previously or photographically. A typical example of the use of a photographic method is in the Debye-Scherrer Powder Camera, which is illustrated, in Figure 16.8. The sample in the

powdered form, applied on any non-crystalline supporting material (such as paper) is placed in the path of the X-rays. The particles of the sample are randomly placed, so it is quite possible that these are oriented in every possible direction relative to the beam of X-rays. This will result in diffracted rays corresponding to all sets of planes on the crystals.

A strip of X-ray film is mounted in a circular position around the sample. Radiations are made to fall on the sample x, but before that it passes through filter F and collimator C. The undeviated central beam passes out through a hole E cut in the film strip P. Diffracted beams fall on the film at various points like d_1, d_2, d_3 etc. When the film is developed, it will show a series of lines on both sides of the central spot (produced by the undeviated beam). Obviously, the distance on the film from the central spot to any line will be a measure of the diffraction angle θ. So, if the wavelength and order n are known, the Bragg equation can be used to calculate the spacing d.

Cameras are usually constructed so that the film diameter has one of three values, 57.3, 114.6 and 143.2 mm. Cameras of larger diameter make it more convenient to measure the separation of lines, provided the lines are sharp. The sharpness of the lines is greatly determined by the quality of the collimating slits and size of the sample. The slit should be able to produce a fine beam, and the sample size should be small to give a point source of the diffracted beam. These will obviously need long exposures and a compromise must be arrived at of all these factors.

The choice of radiation wavelength is an important factor in X-ray diffractometry. Radiation having a wavelength just short of the absorption edge of an element contained in the sample of interest should not be used, because then the element absorbs the radiation strongly. The absorbed energy is emitted as fluorescent radiation in all directions, which increases the background and it becomes difficult to see the diffraction maxima. It is because of this reason that multi-window tubes with anodes of different materials such as tungsten, copper, molybdenum or silver are used in some commercial instruments.

Single crystal diffractometers are quite complex instruments, as they should provide a wide angular range for orienting and aligning the crystal under study and providing a sufficiently large traverse of the detector on a spherical surface. A typical example of a modern diffractometer is that of Norelco Goniometer, whose geometrical features are shown in Figure 16.9.

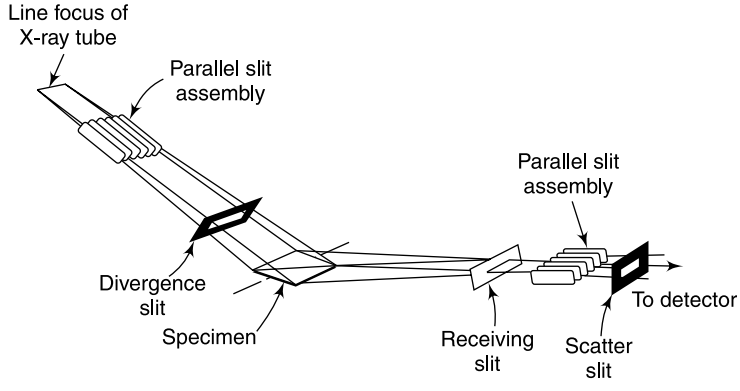


Figure 16.9 *Geometrical features of Norelco goniometer*

The X-ray tube provides a line source 0.06 by 10 mm in size with high intensity. The angular aperture (approximately 1°) of the beam is defined by a single divergence slit. Flat specimens up to 10 by 20 mm can be usually handled. The receiving slit defines the width of the reflected beam, which finally falls on the detector. Two sets of parallel slit assemblies (metal foils equally spaced) limit the divergence of the beam in any plane parallel to the line source. For producing an automatic record, the signal from the detector is amplified and fed into a pen recorder. The recording paper and the detector bearing arm are turned by synchronous motors. The recorded graph gives a plot of the intensity of the diffracted beam versus angle of diffraction, usually denoted by 2θ.

X-ray powder diffraction has been found to be a convenient method for identification of any compound that can be obtained essentially pure in crystalline form. The power of a diffracted beam is dependent on the quantity of the corresponding crystalline material in the sample. It is, therefore, possible to obtain a quantitative determination of the relative amounts of various constituents of a mixture of solids.

More than 90% of all powder diffractometers now sold are automated and are controlled by a personal computer. The major impact of computer automation on improving the accuracy of the measurement of diffraction angles has come from the algorithms which bring much more intelligence to the process than has been conventionally used in manual measurements.

The following are essentially the four elements to the automation of a diffractometer:

- The replacement of the synchronous θ: 2θ motor with a stepping motor and its associated electronics.
- The replacement of a conventional scaler/timer with one which can be remotely set and read.
- The conversion of the various alarms, limit switches and shutter controls to computer-readable signals.
- The creation of an interface which will allow a computer to control the above mentioned functions.

Automated powder diffractometers (APD) can give many hours of unattended and reliable data collection and can greatly assist in the tedious and routine tasks of data analysis, provided one is careful to apply the programs in the correct manner.

16.4 X-RAY ABSORPTION METER

Like other regions of the electromagnetic spectrum, X-rays are absorbed by matter and the degree of absorption is determined by the nature and amount of the absorbing material. The fundamental equation for absorbance of a monochromatic X-ray beam follows Beer's law, which may be expressed as follows:

$$P = P_o e^{-(\mu/\rho)\rho.x}$$

where P = radiant power after passage through an absorbing sample, x cm in length.

P_o = initial power of the radiation

ρ = density of the material

μ = linear absorption coefficient

The term μ/ρ is called the mass absorption coefficient, generally expressed as μ_m. The quantity depends upon the wavelength and the atomic properties of the absorbing substance as follows:

$$\mu_m = \frac{C \cdot Z^4 \cdot \lambda^4 \cdot n}{A}$$

where N is the Avogadro's number, A is the atomic weight of the absorbing material, Z the atomic number, λ the wavelength, n the exponent between 2.5 and 3, and C a constant over a range between characteristic absorption edges. The mass absorption coefficient μ_m is independent of the physical or chemical state of the specimen. In a compound or mixture, it is an additive function of the mass absorption coefficients of the constituent elements. i.e.

$$\mu_m T = \mu_{m1} W_1 + \mu_{m2} W_2 + \cdots$$

where μ_{m1} is the mass absorption coefficient of element and W_1 is the weight fraction and so on for the other elements present.

The variation of mass absorption coefficient with wavelength follows an exponential law. So, if the logarithms are plotted, a straight line should result with slope equal to the exponent of λ. A typical absorption curve of argon is shown in Figure 16.10. This graph shows a discontinuity at $\lambda = 387.1$ pm, which is known as the k-critical absorption wavelength of argon. Radiation of greater wavelength has inadequate energy to eject k-electrons of argon. Hence, it is not absorbed so greatly, as is radiation of greatly shorter wavelength.

X-ray absorption is of most value, where the element to be determined is the only heavy component in a material of low atomic weight. Examples of such analysis are lead in gasoline and chlorine in organic compounds. It may be noted that X-ray absorption spectrophotometers that provide a continuously variable wavelength of X-radiation are not commercially available. Instead, multichannel instruments are available. For industrial control purposes, absorption apparatus is usually designed specifically for each installation.

An atom absorbs an X-ray when the photon energy is sufficient to eject a photoelectron. Below this threshold energy, there is no absorption. Photons with energies greater than the threshold energy to produce a photoelectron are absorbed because the excess energy is conserved by transferring it to kinetic energy of the photoelectron. However, the probability of the absorption occurring decreases as the photon energy increases above the threshold.

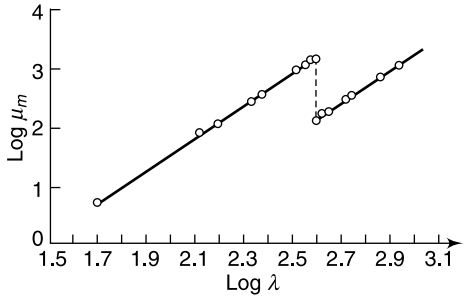


Figure 16.10 *X-ray absorption spectra of argon*

Use of X-ray absorption as an analytical method is not very uncommon because other techniques such as X-ray fluorescence are more sensitive. The absorption of X-rays by a certain element is often used in analytical instrumentation as a filter to block some X-ray wavelengths. For example, the absorption edge of Zr will block K_β and most of the continuum radiation of X-rays from a Mo target as illustrated in Figure 16.3.

16.5 X-RAY FLUORESCENCE SPECTROMETRY

The absorption of electromagnetic radiation by matter quite commonly results in the emission of radiation of the same or longer wavelengths (lower energies) and this phenomenon is referred to as luminescence. If the time over which luminescence occurs is very short, it is termed fluorescence, if longer, phosphorescence. Thus, prompt emission of an X-ray by an atom ionised by a higher energy X-ray is a form of fluorescence. Since such fluorescent emissions are characteristic of a particular element and virtually independent of its state of chemical combination, the measurement of the intensity of fluorescent X-rays can provide a simple, non-destructive means of quantitative analysis. The virtue of fluorescence as a means of X-ray generation is that, unlike electron bombardment, it is not associated with an X-ray continuum as a background to the characteristic lines. Hence, intensity measurement is inherently more sensitive.

X-ray fluorescence (XRF) spectroscopy is an extremely powerful tool for qualitative and quantitative determination of heavy elements in the presence of each other and in any matrix. Characteristic X-ray spectra are excited when the sample is irradiated with a beam of primary X-rays of greater energy than the characteristic X-radiation. XRF is based on the principle that the energy of the emitted X-rays depends on the atomic number of the atom (Z) and their intensity depends on the concentration of the atom in the sample.

Figure 16.11 shows the atomic processes involved in XRF. When a photon or charged particle of sufficient energy interacts with an atom, the atom may be excited releasing a specific electron out of an inner, *K* or *L* shell. In this situation, an outer shell electron can fall into the vacated inner shell, releasing energy as an X-ray. By measuring the photon energy of this fluorescent X-rays, the atom can be identified.

The energy of the emitted radiation depends upon the atomic energy level separation and on the atomic number. In 1913, Mosley showed the relation between atomic number *Z* and the reciprocal of the wavelength λ for each spectral line belonging to a particular series of emission lines for each element in the periodic table. This is given as:

$$C/\lambda = a\,(Z - \sigma)^2$$

where, C = Speed of light

a = constant of proportionality, and

σ = a constant whose value depends on the electronic transition series.

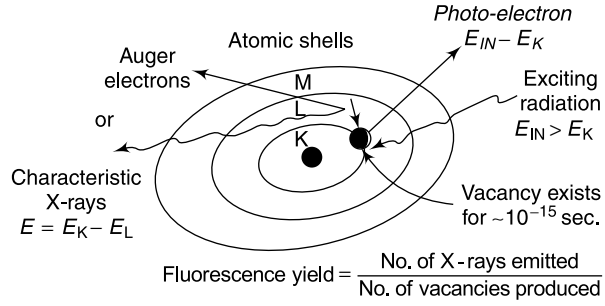


Figure 16.11 *Atomic processes involved in X-ray fluorescence*

The sensitivity and specificity of the XRF depends upon the following fundamental physical factors:

- The probability that the incident radiation will produce the desired excitation.
- The probability that the resulting readjustment of the atom will produce fluorescence X-ray emission.

The relationship between the excitation intensity and the intensity of fluorescence depends upon several factors, such as (a) the spectrum of the incident radiation, (b) angle of radiance, (c) molecular weight and matrix of the analyte and (d) absorption path length.

The intensity of fluorescent X-rays are smaller by a factor of approximately one thousand than X-ray beam obtained by direct excitation with a beam of electrons. Therefore, the fluorescent X-ray method would need high-intensity (tungsten-target) X-ray tubes, very sensitive detectors and suitable X-ray optics. The sensitivity of the analysis will depend on the peak-to-background ratio of the spectral lines. Due to the relative simplicity of X-ray spectra, the problem of spectral interference is not severe.

16.5.1 X-Ray Fluorescent Spectrometer

XRF analysers consist of:

- Excitation source – which bombards the sample with sufficient energy to induce fluorescent X-radiation
- Sample holder
- X-ray spectrometer (energy-analyser)
- Detection system

16.5.1.1 Excitation sources

Any source of radiation capable of producing vacancies in the inner shells of an atom can be used as an excitation source for XRF. It is, however, necessary to use mono-energetic source in order to reduce unwanted background due to scatter occurring over a broad range of wavelengths obscuring the fluorescence of interest. The commonly used sources for obtaining X-ray photon sources are X-ray tubes. Special devices are used to make the X-ray beam from the conventional X-ray tube more nearly monochromatic. This can be done in one of three ways: through the use of a (i) transmission-anode X-ray tube, (ii) secondary fluorescent target, or (iii) filters.

Transmission-anode X-ray tubes operate on the principle that any metal is a good transmission filter for its own X-rays. The anode material is so chosen that it produces characteristic radiation of the desired energy. The accelerating potential is so selected that the characteristic to bremsstrahlung X-radiation is maximum. The thickness of the anode is such that it will filter out the lower energy bremsstrahlung. In *secondary fluorescence*, an X-ray beam from a primary X-ray tube is used to excite secondary fluorescence from a target, whose material is such that it produces the most appropriate exciting radiation. *Filters* make use of a thin metal foil to isolate a more nearly mono-energetic excitation beam. For example, by using a nickel filter which has an absorption edge at 1.488 Å, the K_β of copper (1.392 Å) can be filtered leaving the K_α at 1.541 Å.

16.5.1.2 Spectrometers

The two main types of spectral analysers used in XRF are: (i) energy-dispersive and (ii) wavelength-dispersive type.

The energy-dispersive system is shown in Figure 16.12. It consists of an excitation source, a sample and a semiconductor detector. The fluorescent X-radiation resulting from irradiation of the sample reaches a detector, which produces an electrical pulse proportional to the energy of the X-rays. The energy level indicates the element involved, and the number of pulses counted at each energy level over the entire counting time is related to the concentration of the element.

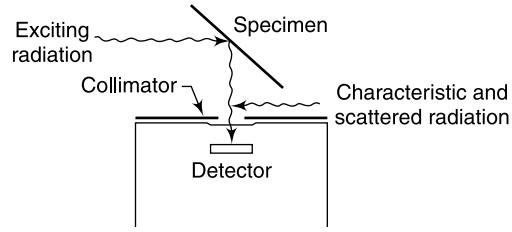


Figure 16.12 *Energy-dispersive system*

In the *wavelength-dispersive* systems, X-rays emitted by the sample are diffracted by a crystal to an angle according to the Bragg equation

$$\sin \theta = n\lambda / 2d$$

The detector receives the diffracted wavelength of interest and counts the pulses over the period of excitation. The range of wavelengths involved is scanned. Wavelengths at which the intensities peak, indicate the types of atoms involved and areas under the peaks are related to the concentration.

The technique derives its name from the fact that the analysis of the X-ray beam by diffraction is similar to spectrum analysis carried out with a diffraction grating. In this case, a crystal is used as a diffracting element. Essentially, the crystal possesses regular three dimensional lattice arrays of atoms and acts as an X-ray grating from which photons can be coherently scattered, so that they are in phase at certain angles. They reinforce each other producing a diffraction pattern.

A wide variety of wavelength-dispersive systems are available. The two commonly used arrangements are shown in Figures 16.13 and 16.14. Basically, the two systems differ only in the shape of the wavelength dispersing crystal, which may be flat, curved or of any other geometries. Details on choice of crystals and the relative advantage of flat, curved and other types of crystals are explained by Liebhafsky (1972).

In the flat-crystal arrangement (Figure 16.13), the primary and secondary slits and the analyser crystal are placed on the focal circle, so that Bragg's law will always be satisfied, as the goniometer is rotated. The detector is rotated by an angle twice the angular change in the crystal setting. The crystal is a flat plate 2.5 cm in width and 7.5 cm in length. The specimen is wildly held in an aluminium cylinder, although plastic material is used for examination of acid or alkaline solutions. Due to absorption of long-wavelength X-rays by air and window materials, some intensity losses of X-rays take place, which can be reduced by evacuating the goniometer chamber; or the air in the radiation - from the sample surface to the detector window may be replaced by helium. In some cases, vacuum spectrometers are also used.

The curved crystal arrangement (Figure 16.14) is more suitable for the analysis of small specimens. In this technique, collimators are not required, but increase in intensity is obtained by focusing the fluorescence lines. The crystal is bent to the diameter of the focusing circle and its inner surface is ground to the radius of the focusing circle. The radiation of one wavelength diverging from the entrance slit will be diffracted for a particular setting of the crystal and converge to a line image

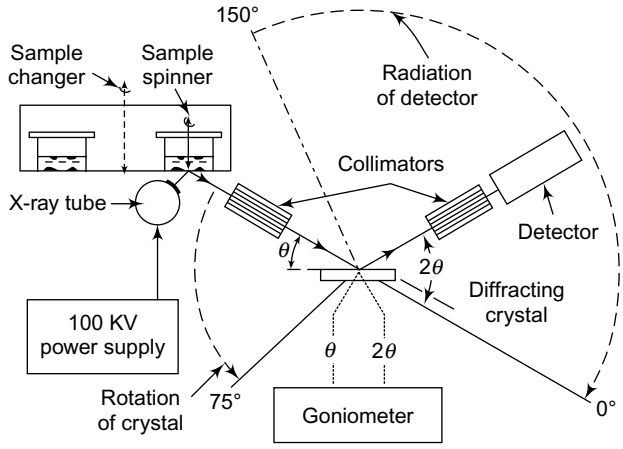


Figure 16.13 *Plane crystal wavelength-dispersive X-ray fluorescence spectrometer*

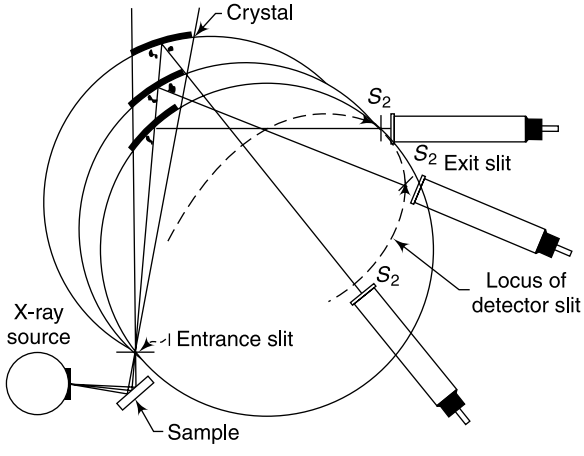


Figure 16.14 *Geometry of curved crystal wavelength-dispersive spectrometer*

at a symmetric point on the focusing circle. The angular velocity of the detector is twice that of the crystal and the two of them move along the periphery of the circle. The X-ray spectral lines are dispersed and detected just as in the flat-crystal arrangement.

16.5.1.3 Detectors

A variety of detectors are used for detection of X-radiation. The most common being the ionisation chamber, gas proportional counters, scintillation detectors and semiconductor devices. For wavelength-dispersive analysis, the resolution is determined by the complex mechanism, which aligns the detector and therefore, it is possible to use scintillation and gas proportional counters, which have relatively poor resolution, but very high count rates as detectors. For energy-dispersive analysis, it is necessary to use a detector which has good resolution and low noise and for this, the semiconductor detector is most suitable. In silicon semiconductor devices [Si(Li)], lithium drifted to compensate for electrically active impurity centres are now the most widely employed detectors for energy-dispersive analysis. They are stable, show a high count-rate capability, have a small size and low background. They give adequate resolution (150 eV at 3.3 keV), which allows the separation of element pairs, as low in the periodic table as carbon/nitrogen. A resolution of 3 eV at 3.3 keV or better is reported with wavelength dispersion systems, which eliminates most line interferences and is able to distinguish between various metallic electronic states. The disadvantage of such detection is that they require low temperatures (say 77 K) for efficient operation and hence, are somewhat inconvenient to maintain. Similarly, germanium detectors, although provide potentially better energy

resolution, have the disadvantage that they must be operated at low temperatures and suffer from high leakage.

For *qualitative* analysis, the angle θ between the surface of the crystal and the incident fluorescence beam is gradually increased. At certain well-defined angles, the appropriate fluorescence lines are reflected. Additional factors for identification of a particular element may be obtained from relative peak heights of fluorescence lines, the critical excitation potential and pulse-height analysis. For *quantitative* analysis, the intensity of a characteristic line of the element is measured. The goniometer is set at the 2θ angle of the peak and counts are collected for a fixed period of time. Alternatively, the time is measured for the period required to collect a specified number of counts. The goniometer is then set to the portion of the spectrum, where only background count is obtained. The net line intensity (peak minus background) in counts per second is then related to the concentration of the element with the help of a calibration curve.

16.5.2 Total Reflection X-Ray Fluorescence Spectrometer

Total reflectance X-ray fluorescence (TX RF) spectroscopy is a modern technique for the determination of ultra-trace amount of elements in various kinds of samples. Here, the specimen is excited by the primary X-ray beam at a glancing angle less then the critical angle at which total external reflection occurs and primary excitation radiation is incident on plane, polished surface serves either as a sample support or is itself the object to be examined. The design of a TXRF is illustrated by Tiwari et al., (2002).

A schematic representation of TXRF spectrometer is shown in Figure 16.15. It essentially comprises of an X-ray generator, a slit-collimator arrangement, a monochromator stage, a sample reflector stage (i.e. sample carrier) and an X-ray detection system. The primary beam emitted by X-ray

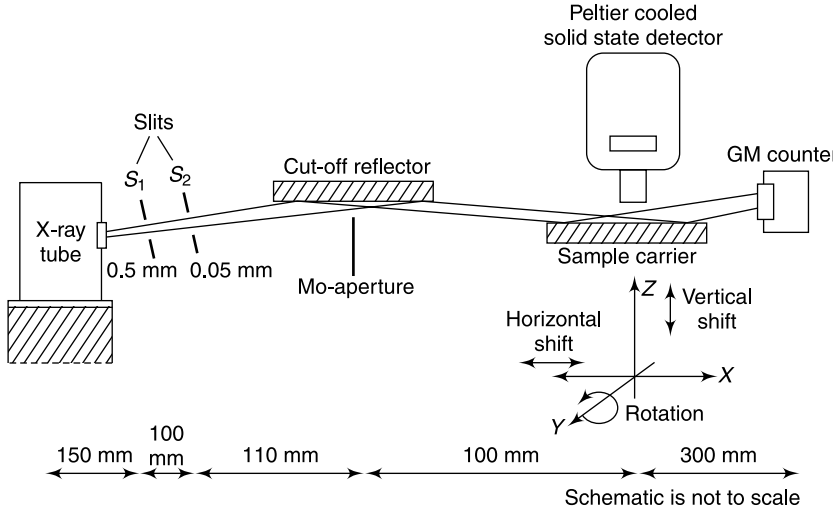


Figure 16.15 *Schematic of the total reflection X-ray fluorescence (TXRF) set up (Adapted from Tiwari et al., 2002)*

tube from its line-focus is collimated by the slit-collimator arrangement, which generates incident X-ray beam. A polished silicon crystal (Si-111) of dimensions 30 × 34 mm, and 0.3 mm thickness, is mounted on the first reflector stage to act as a cut-off reflector. The grazing angle on the cut-off reflector is set to such a value that it cuts off the higher energy part of primary radiation, thereby making the primary X-ray beam quasi-monochromatic.

The suppression of high-energy bremsstrahlung radiation improves the signal-to-background ratio. The second reflector stage serves as sample carrier through which the fluorescence measurement of a sample material can be performed under external total reflection condition. A float glass is mounted as the sample carrier on this stage in the upward looking direction. A 2-mm thick Mo aperture is placed near the cut-off reflector stage to shield the sample reflector from the direct primary X-ray beam.

The detection system for energy-dispersive measurement consists of a peltier cooled solid-state detector, a spectroscopy amplifier and a multi-channel pulse-height analyser installed in a personal computer. The solid-state detector has an energy resolution of 240 eV at 5.9 keV and operates without liquid nitrogen. The detector is placed very close and normal to the sample substrate, so that a large solid angle is intercepted which thereby maximises the counting efficiency. To monitor the intensity of the primary beam, a GM counter is placed in the specular reflection direction.

The slit-collimator assembly comprises of a pair of precisely aligned Mo slits of dimensions 10 × 0.5 mm (S_1) and 10 × 0.05 mm (S_2), respectively and are separated by 100 mm. This slit-collimator assembly makes primary beam dimensions 10 mm wide and 0.05 mm thick with a beam divergence of ~0.01°, which is small enough to obtain total external reflection. The vertical motion (Z-motion) as well as tilting motion (ø-motion) for whole slit-collimator assembly is provided by means of two micro-metres. The angular tilt motion can tilt X-ray beam around X-axis in a controlled manner by using sine-bar arrangement.

The two reflector stages have separate mechanical arrangements for rotation (θ-motion) as well as for the vertical shift (Z-motion). Two micro-metres of least count 1 μm are used for each reflector stage –one for vertical movement and the other for incident angle adjustment using sine-bar arrangement.

All mechanical assemblies including the slit-collimator arrangement, the first reflector stage and the second reflector stage, are mounted on a rigid stainless steel base plate. The TXRF module is connected directly to the window of X-ray generator. The spectrometer has provision for mounting of various types of optical elements such as cut-off-reflector, multi-layer filter or natural crystal at the first reflector stage. Therefore, by choosing appropriate optical element, the energy bandwidth of primary radiation can be tailored to suit the excitation of a given sample.

The most critical aspect of XRF analysis is sample preparation. The major problem is associated with matrix effects, i.e. absorption of both the primary and emitted radiation by the elements present in addition to those of interest. Unless matrix absorption can be made negligible or constant, apparent emission intensities do not vary linearly or reproducibly with concentration.

A number of different procedure may be used to correct for matrix effects. For example, in computer controlled iterative correction, initial raw intensity data may be used to estimate and elemental composition which can then be used to calculate absorption corrections. The cycle is then repeated until consistent results are obtained. The procedure is complex, but where an unknown matrix cannot be avoided, it is the only reliable technique.

Although XRF is, in principle, a non-destructive analysis, appropriate preparation of a sample generally necessitates its destruction. The sensitivity of the method is very dependent on matrix

effects, though under optimum conditions concentrations as low as 1 ppm may be detected. For maximum emission intensity, as large an area as possible (~10 cm^2) is irradiated, the source being ~3 cm. from the sample. New developments such as the synchrotron X-ray microscope do, however, allow levels as low as 1 ppm in a volume as small as 10^{-9} cm^3 to be determined.

XRF technique is inherently very precise and rivals the accuracy of wet chemical methods. It is attractive for elements which lack reliable wet chemical methods, such as tantalum and rare earths, and simultaneous analysis of several elements is possible with automated equipment.

16.6 ELECTRON PROBE MICRO-ANALYSER

The electron micro-probe is basically a combination of an electron microscope and an XRF spectrometer, with a visual microscope attached. Its use offers primarily the only method for detailed elemental analysis of materials which are heterogeneous at the (visual) microscopic level. By bombarding the sample with high-energy electrons in a vacuum, the sample surface may be mapped by detection of scattered electrons (electron microscopy) and its elemental constitution determined by detection of emitted X-rays.

Electron probe micro-analysis is a technique for the non-destructive elemental analysis by utilising a finely focused electron beam to excite X-rays in a solid sample as small as 1 μm in diameter. Figure 16.16 shows a schematic diagram of a typical apparatus, in which three types of optics are employed: (i) electron optics, (ii) light optics and (iii) X-ray optics.

The electron optics consists of an electron gun followed by two electromagnet lenses, which form the electron beam probe. This section of the instrument is very similar to an electron microscope. The specimen is mounted inside the vacuum column of the instrument and under the beam as the target.

The electrons are accelerated by a voltage between 5 and 50 kV maintained stable to at least 1 part in 10^4 and the whole system operates under a high vacuum (< 10^{-10} torr). Electromagnetic lenses focus the electron beam to a diameter between 0.1 and 4 mm, but as at the energies required to cause X-ray generation, the electrons spread laterally and longitudinally in the sample by approximately 1 mm, thus, the effective resolution limits is 1 mm. The micro-probe has the same limits in terms of elements conveniently analysed as an ordinary XRF spectrometer, but its sensitivity is poorer (100–500 ppm) because the characteristic X-ray emissions must be detected against a background of white

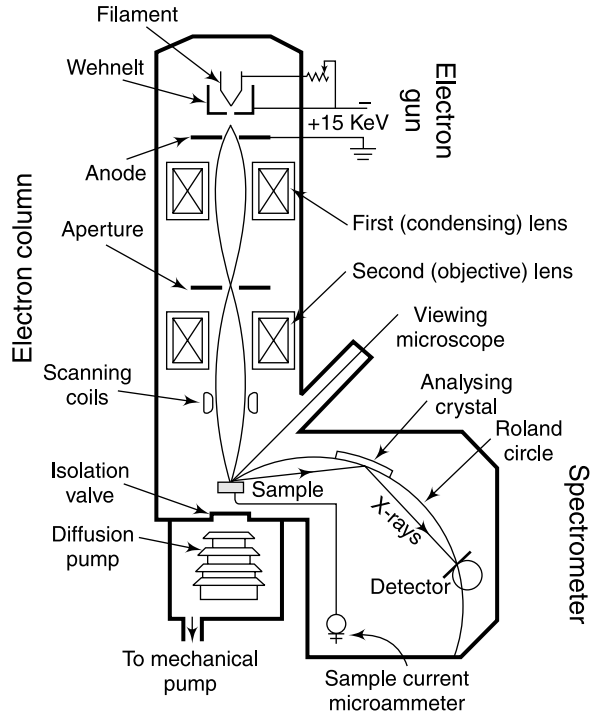


Figure 16.16 *Electron micro-probe analyser*

radiation. This sensitivity difference is offset, however, by the fact that the micro-probe can be used on inhomogeneous material and focused to a very small area, so that as little as 10^{-15} g of material in 10 m^3 can be detected. In conventional XRF analysis, the detection limit is ~10^{-8} g.

The electron bombardment excites characteristic X-rays, which are analysed in an X-ray spectrometer, which is of curved crystal type. The optical microscope helps the operator to locate precisely the desired spot on the sample. Point-by-point micro-analysis is obtained by moving the sample across the beam. This method allows analysis of extremely small objects with the limit of detectability (in a 1 μ size region) about 10^{-14} g. It is particularly useful for phase studies in metallurgy and ceramics for following the process of diffusion in the fabrication of semiconductors and corrosion studies, where excitation is restricted to thin surface layers, as the beam penetrates to a depth of only one or two microns into the specimen.

Matrix corrections are a major problem in quantitative micro-probe analysis and demand computer controlled iterative application of numerous empirically determined correction factors.

While the power of the electron micro-probe in analysis is readily appreciated, its deficiencies in micro-analysis should also be noted. These deficiencies stem from the large irradiation area, the inherent limitations of XRF (to the heavier elements), lack of sensitivity, inability to deal with thin films, and inability to provide information on isotopic composition of an element.

Electron probe micro-analysers are made by several commercial firms. However, an alternative method using lasers for vaporising materials from small focused areas and analysing the vapours by mass spectrometry, optical emission or absorption photometry is gaining popularity.

17

GAS CHROMATOGRAPHS

17.1 CHROMATOGRAPHY

A sample that requires analysis is often a mixture of many components in a complex matrix. For samples containing unknown compounds, the components must be separated from each other so that each individual component can be identified by standard analytical methods. The separation properties of the components in a mixture are constant under constant conditions, and therefore once determined they can be used to identify and quantify each of the components. Such procedures are typical in chromatographic separations.

Chromatography is a physical method of separation of the components of a mixture by distribution between two phases, of which one is a stationary bed of large surface area and the other a fluid phase that percolates through or along the stationary phase. Chromatography was first reported by a Russian botanist Tswett, who separated leaf extract into coloured bands using a column of inulin and a solvent of ligroin. The technique remained largely ignored until the 1930s, when chromatographic separations of carotenes and xanthylls further demonstrated the possibilities of the technique and accelerated its development. The high separating power of gas chromatography has made possible the analysis of samples, which were hitherto regarded as difficult or impossible. This is evident by the wide range of applications found in routine and research work in medical and industrial fields. However, the applicability of the technique is limited to those substances which may be vaporised without decomposition or which may be thermally decomposed in a reproducible manner. Several techniques are used in chromatography as shown in Figure 17.1.

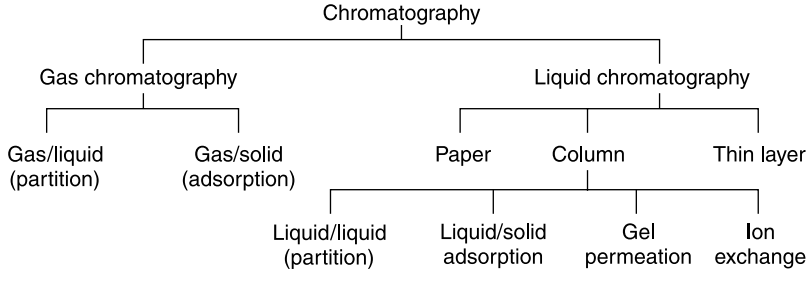


Figure 17.1 *Techniques used in chromatography*

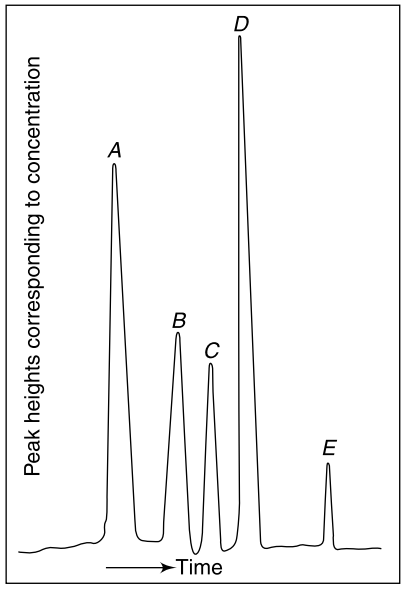


Figure 17.2 *Typical gas chromatogram*

The process of chromatographic separation involves transport of a sample of the mixture through a column. For this purpose, the mixture may be in the liquid or gaseous state. The stationary phase may be a solid adsorbent or liquid partitioning agent. The mobile phase is usually a gas or a liquid and it transforms the constituents of the mixture through the column. During such transport, the material in the column (stationary phase) exercises selective retardation on the various components of the sample. This retardation may be due to adsorption, solubility, chemical bonding, polarity or molecular filtration of the sample. Therefore, the components of the mixture tend to move through at different effective rates, and thereby result in tending to segregate into separate zones or bands. In general, all chromatographic procedures isolate, detect and characterise these bands at some point, usually the column exit. Upon emerging from the column, the gaseous phase immediately enters a detector attached to the column. At this place, the individual components register a series of signals, which appear as successive peaks above a baseline on the recorded curve, called chromatogram. A typical chromatogram is shown in Figure. 17.2. The area under the peak gives a quantitative indication of the particular component and the time delay between injection and emergence of the peak serves to identify it.

The nature of the mobile phase is frequently used to label some of the major forms of chromatography, such as those designated by the acronyms GC (Gas Chromatography) LC (Liquid Chromatography) and SFC (Supercritical Fluid Chromatography).

Other classifications are based on the way the stationary phase is supported. For example, when the stationary phase is spread as a thin, adherent layer on a flat plate of an inert material such as glass or aluminium, the technique is referred to as planar chromatography, the best known variant being Thin Layer Chromatography or TLC. TLC can be used for quantitative analysis and preparative work but is most commonly applied to qualitative analysis of complex mixtures and assessment of purity. In most forms of chromatography important in quantitative analysis, the stationary phase is supported in a long, tubular column usually made of glass on stainless steel. When the acronyms GC, LC and SFC are used, it is usually understood that particular forms of column chromatography are being referred to. Thus, the standard chromatograph is a column into which a mixture, transported by some fluid, is passed and from which, at some later times, the spatially separated components of the mixture emerge.

17.2 BASIC DEFINITIONS

17.2.1 Retention Time

The retention time (t_R) is the total time that a compound spends in both the mobile phase and stationary phase. In other words, the time between sample injection and an analyte peak reaching a detector at the end of the column is termed the retention (t_R). Each analyte in a sample will have a different retention time. Retention time is generally expressed in minutes.

The time taken for the mobile phase to pass through the column is called t_M. This shows in Figure 17.3.

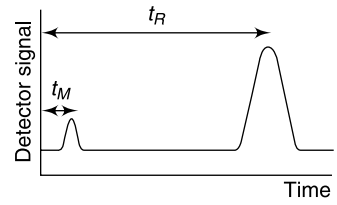


Figure 17.3 *Illustration of 'Retention Time'*

17.2.2 Dead Time

The dead time (t_m) is the time a non-retained compound spends in the mobile phase which is also the amount of time the non-retained compound spends in the column. Dead time is generally reported in minutes.

17.2.3 Adjusted Retention Time

The adjusted retention time (t_R') is the time a compound spends in the stationary phase. The adjusted retention time is the difference between the dead time and the retention time for a compound.

$$T_r' = t_r - t_m$$

17.2.4 Capacity Factor (or Partition Ratio)

The capacity factor (k') is the ratio of the mass of the compound in the stationary phase relative to the mass of the compound in the mobile phase. The capacity factor is a unitless measure of the column's retention of a compound

$$K = \frac{t_r - t_m}{tm}$$

17.2.5 Phase Ratio

The phase ratio (β) relates the column diameter and film thickness of the stationary phase. The phase ratio is unitless and constant for a particular column, and represent the volume ratio β.

$$\beta = r/2.d_f$$

17.2.6 Distribution Constant

The distribution constant (K_D) is a ratio of the concentration of a compound in the stationary phase relative to the concentration of the compound in the mobile phase. The distribution constant is constant for a certain compound, stationary phase and column temperature.

$$K_D = \frac{\text{Concentration of compound in stationary phase}}{\text{Concentration of compound in mobile phase}} = k\,.\,\beta$$

17.2.7 Selectivity (or Separation Factor)

The selectivity (α) is a ratio of the capacity factors of two peaks. The selectivity is always equal to or greater than one. If the selectivity equals one, the two compounds cannot be separated. The higher the selectivity, the more separation between two compounds or peaks.

$$\alpha = KA/KB$$

17.2.8 Linear Velocity

The linear velocity (u) is the speed at which the carrier gas or mobile phase travels through the column. The linear velocity is generally expressed in centimetres per second.

$$u = L/t_m$$

17.2.9 Efficiency

The efficiency is related to the number of compounds that can be separated by the column. The efficiency is expressed as the number of theoretical plates (N, unitless) or as the height equivalent to a theoretical plate (HETP), generally in millimetres. The efficiency increases as the HETP decreases, thus more compounds can be separated by the column. The efficiency increases as the number of theoretical plates increases, thus the column's ability to separate two closely eluting peaks increases.

$$N = 5.545.\ [t_r/W_h]^2$$
$$H = L/N$$

Theoretical plates concept (Figure 17.4) supposes that the chromatographic column contains a large number of separate layers. Separate equilibrations of the sample between the stationary and mobile phase occur in these plates. The analyte moves down the column by transfer of equilibrated mobile phase from the one plate to the next. It may be noted that plates do not really exist, but the concept helps us understand the processes at work in the column. They serve as a way of measuring column efficiency.

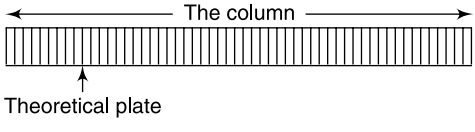


Figure 17.4 *Theoretical plate model of chromatography*

The nomenclature of chromatography normally used in practice is given by Ettre (1979).

17.3 GAS CHROMATOGRAPHY

Gas chromatography (GC) is an analytical technique for separating compounds based primarily on their volatilities. GC provides both qualitative and quantitative information for individual compounds present in a sample. Compounds move through a GC column as gases, either because the compounds are normally gases or they can be heated and vaporised into a gaseous state. The compounds partition between a stationary phase, which can be either solid or liquid, and a mobile

phase (gas). The differential partitioning into the stationary phase allows the compounds to be separated in time and space.

To be suitable for GC analysis, a compound must have sufficient volatility and thermal stability. If all or some of a compound's molecules are in the gas or vapour phase at 400–450°C or below, and they do not decompose at these temperatures, the compound can probably be analysed by GC.

The particular advantage of using a gas as a mobile phase is that high flow rates are possible even with long columns. Another important practical advantage is that several methods of detecting components in a flowing gas stream are available. A further advantage is that it is relatively early to find a chemically innocuous gas which acts as no more than a carrier of various vapours, though this can also be regarded as a disadvantage in that the mobile phase cannot be used to modify discrimination between materials.

In its simplest form, gas chromatography can be regarded as a multiple distillation process, with separations determined by relative volatilities (i.e. differences in the liquid vapour equilibrium of various materials). Temperature variation is the obvious way of controlling volatility and this is basically why temperature control is an important aspect of the operation of gas chromatograph.

Volatility is not, however, the only factor of importance, since specific interactions with the stationary phase, which is usually a liquid adsorbed on a solid but which can be a simple solid, do occur. The separation of simple gases such as O_2, N_2, CH_4, CO and CO_2, for example, can be achieved on a molecular sieve column, where the size of pores within the stationary phase determines whether or not a molecule enters and diffuses within the solid, so being retarded relative to the carrier gas.

One of the recent advances in gas chromatography, in fact, has been a change to capillary glass columns rather than the sometimes chemically reactive stainless steel columns predominant originally. (This use of glass capillaries can be regarded as an offshoot of the development of fibre optic technology.) Another recent development has been the use of open tubular rather than packed columns. In these, the stationary phase is present as a very thin film on the walls only of the column rather than being adsorbed on a finely divided solid which fills the column void. The open column allows high flow rates and the thin film allows very efficient mass transfer, so that extremely small values of H result.

17.4 BASIC PARTS OF A GAS CHROMATOGRAPH

The basic parts of a gas chromatograph are shown in Figure 17.5. It consists of the following parts:

- Carrier gas supply along with pressure regulator and flow monitor
- Sample injection system
- Chromatographic column
- Thermal compartment or thermostat
- The detection system
- Recorder

The carrier gas, normally N_2, Ar or He, is usually available in a compressed form in a cylinder fitted with a suitable pressure regulator. The gas is conducted from the cylinder through a flow regulator, to a sample-injection port maintained at a certain temperature T_1, which is such that it ensures rapid vaporisation, but not thermal degradation of the solute. Gas and liquid samples are

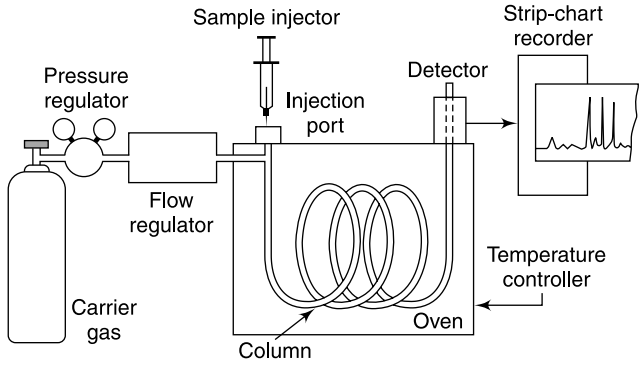


Figure 17.5 *Block diagram of a gas chromatograph*

almost always injected by syringe through a self-sealing silicon rubber diaphragm in the injection port. The solute vapour mixes almost instantaneously with the flowing carrier gas and is swept into the chromatographic column, which is the heart of the chromatograph. It is there that the different solutes in the vaporised sample are separated from each other, by virtue of their different interaction with the column packing. The column is maintained at another temperature T_2. This temperature-determines the time for the passage of the solutes and to some extent, the resolution and efficiency obtained with a particular column. At the end of the column, the solutes emerging individually enter the detector, which produces an electrical signal corresponding to the quantity of solute leaving the column. The detector signal is supplied to a recorder and a plot of the time-signal amplitude called chromatogram, is obtained. This record is used to determine the identity of the components in the mixture and their respective concentrations.

At a fundamental level, acquisition of chromatographic results has been little changed since the early days of GC, though the digital revolution has meant that strip chart recorders, once the mainstay of collecting chromatograms, cannot be found today and only electronic recording-integrators or microcomputers are used. Signal from the detector amplifier is digitised and stored to disk allowing enormous convenience in retrieving and replaying results. This means that peak retention times, peak areas, etc. are automatically reported. In addition, software allows the results to be displayed in an automated manner (i.e. reports can be generated according to standard reporting formats). All this can be economically integrated into the total instrument control and management through computers and is an option on all chromatographs and a standard feature on most instruments.

The various parts of a gas chromatographic system are described below:

17.4.1 Carrier Gas Supply or the Mobile Phase

In a gas chromatograph, the mobile phase is formed by a continuous supply of a carrier gas. This supply is taken from commercially available cylinders, in which they are stored at pressures up to 2,500 lb/sq.in. They pass through the column at low rates of flow (20–50 ml/min), at pressures not much greater than atmospheric pressure. The carrier gas supply system comprises a needle valve, a flowmeter$_1$ a pressure gauge and a few feet of metal capillary restrictors.

17.4.1.1 Types of gases

Several gases like hydrogen, helium, nitrogen, argon and carbon dioxide have been tried as carriers. The carrier gas affects column as well as detector performance. The carrier gas which is best for a particular detector may not always be the best for the required separation. Mostly the choice of the gas is determined by the type of the detector and the ready availability of the gas. For example, helium and hydrogen are preferred when thermal conductivity detection is employed, since their thermal conductivities are much higher than those of the compounds to be separated. Similarly, argon is used with argon-ionisation gauge detectors. Carbon dioxide is used with integral detection systems involving the removal of carrier gas by absorption in alkali solution. On the basis of separation power, nitrogen, argon and carbon dioxide are slightly better than the lighter gases, as the latter have a tendency to enhance axial diffusion of the solutes, a factor that could seriously affect the efficiency of the column. Nitrogen is particularly used where separating power is more important than high detector response.

17.4.1.2 Purity of gases

The presence of contaminants in the carrier gas may affect column performance and detector response, particularly when ionisation detectors are used. Carrier gases may be purified by inclusion of a trap containing a molecular sieve or 5 Å. This is usually adequate for removal of hydrocarbons and water vapour. Ultra-pure N_2 for use with flame-ionisation detector (FID) can be generated by commercially available apparatus. A low oxygen content of the carrier gas is essential for obtaining repeatable retention times and peak widths, because a small amount of oxygen is enough to change the liquid phase and affect the overall partition ratio. In programmed temperature gas chromatography, a high purity carrier gas is even more important than in isothermal operation. The column is saturated at every temperature with the carrier gas impurities. During the heating-up cycle, the saturation level changes and the impurities are given off to a certain extent. These impurities, which are liberated, are eluted and will temporarily increase the zero signal and noise, resulting in a broad peak.

17.4.1.3 The gas flow rate

The rate of the gas flow to be used in a particular analysis, among other factors, depends upon column diameter. The flow is generally in the range 10–400 ml/min – very low and very high flow rates may affect the efficiency adversely. Flow rate should be controlled within 1%, in order to reduce analytical errors. It should also be constant in order to give reproducible retention times. The flow rate of the carrier gas also affects the detector signals, because fluctuations would produce variable heat removal from the Katharometer filaments and hence cause variable filament temperature response and sensitivity.

The flow of the carrier may be maintained constant by inserting a capillary before the column, so that a pressure drop much larger than the pressure drop in the column is created. When this capillary is kept at a constant temperature, the flow in the whole system will be mainly determined by this capillary and not by varying flow resistance of the column. Alternatively, the gas is passed through a short piece of capillary tubing, resulting in a small pressure drop. A regular pressure regulator connected across the capillary keeps the pressure drop constant, resulting in a constant flow. With a view to speed up analysis, flow programming in which the flow through the

column continuously increases can be adopted. This would have the same effect, as in temperature programming of producing closely spaced peaks at the end of the chromatogram. For columns of different sizes, the volumetric gas flow rate should be varied in proportion to the square of the diameters, so as to maintain the average linear rate at approximately the same value.

17.4.2 Sample Injection System and the Size of the Sample

The purpose of the sample-injection system is to introduce a reproducible quantity of the sample to be analysed into the carrier gas stream. The transfer of the sample should be made rapidly to ensure, that the sample occupies the smallest column volume and thus prevents excessive peak broadening, which affects the overall resolution of the system. Samples can be introduced in their gaseous liquid or solid states and many methods have been suggested for the purpose. However, the choice of the method of sample injection depends upon the pressure in the column at the point of introduction, the type of detector to be used and the source of the sample.

The injector basically is a hollow, heated, glass-lined cylinder where the sample is introduced into the GC. The temperature of the injector is controlled so that all components in the sample will be vaporised. The glass liner is about 100 mm long and 4 mm internal diameter.

Theoretically, for optimum separation efficiency, the sample must be introduced into the column as rapidly and in as concentrated a band as possible. This is called Plug insertion that chromatographers consider ideal. The purpose of the sample-injection system is to insert, volatilise and move the resulting gaseous sample into the column. The design of this system is a critical gas chromatograph performance factor, because a column capable of efficient component resolution may appear ineffective, due only to the inadequacy of the sample insertion system.

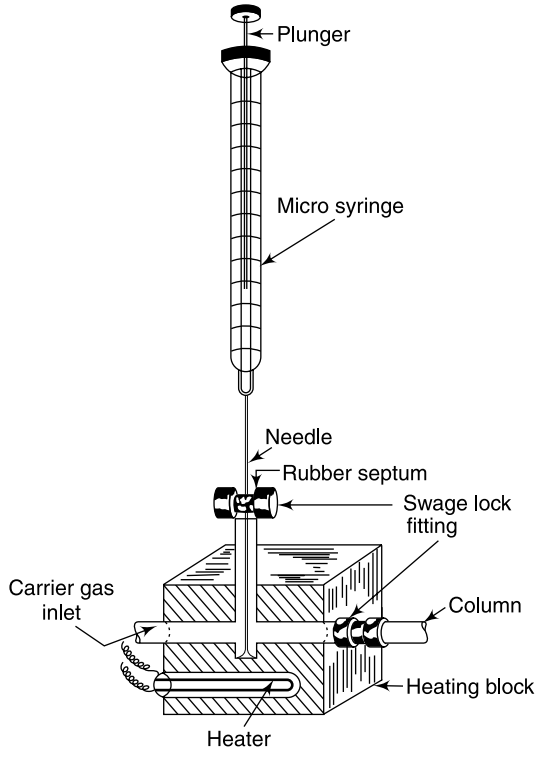


Figure 17.6 *Arrangement for injecting liquid samples*

Theoretical considerations enjoin that the efficiency of separation in GC column improves as the size of the sample is reduced. With a normal analytical column of 44 mm (internal diameter), a liquid sample of 2–20 μ and a gas sample of *0.5–5* ml at atmospheric pressure is generally satisfactory. Exception to this general practice is in the case of trace analysis, when liquid samples up to 200 μl is necessary to obtain sufficient response. Wybrow (1985) describes a microcomputer-based injection system for use in the chromatographic analysis of gases.

17.4.2.1 Liquid samples

The usual method is to inject liquid samples with a micro-syringe, through a silicon rubber septum. Syringes of various capacities are

commercially available and are generally employed for injection of samples between 0.1 and 10 µl. Sample is injected into the hot zone of the column, so that the liquid gets rapidly transferred into the gaseous phase.

A typical arrangement for injecting liquid samples is shown in Figure 17.6. The metal block containing capillary is heated by a controlled resistance heater. Here the sample is vaporised and carried into the column by the carrier gas. Care should be taken to insert, inject and remove the needle quickly.

17.4.2.2 Gas samples

Gas samples are injected by a gas-tight syringe suitable for delivering 0.1–10 ml of sample. They are usually difficult to handle and often cause inaccuracies.

The other method of injecting gaseous samples is the bypass system, also called a stream splitter. This system has been found most valuable and gas sampling valves using this technique are used extensively. The principle of the system is to fill a loop of known volume with the sample. By operating a valve, the loop is placed directly in the carrier gas line. The valves were earlier made of glass, but they have now been replaced by polytetrafluoroethylene designs.

Figure 17.7 shows a schematic of this type of arrangement. Basically, it is an arrangement of three stopcocks, between two of which there is a standard volume, in which gas sample is enclosed. Gas from the bypass capillary loop is introduced into the column by a rotating or sliding valve, so that the loop is connected with the stream of the carrier gas.

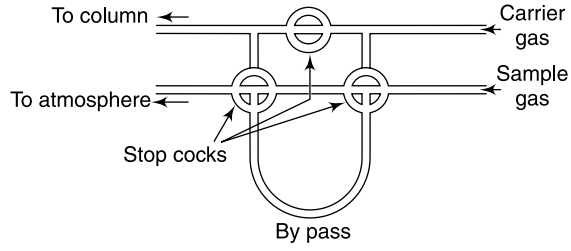


Figure 17.7 *By-pass system for injecting samples*

Figure 17.8 shows the schematic of the most popular split/splitless injector suitable for capillary columns. It has the same requirements as the packed column injectors: carrier gas inlet, a septum, septum purge, injector insert, heater block and column connection, but the improvement is another set of gas lines out of the injector-another path that the vaporised sample can take. This is called the split line. Thus, the carrier gas enters the chamber and can leave by 3 routes, when the injector is in split mode. The sample vaporises to form a mixture of carrier gas, vaporised solvent and vaporised solutes. A portion of this mixture passes onto the column,

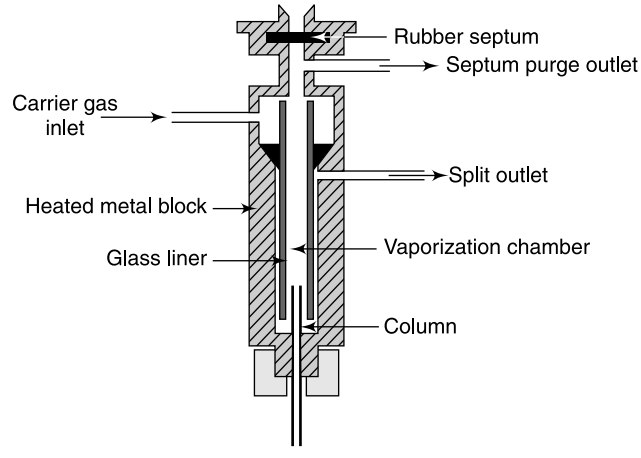


Figure 17.8 *The split/splitless injector (Redrawn after Chasteen, 2000)*

but most of it exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column. In order to prevent column overloading, the amount of sample reaching the column is reduced and very narrow initial bandwidths can be obtained. For maximum sensitivity, the injector can be used in the split less mode, then all the injected sample will reach the column.

17.4.2.3 Solid samples

Solid samples may also be injected by using solid injection syringes, where the sample is deposited on the end of the plunger and withdrawn inside the needle. After piercing the injection septum, the plunger is extended to place the solid in the hot zone of the column, where it is vaporised. Alternatively, the solid is deposited in a glass tube or gauze from a solution. After the evaporation of the solvent, the sample holder is dropped into the column, thus making the injection. Another method is to dissolve the solids in volatile liquids or temporarily liquify them by exposure to infrared heat.

17.4.2.4 Pyrolysis

Pyrolysis offers a technique for injection of certain types of materials, which are low or non-volatile, but may be thermally decomposed in an inert atmosphere to offer a qualitatively and quantitatively reproducible mixture of volatile fragments. The pyrolysis products are transferred to a chromatographic column and separated in the usual manner. Pyrolysis has been accepted as a valuable technique for sample injection in rubber, plastic, polymers and adhesive industries.

17.4.3 Chromatographic Column

The column is the heart of a gas chromatograph, where the fundamental process of separation takes place. Its action is based on the fact that when a sample of gas or vapour is introduced into a column, it spreads by molecular diffusion to yield a concentration profile. As the sample moves through the column, additional spreads takes place, but the band maintains its general shape, which is detected and recorded as the familiar chromatographic peak. The degree of peak broadening with respect to time and column length is an indication of column efficiency. Column performance is usually measured by the number of theoretical plates, which may be determined from the dimensions of peaks.

A theoretical plate is defined as a layer, at right angles to the column of such a thickness, that the solute at its mean concentration in the stationary phase in this layer, is in equilibrium with its vapour in the mobile phase leaving the layer. Laboratory columns of 20–100 plates are widely in use and normally have a height equivalent to a theoretical plate (HETP) of about 1 cm. Longer practical columns of up to 1,00,000 plates have been re-ed in literature.

17.4.3.1 Types of columns

There are two *types of* columns which are commonly used. They are (i) Packed columns, (ii) Capillary columns or open tubular columns.

Packed Column: The packed column is a tube packed with a suitable material, which performs the separations. Columns may be made from any suitable tubing. Glass, stainless steel or copper are the materials most frequently used for making columns. For moderate temperatures,

polyvinyl-chloride tubing is satisfactory. Internal diameter of the column is usually between 4 and 8 mm. The length of the column may be between 1 and 50 m. However, for most of the applications, a length of about 2 m is adequate. Columns longer than 3 m are difficult to pack uniformly. Therefore, very long columns are best constructed by coupling short (less than 3 m) sections, to obtain any desired length. Standard Swagelok unions are adequate for the purpose. Sample sizes for packed analytical columns vary between 0.1 and 10 µ. By increasing the column diameter, greater sample sizes of the order of 100 µl to 3 ml can be used for preparative purpose. For this type of chromatography, the column has internal diameter from 6 to 25 mm and the packing material specifications are also changed to cope with increased loading.

For convenience, the column is made in the form of a U or helix or it can even be straight. Straight and U-shaped columns can be repacked more easily. A helical column is normally difficult to fill. For this reason, copper tubing column is preferred, because it can be filled while straight and then bent to helical shape afterwards. A definite advantage of coiled or helical column is that, they are more compact for a given length of column and therefore easier to heat to an even temperature. The helix type of column is usually of 50–250 mm in diameter and 2 m in length. As long as the column is packed sufficiently tight before coiling, there is no significant difference in performance compared to a straight tube. Columns are quite difficult to pack after they are coiled. Instruments designed for glass columns have tall ovens to accommodate U shapes that can be easily packed for maximum performance. Columns of diameter larger than 12.5 mm are made in straight sections connected by small diameter tubing.

Capillary Column: Capillary columns are open tube columns of tubing approximately 0.25 mm in diameter. Their lengths may run anywhere from 30 to 300 m. Very high efficiencies have been achieved with capillary columns, since the cross diffusion of sample molecules is minimised by the narrow diameter. Capillary columns contain no packing and the stationary phase is coated directly on the inside of tubing. Capillary columns cannot handle samples more than 0.1 µl. Larger samples are handled by the use of inlet splitters. With capillary columns, better separations can be achieved at lower temperatures and in a shorter time.

Capillary columns are of two types: Wall-coated open tubular (WCOT) or support-coated open tubular (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

Figure 17.9 shows a new type of WCOT column called the Fused Silica Open Tubular column. These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

The great analytical strength of capillary gas chromatography lies in its high resolution. Capillary columns have more theoretical plates (a measure of column resolving power

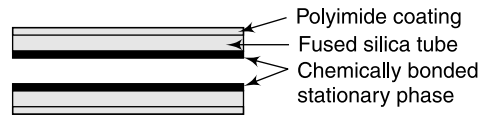


Figure 17.9 *Cross section of a fused silica open tubular column*

or efficiency) per metre as compared to packed columns. The average capillary column (30 m long) has approximately 1,00,000 theoretical plates while the average packed column (3 m) has only 2,500 plates.

They have less resistance to flow and consequently they can be longer than packed columns. Also, capillary column require a smaller amount of sample than packed columns. While the average sample mass of each component in a mixture that is separable by packed column GC can be in the microgram range (10^{-6} grams) per injection, capillary columns routinely only handle 50 nanograms (10^{-9} grams) of a particular component or less.

However, there are some limitations associated with the capillary columns. Since they have smaller diameters (0.05–0.53 mm) than packed columns (2–4 mm), they require relatively specialised injectors and flow controllers.

Capillary column gas chromatography is rapidly developing to meet many of the analytical needs, such as environmental sections, analysis at trace levels for clinical and biomedical diagnostics, the separation of natural products and other complex mixtures.

17.4.3.2 Support material

A support material is used in partition chromatography to provide a thin liquid film, with as large an interface as possible between the gas and liquid phases, so as to facilitate partition between them. A primary requirement of solid support material is that, it should not possess adsorptive properties towards sample components. Besides being inert, the support should have the structure and surface characteristics to hold the liquid phase uniformly over a large area.

A number of supports have been tried, including glass balls, sodium chloride and pumice powder. However, only diatomaceous earth has been found satisfactory. It has been in use under the trade name Celite, with an average particle diameter of 40 μ. Trade names also include GC-22 Super Support, Sil-O-Cell, C-22, Fire brick and Chromosorb P, W or G. Chromosorb is prepared by calcining diatomaceous earth obtained from a marine deposit in Lompoc, California. Celite is the most widely used support material.

Glass beads are also available in a variety of mesh ranges. Because of their low surface area and light liquid-phase loading required, they will elute high boiling-point compounds at a lower temperature than is normally required. Teflon 6 is another material, which is one of the most inert and non-adsorptive. It is best suited for the analysis of highly reactive materials, which are difficult to chromatograph on other supports. However, they offer lower efficiency and a temperature limit of 25°C. Particle sizes may range from 10 to 100 mesh.

The most commonly encountered problem due to support participation with the column is that of peak tailing, which appears as a long tailing edge of a peak due to adsorption. In extreme cases, if the peaks tail badly, separation is impaired and the determinations based on peak area measurements become difficult to make and thus give unreliable results. In these situations, samples may be totally adsorbed. It is possible to minimise tailing effects by modification of the support. The Celite surface, which is prone to cause sample adsorption problems, resulting in peak tailing may be covered in hydroxyl groups. The adsorption effects can also be minimised if the Celite is treated with a silating agent, such as hexamethyldisilazine or dimethyldichlorosilane.

The column must be so loaded that it has an even packing and the gas flow does not vary either across the column or irregularly along its length. Experience is needed to achieve an even packing of the column to obtain a high efficiency.

17.4.3.3 The stationary phase

The separation of the sample into its components is achieved by a partition process involving the stationary phase and the moving carrier gas phase. The stationary phase is either liquid or solid. Therefore, the following are the two possible methods with the gas as the mobile phase:

(a) Gas-liquid chromatography
(b) Gas-solid chromatography

Gas-Liquid Chromatography: Here, the stationary phase is liquid, which is distributed on a solid support material. The stationary phase must be involatile at all temperatures, at which the column will be operated for the analysis and should be coated as a thin even film on to the support. It is chosen for the selective retention characteristics of components in the sample that it will be used to separate. In general, highly polar stationary phases are employed to selectively retard polar compounds. On the other hand, non-polar stationary phases offer little selectivity and components tend to be eluted due to differences in boiling points of the sample components.

At temperatures above 150°C, special difficulties arise, as normal solvents become highly volatile or even unstable. In such situations, substances like silicon polymers may be used, especially for temperatures above 250°C.

An important requirement of the stationary phase liquid is a certain compatibility with the components of the sample under analysis. Generally it is found that a polar substance is most satisfactorily analysed on a polar stationary phase. Similarly, a non-polar compound will give the best results on a non-polar phase. For example, for separations of polar components like alcohols, amines, etc., it is preferable to choose a polar liquid like polyethylene glycol. Nevertheless, departure from the above rule of similarity is quite often necessary.

Normally, for analytical packed columns, 1–10% w/w of stationary phase on the support is employed. The analysis time is approximately proportional to the quantity of stationary phase in the column. Higher rates induce diffusion phenomena that would impair the separation. On the other hand, at low ratios, the inert support might manifest considerable residual absorptivity to cause tailing of elution peaks.

For applications in the biomedical field, for the analysis of sugars, bile acids and steroids, a low percentage of stationary phase may be used to produce a very fast column, which will pass high boiling-point samples at moderate temperatures to avoid thermal decomposition. The choice of stationary phase is extremely important for the successful analysis of each sample mixture. Sometimes, in practice, brief experiments are necessary with a number of trial columns, in order to make a suitable section. It is here, when the experience of the operator would prove useful.

Gas-Solid Chromatography: The stationary phase in this type is a solid material with surface active properties. The separating principle is based on the variation in the extent to which constituents of a mixture are adsorbed on the adsorbent packed in the column. Therefore, the separation is obtained because of the different adsorption affinities which the column packing has towards the sample components. This type of chromatography is used in the analysis of inorganic gases and low-molecular weight hydrocarbon gases. Among the most commonly used adsorbents in gas-solid chromatography are silica gel (SiO_2), alumina (Al_2O_3), charcoal and molecular sieves (sodium or calcium aluminium silicates).

17.4.4 Thermal Compartment

17.4.4.1 Isothermal operation

The column is not normally operated at room temperature, because it would then be suitable only for the analysis of gases or extremely volatile liquids. Therefore, it must be heated in some form of thermostat. Moreover, it is desirable to keep the column at a precisely constant temperature. This is essential, because the quantitative response of the detector is often affected by column temperature. For this purpose the column is housed in an oven, whose temperature is controlled to an accuracy of 0.1°C. Various methods have been tried; namely vapour jackets electrically heated air baths, liquid baths or metal blocks. Usually, an air bath chamber surrounds the column and air is circulated by a blower through the thermal compartment. The temperature of the oven may be controlled accurately, using a proportional temperature controller with a platinum resistance thermometer as a sensing element. The oven is thermally insulated, so that heat loss to the atmosphere is minimised. However, this factor is balanced against the thermal capacity of the insulating material, which if too high, would affect the rate of cooling of the oven.

Normally, the temperature is so chosen that it gives a satisfactory time for analysis. Approximately, a temperature in the vicinity of the average boiling point of the components in the sample will be convenient, so as to effect an elution period of 10–30 min.

Figure 17.10 shows a schematic diagram of oven temperature controller. The temperature sensing is done by the platinum resistance R_1, which is placed in the oven. The temperature setting is

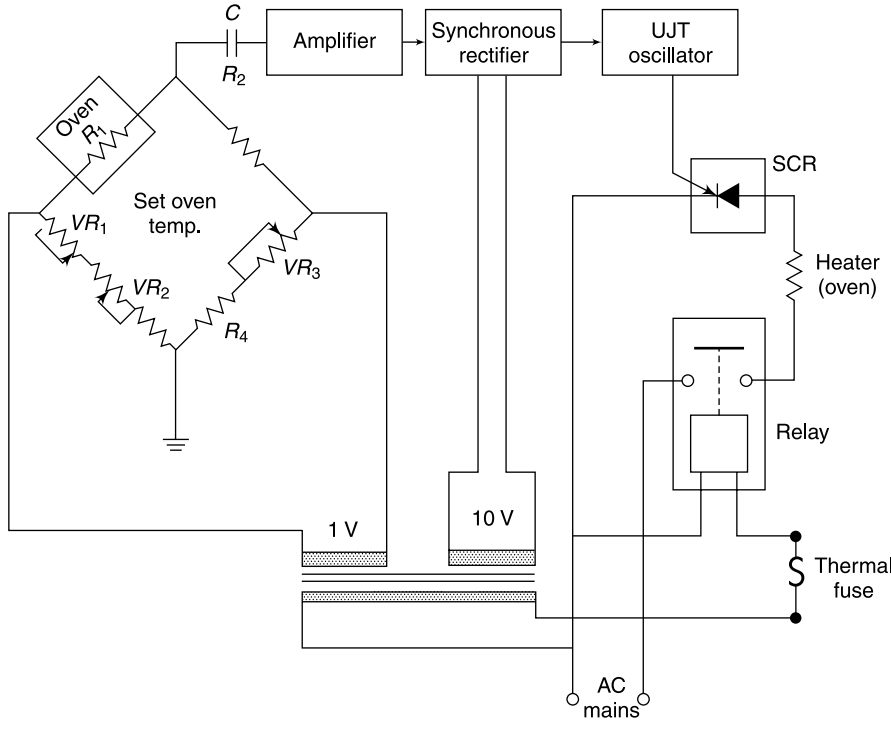


Figure 17.10 *Temperature control circuit for ovens*

done by adjusting the potentiometer VR_1 calibrated in terms of temperature. This control is provided on the front panel of the instrument. When a setting is made, the bridge gets unbalanced and the amplifier, the synchronous rectifier and the UJT oscillator are actuated to open the gate of SCR. Thus, the current is supplied to the oven heater and the oven temperature begins to rise.

As the oven temperature approaches the pre-set value, the sensor resistance becomes higher. The bridge approaches nearer to the balanced state and the heater current decreases. When the oven temperature reaches the pre-set value, the heater current would not flow and the bridge would be balanced. The oven temperature is thus kept constant. A thermal fuse placed in the circuit prevents the oven from overheating

17.4.4.2 Temperature programming

When the column temperature is kept constant, it is difficult to analyse samples having components of a wide boiling range. This difficulty can be overcome by using programmed heating of the column, so that its temperature is not kept at a constant temperature, but is subjected to an exactly controlled temperature rise, while a separation is in progress. The technique combines in it, the advantage of a low temperature for better separation of low-boiling components, with that of high temperature for more rapid elution of high-boiling components, thereby shortening the time of analysis and sharpening the resultant chromatographic peaks. Temperature increase may be programmed to be carried out either continuously or in steps, or abruptly to a predetermined higher level between two peaks. Programs are available, which give linear and non-linear temperature programming of ovens. The temperature can be raised at various rates. Generally, linear rates of temperature programming in the range of 1–20°C/min are used. The rates 5–7°C/min are most typical. Some applications require non-linear temperature programming in exponential or ballistic manner.

For temperature programming, the program according to which the temperature is to be varied, is taped or ink recorded on special Mylar format sheets. Curved rates of temperature rise, linear sections and isothermal operation can be plotted as desired. The recorded sheet is fixed on a rotating drum and the program line is followed by an optical scanner. The scanner is linked to a servo system, which continuously controls the wattage supplied to a proportional heating system. At the completion of a run, column temperature must be dropped from about 300°C to less than 100°C in a few minutes, so that the column may become ready for the next run. Baudean et al. (1977) explains the use of microprocessors for temperature programming.

17.4.4.3 Use of two columns

When using the programmed temperature technique, the behaviour of the column itself is influenced with the change in temperature. There is an increasing tendency for the stationary phase to bleed from the column, as the temperature rises and it is reflected as a baseline drift, with a chromatogram superimposed on it. When working at high sensitivity, it is possible that this drift in base line may severely limit the use of temperature programming. This problem can be partially offset by using two matched columns and operating two detectors in a differential mode. One column is called the sample column and the other as reference column, to which no sample is added. The signals from the detectors are combined to balance the bleed effects and give a straight baseline. However, a careful setting up procedure is necessary to balance the two columns.

The following are important considerations, which are kept in view when designing column ovens:

- The oven must have minimum thermal gradients, so that the temperature is uniform over the whole column.
- The oven must have a fast rate of heating. For this, it must be constructed from low-mass materials. This requirement is particularly significant for changing the column temperature rapidly as in operations involving temperature programming.
- Temperature controlling facility up to 400°C is necessary.
- Power consumption should be kept low. For that, heat loss by all means must be minimised.
- The door of the oven should be large enough to facilitate installation and removal of column and its accessories.

17.4.5 Detection Systems

The detector is placed at the exit of the column. It is employed to detect and provide a quantitative measurement of the various constituents of the sample, as they emerge from the column in combination with the carrier gas. The detector, in fact, acts as a transducer and converts the changes in some physical property to changes in an electrical signal, which can be conveniently recorded.

The choice of a particular type of detector is governed by the following factors:

- The detector should have a high sensitivity, to be sufficient enough to provide an adequate signal for all components with a small sample. It should also permit the use of lower column temperatures.
- It is desirable to be able to measure components from the fractional ppm to almost 100% in one sample. The response of the detector should be linear over the whole range.
- A small internal volume ensures that the resolution of components, which are separated by the column, is not lost and that the shape of peaks is not distorted by the detector.
- Detector temperature should be such that appreciable amount of the eluted vapours does not condense in it.
- The detector should be insensitive to changes in the rate of flow of the carrier gas.
- The detector should give good reproducibility of base line.

There are several detection systems which are used in gas chromatography. Quite often, the fields of application of these detectors overlap to a certain extent, but one of the detectors will usually have characteristics, making it most suitable for a particular analysis.

Signals from various types of gas chromatographic detectors can be conveniently amplified by employing operational amplifiers. With FID, where the signal levels are of the order of 10^{-11} A and even lower, it is necessary to utilise electrometric input operational amplifiers in the input stage. These amplifiers have input bias currents of the order of 10^{-13} to 10^{-14}A. A wide range of signal amplitudes can be handled by a logarithmic electrometric amplifier. Linearity of the overall response is then restored by an exponential converter following the logarithmic amplifier.

17.4.5.1 The Katharometer or thermal conductivity detector

The thermal conductivity detector is a simple and most widely used type of detector. It is based on the principle that all gases have the ability to conduct heat, but in varying degrees. This difference

in heat conduction can be used to determine quantitatively the composition of a mixture of gases. By definition, the thermal conductivity of a gas is the quantity of heat (in calories) transferred in unit time (seconds) in a gas between two surfaces 1 cm^2 in area, and 1 cm apart, when the temperature difference between the surface is 1°C.

In its simplest form, the detector may consist of a hollow tube with an electrically heated coil mounted axially in its centre. Only when the carrier gas flows over it, a thermal balance can be attained at a certain temperature. However, when a gas or vapour differing in thermal conductivity from the carrier gas flows past the heated coil, the temperature of the coil gets altered and a proportionate change in the electrical resistance of the wire takes place. Such changes in resistance arising from the components of the sample are used for detection and estimation of the unknown sample components.

In actual practice, the detector consists of two temperature sensing elements arranged in a Wheatstone Bridge circuit, one in the reference and the other in the measuring arm. The heat sensitive elements are either thermistors or resistance wires, like platinum or tungsten. Figure 17.11 shows a typical circuit arrangement for measuring the changes in the resistance produced in the Katharometer cell elements. Resistances R_1 and R_2 are the Katharometer wires and resistances R_3 and R_4 are the ratio arms of the bridge. Resistances R_7 and R_8 *are used* for making base line adjustment and are made of manganin wire. The output of the bridge is fed *to* the recorder through an attenuator, so that if signal is greater than the span rating of the recorder, full-scale reading may be adjusted.

For the balanced bridge conditions, when the carrier gas flows through the two cells, no current would be flowing between *A* and *C* and

$$R_1/R_2 = R_3/R_4$$

However, when the resistance R_1 changes due to the components of the sample gas, it causes an unbalance current to flow from *A* to *C*. The magnitude of the current serves to detect and measure

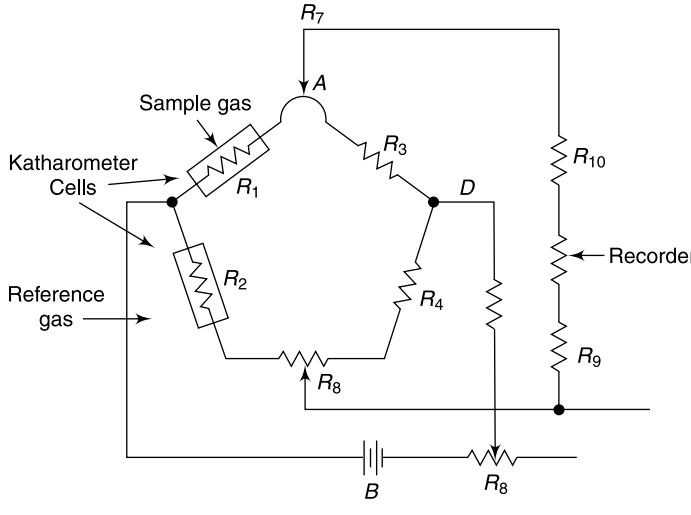


Figure 17.11 *Katharometer cell*

the magnitude of the gas component vapour passing over the measuring cell. If the Wheatstone bridge is excited with an ac current, it can be made many times more sensitive, because the ac signal can be conveniently amplified before it is given to the recorder.

The sensitivity of a thermal conductivity detector depends on the nature of the carrier gas. When helium gas is used, 10^{-7}g of inorganic gases can be detected Katharometer is fairly satisfactory for a wide variety of analytical applications. The technique is non-destructive and therefore, the components of the sample can be further trapped for other forms of analysis.

17.4.5.2 Flame-ionisation detector (FID)

FID is by far the most widely used detector in gas chromatography. It responds with high sensitivity to almost all organic compounds. Its linear dynamic range is approximately 10^7, which is much wider than that obtained from other detectors.

In this detector, the effluent from the column is led into an oxy-hydrogen flame (Figure 17.12). An electrical potential is applied across two electrodes placed in a stainless steel housing. The hydrogen flame burns at the tip of a capillary, which also functions as the cathode and is insulated from the body by a ceramic seal. The collector electrode consists of a loop of platinum and is located at about 6 mm above the burner tip.

The current across the electrodes remains constant, when only the inert carrier gas passes the flame. However, when the vapour of a compound emerging from the column passes the flame, the vapour molecules are broken into ions by the hot flame. These ions result in the ionisation current and there would be a consequent change in the current flowing across the electrodes. The magnitude of the variation in current would be directly proportional to the number of ions or electrons formed in the flame gases, which in turn would be proportional to the carbon content of the organic molecules in the vapour.

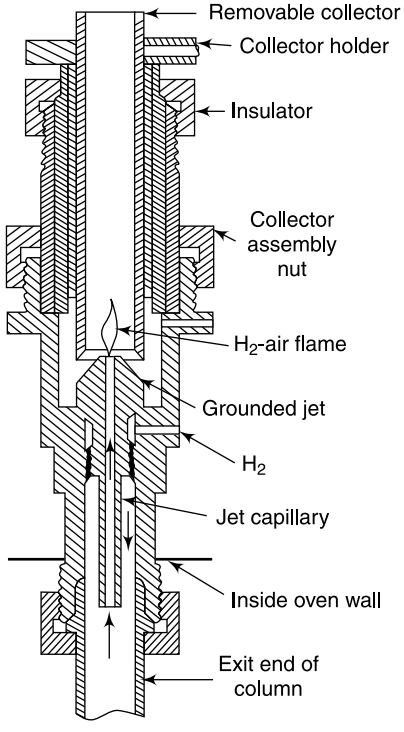


Figure 17.12 *Flame-ionisation detector*

The FID has a high output impedance similar to that obtained with glass electrodes, when making pH measurements. Commercial pH metres can, therefore, be easily adopted for use with this detector. A vibrating reed electrometer is often used in the input stage of the amplifier to attain sensitivities up to 5×10^{-13}A. By placing a set of high resistors across the flame and changing their resistances, enables the sensitivity to be varied. The sensitivity is high, because of the inherently low noise level of this detector.

Figure 17.13 shows a solid-state current amplifier with which a FID can be used at all

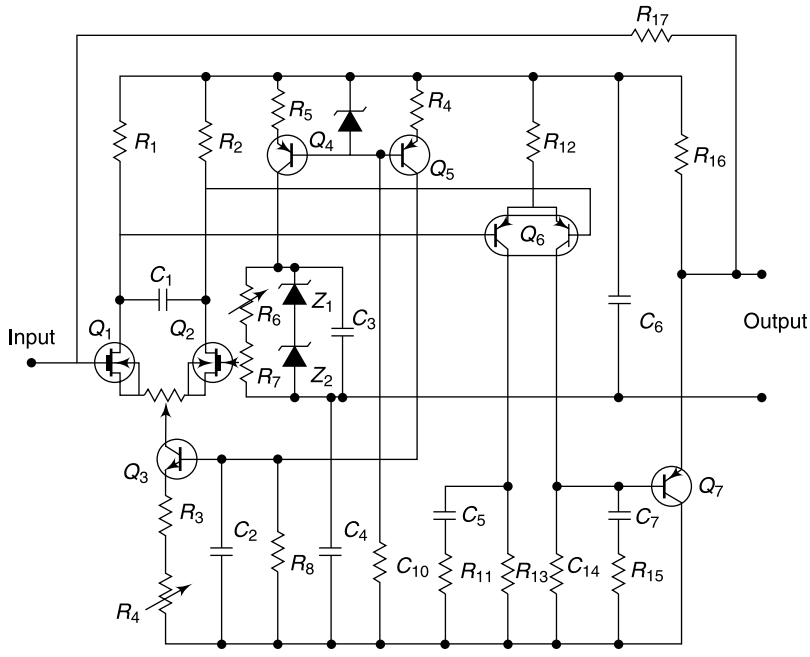


Figure 17.13 *Amplifier circuit used with flame-ionisation detector*

practicable sensitivity levels. The amplifier makes use of metal-oxide-silicon transistors in the input stage in place of an electrometer pentode, which had been usually employed. The amplifier gives a degree of stability significantly better than that achieved with thermionic electrometer valves. The amplifier has a sensitivity of 100 mV pA^{-1} and a noise output equivalent to 2×10^{-15} A rms at the input, thermal drift is typically 1 mV/°C. The input stage of the amplifier consists of a matched pair of MOSFETs, which is followed by a matched pair of p-n-p transistors. The output stage is an emitter follower, while the first stage is a long tailed pair.

There are certain limitations to the use of FID. These are:

- The FID does not respond to inert gases and inorganic compounds.
- The emerging components get destroyed in the flame.
- The response to sample weight has to be separately determined for each component.

In a FID, it is not only the sample components which are ionised in the hydrogen flame, but the liquid phase escaping from the column also participates in producing ions. The ionic current due to the liquid phase is recorded on the chromatogram as a background signal. In case of the programmed temperature analysis, the background signal changes with time, because the amount of liquid phase escaping from the column increases gradually in response to the raised column temperature. This causes great inconvenience to the programmed temperature analysis of trace substances. To eliminate this difficulty, a differential hydrogen FID has been devised. It consists of two FIDs based on the same working principle. The schematic diagram of the DFID is shown in Figure. 17.14.

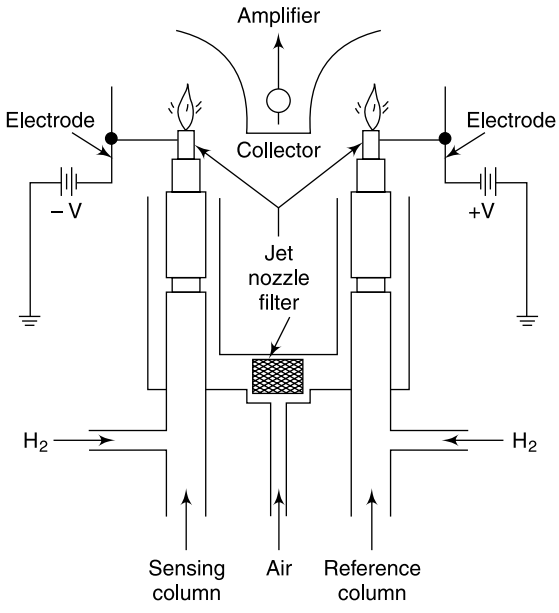


Figure 17.14 *Working principle of differential flame-ionisation detector*

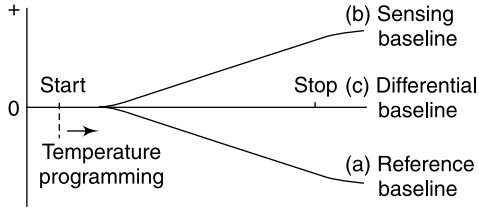


Figure 17.15 *Ideal case of base line compensation in dual FID arrangement*

Two columns are used in this arrangement. If the same packed column as the sample-side one is employed on the reference-side, the carrier gas is fed through the reference-side column at the same speed as on the sample-side, and if the reference-side column is mounted very near the sample-side one, under these conditions, the amount of liquid phase flowing from the reference-side column can be considered of the same as that of the liquid phase flowing from the sample-side. On applying the voltages to the electrodes, the signals produced are shown in Figure 17.15 indicating that the sample-side and reference-side signals offset each other, producing the base lint as straight. However, a certain degree of base line drift cannot be avoided even with DFID, when the sensitivity of FID or the amount of liquid phase differs between the sample-side and the reference-side.

17.4.5.3 Flame photometric detector

Flame photometric detector (FPD) is primarily used for the determination of sulphur or phosphorus-containing compounds. This device uses the chemiluminescent reactions of these compounds in a hydrogen/air flame as a source of analytical information that is relatively specific for substances containing these two kinds of atoms.

Figure 17.16 shows the basic components of gas chromatographic FPD (Lecture Notes by Dr. Chasteen T.G. http://www.shsu.edu/~chemistry/FPD/FPD.html), which are:

- A combustion chamber to house the flame
- Gas lines for hydrogen (fuel) and air (oxidant),
- An exhaust chimney to remove combustion products.
- Thermal (bandpass) filter to isolate only the visible and UV radiation emitted by the flame. Without this, the infrared radiation emitted by the flame's combustion reactions would heat up the PMT and increase its background signal.

The λ_{max} for emission of excited S_2 is approximately 394 nm while it is 510–526 nm for phosphorus compounds in the flame. In order to selectively detect one or the other family of compounds as it elutes from the gas chromatic column, an interference filter is used between the flame and the

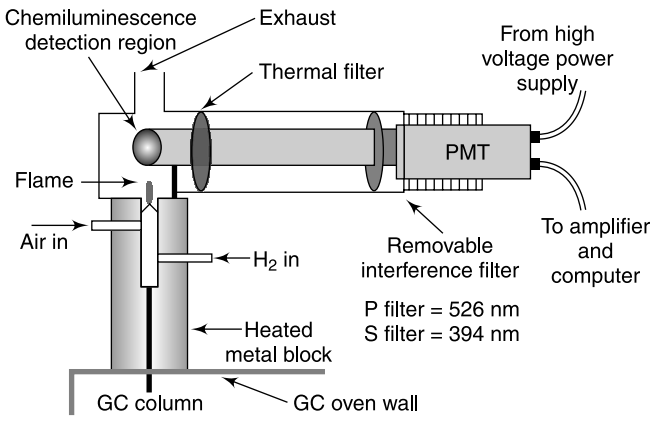


Figure 17.16 *Flame photometric detector (Courtesy: Dr. Sam Houston State University)*

photomultiplier tube to isolate the appropriate emission band. The disadvantage of this detection system is that the filter must be exchanged between chromatographic runs if the other family of compounds is to be detected.

An improvement over the FPD is the pulsed flame photometric detector (PFPD) which is capable of analysing many other elements, besides sulphur and phosphorus. Figure 17.17 shows a schematic of the PFPD. In this arrangement, two different combustible gas flows enter the bottom of the combustion chamber through narrow gas lines, as against one fuel line in the normal FPD. The second incoming gas flow's job in the PFPD is to help fill up the outer volume of the combustion chamber, while the analyte and the primary combustion gas flow into that chamber (Lecture Notes by Dr. Chasteen T.G. http://www.shsu.edu/~chemistry/PFPD/pfpd.html).

The detector contains an ignition wire which stays continuously red hot. When the gases flowing into the combustion chamber, including the analytes exiting the GC column, reach a flammable mixture they are ignited by the ignition wire and the flame propagates back down the combustor. The flame front uses up all of the quickest burning flammable material in the combustion chamber in less than 10 milliseconds, and then the flame goes out. It is after this short flame pulse that the slower burning analytes are excited and emit the light that is characteristic of their elements. After about 300 milliseconds, the flame pulses again as new flammable material fills the combustion chamber from the inlet tubes and GC column and that combination once again constitutes a flammable mixture. In this way about 3 flame pulses are recorded per second. By using a gated amplifier, controlled by a computer, the part of each pulse to be amplified and to be recorded can be chosen.

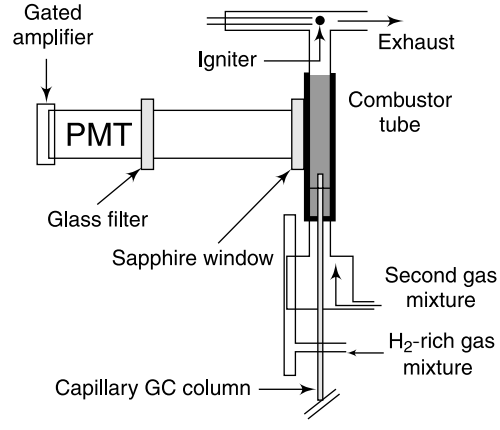


Figure 17.17 *Pulsed flame photometric detection (Adapted from Dr. T.G. Chasteen 2000)*

The analytical discrimination gives the PFPD the ability to selectively, and sensitively detect some analytes co-eluting in the presence of others and the ability to produce element specific chromatograms.

17.4.5.4 Photoionisation detector

The photoionisation detector is mainly used for the selective determination of aromatic hydrocarbons or organo-heteroatom species. The device uses ultraviolet light as a means of ionising an analyte exiting from a GC column. The ions produced by this process are collected by electrodes. The current generated is therefore a measure of the analyte concentration.

If the amount of ionisation is reproducible for a given compound, pressure, and light source then the current collected at the PID's reaction cell electrodes is reproducibly proportional to the amount of that compound entering the cell. The reason for using PID for analysis of compounds like hydrocarbons or heteroatom is that they have ionisation potentials that are within reach of commercially available UV lamps. The available lamp energies range from 8.3 to 11.7 ev, that is, λ_{max} ranging from 150 to 106 nm. Although most PIDs have only one lamp, lamps in the PID are exchanged depending on the compound selectivity required in the analysis. (Lecture Notes by Dr. Chasteen T.G. http://elchem.kaist.ac.kr/vt/chem-ed/sep/gc/detector/pid.htm)

Figure 17.18 shows the schematic of a gas chromatographic photoionisation detector. The major advantage of this detector is that only a small fraction of the analyte molecules are actually ionised in the PID chamber, thus this can be considered as a non-destructive GC detector. Therefore, the exhaust port of the PID can be connected to another detector in series with the PID. In this way, data from two different detectors can be taken simultaneously, and selective detection of PID responsive compounds augmented by response from, say, a FID or an electron capture detector can be done.

17.4.5.5 Electron capture detector (ECD)

This detector works on the principle that the ionisation current set up by certain radioactive sources like Ni^{63} or H^3 gets reduced when an electron capturing compound is introduced into the cell. In effect, the ECD measures the loss of signal due to recombination phenomenon rather than measuring a positively produced electrical current.

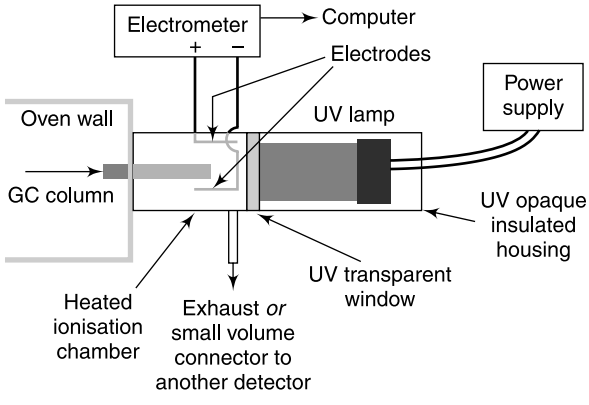


Figure 17.18 *Photoionisation detector (Courtesy: after Chasteen, 2000)*

Ettre (1978) explains the construction of an ECD. The detector consists of two electrodes (Figure 17.19) across which a potential difference of 10 to 100 V can be applied. A radiation source of β-rays (tritium) is mounted on a tantalum wire saturated with the radioactive isotope of hydrogen, so that the emitted β-rays encounter the effluent from the GC column.

As the carrier gas (nitrogen) flows through the detector, β-particles from the tritium source ionise the nitrogen molecules

and form slow electrons. These slow electrons migrate to the anode under a fixed voltage. When these electrons are collected at the collector electrode, they produce a steady current, which provides a base line on the recorder.

The organic compounds containing halogen, nitrogen and phosphorous have the property of capturing electrons, resulting in a variation in the number of electrons reaching the collector electrode; thereby, producing proportionate signals in the detection device.

Detector discrimination can be regulated through the potential applied to the collector electrode. In fact, the response of wetly capturing compounds can even be abolished since the response for different classes ceases at well-defined applied potentials.

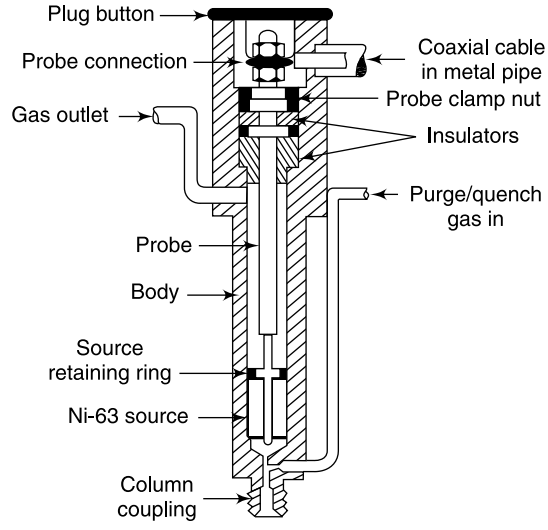


Figure 17.19 *Electron capture detector*

This detector has a sensitivity of 3×1^{-14}A. However, the linear range is limited to less than 10^3. Nitrogen and hydrogen are the best carrier gases with this type of detector. Hydrogen should be used with caution, lest there be an explosion.

17.4.5.6 Argon ionisation detector

For the argon-ionisation detector, argon gas is used as the carrier gas. The detector contains two electrodes placed parallel to each other and a potential difference is applied across them. With the carrier gas emerging out of the GC column, no current passes across the electrodes under normal conditions, as the gas is non-conductor. A radioactive source (tritium) is placed in the approach region to the electrodes, so that the rays emitted by it excite the argon atoms and electrons are produced by this bombardment action. These electrons are accelerated under the influence of a potential of about 1000 V and upon collisions with other argon atoms, raise them to the metastable state. Such metastable argon atoms collide with organic molecules of the sample emerging from the GC column, resulting in these molecules becoming ionised and consequently conducting. This results in the flow of proportionate current across the electrodes and produces signals, the magnitude of which would depend on the quantity of organic samples passing through the detector.

The argon detector responds to most of the organic and inorganic compounds although it is inert to water vapour, oxygen, methane, carbon dioxide and oxygen. The sensitivity of the detector is 0.08 µg/ml and the linear dynamic range is 10^5. The detector is suitable for measurement of organic molecules present over a wide range of concentrations.

17.4.5.7 Cross-section ionisation detector

The cross-section ionisation detector is one of the most useful detectors for gas chromatography, which has proved to be a very reliable method for the separation of gases in mine air. It is precise, reliable, robust, insensitive to changes in the carrier gas flow rate and characterised by a response, which is linearly dependent on the concentrations of the components under investigation, over a

wide range of change in concentration. The vapour concentration for any given molecule can be calculated from the known properties of its constituent atoms.

The ionisation current in the chamber is small, when it is filled with light gas such as hydrogen, but increases with the addition of any other gas. This increase in current is because of the increased total ionisation cross sections of the gas mixture inside the chamber. The cross section ionisation of a gas is a quantity determined by the size of a gas molecule and the number of electrons in the atoms forming the molecule. Therefore, the action of the cross section ionisation detector is based on differences in the cross section affecting the ionisation of the analysed components.

In this type of detector, a radiation source (Sr^{90}) mounted in the approach region of the two electrodes separated by the carrier gas, generates ion-pair from the organic molecules passing with the carrier gas. The application of potential of 300–1,000 V ensures collection of the electrons. A variation in the current flowing across the electrodes leads to proportionate signals and the magnitude of the signal depends on the concentration of the component emerging from the GC column.

In this detector, hydrogen or helium is usually the carrier gas. The detection is non-destructive, though its sensitivity is low (about 10^{-7} g/s). Response to any substance can be calculated from the values of the atomic cross sections of its constituent atoms.

17.4.5.8 Atomic emission detector (AED)

The atomic emission detector has the ability to simultaneously determine the atomic emissions of many of the elements in analytes that elute from a GC capillary column. As eluants come off the capillary column, they are fed into a microwave powered plasma cavity where the compounds are destroyed and their atoms are excited by the energy of the plasma. The light that is emitted by the excited particles is separated into individual lines via a photodiode array. The computer then sorts out the individual emission lines and can produce chromatograms made up of peaks from eluants that contain only a specific element.

The atomic emission detector basically includes the following components which are shown in Figure 17.20:

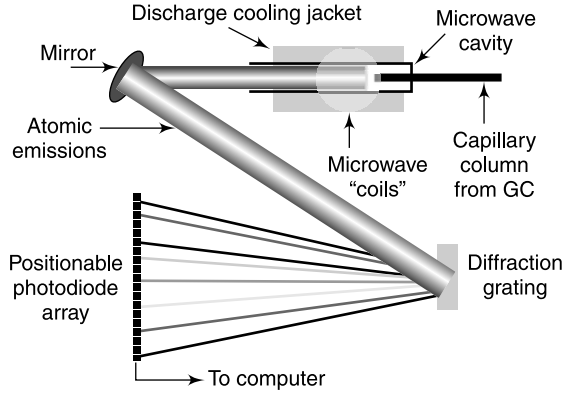


Figure 17.20 *Atomic emission detector (Courtesy: after Chasteen, 2000)*

- An interface for the incoming capillary GC column to the microwave induced plasma chamber.
- The microwave chamber.
- A cooling system for the microwave chamber, which is required because much of the energy focused into the cavity is converted into heat.
- A diffraction grating and associated optics to focus and then disperse the spectral atomic lines.
- A position adjustable photodiode array interfaced to a computer.

This detector is based on the atomic emissions instead of measuring simple gas phase ions created in a flame as with the FID or the

change in background current because of electronegative element capture of thermal electrons as with the ECD (Lecture Notes by Dr. Chasteen, T.G. http://www.shsu.edu/~chemistry/AED/AED.html). It has, therefore, much wider applicability.

17.4.5.9 Chemiluminescence spectroscopy-based detectors

Chemiluminescence uses quantitative measurements of the optical emission from excited chemical species to determine analyte concentration. However, chemiluminescence is usually emission from energised molecules instead of simply excited atoms. The bands of light determined by this technique emanate from molecular emissions and are therefore broader and more complex then bands originating from atomic spectra. Furthermore, chemiluminescence can take place in either the solution or gas phase, whereas AES is almost strictly as gas phase phenomenon.

Though liquid phase chemiluminescence plays a significant role in laboratories using this analytical technique, often in conjunction with liquid chromatography, the instrumental components are somewhat simpler in gas phase chemiluminescence reactions. These detectors are also often used as detectors for gas chromatography.

A schematic of the components necessary for a gas phase chemiluminescence detector interfaced to a capillary gas chromatograph is shown in Figure 17.21.

Like fluorescence spectroscopy, chemiluminescence's strength lies in the detection of electromagnetic radiation produced in a system with very low background. In addition, because the energy necessary to excite the analytes to higher electronic, vibrational, and rotational states (from which they can decay be emission) does not come from an external light source like a laser or lamp, the problem of excitation source scattering is completely avoided. The major limitation to the detection limits achievable by chemiluminescence involves the dark current of the photomultiplier (PMT) necessary to detect the analyte light emissions.

In gas phase chemiluminescence, the light emission is produced by the reaction of an analyte and a strongly oxidising reagent gas. The reaction occurs on a time scale such that the production of light is essentially instantaneous; therefore, most analytical systems simply mix analytes and the reagent in a small volume chamber directly in front of a PMT. If the analytes are eluting from a gas chromatographic column then the end of the column is often fed directly into the reaction chamber itself. Since as much of the energy released by the reaction should be used to excite as many of the analyte molecules as possible, loss of energy via gas phase collisions is undesirable, and therefore a final consideration is that the gas pressure in the reaction chamber be maintained at a low pressure (~1 torr) by a vacuum pump in order to minimise the effects of collisional deactivation.

17.4.5.10 Nitrogen-phosphorus detector (NPD)

The design of a nitrogen-phosphorus detector (NPD) is similar to a FID. The major difference is that the hydrogen/air flame of the FID is replaced by a heated rubidium silicate bead in the NPD. The effluent from the GC column passes through the hot bead. The hot rubidium salt emits ions when nitrogen and

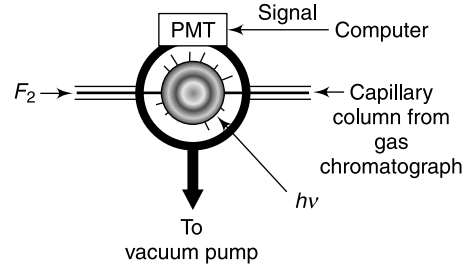


Figure 17.21 *Chemiluminescence detector*

phosphorus-containing compounds pass over it. The ions are collected on a collector above the heated bead to produce a current, similar to the FID.

17.4.5.11 Other types of detectors

A number of new detectors have been introduced recently. There has been a resurgence in the use of electrochemical detectors for gas chromatography. The majority of these include electrolytic conductivity detectors, micro-coulometry and ion-selective electrodes. Some workers have used radiochemical radio ionisation detectors for gas chromatography.

17.4.5.12 Calibration of the detector

Before the analysis of the unknown sample is carried out, calibrate the detector and calculate response factors for components to be determined. This is done by preparing mixtures of known composition by accurate weighing or mixing and then analysing it under the same conditions, which would be used for the unknown samples. From these results, calibration curves can be drawn and the response factors determined, which are applied to the samples to be analysed.

17.4.6 Recording Instruments

The data recorder plots the signal from the detector over time. This plot is called a chromatogram. The retention time, which is when the component elutes from the GC system, is qualitatively indicative of the type of compound. The data recorder also has an integrator component to calculate the area under the peaks or the height of the peak. The area or height is indicative of the amount of each component.

Chromatogram recording is usually done on the self-balancing type potentiometric single-pen graphic recorders. The span of these recorders may be 0.5 or 1 mV. These recorders require a low impedance input and therefore, impedance converters are used with the high impedance detectors. The chart widths are 25–30 cm and response time of about 1 s. For multi-speed operation, a gear box for changing the speed is necessary.

All ionisation detectors generate some background signal (with the carrier gas only) ranging from 10^{-8} to 10^{-11} A. The maximum signal in the presence of vapour is in the range of 10^{-6} to 10^{-8} A. Therefore, the apparatus for measuring current must respond to all current in the range of 10^{-6} to 10^{-13} A. It should also have means of offsetting the background current of the detector in use. The response over large current range is achieved by putting a series of high stability resistors across the input of the potentiometric recorder.

17.4.6.1 Qualitative analysis

The elution of a component from the chromatographic column appears as a peak on the graphic recorder. Under specified column conditions, a component has a characteristic retention time (the time a component is retained in the column) or retention volume (the volume of carrier gas passed during the retention time). This forms the basis of qualitative analysis.

17.4.6.2 Quantitative analysis

Quantitative analysis by gas chromatography depends on the measurement of areas under the component peaks. The area contained by a peak is proportional to the quantity of the component

present in the sample. Every peak is measured and the areas calculated. These are summed and components are expressed as a percentage. Following conditions are necessary for this:

- The flow rate must be constant, so that the time abcissa may be converted to volume of carrier gas.
- The output of the detector system must be linear with concentration.

Retention measurement

The time taken for a given species to pass through the column is referred to as the retention time, T_R for that species. Most desirably, retention times are short while separations are efficient (i.e. resolution is high). Achieving these characteristics for complex mixtures of similar materials is a challenging task, and the extraordinary utility of chromatography lies in its successful application to such tasks.

The identity of an unknown component in a mixture when analysed with GLC can be established by knowing its time of elution or retention volume. It is known that under given operating conditions of column temperature and flow, the time of elution or retention volume for a component is constant. In practice, retention times of known and unknown materials are compared and a tentative identification of the component is established. However, when more than one component has the same retention time, it is possible that wrong results may be reduced.

Relative retention measurement

In this technique, relative retention data are made use of instead of the absolute values of the retention parameters. The method is more reliable, does not depend upon analysis conditions to be set very precisely and is suitable for day-to-day comparisons. Unknown components are compared with the standard to give a relative retention volume. In this way, an operator may build up a library of retention data for future use.

17.5 METHODS OF MEASUREMENT OF PEAK AREAS

Gas and liquid chromatography provides fast and convenient means for analysing the chemical components of complicated mixtures. However, identifying and quantifying the raw chromatographic information obtained on a conventional strip chart recorder requires a major effort. A typical chromatogram would comprise of a series of peaks separated in the time domain, each peak corresponding to a chemical component detected. The time of occurrence of each peak corresponds to the travel time through the column and can be used to identify the corresponding chemical component. The area enclosed by the peak corresponds to the concentration of that chemical.

The area under the chromatographic peaks can be measured by analog or digital techniques. In the analog technique, the detector current is amplified in an operational amplifier and then integrated. It gives a curve with waves, the wave heights corresponding to the peak areas. In the digital method, a circuit shown in Figure 17.22 can be employed.

The output from the pre-amplifier after the detector is brought to resistance R_1 of the integrator. To the E_1 point, is brought a voltage used to eliminate zero shift. As soon as the absolute values of the two voltages differ, charging of the integrating capacitor begins. The output of OP_2 would be a voltage increasing linearly with time, the slope of which will correspond to the difference between the signal voltage and the E_S. As soon as the integrator output voltage reaches or slightly exceeds the voltage value applied to input E_1, the comparator (OP_3) output jumps to the saturation voltage and the relay (R_e) is closed. The integrating capacitor is thus discharged. This process can

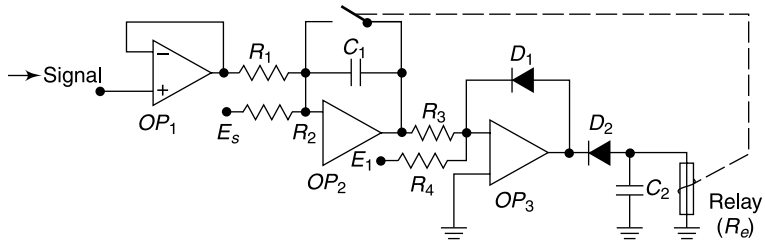


Figure 17.22 *Circuit diagram for measurement of area under the chromatographic peak*

be repeated and the circuit can be used for conversion of an analog signal into a digital signal. As each relay closure is recorded by a counter, the number of pulses registered in the counter gives a measure of the area under the peak.

Chromatographic peaks distorted by tailing can be modified by summing the original signal and its first derivative. The development of electronic integrators speeded up data reduction by automatically computing the areas under the peaks and print the areas and retention time for each peak. Basically, these integrators are voltage-to-frequency conveners that monitor the output of the chromatograph detector and drive a counter activated by, rather complex, peak-recognition logic.

The next development was to derive final results with the aid of a microprocessor working directly from an analog-to-digital converter. This processor provided means of adding automatic calibration, so that the integrator could identify the peak belonging to the calibrating signal and then scale results. It also incorporates means for reducing the effects of detector noise, and for selecting the optimum slope sensitivity automatically; so it can be sensitive to small peaks while ignoring noise peaks.

Modern gas chromatographs are microprocessor-based, which are controlled through a simple keyboard and high resolution video display units. They provide real time screen graphics display of the chromatogram and integral data handling. Such instruments offer flexibility of application through the use of a range of easily interchangeable components. For injection, packed, split/splitless, on-column capillary or programmable temperature vaporiser systems are available, which can be extended by a range of manual and automated gas and liquid-sampling valves.

High speed gas chromatic systems have been developed (Sacks et al.,1998) which combine technologies for dramatically reducing separation times by using electronically controlled inlet systems, temperature and pressure programming with closed-loop control and electronically controlled column selectivity. The data stations receive the detector output, calculate peak areas, and print chromatograms, complete with run parameters and peak data. Chromatographic data may be stored and reprocessed, with integration and other calculation variables being changed as required. Data stations are used also to program the chromatograph, controlling most operational variables and providing for long periods of unattended operation.

17.6 GAS CHROMATOGRAPH-MASS SPECTROMETER (GC-MS)

GC provides an excellent method for separation of the components of a mixture. However, the technique does not provide direct identification of the separated components. The only information presented for each component is its retention behaviour in comparison with that of the other

constituents under a given set of GC operating conditions. The most usual and reliable method of identification is to isolate the compound using spectral methods like IR, UV, NMR and mass spectrometry. Mass spectrometry is the most sensitive of the spectral methods and permits the direct introduction of a gas chromatograph's effluent stream into it. Moreover, both require samples in the vapour state. GC-MS can be thus recognised as an entity in itself rather than just a combination of the two.

Placed at the end of a chromatographic column in a manner similar to the other GC detectors, the mass detector is more complicated than, for instance, the FID because of the mass spectrometer's complex requirements for the process of creation, separation, and detection of gas phase ions. A capillary column most often used in the chromatograph because the entire MS process must be carried out at very low pressures (~10^{-5} torr) and in order to meet this requirement a vacuum is maintained via constant pumping using a vacuum pump. It is difficult for packed GC columns to be interfaced to an MS detector because they have carrier gas flow rates that cannot be as successfully pumped away by normal vacuum pumps; however, capillary columns' carrier flow is 25 or 30 times less and therefore easier to 'pump down'. That said, GC/MS interfaces have been developed for packed column systems that allow for analyte molecules to be dynamically extracted from the carrier gas stream at the end of a packed column and thereby selectively sucked into the MS for analysis. For one type interface, using a silicone membrane, the selectivity for organic molecules (the analyte) over helium (the carrier gas) is 50,000.

One of the most obvious problems encountered in combining the GC and MS is the considerable difference in operating pressures. The pressure at the exit of the column in GC is atmospheric. The flow rate of the carrier gas is 15–50 ml/min; depending upon the type of column used. Mass spectrometers on the other hand accept ion source pressures, generally no higher than 10^{-4} torr (1 torr = 1 mm of Hg). Therefore, the total flow of the GC instruments cannot be introduced into any MS of commercial type. In the earlier days, some sort of splitting arrangement was used, which permitted to feed only a portion of the effluent into the mass spectrometer. More efficient linkages have been reported in literature, which in principle strip the sample from the carrier gas, discard the carriers and introduce the sample into the mass spectrometer. These are known as enrichment devices, or molecular separators, since the sample gets concentrated by passage of the effluent stream through tile separator.

Figure 17.23 shows the principle of a jet type molecular separator, which provides a pressure dip from approximately atmospheric to about 10^{-4} mm of Hg. Substances eluted from the GC column

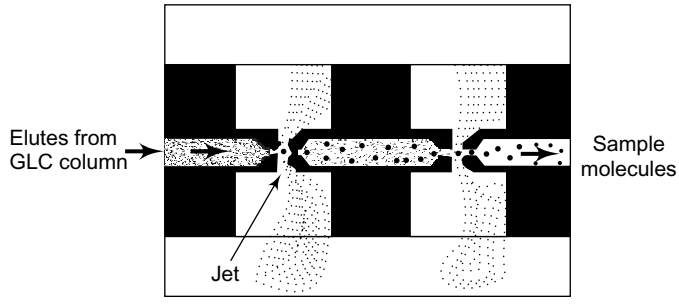


Figure 17.23 *Principle of a jet type molecular separater*

are carried along with helium carrier gas into the system, which in two stages, removes most of the helium (carrier gas), while the sample passes into the ion source chamber in a highly concentrated molecular beam. Each stage consists of an evacuated chamber connected to a pumping system. The column effluent enters the system through a very fine jet, which is aligned with a small exit orifice positioned a short distance from the entrance jet. The column effluent would enter the separator as a very high speed stream of gas, with the entrance jet acting as a restrictor between the GC and the separator. The carrier gas with the low-molecular weight would diffuse at a higher rate than the higher molecular weight sample. Therefore, the carrier gas would diffuse away from the line of flow and would be pumped away. The sample molecules along with the remaining carrier gas then pass into the second stage, when tile process repeats itself. This type of separator is used in most of the commercial GC-MS systems. Other methods for separating out the carrier from the sample include porous tubes, Teflon tube and selective membranes.

The essential components of a basic mass spectrometer are a sample inlet system, an ion source, a mass analyser, a detector, a vacuum system, and a data processing device. Figure 17.24 shows the block diagram of a typical quadrupole GCMS.

The sample inlet system in mass spectrometer permits introduction of a representative sample into the ion source with minimal loss of vacuum. These may include chromatographic inlets or capillary electrophoretic inlets. In GCMS, chromatographic inlets are used where the tip of the capillary column is precisely inserted into the inlet.

The purpose of an ion source is to ionise the molecule to produce gaseous analyte ions. Electron impact ionisation (EI), chemical ionisation (CI) and field ionisation (FI) are used to ionise the analytes.

EI ionisation is the most common ionisation technique used, in which the sample is brought to a temperature high enough to produce molecular vapour, which is then ionised by bombarding with a beam of energetic electrons. Despite certain disadvantages (excessive fragmentation leading to

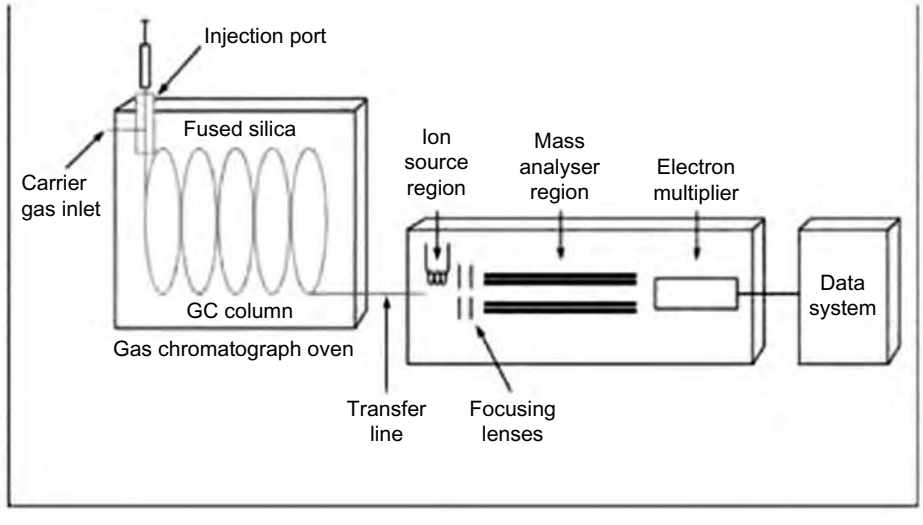


Figure 17.24 *Block diagram of GC-MS (Courtesy: FAO, 2011)*

disappearance of molecular ion peak at times and the need to volatilise the sample), this technique produces a reproducible mass spectra of a compound and is the basis on which many mass spectral libraries are built.

In CI, also termed as soft ionisation, a gaseous sample is ionised by collision with ions produced by electron bombardment of a reagent gas such as methane or ammonia. Collision between the sample molecule (M) and highly reactive reaction products aroused from the reagent gas usually involves proton or hydride transfer leading to formation of either $(M+1)^+$ or $(M-1)^+$ ions. Relative to EI spectrum, CI spectrum is simple and provides molecular weight information.

The mass analyser separates the mass fragments produced by the ionisation sources. The capability of a mass spectrometer to differentiate between masses is usually stated in terms of its resolution (R) which is defined as $R = m/\Delta m$, where Δm is the mass difference between two adjacent peaks that are just resolved and m is the nominal mass of the first peak (mean of the two peaks is sometimes used). Several low and high resolution mass analysers are available which include single-stage quadrupole, triple-stage quadrupole, ion trap, magnetic sector and time-of-flight.

Detectors used in the mass spectrometer include electron multiplier detectors, Faraday cup collector and photomultiplier detectors. Electron multiplier detectors are most commonly used. The data processing devices have the capability to control the instrument as well as process a large quantity of data and provide mass spectrum of compounds.

The optimisation of the parameters controlling ion source sensitivity, mass spectral scan cycle time, and chromatographic elution profile as well, on on-line computer systems to record, display in real time and subsequently evaluate these sets of GC-MS data is mandatory, for full utilisation of potentiality of this combination.

17.7 GAS CHROMATOGRAPHY–INFRARED SPECTROSCOPY

Infrared spectroscopy may be combined with each of a number of possible chromatographic techniques, with gas chromatography–infrared spectroscopy (GC–IR) being the most widely used. GC–IR allows for the identification of the components eluting from a gas chromatograph. In GC, the sample in a gaseous mobile phase is passed through a column containing a liquid or solid stationary phase. The retention of the sample depends on the degree of interaction with the stationary phase and its volatility: the higher the affinity of the sample for the stationary phase, then the more the sample partitions into that phase and the longer it takes before it passes through the chromatograph. The sample is introduced into the column, housed in an oven, via injection at one end and a detector monitors the effluent at the other end. A common method for coupling a gas chromatograph to an FTIR spectrometer is to use a light pipe (i.e. a heated flow cell which allows the continuous scanning of the effluent emerging from the GC column). Figure 17.25 shows a schematic diagram of a typical GC–IR system, while the constructional details of the light pipe are shown in Figure 17.26. Important features are the light pipe dimensions. The volume of the cell is always a compromise between a good GC resolution (small cell volumes needed), and a good sensitivity (requiring large cell volumes).

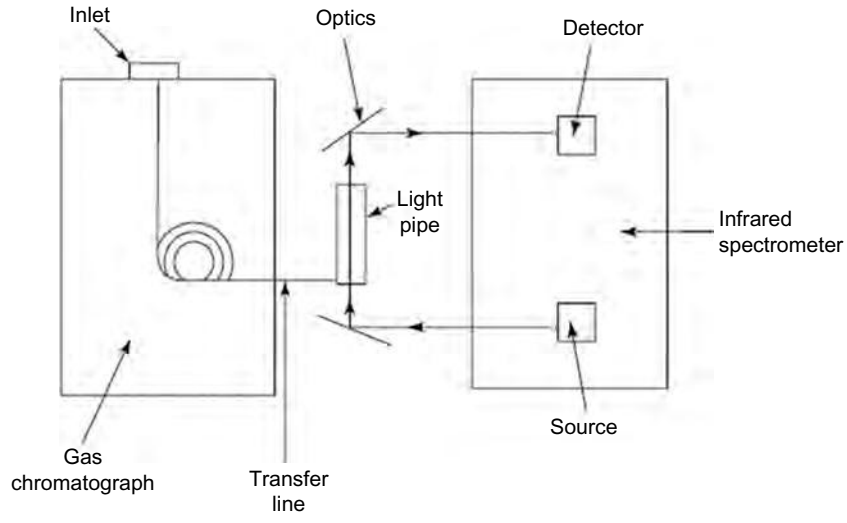


Figure 17.25 *Layout of a typical GC–IR system (Adapted from Stuart, 2004)*

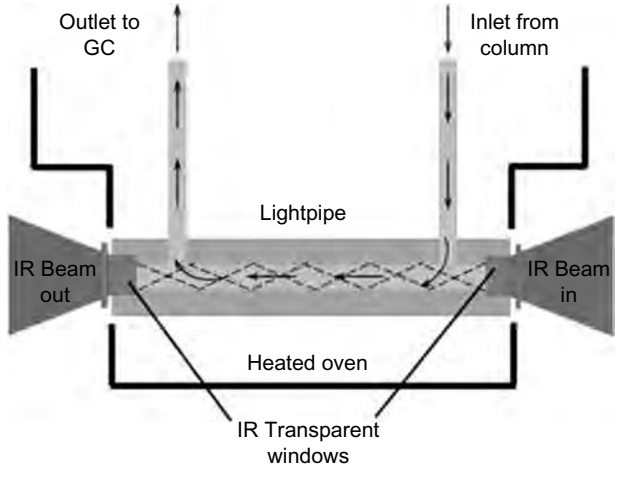


Figure 17.26 *The constructional details of light pipe*

The nature of this technique requires that interferograms are collected over short time intervals. Data can be displayed in real time and are commonly monitored as the changing spectrum of the GC effluent and the changing infrared absorption as a function of time.

18

LIQUID CHROMATOGRAPHS

18.1 LIQUID CHROMATOGRAPHY

Liquid chromatography (LC) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase, the different solutes will interact with the other phase to differing degrees due to differences in adsorption, ion exchange, partitioning, or size. These differences allow the mixture components to be separated from each other by using these differences to determine the transit time of the solutes through a column.

The early separations by chromatographic technique were performed using a bed of solid, powder absorbent, such as alumina or charcoal through which the sample was passed in a stream of solvent. Since these techniques used liquid as the percolating agent, LC can be considered as the oldest of all chromatographic processes. Later developments included liquid/liquid partition chromatography, paper chromatography and the ion-exchange chromatography. Gel-permeation chromatography and thin-layer chromatography were developed in the 1950s.

Simple LC consists of a column with a fritted bottom that holds a stationary phase in equilibrium with a solvent. Typical stationary phases (and their interactions with the solutes) are solids (adsorption), ionic groups on a resin (ion exchange), liquids on an inert solid support (partitioning) and porous inert particles (size-exclusion). The mixture to be separated is loaded onto the top of the column followed by more solvent. The different components in the sample mixture pass through the column at different rates due to differences in their portioning behaviour between the mobile liquid-phase and the stationary phase. The compounds are separated by collecting aliquots of the column effluent as a function of time.

Until a few years ago, LC was not quite commonly used, because of the non-availability of high-sensitivity detection systems. With the introduction of such detectors, the analytical potential of LC is greatly enhanced and sophisticated liquid chromatographs are now commercially available.

Conventional LC is most commonly used in preparative scale work to purify and isolate some components of a mixture. It is also used in ultra-trace separations where small disposable columns are used once and then discarded. Analytical separations of solutions for detection or quantification typically use more sophisticated high-performance LC instruments. HPLC instruments use a pump to force the mobile phase through and provide higher resolution and faster analysis time.

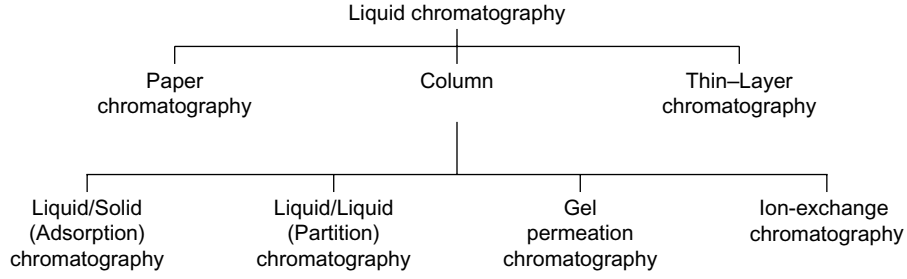
High pressure liquid chromatography (HPLC) is similar to gas chromatography, in that the chemical components of a mixture are separated as the mixture is forced through a column, packed with fine particles. In gas chromatography, the substance is carried through the column in vaporised form by an inert gas, whereas in HPLC it is carried through in liquid form by a solvent. Because the substance need not be vaporised, HPLC can be used on a broad range of substances that are not analysable by gas chromatography.

HPLC presents some unique problems, such as, the maintenance of the solvent flow accurately, for obtaining repeatable results. Problems in controlling solvent flow arise, because the solvents differ in viscosity, compressibility and other characteristics. In addition to this, the volume of the solvent mixture is not necessarily equal to the sum of the volumes of the individual solvents. Also, as the column must be tightly packed with small, uniform particles to obtain adequate separation of the component substances, high pressure of the order of 3000 psi or more is needed to force the substance through the column in a reasonably short time.

LC has been performed in a column and on an open bed (paper chromatography and thin-layer chromatography), whereas HPLC has been performed almost totally in columns. However, thin-film chromatography was introduced recently, as a high-speed method for thin-layer chromatography.

18.2 TYPES OF LIQUID CHROMATOGRAPHY

Liquid chromatography can be classified as under:



18.2.1 Column Chromatography

18.2.1.1 Adsorption chromatography (Liquid/solid)

In adsorption chromatography, a solid adsorbent, usually in powder form, is the stationary phase, through which a mobile liquid-phase carrying the mixture to be analysed is allowed to percolate. Adsorption chromatography is carried out in columns with the adsorbent supported by a plug of glass or cotton wool, or by a sintered glass filter.

The stationary phase in adsorption chromatography are silica or alumina particles. Analytes are separated due to their varying degree of adsorption onto the solid surfaces. The main advantage of adsorption chromatography is in separating isomers, which can have very different physisorption characteristics due to steric effects in the molecules.

18.2.1.2 Partition chromatography (Liquid/Liquid)

In this technique, the mobile liquid-phase is made to percolate through a column containing the stationary liquid-phase, which is deposited on a solid surface as a thin film. Solid supports usually used are silica gel, porous glass and cellulose.

18.2.1.3 Gel-permeation chromatography

Gel-permeation chromatography is a recently developed separation technique in LC. The separation is based on molecular size and shape. The gel permeation column is packed with a stationary phase in the form of a gel which contains pores of a specific size. As the sample is carried through the column bed by the carrier liquid, the sample molecules penetrate the pores in the packing gel, depending upon the size and shape of the molecules. Large molecules do not penetrate the gel and are consequently quickly eluted. Elution takes place in inverse order of their degree of gel permeation and consequently of decreasing molecular size.

18.2.1.4 Ion-exchange chromatography

Ion-exchange chromatography involves the exchange of ions between a solution and a solid insoluble material in contact with the solution. Many naturally occurring solid materials have the ability to exchange ions. Also, many artificial ion-exchange materials have been developed. The ion-exchange process is reversible and this fact is made use of in ion-exchange chromatography. When a sample is introduced at the top of an ion-exchange column (Figure 18.1), the ions exchange rapidly with the ions in the resin. If a mobile phase is used, the sample ions are displaced into the solution again and then re-exchange on to the resin. This process continues until the sample ions emerge from the end of the column. If the various sample ions are held on to the resin to different extents, then the time taken for them to pass through the column will be different and a separation will be achieved.

This type of chromatography depends upon molecular ion-exchange instead of liquid-surface adsorption separation of acidic and basic organic substances from mixtures is achieved by using synthetic resins, which have highly selective action for certain substances, particularly amino-acids and allied compounds. Essentially the process involves interchange of anions and cations between the components of certain resins.

Ion in solution can be detected by measuring the conductivity of the solution. In ion chromatography, the mobile phase contains ions that create a background conductivity,

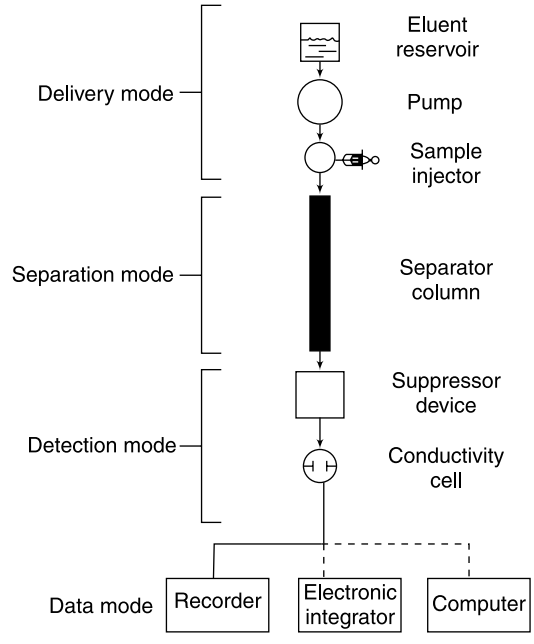


Figure 18.1 *Typical ion-chromatography configuration*

making it difficult to measure the conductivity due only to the analyte ions as they exit the column. This problem can be greatly reduced by selectively removing the mobile-phase ions after the analytical column and before the detector.

In the early stages of development of ion chromatography, dedicated ion chromatographs were considered necessary for the application of the technique. Advances in recent years, particularly in the field of detection systems, have however, eliminated the need for this dedicated approach and enabled ion chromatography to be carried using conventional HPLC instrumentation (Browne, 1986). For identifying and quantifying various ionic species in solution, ion chromatography is a competitor to well established electrochemical methods such as ion-selective electrodes and polarography (George and Adam, 1984).

18.2.2 Thin-Layer Chromatography

In thin-layer chromatography (TLC), the stationary adsorbents are applied to a planar glass or plastic surface and the solvent flows over them. All of the basic types of action, like adsorption, partition, ion exchange, gel-filtration can be used on TLC plates, while solvents are applied in a chamber similar to that used in paper chromatography.

This technique is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products.

TLC consists of a stationary phase immobilised on a glass or plastic plate, and an organic solvent. The sample, either liquid or dissolved in a volatile solvent, is deposited as a spot on the stationary phase. The constituents of a sample can be identified by simultaneously running standards with the unknown. The bottom edge of the plate is placed in a solvent reservoir and the solvent moves up the plate by capillary action. When the solvent front reaches the other edge of the stationary phase, the plate is removed from the solvent reservoir. The separated spots are visualised with ultraviolet light. The different components in the mixture move up the plate at different rates due to differences in their portioning behaviour between the mobile liquid-phase and the stationary phase.

TLC can be automated using forced solvent flow, running the plate in a vacuum-capable chamber to dry the plate and recording the finished chromatogram by absorption or fluorescence spectroscopy with a light source. The ability to program the solvent delivery makes it convenient to do multiple developments in which the solvent flows for a short period of time, the TLC plate is dried and the process is repeated. This method refocuses the spots to achieve higher resolution that in a single run (Poole and Poole, 1994).

18.2.3 Paper-Partition Chromatography

Paper-partition chromatography is a simplified version of column chromatography, which makes use of strips or hollow cylinders of filter paper to hold both the solid and liquid phases. Here, drops of the solutions containing unknown mixtures are applied to a number of parallel strips, a few inches from the end of each test paper and allowed to dry. The strips of paper are placed in a chromatography chamber with a saturating and equilibrating vapour and hung from a solvent reservoir, so that the movement of the solvent downward can be timed and the relative partition of the different substances measured.

18.3 HIGH PRESSURE LIQUID CHROMATOGRAPH (HPLC)

A HPLC chromatograph consists of the following parts as shown in Figure 18.2:

- A high-pressure pump system to force the liquid mobile-phase through the column.
- Gradient elution or solvent programmer.
- The sample injection system.
- The column.
- The detection system including display or recording devices.

Figure 18.3 shows the physical layout of various component of an HPLC system.

As in other chromatographic techniques, the sample is introduced into the column with the help of a sample injection system. Various components of the sample are fractionated during their passage through the column. The detection system senses these components as they elute from the column and produces a signal proportional to the amount of solutes passing through the detection system. The detector determines what separation has taken place and provides data permitting the qualitative and quantitative evaluation of the results. This can be accomplished by simply recording the response of the detector in the form of a chromatogram and/or with the help of a data handling equipment. The individual sample components separated in the column can also be collected.

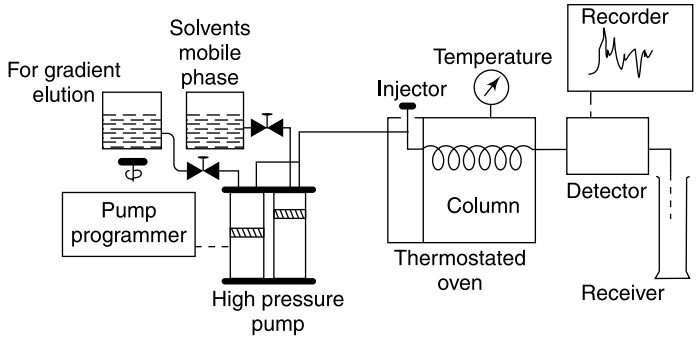


Figure 18.2 *Block diagram of a liquid chromatograph*

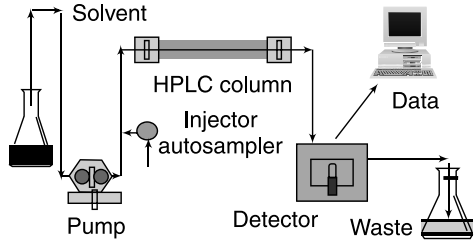


Figure 18.3 *Physical layout of various components of a HPLC (Courtesy: Waters Corp.)*

18.3.1 High-pressure Pump System

Liquid chromatographs of the early type made use of wide diameter columns packed with coarse mesh packing material. They required very little pressure to obtain an adequate flow rate of the mobile-phase liquid. Modern instruments, which employ smaller diameter columns filled with fine-mesh particles, have necessitated the use of high-pressure solvent delivery systems.

The most commonly used methods for solvent delivery are gravity feed system. The gravity feed systems, though simple, are not able to deliver solvent at high pressure. They are therefore, not used with narrow bore columns packed with fine-mesh particles, which need high inlet pressures to yield the required flow rate. Various types of pump systems incorporating piston pumps, peristaltic pumps, diaphragm pumps and syringe pumps, etc. are therefore used in HPLC systems. There are several types of pumps available for use with HPLC analysis, they are: constant flow pumps, reciprocating piston pumps, syringe type pumps and constant pressure pumps.

18.3.1.1 Constant flow pumps

Reciprocating piston pumps consist of a small motor-driven piston which moves rapidly back and forth in a hydraulic chamber that may vary from 35–400 μL in volume. On the back stroke, the separation column valve is closed and the piston pulls in solvent from the mobile-phase reservoir. On the forward stroke, the pump pushes solvent out to the column from the reservoir. A wide range of flow rates can be attained by altering the piston stroke volume during each cycle, or by altering the stroke frequency.

Figure 18.4 shows the schematic of a single-piston type reciprocating pump. Here, a rotating eccentric cam forces the piston to expel liquid through a one-way valve, called the check valve. The pumping rate is usually adjusted by controlling the distance the piston retracts, thus limiting the amount of liquid pushed out of by each stroke. The purpose of the check valve is to assure that

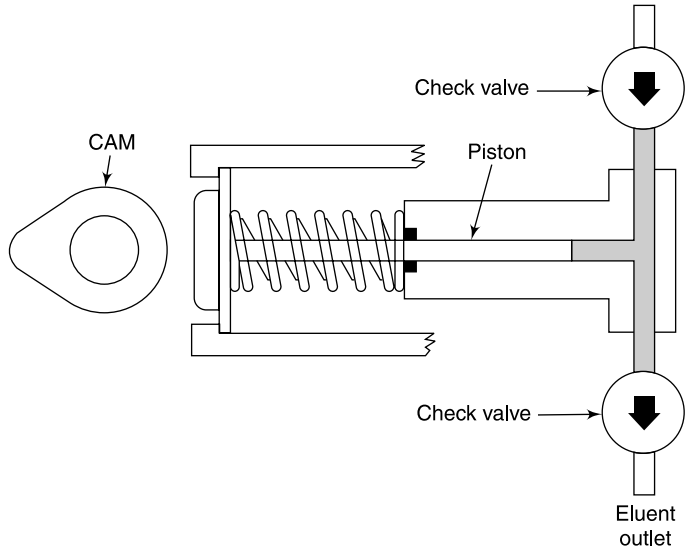


Figure 18.4 *Principle of single-piston reciprocating pump*

liquid moves only in one direction. These pumps, obviously, deliver a series of pulses of the mobile phase, which may disturb the detector. It is thus necessary that these pulses may be eliminated, for which several methods have been developed. Dual and triple head pumps consist of identical piston-chamber units which operate at 180° or 120° out of phase. This type of pump system is significantly smoother because one pump is filling while the other is in the delivery cycle.

The schematic diagram of a dual-head reciprocating pump which provides automatic pulse damping is shown in Figure 18.5. In this arrangement, both the pump chambers are driven by the same motor through a common cam, gears or hydraulic linkage. This common drive allows one piston to pump while the other is refilling, as a result, the two-flow profiles overlap each other, significantly reducing the pulsation, downstream of the pump. Usually, a computer-designed camshaft is used to achieve maximum overlap of pump strokes, resulting in virtually undetectable pulsation or ripple.

Syringe Type Pumps: A most commonly encountered piston-type pump is the syringe pump. In these pumps, a constant and reproducible flow can be obtained by using a gear mechanism. Spring loaded Teflon seals are used in the plungers to minimise leakage around the pistons at high pressure. They are most suitable for small bore columns because this pump delivers only a finite volume of mobile phase before it has to be refilled. These pumps have a volume between 250 and 500 ml. The pump operates by a motorised lead screw that delivers mobile phase to the column at a constant rate. The rate of solvent delivery is controlled by changing the voltage on the motor.

Figure 18.6 shows the schematic of a typical syringe pump. The cylinder holds the mobile phase which is expelled by a piston. The piston is advanced by a motor connected through worm gears, to produce smooth pulseless flow. IN syringe pumps, presence capability is generally quite high

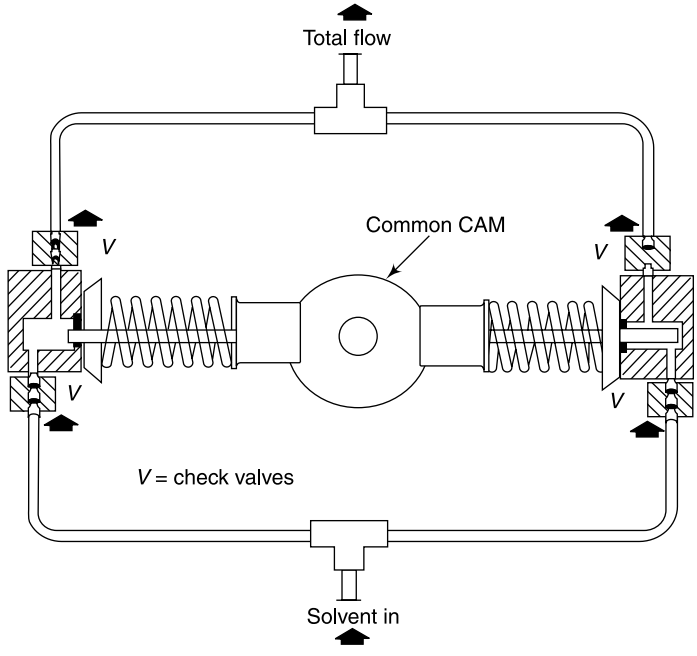


Figure 18.5 *Schematic of a dual-lead reciprocating pump*

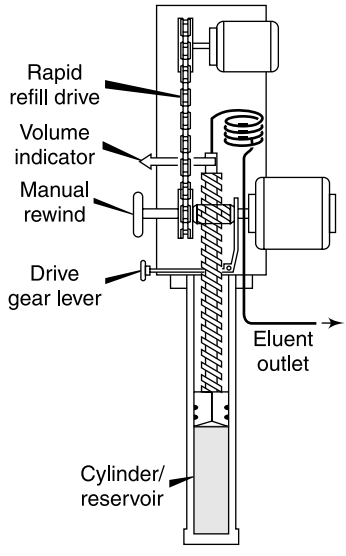


Figure 18.6 *Schematic of a positive displacement syringe pump (after Yost et al., 1980)*

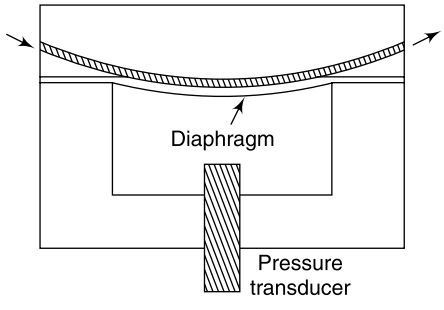


Figure 18.7 *Hydraulic capacitance for smoothening of flow pulsations (after Schrenker, 1975)*

and maintenance is infrequent. However, they have limited reservoir capacity and a slight change of flow rate when extremely high presence compresses the solvent.

18.3.1.2 Constant pressure pumps

In these pumps, the mobile phase is driven through the column with the use of pressure from a gas cylinder. A low-pressure gas source is needed to generate high liquid pressures. The valving arrangement allows the rapid refill of the solvent chamber whose capacity is about 70 ml. This provides continuous mobile-phase flow rates.

High pressures of several hundred atmospheres are required in high resolution HPLC. Varian Aerograph Model 8500 Liquid Chromatograph employs pump system, which gives pressure up to 600 atmospheres. Generally used pumps are the piston type, which provide very high solvent pressures. The flow rate can be set to the desired rate by adjusting the pump stroke length and the motor speed. The pressure is observed as a dependent variable. The flow of solvent from a piston pump is usually in the form of a series of pulses. This type of ripple in the flow is likely to affect column resolution and detector stability. To smooth out the ripple, a long nylon tube of about 1.5 mm diameter may be used between the pump and the chromatographic column. Ripple can also be reduced by using bellows, restrictors or multi-piston pumps, where the action of the individual pistons is arranged at regular intervals of a complete stroke cycle.

The metric unit of presence measurement is the pascal, abbreviated as Pa. One pascal is equal to one Newton per square meter (N/m^2) and, in previously used units, to 1×10^{-5} bar.

Most scientists use the Kilopascal (KPa = 10^3 Pa) or mega Pascal (MPa = 10^6 Pa). Since 1 bar equals 0.9869 atm, 14.5038 psi and 1.0197 Kg/cm^2, one mega Pascal equals 10 bar, 9.869 atm, 145.038 psi and 10.197 kg/cm^2.

A flow control system for HPLC, which maintains constant flow irrespective of differing solvent viscosities and compressibilities, utilises a hydraulic capacitor (Figure 18.7) which smoothens the high-pressure pump pulsations, normally encountered in piston operated pumps. It consists of a rigid vessel filled with fluid of known compressibility. A small fraction of the space is separated from the compressible fluid by an impermeable membrane. The solvent mixture passes through this separated space.

A restriction in the solvent flow path, which could be the chromatographic column itself, is the hydraulic analog of a resistor, so the unit can function analogously to an *R.C.* filter. With a sufficiently large fluid volume (large C), adequate smoothing of the pump pulsations can be obtained with a relatively low value of Eon on the output side. The flow measurement and control system used by HP in their model 1010B HPLC is shown in Figure 18.8.

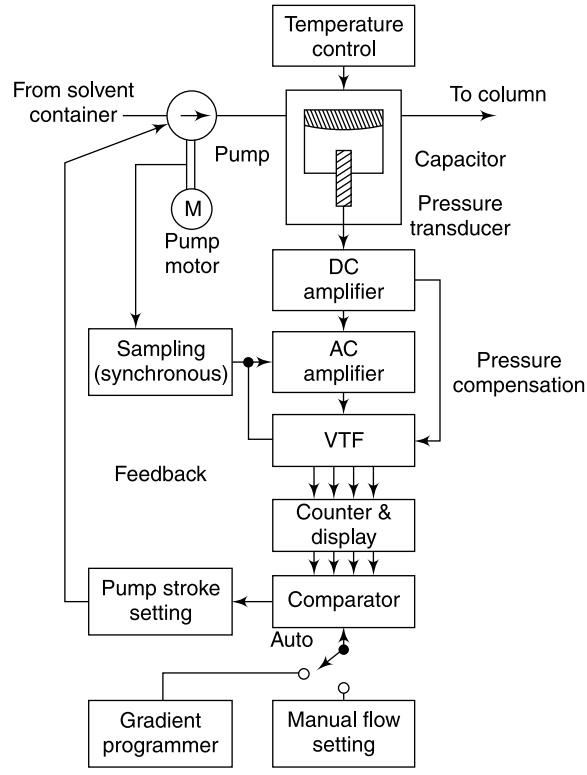


Figure 18.8 *Flow measurement and control system (after Schrenker, 1975)*

A pressure transducer is installed in the hydraulic capacitor. Measurement of the pressure is synchronised with the pump, so that, measurement is made only during the discharge phase. The time integral of the ac component of the transducer output is proportional to changes in pressure and is thus proportional to flow. The ac component is fed to a voltage-to-frequency converter. A counter totals the output of v to f converter, effectively integrating the transducer output. At the end of each 12 s integration period, the counter's contents are compared digitally to the set point. Any error is then used to adjust the pump stroke setting to bring the flow rate to the set point value. The averaged dc output of the pressure transducer represents absolute pressure, and is used as a constant to compensate for the influence of pressure on the compressibility constant of capacitor *C*. The effects of temperature on fluid compressibility are circumvented by using a temperature control system to maintain the capacitor at a constant temperature.

Flow rates in the range of 0.10–9.99 ml/min are controlled by this system with repeatability ± 1%. The flow rates of the two pumps are controlled independently and then outputs are mixed in a low-volume mixing chamber, immediately upstream of the sample injection port.

The use of microprocessors in *LC* pumps has enabled combination of features of uniform flow, accurate solvent proportioning and low system volume. The series 410 microprocessor-based LC pump from Perkin Elmer, whose details are shown in Figure 18.9, is a positive volume proportioning pump, which ensures accurate and repeatable solvent delivery and composition, with no adjustments necessary by the chromatographer. The pump actually measures the volume of solvent delivered by

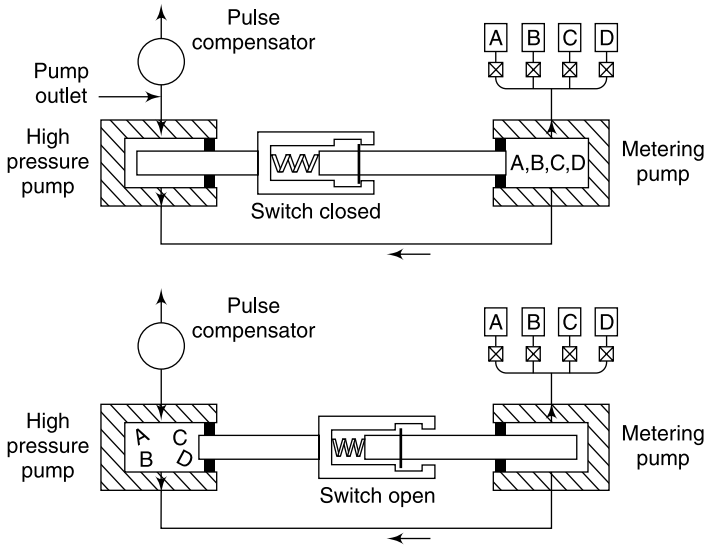


Figure 18.9 *Principle of positive volume proportioning pump (Courtesy: M/s Perkin Elmer, USA)*

the pump. This information is monitored by the microprocessor, which automatically makes any necessary correction to compensate for volume reductions due to solvent mixing. This results in the solvent blend being essentially the same as one, the experienced chromatographer would mix. The pump provides four solvent gradient capability including step, linear, convex and concave gradients to cover the foil range of LC applications, with flow rate ranging from 0.01 to 10.0 ml/min for microprobe, analytical high speed and semipreparatine. The multi-tasking software enables ease of operation by allowing modification of both current and inactive methods, while the pump is running.

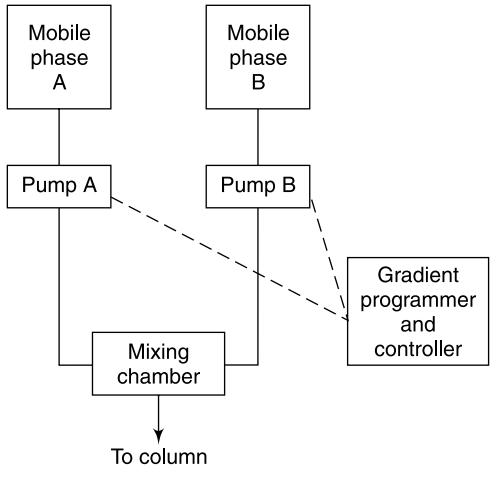


Figure 18.10 *Functional schematic of a system for gradient elution*

18.3.1.3 Gradient elution or solvent programming

In LC, a single substance may be used as a mobile phase during an analysis of the mixture of two or more substances to properly adjust the characteristics of the phase. Also, one may maintain a constant mobile-phase composition during analysis or change it. The first mode is called the Isocratic operation, while the second is culled the gradient elution: Figure 18.10 represents the functional schematic of the system providing the mixed mobile-phase to the column.

Gradient elution is often required to resolve complex mixtures, especially those containing components with significantly different chromatographic behaviour. A solvent

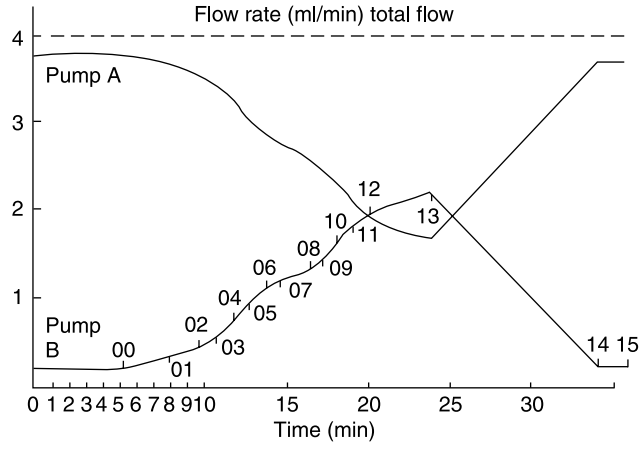


Figure 18.11 *Flow programming of two pumps to give a constant total flow output*

programmer helps to control the composition of the mobile phase according to a predetermined programme as the analysis proceeds. Solvent programming is generally carried out by continuously adding a more polar solvent to the mobile-phase feed reservoir, thereby increasing the polarity of the eluant as a function of time. The technique involves the use of separate pumps, feeding different solvents or solvent mixtures concurrently into the column and programming the output of each pump.

Figure 18.11 shows the arrangement usually employed for solvent programming. The supply of the solvent from the pump is given to a T-connection through solenoid valves. One solvent is used as a feed to the pump and the other is introduced into the bellows assembly. By properly programming the time intervals between opening and closing of the solenoid valves, the desired gradient to the column can be obtained. Standard linear, convex and concave gradients are usually sufficient. However, some manufacturers also provide facilities for non-linear gradients. With the help of this system, it becomes possible to generate gradients of virtually all complexities that one may need to resolve difficult mixtures. The rate of change of solvent composition is selected with the help of suitable programme. The solvent strength can be increased, decreased or held constant. It is held constant at any point or points in the program simply by omitting a rate pin in a given step. This ability to hold constant solvent strength at any point in the program is highly useful for maximising resolution in difficult parts of an analysis, for example, in separating isometric compounds.

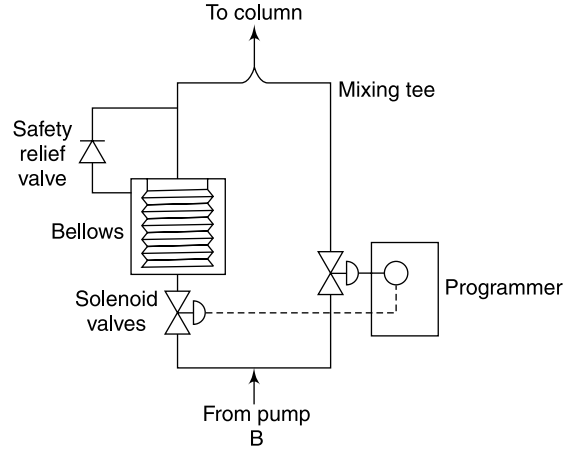


Figure 18.12 *Arrangement employed for solvent programming*

In the recent instruments, a gradient programme is entered by way of a calculator-like

keyboard on the gradient programmer. The programme is entered as a series of linear segments (Figure 18.12) that approximate the desired programme curve. Each segment is specified by these programme entries:

- Flow rate at the beginning of the segment
- Flow rate at the end of the segment
- Time duration of the segment

This information is stored in the digital memory. Because the flow rate programming for each pump is independent of the other, three-programme modes are possible:

- Change the mixing ratio from the two pumps, while maintaining constant column flow by programming equal but inverse flow rate changes for the two pumps (gradient programming).
- Change the column flow rate while maintaining a constant mixing ratio by programming equal percentage changes of flow in A and B (flow programming).
- Combine (i) and (ii), resulting in flow programme super-imposed on a gradient programme.

18.3.2 Sample Injection System

There could be several methods for the introduction of the sample on the top of a LC column. One method is to disconnect the solvent supply, to add the sample in solution and reconnect the solvent supply to the column. The mechanism is simple, but the method is tedious to operate. More recently developed methods fall into two categories namely, the syringe injection method and the injection valve method. Both these methods enable the sample to be introduced directly into the column packing, without interrupting the solvent flow.

18.3.2.1 Syringe injection method

Samples are injected into the HPLC via an injection port. The injection port of an HPLC commonly consists of an injection valve and the sample loop. The sample is typically dissolved in the mobile phase before injection into the sample loop. The sample is then drawn into a syringe and injected into the loop via the injection valve. A rotation of the valve rotor closes the valve and opens the loop in order to inject the sample into the stream of the mobile phase. Loop volumes can range between 10 µl to over 500 µl. In modern HPLC systems, the sample injection is typically automated.

Stopped-flow injection is a method whereby the pump is turned off allowing the injection port to attain atmospheric pressure. The syringe containing the sample is then injected into the valve in the usual manner and the pump is turned on. For syringe type and reciprocation pumps, flow in the column can be brought to zero and rapidly resumed by diverting the mobile phase by means of a three-way valve placed in front of the injector. The method can be used up to very high pressure (Willard et al., 1988).

Syringe injection method basically involves the insertion of the syringe needle through a rubber septum at the top of the column (Figure 18.13). This method, however, cannot be used for the injection of large sample volumes into high-pressure solvent systems. At pressures greater than about three atmospheres, the pressure has to be reduced by turning off the solvent supply before the injection of the sample can be carried but.

18.3.2.2 Injection valve method

In this method, the injection valve containing sample loops are connected in the solvent supply pipe work at the top of the column. The sample loop can be introduced into the solvent stream when desired, without turning off the solvent flow. After sufficient flushing of the loops with solvent has taken place, the sample gets completely carried to the column. The loop can then be removed from the solvent stream for refilling with the next sample. Injection valves can be used for sample introduction into very high solvent pressure systems. By changing the volume of the sample loop, the sample size can be easily varied. Also, this method can be conveniently automated for automatic injection of the samples.

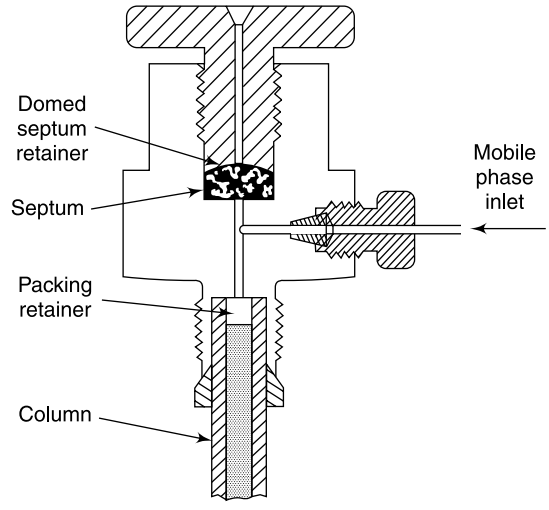


Figure 18.13 *Syringe injection method*

18.3.3 The Column

The column is, by far, the most important part of any chromatographic system, since the ultimate performance of the chromatograph is determined largely by the column. Most of the early analysis work, which was carried out by LC, made use of large columns with an internal diameter of 1 cm or more. With the development of highly sensitive detection systems, it has become possible to analyse minute quantities of sample and to reduce the column diameter. Reduction of sample size and the column diameter result in an improvement in separation efficiency.

There is another factor which necessitates the use of small diameter columns. A large contribution to the band broadening in the chromatographic peaks is known to be due to large-scale unevenness of flow, which becomes worse as the column diameter increases. The effect of uneven flow may be reduced by decreasing the diameter of the column. The columns in current use are generally in the 0.1–2.0 cm internal diameter range. However, there are practical problems when column diameter becomes less than 0.05 cm, because very small particle sizes and very high-pressure drops will have to be used. As regards the column length, they may be 1–4 m long, but most of the applications can be performed on columns of less than 100 cm length.

Various materials have been used for the construction of columns. Glass columns are usually preferred on account of their inert nature and the facility of being able to observe the packing visually. HPLC columns are stainless steel tubes, typically of 10–30 cm in length and 3–5 mm inner diameter, short, fast analytical columns and guard columns, which are placed before an analytical column to trap junk and extend the life time of the analytical column, are 3–10 cm long.

Theoretical considerations have revealed that much smaller particles should be used for column packing in LC than in gas chromatography. Generally, the particle size ranges from 20 to 50 μ. Occasionally, even smaller particle sizes are used. For example, in Varian 8500 LC System, the columns are packed with particles of diameter less than 10 μ. Columns packed with small-sized particles are more efficient, since the solute mass transfer takes place at a very rapid rate. Small

packing particles used in LC, however, present great problems in getting homogeneous packing of the columns. Also, the method of column packing is dependent on the type, regularity and the particle size of the packing used.

In most of the separations, LC columns are operated under ambient temperature conditions. However, some researchers have shown that improvements in column efficiency can be achieved in certain cases by working at elevated temperatures. Columns are therefore placed inside ovens capable of operation up to 25°C. The temperature of these ovens is controllable to a high degree of constancy.

18.3.4 Detection Systems

The detector for an HPLC is the component that emits a response due to eluting sample compound and subsequently produces a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column (Frel, et al., 1985).

High-sensitivity detection systems are necessary for achieving optimum column separating performance, by the use of small sample volumes. The absence of a versatile and economical detector has been one of the main reasons for the limited development of the LC in the past. The earlier methods of detection used in LC were of non-continuous nature. However, continuously monitoring detection systems are more convenient in operation and therefore, all modern LC detectors incorporate this feature.

Several detection systems have been developed, which are mostly dependent upon the measurement of a physical property of the column elute. These physical properties could be changes in ultraviolet absorption, infrared absorption, heat of adsorption, refractive index or electrical conductivity.

In principle, any of these measurement techniques can be used, but in practice there are several constraints when a detector is interfaced to LC (Yeung and Synovec, 1986):

- Good sensitivity is essential to deal with the low concentrations of typical analytes.
- The volume of the detector must be small to avoid additional band broadening due to extra-column effects, particularly in the recently introduced techniques of micro-column LC and super-critical fluid chromatography. Volumes below 1 µl for 1 mm internal diameter (Packed micro-bore) columns and below 1 nl for open micro-tubular columns of 10 µm internal diameter are required.
- The detector must be able to function in the presence of a large background signal and be able to null out this signal and to maintain it at a stable level to reduce noise.
- The response time of the detector must be compatible with the chromatographic event
- Detector selectivity is more important in LC compared with GC, as chances of overlapping in LC are much higher. A selective detector can effectively resolve components without physical separation.

There are many types of detectors that can be used with HPLC. Some of the more common detectors include refractive index (RI), ultraviolet (UV), fluorescent, radiochemical, electrochemical, near-infrared (Near-IR), mass spectroscopy (MS), nuclear magnetic resonance (NMR) and light scattering (LS).

All present-day detectors are on-stream systems, continuously monitoring specific characteristics of the column effluent in a flow-through all. Modern LC systems employ fairly low flow rates and provide high resolution in a short time. The detectors utilise low-volume cells which are efficiently flushed to prevent band broadening and tailing. A measure of the efficiency of these cells

is the so-called 'instrument bandwidth.' The instrument bandwidth, expressed in micro-litres, is the minimum peak volume which the detector produces no matter how small the sample volume introduced into the detector.

Traditional detectors for LC include RI, electrochemical, fluorescence and ultraviolet-visible (UV-Vis) detectors. Some of these generate two-dimensional data; that is, data representing signal strength as a function of time. Others, including fluorescence and diode array UV-Vis detectors, generate three-dimensional data. Three-dimensional data include not only signal strength but spectral data for each point in time.

18.3.4.1 UV-Visible spectrophotometric detectors

With this type of detector system, it is possible to detect and analyse compounds that absorb at any wavelength in the UV-visible range from about 200 to 800 nm with a bandwidth of 5 nm. Almost any spectrophotometer suitable in this range can be modified to work as a detector. The cell in this ease would be of flow-through type, with a path length of 1 cm and cell volume as low as 8–20 µl. Cells may be made of quartz, Teflon and KELF. They are usable up to a pressure of 500 psi. Stainless steel cuvette assemblies are used for higher pressures. When using a recording spectrophotometer, it is possible to stop flow and scan the spectrum of individual peaks in the chromatogram. Just like conventional spectrophotometers, a choice of light sources is usually available, depending on the wavelength desired. Deuterium for the wavelength range of 200–400 nm and Tungsten for 350–800 nm can be selected when required. These instruments are calibrated in Absorbance units over these ranges: 0.01, 0.02, 0.04, 0.1, 0.2, 0.4, 1.0 and 2.0 absorbance units. Three transmission ranges may also be provided to measure 100, 10 and 1% full-scale on any suitable recorder having a full-scale sensitivity of 10 mV. For resolving small events in peaks, the instruments are provided with a 10-turn fine adjustment to achieve zero suppression over the full range of 2 absorption units or 120% transmission range.

Spectrophotometric instruments used in LC are often called spectroflow monitors. Figure 18.14 shows the block diagram of spectroflow monitor Model SF 770 of Schoeffel Inst. Corp., USA, used for UV-visible LC. The system makes use of a double-beam principle and a chopping system to measure the transmission through the sample and reference cells alternately. A Reflection Mode attachment is also available with this instrument. Conventional log function generating amplifiers are employed to get direct readings of optical density. This instrument gives a noise figure which is better than 5×10^{-4} O.D. (at $\lambda = 280$ nm) and stability better than 5×10^{-4} O.D. per hour (at $\lambda = 280$ nm).

Detection systems are also available for the UV range alone (Figure 18.15). Most of these systems operate at fixed wavelengths of 254 or 280 nm. These wavelengths offer excellent sensitivity for many compounds, yet permit the use of a wide range of solvents without interference, even with gradient elution. UV detector employed by Varian Aerograph Model 8500 LC has a special thermal isolation design, which gives a low noise level of only $\pm 5 \times 10^{-5}$ absorbance units equivalent to low nano-gram sensitivity. The output of the detector is linear in absorbance and thus linear in concentration for solutes obeying Beer's Law. The wide linear dynamic range (10^4) enables to measure both trace and major components in the same chromatogram.

The usefulness of spectral information has led to new commercial instrumentation based on diode arrays, rapid scan spectrometers and even Fourier transform (visible) interferometers. Commercial accessories for Fourier transform infrared (FT-IR) detection following LC are also available either as thin flow cells or off-line collection devices based on solvent removal. This is because, unlike GC, the LC eluent absorbs strongly in the IR region to obscure analyte signals. The most

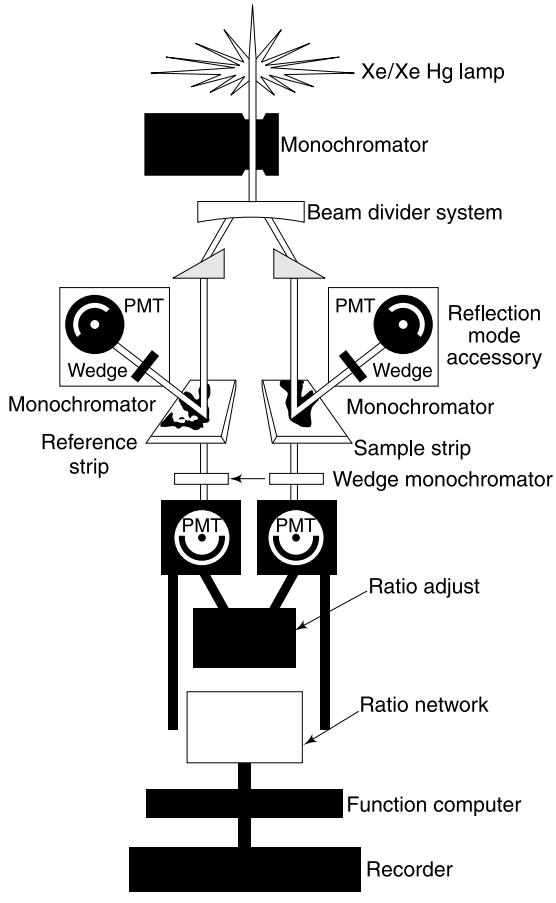


Figure 18.14 *Optical diagram of spectroflow Model SF 770 (Courtesy: M/s Schoeffel Instruments Corp., USA)*

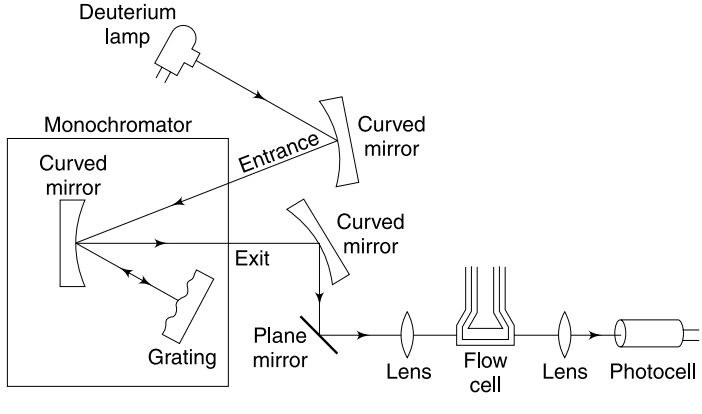


Figure 18.15 *Optical schematic of a typical UV liquid chromatography detector*

widely used detectors currently used in HPLC are the UV, UV-Vis and fluorescence spectrometers. Although normally limited to the detection of only a single wavelength element at a time, these single-channel devices have proved to be sensitive and robust detectors. For multiple component analysis, new techniques such as rapid scanning detectors based on the linear photodiode array (LDA), the silicon vidicon tube or the charge coupled detector presents an alternative technology for rapid wavelength detection in HPLC (Fell, 1985). Currently, there are a number of commercially produced HPLC detectors based on the LDA, usually in combination with a micro-computer. The design uses 256 element diode array and an 8 µl flow cell to acquire spectral data during elution.

Micro-computers provide the necessary memory and speed of operation to handle in real time the data generated by diode arrays, resulting in providing formidable facilities as shown in Figure 18.16 (Miller, 1984). These include comparison of experimental spectrum with a library of spectra for rapid sample identification, subtraction of one spectrum from another; and the calculation of first, second and higher derivative spectra. The graphic display facility enables to have spectrochromatograms, normally presented as isometric projections, which show in three dimensions, axes representing

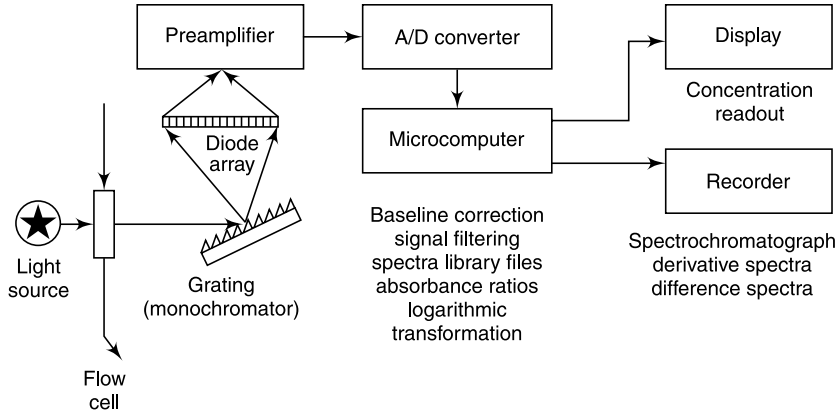


Figure 18.16 *Facilities offered by a diode array spectrometer with a micro-computer (after Miller, 1984)*

time (which can be related directly to chromatographic retention parameters), spectral intensity and wavelength. Spectrochromatograms are thus able to present simultaneous images of the chromatographic separation and the spectral properties of the separated fraction. Moreover, the computer will calculate sections through the total image to generate chromatograms recorded at specific wavelengths, and spectra recorded at particular time.

18.3.4.2 Fluorescence detector

Fluorescence measurements of minute quantities, as encountered in LC, differ greatly in technique and behaviour if compared to standard flow-through absorption monitors or spectrophotometers. In a fluorimeter, the presence of fluorescence emitting substance is measured. The emitting substance is only present occasionally in LC and that its emission of energy is detected by a highly sensitive photomultiplier.

Figure 18.17 shows the optical schematic of a typical fluorescence detector for LC. The commercially available detectors differ in the manner in which the wavelengths are controlled. Less expensive instruments utilise filters, medium priced units offer monochromator control of at least one function, usually excitation wavelength and full capability research-grade instruments provide monochromator control of both excitation and emission wavelengths.

A spectrofluorimeter designed specifically for LC applications offer continuously selectable monochromatic excitation energy over the entire UV-visible spectrum, utilising a highly stabilised deuterium or tungsten-halogen lamp. The monochromator makes use of a grating system. Its continuously variable wavelength extends analytical application far beyond the limited areas, normally offered by line spectra of Hg lamps. Excitation energy from the monochromator enters the cuvette and emission from a 5 µl cavity is collected and directed towards the photomultiplier. A set of easily interchangeable filters is provided to select the emission spectra of interest. The filter set contains filters of wavelengths 370, 389.418, 470, 550 and 580 nm. Transmittance is greater than 0.9 in these areas compared to less than 10^{-5} below the cut-off point and because of this high efficiency, virtually no emission energy gets absorbed before reaching the photomultiplier.

In normal fluorimeters which utilise standard cuvette volumes of several cc's are illuminated by larger excitation light beams of at least 1 cm^2. The emitting material acting as secondary light source to be detected, originates from a much larger area or volume than what is available in the micro-litres type cuvette of fluorimeters used in HPLC. Since emission occurs in all directions from an excited sample surrounding this sample with an efficient optical collector is a must. M/s Schoeffel have introduced a 2 Steradian cuvette (Figure 18.18), which has provided a solution to this problem. This cuvette is of stainless steel and is flow-through type.

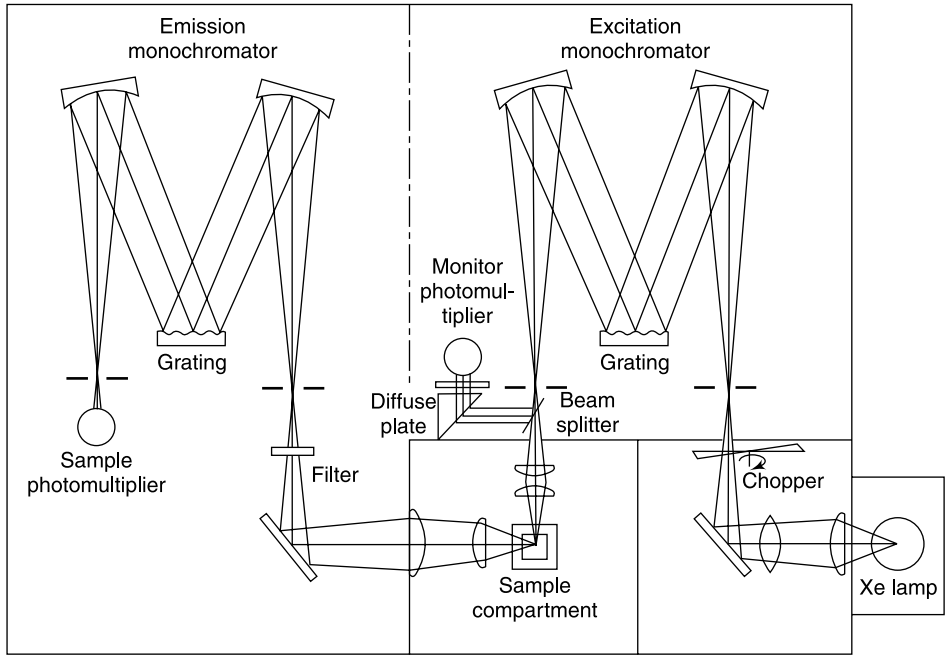


Figure 18.17 *Optical schematic of a typical fluorescence detector for liquid chromatography*

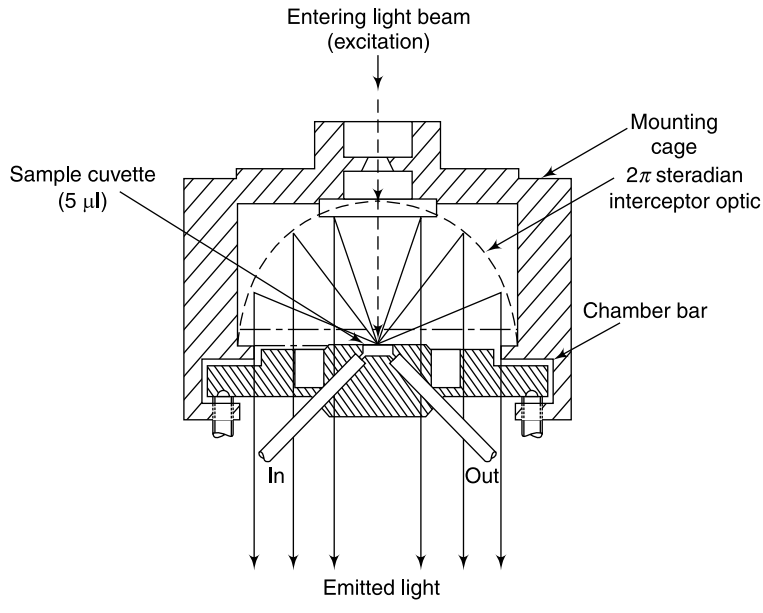


Figure 18.18 *Steradian cuvette used in fluorescence detector*

Fluorescence detection is more selective than absorption detection, because all species that absorb light do not necessarily fluoresce. However, fluorescence detection is limited by the presence of background light, which includes various types of LS, luminescence from the flow cell walls and emission from impurities in the solvent. Although, fluorescence intensity generally increases with excitation intensity, all of the above mentioned factors increase with excitation intensity to produce no net gain. The situation is worse when lasers are used as excitation source, because laser intensities are inherently much less stable than conventional light sources.

The use of laser-excited fluorescence is getting rapidly established, but the developments are really attributable to new designs of flow cells and optics that reduce stray light. Even though fluorescence intensity increases with path length, high power lasers can be used to provide sufficient signal levels over short path lengths, enabling to achieve small detector volumes. The advantage of laser fluorometry is thus the mass detectability, which can be 100 times lower than that obtained from conventional excitation sources. Moreover, better monochromaticity of the lasers can be advantageously used to reject stray light. Yeung and Separiak (1980) have detailed out the designs of flow cells for laser fluorometric detection in HPLC.

18.3.4.3 Refractive index (RI) detectors

The common Rl detectors are based on refraction, reflection or interference of light beams (Yeung arid Synovec, 1986). RI detectors measure the ability of sample molecules to bend or refract light. This property for each molecule or compound is called its refractive index (RI). For most RI detectors, light proceeds through a bi-modular flow cell to a photodetector. One channel of the flow all directs the mobile phase passing through the column while the other directs only the mobile phase. Detection occurs when the light is deflected due to samples eluting from the column. This is read as a disparity between the two channels. Refraction type RI detectors depend on Snell's law at the interface between

the cell in wall (glass) and the flowing liquid to deflect a light beam. Changes in RI are monitored at far field by a position sensor, or as an intensity change on a small area photodiode. Since the effect is produced at the interface, very small volumes are possible if the proper cells are made. Reflection type RI detectors are based on Fresnel's laws at the interface between the cell (glass) and the liquid, which has a smaller RI. Sensitivity will increase as the incidence angle approaches the critical angle. Interference type refractometers provide the best concentration limits of detection in commercial instruments. This is because a longer path length can be used to compensate for lower concentrations.

Figure 18.19 shows the optical schematic of reflective (Fresnel) type detector. Here, the light beam is focused on and reflected from both the liquid-prism cell interfaces and a polished back plate (which forms the near surface of both sample and reference cells) onto the detecting photocell. As the intrusion of sample into the one cell causes light to be refracted at a different angle, this light changes in intensity rather than position, with the unbalance once again being detected as a change in electrical energy. This difference between sample-cell signal and reference-cell signal is given to a recorder. The major advantage of this type of detector includes higher potential sensitivity, ability to operate at extremely low flow rates, with very low-volume flow cells, easy cell accessibility and low cost. Its disadvantage is the need for changing prism to accommodate either high or low RI solvents (Yost et al., 1980).

In the RI detectors, the limit of detection is limited by fluctuations in the RI of the LC effluent. Solvent delivery systems must be pulse-free to avoid pressure effects. Also, thermostated cells must be used to avoid temperature effects.

The use of lasers has helped to reduce detector volumes and has provided added selectivity and sensitivity. The development of small columns to minimise contributions from the solvent and to allow cost-effective use of exotic mobile and stationary phases have also resulted in improvements in detection systems. Therefore, the development of separation processes has in turn benefited from the new generation of detectors.

Compounds without significant UV or visible absorbance can be monitored with a RI detector. This detector may be a dual-bean refractometer of the Fresnel type. These instruments measure the intensity of reflectance, which in turn, is inversely proportional to the RI. Careful control of the temperature is very necessary in these instruments. Small cell volumes of the order of 5 µl are possible.

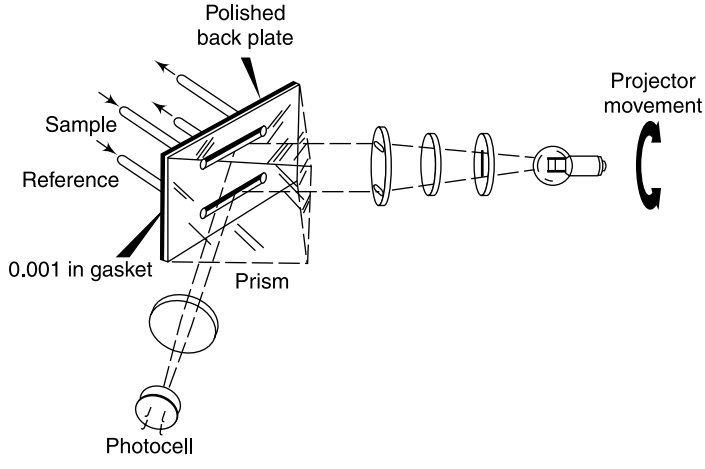


Figure 18.19 *Optical schematic of the Fresnel-type refractive index detector*

Differential refractometers are based on the measurement of the difference in RI between the solution and of the pure solvent. This is usually carried out by measuring the bending of monochromatic light beam as it passes through sample cuvette. Temperature control to within 0.01°C is necessary for measuring absolute refractive indices to within 10^{-5} units. Cell volumes of the order of 75 µl and higher are necessary. The sensitivity of these refractometers alters with variations in flow. The range of RI covered is 1.30–1.60.

18.3.4.4 Adsorption detector

This type of detector depends on the measurement of the evolution of the heat of adsorption and the heat uptake at desorption as the solutes in the effluent stream come into contact with an adsorbent. The measurement of heat is carried out by a thermistor placed in the stream. The detector operates differentially by having another thermistor in the stream and temperature changes as low as 10^{-4}°C can be detected by this system. One detector cavity is packed with an adsorbent, such as alumina, porous glass beads or silica. The other cell, called the reference cell, is packed with inactive glass beads. The thermistors placed in these detectors form a part of the Wheatstone bridge, which gives out electrical signal suitable for recording purposes. These detectors are flow sensitive and therefore, centre discs are used to decrease this effect. The dual-detection system cancels out baseline shifts due to changes in flow rate.

18.3.4.5 Electrical conductivity detector

Electrical conductivity detector is perhaps one of the most important and promising detectors in the future of LC. Areas in which this mode of monitoring are particularly attractive are aqueous and non-aqueous gel-filtration, ion-exchange chromatography and many applications of liquid-liquid chromatography. The conductivity cells used in liquid chromatographs have three electrodes with an internal diameter of under 2 mm and length of 10 cm. The electronics comprises of a stabilised and isolated AC Bridge and phase detector system. The unit is sensitive to a change of approximately 1 part in 10,000 in conductivity and a measuring range of 10–1,00,000 µ mhos/cm.

Figure 18.20 shows a typical arrangement of a conductivity detector for LC applications. It is a three-electrode design. Dimensions of the cell are 10 cm length and 2 mm ID. The cell constant

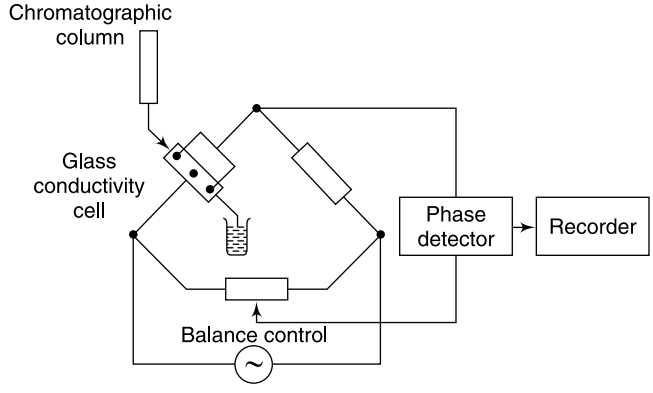


Figure 18.20 *Conductivity detector*

of the cell is 10 cm^{-1}. The total effluent stream passes through the flow-through cell, although the stream can be split to pass only the required amount through it.

18.3.4.6 Thermal detectors

Thermal detectors are also known as micro-adsorption detectors. The principle of operation of these detectors depends on temperature changes taking place due to the heat of adsorption on an active solid source. These detectors are now manufactured by a number of companies. Generally, a portion of the adsorbent column packing is contained in a small chamber at the column outlet A second chamber filled with an inert material such as glass micro-beads is used to achieve a reference signal. This assembly is carefully thermostated. Both chambers contain matched thermistors, which constitute the measuring arms of a Wheatstone bridge. As the elated sample is adsorbed on the solid, a local temperature change takes place, which initiates a signal.

These detectors can be used in applications involving liquid-solid, liquid-liquid, ion-exchange and gel-permeation chromatography. They are non-destructive. However, they are subject to other thermal effects such as thermal conductivity and heat capacity of the solvent. They, therefore, require accurate calibration before use.

18.3.4.7 Mass detector

A mass detector for HPLC based on the scattering of light is described by Turner (1986). It is found that the commonly used UV absorption and RI detectors are unresponsive to certain classes of compounds and are also not useable with certain solvent/solute combinations which again restricts then use. Figure 18.21a shows a schematic of the mass detector, with its cross section shown in Figure 18.21b. In this detector, the solvent stream enters the detector through a small bore hypodermic tube at the top. This is fed into the atomiser assembly where a venturi jet operated by inert gas or air converts the solution into a uniform dispersion of droplets which passes, as a continuous sample into the evaporation.

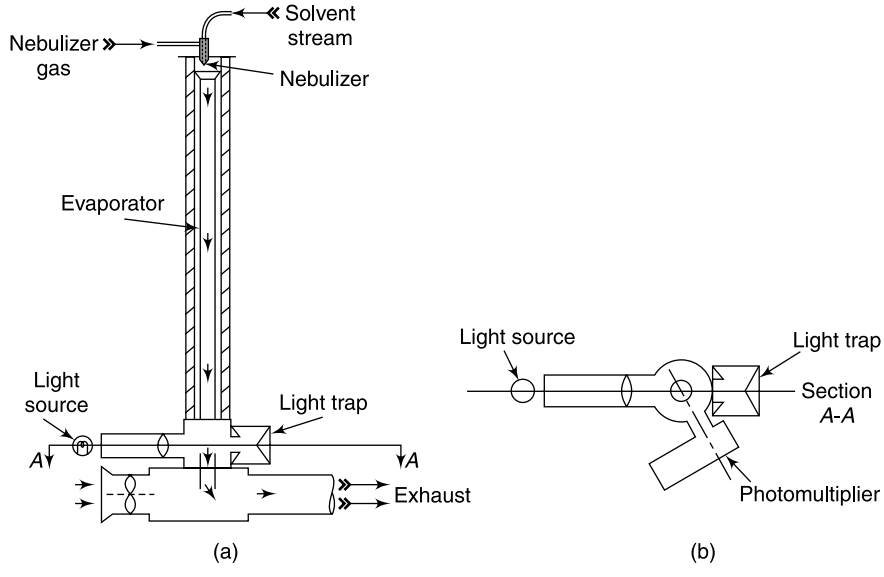


Figure 18.21 *(a) Schematic mass detector (b) cross section at A—A. (after Turner, 1986)*

After nebulisation, the atomised solvent spray passes rapidly down the evaporator. This produces a slightly negative pressure at the base of the evaporator column and ensures that a mixture of gas and solvent present in the evaporator is drawn down and out through the exhaust vent.

Light from a lamp is collimated and passed through the instrument at right angles to the direction of air flow. A light trap is located opposite the source of light to eliminate internal reflection. The amount of light scattered to the photomultiplier does not cause detectable base line drift on the recorder. If, however, solute is present, a particle cloud passes through the light path. Light refracted by this particle cloud is detected by a photomultiplier located at an angle of 120° to the incident light beam. The signal from the photomultipliers amplified and display on a recorder. The output is proportional to the concentration.

18.3.4.8 Radiochemical detection system

These involve the use of radio-labelled material, usually tritium (3_H) or carbon-14 (14_C). It operates by detection of fluorescence associated with beta-particle ionisation and it is most popular in metabolic research.

18.3.4.9 Laser-based detectors

Detectors based on the use of Lasers offer several beneficial properties in chromatographic studies. They offered improved sensitivity and selectivity than other detectors. Also, lasers are capable of better resolution than most LC needs, and have improved signal-to-noise ratio. Some of the types of laser-based detectors are as follows:

- Laser-Induced Absorption
- Laser-induced photoacoustic detector
- Laser-induced fluorescence
- Laser-based refractive index detector
- Laser light scattering detector
- Laser-induced Raman Scattering detector

High energies of lasers can, however, cause thermal distortions and sensitivity can decrease due to scattering at the optical sections of the system. They are not widely used in routine chromatographic practice.

18.3.4.10 Dual-detector systems

In many cases, the use of a single detection might give ambiguous or even incorrect results. This is particularly true for complex, natural samples. For this reason, the simultaneous use of two detectors is often preferred. Following are advantages of using dual-detection system.

- By combining an RI and an UV detector, one can achieve an almost universal detecting capability.
- When a selective detector is combined with a general purpose detector, one can obtain the general fingerprint-type chromatogram of the sample and at the same time, identify certain peaks.
- Combining two UV detectors operated at two different wavelengths (e.g. a fixed wavelength and a variable wavelength detector).

In dual-detector systems, the instrument may be fitted with a second injector and column permitting use of either one column with two detectors in series or two columns each with a separate detector as separate LC systems. Naturally, dual pumps are needed for this purpose.

18.3.5 Programmers and Readouts

Liquid chromatographs are finding online applications for monitoring chemical processes and providing information for desired adjustments. Thus, it must operate automatically. Unless it is computer controlled, it requires a programming unit. The programmer controls the analysis time, injects the sample, selects and measures the peaks and presents the results for display or control. In addition, the programmer controls all column switching functions, including valves used for back flushing and washing. The programme is usually placed in the control room along with strip-chart recorder. The computerised system also includes a keyboard and CRT terminal. A dedicated computer can preferably replace a programmer, which can normally control several LC instruments.

The computer or programmer calibrates the readout for each component of interest. Furthermore, the programming unit controls all functions related to calibration, including re-zeroing the base line at selected times.

18.4 LIQUID CHROMATOGRAPH-MASS SPECTROMETER (LC/MS)

A potentially advantageous area is the development of combinations of LC system with mass spectrometer. For most compounds, a mass spectrometer is more sensitive and far more specific than all other LC detectors. It can analyse compounds that lack a suitable chromosphere. It can also identify components in unresolved chromatographic peaks, reducing the need for perfect chromatography.

Mass spectral data complements data from other LC detectors. While two compounds may have similar UV spectra or similar mass spectra, it is uncommon for them to have both. The two orthogonal sets of data can be used to confidently identify, confirm and quantify compounds.

The problems associated with interfacing LC with mass spectrometer in a practical way are considerable. The simplest method is the direct introduction of the liquid from the LC to the MS ion source region. However, it should be realised that the gas burden from conventional LC flow rates (1 ml/min of water produces 1.2 l/mm of gas) creates nearly 20 times more gas than a cryopumped vacuum system can handle. Therefore, the introduction of total HPLC effluent into a MS is not feasible. Therefore, the interface should be in a position to split the total effluent, so that only 1–5% is introduced in the MS. This, however, would be at the cost of detection limits sensitivity.

Several different types of ion sources are commonly used for LC/MS. Each is suitable for different classes of compounds. Several different types of mass analysers are also used. Each has advantages and disadvantages depending on the type of information needed (Agilent, 2001).

18.4.1 Ion Sources

Much of the advancement in LC/MS over the last 10 years has been in the development of ion sources and techniques that ionise the analyte molecules and separate the resulting ions from the mobile phase.

Earlier LC/MS systems used interfaces that either did not separate the mobile-phase molecules from the analyte molecules (direct liquid inlet, thermospray) or did so before ionisation (particle beam). The analyte molecules were then ionised in the mass spectrometer under vacuum, often by traditional electron ionisation. These approaches were successful only for a very limited number of compounds.

The introduction of atmospheric pressure ionisation (API) techniques greatly expanded the number of compounds that can be successfully analysed by LC/MS. In API, the analyte molecules are ionised first, at atmospheric pressure. The analyte ions are then mechanically and electrostatically separated from neutral molecules. Common API techniques are:

- Electrospray ionisation (ESI)
- Atmospheric pressure chemical ionisation (APCI)
- Atmospheric pressure photoionisation (APPI)

18.4.1.1 Electrospray ionisation

Electrospray relies in part on chemistry to generate analyte ions in solution before the analyte reaches the mass spectrometer. The LC eluent is sprayed (nebulised) into a chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The arrangement is shown in Figure 18.22.

The electrostatic field causes further dissociation of the analyte molecules. The heated drying gas causes the solvent in the droplets to evaporate. As the droplets shrink, the charge concentration in the droplets increases. Eventually, the repulsive force between ions with like charges exceeds the cohesive forces and ions are ejected (desorbed) into the gas phase. These ions are attracted to and pass through a capillary sampling orifice into the mass analyser.

Some gas-phase reactions, mostly proton transfer and charge exchange, can also occur between the time ions are ejected from the droplets and the time they reach the mass analyser.

Electrospray is especially useful for analysing large biomolecules such as proteins, peptides and oligonucleotides, but can also analyse smaller molecules like benzodiazepines and sulphated conjugates.

Large molecules often acquire more than one charge. Thanks to this multiple charging, electrospray can be used to analyse molecules as large as 1,50,000 u even though the mass range (or more accurately mass-to-charge range) for a typical LC/MS instruments is around 3,000 *m/z*. The following is an example:

$$1{,}00{,}000 \text{ u}/10\ z = 1{,}000\ m/z$$

When a large molecule acquires many charges, a mathematical process called deconvolution is often used to determine the actual molecular weight of the analyte.

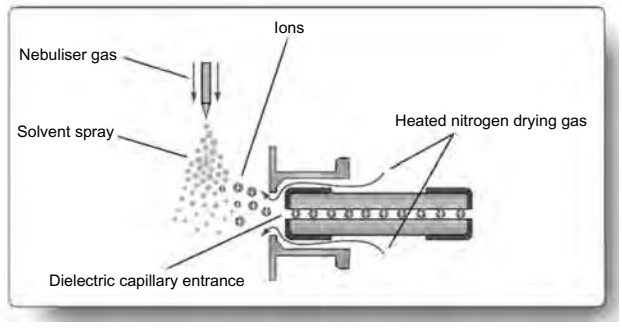


Figure 18.22 *Electrospray ion source (Courtesy: M/s Agilent, 2001)*

18.4.1.2 Atmospheric pressure chemical ionisation

In APCI, the LC eluent is sprayed through a heated (typically 250–400°C) vaporiser at atmospheric pressure. The heat vaporises the liquid. The resulting gas-phase solvent molecules are ionised by electrons discharged from a corona needle. The solvent ions then transfer charge to the analyte molecules through chemical reactions (chemical ionisation). The analyte ions pass through a capillary sampling orifice into the mass analyser, as shown in Figure 18.23.

APCI is applicable to a wide range of polar and non-polar molecules. It rarely results in multiple charging so it is typically used for molecules less than 1,500 u. Due to this, and because it involves high temperatures, APCI is less well suited than electrospray for analysis of large biomolecules that may be thermally unstable. APCI is used with normal-phase chromatography more often than electrospray is because the analytes are usually non-polar.

18.4.1.3 Atmospheric pressure photoionisation (APPI)

Atmospheric pressure photoionisation (APPI) for LC/MS is a relatively new technique. As in APCI, a vaporiser converts the LC eluent to the gas phase. A discharge lamp generates photons in a narrow range of ionisation energies. The range of energies is carefully chosen to ionise as many analyte molecules as possible while minimising the ionisation of solvent molecules. The resulting ions pass through a capillary sampling orifice into the mass analyser.

APPI is applicable to many of the same compounds that are typically analysed by APCI. It shows particular promise in two applications, highly non-polar compounds and low flow rates (<100 µl/min), where APCI sensitivity is sometimes reduced.

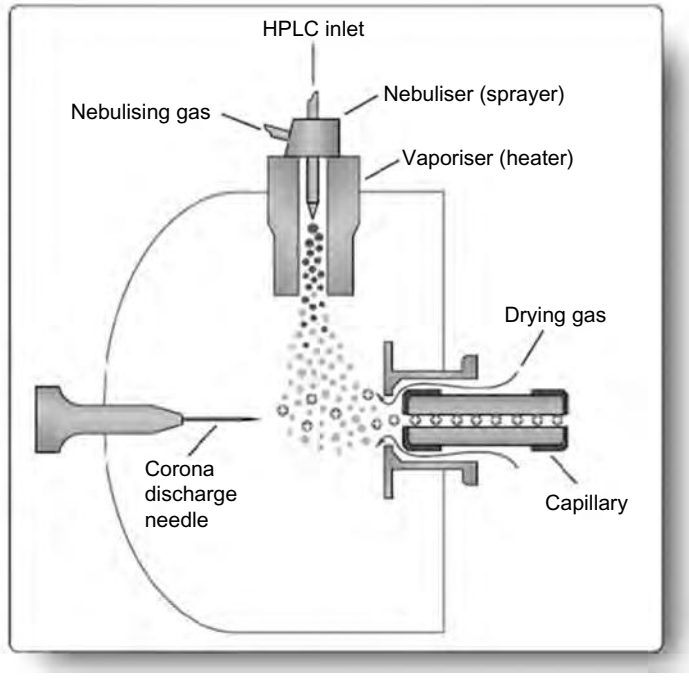


Figure 18.23 *APCI ion source (Courtesy: M/s Agilent, 2001)*

In all cases, the nature of the analyte (s) and the separation conditions have a strong influence on which ionisation technique: electro spray, APCI, or APPI, will generate the best results. The most effective technique is not always easy to predict. The principle of APPI is shown in Figure 18.24.

Covey et al. (1986) review the various techniques used in interfacing LC with MS. The most common interface currently used is the heated nebuliser shown in Figure 18.25. The HPLC effluent passes through the central micro-bore throughput tube of the probe, while the nebuliser gas and make-up gas are introduced coaxially into the heated nebulisation region. The combination of heat and gas flow dissolves the nebuliser droplets, producing a dry vapour of solvent and analyte molecules.

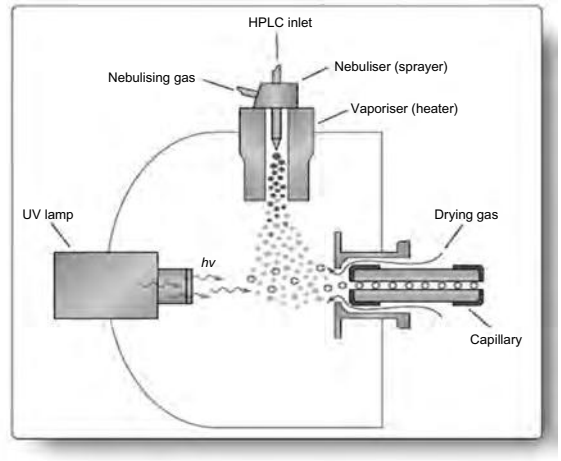


Figure 18.24 *APPI ion source (Courtesy: M/s Agilent, 2001)*

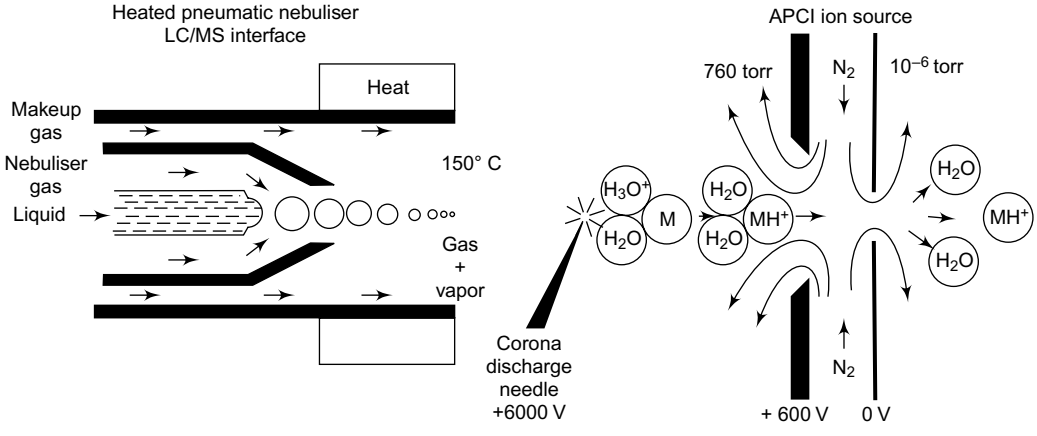


Figure 18.25 *Schematic diagram of a heated pneumatic nebuliser LC-MS interface (after Covey, et al., 1986)*

Ionisation of solvent molecules is produced by a corona discharge at the discharge needle. The solvent ions formed produce analyte ions by APCI source. These ions are focused and declustered through a dry nitrogen region, and then through a 100 µm orifice into the high vacuum analyser region of the MS, where they are mass analysed. Typically, a nebuliser vapour temperature of 125–150°C is maintained, which is suitable for a variety of applications.

19

AUTOMATED CHEMICAL ANALYSIS SYSTEMS

19.1 WHY AUTOMATE CHEMICAL ANALYSIS?

Automation of analysis techniques for either discrete samples for laboratory analysis or online sampling for process monitoring is an important development in the recent decades. It is established that there are a number of possible benefits of an automated technique for a particular procedure, which may include greater accuracy and reproducibility of results due to the facts that samples are analysed in a closed system free from contamination and all are subject to the same constant analytic process. In addition, there are reduced operator errors that often occur in manual analysis or the analysis of results automation uses smaller sample and reagent volumes and this reduces cost.

Automation has enabled procedures to get for greater and more in depth data analysis which have opened up possibilities for completely new procedures. The user has been relieved of the tedious routine procedures normally associated with manual procedures. Similarly, in industrial processes, greater quality control can be obtained through continuous accurate monitoring.

19.1.1 Basic Automatic Analysis System

Whether the application is for discrete laboratory analysis or for online process analysis, there are the following system components:

- Sampling
- Pump and chemistry module
- Detector
- Data recording and analysis

19.1.2 Types of Automatic Analysis Techniques

Basically, there are two types of automatic analysis techniques:

19.1.2.1 Segmented flow analysis (SFA)

Figure 19.1 shows the segmented flow analysis (SFA) technique. The technique involves mixing a liquid sample with reagents pumped in a continuously flowing stream and segmented with bubbles. The sample and reagents are mixed by passing through glass coils (chemistry module) and

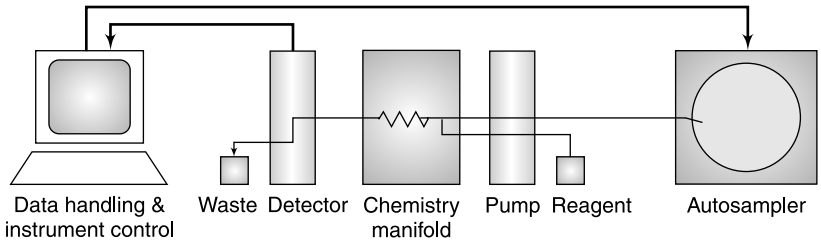


Figure 19.1 *A typical segmented flow analysis system*

also through a temperature controlled heating coil, if required to speed up colour development before detection using a colorimeter, or fluorescence or other detector. A dialyser with a cellophane dialysis membrane is used to remove high molecular weight components contained in samples which interfere with chemical reactions.

SFA is one of the most reliable and widely used methods for analysis in routine and research analytical laboratories. The technique enables up to 16 determinations simultaneously at speeds of up to 120 samples per hour.

19.1.2.2 Flow injection analysis

The principle of flow injection analysis (*FIA*) technique is shown in Figure 19.2. This involves the injection of a liquid sample into a moving non-segmented continuous carrier stream of a suitable liquid, which is usually the colour reagent. A high precision pump is used to pump samples and reagents. The injected sample forms a zone which is then passed though mixing coils and carried to a flow cell in the detector, usually a colorimeter, flame photometer or flourimeter offer the measurement of optical density.

The technique offers advantages such as precision sample introduction, small sample volumes (typical 10–50 ml) low reagent consumption and fast sample throughput.

19.1.3 Benefits of Automation in Chemical Analysis

- Faster analyses up to 120 samples per hour
- Up to 300 samples can be analysed in a batch
- Automatic data recording and preparation

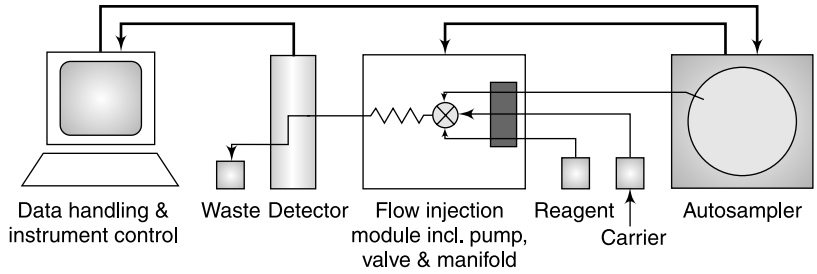


Figure 19.2 *A typical flow injection system*

- Being a closed system, automation reduces contamination, for example, from atmospheric gases
- Greater accuracy and reproducibility of results as all samples are subject to the same processes.
- Smaller sample and reagent volumes; this reduces cost
- Automatic range changing (for over-range samples), drift control and automatic sample preparation
- Out of hours operation with automatic start-up and shut-down procedures.

19.2 AUTOMATED BIOCHEMICAL ANALYSIS SYSTEM

The chemical analysis of blood and other body fluids is one of the earliest forms of diagnostic criteria leading to the investigation of the diseases. In the early years of clinical biochemistry, most of the required analyses were performed on urine as it may be made available easily and in large quantities. However, with the development of semimicro and micro-analytical techniques, analysis can be carried out with minute quantities of samples. Therefore, the analysis of the blood serum or plasma is becoming more frequent. The great increase in the number of determinations coupled with heavy work-load in many laboratories and the development of optical and electronic techniques have stimulated attempts to introduce a high degree of automation in the clinical biochemistry laboratories. A majority of the blood analysis can now be performed by an automated system.

The developments in new concepts and more advanced techniques in analytical methodology have resulted in estimations of blood constituents as a group, whose metabolic roles are related and which collectively provide more meaningful information than the individual analyses. For instance, the group of important anions and cations of the blood plasma (electrolytes) like sodium, potassium, chloride and bicarbonate, which together with serum urea form a related set of tests that are useful to perform on patients with electrolyte disturbances. Another group consists of the several analyses-protein, bilirubin, alkaline phosphatase and SGOT, which together assess liver function. The effect of this trend is in the replacement of single isolated analysis by groups of analyses, all of which are carried out routinely on each sample with highly reproducible and accurate results. With this object in view, automatic analysis equipments have been designed and put to use. Automated analysis systems are available in multi-channel versions and a full description of the detailed working instructions and details of techniques for individual substances are given in literature supplied by the manufacturers to the purchaser of their equipment.

The major benefit of automation in the clinical laboratory is to get rid of the tasks that are repetitive and monotonous for a human operator, which may lead to improper attention that may cause errors in analysis. However, it may be remembered that improvement in reproducibility does not necessarily enhance the accuracy of the results, because accuracy is basically influenced by the analytical methods used.

Any automated system usually has the following steps:

- Specimen identification
- Specimen transport and delivery
- Removal of protein and other interfering components
- Reagent handling and delivery
- Chemical reaction phase
- Measurement procedure

- Signal processing, data handling
- Report preparation

These steps are usually carried out sequentially under the control of microprocessors, which also facilitate to collate results, as well as to perform many statistical and quality control tasks.

The automated systems are usually considered more reliable than normal methods, due to individual variations that may appear m handling various specimens. However, many possibilities exist for mismatching specimens and final results. The risks begin right from the time the sample is collected from the patient and are compounded with every step a specimen undergoes in the analysis chain. The risk is particularly great when accessioning, labelling, re-labelling and preparation of load lists is done by hand transcription. Any incorrect action can cause test results to be attributed to the wrong patient. Even though several codes have been developed to identify the specimen with the right patient, it needs utmost care in those parts, where human action is involved.

19.3 SEGMENTED-FLOW SYSTEM

The segmented-flow type automated system is usually a continuous-flow system, in which individual operations are performed on the flowing stream as it moves through the system. The end-product passes through the colorimeter, where a balance ratio system is applied to measure concentrations of various constituents of interest. The final results are recorded on a strip-chart recorder along with a calibration curve, so that the concentration of the unknowns can be calculated. The output may also be connected to a digital printer along with the graphic record.

The automated system consists of a group of modular instruments (Figure 19.3) interconnected together by a manifold system and electrical systems. The various sub-systems are as follows:

- Sampling unit
- Proportioning pump
- Manifold
- Dialyser
- Heating bath or constant temperature bath
- Colorimeter/flame photometer/fluorometer
- Recorder

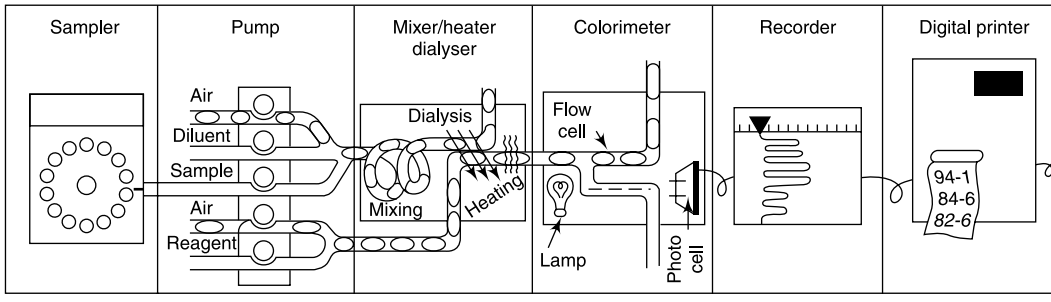


Figure 19.3 *Schematic diagram of a segmented continuous-flow system*

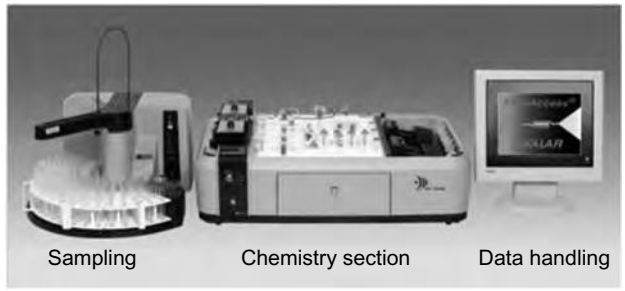


Figure 19.4 *Multi-channel Segmented Flow analyser (Courtesy: M/s Skalar, USA)*

The sample to be analysed is introduced into a stream of diluting liquid flowing in a narrow bore of flexible plastic tube. The stages of the analytical reaction are completed by the successive combination of other flowing stream of liquids with the sample stream, by means of suitably shaped glass functions. Bubbles of air are injected into each stream, so that the liquid in the tubes is segmented into short lengths separated by air bubbles. This segmentation reduces the tendency for a stationary liquid film to form on the inner walls of the tubes and decreases interaction between a sample and the one which follows it. The diluted samples and reagents are pumped through a number of modules in which the reaction takes place, giving a corresponding sequence of coloured solutions, which then pass into a flow-through colorimeter. The corresponding extinctions are plotted on a graphic recorder, in the order of their arrival into the colorimeter cell. The air bubbles are removed before the liquid enters the colorimetric cell or flame emission.

A typical multi-channel SFA is from M/s Skalara, USA. This is shown in Figure 19.4. This is a modular system and can be expanded with ease. The equipment is software controlled and has multi-tasking offering real time analysis where primary data acquisition, graphics, peak detection, calibration and calculation take place, so results are produced immediately upon a peak being detected. Details of the individual units of a multi-channel are described below:

19.3.1 Sampling Unit

The sampling unit enables an operator to introduce unmeasured samples and standards into the auto-analyser system. The unit in its earlier form consisted of a circular turntable (Figure 19.5) currying around its rim 40 disposable polystyrene cups of 2 ml capacity. The sample plate carrying these cups rotates at a predetermined speed. The movement of the turntable is synchronised with the movements of a sampling crook. The hinged tubular crook is fitted at a carrier of the base. The crook carries a thin flexible polythene tube, which can dip into a cup and allow the contents – water, standard or test solution – to be aspirated. At regular intervals, the crook is raised; so that the end of the sample tube is lifted clear of the cup.

Between each sampling, the crook enters a receptacle of water or other suitable wash fluid, to reduce cross-contamination of one sample with another. The ratio of sampling time to wash time is normally 2:1. The plate then rotates a distance sufficient to allow the tube, when it next moves down, to dip into the next cup. One complete rotation of the plate thus presents 40 samples. As the sample plate completes a cycle, a switch is operated, which stops the rotating action of the plate and the sampling action of the sample probe. The sampling rate can be adjusted to 20, 40

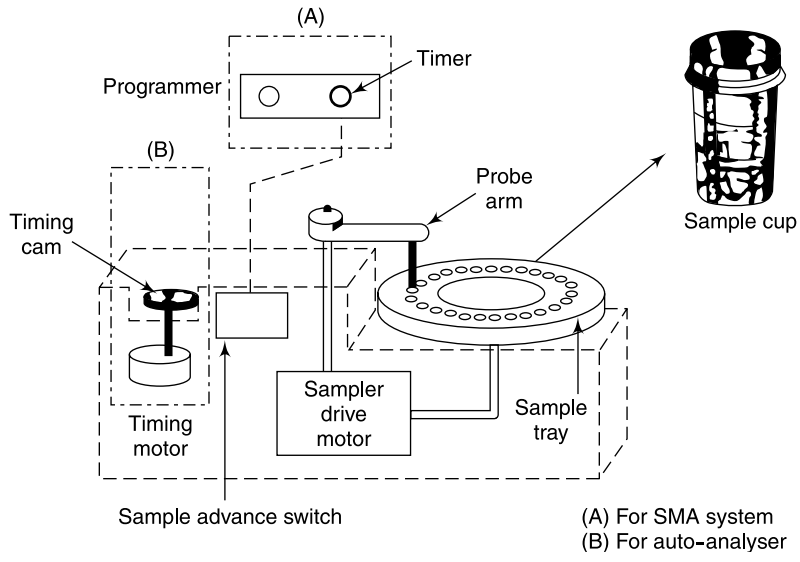


Figure 19.5 *Sampler controls (Courtesy: M/s Technicon Corp., USA)*

or 60 per hour. Accordingly, the above ratio, the time during which a sample is being drawn in, will be 2min, 1 min or 40 s respectively. The volume of liquid taken up in most cases ranges from about 0.2 to 1.0 ml. This depends upon the rate at which the plate is run and the diameter of the pump tube.

The earlier version has been replaced by a more versatile form of the sampler, in which during the time the sample tube is out of the specimen, the crook quickly comes down into water, and thus successive samples are separated by a column of water instead of air. This provides a better separation between them. With this sampler, the sample size may range from 0.1 to 8.5 ml. It utilises cups of sizes 0.5, 2, 3 and 10 ml. The sample plate is kept covered to prevent evaporation, which may sometimes lead to errors up to 5%. Sampling and washing periods are controlled by a programming cam. The sample speed and sample wash cycles are selected by the markings on the cam, such as 40 and 2:1. This implies that the speed is 40 per hour at a sample wash ratio of 2:1.

The samplers are fitted with a sample mixer, which enables the sample to be mixed before and during aspiration. This is important in cases like when whole blood is used for analysis. The mixer prevents separation of the plasma from the cells. The sample base plate is rotated by means of a motor drive gear. When this gear drives the second drive gear for the sample probe assembly, it also rotates a gear chain of internal gears to the Geneva cam. The Geneva cam is an index type cam that may be designed for any acceleration, deceleration or dwell period. During the complete cycle of the sample wash probe, the cam makes one complete turn. As the probe moves back into the wash reservoir, the second point enters the next hole in the drive assembly plate. This rotates the drive assembly, sampler housing and sample plate at a controlled speed. The sample speed is controlled by the sampling rate cam on the programmer. A new sample is aspirated every 2 min and the sample wash ratio of 95% sample and 5% wash is maintained.

Mechanical cams were used in the earlier modules of analysers to initiate and control sample aspiration and wash cycles. Modern systems are microprocessor-based and use electronic timers to do the same function. These timers provide greater flexibility in control of sample-to-wash ratios,

which in turn allows flexibility in setting up parameters for analyses.

The automatic sampler Figure 19.6 employs a different probe washing action between samples. Whereas most automatic samplers operate by dipping the probe alternately into the sample cup and then into a wash pot, the sampler has a probe which simply moves up and down.

The probe washing device consists of a washing chamber through which the probe moves vertically. When the probe is in the sampling position, a 0 ring at the lower end of the internal wash reservoir seals the outlet. Water is pumped through the reservoir by the peristaltic pump used in the analytical system. At this stage, the water flows upwards through the reservoir and then through the outlet at the top of the device. After sampling, the probe moves to the top of the reservoir and the 0 ring seals the outlet. The probe then aspirates water and the excess runs down the probe exit hole, where it is sucked away through the annular channel which surrounds the channel. There is a sub-atmospheric pressure at the probe exit and entry hole, which causes any liquid adhering to the probe to be sucked away as it passes through the chamber. The sample and wash times can be adjusted from 0 to 120 s.

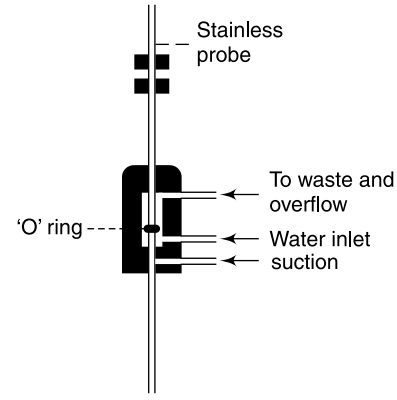


Figure 19.6 *Sampling mechanism of the automatic sampler*

The samplers are usually designed to complement the automatic dilutor and standards preparation unit which automatically creates a range of standards from a single working standard as well as creating all drift and blank controls.

19.3.2 The Proportioning Pump

The function of the proportioning pump is to continuously and simultaneously push fluids, air and gases through the analytical chain. In fact, it is the heart of the automatic analysis system. Here, all the sample and reagent streams, in any particular analysis are driven by a single peristaltic pump, which consists of two parallel stainless steel roller chains with finely spaced roller thwarts.

A series of flexible plastic tubes, one from the sampler and the others from reagent bottles or simply drawing in air, is placed lengthwise along the platen spring-loaded platform. The roller-head assembly is driven by a constant-speed gear motor. When the rollers are pressed down and the motor switched on, they compress the tubes containing the liquid streams (sample, standard and reagents) against the platen. As the rollers advance across the platen, they drive the liquid before them.

The roller head rotates at a constant speed. The different flow rates required in the several streams (0.15 to 4 ml/min) are achieved by selecting tubes of appropriate internal diameter, but of constant wall thickness. Since the proportions of the various reagents are fixed by the tube sizes, no measurements are needed.

Proportioning pumps are available either for single-speed or for two-speed operation. The single-speed pump has the capacitor synchronous gear head utilising 10 rpm output shaft at 50 Hz. The two-speed pump has a non-synchronous 45 rpm motor. The slow speed in this pump is used for the ordinary working during a run and a much quicker one for filling the system with reagents before a run and for rapid washing to clear out reagents after the run. It is also utilised for rapid cleaning of the heating bath, or of the complete system, when fibrin (an insoluble protein)

problems are evident and are disturbing the run. High speed is not used for analysis. Heavy-duty pump is also available, which enables 23 pump tubes to be utilised simultaneously.

The plastic tubes are held taut between two plastic blocks having locating holes, which fit on to pegs at each end of the platen. Before beginning a run, the tubes are stretched. With use and time, the tubes loose elasticity and pumping efficiency is reduced. Therefore, each block has three sets of holes, so that the tubes can be increasingly stretched and the tension thus maintained. The tubes are replaced at the first sign of ageing. In fact, they should be replaced at regular intervals to forestall failure. When not in use, one of the blocks is removed, so that the tubes are not kept in tension.

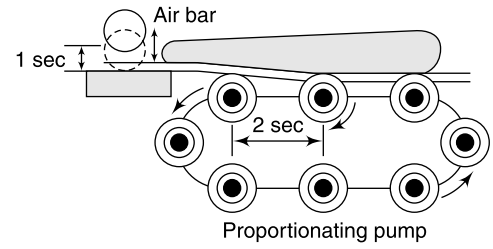


Figure 19.7 *Principle of air segmentation in the continuous-flow system*

Actually the sample or reaction stream is separated by air bubbles into a large number of distinct segments. The air bubbles completely fill the lumen of the tubing conducting the flow, thereby maintaining the integrity of each individual aliquot. In addition, the pressure of the air bubble against the inner wall of the tubing wipes the surface free of droplets which might contaminate the samples which follow. The proportioning include an air bar device (Figure 19.7), which adds air bubbles to the flowing streams in a precise and timed sequence. The air bar is actually a pinch valve connected to the pump rollers that occludes or opens the air pump tubes at timed interval. Every time a roller leaves the pump platen – and this occurs every 2 s – the air bar rises and lets a measured quantity of air through. The release of air into the system is carefully controlled, thereby ensuring exactly reproducible proportioning by the peristaltic pump.

The continuous-flow analysers make use of liquid reagents. Large volumes of reagents are stored in the systems and their quantity is adequate for operation of the analyser for several hours or days. Some automated systems use reagents in a dry tablet form. When required, the tablet is dispensed into a one test reaction vessel and dissolved. The sample is then added for the reaction to take place. This is basically a unit-dose concept, which offers several advantages like less storage space and operator time, long stability of reagents and lesser wastage.

19.3.3 Manifolds

A variety of chemistry manifold types and combinations are generally offered in the automatic analysis systems. A manifold mainly consists of a platter, pump tubes, coils, transmission tubing, fittings and connections. A separate manifold is required for each determination and the change can be effected within a few minutes. The pump tubing and the connected coils are placed on a manifold platter, which keeps them in proper order for each test. The pump tubing are specially made, they are of pre-measured length and are meant to introduce all constituents of an analysis into the system. The physical and chemical properties of the tubing are extremely important in the correct functioning of the pump. It must not be so flexible as to expand beyond its normal internal dimensions on release of pressure, which may lead to variation in the flow, thereby affecting reproducibility and accuracy of the system. The tubes should be chemically inert for the constituents which are expected to flow through the tube. The constant and correct tension also provides the continual delivery of a constant volume. The inside diameter of the pump tubing determines the flow rate per minute.

Several other tubes are required to introduce reagents and to transport the specimen from one module to another. There are five types of such tubings. They are of varying sizes and are to be selected according to the requirements. These are: standard transmission tubing (Tygon), solvaflex tubing, acidflex tubing, polyethylene tubing and glass tubing.

Two types of coils are employed in the system-mixing coils and delay coils. Coils are glass spirals of critical dimensions, in which the mixing liquids are inverted several times, so that complete mixing can result.

Mixing coils are used to mix the sample and/or reagents. As the mixture rotates through a coil, the air bubble along with the rise and fall motion produces a completely homogeneous mixture. The mixing coils are placed in a horizontal position to permit proper mixing. Delay coils are employed when a specimen must be delayed for completion of a chemical reaction before reaching the colorimeter. These coils are selected in length according to the requirements. The standard delay coil is 40 ft long, 1.6 mm I.D. and has a volume of approximately 28 ml. The time delay can be calculated by dividing the volume of coil by the flow rate of specimen plus bubbles.

19.3.3.1 Phasing

With twelve tests to be recorded on each sample and a sampling rate of 60 samples per hour, it follows that 5 s are allowed to record each steady state plateau. The reaction streams in the 12 channels and up to four blank channels must, therefore, be phased to arrive at the colorimeter in waves 5 s apart. For example, if the cholesterol stream arrives at X time, calcium must arrive at X + 5 s, total protein at X + 10 s, albumin at X + 15 s etc. In order to ensure proper sequencing for presentation of the results, a number of devices have been provided to make this adjustment an extremely simple operation. Phasing coils are used to permit the channels to enter the colorimeter in the proper sequence.

Mixing with other reagents begins on leaving the pump. The first reagent with which the test specimen is mixed is usually a simple diluent. The reagent lines are segmented by introducing air through one or more additional tubes into the manifold. This produces a series of bubbles at regular intervals in the liquid stream. This is designated as bubble pattern. A uniform bubble pattern such as shown in Figure 19.8 is very essential for accurate analysis. However, it is not necessary that every bubble be absolutely identical in length, but a firm consistent flowing segmented stream is required.

19.3.4 Dialyser

In analytical chemistry, it is often necessary to remove protein cells to obtain an interference-free analysis. This is accomplished by dialysis. The dialyser module (Figure 19.9) consists of a pair of perspex plates, the mating surfaces of which are mirror grooved in a continuous channel, which goes in towards the centre on itself and returns to the outside. A semipermeable cellophane membrane is placed between the two plates and the assembly is clamped together, similar to the kidney dialyser. The continuous groove channel thus gets divided into two halves and the dialysis occurs across the membrane. A solution containing substance to be analysed passes along one-half, usually the upper one, of the channel, while the

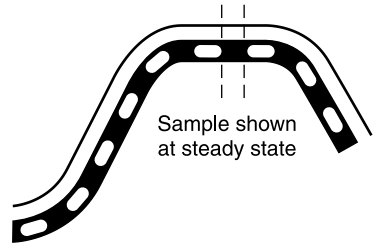


Figure 19.8 *Typical curve showing the steady state conditions when measurements are made in a continuous-flow system*

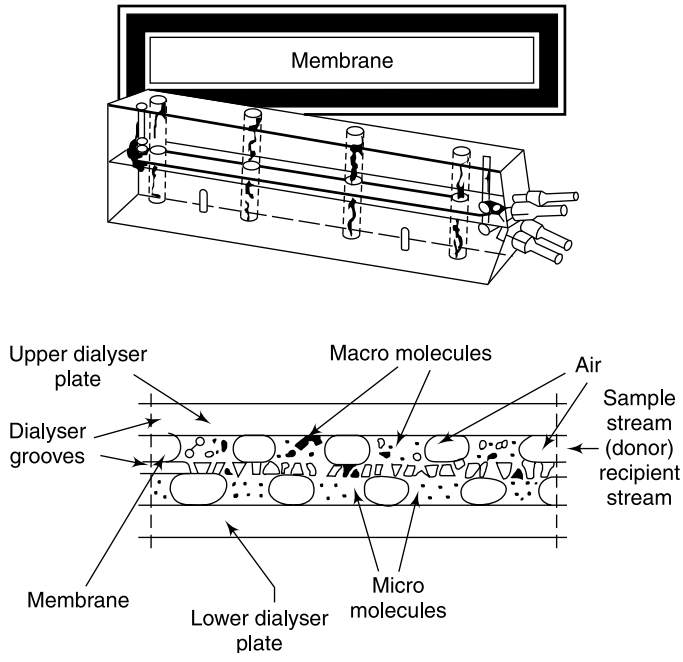


Figure 19.9 *Simplified diagram of the dialysis process*

solvent that is receptive to the substance to be removed enters the other half. The substance to be separated from the sample diluent stream, will diffuse through the semipermeable membrane by osmotic pressure into the recipient stream and the non-diffusable particles will be left behind.

The cellophane membrane usually used in the dialyser has a pore size of 40–60 Å. The rate of dialysis is dependent upon temperature, area and concentration gradient. For this reason, the dialyser unit is usually immersed in a water bath maintained at a constant temperature (37 ± 0.1°C). The temperature is kept constant with a thermostatically controlled heater and a motorised stirrer. Both streams pass through preheating coils, before entering the dialyser unit. The channel path is 2.2 m long, which provides a large surface presentation to the dialysing membrane. The plates of the dialyser must be a matched set. If the plates are not matched set, the channels may be slightly off, causing leakage, poor bubble patterns and loss of dialysing area, which would ultimately result in loss of sensitivity.

The quantity of solute that passes through the membrane in the dialyser is determined by the concentration gradient across the membrane, the duration of contact of the two solutions, the area of contact, the temperature, and by the thickness and porosity of the membrane. Other factors which affect the rate of transfer are the size and shape of the molecules, their electrical charge and the composition of the fluids across the membrane.

A decrease in flow rate of the liquid streams increases sensitivity in continuous-flow systems, since more concentrated samples and thinner membranes can be used. Modern dialysers, therefore, have shallower and shorter grooves, resulting in reduced sample interaction and carryover. Membranes have been found to age with use and time due to protein deposition on their surface and therefore need periodical replacement. In the recent systems, the computer informs the operator to investigate the need for membrane replacement.

19.3.5 Heating Bath

On leaving the dialyser, the stream may be combined by one or more additional reagents. It is then passed to a heating bath. This module is not used in all the tests performed by the analyser. The heating bath is a double-walled insulated vessel, in which a glass heating coil or helix is immersed in mineral oil. A thermostatically controlled immersion heater maintains a constant temperature within ± 0.1°C. Inside the bath, the stream passes along a helical glass coil about 40 ft. long and 1.6 mm I.D., immersed in oil, which is constantly stirred. The heating bath may have fixed temperature, as 95° or 37°C or an adjustable value. Passage through the heating coil takes about 5 min, but it would obviously vary with the rate at which the liquid is moving, which, in turn, depends on the diameter of the tubes in the manifold.

Some systems have dry heating bath which operate at temperatures up to 165°C controlled to ±0.1°C. The dry bath heater uses a standard glass coil, approximately 6 ml volume, with preset digital temperature control adjustable from 37°C. The dry heating bath avoids the use of bulky oil baths and lengthy glass connections.

19.3.6 Measurement Techniques

Automated analysers are mostly using absorption spectrophotometry as the major measurement technique.

The photometers used in the automated systems continuously monitor the amount of light transmitted through the sample. They employ flow-through cuvettes. The flow cell size varies from 6 to 15 mm. The latest designs of flow cells are all of tubular construction and they require a much smaller volume of fluid, so that a smaller volume of sample can be used.

Figure 19.10 (A) shows the construction of the two types of flow cells used in the auto-analyser colorimeter. They are designed to exhibit optimum wash (clean out) characteristics. A debubbler is coupled with the flow cell, so that the entrapped air bubble may leak out. The cell shown in Figure 19.10 (B) is of one piece construction. It has smaller volume, while it maintains the same optical path length.

Some automatic continuous-flow chemical analysis systems incorporate a multi-channel photometer. The colorimeter employs a fibre optics system, using a single high-intensity quartz halogen lamp, which is coupled to a highly stabilised power supply. The working of such type of photometers is given in Chapter 2.

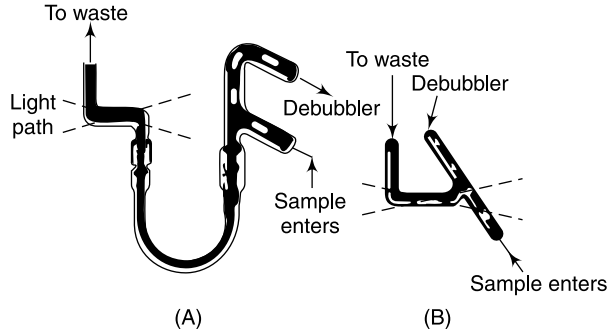


Figure 19.10 *Constructional details of the flow cells (Courtesy: M/s Technicon Corp., USA)*

In addition to photometric detection, a wide range of other detectors can be connected to the analyser such as infrared (IR) detectors, UV detectors, flame photometers, ion selective electrodes (ISE), fluorimeters and amperometric detectors.

19.3.7 Signal Processing and Data Handling

The availability of microprocessors had a major impact on the signal processing and data handling of analytical procedures in automated systems. Real-time acquisition and processing of data, by means of specific algorithms, so that the output is immediately useful and meaningful, has become possible. Transformation of complex, non-linear standard responses into linear calibration curves have allowed automation of procedures, such as reflectance spectrometry.

Specifically, microprocessors are now being used in automated methods for the following functions:

- Complete control of the electromechanical operation of the analyser in relation to transfer of solutions, selection and placement of proper filters and continuous monitoring of operation. This ensures that all functions are performed uniformly, repeatably and in correct sequence.
- Acquisition assessment, processing and storing of operational data from the analyser.
- Providing effective communication between the analyser and the operator through alpha-numeric display on the CRT. Some systems even monitor the equipment function and give out message describing the site and type of problem in a malfunctioning equipment.
- Facility to communicate to main-frame computers through RS-232 interface for integration of instrument with laboratory information.
- Facility to communicate over the telephone lines, using a modem, with the manufacturer's central service department, thereby enhancing ability of the on-site operator to service and repair the analyser.

Modern automatic analysis systems make use of PC as multi-tasking data processor and system controller. However, such multi-tasking is achieved with the use of a microprocessor-based analogue interface card which fits neatly into a standard extension slot inside the computer. Primary data acquisition, graphics, peak detection, calibration and calculation all place in the background, so results are produced immediately upon a peak being detected. These systems have comprehensive software support which includes method development, special functions, interfacing requirements and system control of power values, pump, colorimeters etc. Peaks are captured to have similar dimensions (height and width), independent of the channel or analysis speed, so most peak-picking parameters do not need to be changed from their default values. Up to 16 channels of independent data can be processed simultaneously.

19.4 FLOW INJECTION ANALYSIS TECHNIQUE

The use of air bubbles to separate samples in the SFA technique is an effective method of avoiding sample carryover. An alternative automatic method, which could be operated by the reproducible injection of small samples into a continuously flowing, unsegmented stream of carrier or regent, is described by Miller (1983).

Flow injection analysis (FIA) is a continuous-flow technique for automated wet chemical analysis. The methodology used by the FIA is similar to that used by SFA, with continuously flowing reagent streams, reaction 'manifolds', and flow-through detectors. However, FIA does not use air

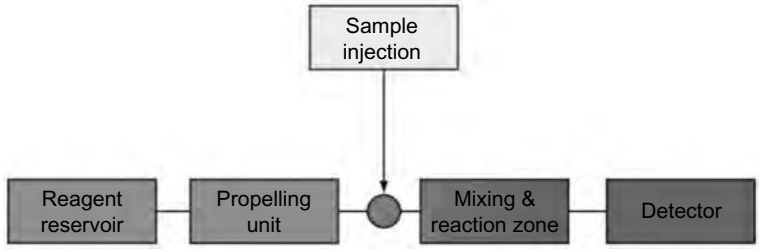


Figure 19.11 *Schematic diagram of a simple flow injection analyser showing its basic components. After its injection into the carrier stream the samples mixes and reacts with the carrier stream's reagents before reaching the detector. (Courtesy: UC Davis ChemWiki)*

bubble segmentation to separate samples and promote mixing. Instead, small diameter tubing is used in the manifolds, resulting in laminar flow conditions in which mixing takes place by axial and radial diffusion, and the manifolds are self-cleaning. Diffusion (dilution and mixing) is controlled by manifold design. A major practical advantage of this technology over that of air segmentation is that analytical results are usually available within a minute or so from the time the sample is aspirated, so any problems in the system can be spotted quickly and corrected, with little wasted time. The overall analysis times also tend to be shorter with FIA, so more samples can typically be analysed in a given period of time. Precision and detection limits are generally comparable between the two technologies.

A schematic diagram detailing the basic components of a FIA is shown in Figure 19.11. The reagent serving as the carrier is stored in a reservoir, and a propelling unit maintains a constant flow of the carrier through a system of tubing that comprises the transport system. The sample is injected directly into the flowing carrier stream, where it travels through one or more mixing and reaction zones before reaching the detector's flow cell.

19.4.1 Propelling Unit

The propelling unit moves the carrier stream through the FIA. Although several different propelling units have been used, the most common is a peristaltic pump. Peristaltic pumps provide a constant flow rate, which is controlled by the drum's speed of rotation and the inner diameter of the tubing. Flow rates from 0.0005 to 40 ml/min are possible, which is more than adequate to meet the needs of FIA where flow rates of 0.5–2.5 ml/min are common. One limitation to a peristaltic pump is that it produces a pulsed flow – particularly at higher flow rates – that may lead to oscillations in the signal.

The peristaltic pumps are provided from manufacturing with multiple channels (usually four) and the general flow rate can be adjusted by adjusting the speed of the rotor and by the pressure degree of the semicylindrical plates on the flexible tubes.

19.4.2 Sample Injection System

The sample, typically 5–200 µl, is injected into the carrier stream. Although syringe injections through a rubber septum are possible, the more common method – as seen in Figure 19.12 – is to use a rotary, or loop injector similar to that used in an HPLC. This type of injector provides for a

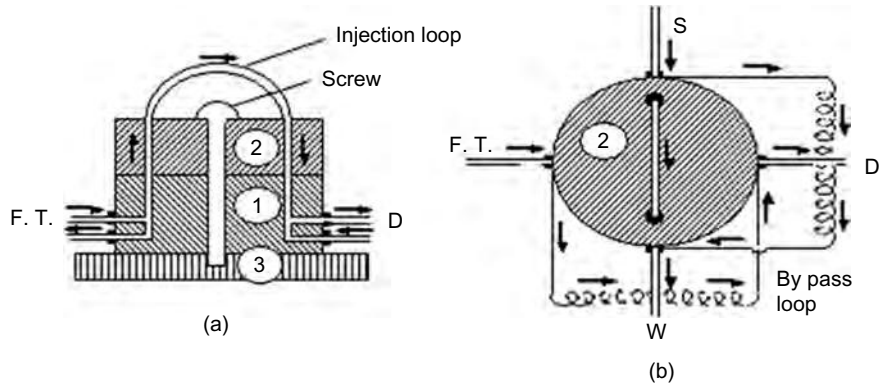


Figure 19.12 *Scheme of a four-way injection valve. a) section view; b) top view. 1 - stator; 2 - rotor; 3 - base. The arrows indicate the moving direction of the fluid flows.*

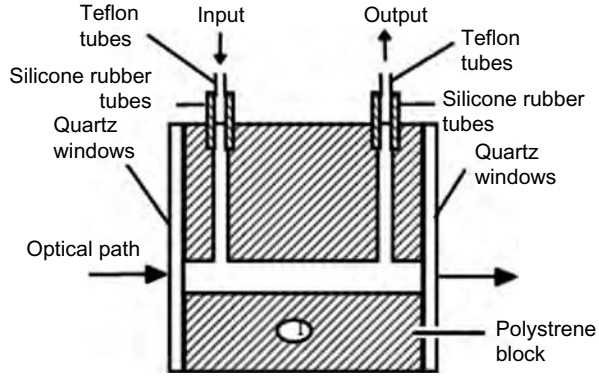


Figure 19.13 *Scheme of a flow cell for spectrophotometric and fluorescence determinations*

reproducible sample volume and is easily adaptable to automation, an important feature when high sampling rates are needed.

The rotating valves used today are available in a variety of constructive types. A necessary condition for an FIA system is that the small volumes of injected sample (10–200 µl) must be inserted with perfect reproducibility, with errors smaller than 1 %. This is required because at the injection of the sample in the flow it is formed a zone that contains the sample to be analysed and it constitutes the starting point in every determination.

Detection System: The most commonly used detectors for FIA are the electrochemical and optical detectors used in HPLC. FIA detectors also have been designed around the use of ion-selective electrodes and atomic absorption spectroscopy

Generally, the detectors are equipped with flow cells that must have the smallest possible volume to avoid the supplementary dispersion and to obtain a better sensitivity (Figure 19.13). In the spectrometric methods for atomic absorption or emission, the sample is sucked directly in the atomisation unit.

19.4.3 Transport System

Two types of tubes used in the FIA systems can be distinguished, as follows: (a) flexible tubes for the peristaltic pump with the internal diameter determining the flow rate of the fluid, the material from which these tubes are made (PVC, modified PVC, silicone rubber, fluoroplast) depends on the type of fluid involved in the FIA determination and they must be changed periodically; (b) the tubes for the connections between the component parts of the FIA systems and the reactors are generally from Teflon, PVC or polyethylene and the internal diameter varies between 0.3 and 2 mm.

The heart of a FIA is the transport system that brings together the carrier stream, the sample, and any reagents that react with the sample. Each reagent stream is considered a separate channel, and all channels must merge before the carrier stream reaches the detector. The complete transport system is called a manifold.

At first sight, it appears that the samples will rapidly spread out in the flowing stream and there will be unacceptable carryover. It has been observed, both theoretically as well as practically that although dispersion of the sample does occur, it is under the control of the laboratory technologist, being regulated by such factors as the carrier stream flow rate, the length and diameter of the flow tubing and so on. This controlled dispersion is the distinctive feature of the FIA.

The major differences between FIA and SFA are that, there is normally only one sample at a time between the injection point and the detector in an FIA system and that by making use of a precision of timing that is impossible in an air-segmented stream. Also, FIA can measure partial responses, i.e. from incompletely formed response peaks in the detector. This means that very high sampling rates can be attained with the FIA technique.

The equipment needed for FIA system is comparatively very simple and cheap. In most FIA systems, the carrier stream is impelled by a simple peristaltic pump. Alternatively, in place of the pump, a constant head device may be used. Flow tubing generally has an internal diameter of 0.5–0.8 mm. Flow rates and tube lengths will vary according to the required dispersion. Depending upon the reaction requirements, the flow manifolds are immersed in a thermostat bath.

Similar to the sequential flow techniques, specialised modules can be incorporated into FIA manifolds. These include dialysis blocks, solvent extraction modules and several types of packed bed reactor.

One of the major advantages of the FIA technique is the possibility of using a wide variety of detector systems. Almost all the common atomic and molecular spectroscopy techniques as well as electrochemical detectors have been employed.

The type of applications in FIA system depend upon the *dispersion* (D) in the system, which is defined as C_o/C, where C_o is the uniform concentration of a sample slug injected into the flowing carrier stream and C is the concentration of the sample by the time it reaches the detector. The dispersion can be measured as H_o/H, where H is the peak height from the detector in the FIA system and H_o is the peak height observed if the sample is directly injected into the detector.

In practice, three ranges of *D* can be considered:

Low Dispersion (D<3): These experiments are of value in studying the intrinsic properties of a sample, e.g. pH, colour, turbidity, etc.

Medium Dispersion (D = 3–10): The medium dispersion mode accounts for the maximum application areas. These experiments involve substantial mixing of the sample with the carrier stream, it normally affects detection sensitivity.

High Dispersion (D>10): This procedure finds fewer applications except for providing means of obtaining calibration curves. It is of particular value when deliberate dilution of a sample is necessary.

FIA technique allows analytical procedures to be carried out in a few seconds, with high precision and minute consumption of reagents. It appears to be one of the most potential and fascinating analytical procedures developed in recent years.

The rate of use of FIA is increasing fast and research papers and reviews are now being published with astonishing frequency.

19.5 SEMIAUTOMATED CLINICAL CHEMISTRY ANALYSERS

Semiautomated analysers have brought about a revolution in the field of clinical chemistry. It has reduced the load on clinical laboratories to a great extent by reducing the time taken in carrying the tests and minimising the involvement of laboratory staff. These systems are used in hospitals to test various blood biochemical parameters. These analysers are used to measure various blood biochemical parameters such as blood glucose, urea, protein, bilirubin, and so forth, and also to measure and observe enzyme growth occurred while performing the other biochemical tests such as ALT (alkaline amino transferase), amylase, AST (aspartate amino transferase), and so forth.

Figure 19.14 shows the basic modules of a semiautomated clinical chemistry analyser (Taneja et al., 2005). The basic modules of the system are; a light source, an optical module, a filter wheel, a quartz cuvette with reaction mixture, a photodetector, and signal processing circuitry based on micro-controller.

Optical module consists of a light source with reflector, condenser system, collimating objectives, flow cell, filter wheel assembly, and photodiode. Halogen lamp has been used as a light source. A constant current power supply is used to power the lamp to reduce the fluctuations in the light output. All optical components have been designed with quartz glass to have good transmission in UV region at 340 nm.

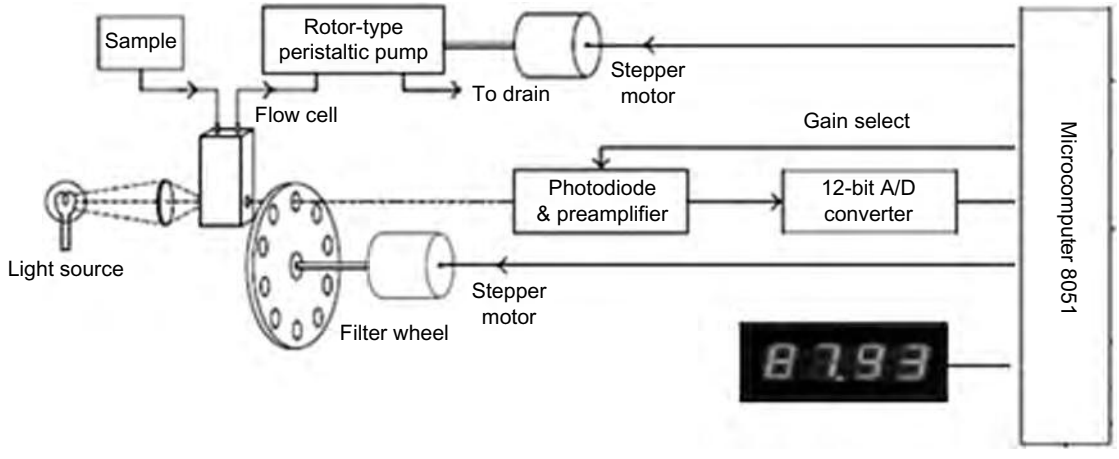


Figure 19.14 *Block diagram of clinical chemistry analyser. (after Taneja et al., 2005)*

To get the required wavelength of light, interference filters of different wavelengths such as 340, 405, 505, 546, 578, and 630 nm, from UV region to visible region spectrum (300–700 nm), are mounted on the filter wheel. These filters are selected automatically depending on the test to be performed. When the filter of required wavelength is selected, the corresponding gain of the amplifier selected automatically. The filter wheel is driven by a stepper motor, which is interfaced with the micro-controller through driver circuit. Pulses are generated according to required sequence to rotate the motor at required angle, which brings the filter in front of photodetector.

Output of photodiode and preamplifier is a voltage which varies directly with the light which is passed through the flow cell and selected wavelength filter. Preamplifier gain is selected automatically as per the selected test parameter and filter. Output of preamplifier is converted into digital value by a 12-bit analog to digital converter. Micro-controller performs calculations on these digital values according to the appropriate calculation algorithms developed.

A stepper motor is driven through a driver hardware that drives the roller type of peristaltic pump which generates the required sequence of pulses for the motor driving hardware. Roller type peristaltic pump used in the system is used for aspirating the required volume of reagents/samples and for washing the flow cell. This pump can be calibrated to aspirate the required quantity of water, reagent, and samples.

Temperature sensor LM335 and peltier device are used to provide and maintain the required temperature for the samples in flow cell. Peltier works in both directions for cooling and heating. This effect is used to control the temperature of the sample.

19.6 LAB-ON-CHIP TECHNOLOGY

A lab-on-a-chip(LOC) is a device that integrates one or several laboratory functions on a single chip of only millimetres to a few square centimetres in size. LOCs deal with the handling of extremely small fluid volumes down to less than picolitres. LOC devices are a subset of MEMS devices and often indicated by 'Micro Total Analysis Systems' (μTAS) as well. LOC is closely related to, and overlaps with, micro-fluidics which describes primarily the physics, the manipulation and study of minute amounts of fluids. However, strictly regarded 'Lab-on-a-Chip' indicates generally the scaling of single or multiple lab processes down to chip-format, whereas 'μTAS' is dedicated to the integration of the total sequence of lab processes to perform chemical analysis.

The concept of these microchip-based systems employs the integration of various chemical operations involved in conventional analytical processes done in a laboratory, such as mixing, reaction and separation, into a miniaturised flow system. (Manz and Beckar, 1998)

A liquid micro-space has several characteristic features different from the bulk scale: for example, short diffusion distances, high interface-to-volume ratio and small heat capacity. These characteristics in micro-space are key to controlling chemical unit operations, such as mixing, reaction, extraction and separation, and constructing the integrated chemical systems. In addition, the transport time takes from several hours to 1 day when the diffusion distance is 1 cm, since the diffusion coefficient of typical molecular ions is of the order of 10^{-5} cm^2/s. In contrast with that case, it takes only several tens of seconds when the diffusion distance is 100 μm. These kinds of scale merits become remarkable below a scale of about 250 μm.

The microchip is generally made from a glass plate, a silicon wafer, ploydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), other polymers, or their combination. Because of the

chemical and physical stability and optical transparency for detection, a glass microchip is preferred for various applications. Glass microchips are fabricated using a photolithographic wet etching method (Hibara et al., 2001). The description of an automated microchip-based platform for fabrication of microchip channel networks, which combines the electro-osmotic pumping and capillary electrophoresis for fluid transport and separation is available on http://www.suffield.drdc-rddc.gc.ca. The system enables the LOC integration of the key elements in analytical processing: injection, mixing, separation, detection and waste elimination within about 3 min. Normally these steps when done manually would require between 30–60 min. Indeed, Lab - on-a- chip has brought about a revolution in biological and medical sciences (Figeys and Pinto, 2000)

The Lab-on-chip technology has reached a stage of commercial production. The Agilent 2100 Bioanalyser which is an automated Analysis System is powered by major advances in microfabrication technology with the development of a fully automated LOC. The chip is designed to integrate sample preparation, fluid handling and biochemical analysis. The LOC technology uses semiconductor-like micro-fabrication techniques to translate experimental and analytical protocols into chip architectures consisting of interconnected fluid reservoirs and pathways. Movement of fluids, or molecules within fluids, is effected by electrodes that create electrokinetic forces capable of driving fluids through selected pathways.

Automated regulation of voltage between these electrodes controls the speed and direction of fluid movement. In this way, it is possible to create the functional equivalent of valves and pumps capable of performing manipulations such as dispensing, mixing, incubation, reaction, sample partition and detection.

Figure 19.15 illustrates the process on the lab-chip integrating, which is given below:

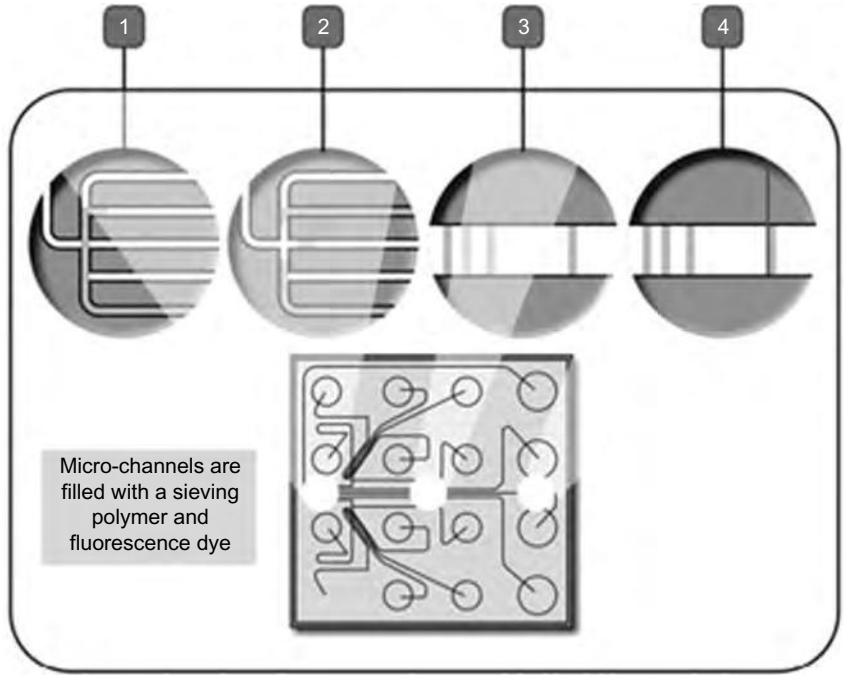
- Sample handling
- Separation and detection
- Data analysis within a single, compact system.

The system is PC-based and primarily developed for DNA, RNA and protein analysis. The software quantitates each DNA fragment automatically against internal standards. This ensures increased accuracy and reproducibility. Similarly, for automated detection of ribosomal RNA contamination, the software integrates the messenger RNA smear and calculates the percentage of ribosomal RNA contamination.

Many merits and uses of microchip systems with pressure-driven flow have been reported. By utilising several characteristics of micro-space, that is, a large specific interface, a short molecular diffusion distance and so on, the performances of several analysis systems were greatly improved by microchip integration. With an LOC, the following advantages have been established:

- Miniaturised fluid pathways give shortened run times
- Strong electrokinetic driving forces improve analyte resolution
- Micro-fabricated chips yield better reproducibility than conventional technologies
- Versatile chip design enables flexible experiment design on one system
- Micro-scale format minimises sample use.

In the near future, these microchip-based systems will be widely spread and highly beneficial to our daily life by further efforts of analytical chemists and engineers. Many researchers believe that LOC technology may be the key to powerful new diagnostic instruments. LOC technology may soon become an important part of efforts to improve global health, particularly through the



1. The sample moves from the sample well through the micro-channels.
2. The sample is injected into the separation channel.
3. Sample components are electrophorectically separated.
4. Components are detected by their fluorescence and translated into gel-like images (bands) and electropherograms (peaks).

Figure 19.15 *Working principle of Agilent 2100 Bioanalyser: Lab-on-chip technology*

development of point-of-care testing devices. The goal of these researchers is to create micro-fluidic chips that will allow healthcare providers in poorly equipped clinics to perform diagnostic tests with no laboratory support.

19.7 DRY CHEMISTRY CLINICAL ANALYSER

Dry chemistry refers to the use of strips impregnated with dry reagents to which the specimen is added. This assessment focuses on quantitative analysis of the chemical reactions by desktop analysers. Applications of the dry chemistry include patient screening, use in health clinics, mobile health services, polyclinics and hospital in-patients including point-of-care testing in critical care areas.

For example, the Ektachem (Eastman Kodak Co) analyser uses multi-layer dry-film reagent approach for colour development (Stockwell, 1985). The system uses 16-mm square slide, in which reagents are dispersed in emulsions which are achieved by diffusion of the sample fluid into the layers. Figure 19.16 shows an exploded view of a typical slide, the type of which is now available for different tests. Slides incorporating miniature ion-selective electrodes for measuring sodium, potassium, carbon dioxide and chloride are currently available.

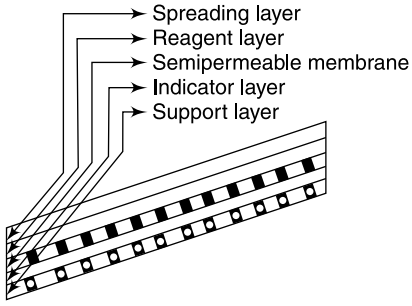


Figure 19.16 *Exploded view of the multi-layer dry-film reagent used in Kodak Ektachem analysers*

The dry reagent systems eliminate dispensing and mixing of liquid reagents. Nevertheless, the Systems still require a mechanism to maintain a stable temperature and to provide accurate positioning of the reaction unit for optical measurements. Thermal control in the Ektachem is provided through intimate contact of the slides, with a heated carousel that supports the slide and rotates it to the measurement position.

M/s FUJI Films announced for the first time in 1980 a dry chemistry analyser based on a multi-layered film method. The initial product was a dry chemistry slide and an analyser for measuring glucose concentration by spotting a 6-micro-litre sample of whole blood. The slide had a special spreading layer made of cloth, which enabled the use of whole blood samples.

Since then, various DRI-CHEM slides and analysers have been developed to fulfil the increasing needs of emergency testing, and a range of peripheral items were also developed. For instance, special pipette tips, now called FUJI CLEAN TIPS, were introduced to enable automatic spotting.

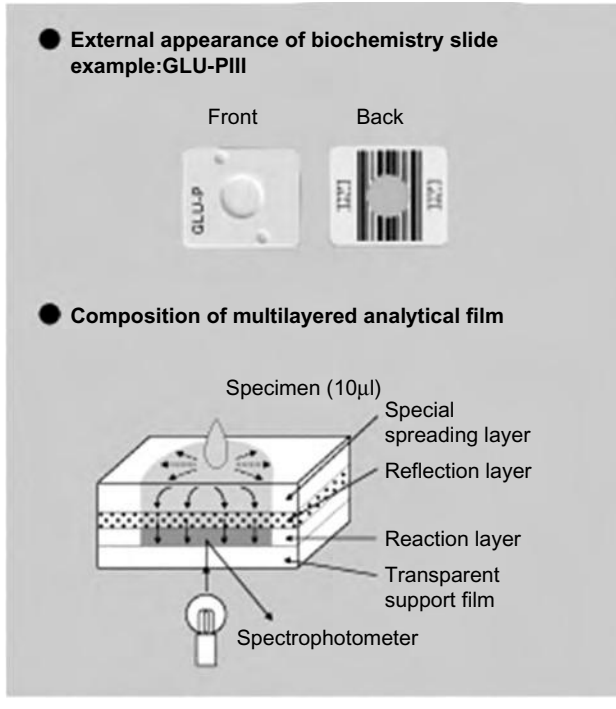


Figure 19.17 *Dry chemistry-based glucose concentration analyser by colorimetric technique (Courtesy: M/s FujiFilms)*

Because the tips do not require wiping, there is little danger of operator contamination from blood samples. The dry chemistry-based systems are now widely used in international market as automatic clinical chemistry analysers.

A typical example of a dry chemistry analyser is that of glucose slide, wherein the actual measurement is conducted as follows. 10 micro-litres of plasma or serum are spotted on a FUJI DRI-CHEM SLIDE GLU-PIII. This is illustrated in Figure 19.17. After spotting, the sample spreads uniformly on the spreading layer and diffuses into the underlying layer. As the process proceeds, large molecular weight components such as proteins or dye components are filtrated, and only small molecular weight components are able to permeate and diffuse into the reagent layer. Glucoseoxidase (GOD) catalyses the oxidation of sample glucose to generate hydrogen peroxide. In the presence of peroxidase (POD), hydrogen peroxide reacts with dye precursors and finally forms red dye. The slide is incubated at 37°C for a specified number of minutes in the FUJI DRI-CHEM analyser and the optical reflection density is measured at 505 nm. The optical reflection density is then converted into the glucose concentration using a calibration curve pre-installed in the analyser.

20

THERMO-ANALYTICAL INSTRUMENTS

20.1 THERMO-ANALYTICAL METHODS

A number of analytical methods have been developed, in which some property of the system is measured as a function of the temperature. In fact, the data are obtained as continuously recorded curves, which may be termed as thermal spectra. These spectra characterise a system, single or multi-component, in terms of the temperature dependence of its thermodynamic properties and physico-chemical reaction kinetics.

Figure 20.1 shows the configuration of a general thermal analysis instrument.

Thermal analysis instrument includes the following:

- *Detection Unit*: Furnace, sample and reference holder, and sensor, heat and cool the sample in the furnace, and detects the sample temperature and property.
- *Temperature Control Unit*: Controls the furnace temperature.
- *Data Recording Unit*: Records the signals of sensor and sample temperature, and analyses them.

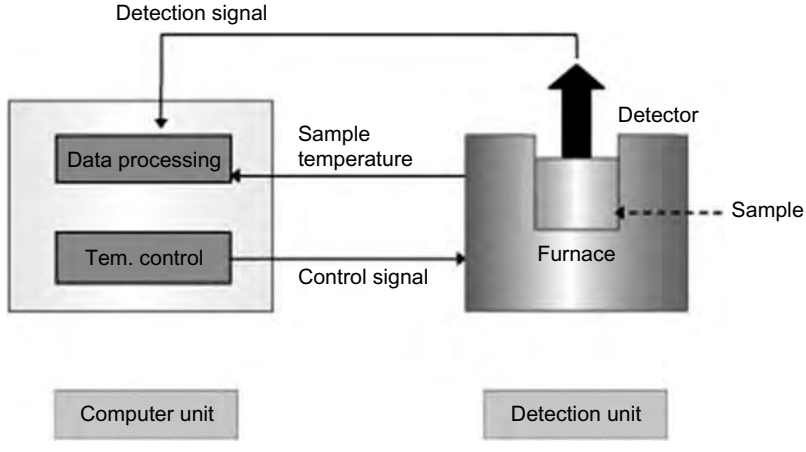


Figure 20.1 *Block diagram of thermal analysis instrument (Courtesy: M/s Hitachi High-Tech Science Corporation)*

Table 20.1 *Measurement techniques in thermal analysis*

TA Measurement Technique	Abbrev.	Property	Unit
Differential Thermal Analysis	DTA	Difference temperature	°C or μV*
Differential Scanning Calorimetry	DSC	Enthalpy	W = J/s
Thermogravimetry	TG	Mass	gram
Thermomechanical Analysis	TMA	Deformation	metre
Dynamic Mechanical Analysis	DMA	Elasticity	Pa = N/m²

In the modern thermo-analytical instruments, temperature control, data recording and analysis are all computer-controlled. The combination of the furnace and sensor enables the various types of the measurement techniques. This computer can be connected to several instruments, which have other types of measurement techniques, and enables simultaneous measurement and analysis. Table 20.1 shows the measurement techniques for each property.

20.2 THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric analysis (TGA) involves the measurement of the weight of a sample under investigation as the temperature is increased at a predetermined rate. The sample may either lose weight to the atmosphere or gain weight by reaction with the atmosphere. The following are the two techniques in TGA:

- *Dynamic thermogravimetry*, in which the sample is subjected to continuous temperature changes, usually linear with time.
- *Static thermogravimetry*, wherein the sample is maintained at a constant temperature for a period of time during which any changes in weight are recorded.

TGA record is generally in the form of an integral curve, with absolute weight (W) as the y-axis and time (t) or temperature (T) as the x-axis.

TGA technique usually finds application in testing materials that are actual or potential analytical standards, and of course in the direct application of the technique to analytical determinations, particularly to evaluate kinetic parameters of weight changes in reactions.

It is important to note that the shape of the thermogravimetric curve is influenced by several factors, the most important among them being the following.

Heating Rate: At any given temperature, the extent of decomposition is greater at a slow rate of heating than for a similar sample at a fast rate of heating. When successive reactions are expected to take place, the rate of heating will determine whether or not these reactions will be separated.

Sample: Equilibrium between sample and product gas and with the furnace temperature will depend on the bulk of the material in the sample cup. Better results are observed if small and finely divided sample is used

Atmosphere: The composition of the atmosphere immediately surrounding lie reacting particles influences the thermogravimetric curve.

With all these factors to influence this analytical technique, the temperatures of salient features on the curves are somewhat different, as observed on different instruments, or on the same instrument at different rates of temperature scanning, or with different size samples, etc.

20.2.1 Instrumentation

The apparatus for TGA comprises the following:

- Precision balance
- Heating arrangement – a furnace programmed for a linear rise of temperature with time
- Temperature control and measurement system
- Recorder

Figure 20.2 shows block diagram of a Thermo-balance

Balance: If very large samples are to be avoided, precision and sensitive balances must be employed to detect the sample weight change. Balances may be either the null point or deflection type instruments. In null type instruments, any deviation of the balance beam is detected by a suitable sensing element. The beam is subsequently returned to the original position by the application of restoring force, which would be proportional to the change in weight. This restoring force is recorded either directly or through a transducer. Deflection type instruments transfer the deviations in beam position into a record of the weight change. These are based on conventional analytical balance, a helical spring, a cantilever beam, a strain gauge or a torsion balance.

Several innovative arrangements have been employed to follow weight change, continuously and precisely. However, most modern systems make use of a torque motor. This is basically a galvanometer with the sample attached to the needle. The weight of the sample is proportional to the current required to restore the needle to some null position. Null is detected by a pair of photocells behind a vane in a light beam.

Thermobalances used in TGA have provision for recording of weight. Those of the non-recording type are intended mainly for the determination of superficial moisture in bulk materials, wherein the sample is heated by an infrared lamp while on the pan of the balance, specially designed to minimise error produced by air currents.

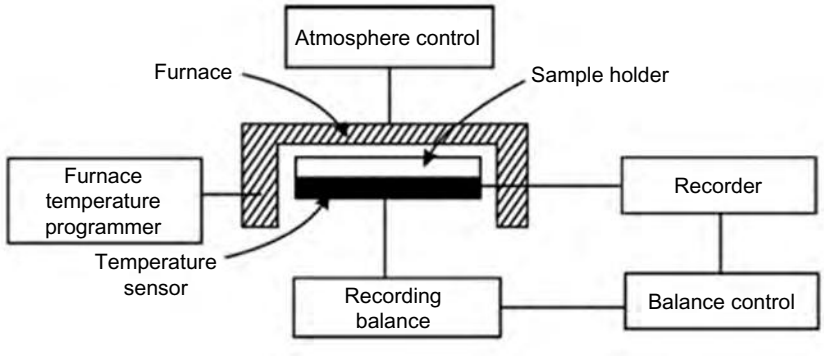


Figure 20.2 *Block diagram of a thermo-balance (Courtesy: M/s ExpertsMind.com)*

Heating arrangement is provided by a furnace, which should maintain either a linear heating program (10–600°C/h) or a fixed temperature. Control is usually achieved using a thermocouple or resistance thermometer placed as close to the furnace winding as possible. The heating material is selected depending on the temperature required. For example, nichrome winding gives a maximum temperature of around 1,100°C, platinum-rhodium up to 1,450°C and a graphite tube furnace for higher temperatures. At higher temperatures, an inert-gas blanket is usually employed.

Temperature measurement of the sample is perhaps one of the most ticklish problems in TGA. For example, during reaction of a sample with the balance atmosphere, the temperature of the surface of the sample is important. The temperature of the gas around the surface of the sample is not important to the reaction kinetics. On the other hand, the temperature of the interior of the sample is important during auto-decomposition. Therefore, it is essential to arrive at a compromise for temperature measurement problems with the rest of the balance system.

Thermocouples are usually employed as transducers for temperature measurement. They can be small enough to be located within the sample. In some cases, use has also been made of bolometers and optical pyrometers for temperature measurement. Perkin Elmer thermobalance makes use of the changes in furnace winding resistance to measure furnace temperature.

Recording system should be able to record both temperature and weight continuously and to make a periodic record of the time.

20.3 DIFFERENTIAL THERMAL ANALYSIS

Differential thermal analysis (DTA) is a technique in which the difference in temperature between the sample and a reference material is monitored against time or temperature, while the temperature of the sample in a specified atmosphere is programmed. It is a fingerprinting technique that provides information on the chemical reactions, phase transformations and structural changes that occur in a sample during a heat-up or a cool-down cycle. The DTA measures the differences in energies released or absorbed, and the changes in heat capacity of materials as a function of temperature.

All materials behave in certain predictable ways when exposed to certain temperatures, so the resulting DTA curve is an indication of the materials and phases present in the sample. For example, the DTA is used to indicate the relative magnitude of reactions and phase transitions of ceramic materials or batches that can be destructive so that safe drying and firing schedules can be determined. The DTA identifies the temperature regions and the magnitude of critical events during a drying or firing process.

DTA basically involves heating or cooling a sample and a reference material in close proximity at a linear heating rate, monitoring and recording the temperature of the furnace, and the difference in temperature between the sample and reference. The differential thermogram consisting of a record of the difference in sample and reference temperature (ΔT) plotted as a function of time (t), sample temperature (T_s), reference temperature (T_r) and furnace temperature (T_f) provide valuable information on phase transitions and chemical reactions. DTA technique is especially suited to studies of structural changes within a solid at elevated temperatures, where few other methods are available.

An endothermic process will cause the thermocouple junction in the sample to lag behind the junction in the reference, and hence develop a voltage, whereas an exothermic process will generate a voltage of opposite sign. Conventionally, the exotherms are plotted upwards, whereas the endotherms are plotted downwards.

Figure 20.3 shows the physical layout of a DTA apparatus from M/s Orton, USA. The sample and the reference are placed symmetrically in the furnace. The furnace is controlled under a temperature program and the temperature of the sample and the reference are changed. During this process, a differential thermocouple is set up to detect the temperature difference between the sample and the reference. Also, the sample temperature is detected from the thermocouple on the sample side.

It uses a pair of ceramic cups that are supported by a pair of vertical thermocouples (differential thermocouple) positioned on the module base. After both cups are placed on top of the differential thermocouple stalks, the furnace is lowered over the cups, and the furnace is heated and cooled according to the programmed thermal cycle. The differential thermocouple output, that is, DTA signal in micro-volts is displayed on a PC monitor, and stored on the PC hard drive as a function of time and temperature for post testing analysis.

The Temperature Control is with a user-programmable PID controller which is used to control the thermal cycle of the furnace. The system includes a Windows 98/2000 compatible data acquisition/analysis software and an analog to digital interface card for the personal computer system. The software displays the test progress on the monitor, stores the data and enables the user to perform the standard DTA analyses on the data after the test is completed.

Figure 20.4 illustrates measurement principles of DTA. Figure 20.4a shows the temperature change of the furnace, the reference and the sample against time. Figure 20.4b shows the change in temperature difference (ΔT) against time detected with the differential thermocouple. ΔT signal is referred to as the DTA signal. Matters that do not change in the measurement temperature range (usually α-alumina) are used as reference.

When the furnace heating begins, the reference and the sample begin heating with a slight delay depending on their respective heat capacity, and eventually heat up in according to the furnace

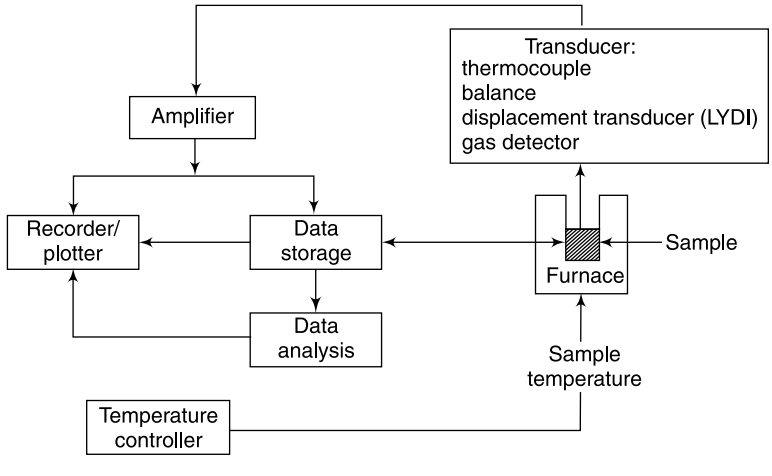


Figure 20.3 *Schematic of a modern thermal analyser instrument (Gill, 1984)*

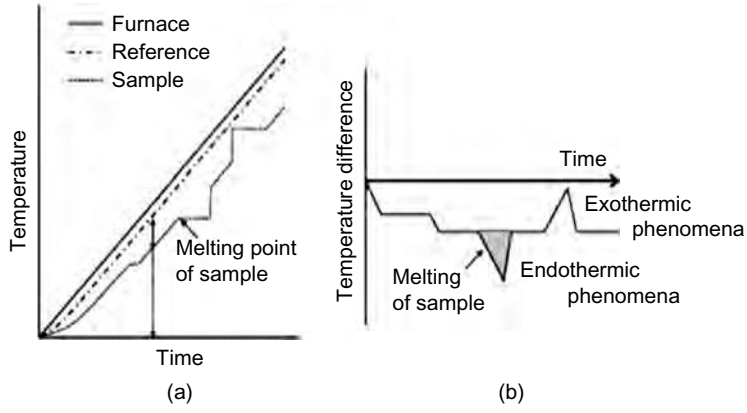


Figure 20.4 *Measurement principles of DTA (Courtesy: M/s Hitachi High-Tech Science Corporation)*

temperature. ΔT changes until a static state is reached after the heating begins, and after achieving stability, reaches a set amount compliant with the difference in heat capacity between the sample and the reference. The signal at the static state is known as the baseline.

When the temperature rises and melting occurs in the sample, for example, the temperature rise stops as shown in graph (a) and the ΔT increases. When the melting ends, the temperature curve rapidly reverts to the baseline.

At this point, the ΔT signal reaches the peak, as shown in graph (b). From this, we can detect the sample superfluous transition temperature and the reaction temperature from the ΔT signal (DTA signal). In graph (b), the temperature difference due to the endothermic change in the sample is shown in a negative direction and the temperature difference due to the exothermic change is shown in a positive direction.

20.3.1 Instrumentation

A modern thermal analyser instrument (Figure 20.5) consist of a furnace for a heating (or cooling) the sample at controlled rate and a selected transducer to monitor changes in the substance. The transducer can be a thermocouple to measure temperature changes (heat flow), a balance to monitor weight changes, or a linear variable differential transducer (LVDT) to detect changes in dimensions. The transducer generates a voltage signal which is amplified, stored on a disc along with a direct temperature response from the sample and recorded on a printer or plotter.

DTA apparatus is available from several companies; their products differ with regard to such parameters as sample, size, temperature range, selectable scanning rates and precision, etc. However, the following are the five basic components in all of them:

- Sample cell with temperature detector
- Furnace assembly
- Temperature programmer
- Amplifier and recorder
- Atmosphere control

The sample cell is just a disposable tube, about 2 mm in diameter which can contain 0.1–10 mg of sample. A similar tube carries the reference material, such as alumina or quartz sand. The two tubes are inserted into a metallic or ceramic block. Very thin thermocouples are inserted in the sample and reference tube. In the low temperature range (–100 to 600°C), the thermocouples used are copper – constantan, iron-constantan and chromel-alumel. For higher temperatures, Platinum/platinum – 10% rhodium thermocouples are necessary. The use of other detectors, such as thermistors and platinum-resistance thermometers, has also been made in some commercial instruments.

The furnace used is generally of high-temperature, pressure-vacuum electric type. Sample temperatures up to 500°C are usual, while maximum temperatures used are around 1000°C. Relatively, small sample volumes minimise thermal gradients and make evacuation easy.

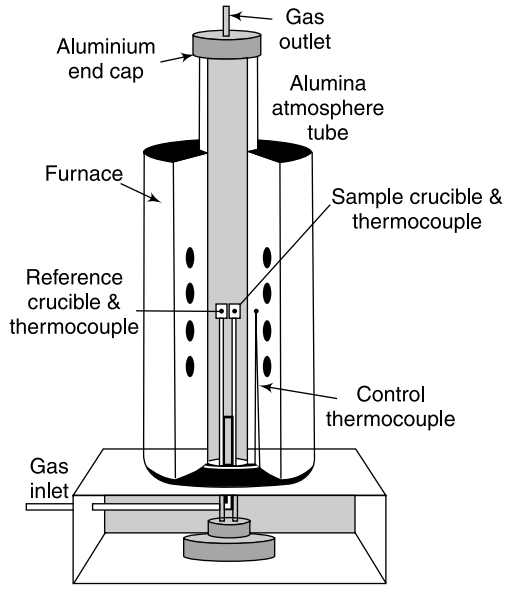


Figure 20.5 *Differential thermal analysis system (Courtesy: M/s Orton, USA)*

Temperature programmer must provide smooth and oscillation-free heating or cooling rate, which should be reproducible to ±0.1°C or better. Heating or cooling rates usually provided on the instruments are 0–30°C/min. The simplest temperature programmer is a variable transformer, in which various programmers consisting of motors attached to the variac shaft are incorporated. However, modern DTA instruments incorporate solid-state electronic temperature controllers. In these controllers, the signal from the thermocouple in the furnace is compared electronically against a reference potential, which can be programmed to correspond to a variety of heating modes and heating rates.

The amplifiers are used for suitable amplification of the dc signal obtained from the thermocouple. The amplifier is necessarily a high gain, low-noise and high input impedance circuit. The amplification before the signal is given to the recorder is about 10,000. The recorder should be dual-pen strip chart recorder, though some systems use two recorders. Usually ΔT (change in temperature) is recorded as Y-axis whereas T (furnace temperature) as X-axis. The recorder sensitivity is usually 1–0.1 mV/inch and a time constant of 1 s or less.

For some types of samples, the atmosphere must be controlled either to suppress an undesirable reaction, such as oxidation, or to learn the nature of a reaction, as for example, by varying the pressure of a gaseous reaction period. Four types of systems are in current use for control of atmosphere: (i) inert-gas purge for the protection of the unencapsulated sample, (ii) inert gas covering within the encapsulation, (iii) inert or reactive gas for thermodynamic and reaction kinetic studies and (iv) flowing inert or reactive gas for study of reaction kinetics.

The cooling system is usually separated from the temperature programmer in most of the commercial instruments. The most satisfactory arrangement for cooling is with a gas as the heat exchange medium.

20.4 SIMULTANEOUS THERMOGRAVIMETRY/ DIFFERENTIAL THERMAL ANALYSIS

A method in which thermogravimetry (TG) and Differential thermal analysis (DTA) are combined and measured simultaneously by a single apparatus. Figure 20.6 shows the balance beams for the sample and the reference are located in the furnace. The masses of the sample and the reference are measured by the sensitivity-calibrated drive coils separately. The mass difference is sent as TG signal. By the differential mass measurement, the effects of the beam expansion, the convection flow, and buoyant force are cancelled. Thus the highly sensitive thermogravimetry measurement is achieved.

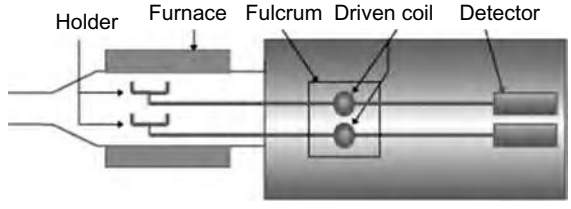


Figure 20.6 *Block diagram of horizontal differential TG/DTA (Courtesy: M/s Hitachi High-Tech Science Corporation)*

The mass measurement of the sample and the reference by the independent drive coils enables the easy adjustment of the TG baseline drift electrically. Also, thermocouple is located in each holder which enables the simultaneous DTA signal output.

TG can be utilised for the analysis of the thermal decomposition, the oxidisation, the dehydration, the heat resistance, and kinetics analysis. By combining with the other measurement technique, variety of information can be achieved from one sample. In particular, TG/DTA simultaneous measurement instrument is most common.

20.5 THERMOMECHANICAL ANALYSIS

The Thermomechanical analysis (TMA) is a technique in which the deformation of a substance under non-oscillatory load is measured as a function of temperature. The mode, as determined by the type of stress applied (compression, tension, flexure or torsion), should always be stated. A special related technique is thermodilatometry (TD), the measurement of a change of dimension of the sample with a negligible force acting on the sample while it is subjected to a temperature regime. The associated thermo-analytical method is thermodilatometric analysis (TDA).

TMA includes several variations according to the force and the way of the force is applied. The principles of compression, penetration, hemispherical indenter, flexure, linear and volumetric extension measurements are generally used. Compression probe is used for applying low load over a wide area of sample for thermal expansion measurements (see TD). Penetration probe applies a high load over a small area for the purpose of measuring softening temperatures. Tension probe is used for measuring thin films and fibres under tension. Volumetric probe is used for measuring the thermal volumetric expansion of irregularly shaped specimens surrounded by an inert packing material (alumina powder or silicone oil).

The most commonly used TMA probe is the expansion probe. This probe rests on the surface of the test specimen under low loading conditions. As the sample expands during heating, the probe is pushed up and the resulting expansion of the sample is measured (Figure 20.7).

The sample is inserted into the furnace and is touched by the probe which is connected with the Length Detector and the Force Generator. The thermocouple for temperature measurement is located near the sample. The sample temperature is changed in the furnace by applying the force onto the sample from the Force Generator via probe.

The sample deformation such as Thermal Expansion and Softening with changing temperature is measured as the probe displacement by the Length Detector. Linear Variable Differential Transformer (LVDT) is used for Length Detection sensor.

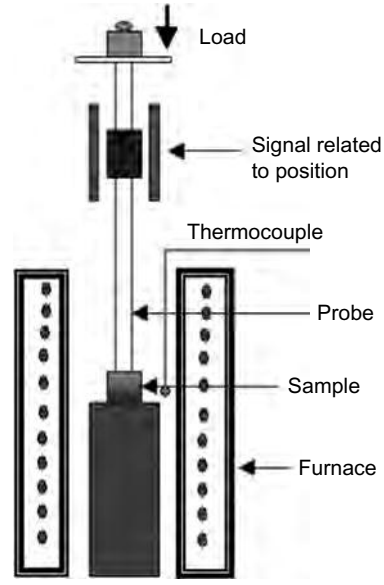


Figure 20.7 *The principle of thermo-mechanical analysis using expansion probe*

There are several types of the probe for TMA as shown in Figure 20.8. The choice is dependent on the measurement purpose.

Expansion/Compression Probe: It is used for the measurement of the deformation by the thermal expansion and the transition of the sample under the compressed force is applied.

Penetration Probe: It is used for the measurement of the softening temperature.

Tension Probe: It is used for the measurement of the thermal expansion and the thermal shrinkage of the sample such as the film and the fibre.

The materials of probes are quartz glass, alumina, and metals. The choice is dependent on the temperature range and/or the measurement purpose.

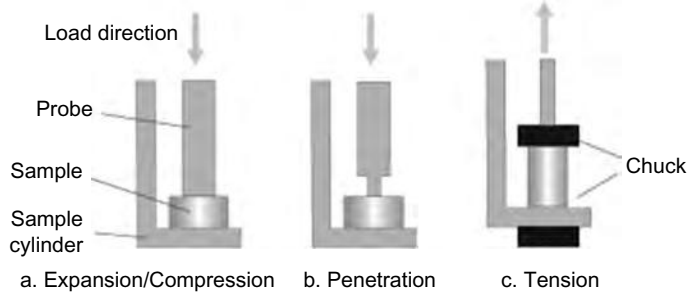


Figure 20.8 *Different types of TMA probes (Courtesy: M/s Hitachi High-Tech Science Corporation)*

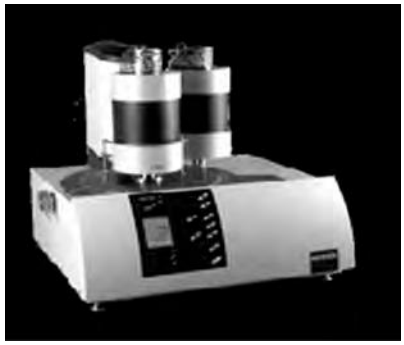


Figure 20.9 *Thermo-mechanical analyser (Courtesy: M/s Netzsch, Germany -www.netzsch.com)*

In case of the TMA which enables the dynamic force control on top of the static force control, the measurement of the stress–strain, the Creep, the stress relaxation, and the DMA measurements can be performed. Measurement of the expansion of solids and liquids caused by various imposed conditions, such as absorption or chemical reaction or other time-related processes, at fixed temperatures is known as dilatometry. When special emphasis is put on recording such dimensional changes as a function of temperature, during a controlled temperature program, the technique is labelled TD.

Irrespective of the selected type of deformation (expansion, compression, penetration, tension or bending), every length change in the sample is communicated to a highly sensitive inductive displacement transducer (LVDT) via a push rod and transformed into a digital signal. The push rod and corresponding sample holders of fused silica or aluminium oxide can be quickly and easily interchanged to optimise the system to the respective application.

Figure 20.9 shows a commercial thermomechanical analyser from M/s NETSZCH. The entire TMA 402 *Hyperion*® measuring system is thermally stabilised via water-cooling. This ensures that the measurement will not be influenced by heat from the furnace or by temperature fluctuations in the local environment. All joints have a vacuum-tight design to allow measurements in a highly pure atmosphere or under vacuum. Pressures of less than 10-4 mbar can be achieved with the use of a turbomolecular pump. In combination with the integrated mass flow controllers (MFC) for purge and protective gases (optional in the TMA 402 ***F3***), measurements in highly pure inert gas or in oxidising atmospheres can be optimally controlled.

Referring to Figure 20.10, the force operating on the sample is generated electromagnetically in the TMA 402 *Hyperion*®. This guarantees a quick response time for experiments with a changing load (e.g. tests on creep behaviour). A highly sensitive force sensor (digital resolution < 0.01 mN) continuously measures the force exerted via the push rod and readjusts it automatically. This sets the TMA 402 *Hyperion*® apart from other instruments, which use only pre-set values.

The electronic control system for the TMA 402 *Hyperion*® allows forces to be set in the mN-range. This enables testing even on sensitive materials such as thin fibres or films. The force operating on the sample can be altered via the software in a stepwise or linear fashion. This makes it particularly simple to carry out such analyses as creep or stress sweep tests. The premium version of the *Hyperion*®, the TMA 402 ***F1***, provides even more capabilities. From single pulse in rectangular or ramp form to continuous modulation with a customisable frequency (up to 1 Hz), every possibility is covered. This model is particularly well suited for determining viscoelastic material properties such as elasticity and creep modulus.

To analyse gases evolving upon heating (EGA), the TMA 402 *Hyperion*® can be coupled to a mass spectrometer and/or FT-IR spectrometer.

TMA is one of the important characterisation techniques in the field of thermal analysis. With TMA, the dimensional behaviours of a sample are measured as the sample is heated, cooled or

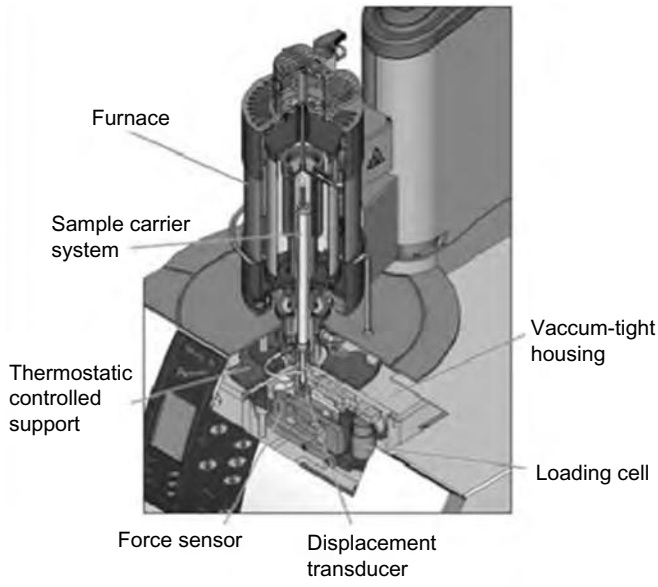


Figure 20.10 *Components of a thermo-mechanical analyser (Courtesy: M/s Netzsch, Germany -www.netzsch.com)*

held under isothermal conditions. The following are the two types of thermomechanical measurement techniques:

- *Dynamic Mechanical Analysis (DMA)*: A technique in which the sample's kinetic properties are analysed by measuring the strain or stress that is generated as a result of strain or stress, varies (oscillate) with time, applied to the sample.
- *Static Viscoelasticity Measurement*: A technique in which the change in stress or strain is measured under uniform stress or strain that remains constant across time.

Figure 20.11 shows the Block diagram of DMA. The sample is clamped into a frame of measurement head and is heated by the furnace. The sample in the furnace is applied the stress from the force generator via probe. To make the strain amplitude constant, the stress is applied as the sinusoidal force. This frequency is one of the measurement conditions. The deformation amount generated by the

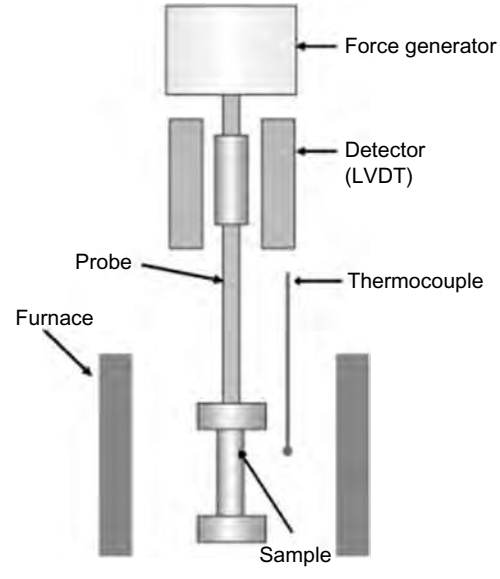


Figure 20.11 *Block diagram of DMA (Tension mode) (Courtesy: M/s Hitachi High-Tech Science Corporation)*

sinusoidal force is detected. Viscoelastic values such as elasticity and viscosity is calculated from the applied stress and the strain and plotted as a function of temperature or time.

DMA can be applied to a wide range of materials using the different deformation modes. DMA deformation mode has tension, compression, dual cantilever bending, three-point bending and shear mode. The deformation mode is selected dependent on the specimen shape and modulus, and the measurement purpose.

The analysis of the glass transition temperature and temperature dependence of the modulus can be measured by the temperature dispersion measurement. By performing the simultaneous measurement of temperature dispersion and frequency dispersion measurement, relaxation phenomena including glass transition can be observed. It enables to obtain the information of molecular structure and molecular motion of polymer.

20.6 DIFFERENTIAL SCANNING CALORIMETRY

DTA is capable of giving good qualitative data about the temperatures and indications of transitions, but it is difficult to obtain quantitative data (i.e. the heat of transition if the purity is known, or the amount of constituent in a sample if the heat of transition is known). This could be due to certain unknown and uncontrollable factors, such as the specific heat and thermal conductivity of the sample before and after the transition. Several other parameters such as the rate of heating, placement of thermocouple and other instrumental parameters also create problems in carrying out quantitative analysis. These difficulties have been solved using another closely related technique to DTA, which is called Differential Scanning Calorimetry (DSC).

DSC is a thermal analysis technique which is used to measure the temperature and heat flows associated with transitions in materials as a function of time and temperature. Such measurements provide qualitative and quantitative information about physical and chemical changes that involve endothermic and exothermic processes or changes in heat capacity on DSC technique usually covers the range from 60° to 1,600°C with variable atmospheres.

In this technique, the sample and reference are mounted on two separate small heaters. The temperatures of the two cells are monitored by platinum-resistant thermometers and the two-heater windings arc supplied with current so that both windings heat or cool at the same rate, which would be up to 80°C/min. The power difference required for two heaters is measured (ΔP) and recorded as a function of the program temperature. This record shows the physical or chemical transitions in the sample and the power difference is equal to the thermal energy absorbed or released during the transition.

DSC differs fundamentally from DTA in that the sample and reference are both maintained at the temperature predetermined by the programme even during a thermal event in the sample. The amount of energy which has to be supplied to or withdrawn from the sample to maintain zero temperature differential between the sample and the reference is the experimental parameter displayed as the ordinate of the thermal analysis curve. The sample and reference are placed in identical environments, metal pans on individual bases each of which contains a platinum-resistant thermometer (or thermocouple) and a heater (Figure 20.12). The temperatures of the two thermometers are compared and the electrical power supplied to each heater adjusted so that the temperatures of both the sample and the reference remain equal to the programmed temperature (i.e. any temperature difference which would result from a thermal event in the sample is 'nulled'). The ordinate signal, the rate of energy absorption by the sample (e.g. millicalories/s.), is proportional to the specific

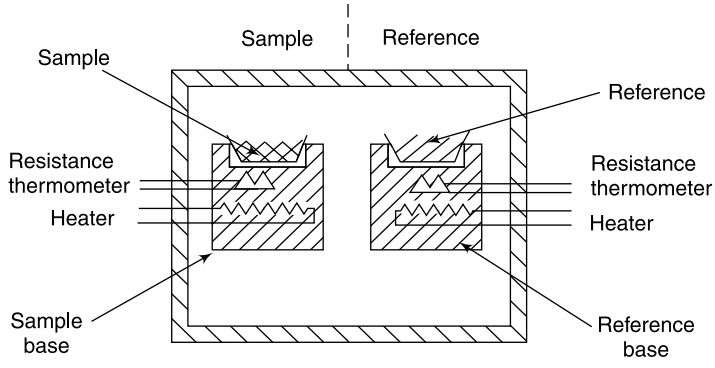


Figure 20.12 *Differential scanning calorimetry set up*

heat of the sample since the specific heat at any temperature determines the amount of thermal energy necessary to change the sample temperature by a given amount. Any transition accompanied by a change in specific heat produces a discontinuity in the power signal, and exothermic or endothermic enthalpy changes peaks, whose areas are proportional to the total enthalpy change (Figure 20.13).

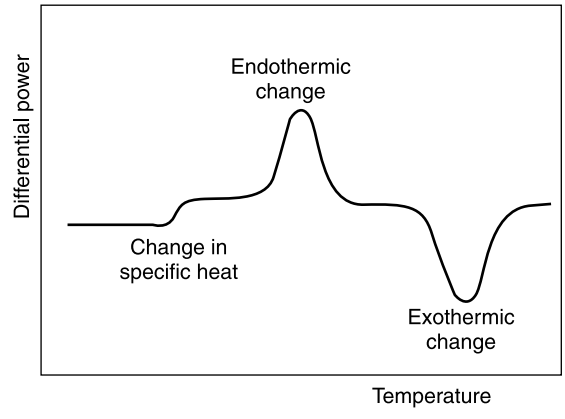


Figure 20.13 *Typical DSC curve*

In other words, in DSC, the measuring principle is to compare the rate of heat flow to the sample and to an inert material which are heated or cooled at the same rate. Changes in the sample that is associated with absorption or evolution of heat cause a change in the differential heat flow which is then recorded as a peak. The area under the peak is directly proportional to the enthalpic change and its direction indicates whether the thermal event is endothermic or exothermic. Donovan (1984) discusses the use of scanning calorimetry for biological studies.

DSC as the generic term for the following two measurement methods, as per JIS (Japanese Industrial Standard):

- *Heat Flux DSCs*
  A technique in which the temperature of the sample unit, formed by a sample and reference material, is varied in a specified program and the temperature difference between the sample and the reference material is measured as a function of temperature.

- *Power Compensation DSC*
  A technique in which difference of thermal energy that is applied to the sample and the reference material per unit of time is measured as a function of the temperature to equalise their temperature, while temperature of the sample unit, formed by the sample and reference material is varied in a specified program.

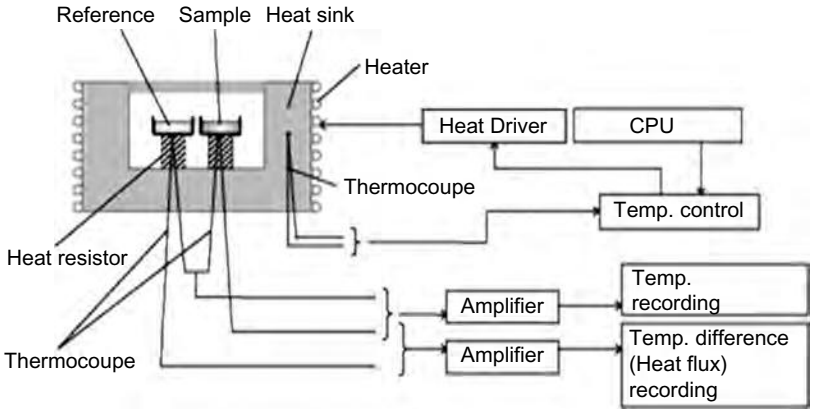


Figure 20.14 *Block diagram of Heat Flux Differential Scanning Calorimetery (Courtesy: M/s Hitachi High-Tech Science Corporation)*

Figure 20.14 shows the block diagram of Heat Flux DSC as an example. Heat Flux DSC comprises the sample and reference holder, the heat resistor, the heat sink, and the heater. Heat of heater is supplied into the sample and the reference through heat sink and heat resistor. Heat flow is proportional to the heat difference of heat sink and holders. Heat sink has the enough heat capacity compared to the sample. In case the sample occurs in endothermic or exothermic phenomenon such as transition and reaction, this endothermic or exothermic phenomenon is compensated by heat sink. Thus the temperature difference between the sample and the reference is kept constant. The difference the amount of heat supplied to the sample and the reference is proportional to the temperature difference of both holders. By calibrating the standard material, the unknown sample quantitative measurement is achievable.

DSC enables the measurements of the transition such as the glass transition, melting, and crystallisation. Furthermore, the chemical reaction such as thermal curing, heat history, specific heat capacity, and purity analysis are also measurable. Recently, with the development of the highly functional polymeric material, these thermal properties analysis needs are increasing dramatically. DTA and DSC detect the temperature differences between the sample and the reference; however, DSC can perform the quantitative measurement of the amount of heat on top.

20.7 SIMULTANEOUS THERMAL ANALYSIS/MASS SPECTROMETER

Simultaneous thermal analysis (STA) techniques comprise both DTA and thermogravimetry (TG). The equipment operates in a manner similar to differential scanning calorimeter (DSC). Two sample crucibles are heated or cooled at a precisely controlled rate in a controlled environment. One crucible contains a standard of known thermal response; the unknown is placed in the second crucible. The differences in the thermal behaviour of the two materials caused by differences in specific heat, occurrence of an exothermic or endothermic reaction or a phase change, result in a temperature difference between the two crucibles.

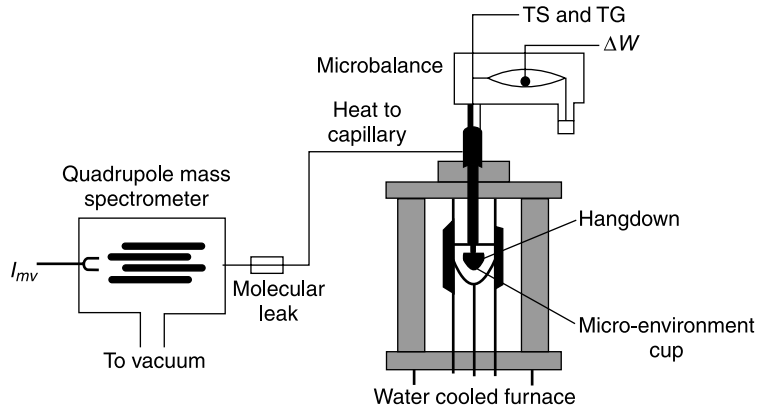


Figure 20.15 *Schematic diagram of simultaneous thermal and mass analyser (Courtesy: www.ms.ornl.gov/htmlhome/tpuc/sta.html)*

Temperature differences are measured with a Pt. vs. Pt-10Rh differential thermocouple that enables properties of the unknown to be determined relative to that of the standard. Simultaneously, any change in mass of the specimen during a heating cycle can be measured with a microbalance as a function of temperature. A mass spectrometer (MS) is attached to the STA instrument for evolved gas analysis. During the thermal cycle, a capillary leak allows samples of the gaseous environment in the immediate vicinity of the specimen to be drawn into a quadruple MS. Evolved gases can thus be identified by the mass-charge ratios of molecules, fragments, or atoms in the gas. Figure 20.15 shows the schematic diagram of STA/MS system.

- The arrangement can be used to make DTA and TG measurements from 25 to 1,500°C with mass change determinations to +/− 0.01 mg.
- Simultaneous quadruple mass spectrometric (MS) enables analysis of evolved gases with sensitivity in ppm level and mass range to 600 amu.

STA can be used to follow the course of chemical reactions, thermal decompositions or phase changes as a function of temperature. The sensitive balance associated with the TG capability of the system allows the mass change of a specimen to be measured as a function of temperature. Simultaneous DTA, TG and MS measurements provide information about the cause of mass changes.

21

ELECTROPHORESIS APPARATUS AND DENSITOMETERS

21.1 ELECTROPHORESIS

Electrophoresis is an old established method of analytical chemistry. It is based on the principle that the individual components of the colloidal solution migrate in a liquid at different speeds when subjected to an electric field. Separations are possible, because particles of similar geometry but different charge, and particles of like charge but different geometry migrate at different rates towards an oppositely charged electrode. Therefore, when the current is passed for a certain time through such a solution, various components present in the solution would move through different distances in their effort to migrate towards the electrodes. Therefore, a substance which may be a mixture is thus separated into its components along the migration distance, according to a definite law. Measurement of the concentration along this migration distance, therefore, would provide the quantitative result of the analysis.

Accordingly, electrophoresis is a separations technique that is based on the mobility of ions in an electric field. Positively charged ions migrate towards a negative electrode and negatively charged ions migrate towards a positive electrode. For safety reasons one electrode is usually at ground and the other is biased positively or negatively. Ions have different migration rates depending on their total charge, size, and shape, and can therefore be separated.

Historically, some of the earliest reports described characteristic electrophoretic mobilities of bio-colloids, such as proteins or enzymes. However, the technique received little notes until 1937, when Tiselius published a paper introducing the moving boundary concept. The moving boundary method utilises the migration of particles in free solution and observation of the various molecular boundaries through sensitive refractometric techniques. With this, the value of electrophoresis in obtaining distinct and measurable fractions of a variety of substances got well established, particularly in clinical laboratories.

Basically, electrophoresis technique separates the molecules based on another size and charge under the influence of an electric field. If E is the strength of the electrical field, Z is the charge on the molecule and F is the frictional force on the molecule, then V the velocity of migration is given by

$$V = \frac{EZ}{F}$$

The frictional force can be defined as

$$F = 6\pi\eta r$$

where η is the viscosity of the medium and 'r' is the stokes radius of the molecule. Therefore,

$$V = \frac{EZ}{6\pi\eta r}$$

This implies that the electrophoretic mobility is proportional to the charge on the molecule and inversely proportional to the radius of the molecule (i.e. larger the radius translates to lower electrophoretic mobility).

Normally, with moving boundary method, only two components of a mixture, one with the highest mobility and the other with the lowest mobility can be separated in pure form. If it is desired to recover components other than those of highest and lowest mobilities in pure form, multiple separations have to be carried out.

Currently, electrophoretic separations are performed by two popular types of methods:

- Slab electrophoresis
- Capillary electrophoresis

21.2 SLAB ELECTROPHORESIS APPARATUS

The slab electrophoresis method is used to separate complex, high-molecular mass species of biological and biochemical interest. The separations are carried out on a thin flat layer or slab of a porous semi solid gel containing aqueous buffer solutions within its pores. The slab has dimensions of a few centimetres on a side and is capable of separating several samples simultaneously. Samples are introduced as spots or bands on the slab. A DC electric field is applied across the slab for a fixed period. When the separations are complete, the electric field is disconnected. The separated spices are then visualised by staining as is done in thin layer chromatography.

The slab electrophoresis apparatus consists of a high-voltage supply, electrodes, buffer, and a support for the buffer such as filter paper, cellulose acetate strips and polyacrylamide gel (Figure 21.1a). A complete paper electrophoresis apparatus (Figure 21.1b) comprises the following parts:

- Electrophoresis cabinet
- Power supply
- Densitometer or scanner

21.2.1 Electrophoresis Cabinet

Electrophoresis cabinet consists of a methacrylate plastic cabinet and gable cover, and a carrier rack of phenolic plastic. The gable cover prevents condensation droplets from falling on paper strips. The carrier supplied for use with paper or cellulose strips up to a total width of 7.25 inches is suitable for supporting paper strips. End surfaces of the carrier are roughened to grip wet paper.

Some electrophoresis baths are designed to take up to six strips, each 34 cm long and 5 cm wide. The strips are supported in pairs on three removable plastic bridges, which are provided with a

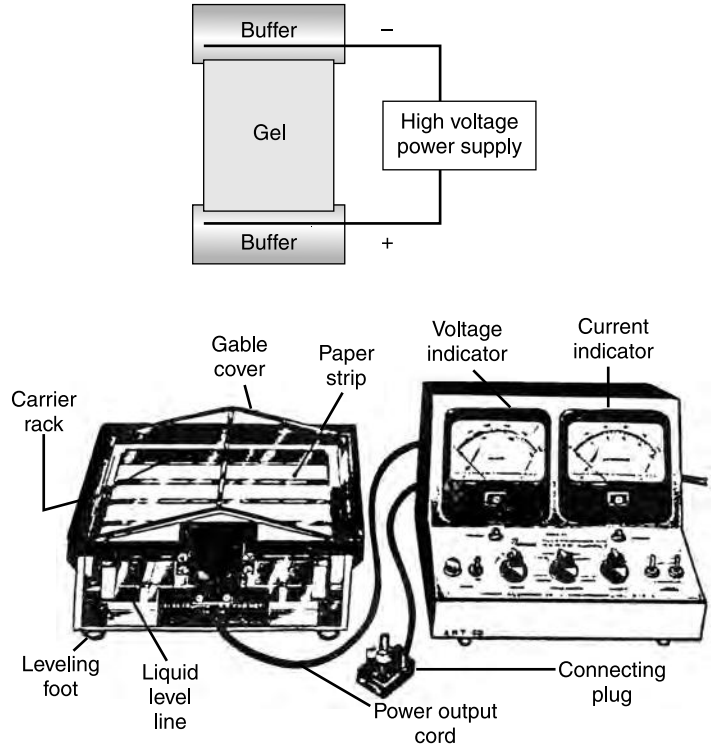


Figure 21.1 *(a) Gel electrophoresis set-up (b) Paper electrophoresis apparatus (Courtesy: M/s Arther H. Thomas, USA)*

number of supports to carry the paper and to prevent undue sagging. At each end of the bath, there are two compartments to contain the buffer solution. The ends of the paper strips dip into the solution in the outer of these two compartments, whilst three platinum electrodes are mounted in the inner compartments. The electrodes are wired to a lead and polarised plug for connecting to the power unit.

The tank should not leak electrically as well as mechanically. To check electrical leakage, the buffer solution is poured into the tank without placing any filter strip. The electrodes are connected to a power supply at 500 V and a micro-ammeter is connected in series with the supply. The current should not exceed 1 μA in a good cell. A tank with a higher her leakage should be discarded or cemented at its inter-surface joint.

Resolution can be greatly improved using isoelectric focusing. In this technique the support gel maintains a pH gradient. As a protein migrates down the gel, it reaches a pH that is equal to its isoelectric point. At this pH the protein is neutral and no longer migrates (i.e. it is focused into a sharp band on the gel).

21.2.2 Regulated Power Supply

Regulated power supply allows to select either constant voltage (0–250 V, 0–500 V or 0–1,000 V) or constant current (0–20 mA, 0–100 mA or 500 mA range). The supplies are provided with two metres, one for indicating current and other for voltage. The polarity reversal facility and overload

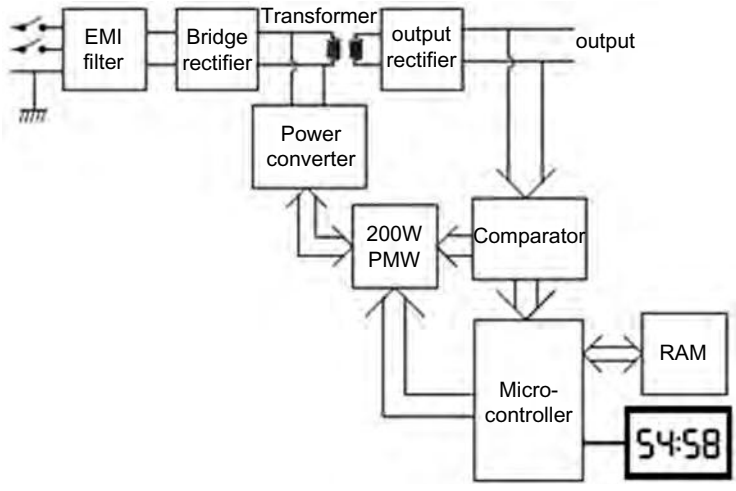


Figure 21.2 *Functional description of electrophoresis power supply*

protection is provided in the power supplies. The ripple content in the power supply should be less than 0.1%.

Power supplies designed specifically for gel electrophoresis offer extensive programming capabilities, including pre-set and customer-preferred settings. They provide constant voltage, constant current or power setting facilities. Most of them are fully protected offering open circuit detection, short-circuit detection, thermal shut down, over-voltage, current wattage detect.

Figure 21.2 is a schematic diagram of a typical 200 V, 200 W, electrophoresis power supply. This is a micro-controller-based fly back converter switched mode power supply. AC power is applied to the bridge rectifier circuit through switch with electromagnetic interference (EMI) filter. The bridge rectifier rectifies the AC power to produce around 320 V DC. The DC is then applied to a power modulator. A pulse-width-modulated (PWM) signal converts the DC to a higher frequency (30 KHz). The high frequency voltage is stepped down for the low voltage DC. It is rectified and filtered to DC at 5 V. The output of the 5 V is sampled, compared to a reference and used to control the PWM generator to provide feedback to control the voltages. Various other controls for over-voltage, current limiting and temperature control are provided through the micro-controller.

High voltage electrophoresis is a rapid and highly effective technique for the separation of a wide range of compounds, especially those with low molecular weights. The high voltages normally employed are 10,000 V at 100 mA or 5,000 V at 200 mA.

21.3 DENSITOMETERS

A number of scanners (densitometers) are commercially available for making direct photometric measurements on stained electrophoretic strips. Then these instruments, the stained paper is made to move across the light source, the recorder will trace curves on the graph paper according to the concentrations of the analyte in the samples. Some instruments include attachments like electronic integrators for measuring peak height, in which the results are available directly in concentration.

Densitometers are available to work in either transmittance or reflectance mode (Greenhalgh, 1983). However, identical samples will give different results when scanned in two modes. Again, the ability of the densitometer to work in fluorescence mode is a desirable optional attachment. It may be noted that the ability of a densitometer to resolve two lines a set distance apart is entirely dependent on the quality of the optical system. The most consistent results are obtained by using a slit width approximating to the width of the bonds on the electrophoretic support material. The light source for most densitometers is a tungsten filament lamp and some densitometers depend on this lamp down to the near UV.

21.3.1 Spectrodensitometers

The introduction of agar gel and subsequently of polyacrilamide gel electrophoresis for fractionation of ribonucleic acids and the need of a direct ultraviolet densitometry of the gels has stimulated the development of electrophoretogram scanning techniques. A double-beam recording spectrodensitometer is generally used for measuring the optical density of dry agar electrophoretograms.

A double-beam ratio type scanning spectrodensitometer incorporates a high intensity continuous xenon light source and a low stray-light quartz prism monochromator. Thin layers, as well as gels and paper strips of all types, and preparative forms can be rapidly and very accurately scanned for quantitative determinations.

The instrument also facilitates liquid samples in static or flow cells for a great number of investigations in the field of column chromatography, spectrodensitometry and kinetic and denaturation research. Figure 21.3 shows the optical system of the instrument. A 150 W xenon high pressure short arc lamp or 200 W xenon-mercury lamp (1) is fitted in a special air-cooled housing, which is equipped with high transmission suprasil optics (2) and a front surface spherical reflector (3) for greater efficiency operation. The reflector is adjustable, so that the primary light image and reflected image can be aligned to be coincident.

A light tube attached to the lamp housing focuses the light beam onto the entrance slit of the monochromator. A slot located just past the focusing adjustment is provided for the insertion of an interference or other type of filter, and a special split ring, which serves to cover this slot while not in use. The light tube also contains the beam diverging optics, all necessary beam aperture and balance adjustments and a deflector mirror to divert the beams down to the exit post (8), adjustment which includes a calibrated slit-width control (9). The details of the optical components of the monochromator are given in (17). The light beam emanating from the source strikes the TLC plate or other media. The media scatters the light, making it as a pair of secondary light sources. These light beams then pass through protective quartz lenses (10) and are maintained separately by a baffle between the lenses which is also in close proximity to the plate. These lenses collect the light and focus it onto the individual photomultiplier tubes (11). The lenses are located in such a way that only the incident beams or the scattered light resulting from the incident beams are directed onto the photomultiplier tubes. The photomultipliers are equipped with individual dynode chain divider circuits and are mounted in a sturdy cast aluminium housing inside the main instrument. They are arranged, so that both angular and lateral adjustments can be made at the factory.

The photomultiplier tubes are supplied with a highly regulated, low ripple power supply. The gain control is adjusted by varying the amount of high voltage being applied to both photomultiplier tubes. The balance control changes the voltage on the signal photomultiplier, with respect

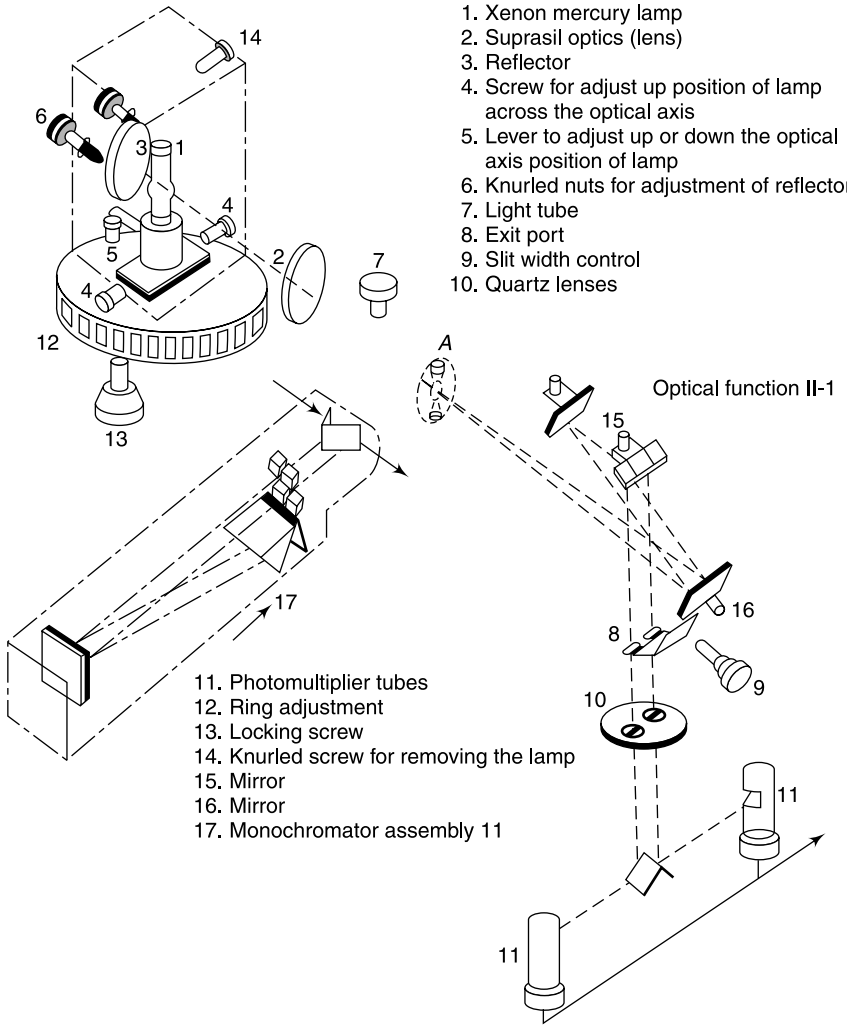


Figure 21.3 *Optical arrangement in a ratio type scanning spectrodensitometer (Courtesy: M/s Schoeffel Instrument Corp., USA)*

to the voltage applied to the reference photomultiplier. The balance control does not affect the reference signal. The amplifiers comprise of high gain amplifiers, which act as current-to-voltage converters. They are adjusted to have equal output from the sample channel and reference channel, with a zero balance setting on the controls. This means the ratio would be 1 (i.e. 100% T or zero optical density). Changes in the sample channel will affect this ratio and can, therefore, be measured. The ratio system compensates for any changes in the light due both to the lamp power supply and characteristics of the lamp itself. In case optical density is to be obtained, the log function of the ratio of the two input signals coming from reference and sample photomultiplier is obtained by using a log amplifier. This gives a linear output for recording and integration.

The monochromator used is calibrated in nm 200–700 in 10 nm increments, linearly. It incorporates high transmission quartz optics in a modified-Littrow mount. The wavelength cam drive can linearise non-linear dispersion over the spectral range. The monochromator can be fitted with a motor drive assembly for spectral scanning. The scanning is done at a speed of 200 nm/min. The wavelength drive should have zero backlash design. A slip clutch permits manual override of the motor drive, permitting any selected wavelength to be set manually. The drive will automatically take over upon release of the manual wavelength control.

21.3.2 Microprocessor-based Densitometer

The inclusion of microprocessors in the densitometers has enabled to perform certain operations while carrying out two-dimensional density scanning of electrophoretic strips. It enables to obtain a graphical representation of the density profile, with trough detection marks, peak positions, peak heights, peak integrals, relative percentages and total integral. Chopra (1984) illustrates the use of a microprocessor-based scanning densitometer, whose block diagram is shown in Figure 21.4.

The apparatus essentially consists of a large flat-bed specimen table which can accommodate a wide variety of samples, as well as providing straightforward sample acquisition. The specimen table is driven in both X and Y axes by incremented stepper motors under microprocessor control, with an incremental step size of 50 μm. Automatic sample acquisition is accomplished by software-based microprocessor control. The densitometer accepts all standard media including paper, acetate, agarose and polyacrylamide gel strips. The scan pattern is fully programmable for efficient processing of all media. Linear scans of up to 200 mm can be carried out for single and

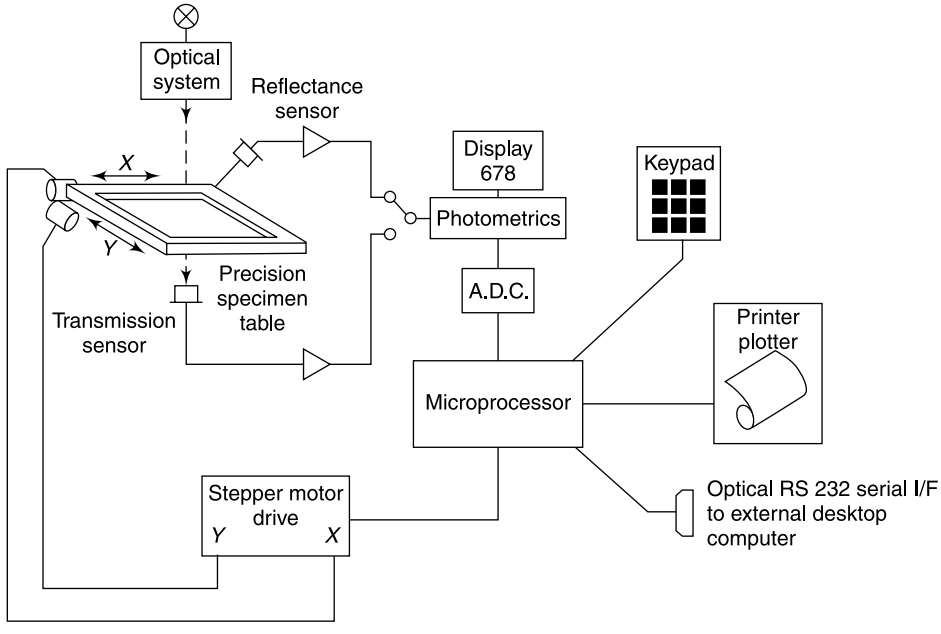


Figure 21.4 *Microprocessor-based scanning densitometer (after Chopra, 1984)*

multi-band samples. For analysis of TLC and HPTLC and multiple-spot traces, raster scans with programmable facilities for raster size reading interval and line interval up to 200 mm. Different detection modes like visible and UV transmission and reflection. In fluorescence and quench fluorescence are used. The scanning aperture is selected by means of interchangeable aperture holders and the scanning wavelength is selected by means of interchargeable filters. The filters may be located for either pre-sample or post-sample filtering. The measurement range is 0–3 AU in the transmission mode and 0–2 AU in the reflectance mode. A background adjustment is provided in the equipment.

The scanning mode and scan parameters can be set up by the user via a push-button control panel, which is interfaced to the microprocessor control and data analysis. The system can be interfaced to an external computer via an RS-232C interface. After scanning, the printer/plotter provides fully annotated graphical hard copy results of each scan. Migration in any electrolyte is influenced by several factors, such as (i) size and shape of the particle, (ii) concentration, (iii) pH, (iv) temperature and viscosity of the electrolyte, (v) intensity and distribution of the electric field and above all (vi) the technique used. The energy in the field tends to produce temperature differences in electrolyte which in turn create mild convection currents. In electrophoresis experiments, these currents will disturb the orderly movement of the particles unless they are controlled.

21.4 CAPILLARY ELECTROPHORESIS

The capillary electrophoresis (CE) is a high-resolution analytical separation technique. The separation of ions is based on the electrophoretic mobility of ions under an applied voltage. The mobility or the migration of ions depends on factors such as charge of the ion, the viscosity, and the size of atom or its radius.

The electrophoresis involves the migration of ions and the opposite charged ions are attracted towards to the oppositely charged electrodes. Thus the movement of oppositely charged ions towards electrodes is the basic phenomena of CE techniques (Petre, 2007).

The rate of migration of particles is proportional to the applied electric field. So the mobility of ions increases with increasing the strength of field. Only the ions migrate while the neutral species remain unaffected. The smaller ions have high migration rate due to their smaller size and so they move with less friction.

The CE is widely used due to the following advantage.

- It is a high-resolution non-chromatography separation technique.
- It has low operating cost and small amount of sample is required.
- The CE is easy method and can be used for small ions to macromolecules like proteins, peptides, chiral compounds and nucleic acid.

One of the major drawbacks of gel electrophoresis is the speed of analysis. Speed could only be improved by increasing the electric current of the system. However, with increase in current a large amount of heat would be generated and an efficient cooling system would be required.

The development of CE has solved the heating problem. Silica fused capillaries ranging from 0.150 to 0.375 mm in outer diameter efficiently dissipate the heat that is produced. Increasing the electric fields produces very efficient separations and reduces separation times.

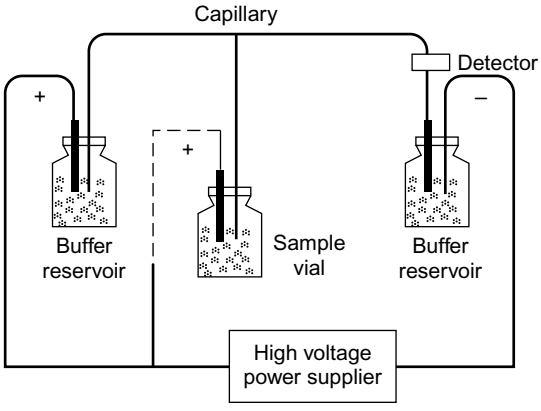


Figure 21.5 *Basic set up of a capillary electrophoresis apparatus(Xu, 1996).*

In a CE separation, a very small amount of sample (0.1–10 nL) is required. The sample solution is injected at one end and an electric field of 100–700 volts/centimetre is applied across the capillary. Proteins in the solution migrate through the capillary due to the applied electric field (electrophoresis). Differing electrophoretic mobility drive each of the components into discrete bands. Quantitative detectors such as fluorescence and absorbance detectors can be used to identify and quantify the proteins in the solution.

Figure 21.5 shows the basic set of a CE apparatus. It consists of a high-voltage power supply (0–30 kV), a fused-silica (Sio_2) capillary, two buffer reservoirs, two electrodes and an on-column detector. Sample injection is done by temporarily replacing one of the buffer reservoirs with a sample vial. A specific amount of sample is introduced by controlling either the injection voltage or the injection pressure.

Capillaries are typically of 50 µm inner diameter and 0.5–1 m in length. CE uses an electromotive force, rather than a pump, to dine the mobile phase through the capillary. Due to electro-osmotic flow, all sample components migrate towards the negative electrode. A small volume of sample (10 nL) is injected at the positive end of the capillary and the separated components are detected near the negative end of the capillary. CE detection is similar to detectors in HPLC, and includes absorbance, fluorescence, electrochemical, conductivity measurements and mass spectrometry. Campana et al. (1997) describe that when combined with CE, chemiluminescence offers excellent analytical sensitivity and selectivity.

UV/Vis detectors are among the most popular. Because absorbance is directly proportional to path length, the capillary tubing's small diameter leads to signals that are smaller than those obtained in HPLC. Several approaches have been used to increase the path length, including a Z-shaped sample cell and multiple reflections. Detection limits are about 10^{-7} M.

21.4.1 Capillary Electrophoresis Instrumentation

One key feature of CE is the overall simplicity of the instrumentation.

A schematic diagram of a generic CE system is shown in Figure 21.6. Briefly, the ends of a narrow-bore, fused-silica capillary are placed in buffer reservoirs. The content of the reservoirs is identical to that within the capillary. The reservoirs also contain the electrodes used to make electrical contact between the high-voltage power supply and capillary. Sample is loaded onto the capillary by replacing one of the reservoirs (usually at the anode) with a sample reservoir and applying either an electric field or an external pressure. After replacing the buffer reservoir, the electric field is applied and the separation performed. Optical detection can be made at the opposite end, directly through the capillary wall.

Capillary: Fused silica capillaries with internal diameters ranging from 10- to 200-µm with a range of outer diameters are generally used, however, 25- to 75-µm id and 350- to 400-µm od are typical.

From an analysis time perspective, capillaries as short as possible should be used. Effective lengths range from as short as 10 cm for gel-filled capillaries and as long as 80–100 cm for complex-sample separations. Most commonly, 50- to 75-cm effective lengths are employed. Ideally the effective length should be as large a percentage of the total length as possible in order to be able to apply very high electric fields and to decrease the time necessary for capillary conditioning, fraction collection, and so on. The total length is generally 5–15 cm longer, depending on the dimensions of the instrument. Similar to GC columns, the capillaries are coated with a protective layer of polyimide to make them strong and easy to handle. For detection, an optical window can easily be placed in the capillary by removal of a small section of the protective polyimide coating.

Capillary thermostating: Effective control of capillary temperature is important for reproducible operation. Temperature regulation to ±0.1°C is beneficial due to the strong viscosity dependence of sample injection and migration time. Further, the system should isolate the capillary from changes in ambient temperature. The two approaches generally used are to bath the capillary in a high-velocity air stream or in a liquid. While liquid thermostating is theoretically more efficient, forced air thermostating at # 10 m/s air velocity is usually sufficient for the quantity of heat generated in CE.

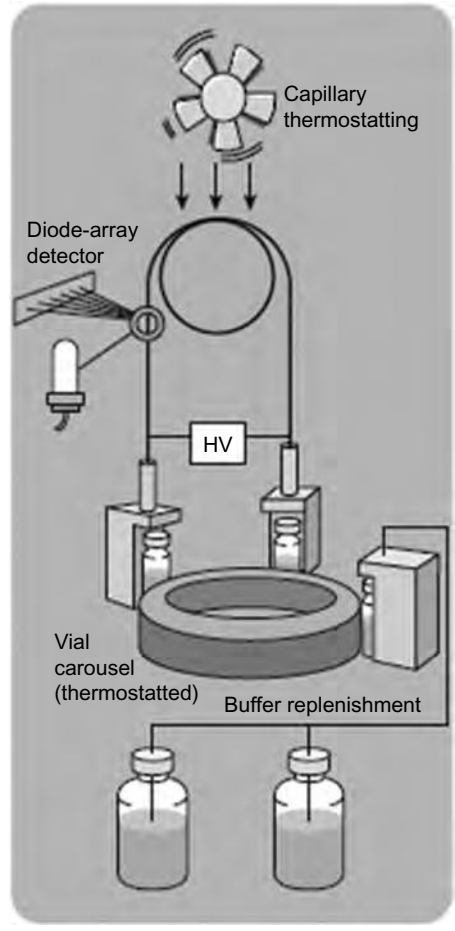


Figure 21.6 *Schematic of CE instrumentation (Courtesy: M/s Agilent)*

High voltage power supply: In CE a DC power supply is used to apply up to about 30 kV and current levels of 200–300 mA. Stable regulation of the voltage (± 0.1 %) is required to maintain high migration time reproducibility.

Another power supply feature is the ability to run voltage, current or power gradients (also called field programming) during an analysis. Field programming can be used to ramp the voltage at the beginning of an analysis to avoid rapid heating, thermal expansion of buffer and expulsion of sample from the capillary. Field programming is also particularly useful for decreasing the analysis time of complex samples and is often necessary for fraction collection.

Detectors: UV-Visible absorption is the most widely used detection method primarily due to its nearly universal detection nature. With fused-silica capillaries, detection below 200 nm up through the visible spectrum can be used.

Figure 21.7 *Capillary electrophoresis system (Courtesy: M/s Agilent)*

Detector design is critical due to the short optical path length. The optical beam should be tightly focused directly into the capillary to obtain maximum throughput at the slit and to minimise stray light reaching the detector. These aspects are important to both sensitivity and linear detection range.

Diode-array detection (DAD) is an alternative to single- or multiple-wavelength detection. Instrumentally a DAD consists of an achromatic lens system to focus light into the capillary. The beam is then dispersed by a diffraction grating and falls on the photodiode array. An array consists of numerous diodes (e.g. 211), each of which is dedicated to measuring a narrow-band spectrum. With a diode array, a whole wavelength range can be selected (e.g. from 190 to 600 nm with a bandwidth of 400 nm). In a single analysis, all solutes absorbing within this range will be detected.

Figure 21.7 shows a commercial CE system from M/s Agilent. The instrument houses a high-voltage power supply, carousel for auto sampling and fraction collection, injection system, on-capillary high-sensitivity DAD, offline buffer replenishment station, capillary cartridge and is capable of accepting an external gas pressure for performing CEC and CGE.

The Agilent CE system is equipped with a 48-position carousel. The carousel can be thermostated simply by connecting an external water bath to the instrument (10–40°C).

The Agilent CE system provides stable and efficient capillary thermostating through the use of high-velocity recycling air flow (10 m/s) which is thermostatically regulated by a Peltier element. Capillary temperature is one of the many operational parameters which can be optimised in CE. The capillary temperature can be regulated between 10° below ambient (minimum 4°C) up to 60°C. This efficient high-velocity air flow thermostating is capable of ensuring stable temperatures and effective capillary cooling even when operating with high concentration, high power buffers.

The Agilent CE system has a power supply capable of applying voltages over the range from –30 kV to +30 kV. The high-voltage power supply is the heart of any CE instrument and as such it must supply a stable voltage during the course of an analytical run. The polarity of the applied voltage is fully controlled by software providing a true positive or negative polarity with the outlet electrode held at ground potential. The software control enables the construction of voltage, current or power gradients, and the application of voltage ramps which is of great importance when analysing heat sensitive analytes in low conductivity solutions.

The Agilent CE system is equipped with a high-sensitivity DAD. In CE the capillary itself acts as the detector flow cell. Wavelength calibration is performed automatically on replacing the capillary cassette and can be performed manually at any time. The response time of the detector is sufficient to detect and acquire spectra of high efficiency peaks with peak widths less than 0.02 min.

The Agilent CE ChemStation built-in DAD and personal computer performs a range of tasks. Simple spectra, isoabsorbance plots, 3-D plots, and other evaluation can take place online while analysis is in progress. The Agilent CE Chemstation, which controls the system, enables full spectral information to be retrieved as well as single wavelength and multiple-wavelength detection.

21.5 PARALLEL CAPILLARY ELECTROPHORESIS FOR DNA SEQUENCING

CE is an attractive technique for DNA analysis because the narrow-bore, gel-filled capillaries provide high-speed, high-resolution separations, increased separation efficiency, and automated gel and sample loading (Khetarpal and Mathies, 1999). The throughput of the system can be increased many times by using capillary array electrophoresis for running multiple capillaries in parallel. The instruments based on this design, which run capillaries are being used in genome centres and pharmaceutical companies for DNA sequencing and analysis. The authors describe details of detection instrumentation used for this purpose which are primarily based on scanning or imaging technology.

Figure 21.8 shows a schematic of parallel CE instrumentation for DNA sequencing. Our platform allows you to achieve the perfect balance of speed and resolution by altering capillary length. Longer arrays can take resolution down to 2 bp for fragments under 300 bp in length. Shorter arrays will get through runs in as little as 15 min for certain applications.

The inlet side of the capillary array is arranged in an 8 × 12 format, with electrodes next to each capillary. This allows direct injection via vacuum or voltage from standard 96-well plates. The capillaries are aligned in parallel in the detection window, which is positioned between the LED light source and the detector assembly. The outlets of the capillaries are bundled to a common reservoir, where they are connected to a ground. The capillary arrays are modular and can be exchanged in less than 5 min. Room temperature air is circulated around the capillaries to modulate the temperature during CE separation.

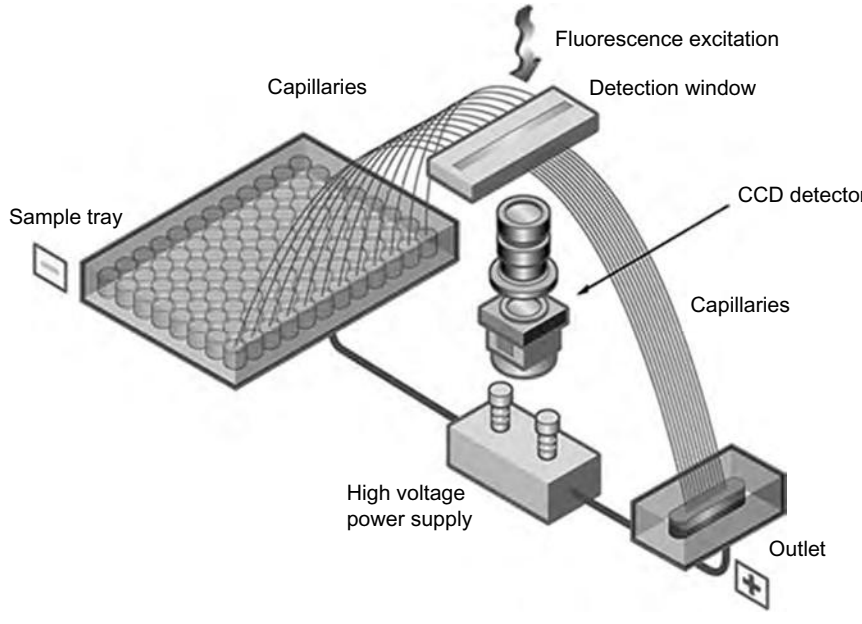


Figure 21.8 *Parallel capillary electrophoresis (CE) for DNA sequencing (Courtesy: M/s Advanced Analytical Technologies, Inc.)*

The optical platform consists of an LED light source (470 nm excitation), camera lens and CCD detector. Fluorescent light from the LED passes through the array window, exciting the intercalating fluorescent dye in the gel matrices. This is captured by the camera lens and spatially imaged onto the CCD detector. Imaging is constant, so you get real time, online detection of separations.

The power supply provides appropriate voltage for electrokinetic sample injection and electrophoresis. The recommended operating limits are +/–16 kV, 3 mA total current (30 μA per capillary). A syringe pump provides controlled pressure or vacuum to the outlet reservoir of the capillary array. Vacuum injection can be especially useful for eliminating the effects of salt on nucleic acid uptake. Built-in software automates capillary conditioning, sample injection, electrophoresis separation and data processing. You can visualise results in your preferred format and share reports easily.

21.6 MICRO-ELECTROPHORESIS

The electrophoresis cell used in normal applications requires a relatively large volume of solution about 20 ml, which might mean as much as 100–200 mg of material, if accurate measurements are desired. Such large material requirements are not easily arrangeable in some biochemical investigations. This requirement has led to the development of methods for micro-electrophoresis, which have the advantage of reducing the volume and concentration of the solution required.

Use of sensitive optical methods can also aid in achieving a reduction in the volume of solution required. One of the methods used for the purpose is Jamin interferometric system. The method is based on the formation of interference fringes, which are produced by the interference of two beams, one of which has passed through some homogeneous reference medium and the other through the cell under investigation. In the Jamin interferometric system, an image of the cell is obtained, which is crossed by fringes. The variation in spacing of the hinges represents the variation in concentration along the cell. The fringes will be crowded at a boundary and in effect, each component of a mixture will make itself seen by a corresponding group of fringes. A simple counting of the fringes gives an approximation of relative concentrations.

A slight variation in the measurement technique using an interferometer is incorporated in the micro-electrophoresis apparatus shown in Figure 21.9.

In this method illustrated in Figure 21.10, the light from the source (A) is split by the first mirror (B) into two coherent light beams, pass through the measuring and reference channels of the cell (C). The two light beams are in the rotatable *mirror* (D). Whenever the two coherent beams encounter variations in optical density in the cell, because of the migration of the proteins into line buffer solution in the measuring channel and the presence of buffer in the reference channel, they pass through optically unequal paths. This difference in optical path length can be compensated for by rotating the mirror (D). Correct compensation can be checked by means of a spectrum and an index line in the eye piece. The mirror is rotated by hand through a micrometer screw, whose movement can be read off and recorded. Rotation of the micro-meter screw corresponds directly to a difference in the concentration of the solutions in the two channels. The channels are measured out at intervals of 0.1 mm from line top downwards, along the protein migration distance.

Figure 21.9 *Micro-electrophoretic apparatus (Courtesy: M/s Rank Brothers Ltd. UK)*

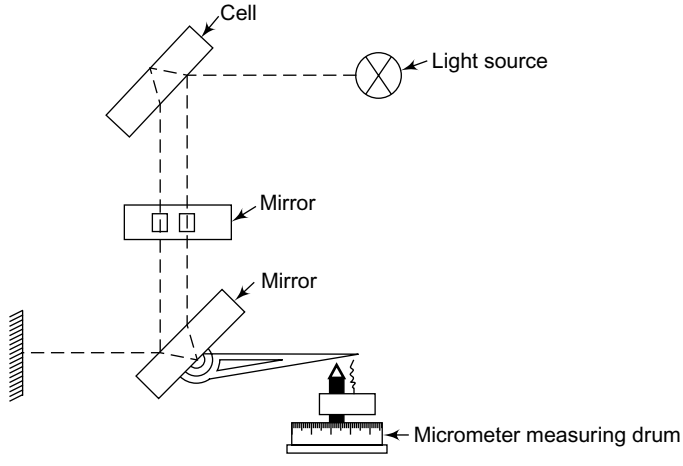


Figure 21.10 *Interferometric method for measurement of density on electrophoretic strips.*

Micro-electrophoresis apparatus can be used to determine the electrophoretic mobility of a wide range of suspended particles. The apparatus uses conventional quartz-iodine illumination unit (which can make particles visible down to about 0.2 μm). For lowering the limit of particle visibility, a laser illuminator (3 mW He-Ne laser) is preferred, which can make particles as small as 0.09 μm visible. The apparatus uses a binocular microscope or closed circuit television system to aid the operator.

22

ELECTROCHEMICAL INSTRUMENTS

22.1 ELECTROCHEMICAL METHODS FOR ANALYSIS

Electrochemical methods are characterised by a high sensitivity, selectivity and accuracy. Analytical sensitivity attainable even exceeds the 10^{-10} molar level and analyses at the sub-nanogram range are possible with these methods. Electrochemical methods have been extensively developed and each basic electrical parameter, namely current, resistance and voltage, has been utilised alone or in combination for analytical purposes. Besides direct analysis, electrical measurements are excellent indicators in all areas of titrimetry. Reagents are added volumetrically by automatic or manual means, to an end point that is conveniently detected electrometrically. Sometimes, even the reagent can be coulometrically generated within the sample to obtain a complete electrochemical system.

22.2 ELECTROCHEMICAL CELL

The devices in which chemical energy released during a chemical reaction is converted into electrical energy are called Electrochemical Cells.

A typical arrangement of an electrochemical cell is shown in Figure 22.1. It consists of two beakers, one of which contains $CuSO_4$ solution and the other one contains $AgNO_3$ solution. A copper rod is dipped in the $CuSO_4$ solution and a silver rod is dipped in the $AgNO_3$ solution. These metallic rods are called Electrodes. The solutions in the two beakers are connected by an inverted U tube containing a saturated solution of KNO_3, which does not undergo any chemical change during the process. This U tube is called a Salt Bridge. The purpose of the salt bridge is to isolate the contents of two halves of the cell while maintaining electrical contact between them. Isolation is necessary to prevent direct reaction between silver ions and copper electrodes. A volt metre is attached between the two electrodes to measure the potential difference between the two electrodes.

There are two types of electrochemical cells: galvanic (voltaic) and electrolytic. A galvanic cell consists of two electrodes and one or more solutions (two half-cells). In these cells, a chemical reaction involving an oxidation at one electrode and a reduction at the other electrode occurs. An electrolytic cell is one in which the electrical energy is supplied from an external source, the cell through which the current is forced to flow. In an electrochemical cell, the following definitions are generally used:

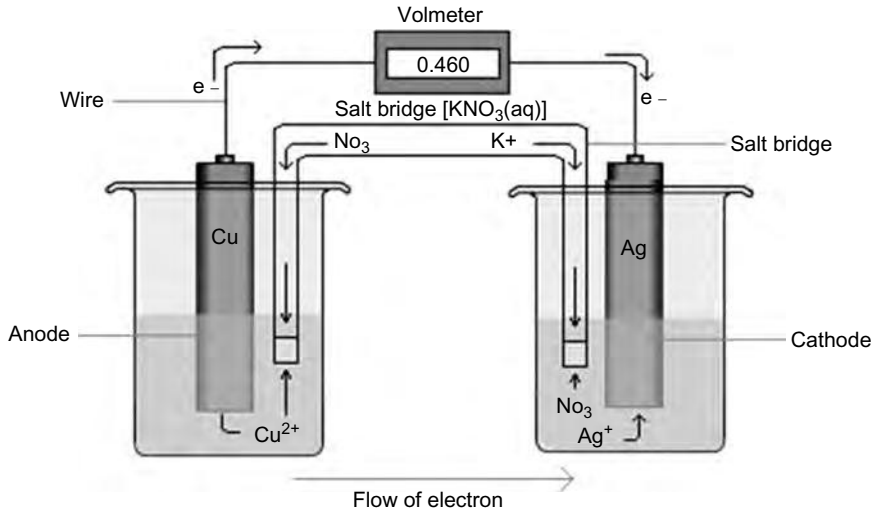


Figure 22.1 *Schematic of an electrochemical cell*

- *Anode*: The electrode where electrons are released or oxidation occurs is called the Anode. It is the negative terminal of the cell.
- *Cathode*: The electrode where electrons are accepted or reduction occurs is called Cathode. It is the positive terminal of the cell.
- *Half cells*: The two containers involving oxidation and reduction half reactions are called half cells.

22.2.1 Types of Electrodes

There are a wide variety of electrodes which are used for different electrochemical techniques. Basically, they can be classified as working, auxiliary and reference electrodes.

22.2.1.1 Working electrode

The working electrode is that electrode at which the desired reaction occurs. Platinum disc, carbon paste and dropping mercury electrode are examples of working electrodes. The primary reaction takes place at the surface and the change in current or potential resulting from this reaction is measured.

Working electrodes are usually small and are manufactured in a solvent-resistant CTFE plastic body (7.5 cm length × 6 mm OD) which is embedded with highly polished disks of various electrode materials (glassy carbon, gold, platinum, palladium, nickel, or silver). The electrode disk diameter is either 1.6 or 3.0 mm, depending on the material chosen. The working electrodes used in electrochemical analytical techniques take a variety of shapes and forms. Figure 22.2 shows typical working electrodes for voltammetry.

A metal pin (0.75 cm) mounted in the plastic body of the electrode is used to connect these electrodes to an appropriate instrument using either an alligator clip or a 0.060" gold-plated connector. Each electrode comes with a silicon O-ring which fits snugly on the plastic body. By rolling the O-ring to different positions, you can change the depth of the electrode mounted in a voltammetry cell.

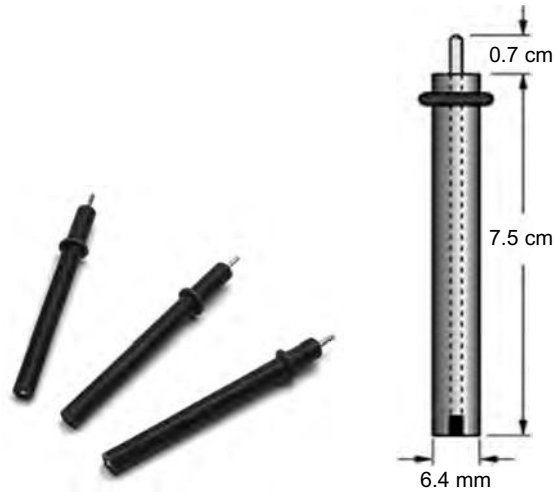


Figure 22.2 *Working electrodes for voltammetry (Courtesy: M/s BASi USA)*

Mercury working electrodes have been used in voltammetry in various forms. The dropping mercury electrode (DME) is the most common. The mercury pool offers about ten-fold increase in sensitivity over the DME, but requires the use of a large pool (2 cm diameter) to minimise changes in curvature and area with change in applied potential due to interfacial tension. The mercury plated electrode provides a very high resolution and operation over extended negative potentials. Nickel, silver and platinum are most often used as the plating surface.

The solid electrode is usually in the form of a wire, disc or wire mesh. Much work has been done with platinum than with my other solid electrode. However, a platinum surface is subject to formation of oxide films on its surface by either chemical or electrolytic oxidation. A gold surface is less susceptible than platinum, to attack by some chemical oxidising agents. Gold is probably a better electrode material than platinum for general utility.

The carbon paste electrode is one of the most practical of solid electrodes and for routine applications. The carbon paste surface is superior to gold or platinum. Vitreous carbon is highly resistant to chemical attack and is relatively insensitive to changes in pH. It has however, higher residual current than the carbon paste electrode.

22.2.1.2 Reference electrode

This reference electrode provides a stable potential which may be taken as reference. Most literature states the potential measurements in relation to a saturated calomel electrode (SCE), which is a convenient electrode to use. Another common reference electrode is the silver-silver chloride electrode. Reference to hydrogen electrode, the difference in potential between a calomel (–0.244V) and silver-silver chloride (–0.200V) electrode is 44 mV.

Depending upon the type of cell, the glass-bodied reference electrodes are available in two styles: The 7.5 cm long and 3 cm long. All electrodes have a 6 mm OD glass body, and use a porous junction (frit) made from either ceramic or CoralPor (porous glass). Both electrodes can be used to suspend three reference electrodes in the salt solution while protecting the

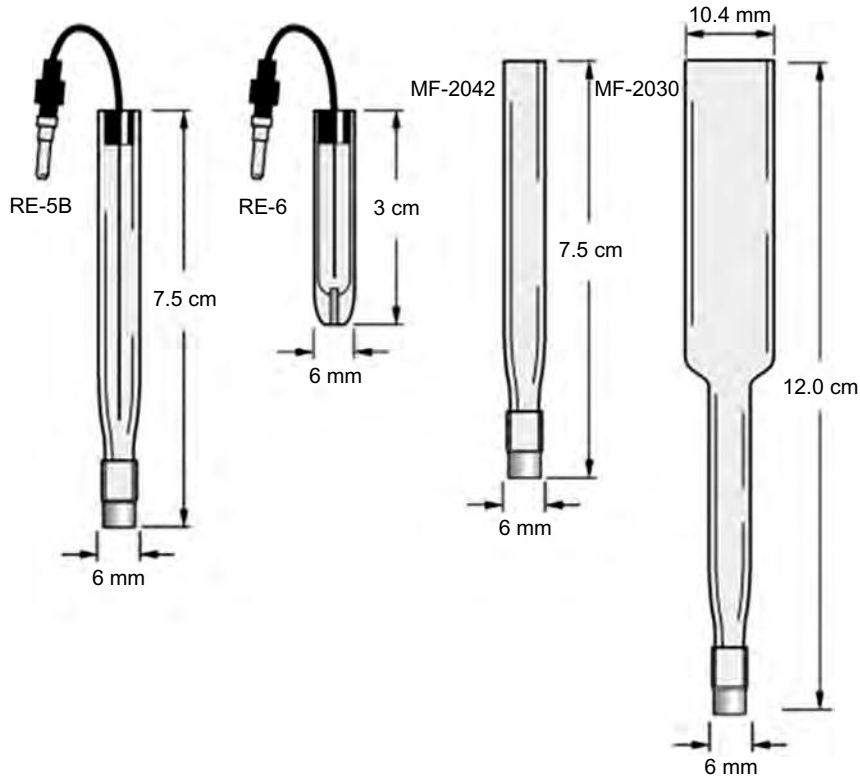


Figure 22.3 *Reference electrodes for voltametery (Courtesy: M/s BASi, USA)*

connecting pins and wires from corrosion. Between uses, reference electrodes should be stored with junction tips in a MF-5275 vial filled with 3M NaCl. Typical reference electrodes are shown in Figure 22.3.

22.2.1.3 Auxiliary electrode

The electrode required for completing the electrolysis cell is called the auxiliary electrode. It performs the reverse reaction of that, which takes place at the working electrode. Usually, a platinum disc may be used as an auxiliary electrode. A frit separates the auxiliary compartment solution and sample compartment containing the working electrode.

Platinum wire auxiliary electrodes (0.5 mm diameter) are available in a variety of lengths (Figure 22.4). Each platinum wire terminates

Figure 22.4 *Platinum wire auxiliary electrode (Courtesy: M/s BASi USA)*

in a gold-plated brass connector. Electrical connection to this pin can be made using either an alligator clip or a 0.040" gold-plated connector (MF-2000).

22.3 POTENTIOSTATS

The heart of an electrochemical analysis system is the potentiostat. A potentiostat is a device which controls the voltage between two electrodes, a working electrode and a reference electrode, to a constant value. Reference electrodes are electrodes, which maintain a constant voltage referred to the potential of the hydrogen electrode. A silver wire, covered with a silver chloride layer, dipping in a chloride solution, is a simple reference electrode. However, as soon as a current passes this electrode, it is polarised. That means, its potential varies with current, and so it is not possible to mountain a constant potential difference between a working electrode and reference. Therefore, another electrode, called the 'auxiliary electrode' is introduced into the system. A typical low-volume electrochemical cell used as a potentiostat with all the electrodes in place is shown in Figure 22.5.

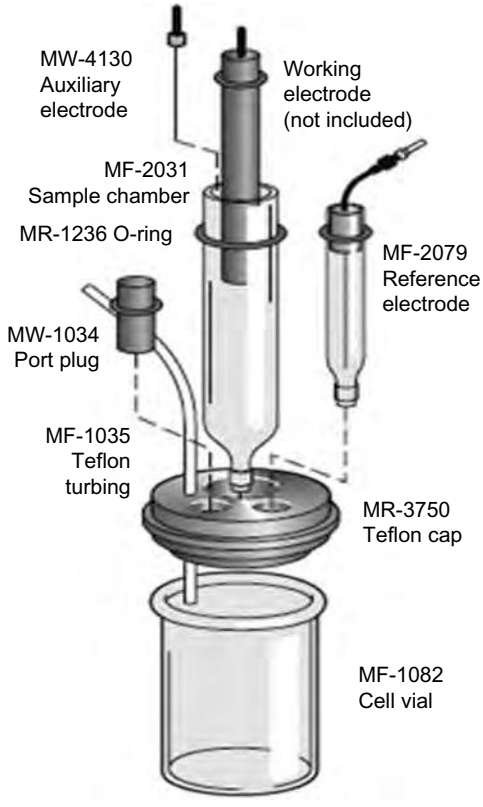


Figure 22.5 *Low-volume cell for electrochemical techniques (Courtesy: M/s BASi USA)*

The potentiostat has two functions:

- To measure the potential difference between working electrode and reference electrode without polarising the reference electrode and
- To compare the potential difference to a present voltage and force a current through the auxiliary electrode towards the working electrode in order to counteract the difference between pre-set voltage and existing working electrode potential.

This can be realised by the arrangement shown in Figure 22.6. Here, the difference voltage between working electrode and reference electrode is amplified and inverted by the operational amplifier. A matching current is fed to the auxiliary electrode. The control circuit is closed by the cell, where the current passes the electrolyte from the auxiliary electrode to the working electrode. This polarises the working electrode exactly so that the difference between the reference electrode input and the working electrode input is set to zero. By doing so, the potential of the working electrode can be kept exactly as the potential of the reference electrode.

A simplified schematic of a typical Potentiostat is shown in Figure 22.7. The electronic circuit

consists of four blocks. The amplifier X1 is a unity gain differential amplifier. The output voltage of this circuit is the difference between its two inputs. The blocks labelled Voltage and Current are the voltage and current signals that are sent to the system A/D converter for digitisation.

The electrometer circuit measures the voltage difference between the reference and working electrodes. Its output has two major functions: it is the feedback signal in the potentiostat circuit and it is the signal that is measured whenever the cell voltage is needed.

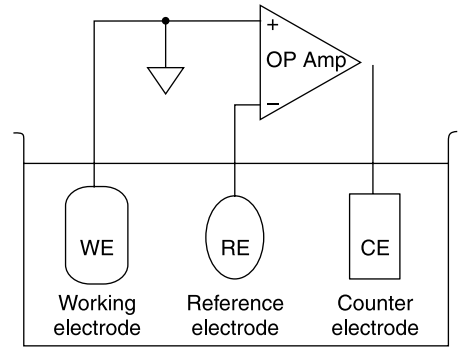


Figure 22.6 *Using an amplifier as potentiostat (adapted from Potentiostat: Bank electronic, GmbH)*

An ideal electrometer has zero input current and an infinite input impedance. Current flow through the reference electrode can change its potential. In practice, all modern electrometers have input currents close enough to zero that this effect can usually be ignored. Two important electrometer characteristics are its bandwidth and its input capacitance.

The electrometer bandwidth characterises the AC frequencies the electrometer can measure when it is driven from a low impedance source. The electrometer bandwidth must be higher than the bandwidth of the other electronic components in the potentiostat.

The electrometer input capacitance and the reference electrode resistance form an RC filter. If this filter's time constant is too large, it can limit the effective bandwidth of the electrometer and cause system instabilities. Smaller input capacitance translates into more stable operation and greater tolerance for high impedance reference electrode.

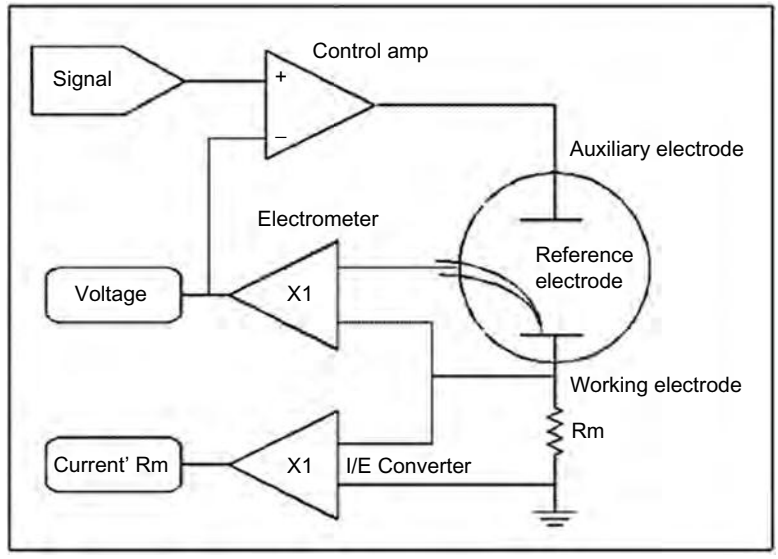


Figure 22.7 *Schematic diagram of a typical potentiostat (Courtesy: M/s Gamry Instruments)*

The current-to-voltage (I/E) converter measures the cell current. It forces the cell current to flow through a current measurement resistor, Rm. The voltage drop across Rm is a measure of the cell current. A number of different Rm resistors can be switched into the I/E circuit under computer control. This allows measurement of widely varying currents, with each current measured on using an appropriate resistor. An 'I/E autoranging' algorithm is often used to select the appropriate resistor values.

The control amplifier is a servo amplifier. It compares the measured cell voltage with the desired voltage and drives current into the cell to force the voltages to be the same. The control amplifier has a limited output capability. Usually the control amplifier provides 20 V output at 300 mA.

The signal circuit is a computer-controlled voltage source. It is generally the output of a digital-to-analog (D/A) converter that converts computer generated numbers into voltages. Proper choice of number sequences allows the computer to generate constant voltages, voltage ramps and even sine waves at the signal circuit output. When a D/A converter is used to generate a waveform such as a sine wave or a ramp, the waveform is a digital approximation of the equivalent analog waveform.

22.4 TYPES OF ELECTROCHEMICAL METHODS

There are five main types of electrochemical methods:

- *Potentiometry*: This measures electrical potential developed by an electrode in an electrolyte solution at zero current flow. The concentration of ions in the solution is calculated by using Nernst Equation.
- *Voltammetry*: This determines concentration of ion in solutions from current flow as a function of voltage when polarisation (depletion of concentration caused by electrolysis of ion occurs around the electrode. When DME is used, the method is called 'Polarography'.
- *Coulometry*: This measures the charge (Q) over a fixed time and controlled voltage (i.e. it involves electrolysis of a solution and use of Faraday's Law relating quantity of electrical charge to amount of chemical change). Faraday's Law states that it takes 9.65×10^4 Coulombs of electrical charge to cause electrolysis of 1 mole of a univalent electrolyte species.
- *Amperometry*: This measures the concentration of an electroactive substance by applying a fixed voltage across the electrodes and then measuring the current passing through the cell.
- *Conductometry*: This measures conductance for controlled concentration.

22.5 POTENTIOMETERS

Potentiometry consists in the measurement of the difference in potential between an indicating electrode and a reference electrode immersed in a solution of the ions to be determined. The potential *E (half-cell* potential) of any electrode is given by the generalised form of the Nernst equation.

$$E = E^0 + RT/nF \log a_{ox}/a_{red}$$

where E^0 = reduction potential of the half-cell under standard conditions.
E = the potential of the half-cell
R = a constant, 8.314 J/deg.
T = absolute temperature
F = Faraday Number (96,494 C)
n = number of electrons transferred in the electrode.

a_{ox} and a_{red} are the activities of the oxidised and reduced forms, respectively, of the electrode action. Substituting concentrations for activities and various other constants and assuming the temperature to be 25°C, the Nernst equation becomes

$$E = E^0 + 0.0591/n \log (\text{ox})/(\text{red})$$

where [ox] is the concentration of the oxidised form of the ion and [red] is the concentration of the reduced form of the ion.

From this equation, it is obvious that measurement of the potential developed by a half-cell serves as a measure of the concentration of the components in a solution. In practice, the half-cell to be measured is connected to a standard or reference half-cell to form a complete cell. The method of potentiometry is used to measure the voltage of the complete cell and the potential of the complete cell is calculated from the relationship

$$E(\text{half-cell}) - E(\text{standard half-cell}) = \text{EMF(observed)}$$

The EMF of the standard half-cell being known, the unknown potential can be determined. A potentiometer is used to measure the voltages instead of voltmeter, as it draws negligible current and hence does not produce my depolarisation during the measurement.

Although potentiometry is useful in several analytical areas, its most common application lies in the determination of pH. Potentiometric measurements are also applied to the detection of the end point of titrations. Another area where potentiometry is being increasingly applied, is that of the use of ions-selective electrodes for the direct measurement of cations and anions.

22.6 CONDUCTIVITY METERS

The conductivity of an electrolyte is a measure of the ability of the solution to carry electric current. The current through the solution takes place through the movement of electrically charged particles called ions. When a potential difference is applied to two electrodes immersed in the solution, ions are almost instantaneously accelerated towards the electrodes. Since the conductance of a solution of electrolyte is related to the concentration of electrolyte, analytical applications of conductance are possible.

Like a metallic conductor, electrolyte solutions obey Ohm's law. The reciprocal of the resistance R of the electrolytic solution (I/R) is called the conductance. It is expressed in reciprocal ohms or

mhos. The resistance of a solution depends upon the length l, area a and the intrinsic properties of the solution. It can be expressed as,

$$R = \rho l / a$$

where ρ *is* known as specific resistance. Since conductance is the reciprocal of resistance

$$1/R = 1/\rho\ (a/l) = K\ (a/l)$$

The constant K is called the specific conductance. It is expressed in ohm^{-1} cm^{-1}.

The specific conductance of an electrolyte is a function of concentration. As the solution is diluted, the specific conductance will decrease. This is because fewer ions are present to carry the electric current in each cubic centimetre of solution. The ability of individual ions to conduct is usually expressed by a function called the equivalent conductance. The equivalent conductance is the conductance of a hypothetical solution containing one gram equivalent of an electrolyte per cubic centimetre of solution. The equivalent conductance (A) is connected with the specific conductance and concentration (in gm equivalent per 1,000 cm^3) as follows:

$$A = 1{,}000\ K/C, \qquad K = AC/1000.$$

The conductance of an electrolyte between two electrodes, when expressed in terms of equivalent conductance and concentration is given by

$$1/R = K\ (a/l)$$
$$= AC\ \mathrm{a}/1{,}000l$$

The equivalent conductance of a salt is the sum of the equivalent ionic conductances of its ions

$$\lambda = \lambda_+ + \lambda_-$$

The total conductance of a solution at infinite dilution is

$$A_\infty = \Sigma\lambda_+ + \Sigma\lambda_-$$

which shows that at infinite dilution, the migration of ions are theoretically independent of each other and are not affected by other ions in the solution. This is, however, not strictly true and there are very slight differences in ionic conductance of an ion in the presence of various other ions.

Units: The unit of conductivity is Siemens per cm (S/cm). Derived units are µS/cm (one millionth of a S/cm) and mS/cm (one thousandth of a S/cm). S/cm is the same as the older unit mho/cm. Certain high-purity water industries, primarily semiconductor and pharmaceutical, use resistivity instead of conductivity. Resistivity is the reciprocal of conductivity. The units are MΩ cm.

22.6.1 Measurement of Conductance

22.6.1.1 Null method

Conductivity is usually determined by measuring the resistance of a column of solution. This is done by using a Wheatstone bridge, in which the conductivity cell forms one arm of the bridge (Figure 22.8). In order to avoid changes in ionic concentrations due to net chemical reactions at the electrodes, alternating current rather than direct current devices are employed. The choice of frequency is not critical and may be anything between 50 and 10,000 Hz. However, most commonly employed frequency is 1,000 Hz. This AC source may be a low-voltage tapping on the 50 Hz transformer or an oscillator.

When the bridge is balanced, and it is assumed that the conductivity cell behaves as a pure resistance, then the voltage between *B* and *D* is equal to zero.

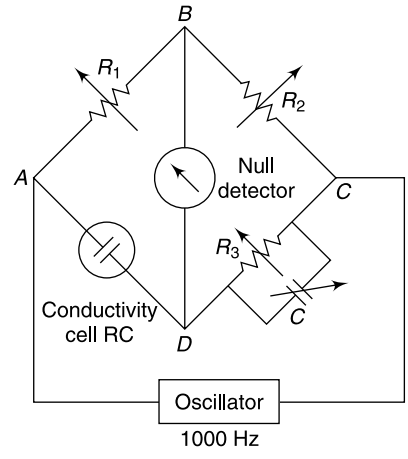


Figure 22.8 *Wheatstone bridge circuit for measurement of conductivity*

$$R_c = R_1/R_2 \times R_3$$

By adjustment of the ratio R_1/R_2, *a* wide range of resistances can be measured. However, whenever possible, this ratio is kept unity. This condition is the most favourable to precise measurements. The arms AB and *BC* represented by resistance R_1 and R_2 are usually in the form of a single calibrated slide wire resistor, with a sliding contact connected to the null detector.

As the conductivity cell contains electrodes separated by a dielectric, an appreciable cell capacitance is invariably present. This capacitance is balanced out by providing a variable capacitor, in parallel with resistance R_3. It is so adjusted that the detector gives a sharply defined balance point.

22.6.1.2 Direct reading method

In practice, direct reading instruments are proffered over the null-balance type instruments. In these instruments, the necessity of converting resistance readings into conductance readings is eliminated. Also, it is much more convenient to work with direct reading instruments.

Figure 22.9 shows a simplified circuit of a typical conductivity metre. It applies an alternating current (I) at an optimal frequency to two active electrodes and measures the potential (V). Both the current and the potential are used to calculate the conductance (I/V). The conductivity metre then uses the conductance and cell constant to display the conductivity.

$$\text{Conductivity} = \text{cell constant} \times \text{conductance}$$

The current source is adjusted so that the measured potential (V) is equal to the reference potential (Er) which is approximately ±200 mV.

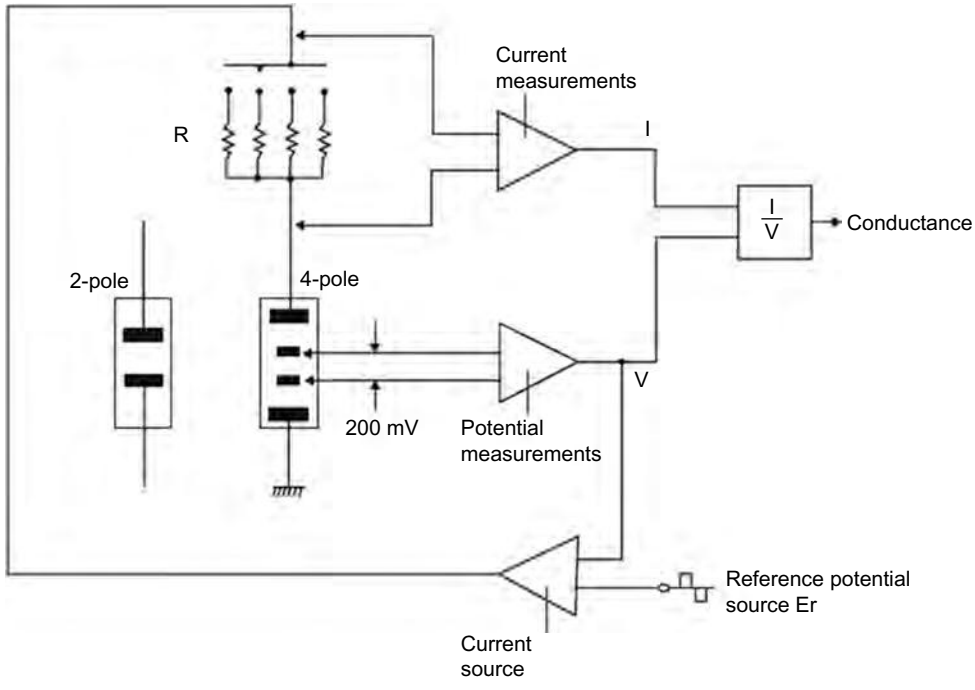


Figure 22.9 *Simplified circuit diagram for measurement of conductivity (Courtesy: M/s Radiometer analytical)*

22.6.2 Conductivity Cells

A conductivity cell comprises of two electrodes, which may be two parallel sheets of platinum fixed in position by sealing the connecting tubes into the sides of the measuring cell. In order to reduce the polarisation effects which produce a large cell capacitance, the effective area of the electrode is greatly increased by coating the electrode with platinum black. This deposit can be obtained by immersing the electrodes in a solution containing 7.5×10^{-2} M chloroplatinic acid and about 8×10^{-4} M lead acetate and applying a direct current, reversing the direction every half-minute. When a black deposit is obtained, the electrodes are washed in water, and the occluded gases are removed by electrolysing in dilute H_2SO_4 for about half an hour with current reversal every minute. Electrodes are washed again and stored in distilled water overnight before using. Also, the electrodes should be stored in distilled water when not in use. This prevents the platinum black from drying out.

Most of the conductivity cells are of such a design that the solution completely surrounds the electrodes. In such cases, the conductance of the cell is given by

$$1/R = \rho\,[a/l], \qquad \rho = 1/R\,(l/a)$$

The term l/a is called the cell constant and may be denoted by θ

$$P = \theta/R$$

The effective value of θ for a cell is not simply related to the cell geometry. However, it has a constant value for electrolytes, measured in that particular cell. The cell constant can be determined by measuring R for a solution of known specific conductance.

Solutions of potassium chloride of known concentrations are invariably employed for this purpose. The specific conductance of these solutions is determined once and for all with a cell, in which the cross-sectional area is uniform and known with accuracy.

Conductivity cells are available in different types, sizes and shapes. The simplest is the dip type, which is immersed in the liquid to be tested. The solution may be an open container and may have volumes in the range of 5 ml. Pipette cells permit measurements of conductivity with small volumes of solution, which may be as small as 0.01 ml. Epoxy cells are employed for high temperature use.

For the majority of applications the cells used are made from a specially developed high density carbon that has the same desirable quality as platinised platinum of eliminating electrochemical errors, but without the need for frequent re-platinisation and recalibration. The annular carbon electrodes are fitted within the tubular bore of the cell. The cell body is moulded from an epoxy resin. The cells are now available for screw-in, flow-line and dip-type installations.

Two-terminal conductivity cells are commonly used. These cells are quite satisfactory in many applications, but with dirty solutions, fatty acids or other sticky deposits, fouling takes place. This modifies the surface area and thus results in change of the cell constant, resulting in incorrect readings. This problem has been largely overcome by the four-terminal conductivity cell. Here, a four-electrode cell is used, two outer electrodes being for current and the two inner ones as voltage electrodes.

22.6.2.1 2-Electrode conductivity sensor

The 2-electrode design represents the classical conductivity measurement arrangement. Initially, electrodes were made of platinum, often plated with platinum black. Gold was another common electrode material. Today's electrodes are typically made of titanium, graphite, or stainless steel. A common design uses concentric rod and cylindrical electrode surfaces rather than square electrodes. An example of a 2-electrode sensor for online use is illustrated in Figure 22.10.

Temperature sensors are always built into all conductivity sensors, with a variety of temperature compensation algorithms appropriate to match the measured solution, selectable within the conductivity metre.

In the 2-electrode design, polarisation can still occur to an appreciable degree at high ionic concentrations even with the use of an AC voltage. The range of applications is dependent on the cell constant. The 2-electrode sensors are typically applied to conductivity measurements not greater 1,000–10,000 uS/cm.

Also, the cell resistance measured is the total of the solution plus any resistive coatings on the electrode surfaces. Therefore, resistive coating on the electrode surface will directly affect the reading. The design is most suitable for the low conductivity solutions.

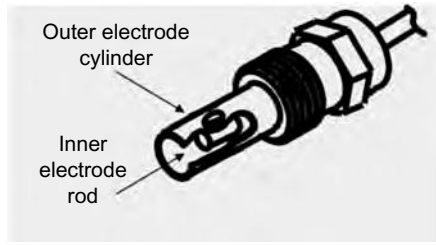


Figure 22.10 *Concentric 2-electrode design (Courtesy: M/s Mettler-Toledo Ingold, Inc)*

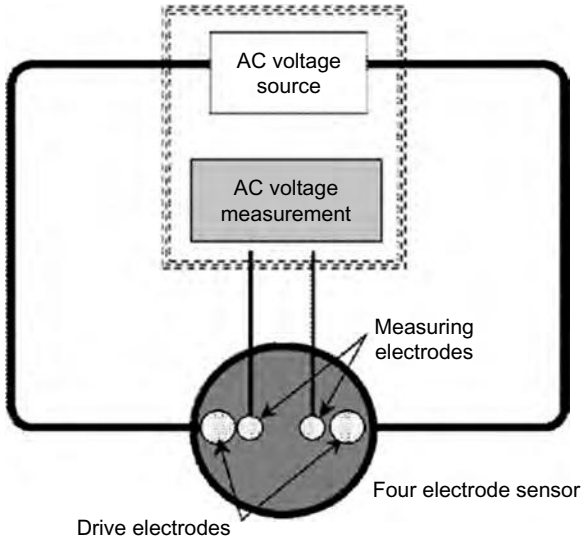


Figure 22.11 *4-electrode sensor design (Courtesy: M/s Mettler-Toledo Ingold, Inc)*

22.6.2.2 4-Electrode conductivity sensor

The 4-electrode sensor design is illustrated in Figure 22.11. An AC voltage is applied across the 2 outside electrodes as with the 2-electrode sensor. However, rather than directly measuring the current between these 2 electrodes, the principle of the 4-electrode sensor is to measure the voltage drop across the 2 inner electrodes. Operating with a known current condition, '*I*', the cell resistance can be calculated by rearrangement of Ohm's law:

$$V = IR,\ R = V/I$$

One limitation of the 2-electrode design is the polarisation effect at high concentrations. In the 4-electrode configuration, there is virtually no current flow at the inner voltage sensing electrodes. Therefore, polarisation does not occur, allowing the 4-electrode design to be used at higher concentrations. A second benefit of the 4-electrode sensor is its tolerance of electrode coating. Since the 4-electrode technique measures potential drop rather than resistance, the measurement remains accurate, despite minor coating. Other advantages of the 4-electrode design are its design permits easier in-process cleaning than 2-electrode designs, and that it can be installed in smaller piping than inductive sensors.

22.6.2.3 Inductive conductivity sensor

The inductive conductivity sensor consists of two toroidal coils encapsulated in an inert polymer body, such as polypropylene. This is shown in Figure 22.12. One coil is energised at a frequency of 10–20 kHz, which, by means of electromagnetic principle, induces an electric field through the centre bore of the sensor (Light, 1990). When placed in a conductive solution, a current loop is generated, which is then sensed by the measurement toroid. The more conductive the medium, the more signal is transferred to the second coil.

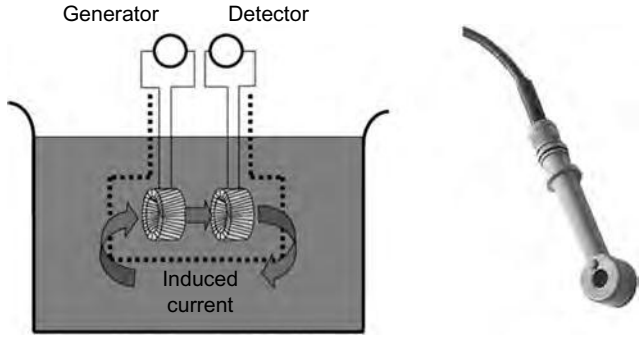


Figure 22.12 *Inductive sensor design (Courtesy: M/s Mettler-Toledo Ingold, Inc)*

The inductive design offers several advantages over other designs: since there are no metallic electrodes contact with the sample solution, corrosion is less of an issue; there are no polarisation effects; and the signal increases with decreasing resistance as compared to opposite with contacting electrodes. Also, to a great extent, the sensor is not affected by resistive coatings on the sensor, including oils and grease. An installation consideration is that the induced field extends several inches beyond the sensor body itself. Therefore, typically larger piping is required for inductive sensors than contacting. If sufficient clearance is not provided, the reading will be impacted. If the piping is non-conductive, the readings will be low, if the piping is conductive, the readings will be high. This effect can be compensated by calibrating the sensor in the configuration in which it will be used.

Due to the nature of the measurement principle, the 'inductive' design is also known by a variety of names including 'non-contacting' conductivity or 'electrodeless' conductivity owing to the fact that there are no metallic electrodes in contact with the solution. Given the sensor construction, it is also referred to as 'toroidal' conductivity cell.

22.6.3 Temperature Compensation in Conductivity Measurements

The conductivity of electrolytic solutions varies with temperature. This is because the ionic mobilities are temperature dependent. The temperature coefficient is usually of the order of 1.5 to 2%/°C at room temperature. Control of temperature is thus very essential in precision work. This is usually done by introducing into the bridge circuit a resistive element, which will change with temperature at the same rate as the solution under test. The temperature compensating resistor may be a rheostat calibrated in temperature, which can be manually adjusted. Automatic temperature compensation can be provided by using thermistor and resistance combination in contact with the solution, which would automatically offset the effect of changes of temperature of the solution under test.

An important accessory to a precision type conductivity metre is a thermostatic bath capable of providing very high long-term temperature stability. A proportional controller is employed in preference over the conventional on-off methods. With this method, a temperature stability of 0.02°C may be achieved. Thermostatic baths are very essential for continuous measurement of electrical conductivity in liquid streams, especially where very small changes in salt concentrations take place.

22.6.4 Conductivity Measurements Using High Frequency Methods

High frequency method of measuring the conductivity of solutions offers the advantage of placing the electrodes outside the solution container and out of direct contact with it. This eliminates the possibility and danger of electrolysis or electrode polarisation.

The method consists in placing the container with the sample to be analysed between the plates of a capacitor, which forms a part of the high frequency generator circuit, functioning at a frequency of a few megacycles per second. Since the capacitor is a part of the oscillator circuit, any changes in the composition of the solution will result in the changes in the plate and grid currents and voltages due to change in the conductance and capacitance of the cell. The frequency of a parallel resonance circuit is given by

$$f = \frac{1}{2\pi}\sqrt{LC}$$

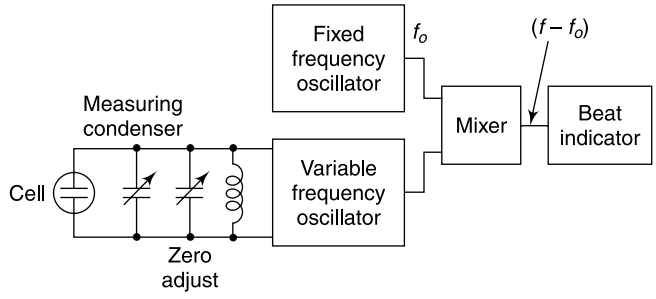


Figure 22.13 *Beat frequency method for measuring conductivity*

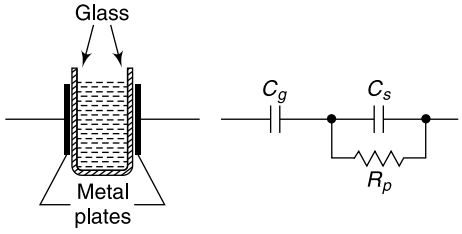


Figure 22.14 *Equivalent circuit of the conductivity cell when used in high frequency method*

The sample cell is usually placed in parallel with a calibrated capacitor. In order to achieve the resonant frequency, the exact amount of capacitance which is added by the sample is removed by adjustment. This gives a measure of the conductivity.

Beat frequency method can also be used to measure the output frequency of two oscillator circuits. One of these circuits (Figure 22.13) contains the sample cell as a part of the oscillator capacitance and the other is of fixed frequency. *The* output of the two when given to a mixer unit, would be the difference of the two frequencies (f–f_o), which would be directly proportional to the changes in high frequency capacitance of the cell. The difference frequency is measured directly with a beat indicator.

A typical sample cell for high frequency conductivity measurement may be composed of two metallic plates sealed on to the wall of a rectangular container. When the solution is put in the container, the metal plates act as a condenser with solution and glass as the dielectric. The equivalent circuit of the cell is as shown in Figure 22.14. C_g represents the capacitance of the glass walls of the cell, C_s is the capacitance of the sample and R_p is the resistance in parallel with C_s. The resistive component is very high and offers negligible contribution. Capacitive effect is the major factor in high frequency measurements, whereas resistive balance is more important in low frequency measurements.

22.7 VOLTAMMETRY

Voltammetry refers to the measurement of current that results from the application of potential to drive an electrochemical reaction. Unlike potentiometry measurements, which employ only two electrodes, voltammetric measurements make use of a three-electrode electrochemical cell. The use of three electrodes (working electrode, reference electrode and auxiliary electrode), along with the potentiostat instrument, allows accurate application of potential functions and measurement of the resultant current (Hacke, 1985).

There are different volumetric techniques which are distinguished from each other primarily by the applied potential function to drive the reaction and by the material used as the working electrode. Two main forms of voltage scans are used – the linearly increasing voltage in linear sweep voltammetry (LSV) and the triangular voltage scan in cyclic voltammetry (CV).

LSV is a general term applied to any voltammetric method in which the potential applied to the working electrode is varied linearly in time. These methods include polarography, CV and rotating disc voltammetry.

Figure 22.15a shows the type of waveform used in LSV. In this figure, the slope of the ramp is called scan rate and is represented ad volts per unit time. The value of the scan rate may be varied from as low as a few mV/sec to several thousand volts per sec.

Cyclic Voltammetry (CV): In CV, the potential is ramped up (Figure 22.15b) to some pre-determined value and then returned at the same rate to the starting potential. The scan rates in CV are much faster than in LSV, which leads to local depletion of analyte around the electrode surface. Once the analyte is consumed around the electrode, the current diminishes to a value determined by the rate at which new analyte gets to the electrode surface from the bulk solution. On the return sweep, a peak is observed as part of the 'cloud' of material generated in the forward sweep is converted back into starting material.

Pulse Voltammetry: Pulse voltammetry can be carried out in two modes; normal-pulse voltammetry and differential pulse voltammetry. Normal pulse voltammetry involves the imposition of square-wave voltage impulses or increasing magnitude upon a constant DC voltage. In normal-pulse voltammetry, the contribution of background capacitance to the current is minimised by eliminating the continuously varying potential ramp, and replacing it with a series of potential steps of short direction. This is shown in Figure 22.15c. Here, each potential steps begins at the same value and the amplitude of each subsequent steps increases in small increments. The method offers excellent discrimination against the background capacitive current. The pulse voltammetry method increases the analytical sensitivity by 1–3 orders of magnitude. In the differential pulse mode, fixed magnitude pulses superimposed on a DC voltage ramp are applied to the working electrode; the currents are measured prior to pulse application and just before termination of the voltage pulse. For a given pulse, the output is recorded as the difference between the two current flows. Figure 22.15d shows the voltage waveform applied in differential pulse voltammetry. This

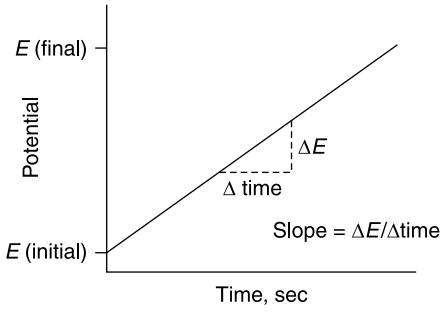


Figure 22.15 (a) *Principle of linear sweep voltammetry (Anderson, 1996 a)*

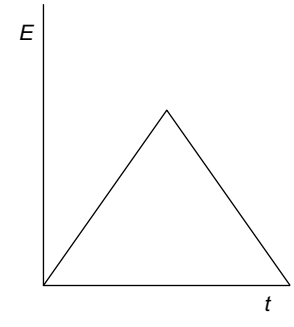


Figure 22.15 (b) *Typical voltage sweep in cyclic voltammetry (CV).*

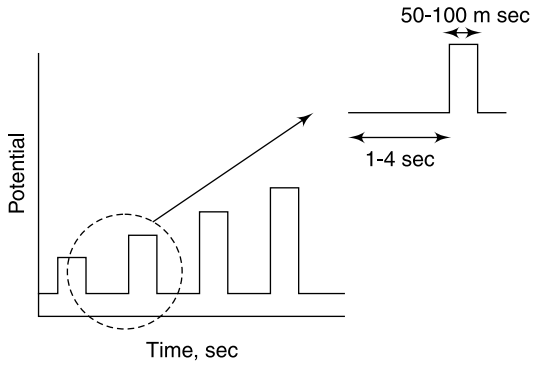


Figure 22.15 (c) *Principle of normal-pulse voltammetry (Anderson, 1996 b)*

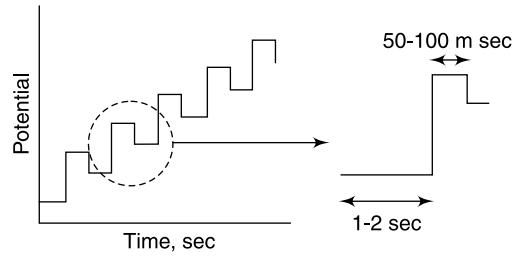


Figure 22.15 (d) *Principle of differential pulse voltammetry (Anderson, 1997)*

method has even better ability to discriminate against capacitive current because it measures a difference current.

Stripping voltammetry: In this technique, the electrode is held at a potential sufficient to reduce any metal ion it encounters for about 60 s before the scan begins. This process will have the effect of reproducibly concentrating the analyte in the vicinity of the electrode. Some analytes coat the electrode surface, others actually dissolve in the mercury. During the scan, all the reduced analyte at the electrode surface will be re-oxidised, thereby giving a much larger signal. This technique is called anodic stripping and it is one of the most widely used techniques for the electrochemical determination of metal ions in water used today.

The instrumentation used for voltammetric measurements is very simple. Figure 22.16 shows the various components of a potentiostat for carrying out voltammetric measurements using linear scan generator. The electrochemical cell is made of three electrodes immersed in a solution

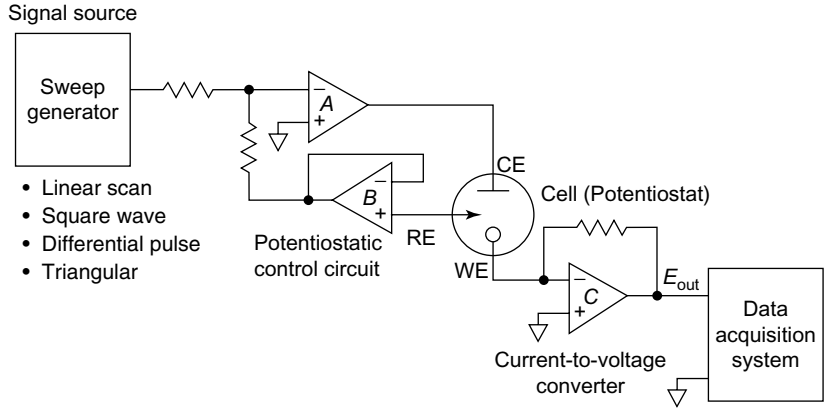


Figure 22.16 *Setup for voltammetric measurements using potentiostat with three-electrode cell (Adapted from Skoog et al. 2007)*

containing the analyte. The three electrodes are: working electrode, reference electrode and counter electrode. The output from the signal source is given to a potentiostatic circuit. The input impedance of the control circuit connected to the reference electrode is very high and is of the order of 10^{12} ohms, thus drawing very little current from the source. The resulting current is directly proportional to the potential difference between the working electrode and the reference electrode is converted into voltage and recorded as a function of time by the data acquisition system.

22.8 POLAROGRAPHS

When a voltage is applied to a pair of inert electrodes, placed in a solution, specific relationship exist between current and voltage, which depends upon the electroactive species present in solution. Current-potential curves can be plotted, which prove useful for chemical analysis. These curves can be plotted by varying the voltage applied to a cell and measuring the current flowing through it. Usually, it is assumed that the ohmic drop in the cell is negligible and the potential of one electrode is independent of current. This electrode is said to be unpolarised and the other electrode as polarised.

Polarography the name given to the technique, in which a DME is used as an indicator electrode. The basic procedure deals with the measurement of current as a function of electrode potential. Recording instruments are available for directly having a plot of these characteristics.

In polarography, the electric potential or voltage is varied in a regular manner between two sets of electrodes (working and reference) while the current is monitored. The shape of a polarogram depends on the method of analysis selected, the type of working electrode used and the shape of the potential waveform that is applied. Figure 22.17 shows different methods of Polarography in which various types of potential ramps are applied to a mercury electrode and the shapes of the resulting waveforms are compared.

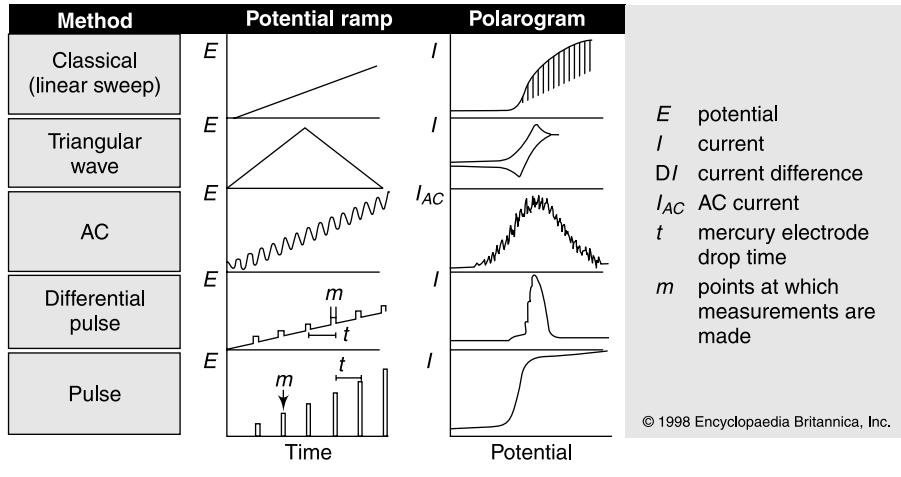


Figure 22.17 *The various potential ramps that can be applied to a mercury indicator electrode during selected forms of polarography, along with their typical polarograms (Encyclopaedia Britannica Inc. 1998)*

22.8.1 Basic Polarographic Instrument

The essential instrumental requirements of a polarograh are few and simple. The arrangement must have:

- means of applying a variable but known voltage ranging from 0 to ±3V to the cell.
- a method for measuring the resultant current which is usually in the range of 0.1–100 μa.

A block diagram of a polarographic set-up is shown in Figure 22.18.

The electrolysis cell is shown in the diagram as *G*. The beaker contains the test solution. *K* represents a DME consisting of a narrow capillary from which mercury emerges at a rate of 20–30 drops per minute. *A* is a non-polarisable electrode (e.g. a mercury pool at the bottom of the beaker). Instead of this, it is common to use a calomel half-cell.

The polarographic method is based on recording of the variations in the current flowing through the electrolysis cell, as the potential between the electrodes is gradually increased from one value to another, say from 0 to 2 V. This process is achieved through a motor, which simultaneously drives the potentiometer and feeds the chart, on which the electrolysis current is recorded as a function of the electrolysis voltage.

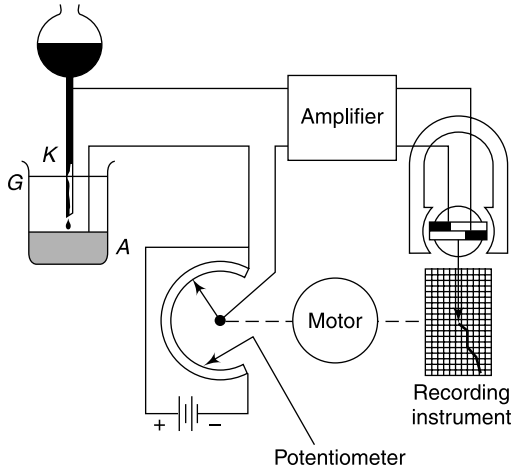


Figure 22.18 *Basic polarographic set-up*

Linearly increasing polarising voltage can be conveniently obtained by using an operational amplifier as an integrator. This enables the potential scan-rate to be varied over a wide range. Also, small currents encountered in voltammetry are advantageously amplified in a high input impedance operational amplifier, without loading the system with an undesirable voltage drop, when connected with grounded positive input and negative feedback. The input bias current of the operational amplifier used as an integrator must be at the most of the order of 10^{-11} A, if integration times of the order of minutes are to be achieved. Figure 22.19 shows a simple 2-electrode polarographic system, with amplifier 1 connected as an integrator, to the output of which the DME is connected. The counter electrode is connected to the input of amplifier 2. The integrator output is monitored on voltmeter *V*. The output of current amplifier is connected to a recorder.

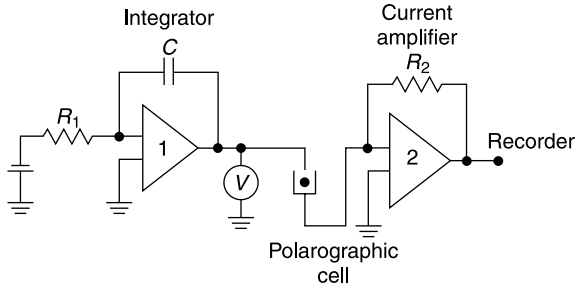


Figure 22.19 *Circuit arrangement for obtaining linearly increasing polarising voltage*

The polarographic current pulsates between zero and a maximum value during the growth and fall of the mercury drops. This effect is controlled by providing a circuit for slight overdamping.

Figure 22.20 *Polarography-voltammetry system with PC support (Courtesy: M/s Topac, USA)*

Modern polarographic instruments are PC based which perform all typical voltammetric and polarographic analysis such as DC polarography, pulse voltammetry and, CV. Figure 22.20 shows a typical PC based instrument. A range of electrodes are available to meet the needs of various applications.

22.8.2 Dropping Mercury Electrode

The DME is exactly as its name implies. It consists of a length of marine barometer tubing with a fine capillary and a head of mercury above it. Mercury, usually under force of gravity, is forced through a section of very fine glass capillary. A mercury drop starts, grows and finally falls off as another drop starts. The measured current will naturally tend to follow this process of increasing steadily, dropping sharply and finally increasing again. The mercury head is so adjusted that it gives a drop time of 2–5 s. The head is generally kept between 40 and 80 cm. The internal diameter of the capillary tube is of the order of 0.03–0.05 mm and the length of the capillary is about 8 cm. A platinum wire is immersed in the mercury reservoir and the dropping mercury electrode is coupled with an unpolarised electrode. This electrode is useful over the range from 0.4 to –2.8 V refereed to the normal hydrogen electrode. Above 0.4 V, mercury dissolves and gives an anodic wave. At potentials more negative than –1.5 V, the electrolytes begin to discharge.

The dropping mercury electrode is a truly elegant electrode and has the following advantages over other types of electrodes:

- Hydrogen has a very large over-potential than mercury. It renders possible the deposition of substances difficult to reduce, as for example, the alkali ions.
- It provides very nearly ideal conditions for obtaining a diffusion controlled limiting current, which is reproducible.
- It provides a continuously refreshed surface, which is conducive to a high degree of reproducibility for the current measurements. The constant renewal of the electrode surface eliminates passivity or poisoning effects.

The dropping mercury electrode, however, cannot be used for dilutions less than 10^{-5} M, due to the presence of a relatively large charging current. Nevertheless, high sensitivity derivative instruments

Figure 22.21 *Controlled Growth Mercury Electrode (Courtesy: M/s BASi)*

may be 200 times more sensitive, as they compensate for the effect of charging current.

Mercury drop electrodes are generated in the following three modes.

Dropping Mercury Electrode (DME): mercury is allowed to flow freely from the reservoir down the capillary and so the growth of the mercury drop and its lifetime is controlled by gravity. 100 μm capillary is generally recommended for this mode.

Static Mercury Drop Electrode (SMDE): The drop size is determined by the length of time for which the fast-response capillary valve is opened, and the drop is dislodged by a drop knocker. The dispense/knock timing is microprocessor-controlled and is typically coordinated with the potential pulse or square-wave waveform. This mode can also be used to generate the Hanging Mercury Drop Electrode required for stripping experiments.

Controlled Growth Mercury Electrode (CGME): The mercury drop is grown by a series of pulses that open the capillary valve. The number of pulses, their duration and their frequency can be varied by PC control, providing great flexibility in both the drop size and its rate of growth. This CGME shown in Figure 22.21 mode can be used for both polarographic and stripping experiments.

A built-in gas control allows purging or blanketing of the sample prior to or during analysis, and there is a magnetic stirrer for experiments requiring convection, such as stripping voltammetry. The cell arm with detachable cell top allows easy access to the electrodes for rinsing and cleaning. The stir motor/cell vial base pivots for easy removal and replacement of the cell vial.

22.8.3 Reference Electrode

A mercury pool at the bottom of the polarographic cell acts as a reference electrode. It has a large area and therefore, the current is generally very small. The concentration overpotential at this electrode is negligible and its potential may be regarded as constant. Though convenient, the mercury pool never possesses a definite known potential. Therefore, the reference electrode is usually a SCE. It is almost an universal practice in voltammetry to express half-wave potentials with reference to this electrode.

22.8.4 Typical Polarographs

Figure 22.22 shows a polarogram of a solution containing copper, cadmium and zinc. Initially, the electrolysis current is practically zero. The current then starts rising rapidly with the voltage. The increase is brought about, as an increasing number of Cu^{2+} ions are discharged at the dropping

mercury electrode. The current soon reaches a constant value, which is governed by the velocity of diffusion at which the Cu^{2+} ions are transferred to the cathode. This difference would reach the maximum value when the Cu^{2+} ions are discharged as fast as they appear at the mercury cathode. This means that the height of the copper wave is proportional to the Cu^{2+} connection. As the potential is increased further, the Cd^{2+} ions will begin to discharge at the cathode, which would result in a wave corresponding to the Cd^{2+} concentration. The Zn^{2+} is recorded at a still higher potential. This figure shows that the height of the wave corresponds to the concentration of the ion concerned, whereas the potential at which the wave is produced, is characteristic of the ion.

The spiked oscillations (Figure 22.23) seen on the recorded polarograms are due to the growth and fall of the mercury drops.

As shown in the inset, the current increase as the drop grows, and then drops sharply when the mercury drop detaches itself and falls.

A decreasing interest in the polarographic methods has been noticed largely due to the concerns about the use of large amount of mercury in the laboratory as well as in the environment, coupled with the cumbersome nature of the apparatus. Also, more convenient instrumental techniques have been developed which are faster and more convenient.

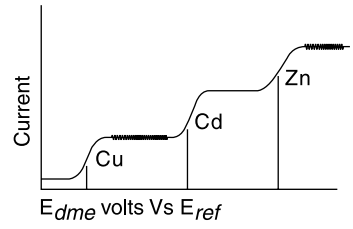


Figure 22.22 *Typical polarogra*

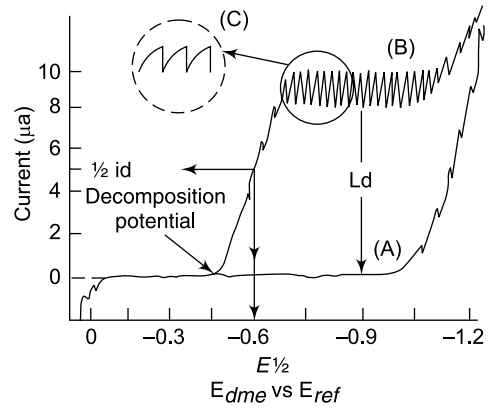


Figure 22.23 *Typical polarogram showing growth and fall of the mercury drop and corresponding change in the current*

22.9 COULOMETERS

Coulometry is an analytical technique for measuring an unknown concentration of an analyte in solution by completely converting the analyte from oxidation state to another. This technique is an absolute measurement similar to gravimetry or titration and requires no chemical standards or calibration.

Thus, coulometric methods of analysis depend on the exact measurement of the quantity of electricity that passes through a solution during the course of an electrochemical reaction. The quantity of reactant formed between the beginning and the interruption of current at the end of the process is directly related to the net charge transferred, Q. Analytical methods based on the measurement of the quantity of electricity are designated by the generic term of coulometry, a term derived from coulomb.

According to Faraday's law, the quantity of electricity involved in the electrolysis of one equivalent of substance is one Faraday or 96,494 coulombs. The weight W of a substance consumed or produced in an electrolysis involving Q coulombs is

$$W = W_m Q/96{,}494\, n \tag{22.1}$$

where W_m is the gram atomic weight or gram molecular weight of the substance being electrolysed. Here, n is the number of electrons involved in the electrode reaction. Equation (22.1) can also be expressed as

$$\mathrm{Q} = nFVC_b$$

where F equals *96,494* coulombs, V equals volume of solution in litres and C_b equals bulk concentration of the electrolysed analyte in moles/litre.

The total amount of electricity (Q) which is required to electrolyse a certain species is the current-time integral.

$$Q = \int_o^t i dt$$

This integral is equal to the area under the *i–t* curve.

Coulometric methods employ two techniques:

- Potentiostatic coulometry or controlled-potential coulometry
- Amperostatic coulometry or controlled-current coulometry

In controlled-potential coulometry, the potential of the working electrode is controlled at a constant value and the electrolysis current is measured against time. Completion of the electrolysis is indicated by decay of the current to a negligibly small value. Typical working electrodes used in this analysis are platinum, silver, silver chloride and mercury.

Figure 22.24 shows a typical current-time curve for controlled-potential coulometry. Before starting the electrolysis, the working electrode potential is chosen, which will electrolyse the species of interest. If this potential is not known beforehand, it can be determined from a polarogram of a standard solution of the given analyte. When the electrolysis is begun, the current increases to a high value initially. It then falls exponentially, as the analyte is consumed. Obviously, it is possible to carry out the electrolytic generation to infinite time. In practical analysis, the electrolysis is complete when the current has decayed to less than 0.1% of the initial current.

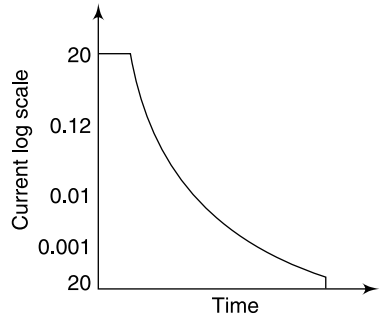


Figure 22.24 *Typical current-time curve for controlled-potential coulometry*

Integration of the *i–t* curve may be done by graphic, mechanical, electromechanical or electronic means. The integration unit may be attached to the potentiometric recorder which draws the *i–t* curve.

The current decay in controlled-potential coulometry is given by the equation

$$i_t = i_o \times e^{-kt}$$

$$\text{or } 2.3 \log (i_o/i_1) = kt$$

where i_0 is the initial current and i_t the current at time t. When the logarithm of the current is plotted as a function of time, the intercept at $t = 0$ is i_0 and the slope is $-k/2.3$.

In a controlled-current titration, the current is set at a working electrode and maintained throughout the titration, with the potential at the indicating electrode measured against time. A mercury or platinum electrode is used as the working electrode, along with a reference and indicating electrode.

The success of most coulometric titrations depends on the ability to attain 100% current efficiency. This implies that the amount of titrant produced in the electrolysis is exactly equal to that predicted by Faraday's law, judicious selection of the current density (current/unit electrode area) can mean the difference between success and failure of a coulometric titration. A value of 0.5 mA, cm^{-2}, mN^{-1} is commonly stated as near the maximum limiting current density for many substances. Coulometry involves only the fundamental quantities *of* current and time. It is thus free from many of the uncertainties and errors associated with standard solutions. It offers excellent precision and accuracy and is useful for analysis at very small amounts, ranging as low as a few hundredths of a micro-gram in volumes of 5–50 ml.

A coulometric cell is an electrochemical cell, in which the two electrodes can be a platinum pair. As electrochemical reactions can occur at either electrode, the two-electrode compartments must be separated by a suitable membrane to prevent interaction between the products. A sintered glass disc or an agar-gel plug is often used for this purpose. The electrode compartment of interest must be suitably stirred. The end points are usually detected with a micro-ammeter.

Coulometric experiments range from chronocoulometry, whereby steps or sweeps of electrode potential produce current transients of less than 10^{-3} s duration to the recording of charge-time relations or total final charge values in electrosynthesis, electrodeposition or controlled-potential coulometry operations, which last several minutes or hours.

22.10 AMPEROMETERS

Amperometry is the method of determining the concentration of an electroactive substance by applying a fixed voltage across an indicator and reference electrode, and then measuring the current passing through the cell. This technique is particularly well suited to trace analysis. The current measured is generally on the diffusion-current plateau of the current-voltage curve, a region where the current is independent of the potential of the indicator electrode. The rate of diffusion and hence the current is proportional to the concentration of diffusing material in the bulk of the solution. The most common use of amperometry is in titrations, where the current is measured as a function of the volume of titrant added. Concentration changes during a titration are reflected in a change in the current.

Amperometric technique may be used with either single polarised electrode or two polarised electrodes. The electrodes in either case are usually small with surface areas of a few tenths of a square centimetre.

In the single-electrode method, a polarised electrode, coupled with an unpolarised electrode (SCE), is immersed in the solution being titrated. The polarised electrode may be a dropping mercury electrode, a rotated electrode or a stationary electrode. The cell is connected to a manual type polarograph. The potential of the polarised electrode is held at a constant value during the titration and the current which flows through the system is observed. The second electrode acts as a reference electrode. Typical titration curves in amperometric titrations with one polarised electrode may take a variety of forms depending on whether the electroactive species is the titrate, titrant or a product of the titration reaction.

In the two-electrode system, two stationary platinum wire electrodes are immersed in the titration cell. The potential of both electrodes vary during the titration, but the potential difference between them is kept constant. The current through the cell is measured during titration. The equivalence point is deduced from the plot of current against volume of titrant. The applied potential difference is relatively small, 0.01 to 0.1 V and the current is generally not as large as in single indicator electrode amperometry. Two electrode system is also called dead stop end point, and is particularly applicable when a reversible oxidation-reduction system is present, either before or after the end point

Amperometric titrations are carried out in a polarographic cell suitably modified to permit entry of a burette stirrer. H-type cells are also convenient, but a wide-mouthed 100 ml flask or beaker fitted with a suitably pierced cover is mostly employed. When oxygen is known to interfere, it may be removed in the usual way. For stirring, gas bubbling is frequently employed. Any polarographic instrument may be employed to carry out amperometric titrations.

Advantages of amperometric titrations are that, the electrode characteristics are unimportant and the method offers greater sensitivity than conductance and potentiometric titrations. The method is applicable in very dilute solutions, even down to 10^{-4} or 10^{-5} M, according to the type of electrode used.

A typical example of application of amperometry is in the measurement of glucose in the blood. The method is based on the use an enzyme (glucose oxidase) that catalyses the glucose reaction within the electrochemical test strip. The measurement schematic diagram is shown in Figure 22.25. A sensor measures the current generated in a chemical reaction from

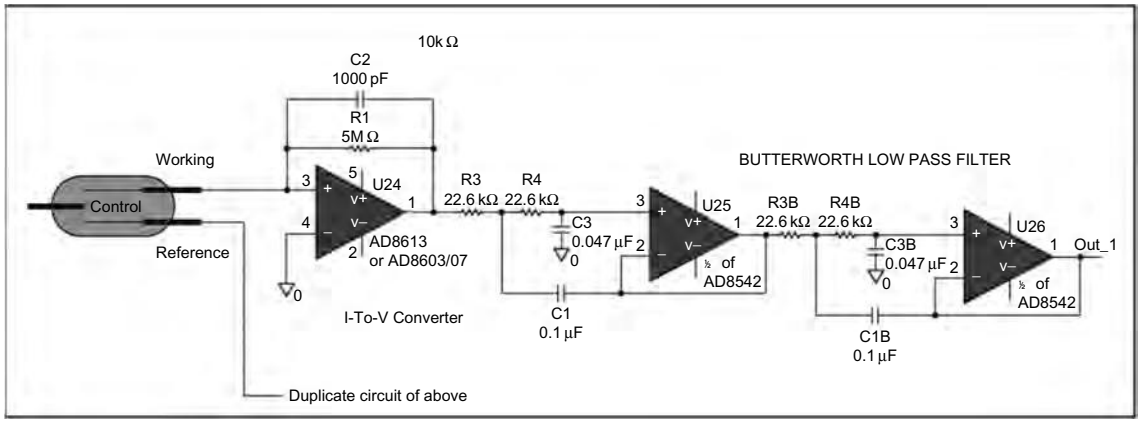


Figure 22.25 *Amperometric method for glucose measurement (Courtesy: M/s Analog Devices)*

electrochemical strips with glucose oxidase enzymes. In general, three electrodes are used: a reference electrode, a control electrode and a working electrode. Signal currents are usually less than 3,000 nA full scale, so the I-to-V converter requires low input bias current. A low-pass filter with a cut-off of 80–100 Hz is desirable to remove extraneous noise. It can be a simple two- or four-pole Butterworth filter design. Low power operational amplifiers with bandwidths of 50–500 kHz are adequate.

The AD8603/AD8607 and AD8613 family of operational amplifiers offer low offset, low noise, very low input bias current and low power consumption. Operating on 2.7 V with bandwidths of 400 kHz at –3 dB, they are ideal for portable applications.

22.11 AQUAMETERS

Aquametry consists in determining small amounts of water in solids, liquids and gases. This is done by titration with Karl Fischer Reagent (KFR) and the end point is determined colorimetrically or electrometrically. The electrometric method is generally preferred, because highly coloured samples obscure the colorimetric end point.

Automated titration equipment have been designed, in which the titrant delivery is controlled by an automatic correcting circuit, that varies the rate of titrant addition to minimise titration time, while maintaining high precision and repeatability. Sensitivity as great as 1 ppm of water in 100 ml of sample is attainable.

Karl Fishcer Titrator essentially comprises of two parts: The *Burette Assembly* consists of a piston travelling in precision bore glass tubing. The inside diameter of the tube is very accurately controlled with a tolerance of ±0.0002 inch. The pistons are coupled with the counters which are driven from the lead screw by a toothed belt. Backlash must be held to less than 0.002 ml. A small DC motor is connected to the lead screw by means of a friction drive and gear set with a speed reduction of 200:1. Limit switches are used to automatically stop the motor at both ends of the piston stroke.

Figure 22.26 shows the block diagram of a typical Karl Fischer Titrator.

A voltage is applied to two platinum electrodes immersed in the sample solution. The resultant current is measured by amplifier A_1 and compared to a reference voltage by amplifier A_2. When the output of A_1 exceeds the reference voltage set at R_2, relay K_1 is energised. The relay contacts turn the burette drive motor on and off. A timer circuit is used to provide selectable end point delays from 10 s to two minutes. The burette drive motor should be speed controlled during the course of the titration, so that time required to reach a precise end point is minimised.

In the Karl Fischer titration, each addition of titrant causes an initial increase in electrode current, which decays rapidly at first, and then slowly as the end point is approached. The end point is reached when the current stays above a set level for some pre-selected time.

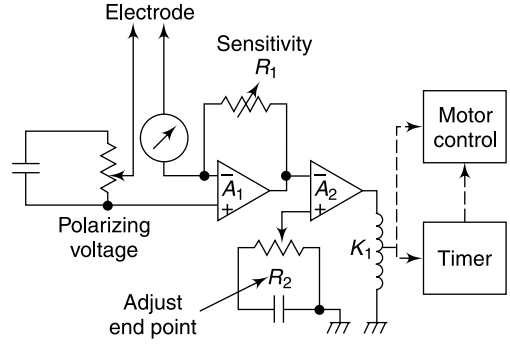


Figure 22.26 *Block diagram of electronic circuit for Karl Fischer titrator*

22.12 GENERAL PURPOSE ELECTROCHEMICAL INSTRUMENTATION

Several electrochemical techniques have proved to be powerful tools in analytical chemistry. For example, for an overview of chemistry, CV is powerful, but normal and reverse pulse voltammetry, rotating electrode methods, or chronocoulometry provide simpler means for obtaining quantitative evaluation of parameters of interest. A greater insight of a sample is obtained by examining it by various types of pulse voltammetry, possibly by AC voltammetry in any of several forms, and perhaps by one or more stripping procedures. The objective, of course, is to find the combination of conditions that leads to optimum sensitivity, precision and flexibility in the format of electrochemical excitation and observation.

Ideally, in an electrochemical laboratory, one would have a full range of options on a single instrument that should be simple, provide common style for selecting and setting up the experiments, regard-less of experimental mode and the time needed to switch between modes ought to be negligible. The only realistic method for achieving this is to place the entire repertoire under the charge of a computer. The computer must have full control of the potentiostat and cell by an automated switching network. It must provide a range of excitation waveforms and schemes for acquisition of data, processing the data for taking certain decisions and communication with the operator (He et al.,1982).

The Model 600E series from M/s Sinsil Instruments is designed for general purpose electrochemical measurements. Figure 22.27 shows the block diagram of the instrument. The system

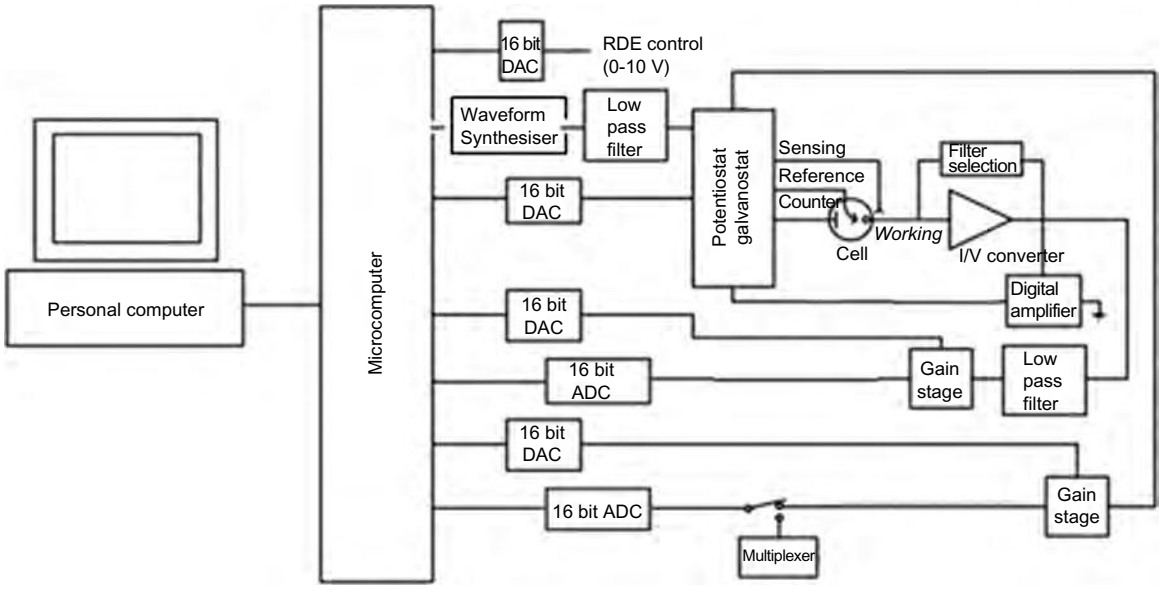


Figure 22.27 *Block diagram of general purpose electrochemical measurements (Courtesy: M/s Sinsil International)*

contains a fast digital function generator, a direct digital synthesiser for high frequency AC waveforms, high speed dual-channel data acquisition circuitry, a potentiostat and a galvanostat. The potential control range is ±10 V and the current range is ±250 mA. The instrument is capable of measuring current down to picoamperes.

The function generator can update at a 10 MHz rate. Two high speed and high resolution data acquisition channels allow both current and potential to be sampled simultaneously at a rate of 1 MHz, with 16-bit resolution. For instance, the scan rate in CV can be up to 1,000 V/s with a 0.1 mV potential increment or 5,000 V/s with a 1 mV potential increment. The potentiostat/galvanostat uses a 4-electrode configuration, allowing it to be used for liquid/liquid interface measurements and eliminating the effect of the contact resistance of connectors and relays for high current measurements. The instrument also includes a true integrator for chronocoulometry.

The instrument is capable of a wide variety of electrochemical techniques, and is available with integrated simulation and fitting software functions for both impedance and CV. These features provide powerful tools for both electrochemical mechanistic studies and trace analysis.

23

pH METERS AND ION ANALYSERS

23.1 WHAT IS pH?

The concept of pH was introduced by Sorensen in 1909. He recognised that hydrogen-ion concentrations, as distinct from total acidities, are frequently of importance in chemical processes. While studying enzymatic reactions, he found it convenient to define a symbol which could represent the concentration of hydrogen ions and called this symbol as pH. It is defined by the following equation:

$$pH = -\log_{10} C_H$$

where C_H is the hydrogen-ion concentration.

$$C_H = 10^{-pH} \tag{23.1}$$

Pure water is known to be a weak electrolyte and it dissociates to form hydrogen ions and hydroxyl ions as shown below:

$$H_2O = H^+ + OH^-$$

Assuming that activity coefficients are unity, the dissociation constant K_w of pure water is given as follows:

$$K_w = C_{H^+} \times C_{OH^-} \tag{23.2}$$

The product of hydrogen and hydroxyl ions in water at 25°C is 1.008×10^{-14} moles^2 litres^{-2} and the concentrations of hydrogen and hydroxyl ions will of necessity be equal. Since the positive and negative electric charges in the solution must balance, each of these concentrations is given by

$$\begin{aligned} C_{H^+} = C_{OH^-} &= \sqrt{K_W} \\ &= \sqrt{1.008 \times 10^{-14}} \\ &= 1.004 \times 10^{-7} \\ C_{H^+} &\approx 10^{-7} \end{aligned} \tag{23.3}$$

Therefore, pH of pure water = 7

It is obvious that the neutral point or the point at which the hydrogen and hydroxyl ions are present in equal concentrations is located at pH 7.

The lower-case letter 'p' in pH stands for the negative common (base 10) logarithm, while the upper-case letter 'H' stands for the element hydrogen. Thus, pH is a logarithmic measurement of the number of moles of hydrogen ions (H^+) per litre of solution. Incidentally, the 'p' prefix is also used with other types of chemical measurements where a logarithmic scale is desired, pCO_2 (Carbon Dioxide) and pO_2 (Oxygen) being two such examples.

The pH of an acidic solution (i.e. $(H^+) > (0H^-)$) at 25°C will be less than 7 and that of an alkaline solution greater than 7. The peculiarity of the logarithmic scale is exemplified by the fact that a 10-fold decrease in hydrogen-ion concentration (pH) corresponds to an increase of one pH unit, whereas a 2-fold increase of the concentration means pH will decrease by log 2 (i.e. by 0.301 units).

The dissociation constant K_W is a function of temperature and therefore, the neutral point will vary as the temperature is changed. The pH for neutral conditions decreases to 6.5 at 60°C from the value of 7 at 25°C and increases to 7.5 at 0°C. The range of the pH scale also depends upon the magnitude of K_W.

The approximate practical range of the pH scale is from –1 to 15 at room temperature, although most of the commercial instruments are designed to measure 0–14 pH.

There is at present a wide utilisation of pH measurements in the chemical laboratories, industries and the clinics. This has been made possible by the discovery of the hydrogen-ion function of glass membranes, which led to the development of convenient, practical glass electrodes, pH metres and controllers that allow the pH of process solutions to be adjusted automatically. The technology of pH instrumentation has been greatly developed and extremely sensitive instruments are now commercially available.

With the developments in chemical thermodynamics, it has gradually become clear that Sorensen's experiments did not, in reality, yield pH. No doubt the numbers obtained depended in a complex manner on the activity of the electrolytes in the solution under investigation, they were not an exact measure of the hydrogen-ion activity and indeed could never be made so. Sorensen's measured value of pH are not, therefore, values of C_{H^+} (pH) as he originally considered them to be. However, the methods of pH measurement have been standardised by convention, in a manner which allows a maximum of theoretical significance to be placed upon the experimental results.

23.2 PRINCIPLE OF pH MEASUREMENT

The measurement of pH in a test solution is made by measuring the potential developed in an electrochemical cell. The electrochemical pH cell consists of a measuring electrode and a reference electrode, both immersed in the solution under investigation. The two electrodes are connected to a measuring instrument and the electromotive force (emf) between these two electrodes is measured. The measuring electrode is pH sensitive and its potential is proportional to the pH of the solution, in which it is immersed while the reference electrode would always develop a constant electrical potential, against which the potential of the glass electrode is measured.

The potential of the measuring electrode may be written by means of the Nernst equation:

$$E = E_0 + 2.3026 \quad RT/F \quad \log C_H$$
$$E = E_0 - 2.3026 \quad RT/F \quad pH_C$$

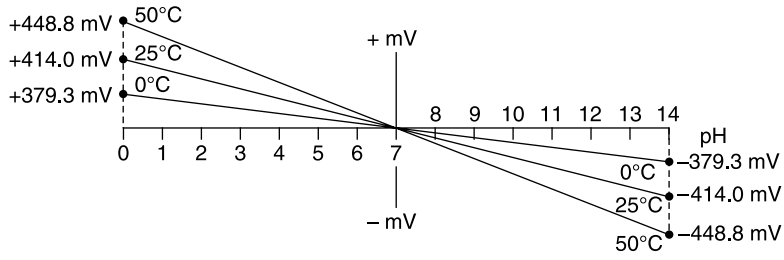


Figure 23.1 *Relationship between pH and emf at different temperatures (Courtesy: M/s Beckman Instruments, USA)*

where E_0 = standard potential
R = *gas* constant
T = absolute temperature
F = Faraday constant
pH_C = pH value deviation from *7*

The above equation shows that the emf developed in the electrochemical pH cell is a linear function of pH_c. Figure 23.1 shows relationship between pH and temperature of a typical glass electrode.

$$\text{Change of pH of one unit} = 58.2 \text{ mV at } 20°C$$
$$= 62.2 \text{ mV at } 40°C$$

The factor –2.3026 *RT/F is* called the slope factor and is obviously dependent upon the solution temperature. It is clear that with 1°C change in temperature, the emf changes by 0.2 mV. Further, pH measurement is essentially a measurement of millivolt signals by special methods.

For measurement of pH, the electrodes are first immersed in a buffer solution of known pH. The pH metre zero reading is adjusted by the standardisation control, until the pH value of the buffer is indicated by the metre. This standardisation automatically compensates for the various potentials in the electrodes system. Subsequently, immersion of the electrodes in a test solution produces a potential that is proportional to the pH of the solution. This potential registers directly as pH on the scale of the pH metre. Temperature compensation knob is set at the temperature of the solution.

23.3 ELECTRODES FOR pH MEASUREMENT

23.3.1 The Hydrogen Electrode

The hydrogen electrode is the primary electrode to which all electrochemical measurements are referred. However, owing to the experimental difficulties associated with it, other electrodes are commonly employed for routine pH measurements. Nevertheless, the performance of all other electrodes is always evaluated in terms of the hydrogen electrode.

The hydrogen electrode consists of an inert but catalytically active metal, surface, most frequently platinum, over which hydrogen is bubbled to achieve electrochemical equilibrium with the hydrogen ions in the solution. The following redox reaction takes place:

$$H^+ + e^- \rightleftharpoons 1/2\, H_2$$

The electrode is immersed in the solution under investigation and electrolytic hydrogen gas at 1 atm pressure is bubbled through the solution and over the electrode, in such a way that the electrode surface and the adjacent solution gets saturated with the gas at all times. Electrode life is 7–20 days before its response becomes sluggish. The potential set-up at the hydrogen electrode by a given activity of hydrogen ions is governed by the Nernst equation. When the partial pressure of the hydrogen is other than 1 atm, correction would have to be applied.

Since the hydrogen electrode is essentially a redox system and as such is affected by the presence of oxidising and reducing agents, it is therefore subject to a number of limitations in its application.

23.3.2 Glass Electrode

The widespread application of pH measurements in the control of industrial processes, as well as in research can be largely attributed to the development of the convenient and versatile glass electrode. Its action is based on the principle, that when a thin membrane of glass is interposed between two solutions, a potential difference is observed across the glass membrane, which depends on the ions present in the solutions. Depending on the composition of the glass, the response may be to H^+ ion or it may be to other cations. The selective response of certain glass compositions to H^+ has led to the development of pH^- responsive glass electrodes.

In construction, the glass electrode consists of a thin walled bulb of pH-sensitive glass sealed to *a* stem of non-pH-sensitive high resistance glass. The pH response is limited entirely to the area of the special glass membrane, thus making the response independent of the depth of immersion. The membrane normally has a thickness of the order of 0.05–0.15 mm, and the bulbs are of the order of 10 mm in diameter. Figure 23.2 shows typical construction of a glass electrode. Both surfaces of the membrane are pH sensitive.

The glass pH electrodes are constructed of special glass to create the ion-selective barrier needed to screen out hydrogen ions from all the other ions floating around in the solution. This glass is chemically doped with lithium ions, which is what makes it react electrochemically to hydrogen ions. Of course, glass is not exactly what you would call a 'conductor'; rather, it is an extremely good insulator. This presents a major problem if it is intended to measure voltage between the two electrodes. The circuit path from one electrode contact, through the glass barrier, through the solution, to the other electrode, and back through the other electrode's contact is, therefore, one of extremely high resistance.

On the inside of the membrane is a system of effectively constant pH. It is composed of a silver-silver chloride or calomel electrode dipped in hydrochloric acid. Changes in electrical

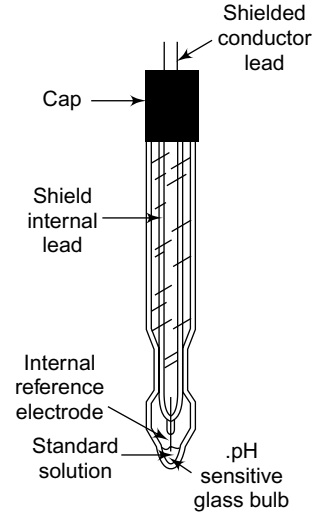


Figure 23.2 *Construction details of Beckman glass electrode*

potential of the outer membrane surface are measured by means of an external reference electrode and its associated salt bridge. The complete pH cell is represented as follows:

Internal Reference Electrode	Internal Electrolyte	Glass Membrane	Test Solution	External Reference Electrode

The ideal pH response of a glass electrode behaving exactly in the same manner as a hydrogen electrode is given by

$$E_2 - E_1 = 2.3026 \quad RT/F\ (pH_2 - pH_1)$$

where E_1 and E_2 are the values of the emf of cell 1 in test solutions pH equal pH_1 and pH_2 respectively.

The equation shows that ideal pH response is 54.2 mV at 0°C, 59.16 mV at 25°C and 73.04 mV at 95°C. Unfortunately, no glass electrode yet constructed has the theoretical response in all types of test solutions and over the entire pH range. The most important characteristics of a glass electrode are, low melting point, high hygroscopicity and relatively high electrical conductivity. For many years, the best pH-sensitive glass available was Corning 015 or Schott 4073 glass.

The useful pH range for glass electrode is generally from pH 1 to pH 11. Below pH 1, acid errors may be expected requiring frequent standardisation against a buffer of a pH close to the actual pH of the test solution. Above pH 11, alkaline errors are observed. The alkaline error depends on the composition of the membrane glass. Some manufacturers like Radiometer (Denmark) supply two different types of glass electrodes, one for 0–12 pH and the other for 0–14 pH. An approximate correction can be made for alkaline errors by means of empirically determined correction for the type of membrane glass used.

Due to large change with temperature of the electrode resistance, glass electrodes will as a rule perform satisfactorily only within a temperature range of about 60°C. However, special glass electrodes covering temperature to about –10°C or up to about +120°C are also available, but they need to be specified.

The useful lifetime of a glass electrode is generally several years, if it is handled with care. The bad condition of the electrode manifests itself in slow response and reduced sensitivity. Usually, a glass electrode may be considered good if it has a fast response (<1 min), gives stable response during measurements on a well stirred solution and exhibits sensitivity better than 95%.

A glass membrane exhibits quite a high electrical resistance and consequently the internal resistance of the cell with a glass electrode is of the order of 50–500 MΩ. The emf measurement therefore, necessitates the use of measuring circuits with high input impedance. Furthermore, the electrodes and the leads from them must be supported on holders made of a good insulating material, in order to eliminate electrical leakage across the outside surface of the glass bulk. Sometimes the upper part of the outside of the glass electrode is rendered water repellent by the application of a silicone oil or paraffin wax. The high resistance of glass electrodes renders them very susceptible to capacitive pick-up from AC mains or charged bodies. In order to minimise such effects, it is necessary to screen the electrode cable. The screen may be connected directly to earth or is grounded to the case of the instrument.

Commercial glass electrodes are available in a wide variety of sizes and shapes. They are designed to operate with samples as small as one drop; others require at least 5 ml of solution. Special micro-cells for the pH measurement of blood are supplied by several manufacturers.

The correct physical dimensions of the electrode are dictated by the sample size and the sample vessel. Electrodes with epoxy bodies and protective cap in place should be used for harsh environment conditions. An electrode with a flat membrane should be used for measurements performed directly on flat surfaces. In short, there is literally an electrode design for almost every possible measuring situation.

Glass electrodes have the following two disadvantages:

- Measuring solutions containing particulate can damage the glass membrane.
- The glass membrane is easily broken. There are alternatives to the glass membrane, though they are used seldom due to other drawbacks, such as limited pH range or long response time. The antimony electrode is used as an alternative, mainly in solutions containing HF. A thin oxide layer formed on the surface of the antimony is sensitive to pH.

Care and maintenance of the glass electrode on a regular basis ensures the following:

- A faster response
- More reliable measurements
- A long lifetime

The entire glass membrane must always be clean. Rinsing the membrane with distilled water will often suffice for aqueous solutions. Rinsing the electrode with a mild detergent solution once a week will be beneficial. An alkaline hypochlorite solution can be used to clean electrode membranes subjected to solutions containing fat or proteins.

Between measurements, store the glass electrode in a pH buffer with pH < 7. High temperature measurements, compounded by constant use in strong alkaline solutions or weak solutions of hydrofluoric acid will drastically reduce the lifetime of the electrode, since the glass membrane will slowly dissolve. Dry storage is recommended if the electrode will not be used for two weeks or more. Before use, the electrode should be soaked well.

New electrodes or those that have been stored dry should be conditioned or activated before use by soaking the bulb for a period of 12–24 h in 0.1 N hydrochloric acid. After overnight soaking rinsing, soaking in a buffer of pH = 4, and again rinsing, the electrode should be ready for use. The relative fragility of glass demands reasonable care in its handling. The pH-sensitive tip of the electrode should not become scratched or cracked through contact with the sample or container. Therefore, it should not be allowed to rest on the bottom of the sample container. The tip should be dried by gentle rubbing with absorbent tissue.

Trapped air bubbles around the inner reference electrode will produce an unstable reading. Swing the electrode in an arc or tap it gently to remove the bubbles. The electrode may have to be heated gently to approximately 60°C in a water bath if the air bubbles are trapped by KCl crystal.

A sluggish response for a glass electrode, even after proper maintenance has been performed, may dictate the need for a slight etching of the outer glass layer of the membrane. The following treatment is only recommended after all other measures have been used to improve response and have failed. Soak the glass membrane portion of the glass electrode in a 20% ammonium bifluoride solution for 1 min, followed by 15 s in 6 M hydrochloric acid. Rinse the electrode well and soak for 24 h in a pH buffer with pH < 7. The electrode deteriorates after 5–7 etchings.

The proper functioning of the glass electrode depends on the hydration of the glass layer that takes place on the surface of the pH-sensitive glass membrane during soaking and measurement

in aqueous solutions. As long as the electrode is frequently rehydrated, accurate measurements in non-aqueous or party aqueous solutions are also possible. This can be accomplished by soaking in a slightly acidic buffer. In non-aqueous solvents completely immiscible with water and before soaking, the electrode should first be rinsed with a solvent which is miscible with both water and the solvent before rinsing with water.

The electrode cable and the electrode plug must be kept clean and dry if reliable measurements are to be obtained because of the very small electrode currents which pass through the glass electrode.

A number of factors dictate the useful lifetime of the glass electrode membrane and is highly individualistic. High temperatures, frequent measurements in alkaline solutions, repeated etchings, and improper maintenance will reduce the electrode's lifetime, whereas proper maintenance will prolong the useful lifetime. The glass membrane will, however, deteriorate gradually even when stored dry. A standard glass electrode, whether a mono-probe or a combination electrode, will usually last for 12–18 months.

23.3.3 Calomel Electrode or Reference Electrode

The purpose of the reference electrode is to provide a stable, reproducible voltage to which the working (detector) electrode potential may be referenced. A reference electrode may be considered a small battery whose voltage is determined by the chemistry taking place between a solid conductor, which is usually a metal salt, and the electrolytic solution around it. Ideally, if a small current is passed through the electrode, the potential change is negligible. Also, the potential value of the reference electrode should not vary with time and should be reproducible from electrode to electrode.

The most common reference electrodes which meet these criteria are:

- Mercury/mercurous electrode (calomel)
- Silver/silver chloride (Ag/Agcl)

Therefore, in order to measure the potential changes of the pH-sensitive electrode directly it is necessary that the pH cell be completed by means of a stable reference electrode, whose potential remains unaffected by changes in the composition of the cell solution. The reference electrode against which the potential of the glass electrode is measured is the calomel electrode. It consists of (Figure 23.3) a metallic internal element, typically of mercury-mercurous chloride (calomel) or silver-silver chloride, immersed in an electrolyte, which is usually a saturated solution of potassium chloride.

The electrolyte solution forms a conductive salt bridge between the metallic element and the sample solution, in which the measuring

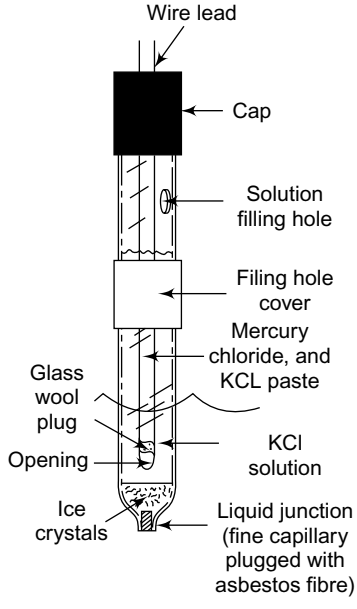


Figure 23.3 *Calomel electrode (Courtesy: M/s Beckman Instruments, USA)*

and reference electrodes are emplaced. For a stable electrical connection between the internal metallic element and the sample solution, a small but constant flow of electrolyte solution is maintained through a liquid junction in the tip of the outer body of the reference electrode. Depending upon the nature of the application, this junction may be formed in several ways. For example, the tip of the electrode could be formed by an embedded linen or asbestos fibre, or by a permeable composition of pressed-sintered carborundum and glass pellets.

$$1/2\ Hg_2Cl_2 + e \rightleftharpoons Hg + Cl^-$$

Since at a given temperature the activity of the mercurous chloride is constant and that of the mercury is unity by definition, it is the chloride ion activity which is potential determining. When this is fixed, the electrode has a fixed potential at a fixed temperature. The most commonly used source of chloride ion is potassium chloride at saturated 3.8 M, 3.5 M or 0.1 M concentrations. Saturated electrodes have largely replaced electrodes with other KCl concentrations. They are used in practical pH measurements at high as well as low temperature. However, their useful life is known to be short at temperatures above 70°C.

Although calomel reference electrode is the most commonly used electrode, it is sometimes necessary to use other types of reference electrodes in special circumstances. For example:

- The Ag/AgCl electrode is recommended as a reference electrode at high temperatures (–10 to + 110°C).
- The *mercury/mercurous sulphate electrode* with a salt bridge solution of potassium sulphate is used *in test* solutions that must not contain chloride ions.
- The *calomel* chloride with a salt bridge solution of saturated lithium chloride (LiCl) is used for measurement is non-aqueous solutions. Li Cl is more suitable in organic solvents than is Kcl.
- A calomel electrode with open liquid junction facilitates measurements in contaminated samples and samples containing solid particles.
- A calomel electrode with duplicate salt bridge (potassium nitrate *as* extra salt bridge) may be used if test solution should not contain chloride ions.

The reference electrode should preferably be stored in a small beaker containing the salt bridge solution for short term storage. For long term storage, the electrode should be rinsed, dried and stored with the end cap on and the rubber band covering the filling hole in place.

The solution in the salt bridge should always be on a higher level than the solution to be measured, as infiltration of the sample solution may occur in the salt bridge, that is, the direction of flow should always be from the salt bridge into the sample solution. If this cannot always be achieved, monthly changing the salt bridge solution should be the norm.

Annular ceramic junctions and porous pin junctions can occasionally become blocked due to crystallisation of the salt bridge filling solution. Soaking in the salt bridge solution usually remedies the situation, but, on those instances that it doesn't, raising the temperature to the maximum allowable for the reference system will often help. A precipitate of solver chloride or sulphide may clog the porous pin. The gentle use of an abrasive paper (e.g. emery cloth) will remove most blockages. Soaking the porous pin for a few hours in acidic solution of thiourea (1 m thiourea in 0.1 M HCl) will usually do what the abrasive paper sometimes fails to do, that is, chemically clean the blockage.

Trapped air bubbles can also cause malfunctions. These bubbles can be removed by gently tapping the electrode or shaking it downward as one would a clinical thermometer.

The useful lifetime of a reference electrode depends on the maintenance and care given to the electrode. The electrode should never be allowed to dry out, the junction should be kept clean, and the salt bridge should always be filled to the level intended by the manufacturer. With proper maintenance, the lifetime for a reference electrode is indefinite, but usually greater than two years. By providing good maintenance care to the electrodes, proper calibration should be able to be performed easily. If there is a continued problem, the electrodes should be replaced or examined again.

When performing a calibration with two buffers, stability should occur within approximately 1 min in each case. The zero point and sensitivity should be written down after each calibration since a large deviation from one calibration to the next indicates a problem.

23.3.4 Silver/Silver Chloride Reference Electrode

Silver/silver chloride (Ag/AgCl) electrode features glass-body construction with a porous ceramic frit at the solution interface. The filling solution is 3M NaCl gel that has been saturated with AgCl, the gel is semi solid and will appear cloudy with occasional particles.

Figure 23.4 shows the basic construction of silver/silver chloride electrode. A porous reference junction separates the filling solution in the electrode from the solution whose pH is to be measured. The filling solution's constant chloride ion concentration generates potential at a pure silver wire with silver chloride on int. The silver wire passes the signal from the solution being measured to the electrodes cable or connector. This configuration of the electrode is called Single Junction Reference.

For most applications, a single junction reference electrode is adequate. However, if samples contain proteins, sulphides, heavy metals or any other material which interacts with silver ions, they may react with the gel, causing a reduction in the reference output. This reaction can lead to erroneous reference signals or to precipitation at the reference junction leading to a short service life. A double junction reference electrode design as shown in Figure 23.5 offers a barrier of protection to combat the above interactions. In this design, the inner chamber contains the usual high (3.5 M or higher) salt concentration solutions so that stable outputs are generated. The outer chamber, which contacts the sample through the porous reference junction, is filled with 0.1 M KCl. This lower ionic strength material more closely matches that of the sample and further reduces spurious potentials.

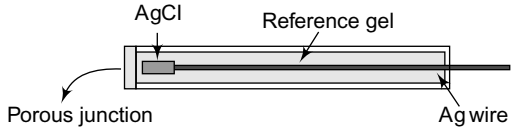


Figure 23.4 *Silver/silver chloride reference electrode-single junction*

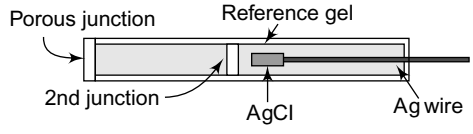


Figure 23.5 *Silver/silver chloride reference electrode-double junction*

The Ag/AgCl reference electrodes are easily spoiled by drying. It is, therefore, advisable to keep the tips wetted at all times and store in 3 M NaCl when not in use. This helps to extend its life time. They usually last for 3–6 months.

The use of a double junction electrode with a second internal junction not containing KCl or a modified internal filling solution can be used. An electrode utilising mercurous sulphate and potassium sulphate is one example. Several other combinations can be found in Table 23.1.

Table 23.1 *Potentials for different reference electrodes*

Type of reference electrode	Salt bridge solution(s)	Potential vs. standard H_2 electrode	Potential vs. sat. calomel electrode
Hg/Hg_2Cl_2	Sat. KCl	244 mV	0 mV
Ag/AgCl	Sat. KCl	200 mV	–44 mV
Hg/Hg_2SO_4	Sat. K_2SO_4	640 mV	408 mV
Calomel	Sat. LiCl	~200 mV	~ –45 mV
Hg/Hg_2Cl_2	Sat. KCl/KNO_3	244 mV	~ 0 mV

23.3.5 Combination Electrode

A combination electrode is one in which the pH-sensitive glass electrode and the reference electrode are built together (Figure 23.6). In this arrangement, the internal reference electrode in the glass electrode is identical with the external reference electrode. Both reference electrodes are the silver/silver chloride type. Also, the inner solutions in the two parts of the electrode are identical and they both are symmetrical electrode chains. The two silver/silver chloride reference electrodes are protected against light by means of ruby-red glass, which absorbs light. The construction ensures that the two electrodes are at the some temperature during operation.

A combination electrode can be used within the temperature range –10°C to +100°C as they have silver/silver chloride reference electrodes. Also, the measuring error due to small temperature differences of the buffer solutions and the sample are negligible.

Combination electrodes containing the electrolyte in the gel format have been introduced, which provide the advantage of low maintenance. They are generally supplied with plastic bodies, and are more rugged than liquid filled glass electrodes. Hence, they are commonly used with portable pH metres as shown in Figure 23.7. The gel used is a non-toxic, USP grade, organic material or it may be polyacrylamide.

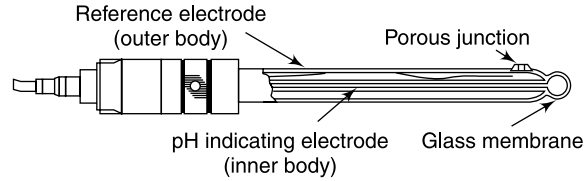


Figure 23.6 *Combination electrode*

Figure 23.7 *Portable pH metre (Courtesy: M/s Hanna Instruments, www.hannainst.com)*

Most gel-filled electrodes utilise a wick or diffusion style junction. These junctions allow the sample to diffuse in and the reference gel to diffuse out, causing a shift in potential. Some samples also react with the silver ion in the gel, which forms a precipitate. Both of these processes can cause clogging of the junction, as manifested in slower and less accurate response over time.

Beckman coulter gel-filled combination electrodes provide a special micro-pore junction

which overcomes the above problems. The junction consists of several small pores in the actual body of the electrode which can be wiped clean each time the electrode is rinsed. There is direct contact between the sample and the gel, thus resulting in faster readings. Additionally, this allows the electrode to be stored dry and eliminates the need for a special storage solution. The gel-filled electrodes operate in a wide temperature range (–5 to 100°C) and are available in various shapes, sizes and lengths to meet varied requirements.

The electrodes should never be stored dry. The preferred choice for storing is KCl solution of any concentration between 2.0 M and 3.8 M. Another good choice is buffer solution (pH4). If it is necessary to clean the probe with acid, caustic, solvent or other cleaning solution, it is best to soak the electrode in KCl solution after cleaning and prior to use or calibration. This will re-condition the bulb and reference, extending the probe life and improving calibration accuracy.

23.3.6 The Asymmetry Potential

If identical solutions are placed inside and outside the bulb of the glass electrode, it is found that in spite of the apparent symmetry of the cell so formed, there exists an emf of a few millivoltt. This potential difference is called the asymmetry potential and is thought to arise from the different states of strain on the inside and outside surfaces of the glass electrode bulb. The asymmetry potential of a glass electrode is not absolutely constant, but may drift slightly from day to day. However, it is not ordinarily subject to large and sudden fluctuations, particularly in glass electrodes, and the existence of this potential is therefore of no practical consequence in the pH measurement. It may be regarded, for a short series of measurements, as a constant of the cell assembly. In most of the commercial pH metres, this constant potential is compensated by adjustment of the standardising control knob or zero adjustor, when the instrument is made to read the pH for the standard solution. The standardisation of the instrument is carried out immediately before the measurements are made on unknown solutions. As an extra precaution, the standardisation procedure can be repeated at the end of the measurement.

23.3.7 Buffer Solutions

A buffer may be defined as a solution whose pH remains nearly constant, despite the addition of a substantial quantity of acid or base. Buffers are employed for the standardisation of pH cells. The cell emf is measured for given buffers and related to the known pH values for the buffers. The pH value of an unknown solution is then derived from this calibration. Many buffer solutions have been reported in literature. There are also British standards and standards of NBS on buffers.

Buffer tablets are commercially available, which when dissolved in an appropriate quantity of fresh distilled water, give buffer solutions. These tablets may contain the buffer material admixed with substance, which aid in tabletting but do not significantly affect the pH. Buffer tablets for pH 4, 7 and 9.2 are available commercially.

Buffers should be protected from exposure to the atmosphere, where gases such as carbon dioxide, ammonia and oxygen are present, as they will tend to change the pH value of the buffer solution. Since air would enter the bottle everything the buffer containing bottle is opened, a buffer solution would have a limited useful lifetime, when reliable pH measurements are required. Also, the pH value of a buffer solution depends upon the temperature and the actual value for the test temperature can be found on the label on the bottle.

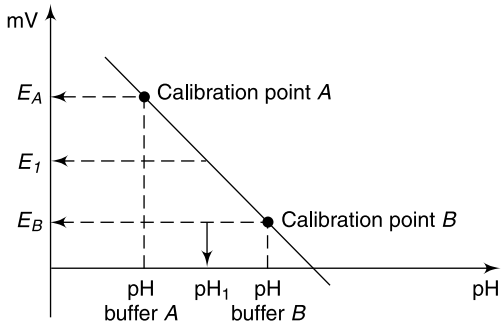


Figure 23.8 *Calibration curve developed using two buffers*

Buffers with high precision have only a limited lifetime, a limited stability. They must be used within a short period of time, governed by how precise the measurements must be. Though alkaline buffers pose the greatest problem because of their tendency to absorb carbon dioxide from the air, a buffer solution in an opened and capped bottle, will last for a limited period of time. Even unopened buffers in thin, plastic bottles have a tendency to absorb CO_2 from the atmosphere. Thick plastic bottles, sealed in cans under a nitrogen atmosphere, have the greatest advantage for maintaining optimal solutions.

High quality buffers contain a small amount of germicide to prevent microbiological growth, since many of the buffers are excellent culture media. Buffers with other additives, including many coloured dyes, could disturb the pH value or the stability of the solution. They may have an adverse effect on the liquid junction.

23.3.8 Calibration

Each electrode has slightly different characteristics. Electrodes with different nominal values are produced by different manufacturers. The zero pH and the sensitivity will vary with time. Calibration matches the pH metre to the characteristics of the electrodes being used and continued calibration of the electrode on a regular basis corrects for continually changing characteristics during the lifetime of the electrodes. The most accurate calibration is performed using two different buffer solutions (Figure 23.8). This enables both pH^0 (zero pH) and the slope (sensitivity) to be determined.

The sensitivity is independent of temperature and is usually stated as a percentage of the theoretical value. The slope, when expressed as mV/pH, is directly dependent on temperature. The slope at 25°C is often used as an alternative to sensitivity in % (100% = 59 mV/pH).

Zero pH (pH^0) is generally used to describe electrode characteristics, thought the potential at pH 0 or pH7 at 25°C can also be given.

In modern pH measuring instruments, it is not normally necessary for the operator to construct a calibration graph and interpolate the results for unknown samples. Most pH electrodes when connected directly to a pH metre perform the calibration automatically. This determines the slope mathematically and calculates the unknown pH value for immediate display on the metre.

23.4 pH METERS

The function of the pH metre is to measure the potential difference (in mV) between the electrodes and convert it to a pH display.

In the earlier days, the pH of a solution was determined with the change in colour observed on pH paper when dipped in the solution of interest. The colour was compared with the colour chart. It is possible to read pH to an accuracy of 0.1 pH by this method.

With the advent of glass and calomel electrodes and with the development of very stable, drift-free DC amplifiers with extremely high input impendence, pH measurements too much better accuracy have become possible.

pH electrodes are basically voltage sources with a very high internal resistance. To avoid errors in the measurement of voltage, no current should flow from the source if possible. This means that the whole measuring circuit, from the electrode via cable, plug, socket, switching element up to the measuring amplifier itself must be very well insulated. Only high-quality insulation material such as PTFE (Teflon), polyethylene, glass, etc., must be used to get insulation resistance of upto 10^{14} ohms. It may be noted that even a small contamination by atmospheric deposits or from split liquids can influence the insulation values.

The following considerations govern the design of pH metres:

- The internal resistance of the electrode is very high. It is of the order of 1,000 MΩ. Therefore, the input impedance must be at least 1,000 times more than the resistance of the pH cell. Also, the measurement should be unaffected by large changes in magnitude of this resistance.
- Current should neither be drawn by the pH metre from the solution nor should any current flow on the electrode, which might result in the polarisation of the electrode. Such polarised electrodes will give rise to erroneous results.
- The metre must have a provision for compensating changes in pH readings due to changes in temperature.

In general, commercial pH metres can be categorised broadly into the following two main types:

- The null-detector type or the potentiometer type
- The direct-reading type

In the instruments of the first type, the procedure followed is essentially that used in potentiometers. An emf equal and opposite to that of the pH cell is applied, so as to give zero reading on a galvanometer.

Direct-reading instruments are similar to voltmeters of the deflection type. The current signal available after the amplifier is used to operate a metre, suitably calibrated in pH units. Of the two types, the null-detector type of instrument is inherently capable of greater accuracy than the direct-reading type. However, the convenience and the usefulness of the direct-reading type metres in following the changes of pH that occur during the course of a reaction have made them more popular.

The pH metres invariably make use of some amplifying device to amplify emf produced in the pH cell. As the amplifier input stage must have an extremely high input impedance, a number of commercial pH metres have been making use of special electrometer tubes. They have a very low grid current and the stray currents emanating from surface leakage emission of photoelectrons by the grid under the action of light and soft X-rays. Quite recently, electrometer tubes have been replaced by solid-state devices, like field-effect transistors (FETs), MOSFET's and integrated circuits having high input impedance. IC 8007 is an example of the integrated circuit having FET input stage and which is quite suitable to be used in the input stage of a pH metre. With modern semiconductor components, a pH metre with an input resistance of up to 10^{17} Ω can be built with little difficulty.

For making pH measurements, the emf of a pH cell is amplified in a direct coupled. DC amplifiers give rise to zero drift errors, which must be eliminated for getting accurate results. Improvements in zero stability are possible by the use of low drift and low input offset voltage integrated circuits and

highly stable power sources. Zero drift can also be reduced by the use of balanced and differential amplifiers. They are so constructed that their responses to external signals are additive, while those to internal noise or drift are subtractive. Several other methods are available, by which zero stability can be achieved to a great extent by using zero corrected dc amplifiers, contact-modulated amplifiers and vibrating capacitor modulated amplifiers. Then methods are discussed in the subsequent sections.

The Nernst equation, on which pH measurements are based contains temperature dependent component. Therefore, arrangements are invariably made for automatic or manual compensation in changes of pH due to changes in temperature in the commercial pH metres. The instrument is calibrated at one temperature (say 25°C) and compensation is applied by suitable adjustments of the output current of the amplifier. The current is adjusted by incorporation of a variable resistance in the output circuit, so that the calibration point may then correspond to the desired temperature. This ensures that for a given pH, the current to the metre is constant.

Automatic temperature compensation is achieved by using a resistance thermometer or thermistor in the output circuit. The thermistor is mounted on the electrode holder and is immersed in the test solution, along with the electrodes. As the temperature of the solution changes, the circuit constants are altered accordingly.

23.4.1 Design considerations for pH Meters

Inherently, bipolar transistors are current amplifying devices with a low input impedance. For these reasons, conventional transistor amplifiers are seldom employed for pH measurements. Instead, FETs are used at the input stage to achieve a high input impedance. Metal-oxide silicon FETs of the insulated gate type have very small input leakage currents. A pair of these can be used to construct a pH metre. Figure 23.9 shows one such circuit. This circuit makes use of two MOSFETs in a differential amplifier configuration. This circuit is suitable to be used with combination type pH electrode. The differential input cancels the common mode errors, such as effects of temperature and supply voltage variations. The metre used is of centre zero type. Initially the input is grounded and the potentiometer R_7 is adjusted to bring the metre pointer to centre of the scale (i.e. pH^7). R_3 is

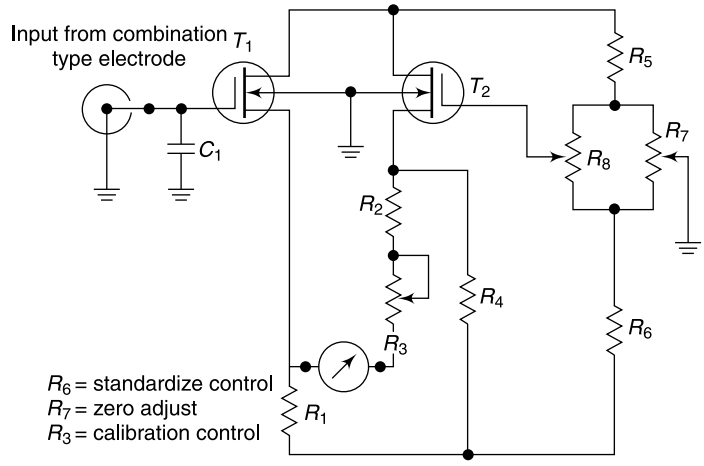


Figure 23.9 *Circuit arrangement of a pH metre using MOSFETs at the input stage*

adjusted to read 0 pH after a simulated electrical signal (for 25°C) is given to the input, corresponding to a change of 7 pH units.

Before the pH of a solution is measured, the pH metre is standardised by dipping the electrode in a solution of known pH and adjusting R_6 (standardise control), until the metre indicates the known pH. After the metre is thus standardised, the probe is rinsed in distilled water, wiped dry and placed in the solution whose pH is to be measured.

The pH readings can be corrected if the measurements are made at temperatures other than 25°C by the following formula:

$$\text{pH error} = (\text{T}-25)\ (\text{pH}_1 - \text{pH}_2)/\text{T} + 273$$

where pH_1 is the instrument reading and pH_2 is the pH of the buffer.

Care should be taken to wire the circuit on high-quality glass epoxy base printed circuit board. As MOSFET's are easily damaged by static electric charges, care should be taken to short their leads with wires during assembly and wiring of the circuit. This circuit is usually employed in pocket pH metres.

Figure 23.10 shows another circuit arrangement which uses a matched pair of FETs housed in a single can. The circuit would provide input impedance greater than 10^{12}.

The emf produced by the measuring electrode is given to the gate of transistor T_1. The potential applied to the input 1 of the operational amplifier would depend upon current, which passes through the transistor and its corresponding resistance R. The potential applied to the gate of transistor T_2 is set by the buffer bias adjustment, which is fed from a zener stabilised potential supply. The potential developed across R_2 would depend on the current through T_2. Resistance R_1 and R_2 are kept equal. Therefore, the output of the operational amplifier will depend upon the difference in potential developed on the measuring electrode and the potential set-up in the instrument. The current flowing through the indicator also flows through the manual or automatic temperature compensating resistor. Thus, potential applied to the reference electrode can be arranged to

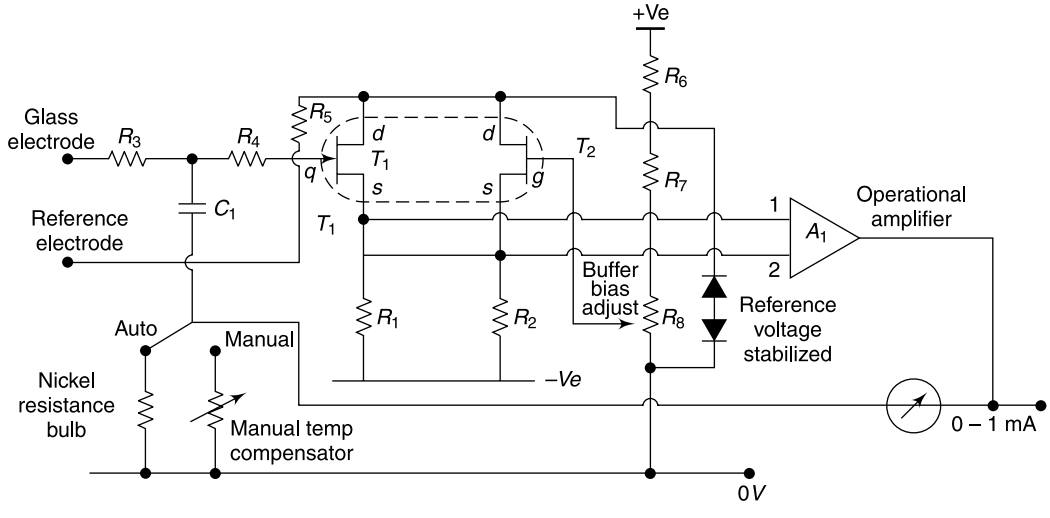


Figure 23.10 *Direct-reading type pH metre with temperature compensation arrangement*

compensate for the change in slope of the pH/temperature relationship, that is, the gain of the system can be changed by the negative feedback *across* the temperature compensator, so as to match the slope of the pH/temperature relationship.

23.4.2 Digital pH Meters

A digital pH metre signifies a major advancement in pH measurements by offering a resolution of 0.1 or even 0.01 mV, thereby enabling much greater accuracy. This means that the stability and reproducibility of the electrode response become the main limiting factor in determining the accuracy and precision.

The amplifier and other circuits must have a small temperature coefficient (i.e. the influence of temperature variations must be under control to attain reliable and consistent results). Operational amplifiers with FET input stage are available in the integrated form. They can be directly coupled to the electrodes to amplify signals from the pH cell.

Figure 23.11 shows a microprocessor-based digital pH metre. The amplifier works under the same conditions all the time and is directly connected to an analog-to-digital (A/D) converter. The input amplifier and the converting circuit must meet certain requirements in order to obtain a correct measurement. A potential difference between the glass electrode and the reference electrode is amplified in the mV amplifier before the A/D converter feeds the signal to the microprocessor for calculation of the result.

The amplifier's input resistance, R_1, must be considerably higher than the inner resistance of the typical glass electrode, that is, higher than 10^8 Ohms. It is also important that the amplifier does not send any current through the glass electrodes as this will give an error potential and could even damage the electrode. The bias current or so-called terminal current, I_{term}, should therefore be below 10^{-12}A.

A microprocessor-based circuit then translates the converter's output and the calculated pH is displayed. A temperature sensor provides both temperature display and a temperature correction. For some microprocessor systems, automatic recognition of calibration buffers and automatic stability control of the electrode signal is possible.

Microprocessor-based instruments contain simple programmes to calculate the slope and intercept from the calibration data and then use these to calculate the sample concentration from the millivolt reading in the sample. The operator can simply enter the concentrations of the standards and measure the millivolts, then immerse the electrodes in the sample and read the sample concentration directly from the metre. These instruments usually have small keypads and are prone to errors in data entry.

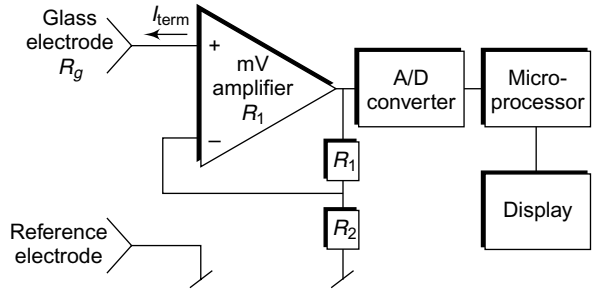


Figure 23.11 *Block diagram of a microprocessor-based digital read out direct-reading pH metre*

23.4.3 pH Sensing Integrated Analog Front End

The Texas Instruments have introduced an integrated analog front end (AFE) module which interfaces with all of today's pH sensors and bridges the gap between sensor and microprocessor. The internal circuit diagram of sensor AFE is shown in Figure 23.12. Its chip form is shown in Figure 23.13 and is designated as LMP91200.

The LMP91200 provides either a differential or single-ended analog output. The modes and configuration are adjusted via SPI. It draws only 50 uA power while measuring pH. The fundamental blocks LMP 91200 are described below:

pH Buffer: The pH Buffer is a unity gain buffer with a very low input bias current which introduces a negligible error in the measurement of the pH. The pH buffer is provided with 2 guard pins in order to minimise the leakage of the input current and to make easy the design of a guard ring.

Programmable Gain Amplifier (PGA): The internal current source is programmable current generator which is able to source different current values in order to well stimulate Pt 100 and Pt 1000 thermal resistor. The selected current is sourced from either RTD pin or CAL pin (pin for reference resistor connection). The voltage across either the thermal resistor or the reference resistor is amplified by the PGA and provided at the VOUT pin when the LMP91200 is set in Temperature measurement mode.

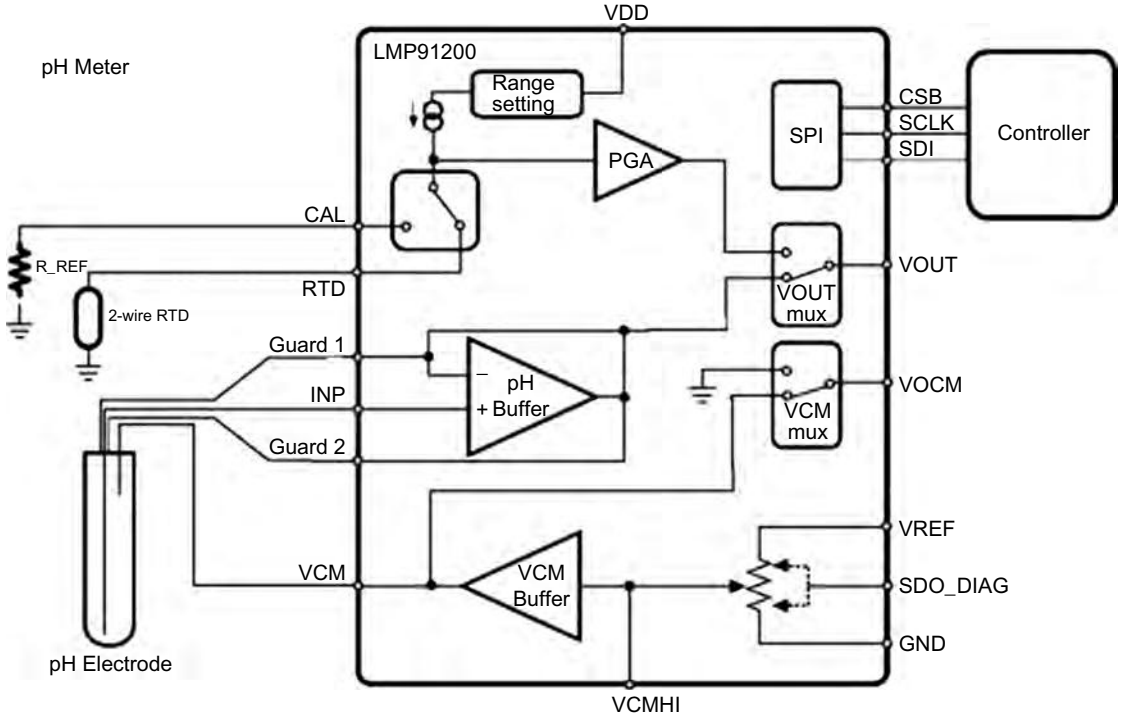


Figure 23.12 *Circuit diagram of LMP91200 pH sensor integrated chip (Courtesy: M/s Texas Instruments)*

Common mode selector and VCM buffer: The common mode selector allows to set different values of common mode voltage according to the applied voltage reference at VREF pin. Both buffered and unbuffered version of the set common mode voltage are available respectively at VCM pin and VCMHI pin. A copy of the buffered version is present at VOCM pin in case of differential measurement.

Figure 23.13 *LMP91200 pH Sensor integrated circuit (Courtesy: M/s Texas Instruments)*

Output Mixer: The output of the LMP91200 can be configured to support both differential and single-ended ADCs. When measuring pH, the output signal can be referred either to VCM or GND. When measuring temperature, the OUTPUT signal is referred to GND. The output configuration is controlled through the SPI interface.

Serial Control Interface Operation: All the features of the LMP91200 (Mode of Operation, PGA Gain, Voltage reference, Diagnostic) are by data stored in a programming register. Data to be written into the control register is first loaded into the LMP91200 via the serial interface. The serial interface employs a 16-bit shift register. Data is loaded through the serial data input, SDI. Data passing through the shift register is output through the serial data output, SDO_DIAG. The serial clock, SCK controls the serial loading process. All sixteen data bits are required to correctly program the LMP91200.

The LMP91200 is a configurable sensor AFE for use in low-power analytical sensing applications. The LMP91200 is designed for 2-electrode sensors. This device provides all of the functionality needed to detect changes based on a delta voltage at the sensor. Optimised for low-power applications, the LMP91200 works over a voltage range of 1.8–5.5 V. With its extremely low input bias current, it is optimised for use with pH sensors. Also in absence of supply voltage, the very low input bias current reduces degradation of pH probe when connected to the LMP91200. The Common Mode Output pin (VOCM) provides a common mode offset, which can be programmed to different values to accommodate pH sensor output ranges. Two guard pins provide support for high parasitic impedance wiring. Support for an external Pt1000, Pt100, or similar temperature sensor is integrated in the LMP91200. The control of this feature is available through the SPI interface. This function tests the sensor for proper connection and functionality. Available in a 16-pin TSSOP package, the LMP91200 operates from –400 C to +1250 C.

23.4.4 Industrial pH Meters

The measurement of pH is carried out in industry to realise either for the proper reaction to take place or for the quality of the end product, and thus contributing to better quality products at lower production costs. Measurements and control of pH normally need to be done either in a tank or on steam in a pipe. For these examples, the electrode systems could be dip-type or mounted online.

Quite often, the electrode system and the control unit could be as far apart as 50 m. There would be a strong possibility of interference due to pick up by the cable connecting the two. Special type of shielded cables are used for this purpose. The second factor is the loss of signal strength in the connecting cable. This problem can be overcome either by compensating the loss by the amplifier

circuit or alternatively fitting the amplifier into the electrode system and the amplified signal is transmitted through the cable to the display system kept at a convenient place. This, however, introduces problems of on-site maintenance.

There could be situations when the measurement is to be carried out in an explosive atmosphere. In this case, the design should be such as not to ignite the combustible gases. Normally, the amplifier part is housed in an explosive proof chamber and it is operated preferably on batteries, thereby minimising chances of sparking in the circuit. Industrial pH measuring instruments are fitted with contacts for operating visual or audible alarms, or activating control valves providing pH control of the process within preselected limits.

The output from the industrial pH amplifier is fed into recorders, controllers, indicators, etc. There is usually a choice of outputs and in many cases these are isolated from earth. Any leakage current from external equipment would be fed back to the circuit via the reference electrode causing an error in reading. An isolator eliminates this problem. The DC output current from the pH metre is periodically reversed by a transistor switch. The resultant square wave is then coupled through a transformer and reconverted to DC by a second synchronised switch. The transformer interrupts the dc path between the pH metre and the external equipment, so that it may be earthed.

23.4.5 Failures in pH Meters

The failures in a pH metre can be classified into three main categories (a) defective electrodes, (b) defective input circuitry and (c) defective electronic circuitry.

If the instrument balances at zero with the input shorted and fails at pH measurement, it is logical to replace the electrodes with new ones. If the instrument works with new electrode, defective electrode must be replaced. Since majority of failures occur because of defective electrodes, it is advisable to have a spare set of electrodes with every instrument and use them by rotation to avoid drying up due to long storage. If the instrument fails even with new electrodes, input circuitry must be checked. The failures in input circuit could be due to poor insulation between glass electrode terminal and common terminal and excessive leakage current in the input circuit. Because of high resistance associated with the glass electrode, input circuit should have high insulation resistance and low leakage currents. These may be impaired at times by collection of dust and other vapours. In such cases, input terminals and function-selector switch should be cleaned with a solvent such as carbon tetrachloride. The capacitor connected at the amplifier input should also be checked for leakage and it should be replaced if defective. If the instrument is still defective it may be due to excessive input current. In such a case, the input stage should be checked.

If the instrument fails to balance at zero, it is most likely that electronic circuitry is defective. Before attempting the repairs, it is advisable to short-circuit the input externally and note the position of the pointer in all measuring ranges. If the pointer is within the scale, coarse buffer adjustments may be tried.

23.5 SELECTIVE-ION ELECTRODES

Over the past decade, the pH metre has been at the centre of a most important change in the field of analytical measurements due to the introduction of selective ion electrodes. As their name implies, these electrodes are sensitive to the activity of a particular ion in solution and quite insensitive

to the other ions present. As the electrode is sensitive to only one ion, a different electrode is needed for each ion to be studied. Approximately 20 types of selective ion electrodes are presently available.

In recent years a number of measuring electrodes, which possess selectivity towards some particular ion, have increased in popularity among many fields of science. These electrodes have been used in such applications as pollution control, biomedical research, clinical medicine, food processing, and metallurgy, to name a few. Various ion-selective electrodes give direct measurement of the activity of many common anions and cations such as sodium, potassium, calcium, chloride, nitrate, sulphide, as well as fluoride and many more. There are about twenty of these types of electrodes. Some electrodes measure dissolved gases such as ammonia, sulphur dioxide and carbon dioxide. In addition, several enzyme selective electrodes have been developed.

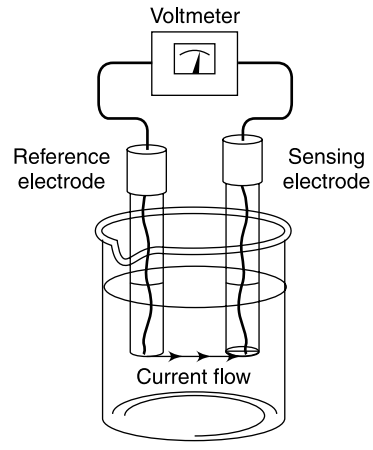


Figure 23.14 *Principle of measurement of specific ions using ion-selective electrodes*

Just like the most common pH electrodes, ion selective electrodes (ISEs) work on the basic principal of the galvanic cell. By measuring the electric potential generated across a membrane by 'selected' ions, and comparing it to a reference electrode, a net charge is determined (Figure 23.14). The strength of this charge is directly proportional to the concentration of the selected ion.

The development of a range of ISEs has stimulated interest in the solid-state chemistry of glass and crystals and in the specificity for metal ions of synthetic and natural organic and complexing agents. Following developments in cation-responsive glasses and precipitate-impregnated silicone rubber membranes, the real impact of ISEs was made by the fluoride ion-selective solid-state lanthanum fluoride crystal membrane electrode. The fluoride electrode was quickly followed by a calcium ion-selective liquid ion-exchanger membrane electrode. These and a whole host of other ISEs in various styles have been applied in fields like environmental and industrial monitoring, reaction rate studies, enzyme reactions, general analysis and non-aqueous media studies.

ISEs come in various shapes and sizes. All consist of a cylindrical tube, generally made of a plastic material, between 5 and 15 mm in diameter and 5–10 cm long. An ion-selective membrane is fixed at one end so that the external solution can only come into contact with the outer surface, and the other end is fitted with a low-noise cable or gold plated pin for connection to the millivolt measuring device. In some cases the internal connections are completed by a liquid or gel electrolyte, in others by an all-solid-state system. Figure 23.15 shows different technologies used in ISEs.

Ion-selective membranes are currently available for a number of commonly occurring ionic species. A perusal of manufacturer's catalogues reveals that the most common are as follows:

CATIONS: Ammonium (NH_4^+), Barium (Ba^{++}), Calcium (Ca^{++}), Cadmium (Cd^{++}), Copper (Cu^{++}), Lead (Pb^{++}), Mercury (Hg^{++}), Potassium (K^+), Sodium (Na^+), Silver (Ag^+).

ANIONS: Bromide (Br^-), Carbonate (CO_3^-), Chloride (Cl^-), Cyanide (CN^-), Fluoride (F^-), Iodide (I^-), Nitrate (NO_3^-), Nitrite (NO_2^-), Perchlorate (ClO_4^-), Sulphide (S^-), Thiocyanate (SCN^-).

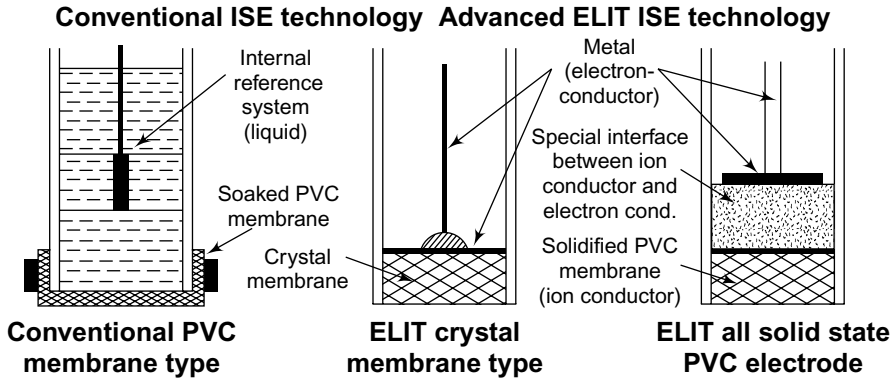


Figure 23.15 *Ion-selective electrode technology (Courtesy: www.nico2000.net)*

The manner in which these different membranes select and transport the particular ions is highly variable and in many cases highly complex. There are two main types of membrane material, one based on a solid crystal matrix, either a single crystal or a polycrystalline compressed pellet, and one based on a plastic or rubber film impregnated with a complex organic molecule which acts as an ion-carrier.

(i) **Crystal-Membrane Electrodes (e.g. Fluoride)**

The Fluoride electrode is a typical example of this type. Here the membrane consists of a single lanthanum fluoride crystal which has been doped with europium fluoride to reduce the bulk resistivity of the crystal. It is 100% selective for F^- ions and is only interfered with by OH^- which reacts with the lanthanum to form lanthanum hydroxide, with the consequent release of extra F^- ions.

(ii) **Impregnated-PVC-Membrane Electrodes (e.g. Potassium)**

The Potassium electrode was one of the earliest developed and simplest examples of this type. The membrane is usually in the form of a thin disc of PVC impregnated with the macrocyclic antibiotic valinomycin. This compound has a hexagonal ring structure with an internal cavity which almost exactly the same size as the diameter of the K^+ ion. Thus, it can form complexes with this ion and preferentially conducts it across the membrane. Unfortunately, it is not 100% selective and can also conduct small numbers of sodium and ammonium ions. Thus, these can cause errors in the potassium determination if they are present in high concentrations.

Several limitations must be kept in mind when using ISEs. First, activity and not concentration is being measured. Because the activity coefficient is dependent upon the total ionic strength of the solution, the activity will deviate from the concentration to a greater extent as the solution concentration increases. Also, the variation in concentration of any other ionic species that happens to be in the solution will affect the total ionic strength and change the activity of the ion being measured. These effects may be swamped by adding high and constant concentrations of non-interfering ions, such as sodium chloride that are available in the Total Ionic Strength adjustment Buffer (TISAB).

Second, the electrode responds only to free ions. The presence of any species, which complexes with the ion being measured, will lower its activity and therefore the electrode response. Finally, the electrode response is selective, not specific. Selectivity means interference from competing ions is possible.

The potential of the ISE arises when there are solutions of a given ion, X, on either side of a membrane. The type of membrane categorises the electrode: there are glass membranes (H^+, Na^+, K^+, Ag^+ selective), crystal membranes (F^-, Ce^{3+}, Pb^{2+} selective), liquid ion-exchange membranes (NO^{3-}, K^+, Ca^{2+} selective) and so forth.

ISEs are classified into the following four major groups:

- *Glass Electrodes*: The first glass ISE developed is the one sensitive to hydrogen ions. Glasses containing less than 1% Al_2O_3 are sensitive to hydrogen ions (H^+) but almost insensitive to other ions present. Glasses of which the composition is Na_2O 11%, $Al_2O_3$18% and SiO_2 71% is highly selective towards sodium, even in the presence of other alkali metals. Glass electrodes have been made that are selectively sensitive to sodium, potassium, ammonium and silver.
- *Solid-State Electrodes*: These electrodes use single crystals of inorganic material doped with a rare earth. Such electrodes are particularly useful for fluoride, chloride bromide and iodide ion analysis.
- *Liquid-Liquid Membrane Electrodes*: These electrodes are essentially liquid ion-exchangers, separated from the liquid sample by means of a permeable membrane. This membrane allows the liquids to come in contact with each other, but prevents their mixing. Based on this principle, cells have been developed that *are* selective to calcium and magnesium. These cells are used for measuring water hardeners.
- *Gas Sensing Electrodes*: These electrodes respond to the partial pressure of the gases in the sample. The most recent of these *to* be developed are the gas sensing electrodes for ammonia and sulphur dioxide. Ammonia or sulphur dioxide is transferred across a gas-permeable membrane, until the partial pressure in the thin film of filling solution between the glass electrode membrane and the probe membrane equals that in the sample. The resultant pH change is measured by a combination pH electrode, a potential is developed related to the partial pressure and hence the ammonia or sulphur dioxide concentration.

The applications using ISEs are many, most are time saving and simple to use. The electrodes are now used in continuous monitoring of ammonia, nitrate, fluoride, chloride, cyanide, sodium, etc., providing vital information for the power industry environmental work and process control industries.

Figures 23.16 shows examples of various types of Orion ISEs.

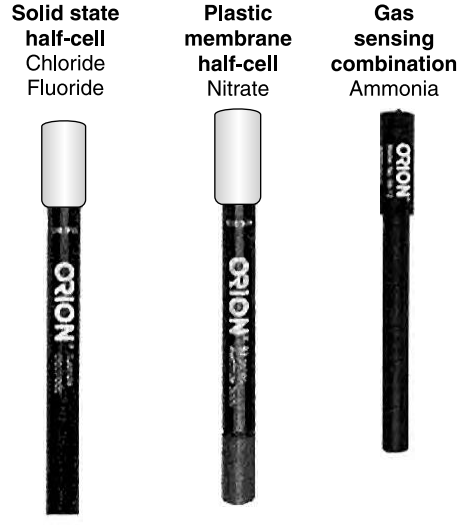


Figure 23.16 *Different types of ion-selective electrodes (Courtesy: M/s Orion, USA)*

23.5.1 Advantages of Ion-Selective Electrode

- When compared to many other analytical techniques, ISEs are relatively inexpensive and simple to use. They have an extremely wide range of applications and wide concentration range.

- The plastic-bodied all-solid-state or gel-filled models are very robust and durable and ideal for use in the field or laboratory environments.
- When measuring ions in relatively dilute aqueous solutions and where interfering ions are not a problem, they can be used very rapidly and easily (e.g. simply dipping in lakes or rivers).
- They are highly useful for the continuous monitoring of changes in concentration (e.g. in potentiometric titrations, monitoring the uptake of nutrients or the consumption of reagents).
- They are particularly useful in biological/medical applications because they measure the activity of the ion directly, rather than the concentration.
- With careful use, frequent calibration, and an awareness of the limitations, they can achieve accuracy and precision levels of ±2 or 3% for some ions and thus compare favourably with analytical techniques which require far more complex and expensive instrumentation.
- ISEs are one of the few techniques which can measure both positive and negative ions.
- They are unaffected by sample colour or turbidity.
- ISEs can be used in aqueous solutions over a wide temperature range. Crystal membranes can operate in the range 0°C to 80°C and plastic membranes from 0°C to 50°C.

The majority of pH electrodes are produced in the form of combination electrodes in which the reference system is housed with some cylindrical body as the sensor head. This arrangement minimises the effect of any stray electrostatic fields. In contrast to pH electrodes, most of the ISEs are produced as mono-electrodes for use with separate reference systems. One reason for this is because ISE membranes have a far lower impedance than pH sensors and are less susceptible to stray electrostate fields. Thus, it is not necessary to screen the sensor head by surrounding it with the reference system.

23.5.2 Problems with ISE Measurements

The main problems with ISE measurements are as follows:

- Effect of interference from other ions in solution.
- Effect of the ionic strength of the solution, thereby reducing the measured activity relative to the true concentration at high concentration.
- Drift in electrode potential during a sequence of measurements.
- Blocked or contaminated ISE or reference electrode by organic molecules.

These problems have led to the definition of the following parameters:

Selectivity Coefficient: The ability of an ISE to distinguish between different ions in the same solution is termed as selectivity coefficient. For example, if the primary ion for which the electrode is sensitive is '*A*' and the interfering ion is '*B*', then a selectivity coefficient of 0.01 would mean that the electrode is 100 times more sensitive to '*A*' than to '*B*'.

Ionic Strength: It is a measure of the total effect of all the ions in a solution. It is the sum of the molar concentration multiplied by the square of the valency of all the ions.

Activity: The effective concentration measured at the electrode head is known as the activity of the ion,

Activity Coefficient: It is the ratio of the activity divided by the concentration. The activity coefficient is always less than one and becomes smaller as the ionic strength increases. Thus, the difference between the measured activity and the actual concentration becomes higher at higher concentration.

23.5.3 Ammonia Electrode

One of the most commonly used ISEs is the ammonia electrode. The electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from the electrode internal solution (Figure 23.17). Dissolved ammonia on the sample solution diffuses through the membrane until the partial pressure of ammonia is the same on both sides of the membrane. In any given sample, the partial pressure of ammonia is proportional to its concentration.

When ammonia diffuses through the membrane, it will dissolve in the internal filling solution and react reversibly with water as follows:

$$NH_3 + H_2O \rightleftharpoons NH^+_4 + OH^- \qquad (23.1)$$

The relationship between ammonia, ammonium ion and hydroxide is given by

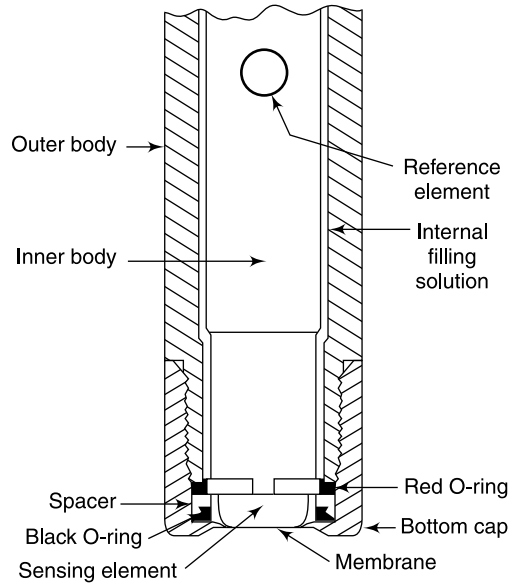


Figure 23.17 *Construction of ammonia electrode*

$$[NH_4^+]\,[OH^-]/[NH_3] = \text{constant} \qquad (23.2)$$

The internal filling solution contains ammonium chloride at a sufficiently high level, so that the ammonium ion concentration can be considered fixed. Therefore

$$[OH^-] = NH_3 \text{ Constant} \qquad (23.3)$$

The potential of the electrode sensing element with respect to the internal reference element varies with changes in the hydroxide level:

$$E = E_0 - S \log [OH^-] \qquad (23.4)$$

where S is the electrode slope

Since the hydroxide $[OH^-]$ concentration is proportional to ammonia concentration [Eq. (23.4)], electrode response to ammonia is given by

$$E = E_0 - S \log [NH_3]$$

The reference potential E_0 is partly determined by the internal reference electrode, which responds to the fixed level of chloride in the internal filling solution.

When plotted on a semi logarithmic paper, electrode potential response as a function of ammonia concentration is shown in Figure 23.18. The response is a straight line with a slope of about 58 mV per decade.

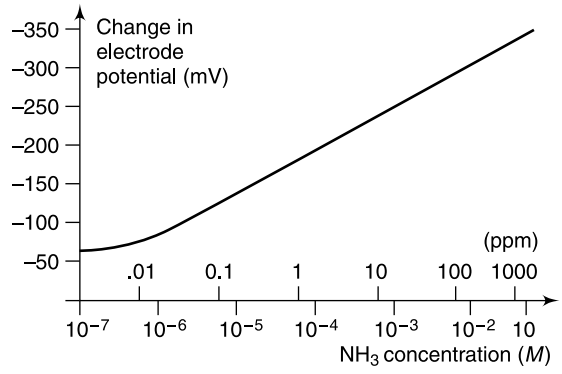


Figure 23.18 *Ammonia electrode potential response as a function of ammonia concentration*

The response time of the electrode is about 1 min (95% of total mV reading) for ammonia concentrations above 6×10^{-5} M (1 ppm NH_3 or 0.8 ppm N). Below this value, response times are usually longer. Also, there could be errors due to ammonia absorption from the air. Samples above 1 M in ammonia concentration should be diluted before measurement.

Temperature changes cause electrode response to shift and change slope. At 10^{-3} M, a 1°C temperature change produces a 2% error. Therefore, it is necessary that the samples and standards should be at the same temperature.

Water vapour is a potential electrode interference, as it can move across the membrane, changing the concentration of the internal filling solution under the membrane. Such changes manifest themselves as electrode drift. The problem is minimised if the total level of dissolved species in solution (osmotic strength) is below 1 M. Therefore, samples with osmotic strengths above 1 M should be diluted before measurement However, dilution should not reduce the ammonia level below 10^{-5} M.

The reproducibility of results in ammonia electrodes is a ticklish problem and is limited by several factors, such as temperature fluctuations, drift and noise. With calibration every hour, electrode measurements to ±2% can be obtained. Within the operating range of the electrode, reproducibility is independent of concentration.

Ion sensitive electrodes are characterised by what is called *electrode slope*. Slope is defined as the change in millivolts observed with every tenfold change in concentration. For ammonia electrode, *the* change in millivolts should be in the range of –54 to –60 mV/decade, when the solution temperature is between 20 and 25°C.

Ammonia electrode allows measurements not only of dissolved ammonia in aqueous solutions, but also it can be used to measure the ammonium ion after conversion to ammonia or organic nitrogen after Kjeldahl digestion of the sample. The performance of the electrode is not affected by sample colour and turbidity. There is no interference from almost all anions, cations and dissolved species, other than volatile amines.

23.5.4 Fluoride Electrode

The fluoride ISE is a solid-state device that measures fluoride ion in water and some organic solvents. The fluoride selective electrode is a solid-state type electrode consisting of a lanthanum fluoride crystal sealed over the end of an inert plastic tube which contains an internal electrode

and filling solution usually of 0.1M NaCI and 0.1M NaF. A potential arises because of the difference in fluoride activity on either side of the crystal. The crystal is an ionic conductor in which only fluoride ions are mobile.

When the membrane is in contact with a solution containing fluoride ions, an electrode potential develops across the membrane. The magnitude of this potential is dependent upon the level of fluoride ion in solution. The potential across the membrane is measured against an external reference electrode with a digital pH/millivolt metre. The potential corresponding to the level of fluoride ions in solution is described by the Nernst equation:

$$E = E^{o} - 0.0591 \log A$$

where E = measured electrode potential
E^{o} = reference potential (a constant)
A = fluoride level in solution

The level of fluoride, A, is the activity or 'effective concentration' of free fluoride ions in solution. The total fluoride concentration, C_{total}, may include some bound or complexed ions as well as free ions. The electrode responds only to free ions, whose concentration is:

$$C_{free} = C_{total} - C_{bound}$$

Where C_{bound} is the concentration of fluoride ions in all bound or complexed forms.

The fluoride activity is related to free fluoride concentration by the activity coefficient. Ionic activity coefficients are variable and largely depend on ionic strength.

The pH range for the fluoride selective electrode is limited at the acid end by the formation of HF and at the basic end by hydroxide interference. Polyvalent cations of Si^{+4}, Fe^{+3}, and AI^{+3} also interfere by forming complexes with fluoride. To avoid problems arising from these possible interferences, TISAB is added in excess and in exactly the same amount to each of the solution that will be measured. This TISAB buffer (Tissue, 2000b) renders all of the solutions to about a pH of 5.0 and contains a complexing agent to complex polyvalent cations. Most importantly, however, it contains a high concentration of NaCI to provide a constant ionic strength to each solution.

The fluoride electrode also responds to metal ions that complex with fluoride (e.g. AI^{3+} and Fe^{3+}). Such ions constitute an interference if present in sufficiently large concentrations. The response of the electrode to another interfering ion can be included in the Nernst equation which is shown below for a general case:

$$E = E^{o} + (0.0591/n) \log(a_A + K_{AB} a_B n/z)$$

Where E^{o}, = a combination of several constants
a_A = activity of species A with charge n
a_B = activity of interferent B with charge z
K_{AB} = the selectivity constant of the electrode for A over B

The selectivity constant is a measure of the extent of the interference posed by a particular ion that might be present in the sample. The selectivity of the fluoride electrode is excellent, OH^{-} being the main interference.

The pH range for satisfactory measurements with the fluoride electrode is from 5 to 7 at 10^{-6} M F^- (up to pH 11 at 0.1 M F^-). In acid solutions with a pH below 5, hydrogen complexes a portion of fluoride in solution, forming undissociated acid HF and the ion HF_2^-. Hydroxide ion interferes with the electrode response to fluoride when the level of hydroxide is greater than one-tenth the level of fluoride ion present.

A calibration curve is prepared for the response of a particular electrochemical cell to a series of standard fluoride solutions. The measured potentials are plotted versus the logarithm of the concentrations or activities of the fluoride ion. The result is a straight line with a slope of 59.16 mV if a Nernstian response is obtained. In neutral solutions, fluoride concentration can be measured down to 10^{-6} M (0.02 ppm) fluoride. The upper limit of detection is a saturated fluoride solution.

The electrode response curve will not only shift but will change slope with changes in temperature. A 1°C change in temperature will result in a 2% error at the millimolar level. Ideally, standards and samples should be kept at a constant temperature, preferably 25°C. The fluoride electrode has a operable temperature range of 0° to 80°C under continuous use.

Solid-state electrodes have a typical life of 1–2 years in the laboratory and 1–3 months when used continuously at elevated temperature. The main advantage of solid-state sensors over silver metal-silver halide electrodes of the second type is their insensitivity to redox interferences and surface poisoning.

23.5.5 Care and Maintenance of ISEs

When handling ISEs, care should be taken to avoid damaging the membrane surface. If the electrodes are in frequent use then they can simply be left hanging in the electrode holder with the membrane surface open to the air but protected by a clean dry beaker. For prolonged storage in a cupboard or drawer, the membrane should be protected by covering with the rubber or plastic cap which is normally provided with the electrode. After extensive use, the membranes may become coated with a deposit or scoured with fine scratches which may cause a slow or reduced response (low slope) or unstable readings.

Crystal membranes can be regenerated by washing with alcohol and/or gently polishing with fine emery paper to remove any deposit or discolouration, followed by thoroughly washing with de-ionised water to remove any debris. After this, they may require soaking in the concentrated standard solution for a few minutes before a stable reading can be re-established. However, that prolonged immersion of crystal membranes in aqueous solutions should be avoided because this can cause a build-up of oxidation products on the membrane surface and thus inhibit performance. Conversely, PVC membranes should not even be touched, let alone polished. The can often be regenerated by prolonged soaking over several days in the standard solution, after removing any deposit with a fine jet of water, or rinsing in alcohol.

23.5.6 Difference Between pH and Other Ion-Selective Electrodes

The differences between pH and other ISEs are as follows:

- In contrast to the pH membrane, the membranes used in the ISEs are not entirely ion-specific. They can permit the passage of some of the other ions, leading to the problem of ionic interference.
- Most ISEs have a much lower linear range and higher detection limit than the pH electrode.

- The calculation of ionic concentration is for more dependent on a precise measurement of the potential difference there is the pH, because the pH depends on the order of magnitude of the concentration rather than the precise value.
- ISEs mostly work effectively over a narrow pH range.

23.6 ION ANALYSER

Ion analysers are basically pH/mV metres (Figure 23.19), which enable the operator to calculate the concentration of specific ions from the potentials developed at the ion-sensitive electrode, when dipped in sample solution. By measuring both the electrodes potential in a standard solution and in the sample solution, it is possible to calculate the unknown solution concentration by solving the following equation:

$$C_X = C_S \times 10\ \Delta E/S$$

where, C_X = concentration of the unknown solution
C_S = concentration of the standard solution
E = difference between the observed potential in the sample solution and the observed potential in the standard solution
S = electrode slope (change in electrode potential per ten-fold change in concentration)

Ion analysers are mostly microprocessor-based instruments, which are programmed to calculate sample concentration from a set of input data, such as electrode potentials, standard concentration, slope and blank correction. The instruments measure relative millivolts, pH and concentration of specific ions. The programme for direct measurement concentration is based on Nernstian electrode response:

$$E_X = E_o + S \log (C_X + C_b)$$

where, E_x = electrode potential
E_o = constant
C_b = blank concentration

The blank correction (C_b) accounts for the finite lower limit of detection of electrodes. If a solid or liquid-membrane electrode is placed in pure water, the membrane dissolves slightly, producing an equilibrium concentration of the measured ion. This concentration is a constant background for all measurements and is represented by C_b. Typical electrode response curves are generally given by the electrode manufacturers. If the sample concentration falls in the linear response region, a blank correction may not be necessary. But, if the sample concentrations are low, and fall in the non-linear region of the response curve, *blank* correction must be applied.

Figure 23.19 *pH metre/ORP/ISE/T Meter Model 290A (Courtesy: M/s Orion)*

Ion analysers need recalibration every two to 3 h. If there is no change in sample temperature, repeat only set standard. If temperature has changed, increase the magnitude of the slope setting by 1 mV (monovalent) or 0.5 mV (divalent ion) per 5°C increase in temperature.

The standard calibration procedure for a specific ion metre is similar to that used to calibrate a pH metre with pH buffers. Two standard solutions are used, which are a decade apart in concentration and approximately bracket the expected concentration range of the unknown sample solution.

ISEs and ion analysers find numerous applications in agriculture, agronomy food technology, medicine, chemistry, biology, physics, geology, oceanography, pharmacology, engineering and environmental sciences. This is because hundreds of different analysers can be made faster and easier through electrode methods.

A block diagram of the ion analyser is shown in Figure 23.20. The first stage is the input buffer amplifier, which provides a very high input impedance and less than lpA input bias current, The electrode potentials are individually buffered by unity gain amplifiers with FET front ends. Figure 23.21 shows the input buffer stage. The two FETs are operated as source followers, each running at a constant drain current determined by its associated op-amp. The voltage at the + input of each op-amp is held constant, and therefore the drain current in FETs will be constant. To do this, the op-amp output voltage must maintain a constant V_{GS} and must therefore follow the input voltage. The op-amps effectively serve the dual purpose of controlling the operating current of the FET and providing current gain. As with other similar circuits, the high input impedance of the buffer amplifier gets degraded by the presence of dirt, moisture or solder flux. Also, the input FET is delicate and will get destroyed by static discharge. When the inputs are not being driven by a signal, they must be grounded with shorting straps. The input amplifier is followed by a differential amplifier, before the signal is given to an A/D converter (Jain, 1979).

The A/D converter could be a dual-slope integrator type. The results of the A/D converter are held in the A/D data latch by using shift-registers and the loading function is controlled by the A/D converter. The output of the latch remain in high impedance state, until they are enabled by a signal from the control port decoder. Thus, the loading and reading of data from the A/D are independent. The microprocessor may read data from the A/D converter, regardless of the timing of the A/D conversion cycle.

The microprocessor sends and receives information through the input-output (I/O) bus. The bus is driven by only one source at a time and all other sources must be disabled (i.e. kept in a high impedance state). The bus may be driven by the CPU, A/D converter, slope switches, standard value switches and mode switches. The CPU and display receive data from the bus.

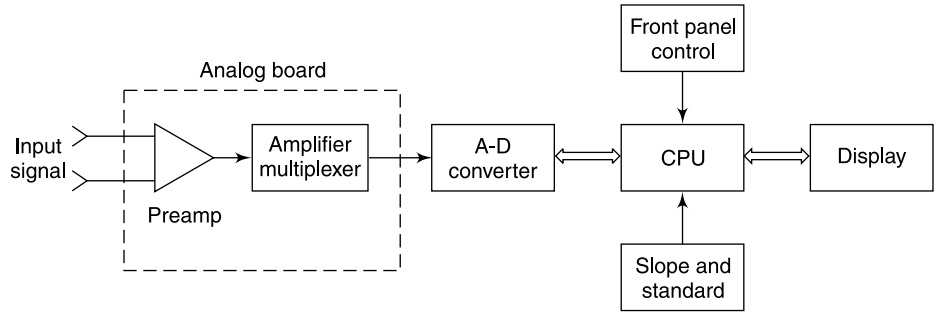


Figure 23.20 *Block diagram of a microprocessor-based ion analyser*

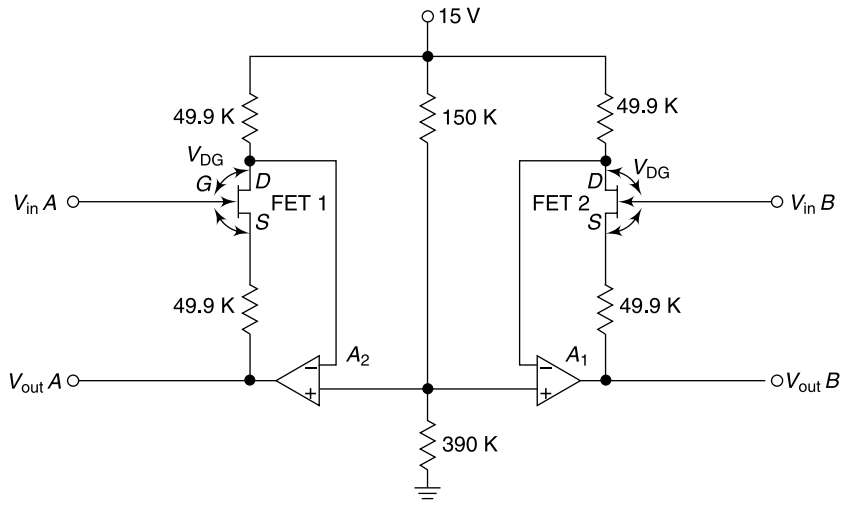


Figure 23.21 *Input-buffer amplifier of an ion analyser*

Under program control, the microprocessor generates signals on the control port to select the path along which data will flow on the I/O bus. The CPU communicates with the memory and the memory interface through the microprocessor data bus. Through this bus instructions and numerical constants flow from the memory outputs into the CPU. The memory interface performs the task of generating the address for each instruction stored in memory. It does this by maintaining a program counter according to commands from the CPU. The timing for the microprocessor and for all signals on the buses is generated by the CPU clock.

Because of the low level of signal generated and high impedance of the ISEs, the grounding system is designed very carefully. Usually, the ion-analysing instruments have the following three grounds: (i) The chassis and the electrostatic shield in the power transformer are connected to earth ground through the third wire of the AC line. This provides isolation from line noise; (ii) digital ground provides the return path for all the logic signals, including the microprocessor signal and the display current and (iii) analog ground provides a reference point for electrode input signals and return path for all analog current.

The analog and digital grounds are kept separate, so that digital signal return currents never flow through the same conductor as analog signal returns. The earth ground is not connected either digital or analog ground.

Kollman and Reddish (1983) illustrate a continuous ion selective analyser for monitoring of gases, detectable via ISEs.

23.6.1 PC-based pH Meter Ion Analysers

The development of computer interfaces for connecting the sensor electrodes directly to a personal computer, without the need for a metre to measure the voltage, has revolutionised ISE data acquisition and processing.

Specially designed electronic hardware can be used with any type of electrode plugged into the interface with a standard connector. The electrode signals are processed by a low-noise amplifier

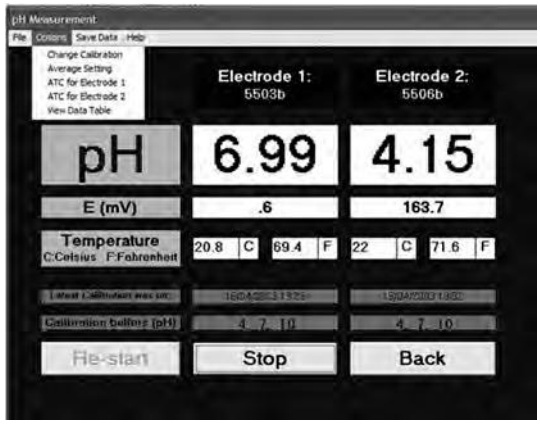


Figure 23.22 *Typical screen display on a PC-based pH metre (Courtesy: M/s Nico 2000 Ltd., UK)*

and A/D converter for direct transfer into any computer. Versions are available for connecting to either the serial or parallel printer port using standard cables. The visual basic software is designed to run on any computer capable of running Windows 3.1 or later version operating system. Apart from the ease and speed of operation, the great advantage of this type of measuring system is that it virtually eliminates any possibility of operator error in recording and transferring data. It also facilitates far more sophisticated data processing, and display and archiving of results once the raw data are securely recorded in the computer.

Data processing software is designed to carry out most of the functions previously done by the operator, and much more. The software basically carries out the following functions:

- *Hardware set-up*: This is meant to configure the system for the sensors and reference electrodes being used (e.g. temperature, pH, Redox, or any ISE) and give a full documentation of electrode types, serial numbers, operator details, date and time, etc.
- *Signal measurement*: The software interprets the signal from the interface and uses a calibrating factor to produce an accurate display of the millivolts. The operator can select to take single readings, or the average of multiple readings. A time delay after immersion of the electrodes can also be specified before taking a reading, if required.
- *Calibration*: The only operator entry required is of the concentration (or pH) of the calibration standards. For ISE measurements, calibration graphs are plotted automatically and instantaneously and can be assessed with various line-fitting techniques.
- *Sample Calculation*: The sample results are calculated instantaneously with no possibility of operator error. All results are saved and displayed in data tables where sample numbers and comments can be added. Concentration results can be reported as ppm or moles/l.
- *Display, reporting and archiving of results*: All data can be stored, printed or imported to other software packages. Continuous recording of pH measurements can be displayed graphically, in real-time, to facilitate such activities as pH titrations or process monitoring. Figure 23.22 shows a typical screen display in a PC-based system.
- *Help and Advice for the Operator*: The software contains several help menus and drop-down hints and warnings which guide the operator reliably through the analysis.

23.7 CHEMICALLY SENSITIVE SEMICONDUCTOR DEVICES

Considerable effort has recently been directed towards the development of ion-sensitive electrodes based on a modification of the metal-oxide semiconductor FET. In these devices, the chemical sensitivity is obtained by fabricating the gate insulation of the FET out of ion-sensitive materials,

usually a polymer or SiO_2. These devices are called ISFETs (ion-selective FETs). A simple review which discusses the chemistry and physics of chemically sensitive semiconductor devices is due to Janata (1989).

In these devices, the ion-sensitive material is bonded to the FET itself. This requires the material and its method of fabrication to be compatible with the substrate (high purity silicon). This very significant requirement puts a severe limitation on the use of some of the best-characterised membrane materials, including ion-sensitive glasses. Donald et al., (1976) report the development of pH-sensitive electrode by means of thick-film screening techniques. This electrode retains the advantages of ion-sensitive FET transducers, but eliminates the restrictions on membrane selection and fabrication. Here, the ion-sensitive structure is physically separated from the FET. In this way the ion-sensitive membrane can be fabricated on a compatible substrate and the FET can then be attached approximately and placed in close proximity to the ion-sensitive membrane. A hybrid electrode structure permits the incorporation of a source follower FET amplifier, directly adjacent to the pH membrane, significantly reducing response time and noise pick-up (Bergveld, and Rooji, 1979).

A chemically sensitive ISFET, essentially consists of a conventional insulated gate FET, that has its metallic gate contact replaced by a chemically sensitive coating and a reference electrode. In solution, the gate region can be coated with an ion-sensitive membrane. Interaction of ions in solution with the membrane results in a change of the interfacial potential and corresponding alteration of drain current. By this technique, numerous cations and anions have been sensed (H^+, K^+, Ca^{2+}, Cl^-, I^- and CN^-). The ISFET has advantages in its small size (less than 1 mm^2) and low output impedance, which makes it ideal for *in vivo* monitoring or analysis of small sample volumes. However, problems like ion-selective coating adhesion and device encapsulation have prevented large scale use of ISFETs (Chang-Soo Lee, 2009).

The construction and working of micro-sensor devices have become possible as a result of extraordinary advances in micro-electronics. These devices fall into two categories. The first category consists of devices that sense chemical species, whose presence modulates the transport of electronic charge in the device. Examples of micro-sensors in this category include CHEMFETs, ion-controlled diodes, Schottky diodes, thin-film tin oxide gas sensors and chemiresistors, etc. The second category consists of all micro-sensor devices that do not fit into the first category. These devices which are based on unique techniques, include surface acoustic wave devices, potentiometric gas Sensors and pyroelectric enthalpimetric sensors. Seitz (1984) discussed several other chemical Sensors based on fibre optics.

On the basis of this principle, a urea bio-sensor can be constructed by the addition of the specific bio-component to the ISFET (i.e. the enzyme urease), which reacts with urea, splitting it into carbon dioxide and ammonia, which in turn are in equilibrium with bicarbonate ion and ammonium ion. These two chemicals can be detected directly as gases by the appropriate gas-sensitive FET. Alternatively, bicarbonate and ammonium ion can be detected with appropriate ion-sensitive, polymeric membrane on an ISFET. Due to the use of enzyme, the dense is referred to as an ENFET (enzyme FET). Its constructional details are shown in Figure 23.23.

While time saving is the major benefit of ISFETs, they are notorious for their sensitivity to changes in temperature, external pH, ionic strength, characteristics of the reference electrode, necessitating calibration before every measurement. pH probes based on ISFET are now commercially available as a replacement fragile glass electrodes. These probes are virtually unbreakable and give faster and more reliable measurements. They can be stored dry and regime minimal routine maintenance.

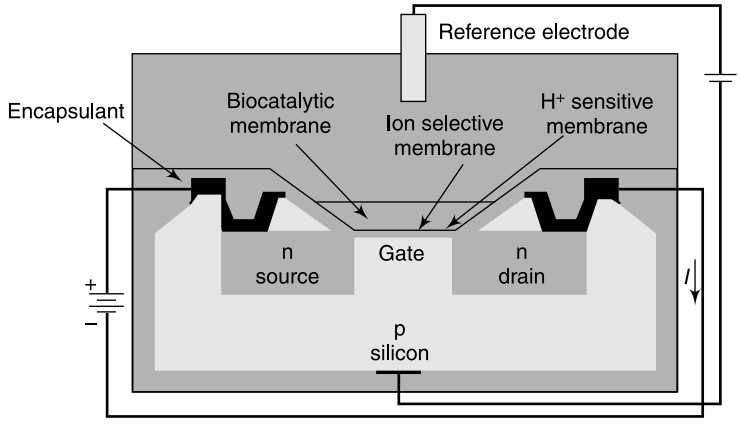


Figure 23.23 *Schematic diagram of an ENFET (Frazer, 1995 a)*

The associated electronics is embedded in the probe itself. The pH related and temperature signals are so processed that they match the glass pH electrode signal thus enabling a perfect match with pH metres facilities such as automatic temperature compensation, self-diagnostics, auto shut off and calibration.

23.8 BIOSENSORS

Modification of the ISE, in which an enzyme is immobilised on the surface of the glass electrode behind a polymer membrane, would result in bio-molecule specificity. Such type of sensors are known as biosensors, a term which is used to describe a wide variety of analytical devices based on the union between biological and physico-chemical components. A biosensor is therefore, an analytical device which converts a biological response into an electrical signal (Figure 23.24). The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilise a biological system directly. A more practical definition states

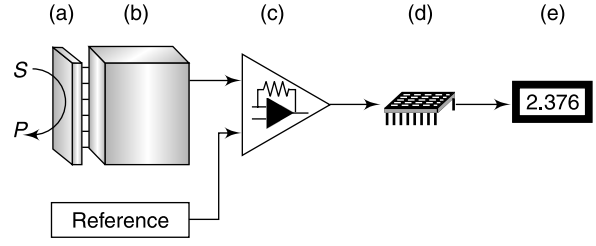


Figure 23.24 *Schematic diagram of the main components of a biosensor. The biocatalyst (a) converts the substrate to product. The reaction is determined by the Transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e) adapted from Chaplin, 2004*

A biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element.

The biological response of the biosensor is determined by the biocatalytic membrane which accomplishes the conversion of reactant to product. Immobilised enzymes possess a number of advantageous features which makes them particularly applicable for use in such systems. They may be re-used, which ensures that the same catalytic activity is present for a series of analyses. This is an important factor in securing reproducible results. Many enzymes are intrinsically stabilised by the immobilisation process, but even where this does not occur there is usually considerable apparent stabilisation.

The biological component can consist of enzymes, antibodies, whole cells or tissue slices and is used to recognise and interact with a specific analyte (Figure 23.25). The physico-chemical component, often referred to as the transducer, converts this interaction into a signal, which can be amplified and which has a direct relationship with the concentration of the analyte. The transducer may use potentiometric, amperometric, optical, magnetic, colorimetric or conductance change properties.

Some typical examples of bio-sensors are as follows:

- The heat output (or absorbed) by the reaction (calorimetric biosensors)
- Changes in the distribution of charges causing an electrical potential to be produced (potentiometric biosensors)
- Movement of electrons produced in a redox reaction (amperometric biosensors)
- Light output during the reaction or a light absorbance difference between the reactants and products (optical biosensors)
- Effects due to the mass of the reactants or products (piezoelectric biosensors).

The best-known example of a biosensor is the urea electrode, which employs an ammonium ISE coated with urease behind a Teflon membrane. Urea diffuses through the membrane and is converted to NH^{4+} by the reaction with urease and can then be determined potentiometrically at the glass membranes. Another important biosensor developed is for measuring blood glucose in diabetic

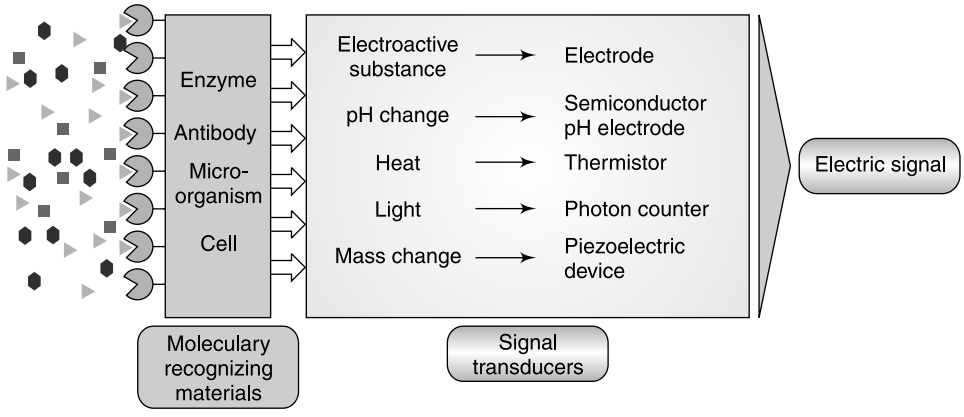


Figure 23.25 *Principle of Biosensors (www.jaist.ac.jp/~yokoyama/biosensor)*

patients. The biosensor combines glucose oxidase with an electrode to measure the oxygen generated by the reaction. Alternatively, glucose oxidase has been coupled to an electrode by ferrocene derivatives, to facilitate electron transfer between the two. These biosensors measure glucose concentrations between 10^{-1} and 10^{-7} M, but generally have a linear response in the range of 10^{-2} to 10^{-4} M.

The critical areas of biosensor construction are the means of coupling the biological component to the transducer and subsequent amplification system. Most of the early biosensors immobilised enzymes on selective electrodes, such as the Clark O_2 electrode, which measured one of the reaction products (e.g. O_2) of the enzyme-analyte interaction. Figure 23.26 shows the construction of this type of electrode. If the immobilised enzyme is soluble glucose oxidase between the two membranes, it becomes a glucose sensor. It works on the principle that in the presence of glucose, oxygen is electrode consumed, providing a change in the signal from a conventional oxygen electrode. For constructing the sensor, glucose oxidase entrapped in a polyacrylamide gel was used. In general, the response times of such types of bio-electrodes were slow and subsequent work has concentrated on closer coupling of the biological component to the transducer. The present research in biosensors attempts to dispose of the coupling agent by direct immobilisation of the enzyme onto an FET. If such a system is developed for glucose, it will enable the biosensor to be attached to cathodes and injected into patients to measure blood glucose directly.

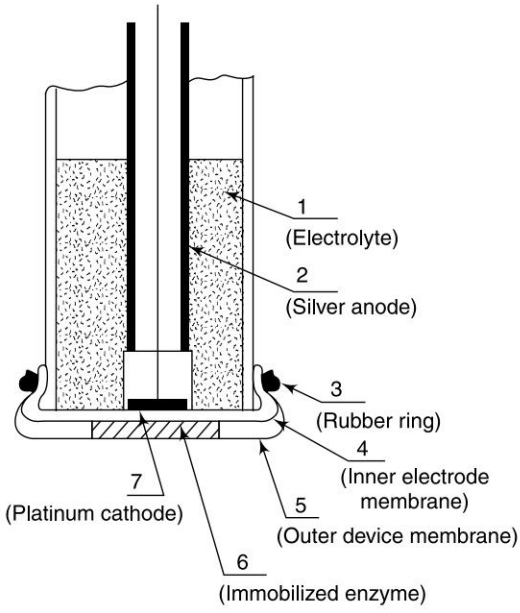


Figure 23.26 *Constructional details of an enzyme utilising sensor with oxygen electrode as the underlying analytical tool*

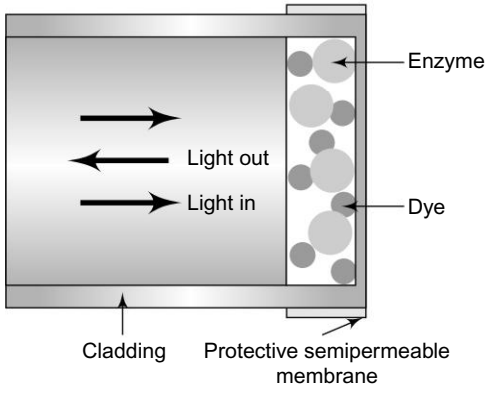


Figure 23.27 *Optical biosensor (Frazer, 1995b)*

The transducers employed in the bulk of enzyme electrodes use either the potentiometric principle, or the amperometric principle.

After electrochemical biosensors, the second major family of biosensors are optical biosensors which have been exploited commercially. These are based on advances in fibre optic technology, laser miniaturisation and reproducible manufacture of prisms and waveguides. These can be considered as miniature traditional spectrophotometers. Figure 23.27 shows a typical arrangement of optical biosensor (Sloper and Flanagan, 1994). The fibre optic cable is coated with a biological

element. Light is introduced into the sample via the fibre. Depending on their number and type, molecules present at the surface of the fibre will absorb certain wavelengths of incoming light. The light that traverses or scattered by the biological layer is measured. The difference between incoming and outgoing intensity of light when measured and compared to a calibration or reference enables to determine the level of the analyte.

Tan et al. (1999) discuss about the development of a new device called nano-scopic optical biochemical sensor, with dimensions in the micro-metre to nano-metre range, which offers fast response time and excellent biochemical sensitivity. The device uses the biochemical selectivity of optically sensitive dye molecules or bio-molecules, such as enzymes, antibodies, DNA molecules or living cells to recognise substances of interest. These ultra-small sensors are expected to have increasing applications in the biomedical field at intracellular and extra cellular levels.

Biosensors thus combine the selectivity of biology with the processing power of modern microelectronics and opto-electronics to offer powerful new analytical tools with major applications in medicine, environmental diagnostics and the food and processing industries. Biosensors can benefit bioprocesses in several ways, such as

Specificity: Due to remarkable ability of biological sensing to distinguish the analyte of interest and similar substances.

Speed: Enable direct and instantaneous measurement of analytes as compared to normal lengthy analytical procedures.

Simplicity: Dispenses with conventional methods of analysis which requires many steps to treat the sample, as the receptor and transducer are integrated into one single sensor.

Continuous Monitoring Capability: This is because in enzyme-based biosensors, the immobilised enzyme can be used for repeated assays.

Biochips and bio-computers are situated at the extreme limits of biosensor technology within the domains of bioelectronics, nanotechnology and micro-machining. It is being attempted to replace those components of computers presently made from doped inorganic semi conducting oxide and insulating nitrides (silicon chips) by engineered protein molecules, capable of communicating via electron and light exchange. The advantage would mainly be one of size and speed. While photolithography can create sub-micron features in inorganic material and organic polymers, proteins are usually on the nano-metre scale. Intense research activity is presently underway in the field of nanotechnology.

Besides the medical field, biosensors have tremendous applications in food and beverage industries. Although several biosensors have been developed over the past few years and there are already numerous working biosensors, various problems still need to be resolved. Most complex problems awaiting solution are their limited lifetime, which restrict their commercial viability, necessitating improvements in their stability.

At present, most biosensors are based on a layer of enzyme setting on top of an electrode (i.e. such a device is an enzyme electrode). Commercially, the most successful enzyme electrodes have been those based on glucose oxidase. These are used by diabetics as a glucometer – a portable and home device to measure the concentration of glucose in the blood.

23.9 POINT-OF-CARE INSTRUMENTS

23.9.1 Point-of-Care Testing (POCT)

Point-of-care testing (POCT) or bedside testing is defined as medical testing at or near the site of patient care. These are simple medical tests which can be performed at the bedside. Simple tests such as those found in medical examinations, urine test strips and even simple imaging such as with a portable ultrasound device, as well as regular observations such as ECGs, O_2 saturation and heart rate. The driving philosophy behind POCT is to bring the test conveniently and immediately to the patient. This increases the likelihood that the patient, physician, and care team will receive the results quicker, which allows for immediate clinical management decisions to be made. POCT is often accomplished through the use of portable and handheld instruments, and tool kits. One of the most popular POCT is blood glucose monitor used by diabetic patients.

23.9.2 Blood Glucose Monitor

A glucose metre or glucometer (Figure 23.28) is a medical device for determining the approximate concentration of glucose in the blood. It is a key element of home blood glucose monitoring by people with diabetes mellitus or hypoglycaemia. A small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip that the metre reads and uses to calculate the blood glucose level. The metre then displays the level in mg/dl or mmol/l.

Test strips: A consumable element containing chemicals that react with glucose in the drop of blood is used for each measurement. For some models, this element is a plastic test strip with a small spot impregnated with glucose oxidase and other components. Each strip is used once and then discarded.

The principle behind blood glucose metres is based on reactions that are analysed by electrochemical sensors. On each strip, there may be about 10 layers, including a stiff plastic base plate, and other layers containing chemicals or acting as spacers. For instance, there is a layer containing two electrodes (silver or other similar metal). There also is a layer of the immobilised enzyme, glucose oxidase, and another layer containing micro-crystalline potassium ferricyanide. Specifically, the reaction of interest is between glucose and glucose oxidase. The glucose in the blood sample reacts with the glucose oxidase to form gluconic acid, which then reacts with ferricyanide to form ferrocyanide. The electrode oxidises the ferrocyanide, and this generates a current directly proportional to the glucose concentration.

Figure 23.28 *Blood Glucose Monitor*

Currently, there are many metres on the market that give results as 'plasma equivalent,' even though they are measuring whole blood glucose. The plasma equivalent is calculated from the whole blood glucose reading using an equation built into the glucose metre. It is important for patients and their health care providers to know whether the metre gives its

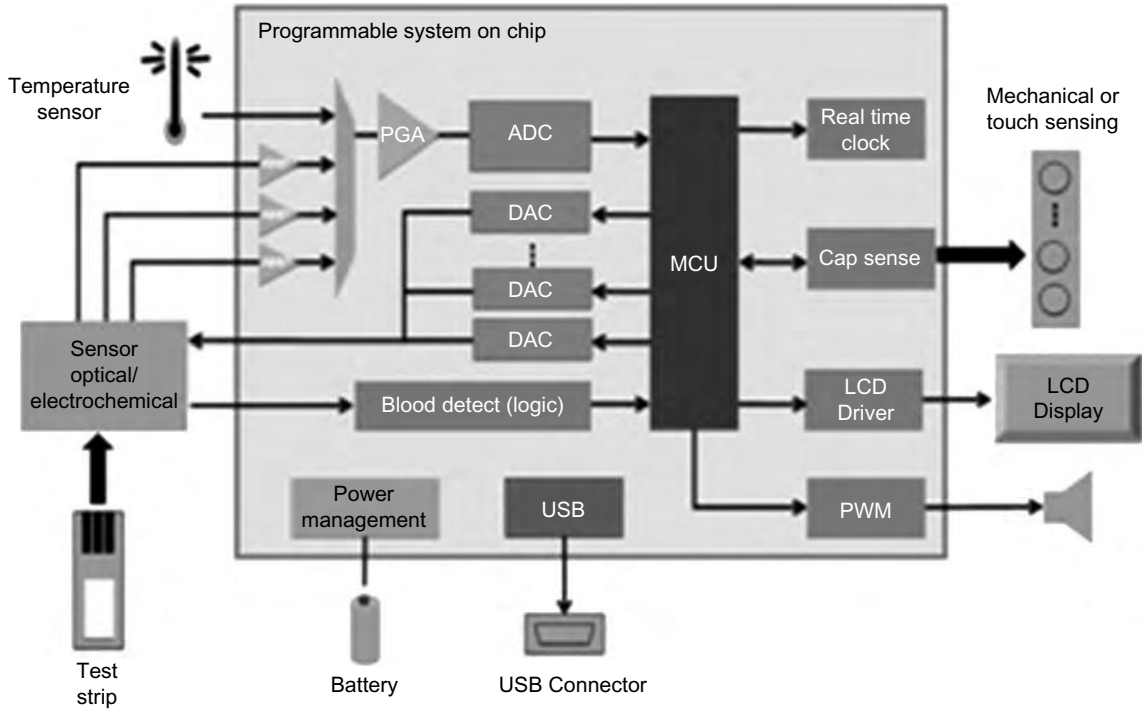


Figure 23.29 *Block diagram of blood glucose monitor (Courtesy: M/s Cypress Semiconductor Corporation)*

results as 'whole blood equivalent' or 'plasma equivalent' since glucose levels in plasma are generally 10–15% higher than glucose measurements in whole blood.

Figure 23.29 illustrates block diagram of a Blood Glucose Meter. Glucose metres require a precision analog front end to interface to the optical or bio-sensor-based glucose sensor. The sensor detects the optical properties of the chemical strip as it reacts with a blood sample. The analog front end includes up to four channels of trans impedance amplifiers with a gain stage, a precision ADC, and a very accurate voltage reference. Multiple precision DACs are also required to properly bias the sensor. Other design requirements include LCD drive, real-time clock (RTC), very low power consumption and user interface. Consistent performance across temperature (0–50°C) is also essential to ensure accurate diagnosis of unsafe blood glucose levels.

Programmable System-on-Chip (PSoC) enables a scalable one chip glucose metre solution that flexibly integrates all the basic and advance requirements of a glucose metre. Furthermore, the programmable analog front end enabled in PSoC provides a more flexible solution for interfacing to the glucose sensor. This enables a more scalable platform that can be utilised across multiple glucose metre designs and strip technologies. Finally, PSoC can enable more advanced features for innovating including USB, and Graphics Display.

24

BLOOD GAS ANALYSERS

Blood gas analysers are used to measure pH, partial pressure of carbon dioxide (pCO_2) and partial pressure of oxygen (pO_2) of the body fluids with special reference to human blood. The measurements of these parameters are essential to determine the acid-base balance in the body. A sudden change in pH and pCO_2 could result in cardiac arrhythmias, ventricular hypotension and even death. This shows the importance of the maintenance of physiological neutrality in blood, and consequently the crucial role the blood gas analysers play in clinical medicine.

24.1 ACID-BASE BALANCE

The normal pH of the extra-cellular fluid lies in the range 7.35–7.45, indicating that the body fluid is slightly alkaline. When the pH exceeds 7.45, the body is considered to be in a state of alkalosis. A body pH below 7.35 indicates acidosis. Both acidosis and alkalosis are disease conditions widely encountered in clinical medicine. Any tendency of pH of blood to deviate towards these conditions is dealt with by the following three physiological mechanisms: (i) buffering by chemical means, (ii) respiration and (iii) excretion, into the urine by kidneys.

The blood and tissue fluids contain chemical buffers, which react with added acids and bases and minimise the resultant change in hydrogen ions. They respond to changes in carbon dioxide concentration in seconds. The respiratory system can adjust sudden changes in carbon dioxide tension back to normal levels in just a few minutes. Carbon dioxide can be removed by increased breathing and therefore, hydrogen concentration of the blood can be effectively modified. The kidney requires many hours to readjust hydrogen ion concentration by excreting highly acidic or alkaline urine to enable body conditions to return towards normal.

Arterial blood has a pH of approximately 7.40. As venous blood acquires carbon dioxide, forms carbonic acid and hydrogen ions, the venous blood pH falls to approximately 7.36. This pH drop of 0.04 units occurs when CO_2 enters the tissue capillaries. When CO_2 diffuses from the pulmonary capillaries into the alveoli, the blood pH rises 0.04 units to bring the normal arterial value of 7.40. It is quite difficult to measure the pH of fluids inside the tissue cells, but from estimates based on CO_2 and $(HCO_3)^-$ ion concentration, intracellular pH probably ranges from 7.0 to 7.2.

In order to maintain pO_2, pCO_2 and pH within normal limits, throughout the wide range of bodily activity, the rate and depth of respiration vary automatically with changes in metabolism.

Control of alveolar ventilation takes place by means of chemical as well as nervous mechanisms. The three important chemical factors regulating alveolar ventilation are the arterial concentrations of CO_2, H^+ and O_2. Carbon dioxide tension in the blood stream and cerebrospinal fluid is the major chemical factor regulating alveolar ventilation. The carotid and aortic chemoreceptors stimulate respiration when oxygen tension is abnormally low. In fact, so many organs participate in the control of respiration, it is difficult to include all aspects in the text. The readers may like to read any standard textbook on human physiology to appreciate the mechanism of respiration control and maintenance of physiological neutrality of the blood.

Table 24.1 lists out the normal range for pH, pCO_2, pO_2, total CO_2, Base Excess and bicarbonate, all measurements made at 37°C (Gambino, 1967).

Table 24.1 *Typical expected values of blood gas parameters*

Plasma parameter		Arterial or arterialised capillary blood	Venous plasma (separated a 37°C)
pH		7.37–7.44	7.35–7.45
pCO_2	Men	34–35 mmHg	36–50 mmHg
	Women	31–42 mmHg	34–50 mmHg
pO_2	Resting adult	80–90 mmHg	
	Resting adult over 65 years	75–85 mmHg	25–40 mmHg
Bicarbonate	Men	23–29 mmol/l	25–30 mmol/l
	Women	20–29 mmol/l	23–28 mmol/l
Total CO_2 (plasma)	Men	24–30 mmol/l	26–31 mmol/l
	Women	21–30 mmol/l	24–29 mmol/l
Base Excess	Men	–2.4 to + 2.3 mmol/l	0.0 to + 5.0 mmol/l
	Women	–3.3 to + 1.2 mmol/l	–1.0 to + 3.5 mmol/l

24.2 BLOOD PH MEASUREMENT

When making pH measurements, the Sorenson's definition for pH of blood and plasma is adopted, which is given by

$$pH = -\log_{10} (H^+)$$

where H^+ designates the concentration of hydrogen ions.

According to this relation, whole blood with a (H^+) of 4×10^{-8} moles/l would have a pH of 7.4. This implies that an increase in the (H^+) to 1×10^{-7} moles/l would correspond to a decrease in pH to 7.0.

Blood or plasma pH is a fairly constant number. The range of pH in health and disease is relatively narrow: in health 7.36–7.42, in disease from about 7.00–7.80. Any greater fluctuations of pH may result in acidosis or alkalosis and death may occur on either side of variation.

Electrochemical pH determination utilises the general method of measuring the difference in potential occurring between two solutions of different pH, using the glass electrode. However, there are special problems associated with the measurement of pH of blood. Particular attention must therefore be given to the problems mentioned below.

24.2.1 Electrodes for Blood pH Measurement

Several types of electrodes have been described in literature for the measurement of blood pH. They are all of the glass electrode type but made in different shapes so that they may accept small quantities of blood and yield accurate results. The most common type is the syringe electrode, which is preferred for the convenience of taking small samples of blood anaerobically. The small 'dead space' between the electrode bulb and the inner surface of the syringe barrel is usually filled with dilute heparin solution to prevent blood coagulation. Before making measurements, the syringe should be rolled between the hands to ensure thorough mixing.

Micro-capillary glass electrodes are preferred when it is required to monitor pH continuously (e.g. during surgery). These types of electrodes are especially useful when very small volume of the sample is to be analysed.

Typically, a micro-electrode for clinical applications requires only 20–25 µl of capillary blood for determination of pH. The electrode is enclosed in a water jacket with circulating water at a constant temperature of 38°C. The water contains 1% NaCl or shielding against static interference. The capillary is protected with a polyethylene tubing. The internal reference electrode is silver/silver chloride and the calomel reference electrode is connected to a small pool of saturated Kcl, through a porous pin. An accuracy of 0.001 pH can be obtained with this electrode against a constant buffer. Figure 24.1 shows the constructional details of a typical blood pH electrode and the measurement set up used in practice.

Quite often combination electrodes comprising both measuring and reference electrodes offer single-probe convenience for all pH measurements. Several instruments offer the ability to measure pH in small containers with as little as 250 µl of sample. Ahn et al. (1975) bring out the drawbacks of the conventional macro- and micro-size pH electrodes when used for biomedical applications. These are due to the relatively large size of the macro-electrode and the fragility of the micro-electrode

24.2.2 Effect of Blood on Electrodes

Glass electrodes deteriorate if allowed to remain in contact with blood for a long time. This results in change of emf-pH slope. The poisoning effect appears to be due to protein deposition. Therefore, as a precautionary measure, in an apparatus where blood necessarily remains in contact around the electrode for long periods (more than 20 min.) the response must be checked frequently against buffer solutions. The poisoning effect can be reduced by putting the electrode in pepsin and 0.1 N HCl, followed by careful wiping with tissue paper.

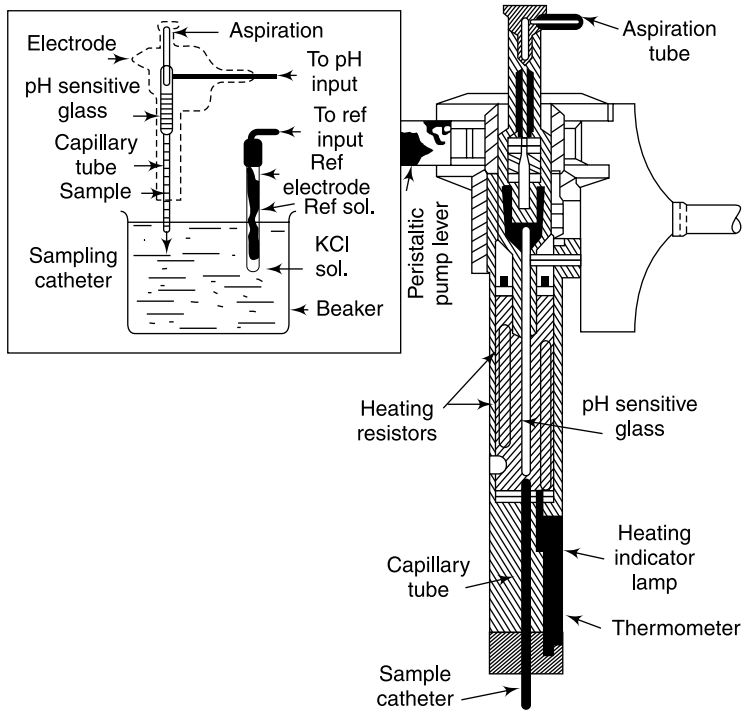


Figure 24.1 *Constructional details of a blood pH electrode (Courtesy: Corning Scientific Instruments, USA)*

The pH of blood is found to change linearly with temperature in the range 18° to 38°C. The temperature coefficient for the pH of blood is 0.0147 pH unit per degree centigrade. This necessitates the use of a highly accurate temperature-controlled bath to keep the electrodes with the blood sample at 37°C ± 0.01°C. A circuit diagram for controlling temperature of the bath is shown in Figure 24.10.

Another important point to be kept in mind while making blood pH measurements is that because of the possible individual variations in the temperature coefficient of blood pH, the method of measuring at some temperature other than 37°C followed by correction is not recommended. It is advisable to keep both the glass as well as the reference electrode at the temperature of measurement.

24.2.3 Buffer Solutions

Buffer solutions are primarily used for (i) creation and maintenance of a desired, stabilised pH in a solution and (ii) standardisation of electrode chains for pH measurements. A buffer is, therefore, a substance which by its presence in a solution is capable of counteracting pH changes in the solution as caused by the addition or the removal of hydrogen ions. Buffer solutions are characterised by their pH value. They are available in tablets of pH value 4.7 and 9.2.

Buffer solutions used in blood pH measurements are the following:

- 0.025 molar potassium dihydrogen phosphate with 0.025 molar disodium hydrogen phosphate. This solution has a pH value of 6.840 at 38°C and 6.881 at 20°C.
- 0.01 molar potassium dihydrogen phosphate with 0.04 molar disodium hydrogen phosphate. This buffer has a pH value of 7.416 at 38°C and 7.429 at 20°C.

These buffers should be stored at a temperature between 18° and 25°C. To maintain an accurate pH, the bottles containing them should be tightly closed.

24.3 MEASUREMENT OF BLOOD pCO2

The blood pCO_2 is the pCO_2 of blood taken anaerobically. It is expressed in mmHg and is related to the percentage CO_2 as follows:

$$pCO_2 = \text{Barometric pressure} - \text{water vapour pressure} \times \frac{\%CO_2}{100}$$

At 37°C, the water vapour pressure is 47 mmHg, so at 750 mm barometric pressure, 5.7% CO_2 corresponds to a pCO_2 of 40 mm.

All modern blood gas analysers make use of a pCO_2 electrode of the type described by Stow et al. (1957). It basically consists of a pH-sensitive glass electrode having a rubber membrane stretched over it, with a thin layer of water separating the membrane from the electrode surface. The technique is based on the fact that the dissolved CO_2 changes the pH of an aqueous solution. The CO_2 from the blood sample defuses through the membrane to form H_2CO_3, which dissociates into (H^+) and (HCO_3) ions. The resultant change in pH is thus a function of the CO2 concentration in the sample. The emf generated was found to give a linear relationship between the pH and the negative logarithm of pCO_2. Although the electrode could not provide sensitivity and stability required for clinical applications, it made way for realising a direct method for measurement of pCO_2.

The basic construction of the electrode was modified by Severinghaus and Bradely (1958) to a degree that made it suitable for routine laboratory use. In the construction worked out by them, water layer was replaced by a thin film of an aqueous sodium bicarbonate ($NaHCO_3$) solution. The rubber membrane was also replaced by a thin Teflon membrane, which is permeable to CO_2 but not to any other ions, which might alter the pH of the bicarbonate solution. The CO2 from the blood diffuses into the bicarbonate solution. There will be a drop in pH due to CO_2 reacting with water forming carbonic acid. The pH falls by almost one pH unit for a tenfold increase in the CO_2 tension of the sample. Hence, the pH change is a linear function of the logarithm of the CO_2 tension. The optimum sensitivity in terms of pH change for a given change in CO_2 tension is obtained by using bicarbonate solution of concentration of about 0.01 mole/l. The electrode is calibrated with the known concentration of CO_2. The response time of the CO_2 electrode is of the order of 0.5–3 min. This electrode is twice as sensitive and drifted much less than the Stow's electrode. Figure 24.2 shows the construction of a typical pCO_2 electrode.

Further improvements in stability and response time were achieved by Hertz and Siesjo (1959). They used a dilute solution of $NaHCO_3$ (0.0001 N) which helped in reducing the response time but the drift introduced posed serious problems. The compromise between response time and drift was achieved by using 0.001 N solution of $NaHCO_3$. Silver/silver chloride reference electrode was

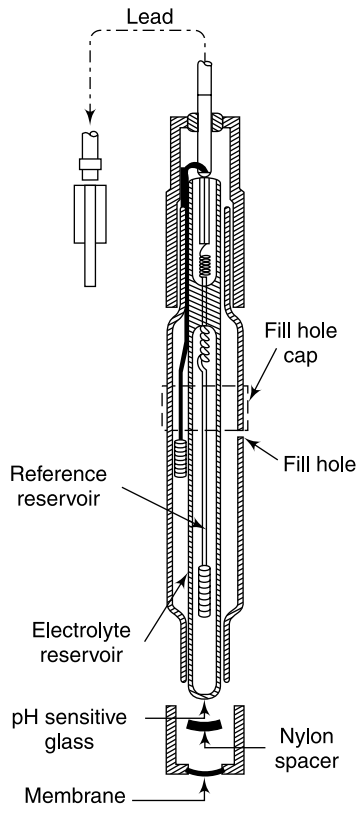


Figure 24.2 *Parts of blood pCO_2 electrode (Courtesy: M/s Corning Scientific Instruments, USA)*

replaced by a calomel cell which was made an integral part of the electrode.

Severinghaus (1962) made a further improvement upon the earlier Severinghaus-Bradley electrode in the low pCO_2 range by replacing the cellophane spacer with a very thin nylon mesh. Glass fibres or powdered glass wool were also found to be good separators. He used a membrane of 3/8 mil Teflon and glass wool for the separator. Electrodes with 95% response in 20 s were constructed in this way.

Reyes and Neville (1967) constructed a pCO_2 electrode using 0.5 mm polyethylene as a membrane and used no separator between the glass surface and this membrane. They added carbonic anhydrase to the electrolyte. The response time was found to be 6 s for 90% of a step change from 2 to 5% CO_2. Use of pCO_2 electrode for measuring blood or plasma pCO2 has been studied repeatedly and has been found to be accurate, precise and expedient, Hill and Tilsley (1973). An extension of the miniature pH electrode (Ahn et al. 1975) is the miniature pCO_2 electrode described by Lai et al. (1975).

24.3.1 Performance Requirements of pH Meters Used for pCO_2 Measurement

The emf generated by pCO_2 electrode is a direct logarithmic function of pCO_2. It is observed that a tenfold change in pCO_2 causes the potential to change by 58 ± 2 mV. The pH versus log pCO_2 relationship is linear within ±0.002 pH unit from 1 to 100% carbon dioxide. Since 0.01 unit pH change corresponds to a 2.5% change in pCO_2 or 1 mmHg in 40 mmHg, for achieving an accuracy of 0.1 mmHg, it is desirable to read 0.001 pH unit (i.e. a resolution of 60 μv). This order of accuracy can be read only on a digital readout type pH metre or on analog metre with expanded scale. The instrument should have a very high degree of stability and a very low drift amplifier. The input impedance of the electronic circuit must be at least 10^{12} Ω.

It is essential to maintain the temperature of the electrode assembly constant within close limits. It is experimentally shown that variation in the temperature of ±1°C produces an error of ±1.5 mmHg or about ±3% at 5 mm pCO_2. The combined effects of temperature change upon the sensitivity of the pH electrode and upon the pCO_2 of the blood sample amount to a total variation in sensitivity of 8% per degree centigrade.

Calculated Bicarbonate, Total CO_2 *and Base Excess*: Acid-base balance determinations are based on several calculations, which are routinely used in conjunction with blood pH and gas analysis. An accurate picture of acid-base balance can be determined from the equilibrium.

$$CO_2 + H_2O \rightarrow H_2CO_3$$
$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

which for bicarbonate has an equilibrium constant as follows:

$$K_{H_2CO_3}/HCO_3^- = \frac{[H^+][HCO_3^-]}{H_2CO_3}$$

where (H^+), (HCO_3^-) and (H_2CO_3) refer to the concentration of these substances.

Since $H_2CO_3 = 0.03\ pCO_2$

and since $pH = -\log [H^+]$

Therefore, $$pH = pK + \log \frac{[HCO_3^-]}{0.03\ pCO_2}$$

where pK equals 6.11 for normal plasma at 37°C. This formula is used in blood gas analysers for calculating actual bicarbonate.
Total CO_2 is calculated from the following relationship:

$$[HCO_3^-] + (0.03 \times pCO_2) = \text{total } CO_2 \text{ in milli-moles/l}$$

Base Excess is calculated from the formula described by Siggaard-Andersen (1963).

$$\text{Base Excess} = (1–0.0143 \times Hb)\ [HCO_3^-] – (9.5 + 1.63\ Hb) \times (7.4 – pH) – 24$$

where Hb represents the patients' haemoglobin value.

Base Excess is the number of milli-equivalents of strong acid or base which would be required per litre of blood to restore it to a pH of 7.400 at 37°C with pCO_2 held at 40 torr. This is usually estimated from pH and pCO_2 measurements done at 37°C in a sample of blood using Siggaard-Andersen's alignment monogram (Siggaard-Andersen 1963).

24.4 BLOOD pO2 MEASUREMENT

The pO_2 in blood or plasma indicates the extent of oxygen exchange between the lungs and the blood, and normally, the ability of blood to adequately perfuse the body tissues with oxygen. The pO_2 is usually measured with polarographic electrode. There is a characteristic polarising voltage at which any element in solution is predominantly reduced and in the case of oxygen, it is 0.6–0.9 V. In this voltage range, it is observed that the current flowing in the electrochemical cell is proportional to the oxygen concentration in the solution.

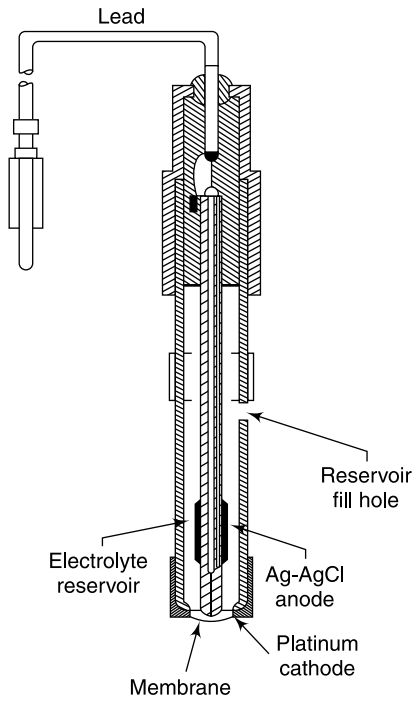


Figure 24.3 *Constructional details of pO_2 electrode (Courtesy: M/s Corning Scientific Instruments, USA)*

Most of the modern blood gas analysers utilise an oxygen electrode first described by Clark (1956) for measuring oxygen partial pressure. This type of electrode consists of a platinum cathode, a silver/silver chloride anode in electrolyte filling solution and polypropylene membrane. The electrode is of single unit construction and contains the reference electrode also in its assembly. Figure 24.3 shows the construction of a typical Clark type oxygen electrode. The entire unit is separated from the solution under measurement by the polypropylene membrane.

Oxygen from the blood diffuses across the membrane into the electrolyte filling solution and is reduced at the cathode. The circuit is completed at the anode, where silver is oxidised, and the magnitude of the resulting current indicates pO_2. The reactions occurring at the anode and cathode are:

Cathode reaction:

$$O_2 + 2H_2O + 4e^- \rightarrow 40H^-$$

Anode reaction:

$$4Ag \rightarrow 4Ag^+ + 4e^-$$

The Clark electrode for measuring pO_2 has been extensively studied and utilised. It is found to be of particular advantage for measuring blood samples. The principal advantages are: (i) sample size required for the measurement can be extremely small, (ii) the current produced due to pO_2 at the electrode is linearly related to the pO_2, (iii) the electrode can be made small enough to measure oxygen concentration in highly localised areas and (iv) the response time is very low, so the measurements can be made in seconds. As compared to this, it takes a very long time if the measurements are made by chemical means.

McConn and Robinson (1963) observed that zero electrode current was not given by a solution having zero oxygen tension, but occurred at a definite oxygen tension, which they called the 'electrode constant'. So, for calibrating the electrode it is necessary to know this constant for that particular electrode. They further showed that when the straight line calibration curves (Figure 24.4) were extended backwards, they did not pass through the origin, but intersected the oxygen tension axis at a negative value. To obtain a true zero current (less than 10 nA), the electrolyte of the electrode is deoxygenated by bubbling nitrogen through it for about half an hour and then placing the electrode in water redistilled from alkaline pyragallol.

The platinum cathode of the oxygen electrode tends to become contaminated or dimensionally unstable with time and use. The result is usually an inability to calibrate and slope the electrode on any pO_2 range. The manufacturers usually recommend application of ammonium hydroxide on the tip of the electrode (10% solution), with a gentle, rotary motion using a swab. The silver chloride gets dissolved in ammonium hydroxide. It is then flushed with distilled water.

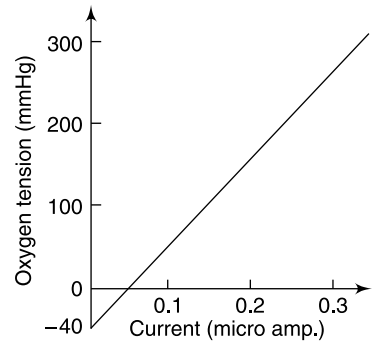


Figure 24.4 *Calibration curve used to obtain a calibration constant of pO_2 electrode (after MConn and Robinson, 1963)*

The polarographic electrodes usually exhibit ageing effect by showing a slow reduction in current over a period of time, even though the oxygen tension in the test solution is maintained at a constant level. Therefore, it needs frequent calibration. It is probably associated with the material depositing itself to the electrode surface. The effect due to ageing can possibly be avoided by covering the electrode with a protective film of polyethylene, but it has the undesirable effect of increasing the response time.

The measurement of current developed at the pO_2 electrode due to the pO_2 presents special problems. The difficulty arises because of the extremely small size of the electrical signal. The sensitivity (current per torr of oxygen tension) is typically of the order of 20 pA per torr for most commercial instruments. It is further subject to constant drift and is also not independent of the sample characteristics. Measurement of oxygen electrode current is made by using high input impedance, low noise and low current amplifiers. Field effect transistors usually form the input stage of the preamplifiers.

Hahn (1969) used a field-effect transistor operational amplifier to measure small polarographic currents. The op-amp is connected as a transresistance converter, the output of which can be read directly by a digital voltmeter. Figure 24.5 shows the circuit in which the polarising voltage is supplied by the cell B (1.3 V) and variable resistance *VR*1. The standing current from the electrochemical cell is cancelled by means of *VR*2, Battery *B* and 1 GΩ resistance. Capacitor *C* (100 pF) is included to limit the bandwidth of the amplifier to reduce noise and to ensure good dynamic stability.

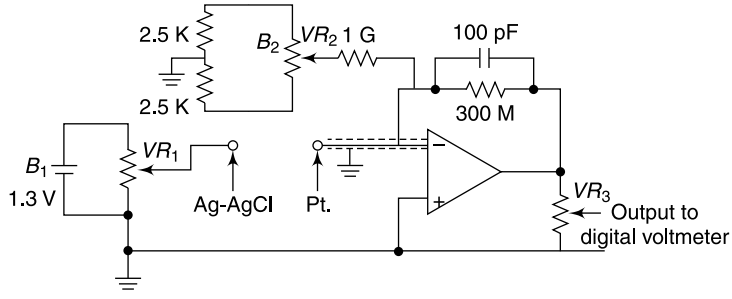


Figure 24.5 *Circuit diagram of a current amplifier for use with pO_2 electrode*

24.5 A COMPLETE BLOOD GAS ANALYSER

Blood gas analysers are designed to measure pH, pCO_2 and pO_2 from a single sample of whole blood. The size of the sample may vary from 25 µl to a few hundred micro-litres. The estimations take about 1 min. With built-in calculators, the instruments can also compute total CO_2, HCO_3 and Base Excess. A typical block diagram of a blood gas analyser machine is shown in Figure 24.6. In this machine separate sensors are used for pH, pCO_2 and pO_2.

The outputs from multiple sensors and calculators are driven through a multiplexer to an analog-to-digital converter (ADC). The data is processed in the micro-controller, which is connected to a PC are other instruments through RS-232, USB, or Ethernet. A digital-to-analog converter (DAC) is often used to calibrate the sensor amplifiers to maximise the sensitivity of the electrodes.

Modern blood gas analysers increasingly employ a touch screen in combination with a graphical user interface (GUI) to make the programming process more intuitive.

The instrument contains three separate high input impedance amplifiers designed to operate in the specific range of each measuring electrode. A separate module houses and thermostatically controls the three electrodes. It also provides thermostatic control for the humidification of the calibrating gases. A vacuum system provides aspiration and flushing service for all three electrodes. Calibrating gases are selected by a special push button control and passed through the sample chamber when required. Two gases of accurately known O_2 and CO_2 percentages are required for calibrating the analyser in the pO_2 and pCO_2 modes. The gases required are: O_2 value of 12% Cal and 0% Slope and CO_2 value of 5% Cal and 10% slope. These gases are used with precision regulators for flow and pressure control. Two standard buffers of known pH are required for calibration of the analyser in the pH mode. The buffers that are used are 6.838 (Cal) and 7.382 (Slope). It is generally recommended that the sample chamber should control 7.382 buffer when in the standby mode.

Input signal to (HCO_3^-) calculator (Figure 24.7) comes from the outputs of pH and pCO_2 amplifiers. The outputs are suitably adjusted by multiplying each signal by a constant and are given to an adder. The next stage is an anti-log generator similar to the one used in pCO_2 amplifier. The output of this circuit goes to ADC for display. Resistance *R* is used to adjust zero at the output.

Total CO_2 is calculated (Figure 24.8) by summing the output signals of (HCO_3^-) calculator and the output of the pCO_2 amplifier. The facilities for adjusting slope and zero at the output are available.

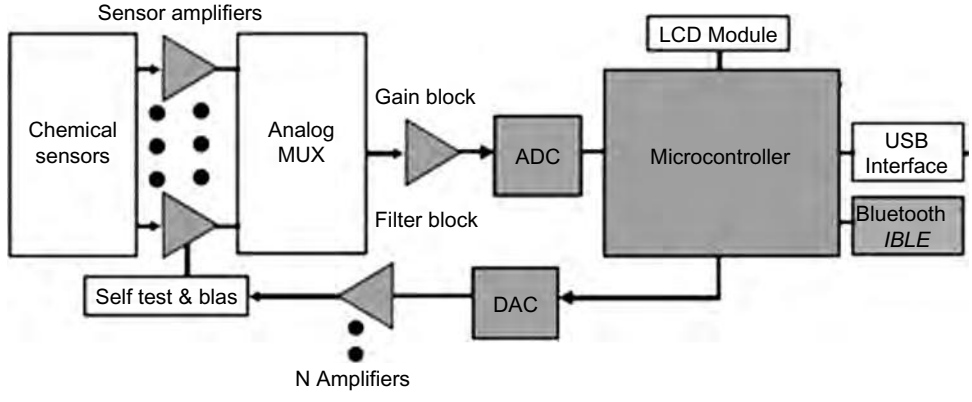


Figure 24.6 *Block diagram of blood gas analyser (Adapted from M/s Texas Instruments)*

The Base Excess calculator (Figure 24.9) consists of three stages. In the first stage, the output of the pH amplifier is inverted in an operational amplifier whose gain is controlled with a potentiometer (Haemoglobin value) placed on the front panel. The output of the HCO_3^- calculator is inverted in the second stage. The third stage is a summing amplifier A_3 whose output is given to an ADC.

The three electrodes (pH, pO_2 and pCO_2) are housed in a thermostatically controlled chamber. It also provides thermostatic control for the humidification of the calibrating gases. The thermal block and the humidifier block heat control circuits are of the same type (Figure 24.10). The temperature is set with a potentiometer for exactly 37°C. The heater circuit is controlled by a thermistor in the block, which acts as a sensor. As the heat increases, the resistance of the thermistor decreases. At 37°C, the thermistor is calibrated to have a resistance of 25 KΩ.

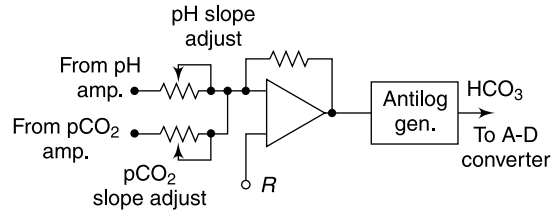


Figure 24.7 *Circuit diagram for computation of Bicarbonate (HCO^-_3)*

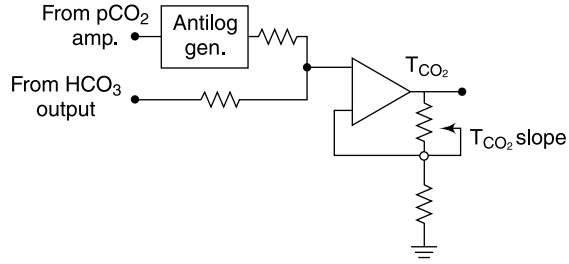


Figure 24.8 *Circuit diagram for computation of total CO_2*

Supposing the temperature of the block decreases, the resistance of the thermistor will increase. The increase in resistance will cause the voltage at inverting input of op-amp to become more negative. This results in the output voltage to go to more positive, increasing the base current of transistors T_1 and T_2. The increase in base current increases the collector current, which goes directly to the heater resistor on the block. As the heater resistor heats up the block, the thermistor will decrease until it returns to 25 KΩ.

Many of the blood gas analysers have a provision for checking the membrane of po_2 and pCO_2 electrodes. In the check position, a potential is applied across the membrane. Any leak in the membrane of sufficient magnitude will result in a considerable lowering of the resistance, which may range be from 100 MΩ to 500 KΩ. The change in resistance can be used to have a change of potential to switch on a transistor, which would cause a lamp to light on the front panel of the instrument. This would indicate that a new membrane is needed.

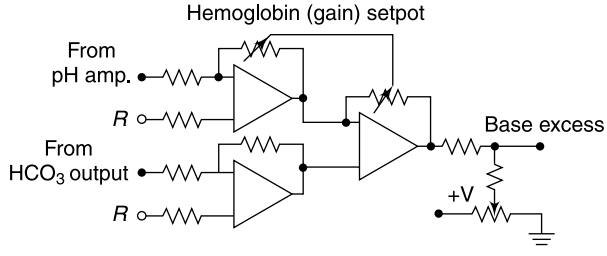


Figure 24.9 *Circuit diagram for computation of Base Excess*

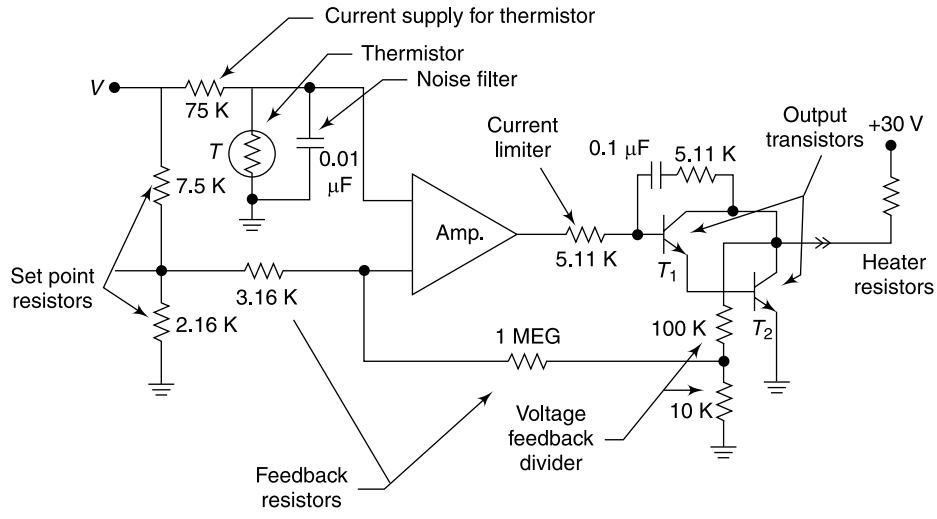


Figure 24.10 *Temperature control circuit for thermostated chamber (Courtesy: Corning Scientific Instruments, USA)*

24.5.1 Fibre Optic-based Blood Gas Sensors

For *in vivo* measurements and reliably analysing blood gases, a small, stable, accurate and bio-compatible sensor is required which could be inserted in the blood flow of an artery through an arterial cannula and remain in place for several days. In addition, it has to be low cost so that it could be used as a disposable item. Advances in fibre optics and the development of pH and oxygen-sensitive dyes have made such a sensor possible. Blood gas analysers based on such sensors are now commercially available.

Figure 24.11 shows the schematic diagram of a fibre optic-based blood gas analyser (Soller, 1994). The sensors are interfaced with an electro-optic monitor. The monitor supplies the excitation light, which may be from a monochromatic source such as a diode laser or a broadband source like xenon lamp whose light is filtered to provide a narrow bandwidth of excitation. Two wavelengths of light are provided, one wavelength is sensitive to

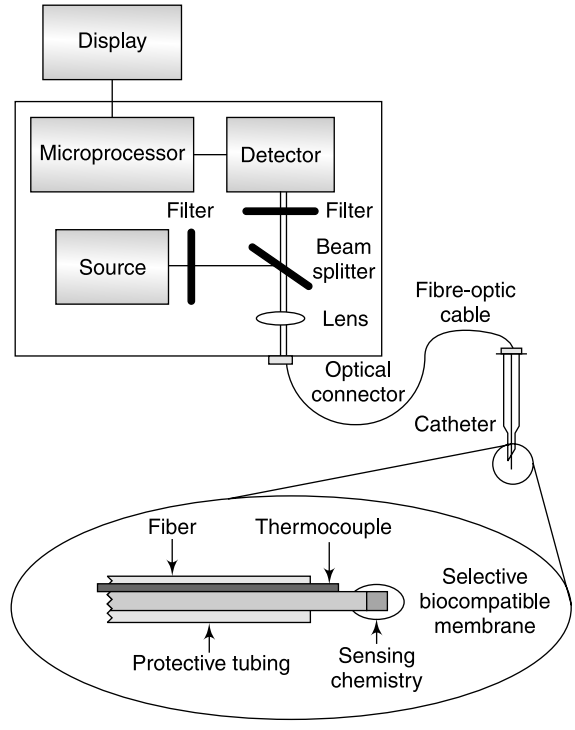


Figure 24.11 *Block diagram of fibre optic-based blood gas sensor and monitor (after Soller, 1994)*

changes in the species to be measured, while the other wavelength is unaffected by changes in the analyte concentration. The unaffected wavelength serves as a reference and is used to compensate for fluctuations in source output and detector efficiency. The light output from the monitor is coupled into a fibre optic cable through appropriate lenses and optical connectors. The cable is sufficiently long to permit easy patient access by allowing the monitor to be placed at a distance.

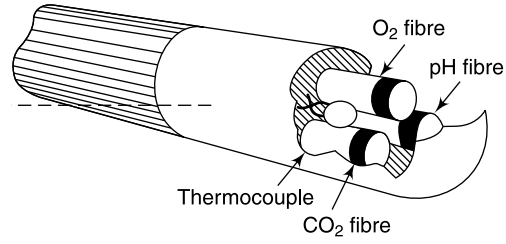


Figure 24.12 *The sensor assembly contains three fibres that measure pH, pCO_2 and pO_2 bundled together with the thermocouple*

Within the sensor assembly (Figure 24.12) are three optical fibres - one each for measuring blood O_2, CO_2 and pH. The optical fibre is approximately 10 cm long and also has a thermocouple or thermistor wire running alongside the fibre to measure temperature near the sensor tip. Temperature correction is necessary for optical blood gas sensors. The solubility of the gases, O_2 and CO_2 in the sensing material is a function of temperature and the optical properties of the sensing chemistry also change as the temperature varies. The fibres and the temperature sensor are encased in a protective tubing to contain any fibre fragments in case of sensor breakage.

Each fibre is as thin as human hair and coated at the tip with a specific chemical dye (Figure 24.13). When light of a known wavelength strikes the dye, the dye fluoresces, giving off light of a different wavelength. The fluorescent emission changes in intensity as a function of the concentration of the analyte (O_2, CO_2 or pH) in the blood. The emitted light travels back down the fibre to the monitor where it is converted into an electrical signal by using a solid-state detector or a photomultiplier. The signal is amplified before it is given to a digitiser. Signal processing to relate the light intensity to the analyte concentration is achieved using a microprocessor and digitally displayed.

Considerable effort has gone into identifying organic molecules, which would make suitable sensors. These molecules must have high fluorescent intensity at excitation and emission wavelengths

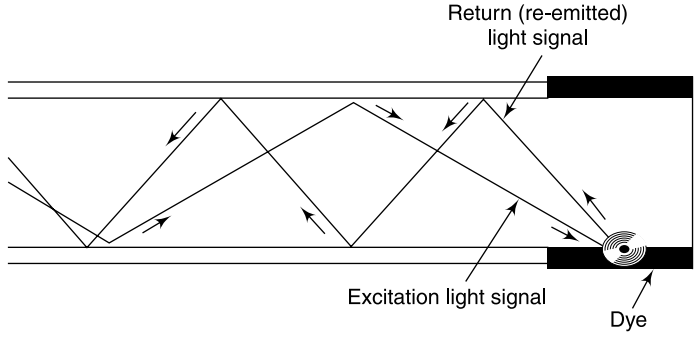


Figure 24.13 *Within each fibre's core, excitation light reflects along the fibre towards the fluorescent dye at the fibre's tip. The dye at the tip reacts to the excitation light and analyte concentration by fluorescing. The fluorescent signal then returns in the same fibre to the monitor, which measures the intensity of the signal*

that match the available light sources and detectors. They must be photostable (i.e. their emission properties should not change as they are continually illuminated by the excitation source). Sensors based on fluorescence quenching of organic dyes such as perylene dibutyrate have been reported for measurement of pO_2. Oxygen sensors based on the phosphorescence quenching of metal – loporphyrins and terbium complexes have also been successfully tried.

It has been found from the experimental studies that as the pO_2 increases, the sensitivity decreases. The best sensitivity is achieved in the region of 30–150 mmHg, but drops off considerably by higher pO_2, making it difficult to resolve small changes in pO_2, when the oxygen partial pressure is greater than 200 mmHg. Further, at high pO_2 as the quenching increases, the light reaching the detector decreases. A compromise is thus required to be made in selecting a sensing material that provides adequate sensitivity over the required pO_2 measurement range and simultaneously offers good signal-to-noise ratio at the detector. The performance range of the sensor is normally limited to under 300 mmHg in order to achieve both good sensitivity and adequate light detection.

pH sensor designs are based on dye molecules whose optical properties change as the pH is varied between 6.8 and 7.8. At any pH in the range of interest, both the acid and base forms of the dye molecule are present and each form has distinct optical characteristics. pH sensors have been developed which take advantage of the fact that the excitation wavelength for fluorescence emission of some dyes is different for the acid and base forms and ratio of emission excited at these two wavelengths can be used to calculate pH. Additionally, sensors have been developed which utilise the difference in absorption maxima for both the acid and base forms of the dye.

The commonly used pH-sensitive dye is phenol red whose absorption spectra is shown in Figure 24.14. The largest peak is observed from base form of phenol red at 560 nm and is used to measure pH because it is more sensitive to pH changes than the acid peak at 430 nm. A wavelength, which is insensitive to pH changes, is used as a reference, either a wavelength greater than 600 nm or the isobestic point at 480 nm. The relationship between pH and base form of the dye is given by Henderson-Hasselbalch equation:

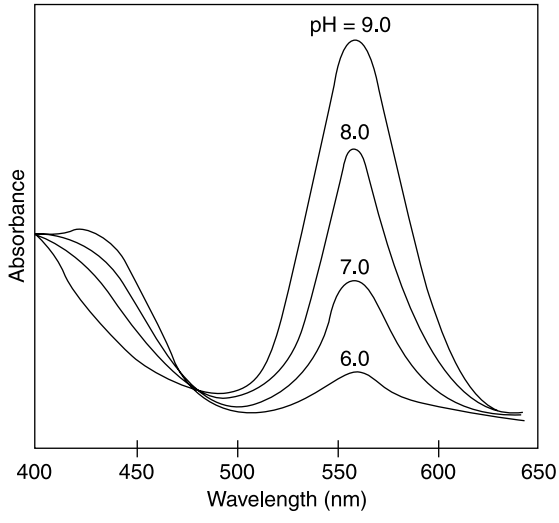


Figure 24.14 *Absorption spectra of pH-sensitive dye (Phenol red)*

$$\text{pH} = pK_a - \log \frac{[\text{HA}]}{\text{A}^-}$$

where pH is the negative logarithm of the hydrogen ion concentration and pK_a is the negative logarithm of the equilibrium constant k_a, which describes the dissociation of the acid, HA.

One of the difficulties in designing a pH sensor is to achieve resolution of 0.01 pH units over the range of 6.8–7.8. An effective way to achieve this is to optimise the pK_a of the dye material. This can be done through proper choice of a functional group attached to the dye molecule or by immobilising the pH-sensitive material on a polymer with the appropriate ionic characteristics.

Most fibre optics sensors for measuring pCO_2 use the same approach as pCO_2

electrode. A pCO_2 sensor is fabricated by surrounding a pH sensor with a gas permeable membrane containing a bicarbonate ion (HCO_3^-) buffer. The membrane allows gaseous CO_2 and water vapour to enter the sensor, and they combine to form carbonic acid as per the following equations:

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

$$H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

$$HCO_3^- \longrightarrow H^+ + CO_3^-$$

The partial pressure of CO_2 can be related to the measured pH through the equilibrium constants for the above reactions and the equation is

$$pH = \log N + pK_1 - \log (K_s\, pCO_2)$$

where

N = concentration of bicarbonate ion in the sensor
pK_1 = negative log of the acid dissociation constant for H_2CO_3 times the hydration constant for CO_2.
K_s = solubility coefficient for CO_2

This principle for the design of pCO_2 sensors has been implemented using both fluorescence-based and absorption-based pH sensors (Vurek et al. 1983).

The methods for measuring pH, CO_2 and O_2 are similar, except that the wavelength of light used for different blood gas parameters vary. The optics is composed of three channels, each for measuring one of the parameters. Provision for calibration is made in the measuring system to compensate for individual physical variations between sensors and monitors. The calibration technique involves placing the sensor in a calibration solution, then bubbling precision mixtures of O_2, CO_2 and nitrogen (N_2) through the fluid. When equilibrium is reached, there are known partial pressure of pO_2 and pCO_2 in the solution. The pH is also known from the gas tensions and the chemical composition of the solution. The bubbling is repeated with a second gas mixture to provide a second calibration point. Using both calibration point the monitor can calculate the appropriate calibration factors for that sensor.

With the development of fibre optic-based blood gas sensors routine electrode membraning and maintenance have become history. Continuous self-monitoring provides clear and immediate information of instrument performance. The keyboard-based user interface provides advanced analytical performance and data processing capabilities. Along with measurement of the blood pH, pO_2 and pCO_2, some instruments like the AVL OPTI Critical Care Analyser (Figure 24.15)

Figure 24.15 *Critical care analyser for measuring blood gases and other parameters (Courtesy: M/s AVL Medical Instruments).*

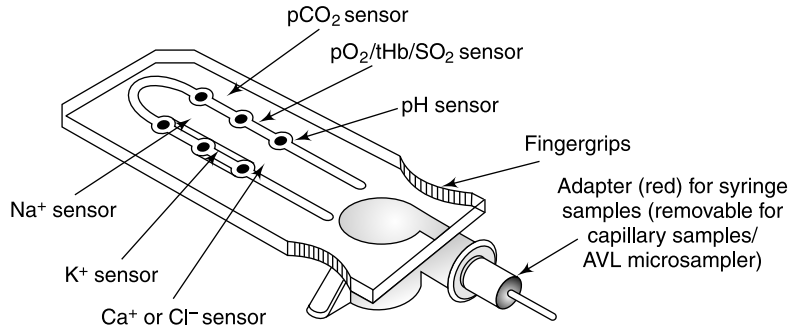


Figure 24.16 *Sensor cassette (Courtesy: M/s AVL Medical Instruments)*

also include facilities for measuring other important ions such as Na+, Ka+, Ca++ and Cl^- in the blood. This is possible with the development of optical sensors based on fluorescence emission. All the sensors are mounted on a cassette shown in Figure 24.16. The syringe adapter shown at the right side of the cassette allows the automatic aspiration of a sample directly from a syringe. Removing the adapter allows for direct sample aspiration from a capillary or micro-sampler. The sensor calibration is verified by the system automatically after insertion of the cassette. The cassette is removed after sample analysis.

The optical sensors in the cassette are designed in a way that the analytes bind with the fluorescent sensor molecule. The sensor molecule is selective for the specific analyte (i.e. the pH sensor molecule reacts only with 'H^+', the O_2 sensor only with O_2 molecules, etc.). The intensity of the emitted fluorescent light varies with the concentration of the ions (H^+, Na^+, K^+) or the partial pressure of the gas molecules (O_2 and CO_2) in the sample. The relationship is specific for each analyte. The corresponding calibration information for each component is encrypted in the bar code. Before the analyte can bind to the fluorescent molecule, it is made to pass through an optical isolator. The isolator prevents interference by unspecific light with the light detection system. The pO_2 sensor also enables to measure and compute total haemoglobin and oxygen saturation. The equipment works on a minimum sample size of 125 µl.

With the miniaturisation of the direct reading electrodes, it is possible to combine them into a single cuvette so that a complete blood gas determination could be made on a single small sample. The introduction of the micro-controller and its use in blood gas analysers free the medical personnel from monitoring the reaction in the electrode chamber and from the tedious chores of calculating and copying the results.

All commercial blood gas analysers make use of the same basic electrodes and signal conditioner circuitry. The main differences between instruments manufactured by various companies are not the measurements of the parameters but the degree of automation and the technique by which the sample is presented to the electrodes.

25

INDUSTRIAL GAS ANALYSERS AND PROCESS INSTRUMENTATION

25.1 TYPES OF GAS ANALYSERS

Several physico-chemical properties have been utilised for analysis of gases in simple or multi-component mixtures. However, most of the commercially available analysers work on the measurement of quantities, such as infrared absorption, paramagnetism, thermal conductivity and the gas density. In addition to these, many of the methods described in other chapters can also be used for the determination of gaseous components. These methods include visual and ultraviolet spectrophotometry, infrared spectrophotometry, mass spectrometry and various electrochemical methods.

All molecules, with the exception of the noble gases, consist of several atoms which exhibit a regular three-dimensional structure by chemical forces and whose valence electrons can attain defined energy states. The molecules are primarily in rotational and translational motions, relative to their surroundings, thus giving Brownian molecular motion. Also, the single atoms within a molecule can vibrate mutually and the shells of the valence electrons within the molecule can reach different states of energy. Eventually, analysers can be designed, so that the molecules of measuring gases may be made to give physical or chemical reaction, which may reveal their nature and extent. Three different interactions are generally utilised, which form the basis of various gas analysers. These are shown in Figure 25.1.

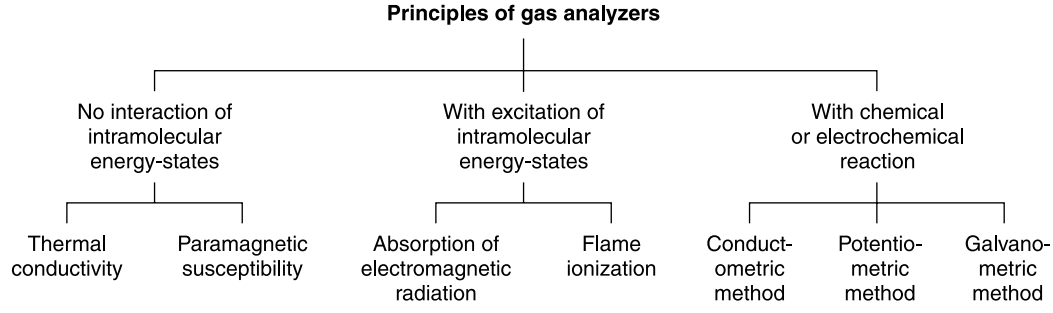


Figure 25.1 *Principle of gas analysers*

25.2 PARAMAGNETIC OXYGEN ANALYSER

Oxygen has the property of being paramagnetic in nature (i.e. it does not have as strong magnetism as permanent magnets, but at the same time it is attracted into a magnetic field). Nitric oxide and nitrogen dioxide are other two gases which are paramagnetic in nature. Most gases are, however, slightly diamagnetic (i.e. they are repelled out of a magnetic field).

Figure 25.2 shows relative paramagnetism exhibited by different gases. The magnetic susceptibility of oxygen can be regarded as a measure of the tendency of an oxygen molecule to become temporarily magnetised when placed in a magnetic field. Such magnetisation is analogous to that of a piece of soft iron in a field of this type. Similarly, diamagnetic gases are comparable to non-magnetic substances. The paramagnetic property of oxygen has been utilised in constructing oxygen analysers.

The paramagnetic oxygen analyser was first described by Pauling et al. (1946). Their simple dumb-bell type of instrument has formed the basis of more modern instruments. Figure 25.3 shows the schematic of a paramagnetic analyser from Beckman. The arrangement incorporates a small glass dumb-bell suspended from a quartz thread between the poles of a permanent magnet. The pole pieces are wedge-shaped in order to produce a non-uniform field.

Referring to Figure 25.4, when a small sphere is suspended in a strong non-uniform magnetic field, it is subject to a force proportional to the difference between the magnetic susceptibility of this sphere and that of the surrounding gas. The magnitude of this force can be expressed as follows:

$$F = C\,(K - K_o)$$

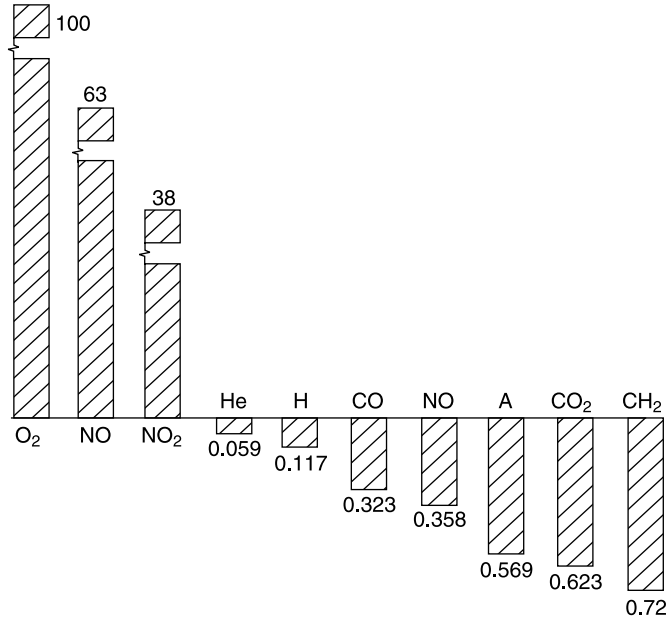


Figure 25.2 *Relative paramagnetism of various gases*

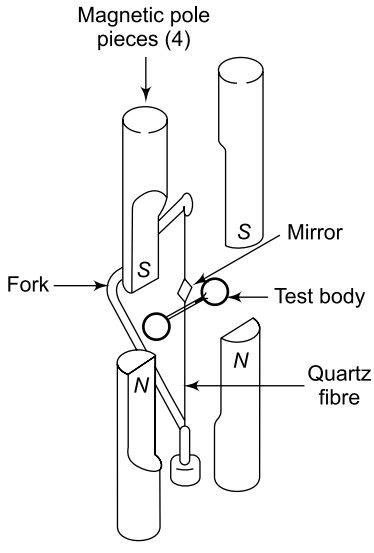


Figure 25.3 *Arrangement of magnets in paramagnetic oxygen analyser (Courtesy: M/s Beckman, USA)*

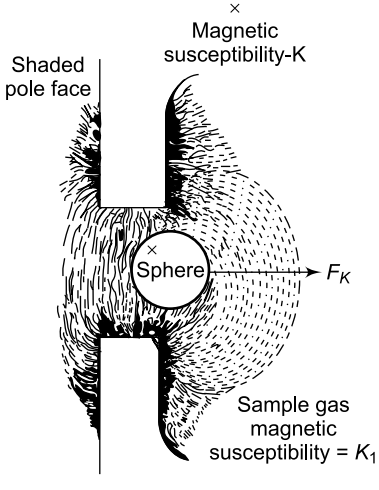


Figure 25.4 *Sphere in non-uniform magnetic field (Courtesy: M/s Beckman, USA)*

where C = a function of the magnetic field strength and gradient,
K_o = magnetic susceptibility of the sphere,
K = magnetic susceptibility of the surrounding gas.

The forces exerted on the two spheres of the test body are thus a measure of the magnetic susceptibility of the sample and therefore of its oxygen content.

The magnetic forces are measured by applying to one sphere an electrostatic force equal and opposite to the magnetic forces. The electrostatic force is exerted by an electrostatic field established by two charged vanes mounted adjacent to the sphere (Figure 25.5). One vane is held at a higher potential than the test body, the other at a lower potential. Since the glass test body must be electrically conductive, it is sputtered with an inert metal.

The test body is connected electrically to the slider of Null Adjust potentiometer R_{20}. This potentiometer is part of a voltage-dividing resistor network connected between ground and B^+. Potential to the test body can be adjusted over a large range.

An exciter lamp directs a light beam on to the small mirror attached to the test body. From the mirror, the beam is reflected to a stationary mirror and then on to a translucent screen mounted on the front panel of the instrument. The geometry of the optical system is so arranged that a very small rotation of the test body causes an appreciable deflection of the image cast by the beam.

Zero control of the instrument is provided by ganged R_{13}-R_{15} setting, which changes the voltage present on each vane with respect to ground, but does not change the difference in potential existing between them. This adjustment alters the electrostatic field. Rheostat R_{19} sets the upscale standardisation point, i.e. provides span or sensitivity control.

When no oxygen is present, the magnetic forces exactly balance the torque of the fibre. However, if oxygen is present in the gas sample drawn in the chamber surrounding the dumb-bell, it would displace the dumb-bell spheres and they would move away from the region of maximum magnetic

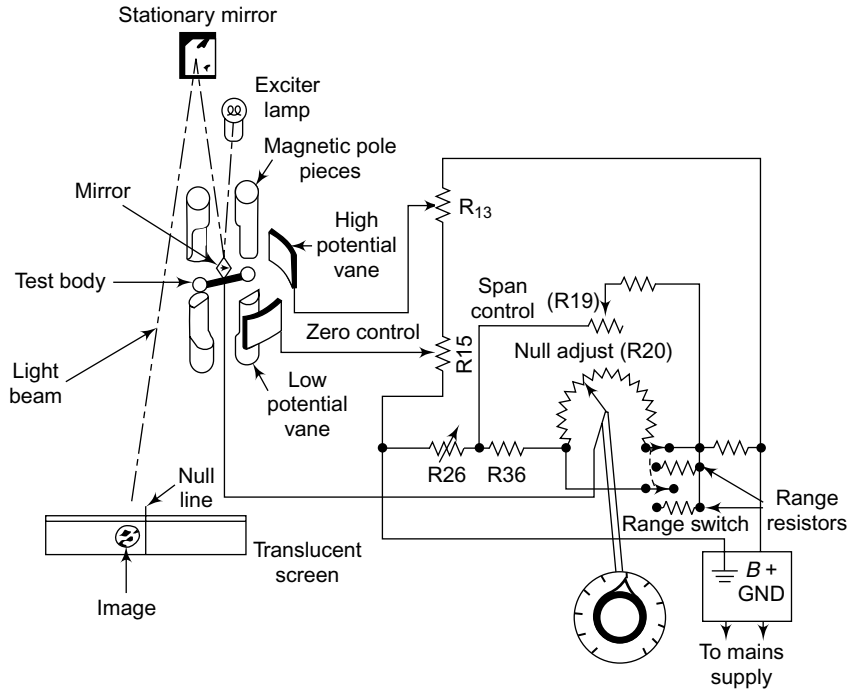


Figure 25.5 *Functional diagram of Beckman paramagnetic oxygen analyser*

flux density. The resulting rotation of the suspension turns the small mirror and deflects the beam of light over a scale of the instrument. The scale is calibrated in percentages by volume of oxygen or partial pressure of oxygen. Paramagnetic oxygen analysers are capable of sampling static or flowing gas samples.

Only a few improvements have been suggested and carried out in the development of oxygen analysers based on paramagnetism. The original quartz suspension has been replaced largely with a platinum-iridium suspension, which is more robust. Instead of measuring the deflection of the dumb-bell, a null-balance system is preferred, where in the deflection is off-set by passing a current through a coil of wire attached to the dumb-bell. The current required is proportional to the deflecting couple and thus to the oxygen tension of the gas. The control of current is carried out by a helical potentiometer which is duly calibrated.

Displacement of the dumb-bell results in unbalancing the output from a pair of photocells. The difference in their output signals is fed to a differential amplifier, which supplies its output current to the dumb-bell coil to null the deflection. The current is indicated on a metre. Oxygen analysers are available with continuous readout 0–25% or 0–100% oxygen. The instruments are calibrated with the references gas specified. Standard cell volume is 0–10 ml and response time is about 10 s.

The recommended flow rate in the Beckman instrument is 50–250 cc/min, when the sample enters the analysis cell through a porous diffusion disc. If the sample enters directly for rapid response, the flow rate is 40–60 cc/min.

Before the gas enters the analyser, it must be pressurised with a pump and passed through a suitable cleaning and drying system. In many cases, a small plug of glass wool is sufficient for

cleaning and drying functions. The entry of moisture or particulate matter into the analyser will change instrument response characteristics. Therefore, the use of a suitable filter in the sample inlet line is recommended.

Any change in the temperature of the gas causes a corresponding change in its magnetic susceptibility. To hold this temperature of the gas in the analysis cell constant, the analyser incorporates thermostatically controlled heating circuit. Once the instrument reaches temperature equilibrium, the temperature inside the analysis cell is approximately 140°F. The sample should be admitted to the instrument at a temperature between 50 and 110°F. The sample may not have time to reach temperature equilibrium before entering the analysis cell.

Calibration of the instrument consists of establishing two standardisation points (i.e. a down-scale and an up-scale standardisation point). These two points can be set by passing standard gases through the instrument at a fixed pressure, normally atmospheric pressure. First a zero standard gas is admitted and the zero control is adjusted. Then the span gas is admitted and the span control is adjusted. Alternatively, the required practical pressures of oxygen are obtained by filling the analysis cell to the appropriate pressures with non-flowing oxygen or air. If the highest point is not greater than 21% oxygen, dry air is used to set the span point. If the point is greater than 21% oxygen, oxygen is used to set this point.

The instrument should be handled carefully, as the fine quartz fibre supporting the test body may break.

25.3 MAGNETIC WIND INSTRUMENTS

Figure 25.6 shows a paramagnetic oxygen analyser based on the phenomenon of magnetic wind, which rises due to the motion of paramagnetic oxygen molecules into the non-uniform magnetic field. The flowing gas is made to pass over a heated filament connected as one arm of a Wheatstone bridge circuit. The gas cools the filament and unbalances the bridge circuit. The output voltage from the bridge can be fed to a recorder, to show a continuous indication of oxygen concentration in the sample.

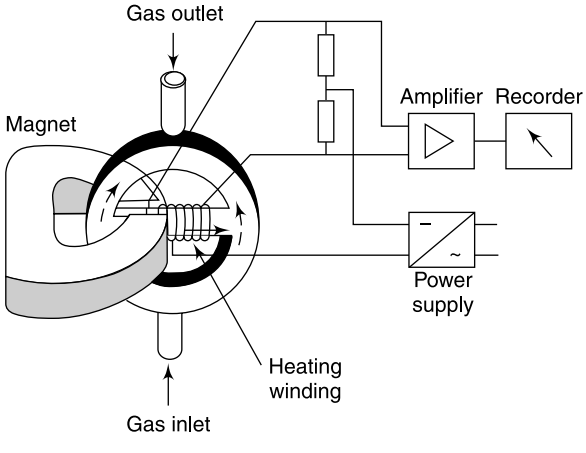


Figure 25.6 *Principle of Hartman and Braun oxygen analyser*

The Hartman and Braun oxygen analyser type Magnos 2T and Magnos 5T work on the principle of thermomagnetic action. The method consists in making use of the dependency of paramagnetic susceptibility on the temperature according to the Curie law.

The analyser comprises of a ring chamber, which consists of a metallic hollow ring arranged in vertical plane, with a gas inlet below and a gas outlet above. The gas is conducted in the upward direction. The two halves of the ring chamber are connected by a glass tube. The transverse tube has taped wire winding on its outside. The winding forms a part of a Wheatstone bridge circuit, whose one-half is placed in the field of a permanent magnet.

In case the sample gas does not contain any oxygen, there is no flow in the horizontally placed transverse tube. If, however, the sample gas contains oxygen, it is attracted into the magnetic field. This gas flow is heated and due to the increase of the temperature, the susceptibility of the paramagnetic oxygen molecules decreases. In this way, the heated gas is pushed away by the cold gas coming from the left-hand side. A gas flow thus arises inside the transverse tube. This flow is called the Magnetic Wind, whose velocity depends upon the oxygen concentration in the sample gas. The magnetic wind cools down the left side of the heated winding more than the right side. Therefore, a change in the bridge balance takes place, which depends on the temperature gradient. The voltage difference of the bridge is proportional to the oxygen concentration in the measuring gas.

The instrument suffers from several major sources of error, which are given below:

(i) The filament temperature is affected by changes in the thermal conductivity of the carrier gas. Thus, the calibration is correct for only one gas mixture, which must be specified for each analyser.

(ii) Hydrocarbons and other combustible gases in the sample stream react on the heated filaments causing changes in temperature and therefore their resistance, which results in extremely large errors. These hydrocarbons have to be removed by means of a cold trap, but if the percentage in the gas is high, an error will result due to the change in sample volume.

(iii) The cross tube must be horizontal, to avoid an error due to gravitational chimney-flow effects.

It is important to note that magnetic wind and thermo-magnetic analysers are synonymous. The first name is generally used in Europe and the second in the United States. In some instruments, the paramagnetic type of sensor works by passing the unknown gas between two powerful magnetic poles, wherein a thin aluminium membrane is suspended. The magnetised oxygen is pulled into the space between one of the magnetic poles and the membrane, thereby displacing the membrane in the opposite direction. The displacement of the membrane can be conveniently measured by using LVDT or strain gauge transducer. The extremely low inertia of the thin membrane in the oxygen sensor allows it to come to equilibrium in 0.25–0.5 s. This enables the recording of rapid changes in oxygen concentration. This is particularly useful for making measurements during each breathing cycle, while making measurements of respiratory gases.

Oxygen analysers are employed in the areas of oxygen absorption studies on plants and tissues, food processing, air pollution, respiratory studies and anaesthesiology.

Figure 25.7 shows the arrangement of a rapid oxygen analyser OXYMAT-M from Siemens used for medical applications.

The measuring procedure is based on the pressure difference which develops between two gases having different oxygen concentration in a magnetic field. One of the two gases, usually ambient air, serves as the reference gas, which flows in the channels, both of which open into a single measuring chamber.

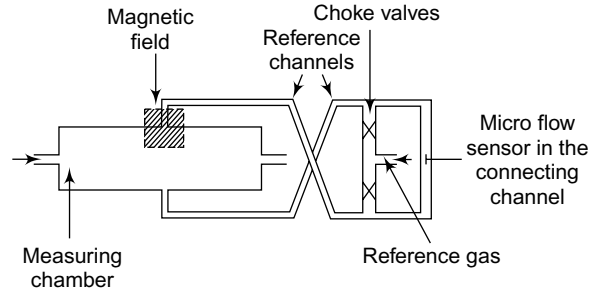


Figure 25.7 *Schematic representation of the measuring system of Siemens OXYMAT—M oxygen analyser for medical applications*

One channel is located within the magnetic field, the other in a position which is virtually free from magnetic influences. The respiratory air to be analysed is sucked directly through the measuring chamber. On account of the paramagnetic property of oxygen, it is subjected, in the inhomogeneous parts of the magnetic field, to forces which act on the oxygen molecules in the direction of higher field strengths. If the oxygen concentrations of the two samples of gas differ, a pressure difference develops between the two points of entry of the reference gas into the measuring chamber. This pressure difference is compensated via a connecting channel, in which a micro-flow sensor converts the stream of gas into an electrical signal, which is proportional to the difference in oxygen concentration between the gas to be analysed and the reference gas.

The instrument employs an electromagnet with changing flux intensity. As a result, an alternating pressure is created in the measuring chamber, and an alternating electric voltage of 25 Hz is developed at the micro-flow sensor, which is rectified and amplified in the electronics part of the equipment. The measurement of alternating pressure has the advantage of avoiding any unsymmetry and drift phenomena.

25.4 THE ELECTROCHEMICAL METHODS

Analysers based on the electrochemical methods are mostly used for the determination of oxygen content of a gas. They utilise an electrolytic cell and can be broadly classified as galvanic, polarographic and conductometric methods.

25.4.1 Galvanic Methods

Galvanometric methods are based on the fact that the electrical current of a galvanic cell, which is equipped with appropriate electrodes and an appropriate electrolyte, would depend upon the oxygen concentration, it being related to rate of oxygen uptake by such a cell. It is the reverse of electrolysis in which oxygen is evolved at the anode and hydrogen at the cathode, when electrical current is made to pass through the cell. These analysers are used for measurement of small oxygen concentrations.

The galvanic cell has two electrodes, one of which is made of noble metal such as silver and the other is made of a base metal such as lead. The oxygen contained in the sample gas is made to bubble through the electrolyte. A magnetically driven stirring system helps to ensure a quick and efficient mixing of the reaction liquid and the gas.

Analysers based on this principle are used to measure the content of dissolved oxygen (DO) in boiler-feed water. For this purpose, the boiler-feed water flows through the cell and acts as an electrolyte and the cell is used for continuous monitoring.

There is a need to control the oxygen uptake at the cathode. This is generally done by having a porous carbon cathode and semi permeable membranes. Since the electrons are supplied by the dissolving anode, the lift of the cell is limited. The cell is affected with a very high temperature gradient, approximately 4%/°C. This is compensated using a combination of negative temperature-coefficient (NTC) and positive temperature-coefficient (PTC) thermistors.

The speed of response and sensitivity is improved by using silver gauze instead of a smooth electrode and reducing the volume of electrolyte. The cell could be cylindrical in shape with a central anode of porous material like lead saturated with electrolyte and dipped in a reservoir, over which the sample gas flows. The cathode is formed by gauze which surrounds the anode. This arrangement is known as Hersch cell. Several improvements have been suggested in the basic

form of the cell to increase its life and sensitivity. One common type is that, in which diffusion of the oxygen through a Teflon membrane causes a current flow between two electrodes separated by a liquid or gel electrolyte.

The noble metal cathode if used is not attacked by the electrolyte. Therefore, the drifts and instability associated with porous cathodes are eliminated. Also the cell has no output in the absence of oxygen, and a definite zero is obtained which does not need calibrating. Current in the galvanic cell obeys Faraday's law, which is given by the following relations:

$$I = 0.263\ CFP\ (298/\mathrm{T})$$

where I is the expected current in micro-amps, when a gaseous sample containing C ppm of oxygen by volume passes through the cell at a flow rate F cm^3/min measured at P atmosphere and T K. The expression assumes that the perfect gas laws apply. With a sample flow rate of 100 cm^3/min at one atmosphere pressure of 298 K, the theoretical sensitivity is 26.3 μA/ppm.

These instruments are generally slow in operation and the sample gas must be scrubbed to remove CO_2, SO_2, H_2S or any acidic gas, but one attraction is that, they can be used to measure DO in liquids.

Another type of electrochemical analyser employs the high temperature galvanic cell. This cell is manufactured by a number of companies. All of these cells consist of a calcium stabilised zirconium oxide electrolyte, with platinum electrodes. At the operating temperature, oxygen molecules on the side of the cell exposed to a high partial pressure of oxygen (the anode) gain electrons. Simultaneously, oxygen molecules are formed by the reverse action, at the other electrode (the cathode).

For cell operating at 850°C, the standard Nernst equation for an oxygen cell is as follows:

$$\text{EMF (open circuit)} = 55.7 \log_{10} (P_a/P_b)$$

where P_a is the partial pressure of oxygen within the cell, and P_b is the partial pressure of oxygen outside the cell. Since this effect is specific for oxygen, the instrument output is not affected by the presence of water or CO_2. However, hydrocarbons, hydrogen and other combustible gases will burn at the operating temperature and result in an indication of less oxygen than is actually present. The response of analysers using such cells is very fast.

The probe can be used for direct determination of oxygen in the flue, provided that the flue temperature is less than 800°C. The instrument can be calibrated only by removing the probe from the flue and inserting it into an enclosed container, which can be filled with calibration gases.

The instrument can be mounted outside the flue. The sample gas is drawn through a short sample tube to the measuring cell by an air ejector pump. This analyser provides a means of introducing calibration gases for setting zero and span. It is important to note that both these types of instruments require a supply of clean, dry air at the reference side of the zirconium electrolyte.

25.4.2 Polarographic Cells

Polarographic cells are generally used to measure the partial pressure or percentage of oxygen from injected samples, continuous streams or in static gas monitoring. They find maximum utility in the respiratory and metabolic laboratories. Polarographic cells are based on the redox reactions, in a cell having both the electrodes of noble metals. When a potential is applied, oxygen is reduced at the cathode in the presence of KCl as the electrolyte and a current will flow. The cathode is protected by an oxygen permeable membrane, and the rate at

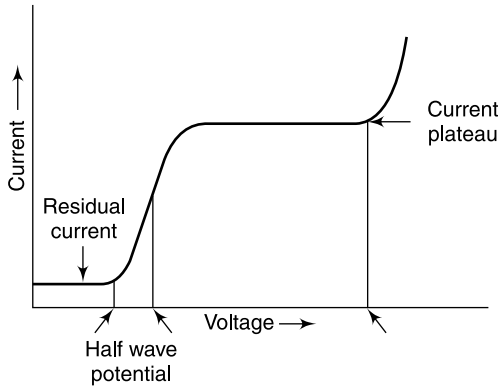


Figure 25.8 *Response of polarographic sensor*

which oxygen reaches the cathode will be controlled by diffusion through the membrane. The voltage-current curve will be a typical polarogram (Figure 25.8). A residual current flows in the cell at the low voltages. The current rises with the increase in voltage, until it reaches a plateau where it is limited by the diffusion rate of oxygen through the membrane. For a given membrane and at a constant temperature, this would be proportional to the partial pressure of oxygen across the membrane. When the voltage is applied in the plateau region, the current in the cell is proportional to oxygen concentration.

Polarographic cells are temperature sensitive, as the diffusion coefficient changes with temperature. The temperature coefficient is usually 2–4%/°C. Therefore, temperature compensation circuits are used to overcome this problem. Polarographic oxygen cells are used mainly for portable gas detectors, where simplicity, low cost and light weight are important. They are preferably used for measuring oxygen in liquids, especially in water pollution and medical work.

The oxygen analyser incorporates oxygen sensor, which contains gold cathodes, silver anode, potassium chloride electrolyte gel and a thin membrane. The membrane is precisely retained across the exposed face of the gold cathode, compressing the electrolyte gel beneath, into a thin film. The membrane, permeable to oxygen, prevents airborne solid or liquid contaminants from reaching the electrolyte gel. The sensor is insensitive to other common gases. A small electrical potential (750 mV) is applied across the anode and cathode.

Although the composition of the atmosphere is remarkably constant from sea level to the highest mountain (i.e. oxygen 21% and nitrogen 79%), there is a great difference in the partial pressure of oxygen at different altitudes. The polarographic sensor, which actually senses partial pressure, would therefore require some adjustment to read approximately the percentage oxygen at the altitude at which it is used. Humidity can also effect oxygen readings, but to a lesser degree. Water vapour in air creates a water vapour partial pressure that slightly lowers the oxygen partial pressure. Therefore, for precision work, it is often desirable to use a drying tube on the inlet sample line. Also, care should be taken to calibrate and sample under the same flow conditions, as required for the gas to be analysed. The range of the instrument is 0–1,000 mm Hg of O_2 and the response time is 10 s for 90%, 35 s for 99% and 70 s for 99.9%. The instrument can measure oxygen against a background of nitrogen, helium, neon, argon, etc., with no difficulty. The sensor is very slightly sensitive to carbon dioxide and nitrous oxide, with typical error less than 0.1% oxygen for 10% carbon dioxide and 4% oxygen for 100% nitrous oxide.

25.4.3 Conductometric Method

The conductometric method is convenient and is the most widely used method for trace gas analysis. In practice, the sample gas is passed through a cell containing a liquid reagent, which can react with the gas of interest. The conductivity of the liquid is measured before and after the reaction with the gas. The difference in conductivity is proportional to the gas concentration.

In order to obtain reproducible results, flow of gas and reagent must be kept constant. Therefore, the measuring gas must enter the analyser at a constant velocity, which is generally adjusted by a pneumatic bypass and indicated by a capillary flowmeter. A slow stream of reaction solution enters the reaction cell via a second capillary, and its flow rate is also kept constant. In some analysers, the chemical reaction between the measuring gas and the reagent takes place quantitatively in a spiral reaction cell, where the gas is separated from the liquid. The liquid is then passed through the conductivity measuring cell, while the gas is vented from the analyser.

In order to minimise errors due to change in ambient temperature, the measuring cell is kept in precisely controlled temperature environment.

By proper selection of the reagent, analysers based on this principle can be made specific for various gas components. The method is especially suitable for measuring traces of H_2S, SO_2, NH_3 and H_2O in the ppb range.

25.5 INFRARED GAS ANALYSERS

For their operation, infrared gas analysers depend upon the fact that some gases and vapours absorb specific wavelengths of infrared radiation. One of the most commonly measured gases using infrared radiation absorption method, is the carbon dioxide. The technique used for this purpose is the conventional double-beam infrared spectrometer system having a pair of matched gas cells in the two beams. One cell is filled with a reference gas, which is a non-absorbing gas like nitrogen, whereas the measuring cell contains the sample. The difference in optical absorption detected between the two cells is a measure of the absorption of the sample at the particular wavelength. Since the vibration excitation occurs only if we have hetero-atomic molecules, the infrared absorption principle is not applicable for the analysis of gases, whose molecules are formed by two identical atoms like oxygen, hydrogen and nitrogen.

Infrared analysers are used for the determination of a large number of components, including CO, CO_2, SO_2, NH_3, H_2O, nitric oxide as well as most gaseous hydrocarbons, The selectivity is however restricted by the fact that the absorption bands partly overlap mutually. This can be eliminated by providing a filter cell, which is filled with the interfering component. The selectivity can also be enhanced by the negative filtering, so that it is possible to distinguish interfering components.

A simple method of using the infrared technique for gas analysis is shown in Figure 25.9. The solid-state detector is PbSe. The chopper has a high speed of 3000 rpm and provides response times up to 100 ms for 90% reading. The infrared source operates at a temperature of about 830°C, where it emits infrared energy optimised for the spectral bands of interest and long life. The infrared energy source is located at the focal plane of a parabolic reflector, so that the reflected energy from the reflector is effectively collimated. The collimated energy is chopped by the coaxial chopper, which allows the energy to pass alternately through the reference and sample tubes. Since the energy is collimated, it passes through these tubes without internal reflections, so that gold foil coatings on the inside of these tubes are not necessary. The sample tube length can be selected according to the absorption strength and concentration of the sample gas. At the output end of the two tubes, a second parabolic reflector images the energy onto the detector filter assembly. The filter is a narrow bandpass interference filter, with bandpass characteristics matched to the absorption spectra of the gas of interest.

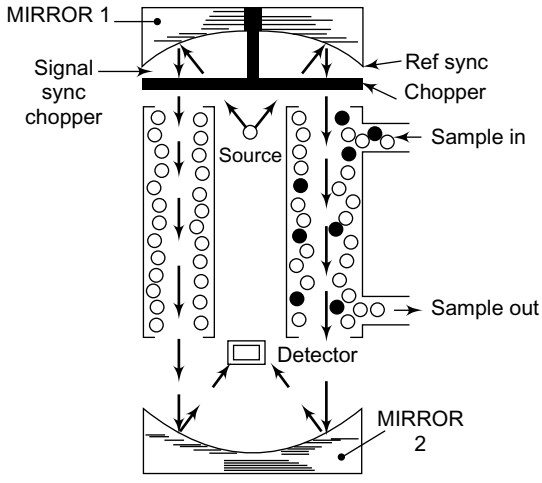


Figure 25.9 *Principle of infrared gas analyser (Courtesy: M/s Infrared Industries, USA)*

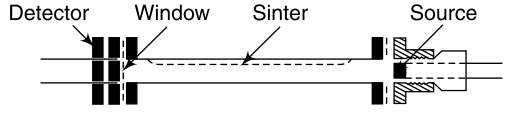


Figure 25.10 *Infrared gas analyser for detection of hydrocarbons (after Jones et al., 1971)*

Infrared gas analysers are particularly useful for measuring carbon dioxide in respired air in the medical field. In these instruments, two types of samples are employed: a micro-catheter cell and a breathe-through cell. The micro-catheter cell is used with a vacuum pump to draw off small volumes from the nasal cavity or trachea. Its typical volume is 0.1 ml and it is particularly useful when larger volumes could cause patient distress. The breathe-through cell accepts the entire tidal volume of breath with no vacuum assistance. It can be connected directly into the circuit of an anaesthesia machine. These instruments have a typical response time of 0.1 s and a sensitivity range of 0 to 12% CO_2.

Infrared sources applied in early approaches of infrared analysers had significant power and used opto-acoustic detectors, characterised by a relatively small sensitivity. The method of modulation of radiation emitted by such sources was to apply mechanical choppers. Thermal or photon detectors equipped with interference filters are more often used in contemporary analysers. They have greater sensitivity and they can operate with sources of lower power and small thermal mass. The radiation sources are now modulated directly by using pulsed circuits, thus offering a high reliability as no moving parts are involved (Puton et al., 2002).

A schematic diagram of a simple infrared gas analyser developed especially for detection of hydrocarbons is shown in Figure 25.10. This analyser employs a partially selective source and a partially selective detector, so chosen that their combined characteristics limit the sensitivity of the combination to a narrow spectral region, with the absorption band centred at 3.4 μm. The indium arsenide photovoltaic detector operating at ambient temperature has maximum detectivity at about 3.4 μm, a rapid decrease in sensitivity at longer wavelengths and no response to radiation of wavelength greater than 4 μm. It is used in conjunction with a source of radiation consisting simply of a bead (about 3 mm diameter), of borosilicate glass encapsulating a platinum-rhodium heating coil. The optimum temperature of operation was found to be in the region 400–500°C by these workers.

The instrument is very simple in construction. It consists of a single-absorption cell, the inner wall of which is silvered, with the source at one end and the detector at the other. The gas is made to diffuse into the cell through a fine stainless steel sinter. The change in the dc signal from the detector is amplified and can be displayed either on a suitably calibrated metre or chart recorder. No chopper arrangement is necessary.

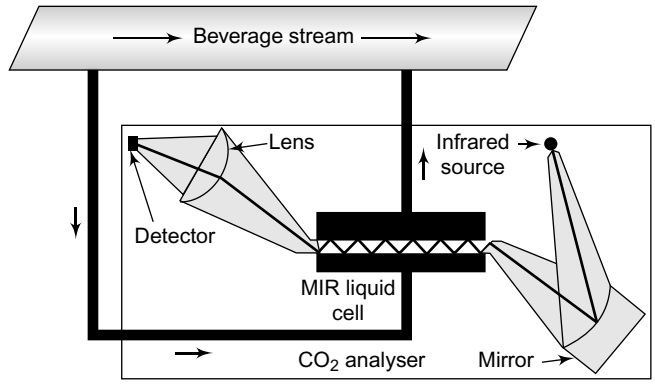


Figure 25.11 *Infrared gas analyser for process applications (after Frant, 1980)*

A similar system comprising a heated quartz source and an indium antimonide detector, which would cover the spectral region from 4 to 6 μm, could be used for the measurement of high concentrations of carbon monoxide and carbon dioxide. To determine either gas unambiguously, a filter cell would be required; this would be filled with carbon dioxide to eliminate the unwanted radiation when detecting carbon monoxide and vice versa.

For process applications, multiple internal reflections technique is used (Frant, 1980). A block diagram of the equipment based on this technique is shown in Figure 25.11. The scheme is basically applicable for measuring CO_2 in the flowing beverage. A portion of the main beverage stream is brought through a small stainless steel pipe to the analyser and then returned to the main stream. The measurements are made using two IR sensors, one measuring sugar and the other measuring CO_2 content.

25.6 THERMAL CONDUCTIVITY ANALYSERS

The thermal conductivity of a gas is defined as the quantity of heat (in calories) transferred in unit time (seconds) in a gas between two surfaces 1 cm^2 in area, and 1 cm apart, when the temperature difference between the surfaces is 1°C. The ability to conduct heat is possessed by all gases, but in varying degrees. This difference in thermal conductivity can be employed to determine quantitatively the composition of complex gas mixtures. Changes in the composition of a gas stream may give rise to a significant alteration in the thermal conductivity of the stream. This can be conveniently detected from the rise or fall in temperature of a heated filament, placed in the path of the gas stream. The changes in temperature can be detected by using either platinum filament (hot wire) or thermistors.

Figure 25.12 shows the relative thermal conductivity of a series of gases of interest for analysis. A gas analysis based on the thermal conductivity procedure presupposes binary gas mixtures or such gas mixtures, respectively, which include a measuring component, whose thermal conductivity differs sufficiently from the thermal conductivity to the carrier gas. Typical examples of application are the measurement of hydrogen in blast furnace gases, the determination of argon in oxygen in the process of air decomposition and of sulphur dioxide in roasting gases in the production of sulphuric acid.

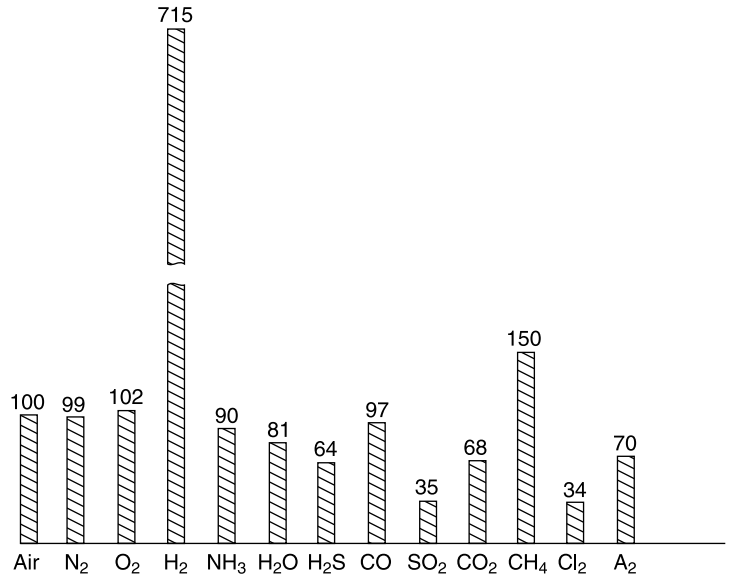


Figure 25.12 *Relative thermal conductivity of different gases*

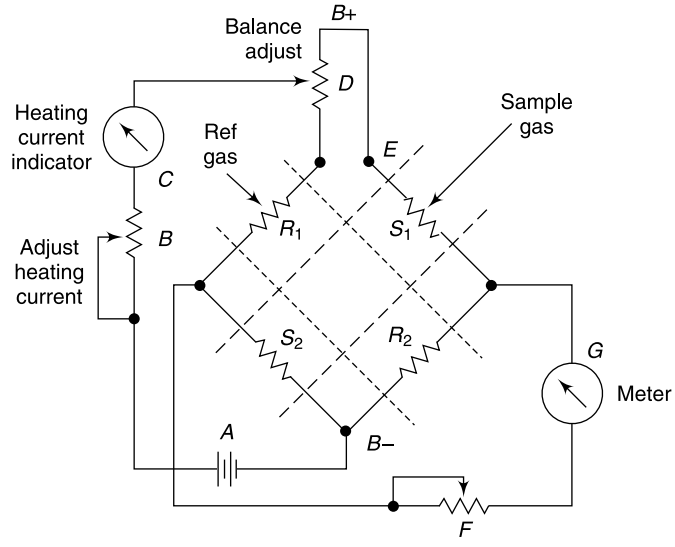


Figure 25.13 *Schematic diagram of a hot wire thermal conductivity analyser*

In a typical hot-wire cell thermal conductivity analyser, four platinum filaments (Figure 25.13) are employed as heat-sensing elements. They are arranged in a constant current bridge circuit and each of them is placed in a separate cavity in a brass or stainless steel block. The block acts as a heat sink. The material used for construction of filaments must have a high temperature-coefficient of resistance. The materials generally used for the purpose are tungsten, Kovar (alloy of Co, Ni and Fe) or platinum.

Two filaments connected in opposite arms of the Wheatstone bridge act as reference arms, whereas the other two filaments are connected in the gas stream, which act as measuring arms. The use of four-cell arrangement serves to compensate for temperature and power supply variations.

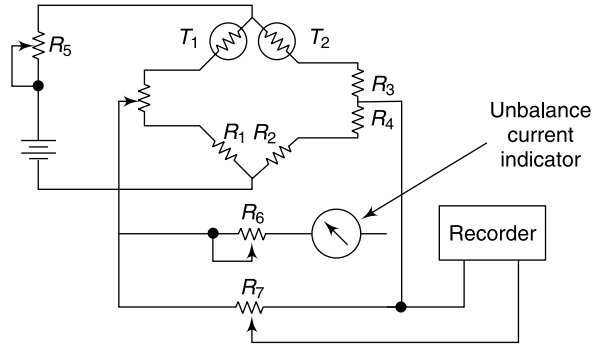


Figure 25.14 *Schematic diagram of thermal conductivity analyser using thermistors*

Initially, reference gas is made to flow through all the cells and the bridge is balanced precisely with the help of potentiometer D. When the gas stream passed through the measuring pair of filaments, the wires are cooled and there is a corresponding change in resistance of the filaments. The higher the thermal conductivity of the gas, the lower would be the resistance of the wire and vice versa. Consequently, the greater the difference in thermal conductivities of the reference and sample gas, the greater the unbalance of the Wheatstone bridge. The unbalance current can be measured on an indicating metre, or on a strip-chart recorder.

Thermistors can also be used as heat-sensing elements arranged in a similar manner, as hot-wire elements in a Wheatstone bridge configuration. Thermistors possess the advantage of being extremely sensitive to relatively minute changes in temperature, and have a high NTC. When used in the gas analysers, they are encapsulated in glass. Thermistors are available which are fairly fast in response. The circuit arrangement is shown in Figure 25.14.

Thermal conductivity gas analysis is inherently non-specific. Therefore, the simplest analysis occurs with binary gas mixtures. A thermal conductivity analyser can be used in respiratory physiology studies to follow CO_2 concentration changes in the individual breaths of a patient. A high speed of response necessary for this purpose can be obtained by reducing the pressure of the gas surrounding the filaments, to a few millimetres of mercury absolute. The variations in the proportions of oxygen and nitrogen in the sample stream will have little effect, since they both have almost the same thermal conductivity. The effect of changes in water vapour content can be minimised by arranging to saturate the gas fed to both the sample and reference filaments.

An analysis of a multi-component mixture is possible, if all components but one have almost the same thermal conductivity, so that it can be treated as a binary mixture. Similarly, analysis is also possible if all components of the mixture other than the one being measured vary in the same ratio from each other.

25.7 ANALYSERS BASED ON GAS DENSITY

It is known that the density of an ideal gas has a direct linear relation with the molecular weight of that gas. Fortunately, all real gases behave as ideal gases at room temperature and normal atmospheric pressure. Instruments based on the principle of gas-density balance are commercially available.

Figure 25.15 illustrates the principle of operation of a gas-density balance. The reference gas enters the balance at *A*, where it splits itself into two streams and leaves the balance at *D*. Two

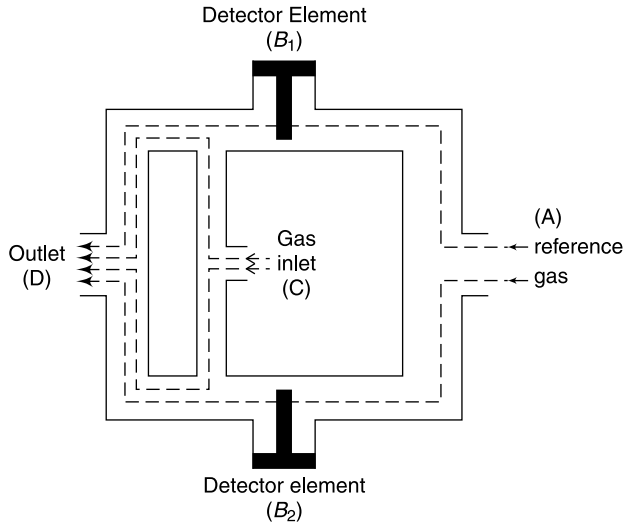


Figure 25.15 *Principle of gas analyser based on gas density*

detectors (B_1 and B_2), which may be either hot wires or thermistors and mounted in the path of the two streams, are connected as two arms of a Wheatstone bridge. When the reference gas flows such that the flow is balanced, the two detectors are equally cooled and the recorder would indicate a zero base line.

The sample gas enters the balance at *C* and it also splits into two streams. It mixes with reference gas in the horizontal conduits and leaves at *D*. If the sample gas has the same density as the reference gas, there will be no unbalance of reference streams or of the detector elements. If the sample carries a gas having a higher density than the reference gas, it will cause a net downward flow, partially obstructing the flow in the lower path, like A-B_2-D. This would result in raising the temperature of the detector element B_2. This in turn, increases the flow in the path A-B_1-D and causes more cooling of the element B_1. This temperature differential causes an unbalance in the bridge, the unbalance being linearly proportional to the gas-density difference between the reference and the sample gas. If the detectors used are hot wires, it may require some factor of amplification before the signal can be given to a recorder. The use of thermistor generally eliminates the requirement of amplification. The effective sample volume is typically 5 ml.

25.8 METHOD BASED ON IONISATION OF GASES

The spectral regions for maximum radiation absorption for different gases are of different wavelengths.

For example:

N_2	less than 900 Å (far ultraviolet)
O_2	1,450 Å (ultraviolet)
CO_2	2.73, 4.25 and 14.93 μ
Water Vapour	2.6, 20 and 52 μ (infrared)

Neither nitrogen nor oxygen analyses are routinely done using these absorption bands. However, with sufficient electrical excitation and at suitable pressures, gases emit radiation in different ways (like spark, arc, glow discharge in different parts of the radiation spectrum). Measurement of the emitted radiation can help in the determination of unknown concentration of a gas in a mixture. This technique has been utilised for measurement of nitrogen gas, particularly in respiratory gases.

The measuring technique utilised for measuring nitrogen is essentially that of a photospectrometer, wherein a gas sample is ionised, selectively filtered and detected with a photocell, which provides an appropriate electrical output signal. The presence of nitrogen is detected by the emission of a characteristic purple colour, when discharge takes place in a low pressure chamber containing the gas sample. Nitrogen metres are usually employed in the medical field for measurement of nitrogen concentration, to follow breath-by-breath variation in respiratory gases and other nitrogen gas analysis applications.

The instrument generally operates in two parts. The sampling head contains the ionising chamber, filter and the detector. The other part contains the power supply, amplifier and display system. The ionising chamber or the discharge tube is maintained at an absolute pressure of a few torr. A rotary oil vacuum pump draws a sample and feeds it to the discharge. The voltage required for striking the discharge in the presence of nitrogen is of the order of 1,500 V dc. This voltage is generated by using a DC-DC convertor, or rectifying the output of a high voltage transformer.

The light output from the discharge tube is interrupted by means of a rotating slotted disc (Figure 25.16), so that a chopped output is obtained. This light is then passed through optical filters to the wavelength corresponding to the purple colour. The intensity of light is measured with a photocell and an amplifier specifically tuned to the chopping frequency. The light intensity is proportional to the nitrogen concentration.

Modern nitrogen analysers measure and display digitally the concentration of nitrogen. The sampling rate in these instruments is adjusted with the help of a needle valve, which is normally set at 3 ml/min. The vacuum system provides 600–1,200 μ of Hg. The instrument is calibrated for

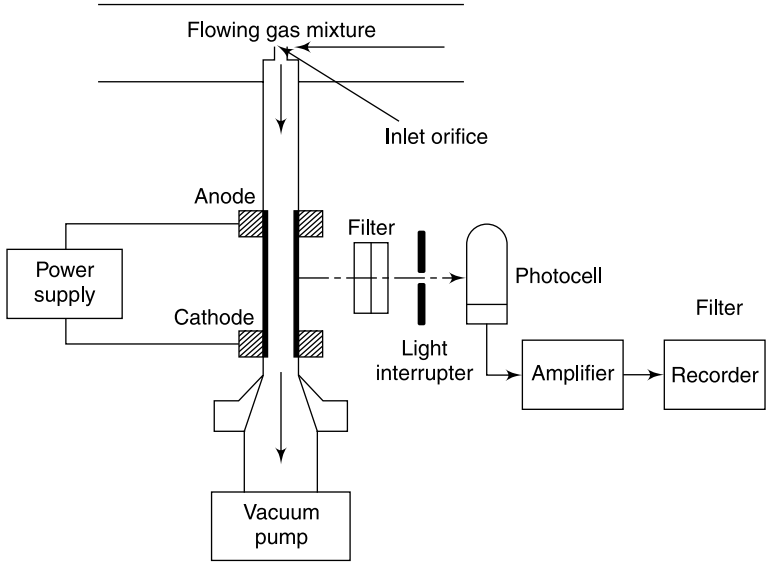


Figure 25.16 *Schematic diagram of a nitrogen meter (Courtesy: M/s Med Science, USA)*

water saturated mixtures of nitrogen and oxygen, as a reading error up to 2% can be expected with dry gases. Compensation for this error can be simply made by adjusting the sampling head needle valve, if it is desired to monitor dry gases.

25.9 PROCESS ANALYSERS

Process analysers are on-line tools for industrial process analytics. They are used to determine the chemical composition or physical properties of substances involved in industrial processes. They enable process optimisation, asset protection, and compliance with environmental regulations.

The need for process analysers arises when we need to monitor an analyte's concentration over time. In that case, it may not be possible to physically remove samples for analysis. This is often the case, for example, when monitoring industrial production lines or waste lines, or when monitoring a patient's blood, or when monitoring environmental systems.

On-line or process analysers are available for pH, conductivity, DO, combustion oxygen, humidity, liquid and gas density and process gas chromatography.

Miniaturised chemical sensors can deliver real-time and on-line information on the presence of specific compounds or ions in complex samples. Usually an analyte recognition process takes place followed by the conversion of chemical information into an electrical or optical signal. Among various classes of chemical sensors ion-selective electrodes (ISE) are one of the most frequently used potentiometric sensors during laboratory analysis as well as in industry, process control, physiological measurements and environmental monitoring. The principle of ISE operation is explained in Chapter 23.

Gas Analysers: Real-time gas analysers are useful to enhance efficiency, safety, throughput, product quality, and to ensure environmental compliance. Gas analysers utilise various technologies such as tuneable diode laser spectroscopy (TDLS), zirconia oxygen analysis, infrared gas analysis, stack gas analysis, dust monitoring, process gas chromatography, continuous emissions monitoring systems (CEMS), hydrogen purity analysis and gas-density systems. Selecting the correct technology is critical to measurement accuracy, reliability, and durability.

Process gas chromatography is used for separating and analysing chemical compounds in the gas phase of industrial processes. GC solutions have proved useful to the oil and gas, refining and petrochemical industries around the world. Over the past 50 years, the GC products of Yokogawa have continued to evolve to meet the ever changing needs of the process industry.

Tuneable Diode Laser Spectrometer (TDLS) is a laser-based gas analyser which enables measurements for process gas, flue gas, impurity analysis, safety, with in situ and extractive methods supported.

Oxygen Analysers: Oxygen analysers provide valuable measurements in combustion control, process quality, safety and environmental applications. These are used in a variety of measurement scenarios including, but not limited to, boiler trimming in power plants, furnace optimisation in refinery and petrochemical applications, process safety in vent headers, and product quality in ethylene production.

Liquid Analysers: Liquid analysers are used for monitoring process chemistry including water quality, providing process optimisation and control. Common measurements include pH, ORP, conductivity, resistivity, percentage of concentration and DO. Liquid analysis is essential for

protecting capital assets including boilers and cooling towers, by preventing corrosion, minimising maintenance, and maximising uptime.

pH Analysers: pH metres, analysers and transmitters are used for continuous process monitoring of pH to ensure water/product quality, monitor effluent discharge, batch neutralisation, pulp stock, scrubbers, cooling towers, chemical, water/wastewater treatment and many other applications.

ORP Analysers: Oxidation reduction potential (ORP or redox potential) instruments are used for continuous process monitoring of ORP to ensure corrosion control, biocide disinfectant, scrubber efficiency, chromium reduction, cyanide destruction, textile dyeing, ozone monitoring, and others.

Conductivity Analysers: Conductivity metres, analysers and transmitters are used for continuous process measurement and monitoring of conductivity, resistivity, de-mineraliser water, RO water, are boiler blow-down, etc.

Dissolved Oxygen (DO) Analysers: Dissolved oxygen (DO) is a liquid process measurement for determining the amount of oxygen dissolved or carried in the process liquid. There are three common technologies for DO measurements: polarographic, galvanic and optical.

Liquid Density Analyser: The liquid density analyser measures liquid density of general process liquids. It has a measuring density range of 0–2.0 g/cm^3, and is unaffected by flow rate and viscosity. Besides general-purpose sensor, explosion-proof sensor is also available.

25.9.1 Process Photometers

Process photometers are designed to provide on-line measurements of gas or liquid components, in simple or complex process streams, for process control, product quality assurance, safety, catalyst protection and area monitoring.

The instrument is generally a fixed filter photometer that utilises optical filters to make continuous measurements. The single-beam dual-wavelength concept used compensates for source and detector ageing and the obstruction of cell windows, while allowing the sample cell to be isolated from the electronics. Figure 25.17 illustrates the optical schematic of process photometer.

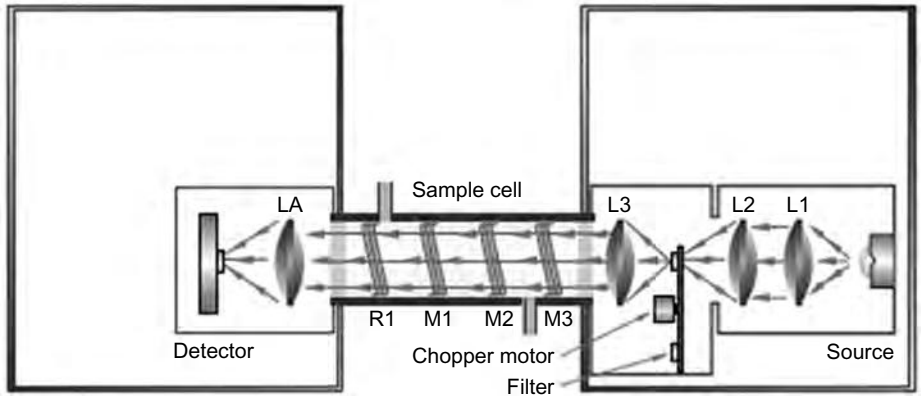


Figure 25.17 *Optical schematic of process photometer (Courtesy: M/s Teledyne Analytical Instruments, USA)*

The design has up to eight filters to the filter wheel. The use of multiple wavelengths enables the instrument to compensate for various interferences and perform multiple component applications. The photometers cover infrared (2.50–14.5 um), near-infrared (800–2,500 nm), ultraviolet (200–400 nm) and visible (400–800 nm) spectral regions.

The process analyser network is a local area network that supports data interchange from process analysers to the Distributed Control System (DCS), in a dedicated and secured manner. It provides seamless connectivity to the plant operating system (Plant LAN) through local area network. Through the remote user-interface, the user can configure, operate or troubleshoot the operation of the analyser from a remote PC. With this remote access capability, all operator functions may be performed from a distance.

25.9.2 CHN/O/S Analyser

Figure 25.18 shows flow schematic of the CHN/O/S Analyser, with which the carbon, hydrogen and nitrogen content in organic and inorganic compounds can be determined. The measuring principle is that of thermal conductivity. Combustion of the weighed sample (typically 1–3 mg; in certain cases up to 500 mg) occurs in pure oxygen under static conditions. With a conversion kit, oxygen and sulphur can be analysed. Helium is used to carry the combustion products through the analytical system to atmosphere, as well as for purging the instrument. Helium is selected for this purpose because it is chemically inert relative to tube packing chemicals, and it has a very high co-efficient of thermal conductivity. Solenoid valves A through G control the gas flow through the system; valves H and I are used for automatic leak testing and other maintenance purposes.

The products of combustion are passed over suitable reagents in the combustion tube to assure complete oxidation and removal of undesirable by-products such as sulphur, phosphorous, and halogen gases. In the reduction tube, oxides of nitrogen are converted to molecular nitrogen and residual oxygen is removed. In the mixing volume the sample gasses are thoroughly homogenised at

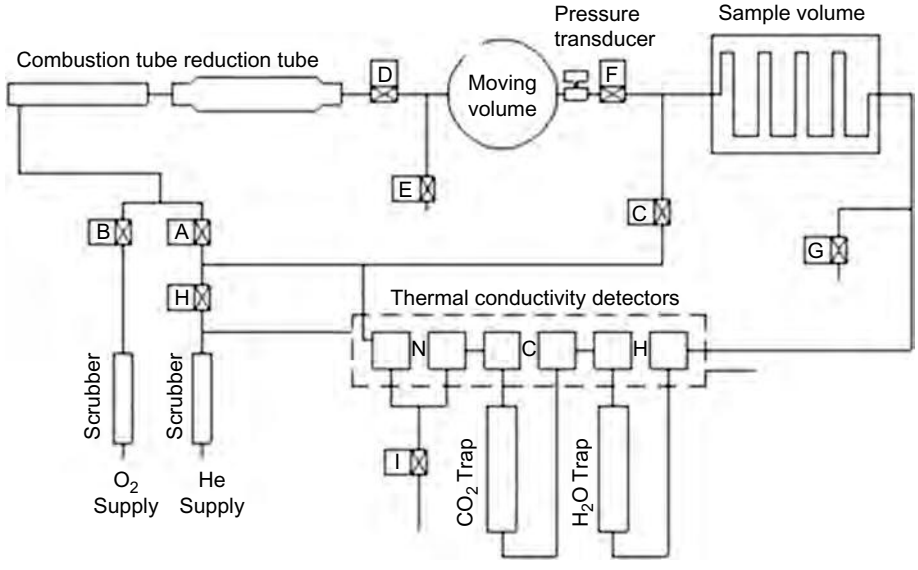


Figure 25.18 *Flow schematic of elemental analyser (Courtesy: M/s Exeter)*

Figure 25.19 *Elemental analyser (Courtesy: M/s Exeter Analytical Inc.)*

precise volume, temperature, and pressure. This mixture is released through the sample volume into the thermal conductivity detector. Between the first of three pairs of thermal-conductivity cells, an absorption trap removes water from the sample gas. The differential signal read before and after the trap reflects the water concentration and, therefore, the amount of hydrogen in the original sample.

A similar measurement is made of the signal output of a second pair of thermal-conductivity cells, between which a trap removes carbon dioxide, thus determining the carbon content. The remaining gas now consists only of helium and nitrogen. This gas passes through a thermal-conductivity cell and the output signal is compared to a reference cell through which pure helium flows. This gives the nitrogen concentration.

For oxygen analysis, the combustion tube is replaced by a pyrolysis tube containing platinised carbon. The reduction tube is replaced by an oxidation tube containing copper oxide. The sample is handled and run as before, but is now pyrolysed in helium so that carbon monoxide is formed from oxygen in the sample. The carbon is oxidised by the copper oxide to form carbon monoxide, which is detected and measured in the same manner as the carbon analysis.

For sulphur analysis, the combustion tube is replaced with one containing a tungstic oxide packing plus a dehydration reagent. The water trap is removed and replaced with silver oxide to absorb SO_2. The sample is handled and run as before, but the sulphur from the sample is oxidised to form SO_2, and the water formed is removed. The SO_2 is detected and measured in the same manner as the hydrogen analysis.

Based on the above illustrated scheme, the Model 440 Elemental Analyser from Exeter Analytical Inc. (Figure 25.19) is capable of routinely analysing almost any sample type including organic compounds, pharmaceuticals, petrochemicals, polymers, refractory, environmental or natural materials (soil, plants or insects). Its horizontal furnace design, allowing for removal of residues between runs, prevents cross contamination and allows for a greater number of samples between combustion column changes.

Employing a combined static/dynamic combustion technology enables it to precisely analyse sample types from volatile to difficult to combust materials including nitrides, graphite fibres, ceramics and even carbides with melting points of over 2,000°C.

25.9.3 Element Analyser Based on Tuneable Diode Laser Spectroscopy (TDLS)

The TDLS analyser is capable of measuring a number of near-infrared absorbing gases in difficult process applications. TDLS has the capability of measuring at very high temperature, high pressures and under difficult conditions (corrosive, aggressive, high particulate service). Most applications can be measured in situ, reducing installation and maintenance costs. The measurements are

rapid, take around 5 s and are interference free, offering improved accuracy when compared to other process analysers.

Typical gases measured by TDLS technique are as follows:

- Oxygen in process applications and combustion applications. Process temperatures can be as high as 1,500°C, pressures can be as high as 10 bar. Measurement span is typically between 1 and 100% oxygen.
- Carbon Monoxide in process and combustion applications. Process temperatures can be as high as 1,500°C.
- Moisture measurements in aggressive process streams at sub-parts per million detection limits are possible in corrosive and aggressive process streams.

TDLS measurements are based on absorption spectroscopy and operate by measuring the amount of laser light that is absorbed (lost) as it travels through the gas being measured. In the simplest form, a TDLS analyser consists of a laser that produces infrared light, optical lenses to focus the laser light through the gas to be measured and then on to a detector, the detector, and electronics that control the laser and translate the detector signal into a signal representing the gas concentration. Figure 25.20 shows tuneable diode laser assembly.

Gas molecules absorb light at specific wavelengths, called absorption lines.

TDL analysers are effectively infrared analysers which obey the Beer-Lambert Law. The circuitry for the TDL system from M/s Spectra Sensors is shown in Figure 25.21. Algorithms developed for the gas measurement are developed using LabVIEW and MATLAB.

An infrared beam is emitted by the tuneable diode laser into a cell with the gas sample and then measured at the receiver. The input laser signal is a sawtooth wave with a superimposed sinusoidal

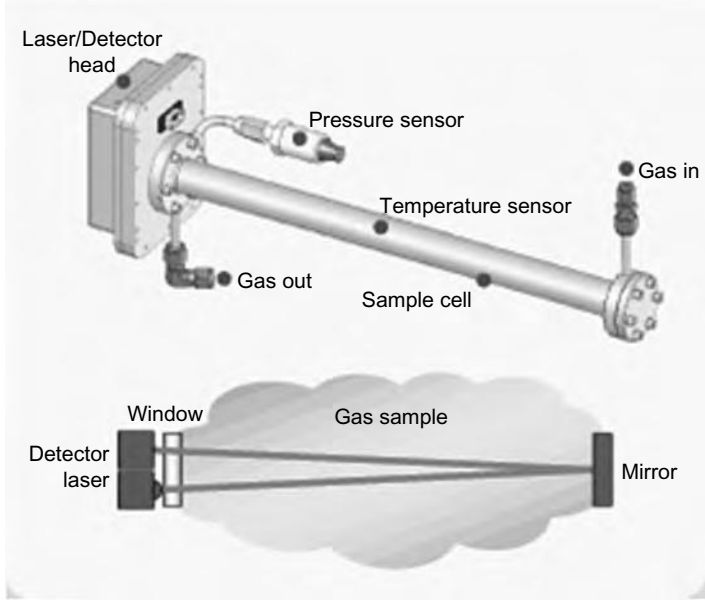


Figure 25.20 *Tuneable diode laser (Courtesy: M/s Spectra Sensors, USA)*

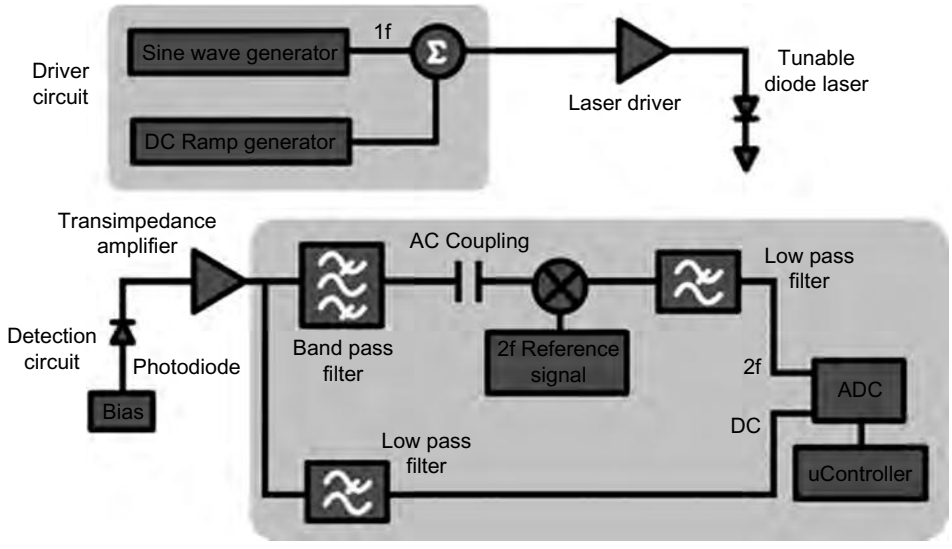


Figure 25.21 *Block diagram of tuneable diode laser spectrometer (Courtesy: M/s Spectra Sensors, USA)*

wave. However, the received signal will differ from the input because the gas molecules absorb some of the electromagnetic radiation. This is caused by the vibro-rotational energy state transition the molecules experience when the frequency of the laser beam matches the resonant frequency of the molecule. Taking the Fourier Transform of the received signal reveals coefficients of the higher order harmonics. The peak height of the 2f harmonic signal is directly proportional to the gas concentration. Thus, the system is able to measure the concentration of the gas sample in the cell.

Using a TDLS as a light source for spectroscopy has the following benefits:

- *Sensitivity*: Application dependant. sub-ppm in some applications.
- *Selectivity*: The narrow line width of the laser is able to resolve single-absorption lines.
- *Power*: Diode lasers have power ranging from 0.5 to 35 mW only.
- *Monochromatic*: No dispersive element (filter, etc.) required. Light source itself is selective.
- *Tuneable*: Wavelength can be swept across the entire absorption feature, this allows resonant (peak) and non-resonant (baseline) measurement during every scan. By measuring the baseline and peak, power at the detector can fluctuate rapidly by large amounts without affecting the measurement. This is useful for high particulate applications.

25.10 LABORATORY ROBOTS FOR PROCESS INDUSTRY

When setting up a laboratory automation system, the first issue confronting the user is to choose between a robot or workstation. Workstations can perform only one type of task but with minimal programming and high reliability, whereas the robots can perform a greater range of tasks but involve higher levels of complexity.

The first lab robot was introduced by Zymark Corporation in 1982. The Zymate robot was used to move labware between various instruments in a 'pie'-shaped work area, simulating the same

procedures followed by *theoretically* higher priced lab researchers and their assistants. Fast forward 30 years and the term lab robot can be further applied to several unique devices;

- *Liquid Handlers*: XYZ robots that pipette reagents, some can move plates using gripper hands. These devices can pipette in a variety of ways from one single channel, 4–12 channels for row or column work or 96 or 384 channels for whole plate transfers.
- *Plate Movers*: Essentially bench top robots that are specifically designed to transport micro-plates. Unlike more flexible industrial robots, these units are pre-tooled for handling micro-plates and come with plate-gripper hands and plate storage racks.
- *Industrial Robots*: While designed for a host of applications from electrical/mechanical manufacturing to painting, welding and sorting, a number of industrial strength robots can be found at the heart of fully integrated systems.

Whatever their work, laboratory robots have some important differences from their industrial counterparts. Most scientists do not have experience in programming motor movements, motion controls, and feedback sensors. The control computers software is designed to make these processes invisible to the average user.

Figure 25.22 is an example of a Zymark Pi system. This type of system was known as a Pi system because the components are arranged in circle. The robot in the centre passes consumables and samples from one workstation to the next.

And while it may be obvious to most, several of the main reasons for automating lab applications remain constants over time.

- *Increased Throughput*: Process more samples without human intervention. This makes lab workers more productive by freeing up time to work on other critical tasks.
- *Repeatability*: Many lab techs can pipette just as good as any liquid handler; however, pipetting is time-consuming and its repetitive nature can make it an error-prone operation. Liquid handling robots largely eliminate human variability and human error, resulting in more reliable data (that's the whole point of an assay, n'est pas?)
- *Human Safety*: Operator exposure to dangerous pathogens, reagents or radioactive chemistry can be minimised with automation. *(Think of the garlic smell of DMSO to skin exposure maybe not life threatening, but certainly a potential social stigma)*
- *Assay Integrity*: While human safety is a major concern for many labs, protecting assay integrity is equally important. Environmental enclosures around automation help minimise assay contamination due to human interaction.

Figure 25.22 *Typical laboratory robot-Zymark Pi system (Courtesy: M/s Caliper Life Sciences, formerly Zymark)*

Today, pharmaceutical applications dominate the laboratory robotics field. A smaller number of systems are found in the food, biotechnology, chemical and household product industries.

26

PARTICLE SIZE ANALYSERS

26.1 PARTICLES AND THEIR CHARACTERISTICS

A particle is a discrete sub-portion of a substance. Particles include solid particles, liquid droplets or gas bubbles. The most common types of materials consisting of particles which are of interest are as follows:

- powders and granules (e.g. pigments, cement, pharmaceutical ingredients)
- suspensions, emulsions and slurries (e.g. vaccines, milk, mining muds)
- aerosols and sprays (e.g. asthma inhalers, crop protection sprays)

Particle size is a geometric characteristic that is usually assigned to material objects with sizes on scale from sub-nano-metres to several millimetres. There are multiple definitions of the particle size depending on the method of its measurement. In order to reflect innate basis for a particular particle size measurement, three different bases are used: numerical, area and weight.

Particle size measurement is extremely important method for research and development and for quality control in many industries. This includes pharmaceuticals, cement, ceramics, paints, emulsions, etc.

Most practitioners in these industries and other fields have reason to know the sizes of particles in a given powder or slurry sample. A fundamental concept to understand is that any accumulation of particles larger than molecular scale actually contains many different sizes of particles. Thus, the term distribution is applied due to the presence of a range of particle sizes in a particular sample. In this way, it is acknowledged that a collection of particulate contains a continuous range of sizes. A collection of particles having a single size is only attainable with meticulous classification effort.

26.1.1 Which Particle Properties Are Important to Measure?

In addition to chemical composition, the behaviour of particulate materials is often dominated by the physical properties of the constituent particles. These can influence a wide range of material properties including, for example, reaction and dissolution rates, how easily ingredients flow and mix, or compressibility and abrasivity. From a manufacturing and development perspective, the following are some of the most important physical properties to measure:

- Particle size
- Particle shape

- Surface properties
- Mechanical properties
- Charge properties
- Microstructure.

Depending upon the material of interest, some or all of these could be important and they may even be interrelated (e.g. surface area and particle size). The two of the most significant and easy to measure properties are particle size and particle shape.

26.1.2 How Do We Define Particle Size?

Particles are 3-dimensional objects, and unless they are perfect spheres, for example, emulsions or bubbles, they cannot be fully described by a single dimension such as a radius or diameter. In order to simplify the measurement process, it is often convenient to define the particle size using the concept of equivalent spheres as shown in Figure 26.1. Here, the particle size is defined by the diameter of an equivalent sphere having the same property as the actual particle such as volume or mass, for example. It is important to realise that different measurement techniques use different equivalent sphere models and therefore will not necessarily give exactly the same result for the same particle diameter.

In order to represent the size range, a frequency diagram or histogram is generated. The histogram is a bar graph, wherein the x-axis represents the particle size. The vertical height of the bars (the y-axis) represents the relative amount of matter contained at that size, or the frequency of occurrences.

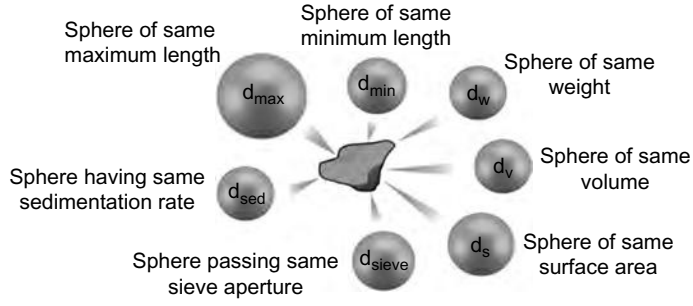


Figure 26.1 *Illustration of the concept of equivalent spheres (Courtesy: M/s Malvern Instruments World Wide)*

26.1.3 Distribution Statistics

In order to simplify the interpretation of particle size distribution (PSD) data, a range of statistical parameters can be calculated, displayed and reported. The choice of the most appropriate statistical parameter for any given sample will depend upon how that data will be used and with what it will be compared. For example, if it is required to report the most common particle size in the sample, the following parameters, as illustrated in Figure 26.2, can be chosen:

- Mean – 'average' size of a population
- Median – size where 50% of the population is below/above the average
- Mode – size with highest frequency.

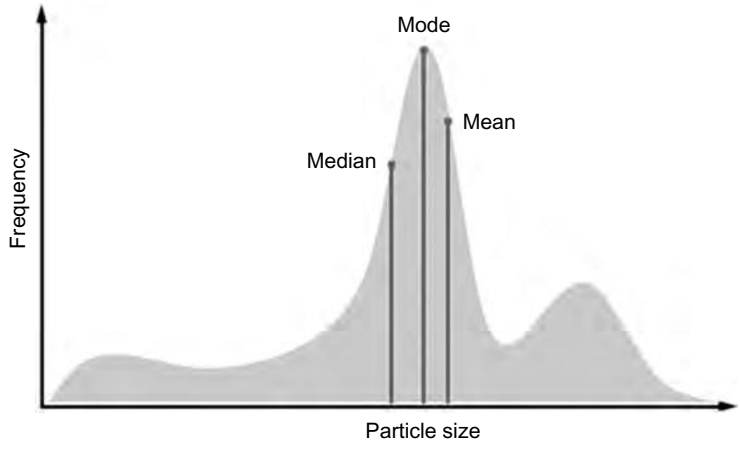


Figure 26.2 *Illustration of the median mode and mean for a particle size distribution (Courtesy: M/s Malvern Instruments, UK)*

If the shape of the PSD is asymmetric, as is often the case in many samples, it is not expected that these three values will be exactly equivalent.

26.2 PARTICLE SIZE MEASUREMENTS

26.2.1 Imaging vs. Non-imaging Techniques

Imaging techniques include optical and electron microscopy, video, holography and photography. Instruments based on imaging are potentially capable of measuring shape, structure and texture in addition to concentration and size. They can, ideally, distinguish between different compositions. Image analysers are often thought of as the primary method of particle size analysis.

However, image analysis has many disadvantages and difficulties. Typically, too few particles are measured to give reliable statistical results. Manual image analysis is subjective, slow and labour intensive. Like other single particle counters, image analysers may suffer from coincidence effects. When automated and computerised, the cost rises and coincidence effects may be more difficult to recognise.

Non-imaging techniques based on equivalent spherical diameters (ESD) reveal information on the shape, structure or texture of the particle. Nevertheless, if definitive information on these parameters is required, then an image analyser is necessary.

Several techniques such as laser diffraction, dynamic light scattering and dynamic image analysis are available for particle size measurements. Each of these techniques generates results in both similar and unique ways. Most techniques can describe results using standard statistical calculations such as the mean and standard deviation.

26.2.2 Laser Diffraction Particle Sizing

Laser diffraction measures PSDs by measuring the angular variation in intensity of light scattered as a laser beam passes through a dispersed particulate sample. Figure 26.3 shows the interaction of the laser light with particles. Diffraction, refraction, reflection and absorption result in

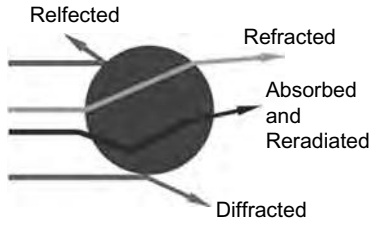


Figure 26.3 *During the interaction of the laser light with particles, diffraction, refraction, reflection and absorption result in light-scattering patterns characteristic for the particle size.*

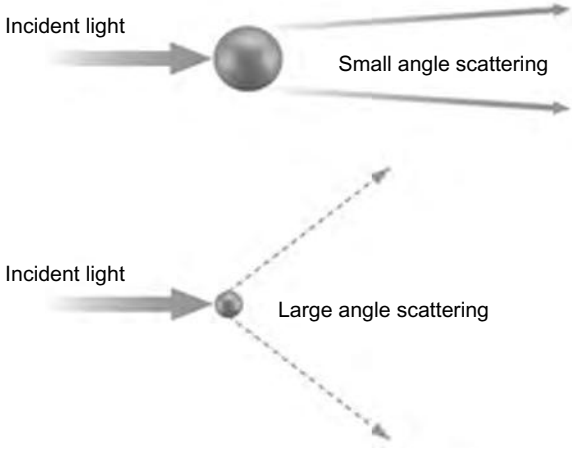


Figure 26.4 *Scattering of light from small and large particles (Courtesy: M/s Malvern Instruments World Wide, UK)*

light-scattering patterns characteristic for the particle size. Laser diffraction is also called Static laser light-scattering, laser diffractometry, Fraunhofer diffraction or Mie scattering.

If the particle size is considerably bigger than the wavelength of the incident light (for particle sizes from several micro-metres upwards), this preferentially results in light-scattering patterns caused by diffraction. The information about the particle size is contained in the small diffraction angles. This phenomenon is described by the Fraunhofer theory and is also called Fraunhofer diffraction. Laser diffraction devices for the determination of particle size used this model first of all by measuring the intensity distribution in the forward direction for small angles (<35°) and used this information for calculating the PSDs.

If the particle size is similar or smaller than the wavelength of the incident light, the light is increasingly scattered with large angles to the side and backwards. The Mie theory describes this phenomenon considering the optical properties (refraction and absorption) of the particles. The smaller the particles are the higher is the contribution of refraction and absorption to the light-scattering pattern.

Large particles scatter light at small angles relative to the laser beam and small particles scatter light at large angles, as illustrated in Figure 26.4. The angular scattering intensity data is then analysed to calculate the size of the particles responsible for creating the scattering pattern, using the Mie theory of light scattering. The particle size is reported as a volume equivalent sphere diameter.

For the measurement of such PSDs, the scattering pattern should be detected over the entire angle range. The interpretation of light-scattering patterns by means of the Mie theory applies to all PSDs, including the Fraunhofer diffraction as a special case. If all of the particles in the sample are larger than the wavelength of the incident light, the Fraunhofer part contained in the Mie theory dominates for the calculation of the PSD. The calculation according to Mie therefore allows the use of only a single-evaluation method for the entire size spectrum.

To calculate the PSD, Mie theory requires knowledge of the optical properties (refractive index and imaginary component) of both the dispersant and the sample being measured. Usually the optical properties of the dispersant can be found from published data, and many modern instruments have in-built databases that include common dispersants.

A typical laser diffraction instrumentation system is made up of the following three main elements:

- *Optical bench*: A dispersed sample passes though the measurement area of the optical bench, where a laser beam illuminates the particles. A series of detectors then accurately measure the intensity of light scattered by the particles within the sample over a wide range of angles. The laser uses a detector system located at a precise distance from the point where the particles interact with the light. A series of small silicon detectors produces electrical current when light illuminates them (Figure 26.5). The detectors also respond to the amount of light (intensity) reaching them, which is related to the amount present of a particular particle size.

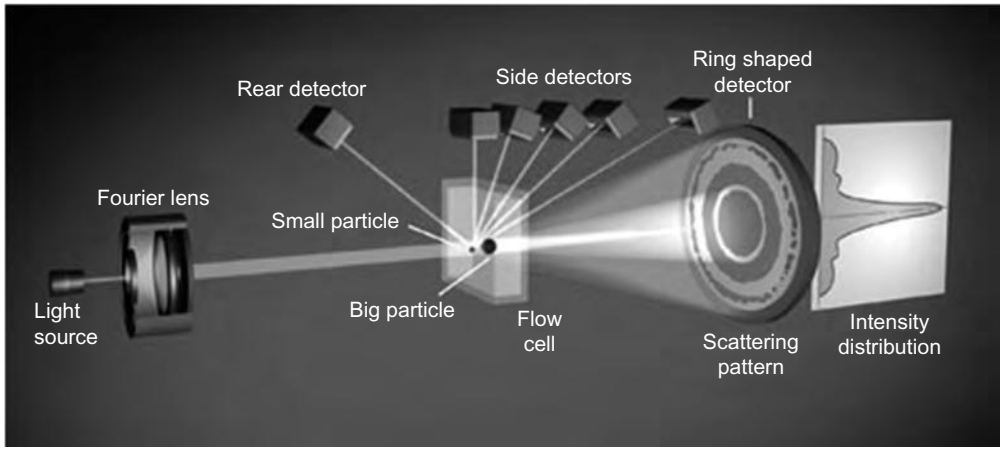


Figure 26.5 *Optical diagram of particle size analyser based on static laser scattering (Courtesy: M/s Retsch Technology)*

The angles of light determined from the illuminated detectors and the intensity of the current produced provide the basis for providing the distribution of particle sizes as well as the quantity of each present. The laser light ($\lambda = 780$ nm) allows for measurement of larger particles by detecting the light scattered over an angular range of 0.02 to approximately 45°. Very small particles scatter light at very wide angles. (Trainer, 2001)

- *Sample dispersion units*: Sample handling and dispersion are controlled by sample dispersion units designed to either measure the wet or dry sample. These ensure the particles are delivered to the measurement area of the optical bench at the correct concentration and in a suitable, stable state of dispersion.

Wet sample dispersion units use a liquid dispersant, aqueous or solvent-based, to disperse the sample. In order to keep the sample suspended and homogenised, it is re-circulated continuously through the measurement zone.

Dry powder sample dispersion units suspend the sample in a flowing gas stream, usually dry air. Normally the entire sample passes once only through the measuring zone, therefore it is desirable to capture data at rapid speeds, typically up to 10 kHz, in order to ensure representative sample measurement.

- *Instrument software*: The instrument software controls the system during the measurement process and analyses the scattering data to calculate a PSD. Results can be displayed on a volume, surface area or number basis. Statistical calculations such as standard deviation and variance are available in either arithmetic or geometric forms. In more advanced instrumentation, it also provides both instant feedback during method development and expert advice on the quality of the results.

Laser diffraction is a widely used particle sizing technique for materials ranging from hundreds of nano-metres up to several millimetres in size. The main advantages of this method are as follows:

- Wide dynamic range – from sub-micron to the millimetre size range
- Rapid measurements – results generated in less than a minute
- Repeatability – large numbers of particles are sampled in each measurement
- Instant feedback – monitor and control the particle dispersion process
- High sample throughput – hundreds of measurements per day
- Calibration not necessary – easily verified using standard reference materials

26.2.3 Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS), sometimes referred to as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS), is a non-invasive, well-established technique for measuring the size of particles and macromolecules typically in the sub-micron region down to below 1 nm. It can be used to measure samples which consist of particles suspended in a liquid (e.g. proteins, polymers, micelles, carbohydrates, nano-particles, colloidal dispersions, and emulsions, etc.).

Principle: Particle size analysers based on DLS are based on the property of the particles in suspension which undergo Brownian motion caused by thermally induced collisions between the suspended particles and solvent molecules.

If the particles are illuminated with a laser, the intensity of the scattered light fluctuates over very short timescales at a rate that is dependent upon the size of the particles; smaller particles are displaced further by the solvent molecules and move more rapidly. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship.

Particles suspended in liquids are in Brownian motion due to random collisions with solvent molecules as illustrated in Figure 26.6. This motion causes the particles to diffuse through the medium. The diffusion coefficient, D, is inversely proportional to the particle size according to the Stokes-Einstein equation:

$$D = \frac{k_B T}{3\pi\eta_o d}$$

D: diffusion coefficient
k_B: Boltzmann's constant
T: absolute temperature
η_o: viscosity
d: hydrodynamic diameter

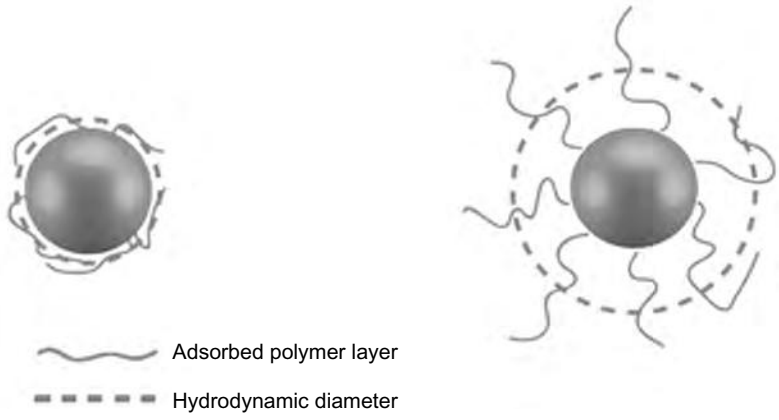


Figure 26.6 *Illustration of the reported hydrodynamic diameter in DLS being larger than the 'core' diameter (Courtesy: M/s Malvern Instruments World Wide, UK)*

This equation shows that, for large particles, D will be relatively small and thus, the particles will move slowly, while for smaller particles, D will be larger and the particles will move more rapidly. Therefore, by observing the motion and determining the diffusion coefficient of particles in liquid media, it is possible to determine their size. The calculations are handled by instrument software.

The above equation shows that sample temperature is important, as it appears directly in the equation. Temperature is even more important due to the viscosity term since viscosity is a stiff function of temperature. Finally, and most importantly, the particle size determined by DLS is the hydrodynamic size. That is, the determined particle size is the size of a sphere that diffuses the way as your particle.

The diameter measured in DLS is called the hydrodynamic diameter and refers to the way a particle diffuses within a fluid. The diameter obtained by this technique is that of a sphere that has the same translational diffusion coefficient as the particle being measured. It is important to note that DLS produces an intensity-weighted PSD, which means that the presence of oversized particles can dominate the particle size result.

Instrumentation: When laser light is directed onto the particles, light is scattered in all directions. The scattered light that is observed comes from a collection of scattering elements within a scattering volume that is defined by the scattering angle and detection apertures. The observed intensity of the scattered light at any instant will be a result of the interference of light scattered by each element; and thus, will depend on the relative positions of the elements. If the particles are in motion, the relative positions of particles will change in time; and thus, fluctuations in time of the scattered-light intensity will be observed.

A conventional DLS instrument (Figure 26.7) consists of a laser light source, which converges to a focus in the sample using a lens. Light is scattered by the particles at all angles and a single detector, traditionally placed at 90° to the laser beam, collects the scattered-light intensity. Because particles in Brownian motion move about randomly, the scattered intensity fluctuations are random. The fluctuations will occur rapidly for smaller, faster moving particles and more slowly for larger, slower moving particles. The intensity fluctuations of the scattered light are converted into

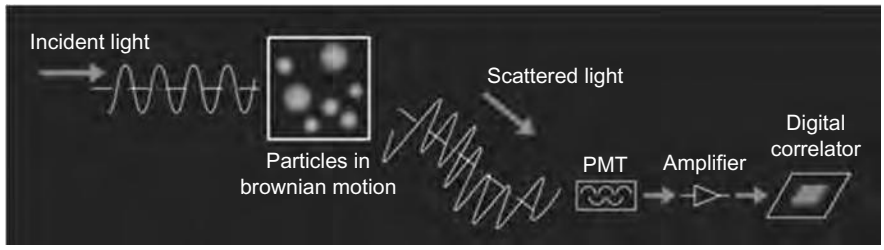


Figure 26.7 *Principle of measurement of particle size based on dynamic light scattering (Courtesy: M/s Horiba)*

electrical pulses, which are fed into a digital correlator. This generates the autocorrelation function, from which the particle size is calculated.

Interference occurs within the light which is scattered by the different particles. This interference can be either constructive (intensification) or destructive (cancellation). Because particles are not bound to a location in liquids, the interference changes over time and this leads to variation of the scattered-light intensity. This intensity is recorded by a highly sensitive photomultiplier (PMT).

The time dependency of the scattered-light intensity therefore depends on the motion speed of the particles and thus also on particle size, which is usually analysed via autocorrelation. Hence, the method is also called photon correlation spectroscopy.

In modern instruments, non-invasive backscatter (NIBS) technology extends the range of sizes and concentrations of samples that can be measured. The sizing capability in these instruments detects the light scattered at 173°. This is known as backscatter detection. In addition, the optics are not in contact with the sample and hence, the detection optics are said to be non-invasive.

Analysers using this method record the scattering light at 90° angle to the incident laser light or they record the back scattering (173°–177°) as shown in Figure 26.8.

Light from the laser light source illuminates the sample in the cell. The scattered-light signal is collected with one of two detectors, either at a 90° (right angle) or 173° (back angle) scattering angle. The provision of both detectors allows more flexibility in choosing measurement conditions. Particles can be dispersed in a variety of liquids. Only liquid refractive index and viscosity needs to be known for interpreting the measurement results.

The signal obtained from the scattered light is fed into a multi-channel correlator that generates a function used to determine the translational diffusion coefficient of the particles analysed. The Stokes-Einstein equation is then used to calculate the particle size. A range of sample cells are available depending on the sample volume and goals of the measurement.

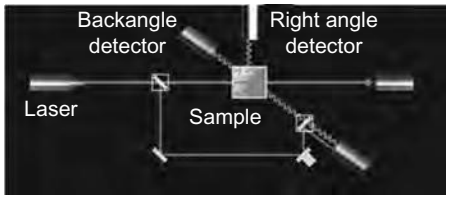


Figure 26.8 *Optical setup for dynamic light scattering (DLS) nano-particle size analyser (Courtesy: M/s Horiba)*

Depending on the physical properties of the sample, the dynamic range is 0.3 nm–8 μm. The lower limit is influenced by concentration, how strongly the sample scatters light and the presence of large, unwanted particles. The upper limit is influenced by the density of the sample since DLS is modelled on all motion coming from Brownian motion, and not gravitational settling.

The following are the key advantages of DLS technique:

- Particle size range ideal for nano- and biomaterials
- Small quantity of sample required
- Fast analysis and high throughput
- Non-invasive allowing complete sample recovery.

26.2.4 Electrophoretic Light Scattering

Electrophoretic Light Scattering (ELS) is a technique used to measure the electrophoretic mobility of particles in dispersion, or molecules in solution. This mobility is often converted to zeta potential to enable comparison of materials under different experimental conditions.

The fundamental physical principle is that of electrophoresis. A dispersion is introduced into a cell containing two electrodes. An electrical field is applied to the electrodes and any charged particles or molecules will migrate towards the oppositely charged electrode. The velocity with which they migrate is known as the electrophoretic mobility and is related to their zeta potential.

This velocity is measured by the laser Doppler technique, of which there are two implementations: one to determine a frequency shift, which can give a full zeta potential distribution, and the second, phase analysis light scattering (PALS), where the phase shift is measured. PALS is a more sensitive method, but only gives an average zeta potential value.

Instruments based on the DLS can be used as particle size analysers, and to measure zeta potential and molecular weight. Typical applications include nano-particles, colloids, emulsions and sub-micron suspensions.

The charge on the surface of particles is characterised by measuring the zeta potential of a suspension. The sample is injected into a disposable cell and a measurement of the particle electrophoretic mobility results in the calculated zeta potential (Figure. 26.9). The zeta potential of the sample is most often used as an indicator of dispersion stability. Large magnitude zeta potential values indicate that an electrostatically stabilised suspension will remain stable. The zeta potential is often measured as a function of pH or other change in the chemistry to help formulators create

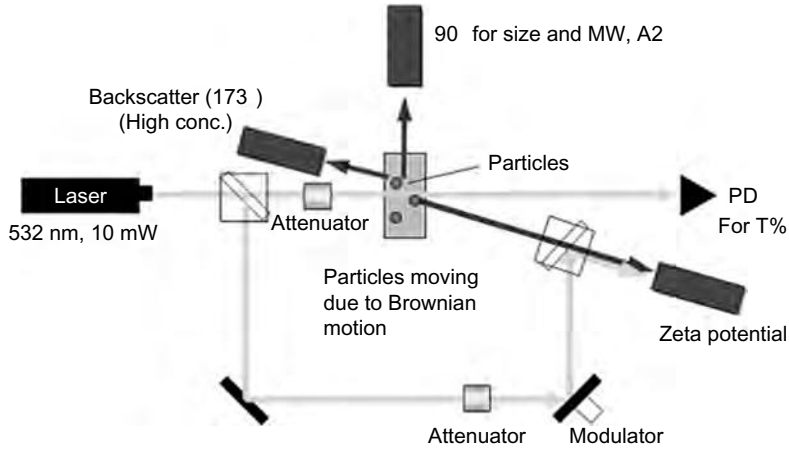


Figure 26.9 *Particle size analyser based on dynamic light scattering (Courtesy: M/s Horiba)*

new products with a long shelf life. Conversely identifying conditions at which the zeta potential is zero (i.e. the sample is at the isoelectric point) allows one to choose optimum conditions for flocculating and separating particles.

26.2.5 Acoustic Spectroscopy for Particle Sizing

Ultrasonic attenuation spectroscopy is another method to measure the PSD of colloids, dispersions, slurries and emulsions. The basic principle is to measure the frequency-dependent attenuation or velocity of the ultrasound as it passes through the sample. The attenuation includes contributions from the scattering or absorption of the particles in the measuring zone, and is depending on the size distribution and the concentration of the dispersed material.

In a typical set-up as shown in Figure 26.10, a high frequency generator is connected to a piezoelectric ultrasonic transducer. The generated ultrasonic waves are coupled into the suspension and interact with the suspended particles. After passing the measuring zone, the ultrasonic plane waves are received by an ultrasonic detector and converted into an electrical signal, which is amplified and measured. The attenuation of the ultrasonic waves is calculated from the ratio of the signal amplitudes on the generator and detector side.

PSD and concentration can be calculated from the attenuation spectrum by either using theoretical calculations requiring a large number of parameters or an empirical approach using a reference method for calibration.

This method can be used for a wide size range of particles covered from below 10 μm to above 3 mm. PSDs can be measured at very high concentrations (0.5% to >50% of volume) without dilution. This eliminates the risk of affecting the dispersion state and makes this method ideal for in-line monitoring.

Current instruments use different techniques for the attenuation measurement: with static or variable width of the measuring zone, measurement in transmission or reflection, with continuous or sweeped frequency generation, with frequency burst or single-pulse excitation.

For process environment, probes are commercially available with a frequency range of 100 kHz to 200 MHz and a dynamic range of >150 dB, covering 1–70% of volume concentration, 0–120°C, 0–40 bar, pH 1–14 and hazardous areas as an option.

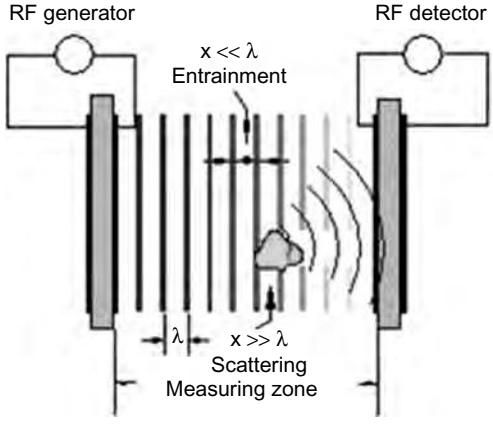


Figure 26.10 *Set up of an ultrasonic attenuation system for particle size analysis (Courtesy: M/s Sympatec Gmbh)*

Acoustic spectroscopy makes possible particle size measurement in concentrated dispersions and emulsions with no dilution and no sample preparation. This is critical in many cases when dilution affects PSD. Also, this method can be applied for structured dispersions. Structure contributes to ultrasound attenuation by itself, but this contribution can be subtracted using appropriate existing theory. In addition, acoustic spectroscopy could resolve PSDs of different species of particles in mixed dispersions, assuming that they have different densities.

This method can be used for continuous monitoring of particle size during milling,

crystallisation and other industrial processes. One of the most important applications is particle sizing of nano-dispersions and other nano-particulates. Acoustics can monitor presence of nano-particles with precision of 1%.

26.2.6 Automated Imaging

Automated imaging is a high-resolution direct technique for characterising particles from around 1 micron up to several millimetres in size. Individual particle images are captured from dispersed samples and analysed to determine their particle size, particle shape and other physical properties. Statistically representative distributions can be constructed by measuring tens to hundreds of thousands of particles per measurement. Static imaging systems require a stationary dispersed sample, whereas in dynamic imaging systems the sample flows past the image capture optics as illustrated in Figure 26.11.

A typical automated imaging system is composed of the following three main elements.

- *Sample presentation and dispersion*

  A range of sample presentation methods is available depending upon the type of sample and instrumentation employed. Static imaging techniques use a flat surface such as a microscope slide, a glass plate or a filter membrane. Dynamic imaging instrumentation uses a flow cell through which the sample passes during the measurement.

- *Image capture optics*

  Images of individual particles are captured using a digital CCD camera with appropriate magnification optics for the sample under study. In dynamic imaging systems the sample is typically illuminated from behind the sample, whereas static imaging systems offer more flexibility in terms of the sample illumination (e.g. episcopic, diascopic, darkfield, etc.). Polarising optics can also be used for birefringent materials such as crystals. The advanced dynamic imaging systems use a hydrodynamic sheath flow mechanism to achieve consistent focus for even very fine particles.

- *Data analysis software*

  Typical instruments measure and record a range of morphological properties for each particle. Graphing and data classification options in the software enable extracting the relevant data from the measurement. Individually stored grey-scale images for each particle provide qualitative verification of the quantitative results.

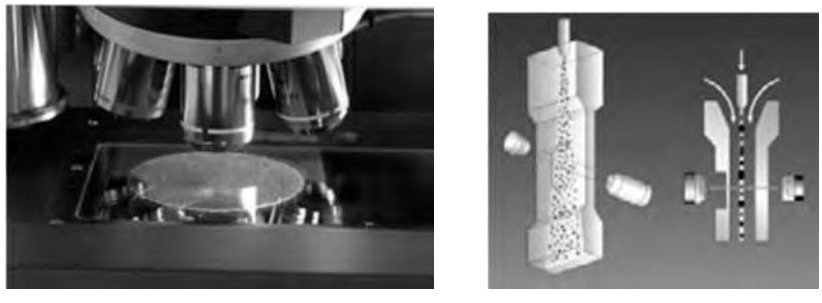


Figure 26.11 *Illustration of static imaging (left) and dynamic imaging (right) optical arrangements (Courtesy: M/s Malvern Instruments World Wide, UK)*

In many applications, particle size and shape information provide an important process and quality indicator. Based on digital image processing, the CAMSIZER/CAMSIZER XT from M/s Horiba is immediately able to analyse the particle shape of the sample material in a detailed and representative manner.

The sample is fed in from the feed channel so that all particles fall through the measurement field. During the measurement procedure the two digital cameras (CCD) perform different tasks. The basic camera records large particles, the zoom camera records the small ones. The contact-free optical measurement is carried out in real time and simultaneously obtains all the required information about particle size and particle shape.

The patented measuring setup – two digital cameras as an adaptive measuring unit – improves and optimises particle analysis by digital image processing. Thus, it is possible to measure a wide range of particles from 1 µm to 30 mm extremely accurately, without having to switch measuring ranges or make adjustments.

26.3 PARTICLE COUNTERS

26.3.1 Coulter Principle Method

Particle counters, operating on the principle of conductivity change, which occurs each time a particle passes through an orifice, are generally known as Coulter Counters. The method was patented by Coulter in 1956 and it forms the basis of several particle counting instruments manufactured by a number of firms throughout the world. The technique is extremely useful for determining the number and size of the particles suspended in an electrically conductive liquid.

The Coulter Principle, also known as electrical sensing zone method (ESZ), provides number, volume, mass and surface area size distributions in one measurement, with an overall sizing range of 0.4–1,200 microns (practical limits for most industrial powders are from 0.06 to 200 microns). Its response is unaffected by particle colour, shape, composition or refractive index.

Particles suspended in a weak electrolyte solution are drawn through a small aperture, separating two electrodes between which an electric current flows. The voltage applied across the aperture creates a 'sensing zone'. As particles pass through the aperture (or 'sensing zone'), they displace their own volume of electrolyte, momentarily increasing the impedance of the aperture.

This change in impedance produces a pulse that is digitally processed in real time. The Coulter principle states that the pulse is directly proportional to the 3-dimensional volume of the particle that produced it. Analysing these pulses enables a size distribution to be acquired and displayed in volume (µm3 or fL) and diameter (µm). In addition, a metering device is used to draw a known volume of the particle suspension through the aperture; a count of the number of pulses can then yield the concentration of particles in the sample.

The instrument based on the Coulter principle works most satisfactorily when the average diameter of the particles ranges between 2 and 40% of the diameter of the measuring hole. Therefore, the following condition must be met for the measuring range:

$$D/50 \leq d \leq D/2$$

where d = maximum particle size
D = diameter of the measuring aperture

The lower limit of measurement in the system is governed by the noise sources involved. The noise sources include the thermal noise of the detector due to the resistance of the fluid flowing across the orifice and the noise inherent in the electronic circuits.

Particles of sizes larger than the diameter of the measuring aperture can only pass through the aperture if their longest dimension is parallel to the axis of the measuring aperture; otherwise they cause the clogging of the aperture. The upper limit of measurement is thus imposed by the increasing size of the particles. When the size of the particle approximates the diameter of the aperture, an amplitude linearity error is produced. Therefore, to count particles of different sizes, the diameter of the measuring aperture must be chosen in such a way as to meet the conditions of measurement. Simple procedures enable the extension of the range as needed via the use of successive aperture size – the total range covered is from about 0.5 microns to upwards of 500 microns.

A wide range of particle counting instruments based on the Coulter principle have been designed to meet a wide variety of requirements. These instruments range from the small counters used primarily for red and white cell counts in very small hospitals and clinics, to the multi-parameter microprocessor-controlled instrument featuring fully automatic diluting of samples and printing of results.

In the basic instrument as shown in Figure 26.12, a glass measuring tube 'C' provided with an aperture 'A' is immersed into the suspension. The pressure difference created between the two sides of the aperture draws the suspension to flow through the aperture. This pressure difference is generated by a simple mechanical pump consisting of a syringe, a relay and other parts.

A constant current is normally passed between the electrodes E_1 and E_2. Therefore, the electric resistance of the liquid measured between these two electrodes changes rapidly when a particle having electric conductance differing from the conductance of the electrolyte passes through the aperture. This results in the generation of a voltage pulse, which is amplified in a pre-amplifier of

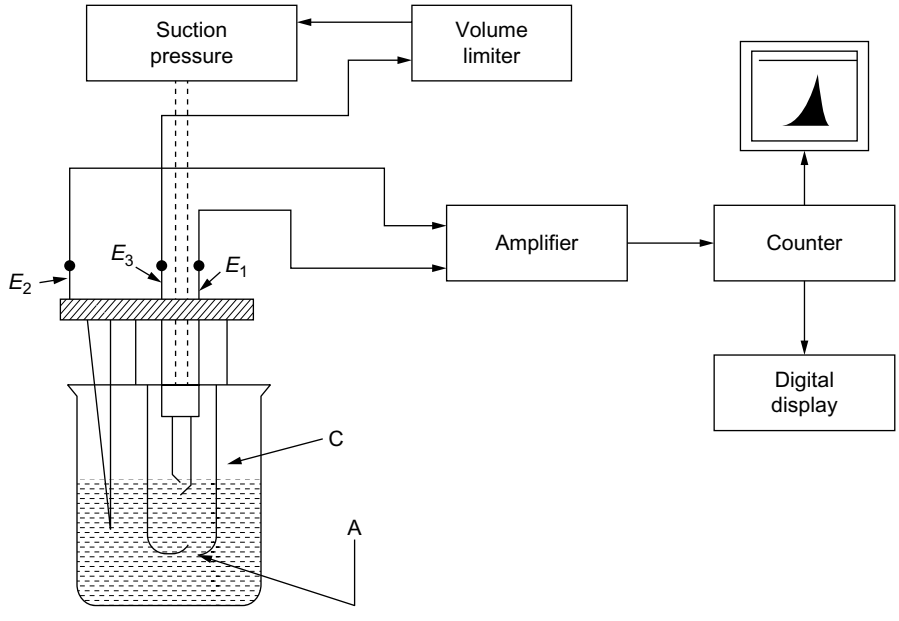


Figure 26.12 *Block diagram of a Coulter counter*

high gain and low noise level. The output signal of this stage goes to a discriminator, which compares the amplitude of the pulse arriving at its input with the pre-set triggering level. If the input signal exceeds the triggering level, the discriminator gives out a pulse of constant shape and amplitude. These pulses go to a counting circuit for the display of the measured parameter. Figure 26.13 shows the sequence of building up the pulse in terms of increase in resistance at different positions of the cell with respect to the orifice.

To enable the instrument to count only those pulses which fall within certain pre-set size limits, the threshold facility is required. The threshold is also necessary to enable the instrument to ignore any electronic noise, which may be present in the system. The lower threshold sets an overall voltage level, which must be exceeded by a pulse before it can be counted. The upper threshold will not allow pulses to be counted which exceed its pre-set level.

The measuring tube C is provided with a third electrode E_3 which helps to monitor the suction of a limited volume of the suspension. When the liquid level reaches E_3, the pump is changed over from the suction phase to the pressure phase. The counting process also occurs during the time the electrolyte is forced out of the measuring tube. After the liquid loses contact with the electrode E_1, the counting automatically stops and the unit becomes ready once again for the operation. The manufacturers recommend that to increase the reliability of the results, it is preferable to repeat the measurements several times, and calculate the mean value based on these measurements.

The Coulter counters are usually provided with a LCD to display the pulse information, which has passed through the amplifier, and acts as a visible check on the counting process indicating instantaneously any malfunctions such as a blocked orifice. In particular, it provides information regarding (i) relative cell size, (ii) relative cell size distribution, (iii) settings of the threshold level control, and (iv) means to check the performance of the instrument for reliability of counts. The voltage pulses produced each time a cell passes through the orifice are displayed on the screen as a pattern of vertical spikes.

Coulter counters also help to give an idea of the size distribution of various types of particles. It has been stated that the pulse height is to a first approximation, proportional to the volume of the particle. Converting the pulse height into a digital number, through an A-D converter, and storing it in memory can help to obtain a plot of the number of particles as a function of their size (McGann et al. 1982).

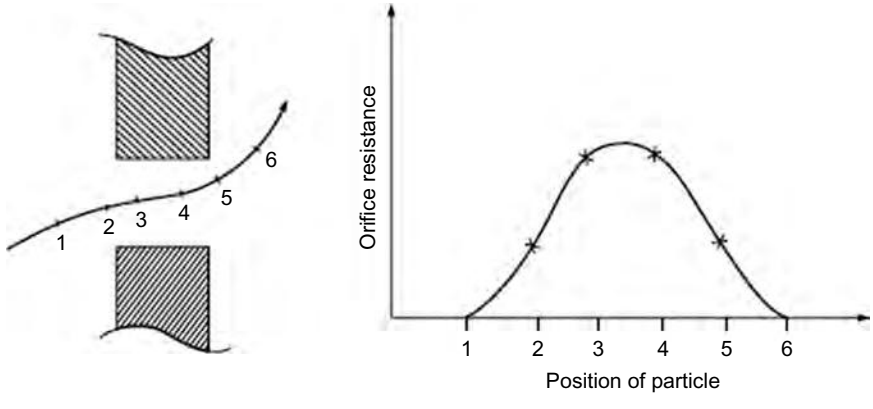


Figure 26.13 *The sequence of building up the pulse in terms of increase in resistance at different positions the cell has with respect to the orifice*

The particle size analysers based on the principle of change in impedance (Coulter Principle) particularly find applications in haematology as blood cell counters.

26.3.2 Blood Cell Counters

26.3.2.1 Types of blood cells

Changes in the normal functioning of an organism are often accompanied by changes in the blood cell count. Therefore, the determination of the number and size of blood cells per unit volume often provides valuable information for accurate diagnosis. The blood constitutes 5–10% of the total body weight and in the average adult, it amounts to 5–6 l. Blood consists of corpuscles suspended in a fluid called plasma in the proportion of 45 parts of corpuscles (cells) to 55 parts of plasma. The percentage of cells in the blood is called the haematocrit value or packed cell volume (PCV). The majority of the corpuscles in blood are red blood cells (erythrocytes), others being white blood cells (leucocytes) and platelets (thrombocytes).

Erythrocytes (Red Blood Cells): Red blood cells have the form of a bi-concave disc with a mean diameter of about 7.5 μ and thickness of about 1.7 μ. The mean surface area of the cell is about 134 μm^2. There are about 5.5 million of them in every cubic millimetre of blood in men and nearly 5 million in women. In the whole body, there are about 25 billion erythrocytes and they are constantly being destroyed and replaced at a rate of about 9,000 million per hour. The normal red cell lasts approximately 120 days before it is destroyed.

Leucocytes (White Blood Cells): Leucocytes are spherical cells having a nucleus. There are normally 5,000–10,000 white cells per cubic mm of blood but their number varies during the day. They live for 7–14 days and there is a rapid turnover, with constant destruction and replacement. Leucocytes form the defence mechanism of the body against infection. They are of two main types: the neutrophils and the lymphocytes. Neutrophils ingest bacteria and lymphocytes are concerned with immunological response.

Thrombocytes (Platelets): Platelets are usually tiny, round, oblong or irregularly shaped cells of the blood with an average diameter of approximately 2 μ. They play an important role in the blood coagulation process. There are usually 2,50,000–7,50,000 platelets in every cubic mm of blood.

26.3.2.2 Special considerations for blood cell counting

The underlying principle of the blood cell counter based on Coulter principle is that blood is a poor conductor of electricity, whereas certain diluents are good conductors. For a cell count, therefore, blood is diluted and the suspension is drawn through a small orifice. By means of a constant current source, a direct current is maintained between two electrodes located on either side of the orifice. As a blood cell is carried through the orifice, it displaces some of the conductive fluid and increases the electrical resistance between the electrodes. A voltage pulse of magnitude proportional to the particle volume is thus produced. The resulting series of pulses are electronically amplified, scaled and displayed on a suitable display.

To achieve optimum performance and to enable the relationship of change in resistance with volume of the cell to hold good, it is recommended that the ratio of the aperture length to the diameter of the aperture should be 0.75:1 (i.e. for an orifice of 100 μ diameter the length should be 75 μ).

One great advantage of this instrument is that the clogging of the capillary is greatly eliminated by applying a bi-directional flow during the measurement procedure. The number of particles N in a unit volume is determined from the following relation:

$$N = \frac{HLE}{V}$$

where

H = factor of dilution
L = scaling factor of the counter
V = measured volume
E = result displayed on the digital display.

For example, if the diluting factor is 63,000 (typical for red cell count in this instrument) and 520 appears on the display, L being 60 and V equal to 0.378 cm^3, then

$$N = \frac{\left(6.3 \times 10^4\right) \times (60) \times \left(5.20 \times 10^2\right)}{\left(3.78 \times 10^2\right)}$$
$$= 5.20 \times 10^6 \text{ per mm}^3$$

In other words, the solution contains 5.2 million blood cells per cubic millimetre.

The capillary diameter for red cell count is 72 μm and the dilution factor is 63,000. For white cells, the diameter is 102 μm, and the dilution factor is 630. For platelet count, the diameter of the capillary is 72 μm and a dilution of 6,300 is used.

A multi-parameter Coulter (Figure 26.14) counter provides the universally accepted profile of white cell count, red cell count, mean cell volume, haemotocrit, mean cell haemoglobin concentration, mean cell haemoglobin and haemoglobin. Besides this, the following five parameters are presented: platelet count, red cell distribution width, mean platelet volume, plateletcrit and platelet distribution width. Such instruments are microprocessor-controlled and provide the flexibility of expressing in various forms the available count and size data stored in memory. A specially designed ticket is provided showing results of the measurements and computations. All this information is presented simultaneously so that the data can be quickly reviewed just by scanning the display.

Figure 26.14 *Typical multi-parameter blood cell counter (Courtesy: M/s Jinan meiyilin electronic instrument Co.,Ltd.)*

26.3.3 Errors in Electronic Counters

There are a number of errors that may occur in the electronic cell counting technique. Briefly, these errors are categorised as follows:

- Aperture clogging
- Uncertainty of discriminator threshold

- Coincidence error
- Settling error
- Statistical error
- Error in sample volume
- Error due to temperature variation
- Biological factors
- Dilution errors
- Error due to external disturbances

The sources of errors enumerated above originate from random factors and since the results too would contain random errors, different results may be observed in repeated tests. The actual value should therefore be determined with due consideration of the fluctuations, measurement losses and other circumstances (Khandpur, 2014).

26.4 PORTABLE COULTER COUNTERS

26.4.1 Handheld Automated Cell Counter and Analyser

The automated blood cell counters are generally of bench type. However, the Scepter™ cell counter (Figure 26.15**)** from M/s Millipore provides true automation based on Coulter Principle in a miniaturised, handheld, format-enabling rapid cell counting. The instruments are based on Coulter principle. With its micro-fabricated, precision-engineered sensor, the Scepter™ cell counter delivers accurate and reliable cell counts in less than 30 s and use the 60 µm sensor for particles between 6 and 36 µm, and 40 µm sensor for particles between 3 and 17 µm. The user prepares a dilution of the cell culture of interest and uses the cell counter to aspirate a sample of this dilution into the sensor.

The cell counter's screen displays the following:

- Cell concentration
- Average cell size

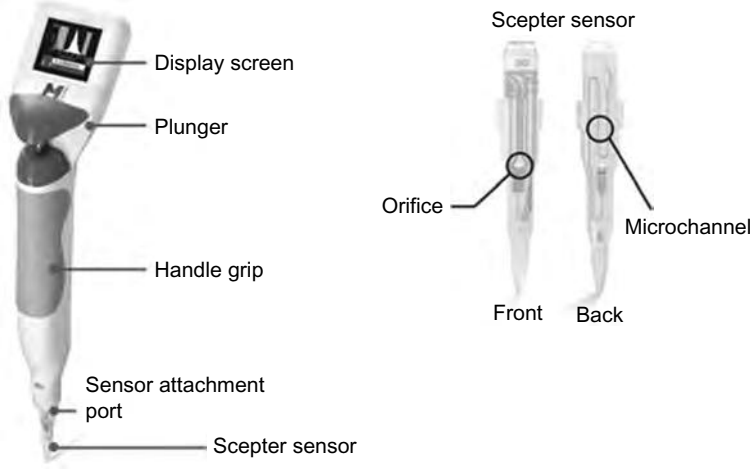


Figure 26.15 *Handheld automated blood cell counter (Courtesy: M/s Millipore)*

- Average cell volume
- Histogram of size or volume distribution
- Precise volumes are drawn into the Scepter™ sensor.

As cells flow through the aperture in the sensor, resistance increases. This increase in resistance causes a subsequent increase in voltage, and voltage changes are recorded as spikes with each passing cell. Spikes of the same size are bucketed into a histogram and counted. This histogram gives you quantitative data on cell morphology that can be used to examine the quality and health of your cell culture.

The Scepter™ cell counter detects every cell and displays the population as a histogram of cell size distributions. From the histogram, you count all the cells or use the gating function to count a chosen sub-population. By monitoring changes in the histogram, the health and quality of the cell culture can be gauged from one experiment to the next.

26.4.2 Blood Cell Counter for Point-of-Care Testing (POCT)

The demand for immediate Point-of-Care Testing (POCT) in diagnosis has been increasing. To meet this demand, a palmtop-type model of blood cell counter has been developed based on MEMS technology (Miyamura, 2004). The instrument uses chip-type sensor including a cartridge-type pre-processing part for dilution. Furthermore, the cartridge is designed to be disposable to save the need of cleaning and to eliminate the possibility of contamination by other samples.

The blood cell counter sensor (Figure 26.16) is basically structured by silicon substrate and glass substrate which are bonded together. The flow channel is fabricated by creating microscopic grooves and an aperture on the silicon, patterning electrodes on both sides of the aperture, then bonding the glass on which a fluid contact hole is created. Sample enters the contact hole, and passes through the flow channel on the silicon groove and through the aperture. At this time, pulse signals are output due to change of impedance between the electrodes. Then the sample is discharged from the other end of the contact hole.

The palmtop blood cell counter consists of three parts: main body, cartridge and dilution tank. The cartridge includes glass capillary for sampling, sensor for blood cell counting and fluid-level

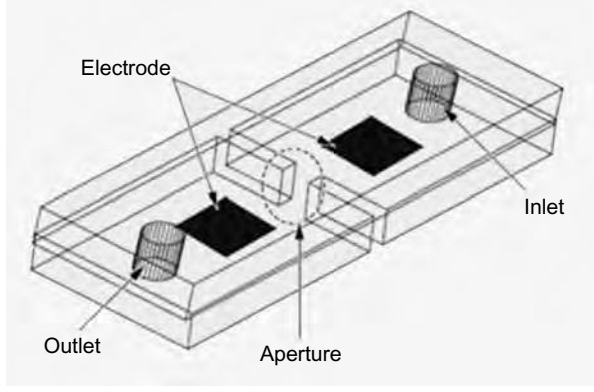


Figure 26.16 *Point-of-care blood cell counter-sensor using MEMs technology (Miyamura, 2004)*

sensor for detecting the end of measurement. The main body basically includes cartridge socket, valve, pump, electronic circuit and LCD for display. The dimensions of the cell counter are $7.5 \times 14.5 \times 5$ cm.

For measurement, 1 μl of blood sample is collected with glass capillary by pricking a fingertip of patient. The dilution tank is then inserted into the tip of sampling part. The cartridge is then inserted with the tank into the main body. The device power turns on as the cartridge is inserted. The blood sample collected with glass capillary flows into the dilution tank which contains a dose of diluted solution by applying pressure, then the mixing starts for approximately 20 s. After mixing, the sample is introduced to the sensor with the pressure reduced and the measurement starts. The waste fluid is accumulated in the cartridge gradually up to the fluid-level sensor. When it reaches the fluid-level sensor, measurement is completed in about 1 min. After calculation, the measurement result is displayed. Wu et al. (2011) describe the design, fabrication and characterisation of MEMS Coulter counter for dynamic impedance measurement of cells.

27

ENVIRONMENTAL POLLUTION MONITORING INSTRUMENTS

The awareness of and concern about the deteriorating environment is increasing world over. It is necessary to monitor changes taking place in the quality of the environment, for initiating efforts to control it. There are a large number of instruments required to accomplish the environmental pollution monitoring program. Representative sampling of pollutant concentrations at the point of discharge, or in the environment requires an understanding of the pollutant characteristics as well as specific objectives, in considerations like monitoring and control. Only then, appropriate measurement techniques can be identified and employed. Pollution monitoring is thus a very complex task, which involves systematic collection and evaluation of physical, chemical, biological and related information pertaining to environmental quality and effluent discharges.

27.1 AIR POLLUTION MONITORING INSTRUMENTS

Rapid industrialisation and steadily increasing vehicular traffic on the roads have generated an acute problem of air pollution. At places, gaseous and dust pollutants in the atmosphere reach such a magnitude, that suitable measures to limit the emission of pollutants become imperative. One of the basic measures to environmental quality management lies in the ability to continually monitor environmental characteristics and provide reliable, accurate and automatically recorded data for timely interpolation. Pollutants are required to be monitored, not only in the environment but also as they are discharged from the multitude of stacks, exhaust pipes from industrial establishments and vehicles. Today, the pollutants that must be measured are many in number and they are emitted from a still larger number of sources.

The most reliable and useful information on the degree of pollutant concentrations is obtained by continuous sampling using officially accepted methods. The data about the peak concentrations obtained from such sampling is indispensable, when monitoring the air for potentially hazardous pollutants. For some specific purposes, however, samples collected intermittently may be useful, as a basis towards a more meaningful monitoring programme. Regardless of whether one is involved with continuous monitoring or monitoring on an intermittent basis, the analysis of the air we breathe has become a necessity for the survival of mankind.

27.1.1 Representation of Concentration of Gases

Gas concentrations in the atmosphere are generally represented as parts per million by volume (ppm/V or simply ppm) or parts per hundred million (pphm) and parts per billion (ppb). These units express the number of molecules of pollutant found in a million (10^6), a hundred million (10^8), a billion (an American billion is 10^9, British billion is 10^{12}) or a trillion (10^{12}) molecules of air, respectively.

On the other hand, toxicological data is generally represented on a gravimetric basis (e.g. micrograms per cubic metre or milligrams per litre). Conversion from volumetric to gravimetric concentration can be obtained by applying gas laws, the general equation for this being:

$$\mu g/m^3 = (\text{ppm}) \times PM/RT \times 10^3$$

where P = total pressure (atm)
M = molecular weight of gas of interest
R = gas constant = 0.0821 1-atm/(mole) (K)
T = absolute temperature, K

27.1.2 Types and Concentration of Various Gas Pollutants

Many types of pollutants are present in the atmosphere. However, for many reasons, it is not simple to make definite statements about pollutant concentrations. This is because of the extreme variability of the pollutant concentration themselves, which vary constantly with air turbulence and the strength of emissions. The major gas pollutants are carbon monoxide, sulphur oxides, hydrocarbons, nitrogen oxides and particulates.

27.1.2.1 Carbon monoxide

Carbon monoxide is colourless, odourless, poisonous gas that has an affinity for haemoglobin, which 210 times that of oxygen. By combining with haemoglobin in the blood, it inhibits the delivery of oxygen to the body's tissues, thereby causing asphyxia or shortness of breath.

Carbon monoxide is an especially hazardous pollutant with which even healthy individuals get affected. It is a by-product of combustion processes, in which incomplete oxidation of fossil fuels takes place. It is basically associated with automotive exhaust and deep mining operations. Industrial processes also contribute to carbon monoxide pollution levels. The average concentrations of this gas are found to be much below 200 ppm.

27.1.2.2 Hydrocarbons

Hydrocarbons enter the atmosphere from a wide variety of sources, like petroleum-refining processes, incomplete combustion of organic fuels and evaporation of fuels and solvents. Gasoline is the major source of their emission from internal combustion engines, since they exhaust unburned and partially burnt hydrocarbons. These are also important because of their reaction in the atmosphere in the formation of ozone with nitrogen oxides and sunlight, and also to form photochemical smog. Methane constitutes the major component of the total hydrocarbon emission.

27.1.2.3 Sulphur oxides

Sulphur dioxide is the most common and the most abundant gaseous pollutant. The major health effects associated with its high exposure include breathing and respiratory problems. Sulphur dioxide damages trees, plants and agricultural crops. It can also accelerate corrosion of natural and man-made materials. It thus, damages ecosystems and a major precursor in the formation of acid rain. It is emitted into the atmosphere from heating and from industrial plants using high-sulphur coal and other sulphur-containing fossils. It is also associated with the most serious urban pollution disasters. Sulphur trioxide, which is estimated to be one-hundredth the concentration of sulphur dioxide, is formed in a secondary reaction and becomes sulphuric acid aerosol. It is reported that SO_2 is present at an average level of 0.024 ppm in urban areas.

27.1.2.4 Nitrogen oxides

Nitrogen oxides are a class of pollutants formed when fuel is burned at a very high temperature (above 1,200°F), such as in automobiles and power plants. For an pollution purposes, the main pollutants are nitric oxide (NO), nitrogen dioxide (NO_2) and other oxides of nitrogen. NO_2, for example, is a highly toxic, reddish brown gas that is formed through the oxidation of NO emitted primarily from the combustion of fuels. It basically causes an odorous, brown haze that irritates the eyes and particularly affecting sufferers of respiratory disease.

The emissions of nitrogen oxides are chiefly from the products of fuel combustion in furnaces and engines. It has been observed that the distribution of nitrogen oxides closely follows the population concentrations. Its level ranges from 0.5 to 0.12 ppm on an annual average basis.

27.1.2.5 Oxidants (Ozone)

The presence of oxidants in air can have a significant effect on ambient air quality. The major component of total oxidants is ozone that has damaging effect on plants, animals and material if present in higher concentrations.

Other pollutants which may be present are hydrogen sulphide, ammonia, halides (chlorine, fluorine and bromine) and carbon dioxide (CO_2).

27.1.3 Instrumental Techniques and Measurement Range

Gas	Full-scale range	Measurement technique
Carbon monoxide	0–50 ppm	Infrared absorption
	0–200	Gas chromatography (flame ionisation detector)
Hydrocarbons	0–80 ppm	Ultraviolet absorption
		Gas chromatography (flame ionisation detector)
		Mass spectrometry
Sulphur oxides	0–2 ppm	Ultraviolet absorption
		Infrared absorption

Gas	Full-scale range	Measurement technique
		Gas chromatography (flame photometric detector)
		Colorimetric method
		Conductimetric method
		Coulometric method
		Electrochemical transducers
Nitrogen oxides	0–1 ppm	Colorimetric method
		Coulometric method
		Chemiluminescence method
		Electrochemical transducers
		Infrared spectroscopy
		Lasers
Oxidant	0–500 ppb	Chemiluminescence method
		Coulometric method
		Colorimetric method
		Ultraviolet absorption method

27.2 AIR POLLUTION MONITORING STATIONS

When carrying out air quality measurements, it is important to choose precisely the site locations, measuring stations and methods. When measuring pollutant gases in the air, there is a difference between mobile measurements and stationary measurements stations (Ionel and Popescu, 2010).

Mobile stations (laboratories) measure at random and change locations, as per pre-planned programme, to determine the spatial distribution of the air pollutants.

Stationary measurements continuously record the temporal distribution of air pollutants in fixed points of a certain area. Stationary measurements are generally carried out at representative points of the investigated area (Erste allg. 1986).

If it is desired to determine the spatial distribution of the ambient air pollutants, it can be done only with mobile measurements at the corner points, say of a 1×1 km grid over the area to be investigated.

Special guidelines have been drawn up so that the site locations for automatic measuring stations are chosen according to standardised criteria. Selection of the right sampling site can be of great importance for stationary as well as for mobile measurements.

According to general international methods, the main pollutants to be measured are: NO_x (NO, NO_2), CO, SO_2, PM10, O_3, TOC (Total organic carbon) and VOC (Volatile organic compounds).

Figure 27.1 shows a scheme of a set-up of a typical measuring station for air pollutants. The measuring gas suction hoods and different meteorological measuring instruments are installed on a 10-m high altitude. In addition to the instruments for the specific measurement of pollutants, there are several other instruments for recording of the meteorological parameters such as wind direction and wind speed, temperatures, global radiation, duration of rain, amount of rain, etc., as well as

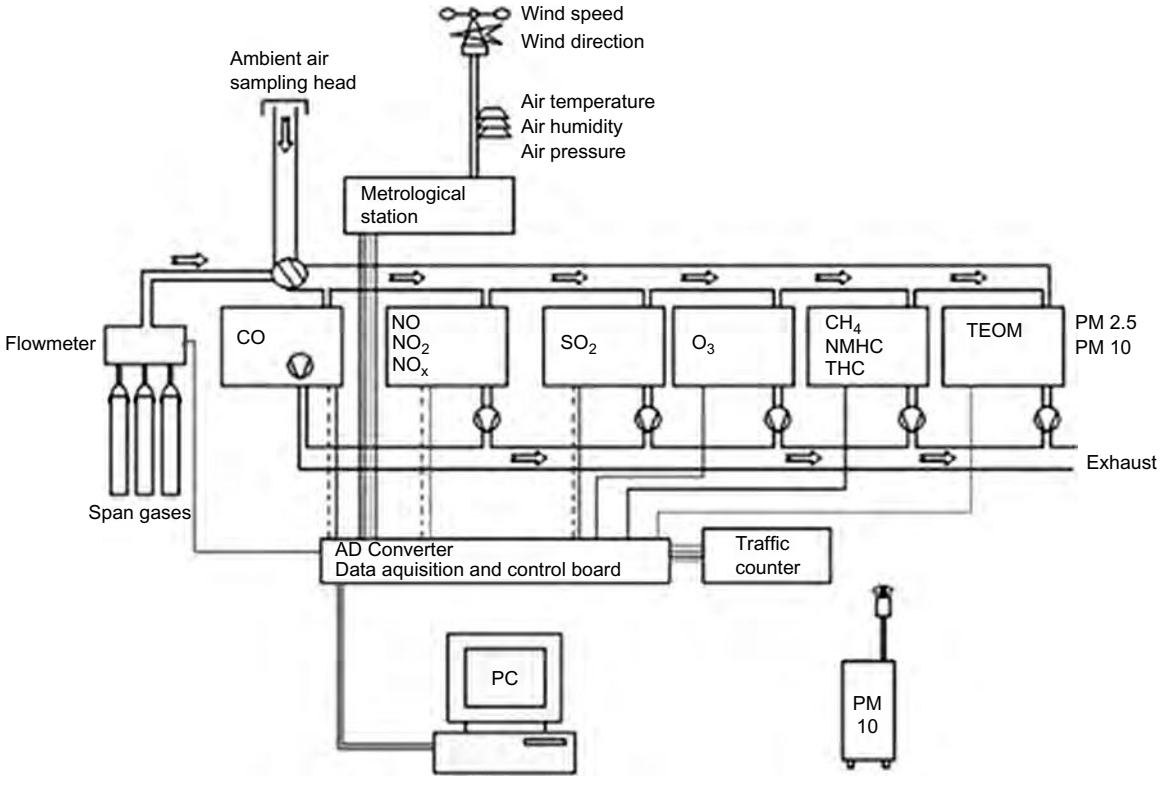


Figure 27.1 *Set-up of the sampling system of an air quality station with air suction through tubes and with calibration gas switching (www.mediu.ro)*

necessary electronics and a computer, to record, calculate and store the measured values. The computer also controls the valve timing and records the measured values according to the given valve position. Mean values of the measurand are calculated and are stored in the memory or/and printed out. Some measuring stations work with direct data transmission to a central computing station.

The sampling system for gaseous pollutants consists generally of a sampling nozzle, a guidance tube, a central sampling tube, sampling connections leading from the central sampling tube to the individual measuring instruments and a fan or a pump. The sampling nozzle might be constructed as a pre-separator for particles and precipitation.

The measurements are carried out for various pollutants in the ambient air using standard and well-established techniques as given below:

- NO_x detection and monitoring: chemiluminescence principle.
- SO_2 concentration: UV by fluorescence method.
- CO detection and measurement: non-destructive infrared technique (NDIR)
- PM10: Sampling analyser based on the filtration and mass determination of a specific suspension by means of gravimetric principles.
- O_3 measurements: UV photometry.
- CH_4 monitoring: flame ionisation detector NMHC.

Calibration and adjusting are of major importance for the correctness of the measurements. Adjusting means setting or trimming a measuring instrument as accurately as possible, while calibrating signifies determining the deviation measured as compared to the accurate value. In air pollution measurements, calibration conditions are set up with the help of calibration gas mixtures. These are gas or gas mixtures having a composition pre-determined with sufficient certainty by measuring basic parameters such as mass, volume, time and amount of substance (molar number) by applying independent analysis methods. During calibration, the calibration gases are entered into the measuring setup, the values indicated are read and compared with the values of the calibration gases assumed correct and then the deviations are recorded. The most convenient way of calibrating is with commercially available calibration gases in pressure gas cylinders (Ionel and Popescu, 2010).

The data acquisition system of the station is automatic and the data are stored in computers. The operator generally uses the data to perform graphics for each pollutant and to compare their concentration with law-regulated concentrations.

27.3 CARBON MONOXIDE

27.3.1 Non-dispersive Infrared Analyser

Non-dispersive infrared analysis depends on the characteristic energy of absorption of CO molecule at wavelength of 4.6 μ. Infrared energy is also absorbed by other gases like CO_2, H_2O, SO_2 and NO_2. The differentiation of CO from such type of gases depends on the difference in energy absorbed, as infrared radiation is passed through a sample cell containing CO and a reference cell containing a fixed quantity of nitrogen, CO and water vapour.

Figure 27.2 shows block diagram of CO monitoring device from M/s Horiba. The device uses the modulation effect that occurs with infrared absorption of sample gas itself when sample gas and reference gas are alternately sent to the measuring cell at a pre-determined flow rate. This is achieved using a solenoid valve which is actuated at a frequency of 1 Hz. Unless the gas concentration of the measured component is changed in the cell, the output from the detector essentially becomes zero.

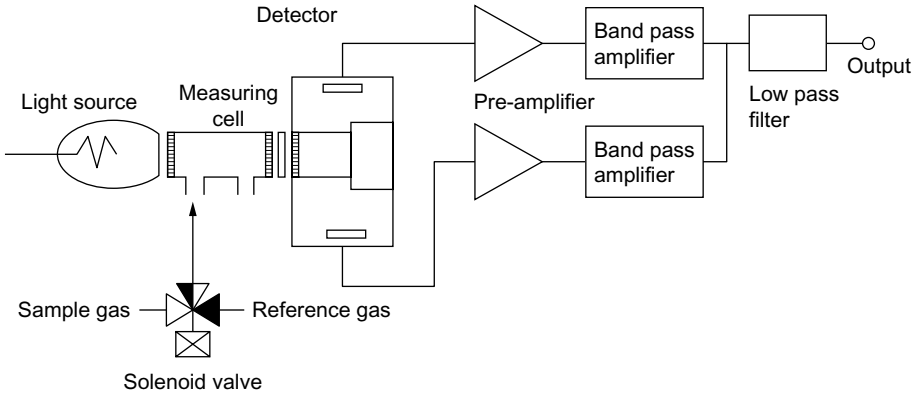


Figure 27.2 *CO monitoring device schematic (Courtesy: M/s Horiba)*

The radiation source is an infrared radiation emitter. The radiation is modulated by a chopper which passes through a chamber containing the probe and in parallel a chamber containing a reference gas. To reduce the influence of interfering gases, optical filters are used. The content of the measurement cell receives periodically infrared radiation with different strength, which results in different temperature and pressure effects. The pressure effects are transferred to electrical signals by a sensor, and this signal correlates to the measured CO concentration. Some systems use the pressure waves for detection, while others use directly the IR receiving an optical detector to measure the CO concentration.

The effects of interfering gases like CO_2 and water vapour can be further minimised by placing optical filters ahead of the sample cell, so that IR radiation window is limited to a range, where radiation absorption by these does not significantly take place.

Non-dispersive infrared absorptiometry has the following advantages:

- The effect of the flow rate is small
- The response speed is high
- High sensitivity measurements are possible
- The effect of interfering components are small
- The equipments are easy to maintain

27.4 SULPHUR DIOXIDE

27.4.1 Conductivitimetry

Analysers based on measuring the change in conductivity of a solution, when a sample of air containing sulphur dioxide is bubbled through it, are the oldest and most commonly utilised instruments for ambient air monitoring. The solution consists of sulphuric acid and hydrogen peroxide. The change in electrical conductivity takes place due to the formation of sulphuric acid by oxidation of the sulphur dioxide.

$$H_2O_2 + SO_2 \rightarrow H_2SO_4 \rightarrow H^+ + HSO_4^-$$

These instruments are characterised by fast response and high sensitivity. However, their performance gets affected due to interference by non-SO_2 gases, that produce or remove ions In solution. Therefore, these analysers are employed only if interfering gases are not in high concentrations, or if they can be effectively removed from the air sample.

Figure 27.3 describes the construction of a conductivity cell for the continuous measurement of sulphur dioxide in air. The cell *C* is made of glass, 1 cm inside diameter with a jet *J*, orifice 0.5 mm diameter and located 1 cm above the reagent surface.

Two electrodes *E* made of 18 SWG stainless steel wire are inserted through a perspex cap *P*. The cap is sealed to the base of the cell. Reagent enters the cell from a central tube inserted in the perspex cap. A small glass bead *B* in the cell acts as a non-return valve on the entry of the feed tube and prevents sulphuric acid diffusing from the cell. The end of the jet is made from a piece of capillary tube. A filter is placed before the jet to prevent blocking due to solid materials. The flow of air through the jet is maintained at approximately 200 ml/min. The cell is of small size (1.5 ml) and its capacity to absorb SO_2 is thus limited. It is therefore operated intermittently, the electrolyte being discharged and replaced at regular intervals of 15 min. The resulting output is recorded as a sawtooth waveform.

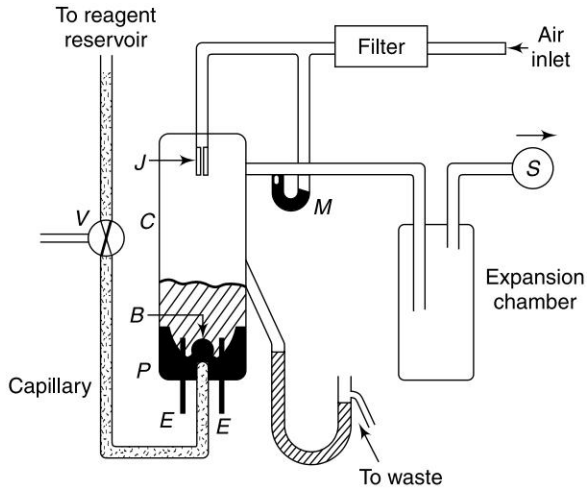


Figure 27.3 *Conductivity method for the measurement of SO_2 in air (After Killick, 1969)*

To measure the conductivity of the cell, 5 V alternative current is applied across the electrodes. Alternating current avoids polarisation. When normal urban concentrations of SO_2 are measured, the current through the cell increases from its zero value of 20 μA to up to 40 μA at the end of 15 min. sampling period. In conditions of heavy pollution, it may go to 2 mA. Because the current is recorded every 15 min., the concentration of sulphur dioxide at any instant is proportional to the slope of the sawtooth at that instant. The calibration is carried out by using known concentrations of sulphur dioxide in air.

27.4.2 Ultraviolet Fluorescence Method

This method is based on the principle that when a sample containing SO_2 molecules is irradiated with ultraviolet (215 nm), SO_2 emits the light of a different wavelength (240 to 420 nm) from that irradiated. The former, irradiated light is referred to as excitation light, and the later, emitted light is referred to as fluorescence. In the fluorescence method, fluorescence, which radiates in all directions, is usually detected at the right angles to the excitation light in order to prevent interference by the excitation light.

Figure 27.4 shows a basic block diagram of a fluorescence SO_2 analyser (Zolner, 1984). The sample gas is continuously drawn into a cylindrical Teflon-coated reaction cell at near ambient pressures. The atmospheric gas is irradiated by UV light in the wavelength range of 190–320 nm that has been mechanically modulated and filtered to 214 nm. If present, SO_2 gives off a fluorescence radiation of 320–380 nm. The higher the SO_2 concentration, the greater the fluorescence. The fluorescent secondary emission of the SO_2 molecules present in the gas is measured by a photomultiplier tube (PMT).

The PMT is located at 90° from the UV lamp source on the axial centre line of the reaction cell. The filtered UV light passes through a collimating lens that focuses the light energy at the centre of the cell. The PMT is optically tuned to measure the fluorescent emission and outputs the signal through an amplifier to a synchronous demodulator. Simultaneously, the UV light source constancy is measured by a reference photodetector tube, located directly across the reaction cell

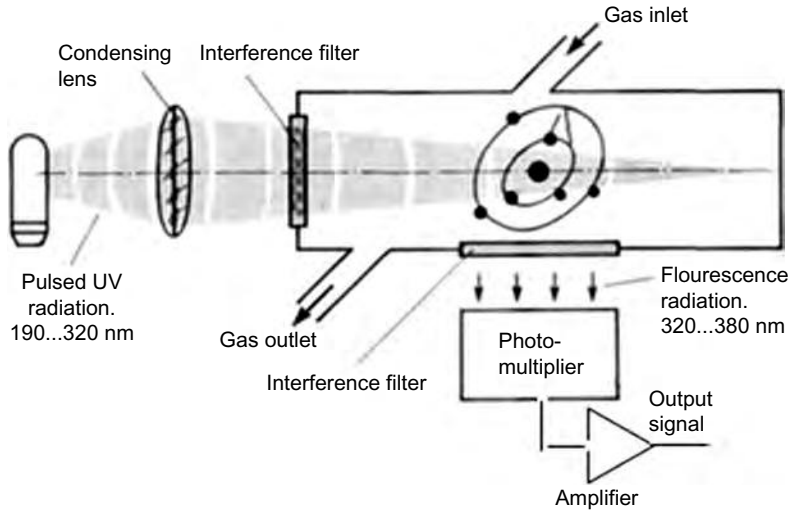


Figure 27.4 *Principle of UV fluorescence measurement (Zolner, 1984)*

from the lamp. The light travels down an optically designed dump to the phototube, whereupon is output is amplified and processed through a nearly identical synchronous demodulator. The mixer board electronics then uses this signal to compensate for any variation in the UV light source. Due to the use of an appropriate interference filter, only a radiation of this wavelength is recorded by the detector (photomultiplier); thus, the measuring principle is strictly selective.

The first stage in the instrument is a hydrocarbon scrubber which removes hydrocarbons contained in ambient air, which are also excited by the light source and consequently emit fluorescence. The SO_2 molecule passes through the hydrocarbon unaffected.

27.5 NITROGEN OXIDES

27.5.1 Chemiluminescence

The phenomenon of emission of radiation from chemi-excited species is known as chemiluminescence. It results due to the formation of new chemical bonds. The species in the excited state possess higher energy levels than the ground state and usually have a very short life. This phenomenon is very useful for measurement of air pollutants, particularly NO and NO_2. Instruments based on the measurement of chemiluminescent emission, based on the following reaction, have been developed:

$$NO + O_3 \rightarrow NO_2 + O_2$$

$$NO_2 \rightarrow NO_2 + h\upsilon \ (\lambda_{max} = 6{,}300 \text{ Å})$$

Since NO_2 reacts only slowly with ozone and the reaction which produces NO_3 is not accompanied by chemiluminescence, it is necessary to reduce NO_2 to NO before admission into the reactor.

$$NO_2 \xrightarrow{\text{energy}} NO + 1/2\ O_2$$

NO and ozone containing gas stream are mixed in a vessel at sub-atmospheric pressure of about 2 mm of Hg. Light emission is measured with a photomultiplier. With the use of high gain, low dark current PMTs, extremely low levels of radiation can be measured. The response of the instruments based on chemiluminescence is linear from 1 ppb to 1,000 ppm of NO. The technique is extremely useful for measurement of NO in automotive exhaust gases.

Figure 27.5 shows the measuring principle for NO_x using chemiluminescence method. The instrument provides continuous and unattended monitoring of NO, NO_2 and NO_x with individual determinations. An internal NO_2 to NO converter permits NO_x analysis and an integral ozone supply system which puts filtered, de-humidified ambient air through an ozonator to generate the ozone necessary for reaction with NO to give chemiluminescence reaction. The instrument has a flow-chopping modulation system to give continuous NO_x and NO analysis. With this system, the sample gas is divided into two separate lines. One sample gas line passes through the NO_2 to NO converter, while the other leads directly to the detector. Also a permeation tube in which only moisture is passed through is used for the sample line is needed. This tube functions so that an influence from the moisture is reduced by minimising difference of moisture concentration between sample gas and reference gas.

Inside the reaction chamber NO reacts with ozone to form NO_2. The NO_2 is excited to a higher electronic state. This chemiluminescence is measured through an optical filter by a photodiode. The modulated hybrid signal from the detector is demodulated to give continuous NO_x and NO signals at the same time.

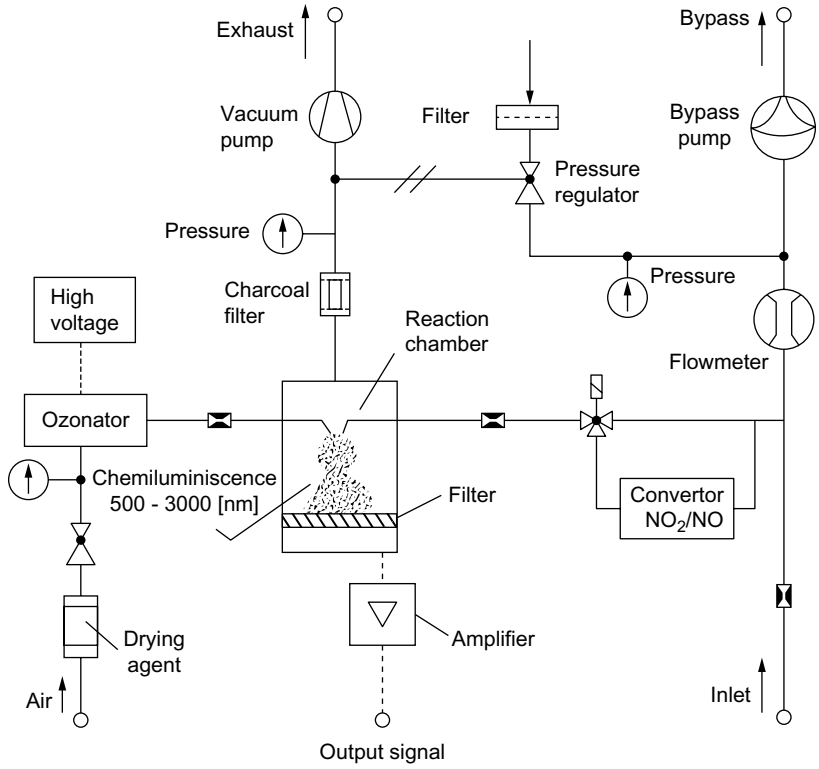


Figure 27.5 *NO_x monitoring device schematic (Courtesy: M/s HORIBA)*

Filtered sample gas is divided into two branches. In one branch, the sample gas flows through an integral converter which reduces NO_2 to NO. In the other branch, the sample gas remains as it is. The sample gas is switched to NO line, reference line, NO line and to reference line again by the solenoid valve with 0.5 s interval. Then it is introduced into respective reaction chamber. Luminescence due to reaction of the sample and O_3 occurred in the chamber is detected by a photodiode. By electrically processing the output of photodiode, it is possible to take out continuous signal in NO line and NO line, respectively.

Flow to the detector unit is controlled by capillaries. Ozone is supplied to the reaction chamber at a constant rate by an internal ozonator which uses de-humidified ambient air as feed gas. The dryer unit has two dryer cylinders. When one cylinder is under operation, the other is regenerated. For regeneration, first heat the tube to 120°C for 135 min to evaporate all the water, and then cool the tube for 45 min. It is possible to perform continuous drying by changing over the line of use and regeneration every 180 min.

27.5.2 Use of CO Laser

Figure 27.6 is a block diagram of an apparatus for detecting NO in 0.25 ppm concentration. The apparatus consists of a CO laser, which emits radiation that is absorbed by the NO in the mixture, the amount of absorption being proportional to the concentration of NO present. The wavelength match between laser and NO is made exact, and hence the absorption is enhanced by placing the NO in a magnetic field of a few kG intensity. The field shifts the absorption wavelength of the NO into coincidence with the fixed laser wavelength.

The CO laser used is a DC excited continuous working laser, which operates at a single wavelength of 5.307 μ and at liquid nitrogen temperature. A diffraction grating is used at one end of the cavity as a line selector. The laser yields 5–30 mW of single line power.

The absorption cell is made of Pyrex and is of 15 mm diameter and 90 cm length. It is evacuated to a pressure of 10^{-6}–10^{-5} torr. In order to produce modulating audio frequency magnetic field along the axis of the cell, insulated wire is closely wound around the outside of the cell over about half its length. The coil is excited with a current of 1 A in the frequency range of 5–150 kHz. This produces a varying magnetic field of about 50 G peak-to-peak intensity. The DC magnetic field is produced by a solenoid, which produces a field up to 2.5 kG.

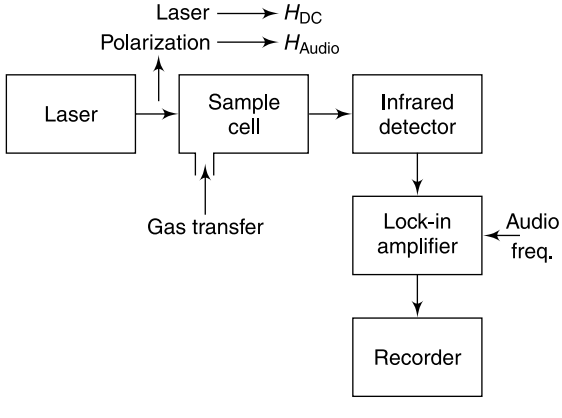


Figure 27.6 *Measurement of nitric oxide using CO laser (After Bonczyk, 1975)*

The detector is a liquid nitrogen cooled Ge–Au element. The signal is amplified in a lock-in amplifier, before it is given to the recorder. The signal amplitude varies linearly with the concentration of NO in the sample.

27.5.3 Laser Opto-acoustic Spectroscopy

Opto-acoustic detectors, in conjunction with thermal IR sources have been widely used in gas detection and measurement systems.

These have been developed with the intention of their application to air pollution measurement. This technique has been used to measure trace amounts of nitrogen oxides in the stratosphere. The opto-acoustic involves the absorption of an amplitude modulated beam of IR by a gas which results in the generation of sound. Energy absorbed by the gas molecules from the IR beam excites the molecules to the rotational-vibrational energy levels above the ground state. The main path for decay of these excited states is collisional de-excitation, which results in the transfer of absorbed energy into heat and raises the gas temperature. The temperature rise causes a corresponding pressure rise in the gas. When the beam intensity is modulated, the gas temperature and pressure change accordingly. This periodic pressure variation in the gas results in the generation of sound.

A block diagram of a laser opto-acoustic detector is shown in Figure 27.7. The arrangement makes use of a CO_2 laser that is tuned by rotating a diffraction grating at one end of the laser cavity. It is tuneable to 64 different emission lines in the range from 927 to 1,085 cm^{-1}. The laser beam is brought to a focus at the chopping wheel and then refocused into the detector. The chopping frequency is selected to optimise the signal-to-noise ratio. The microphone is commercially available, model 4144 manufactured by Bruel and Kjaer Instruments Inc. The acoustic signal is amplified in a pre-amplifier and displayed. The detector is highly sensitive and sample amounts as small as 20 pg may be detected.

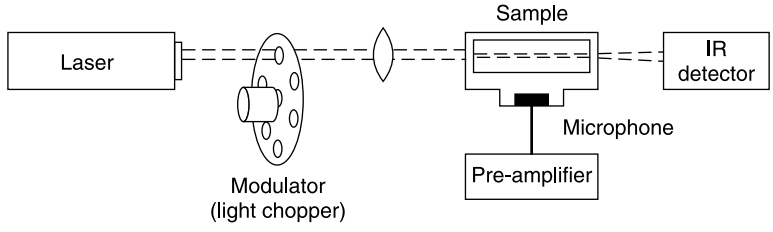


Figure 27.7 *Laser opto-acoustic detector for nitric oxides (After Kreuzer, 1978)*

27.5.4 UV-based NO Analyser

An UV absorption photometer for measurement of NO is schematically shown in Figure 27.8 (Hartmann and Braun, 1982). It uses a hollow cathode lamp which is filled with nitrogen and oxygen at reduced pressure where excited NO molecules are formed in an electrical discharge. The energy of the excited molecules is dissipated by emission of characteristic luminescence radiation. The source of radiation is selective; it produces an emission range which corresponds precisely to the absorption range of NO in the measuring cell. This is called resonance absorption. One peculiarity of the radiation excited by electrical discharge is that two groups of NO-specific lines are emitted: (i) Measuring radiation: 'cold' emission lines – this is the group absorbed by the NO to be determined in the measuring cell, (ii) Reference radiation: 'hot' emission lines – that group of radiation showing lines in the neighbouring range and meeting the detector not influenced by NO.

The radiation is modulated by a chopper wheel which then passes through the measuring cell via a condensing lens. It reaches the radiation detector, a photomultiplier, via an interference filter where interfering radiation is removed. If NO is present in the measuring cell, then the radiation is attenuated by absorption according to the Lambert-Beer law. For this measuring technique, the blank value of extinction is compensated for by alternately setting the chopper wheel to a position where all radiation (hot and cold emission lines) is passed through and to a position with a gas filter.

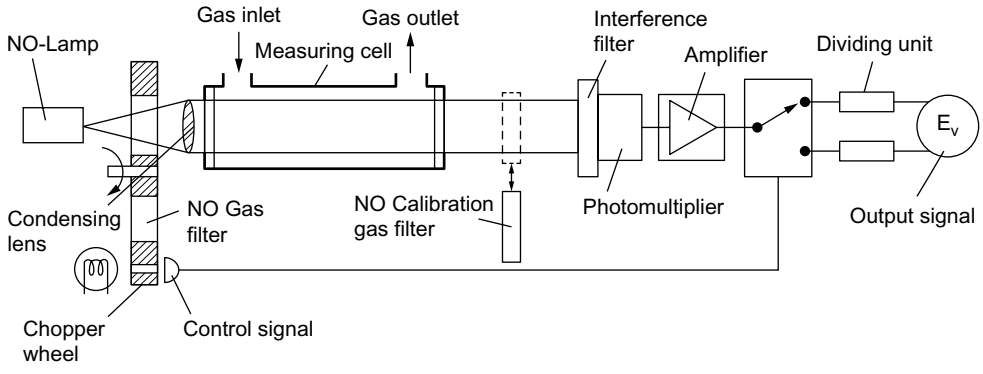


Figure 27.8 *Diagram of a UV gas analyser for the detection of NO with blind value compensation by wavelength comparison (Adapted from Hartmann and Braun AG., 1982)*

Figure 27.9 *Nitric oxide monitor (Courtesy: M/s 2 B Technologies, USA)*

The gas filter contains NO in high concentrations which completely absorbs the cold emission lines. The hot emission lines, however, which are in the immediate neighbourhood range, pass through it as reference radiation. Just like the measuring radiation, they are influenced by the in-line optics, by the cell windows but mainly by the wideband interfering components to produce the intensity reference value at the photomultiplier.

Based on the UV absorption principle is the Model 410 Nitric Oxide Monitor™ (Figure 27.9) from M/s 2B technologies, USA. It is designed for the measurement of atmospheric NO in the concentration range from 0 to 2 ppm with a precision of ±1.5 ppb. The detection principle of the Model 410 is based on the selective reaction of NO with ozone. The resulting ozone depletion is measured using the absolute method of UV absorbance and thus requires only infrequent calibration. By comparison, chemiluminescence NO_x instruments require nearly continuous calibration using a standard gas.

27.5.5 Combined SO_2 and NO Analyser

Fewer compounds absorb in the UV region than in the IR region, and the UV absorption pattern of a compound is not as distinctive or not as narrow as is its IR 'fingerprint'. On the other hand, UV analysers provide better selectivity in applications in which the sample contains air and humidity, because these materials do not absorb in the UV region. UV analysers are also more sensitive than IR detectors. Figure 27.10 shows the absorbance of sulphur and NO_2 in the UV region (GEC, 2014). On an equal path-length basis, the UV absorbance of liquids is stronger than that of vapours in proportion to their densities.

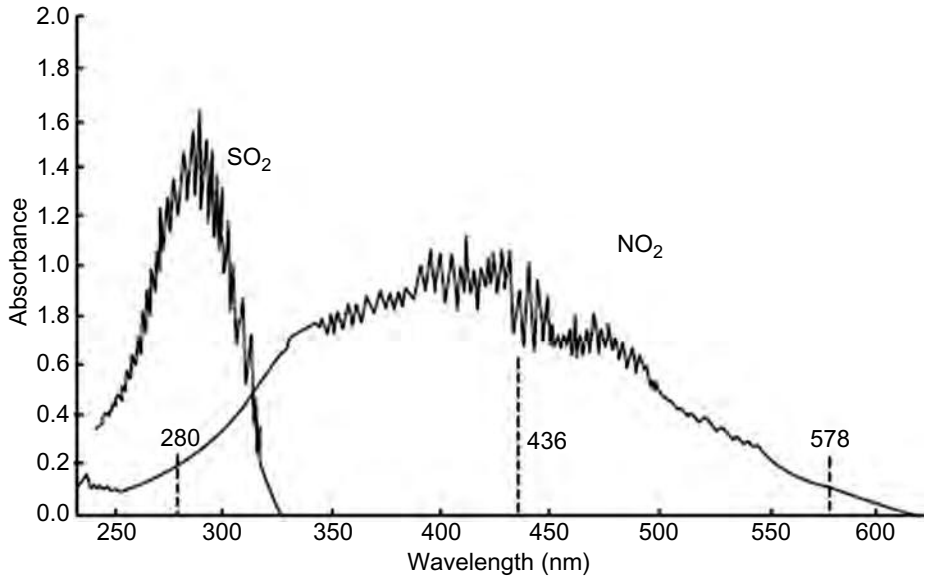


Figure 27.10 *The absorbance characteristics of both SO_2 and NO_2 in the ultraviolet range (Courtesy: GEC, 2014)*

Figure 27.11 illustrates a microprocessor-based UV analyser probe, which can be used for monitoring both sulphur dioxide and nitrogen oxide in stack gas. In this design, the UV light from a deuterium source is projected through the gas measurement cavity inside the filter at the end of the probe. The retro reflector at the tip of the measurement cell returns the measurement beam to a point at which a monochromator separates the light into discrete spectral bands (Liptak, 1994).

The monochromator has two exit slits, one for sulphur dioxide and the other for nitrogen oxide, that allow their corresponding wavelengths to impinge on the detector. By using the second derivative of the absorption spectra, the measurement is compensated for source ageing, line voltage

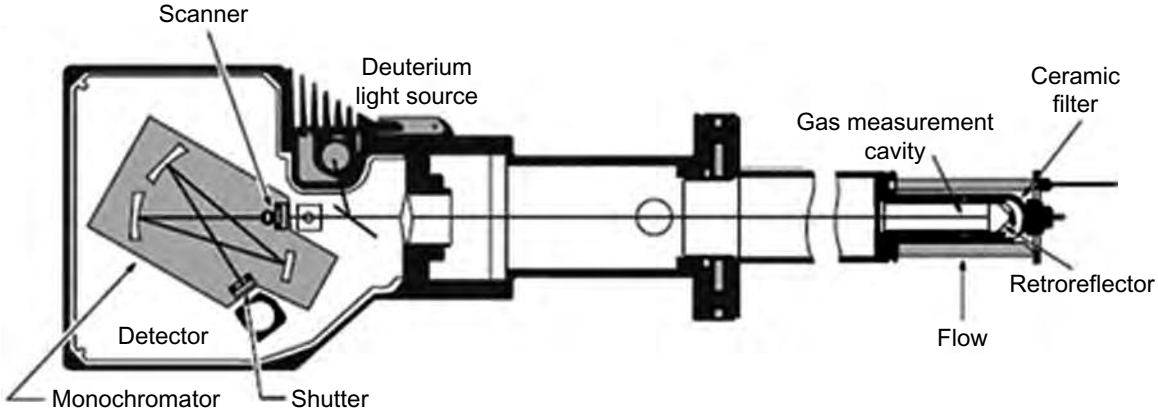


Figure 27.11 *Self-calibrating, self-cleaning and self-drying probe-type analyser simultaneously detects the concentrations of SO_2 and NO_2 at temperatures up to 800°F (Liptak, 1994)*

variations, broadband absorption, dirt build-up on the optics and optical misalignment. A small shutter in the monochromator moves back and forth between the NO and the SO_2 beams, providing sequential reading of both at 1 min intervals.

The analyser is provided with self-calibration capability, including auto-zero correction, auto-span check and correction. The ceramic filter at the probe tip is self-cleaning and self-drying; at pre-set intervals, a blast of hot, pressurised instrument air is forced into the measurement cavity, which forces the entrained water or tar out of the probe and dries the filter. The analyser is suitable for stack gas applications where the particulate loading, water vapour content and temperature are all high (up to 800°F, or 427°C). The analyser measures only NO, so, if evaluations of NO_2 or NOx are needed, additional measurements must be provided. The life of the deuterium lamp can be as short as 3 months.

27.6 HYDROCARBONS

27.6.1 Flame Ionisation Detector

Organic compounds easily pyrolyse when introduced into an air-hydrogen flame. The pyrolysis produces ions that can be collected by having a cylindrical grid surrounding the flame. The detector response would be in proportion to the number of carbon atoms in the chain. For example, propane would roughly give three times the intensity of response as compared to methane. The ions collected on the positively charged grid are amplified in a high input impedance amplifier, whose output is given to a chart recorder. The variation in the ion intensity resulting from flame ionisation of any organic compound in the air sample is recorded on the chart. Generally, 0–20 ppm range is adequate for atmospheric sampling.

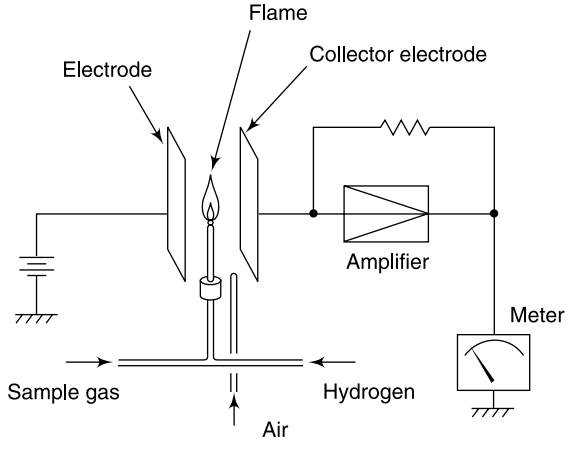


Figure 27.12 *Schematic diagram of a measuring system for hydrocarbons based on flame ionisation detection principle*

Figure 27.12 shows a block diagram of the apparatus used for measuring hydrocarbons based on flame ionisation detector (FID) principle. The sample gas containing hydrocarbon controlled at a constant flow rate is mixed with hydrogen for fuel use. The mixed gas is burned at the end of a very fine nozzle. Two electrodes are placed on either side of the flame and an appropriate electric field is applied to them. Ionic current will flow between the collector electrode and the other electrode. The current is then amplified and displayed on a metre-analog or digital.

It may be noted that the FID method generally shows responses in proportion to carbon numbers. However, if carbon coexists with oxygen, the response may differ depending on the kind of the hydrocarbon. The analyser, in general, provides good linearity across a broad range of concentrations, high sensitivity and fast response.

27.7 OZONE

27.7.1 Chemiluminescence

The chemiluminescene method is based on the emission of light from solid organic dye samples, due to the passage of ozone over the surface. Very low zone concentrations can be detected. However, periodic calibrations are necessary, both for the instrument and for the ozone source used.

Other chemiluminescene reactions due to ozone have also been used to determine ozone concentrations. A portable ozone metre, which utilises the reaction between O_3 and ethylene, has been constructed. This instrument depends on the reaction that occurs, when air and ethylene are drawn into a Pyrex container and mixed directly in front of a Pyrex window. A PMT is mounted behind this window, which produces an electrical signal due to photons of chemiluminescene energy and is proportional to the total ozone present in the sample. The concentrations as low as 0.001 ppm are detectable.

Ray et al. (1986) describe an apparatus for fast chemiluminescene method for measurement of ambient ozone. The design of the instrument is shown in Figure 27.13. Sample gas is drawn through the detector cell at 1–7 l/min by a diaphragm pump. Fluid and air are separated at the reservoir, and the dye solution is circulated. Sample gas flows across a fibre pad of either paper or glass mat that is saturated with the organic dye dissolved in an alcohol solvent.

The air and dye solution enters at the top of the cell through separate holes. Both air and fluid exit at the bottom together. The detection cell also houses a PMT that responds to the chemiluminescence, as the ozone reacts with the dye. The photocurrent is amplified by an electrometer and the signal output is recorded on a strip-chart recorder. The dye solution is pumped into the cell at 1 ml/min. Response of ozone is from 1 to 10 nA/ppb, depending on dye type and solvent.

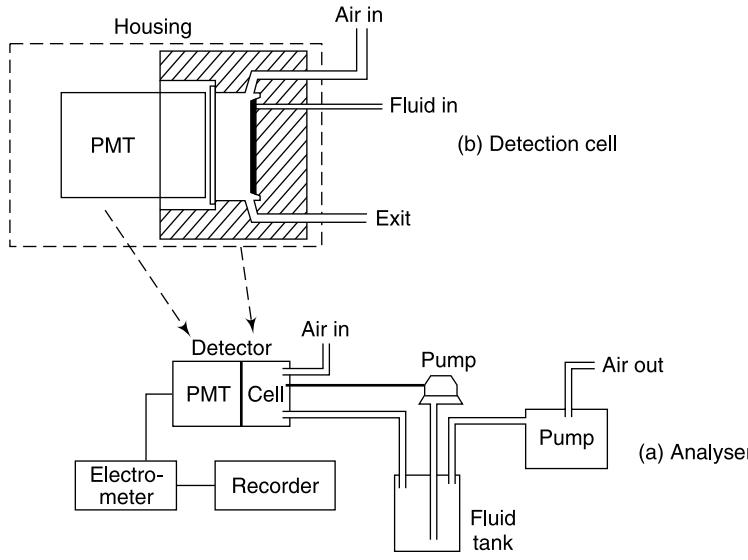


Figure 27.13 *Chemiluminescent ozone analyser and design of the ozone detection cell*

27.7.2 Conductivitimetry

A wet-chemical method which uses the oxidising properties of ozone(O_3) can be employed to construct a sensitive automatic metre for continuous sampling of contaminating oxidants in the atmosphere. The ozone containing air is bubbled into a potassium iodide solution and the resulting iodine determined by measuring the current through the cell. The current is related to ambient O_3 levels by previous calibration with a known ozone source. This technique has been employed to construct an air-ozone metre, which measures and records instantaneous ozone concentrations.

The accuracy of this method has been improved by determining the concentration of iodine and hence ozone by titrating the solution with sodium thiosulphate. The apparatus (Figure 27.14) consists of an hermetically sealed glass jar containing 150 cm^3 of buffered 10% KI solution and about 0.5 cm^3 sodium thiosulphate of known concentration. Two spiral platinum electrodes dip into the solution and a bias voltage of 30 mV is applied across them. The air above the solution is evacuated, whereas the outside is let in through a Tygon tube. which is inert to ozone.

When ozone enters the solution, the following reaction takes place:

$$O_3 + 2I + H_2O \rightarrow I_2 + O_2 + 2OH^-$$

The iodine then reacts with the thiosulphate

$$I_2 + 2(S_2O_3)^- \rightarrow 2(I^-) + S_4O_6^-$$

Reaction continues so long as there is thiosulphate in the solution. When all the thiosulphates have been reacted, free iodine appears and reacts at the electrodes.

The electrical resistance is high, so long as there is excess of thiosuiphate. The resistance decreases when it is used up. This change is used to control the operation of the instrument The voltage drop across 20 KΩ resistance, which is in series with the electrode, is used to operate the recorder as well

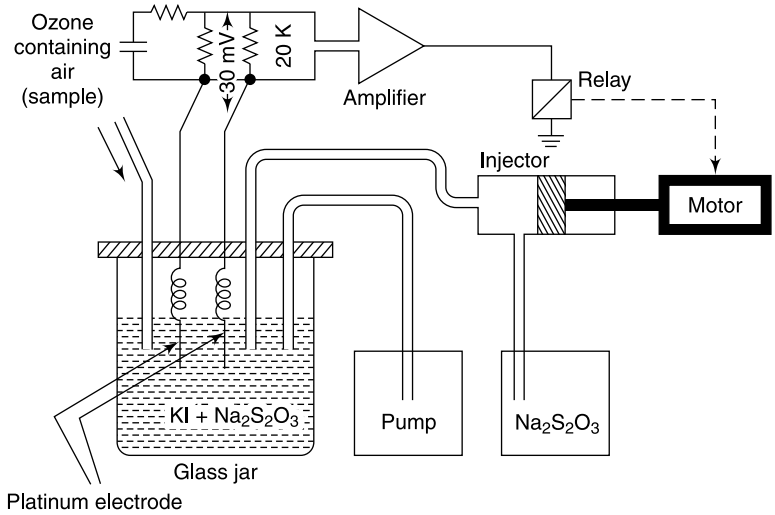


Figure 27.14 *Arrangement for measuring ozone in air using the oxidising properties of ozone (After Steinberger and Goldwater, 1972)*

as a relay, which controls a motorised injector, which injects 0.5 cm^3 thiosulphate in each operation. The recorder serves mainly to indicate as to when the injection was made, and thus the average ozone concentration between any two injections can be calculated. Since the pumping rate is known, knowledge of the time intervals gives the total volume of air sampled.

27.8 AUTOMATED WET-CHEMICAL AIR ANALYSIS

Wet-chemical methods are the accepted means for obtaining data in the majority of environmental studies. Specific chemistries are recommended for most of the common pollutants in the atmosphere. Basically, an automated wet-chemical analysis system would be as shown in Figure 27.15.

In this scheme, the sample is automatically introduced and prepared. Reagents are then added in proper quantities and sequence. A chemical reaction will take place. The presence of a particular constituent is then detected, displayed and recorded.

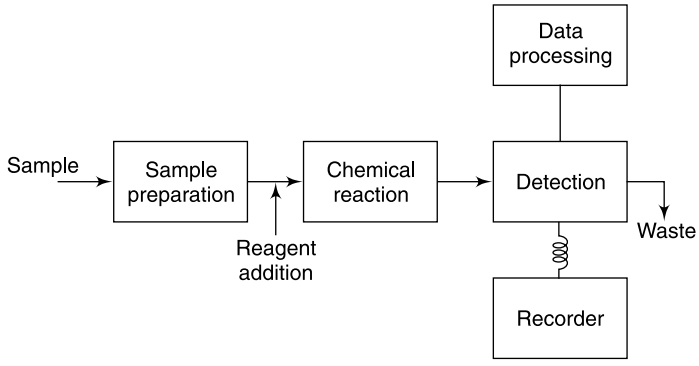


Figure 27.15 *Basic automated air analysis system*

In any air analysis system, the equipment must be capable of obtaining a proper sample. This is essential, in order to obtain correct results. In automated wet-chemical methods, the accuracy of the sampling system is dependent upon maintaining a constant ratio between the amounts of sample ratio and absorbing solution. The rate of flow of the absorbing solution is maintained constant by a proportionating pump.

27.9 MEASURING METHODS FOR PARTICULATE MATTER

Particulate Matter (PM) consists of minute solid or liquid particles (Figure 27.16) that are blown into the air. They are measured as either PM10 or PM 2.5 which refers to particles that are that number of microns in size or less. Various scientific studies have demonstrated the PM link to aggravated respiratory diseases such as asthma, bronchitis and emphysema, and to various forms of heart disease.

When examining PM in ambient air, the following factors must be taken into account:

- Total mass concentration of the PM
- Concentration of fine particles
- Size distribution
- Chemical composition.

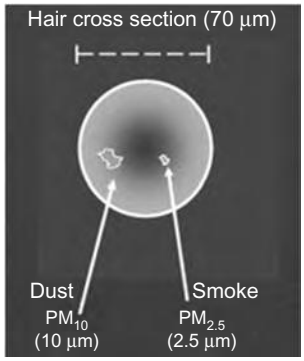


Figure 27.16 *Particulate matter – a complex mixture of extremely solids and liquids droplets*

For the air quality, sedimentation as well as non-sediment suspended PM is of interest, particularly the latter, as it is respirable and can thus carry pollutants into the human body.

PM is a medium which consists of a lot of different substances regarding chemical composition and size distribution. Relevant for human health are PM with an aerodynamic diameter smaller 10 µm (PM10) with a tendency to smaller sizes (e.g. PM2.5).

PMs could be measured with many techniques but the most relevant are gravimetric techniques.

27.9.1 Gravimetric Method

The instrument based on gravimetric principle draws ambient air through a filter at a constant flow rate, continuously weighing the filter and calculating near real-time mass concentrations.

When the instrument sample is ready, the ambient air stream first passes through an optional size-selective inlet and continues down the heated sample tube to the mass transducer (Ionel and Popescu, 2010). Inside the mass transducer, this sample stream passes through a filter made of Teflon-coated borosilicate glass. The instrument measures the mass of this filter every 1.68 s. The difference between the filter´s initial weight and the current mass of the filter gives the total mass of the collected particulate. Next, the mass rate is calculated by computing the increase in the averaged total mass between the current reading and the immediately preceding one, and expressing this as a mass rate in g/sec. Finally, the mass concentration in $\mu g/m^3$ is computed by dividing the mass rate by the flow rate. Internal temperatures in the instrument are controlled in order to minimise the effects of changing ambient conditions. The sample stream is preheated before entering the mass transducer (usually to 50°C) so that the sample filter always collects under conditions of very low and therefore relatively constant humidity.

Figure 27.17 is a schematic diagram showing the flow of the sample stream through the instrument in the case of a PM10 configuration. The particle size separation at 10 µm diameter takes place as the sample proceeds through the PM10 inlet. The flow splitter separates the total flow (16.7 l/min) into two parts: a main flow of 31/min that enters the sensor unit through the sample tube and the bypass flow of 13.7 l/min. The main flow passes through the exchangeable filter in the mass transducer and then proceeds through an air tube and in-line filter to a mass flow controller. The bypass flow is filtered in the bypass fine particulate filter and again in an in-line filter before it enters a second mass flow controller. A single pump provides the vacuum necessary to draw the sample stream through the system.

The weighing principle used in the mass transducer is basically different from that on which most other weighing devices are based. The tapered element at the heart of the mass detection system is a hollow tube, clamped on one end and frees to vibrate at the other. An exchangeable filter cartridge is placed over the tip of the free end. The sample stream is drawn through this filter and then down the tapered element. This flow is maintained at a constant volume by a mass flow controller that is corrected for local temperature and barometric pressure. The tapered element vibrates precisely at its natural frequency, much like the tine of a tuning fork. An electronic control circuit senses this vibration and, through positive feedback, adds sufficient energy to the system to overcome losses.

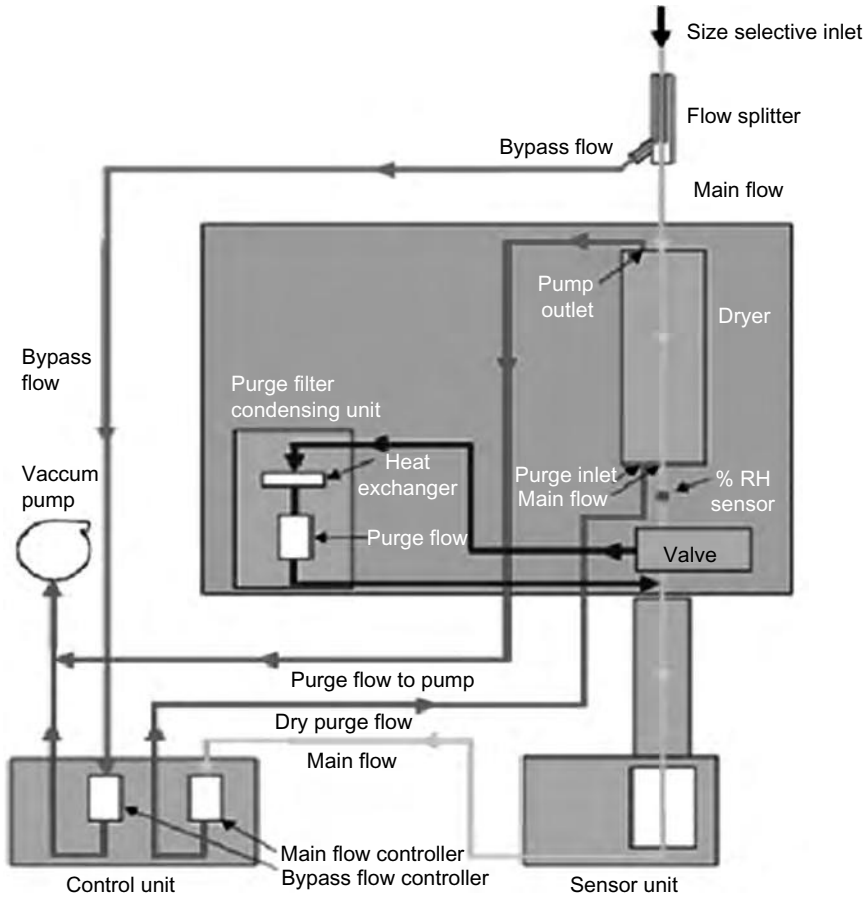


Figure 27.17 *PM measurement device TEOM (Courtesy: M/s Thermo Scientific, User manual TEOM Monitor, Series 1400ab)*

An automatic gain control circuit maintains the vibration at constant amplitude. A precision electronic counter measures the frequency with a 1.68 s sampling period. The tapered element is in essence a hollow cantilever beam with an associated spring rate and mass. As in any spring-mass system, if additional mass is added, the frequency reads out on the screen of the computer.

27.9.2 Beta Attenuation Monitoring (BAM)

One of the widely used air monitoring techniques is based on the absorption of beta radiation by solid particles extracted from air flow. This technique allows for the detection of PM10 and PM2.5. When the high-energy electrons emanating from the radioactive decay of ^{14}C (carbon-14) interact with matter, they lose their energy and, in some cases, are absorbed by the matter. These high-energy electrons emitted through radioactive decay are known as beta rays and the process is known as beta ray attenuation.

When matter is placed between the radioactive ^{14}C source and a device designed to detect beta rays, the beta rays are attenuated. This results in a reduction in the number of beta particles detected.

The magnitude of the reduction in detected beta particles is a function of the mass of the absorbing matter between the ^{14}C beta source and the detector, which is a PMT with a scintillation device.

For measurement, a glass filter tape passes through the gap between the ^{14}C source and the detector. At the start of every measurement cycle, the flux of beta rays is measured across clean filter tape, resulting in the measurement of I_o. After the measurement of I_o, the filter tape is advanced and ambient air is sampled thereby impregnating the tape with PM 2.5. After the sampling is complete, the tape retracts and the beta ray flux through the particulate-impregnated tape is measured (Gobeli, 2008).

The beta ray flux reaching the detector depends on the particulate mass deposited onto the filter tape. The beta ray flux reaching the detector decreases nearly exponentially with the mass through which it must pass. Detector is simply a Geiger–Mueller counter. To a very good approximation, Equation 27.1 shows this relationship.

$$I = I_o e^{-\mu x} \tag{27.1}$$

where I is the measured beta ray intensity (counts per unit time), of the attenuated beta ray (dust-laden filter tape), I_o is the measured beta ray intensity of the non-attenuated beta ray (clean filter tape), μ is the absorption cross section of the material absorbing the beta rays (m^2/kg) and x is the mass density of the absorbing matter (kg/m^2).

Equation 27.1 may be rearranged to solve for x, the mass density of the absorbing matter. This is shown in Equation 27.2.

$$x = \frac{1}{\mu} \ln\left[\frac{I_o}{I}\right] \tag{27.2}$$

The absorption cross section, μ, is a physical constant of the material through which the beta rays are passing. Once I and I_o are measured, the mass density may be determined.

Figure 27.18 *Particulate matter monitor (Courtesy: M/s Met One Instrument Inc. USA)*

In practice, ambient air is sampled at a constant flow rate (Q) for a specified time t. This sampled air is passed through a filter of surface area A. Once x, the mass density of collected particles, has been determined, it is possible to calculate the ambient concentration of PM (in kg/m^3).

The radiation source can be a gas chamber, filled with ^{86}Kr gas or pieces of ^{14}C-rich polymer plastic. The key to the success of the beta attenuation monitor is in part due to the fact that μ, the absorption cross section, varies little among commonly sampled PM. This permits the device to be calibrated during the manufacturing process and permits the user to accurately measure PM2.5 concentrations without having to know in advance the chemical composition of the aerosols being sampled.

Figure 27.18 shows a PM monitor based on beta attenuation technique. For operation, at the beginning

of each sample hour, a small ^{14}C (carbon-14) element emits a constant source of high-energy electrons (known as beta rays) through a spot of clean filter tape. These beta rays are detected and counted by a sensitive scintillation detector to determine a zero reading. The BAM-1020 then advances this spot of tape to the sample nozzle, where a vacuum pump pulls a measured and controlled amount of outside air through the filter tape, loading it with ambient dust. At the end of the sample hour, this dust spot is placed back between the beta source and the detector, thereby causing an attenuation of the beta ray signal which is used to determine the mass of the PM on the filter tape. This mass is used to calculate the volumetric concentration of PM in ambient air.

27.10 REMOTE MONITORING

Remote sensing systems are used for detection of airborne pollutants. These systems deliver information about the concentrations in a certain region which is covered from a light beam between emitter and receiver. This results in an average value which represents mostly better pollution level in a particular area than a point measurement. In addition, they can be used for monitoring of air pollutants at industrial sites. The path length can vary from some centimetres to some hundreds of metres.

Remote sensing devices offer a number of advantages over competing technologies. The value of remote sensing instrumentation has been proven in applications including transport, power generation, chemical processing and air quality monitoring, to monitor gaseous emissions for the protection of the environment or the safety of citizens. This arrangement also offers the advantages of eliminating the possibility of sample degradation during passage to and through an instrument, and of integrating the concentration over a region of space rather than sampling at one point.

27.10.1 LIDAR

Light detection and ranging (LIDAR) describes a family of active remote sensing methods. A block diagram of principle of functioning of LIDAR system is shown in Figure 27.19. The most basic technique is long-path absorption, in which a beam of laser light is reflected from a distant retro reflector and returned to a detector which is co-located with the source. The wavelength of the radiation is chosen so that it coincides with an absorption line of the gas of interest. The concentration of that gas is found by applying the Beer-Lambert law to the reduction in beam flux density over the path length (Richter, 1994).

LIDAR effectively detects and characterises air contaminants, with best spatial and temporal resolution, locates the pollution sources while also helping in developing perspective strategies. Complementary, by applying a trajectory model or different dispersion models, one can characterise, at regional scale, the pollution regime as well as the dynamics of the pollutants. The most obvious use is to track the evolution of a pollutant over time.

LIDAR has a unique ability to detect particles in both water and air. As LIDAR uses short wavelengths of light in the visible spectrum, typically ultraviolet, visible or near infrared, is it possible to image an object or feature only about the same size as the wavelength or larger. This makes it particularly sensitive to aerosols, cloud particles and air molecules. Pollutants such as CO_2, sulphur dioxide and methane are all detectable with LIDAR. Combined with a building or terrain model, this allows researchers to monitor and effectively reduce pollutant build up in certain areas.

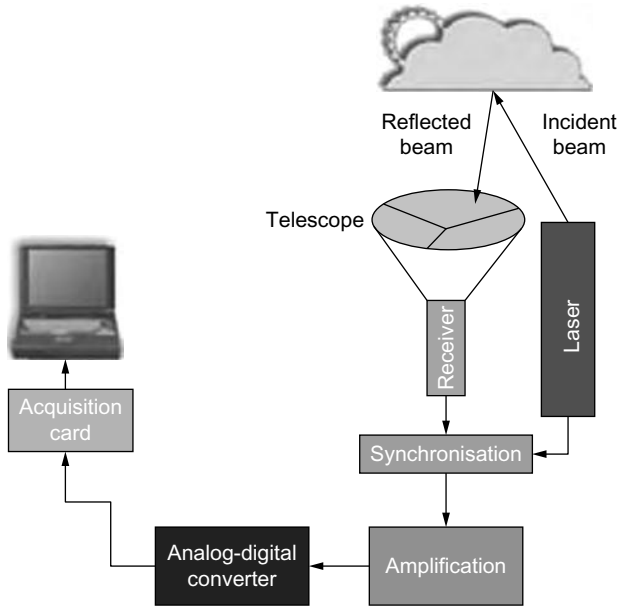


Figure 27.19 *Principle of functioning for LIDAR systems (Adapted from Vetres, 2009)*

27.11 WATER POLLUTION MONITORING INSTRUMENTS

Pollution of water sources can occur from a very large number of compounds, which have varying degrees of potential pollution. Their access to the water resources can adversely affect the quality of water and may render it unsuitable for drinking purposes, industrial use, production of fish and aquatic foods, irrigation, and even transport through waterways. Sometimes, the constituents of industrial wastewaters may present health hazards, even when present in minute amounts. The problem of water pollution is thus, one of considerable importance for the society.

The quality of water can be determined by measuring the quantity of specific pollutants present in it. The measurements generally include elemental analyses, physical determinations, microbiological and bacteriological examinations, radiochemical analyses, etc. Water analyses was earlier carried out either at the field site or in the laboratory, on samples gathered from the site. It is obvious that taste, smell and visual inspection are inadequate to determine the chemical, physical and biological characteristics of water, and therefore, instrumental methods have been adopted to perform this work. Instruments such as pH metre, colorimeter, conductivity metre and turbidity metre are routinely used in water analysis laboratories. Modem instruments, such as atomic absorption spectrophotometer, gas chromatograph, thin-layer chromatograph and polarograph are increasingly used for detecting trace metals, pesticides and toxic organics. Instruments are preferred over wet-chemical methods, due to their rapidity of estimates, greater precision and accuracy of measurements. With these instruments, it is possible to estimate several pollutants at microgram and nano-gram level.

General purpose water quality testing instruments are used to test water for chemical and biological agents, and to measure variables such as clarity and rate of movement. These instruments provide a standard tool that can be used to collect information from various water sources. Water

quality testing instruments can monitor water temperature, dissolved oxygen (DO), pH, conductivity, nitrogen/phosphorus concentration, turbidity, and levels of pesticides and toxic chemicals.

- *Conductivity and Resistivity Metres*: Conductivity metres, dissolved solids metres and resistivity metres are analytical instruments that measure the conductivity, dissolved solids and/or resistivity of a liquid sample.
- *Dissolved CO_2 Instruments*: Dissolved CO_2 instruments are analytical devices that measure the amount of CO_2 dissolved in a liquid sample such as water. They typically include a submerged probe that is covered by a thin organic membrane.
- *Dissolved Oxygen Metres*: DO metres are analytical instruments that are used to measure the amount of oxygen dissolved in a liquid sample.
- *Ion Specific Electrode Metres*: Ion specific electrode metres are millivolt metres that interface with ion selective electrodes (ISEs). These metres take the potential generated by the electrode and convert it into units of concentration.
- *Oil in Water Monitors*: Oil in water monitors are used to detect the presence of hydrocarbons in water.
- *pH Instruments*: pH instruments are used to measure or monitor potential of hydrogen (pH) in a solution.
- *Turbidity Instruments*: Turbidity instruments measure the average volume of light scattering over a defined angular range. Both particle size and concentration of suspended solids as well as dissolved solids can affect the reading.

27.11.1 Types of Pollutants and Techniques

Different types of investigations would be required for monitoring pollutants, depending on the purpose of the monitoring programme. Following standard parameters are determined for assessment of normal pollution characteristics of wastewaters: alkalinity, biological oxygen demand (BOD), ammonia, nitrates, kjeldahl nitrogen, total phosphorous, and total dissolved suspended and volatile solids. Several other parameters are monitored in case of industrial effluents and similarly for water used for drinking or domestic purposes.

27.11.2 Conductivity

Conductivity is the measure of dissolved ionised solids in water. Conductivity is temperature dependent and therefore, it is standardised at 25°C. It is non-specific and measures to some degree all ions present in water. A sudden increase in conductivity is an indication of pollution by strong acids, bases or other highly ionised substances. Conductivity was expressed in micromhos (μ mhos). However, it is now expressed as microSiemen. High conductivity values can be expressed as milliSiemens. For ultrapure water, the conductivity is 0.055 μ mhos/cm at 25°C. Conductivity, in simple terms, is a measurement of the ability of a solution to conduct an electric current. An instrument measures conductivity by placing two plates of conductive material with known area and distance apart in a water sample. Then a voltage is applied and the resulting current is measured. Using Ohm's Law,

$$V = I/R$$

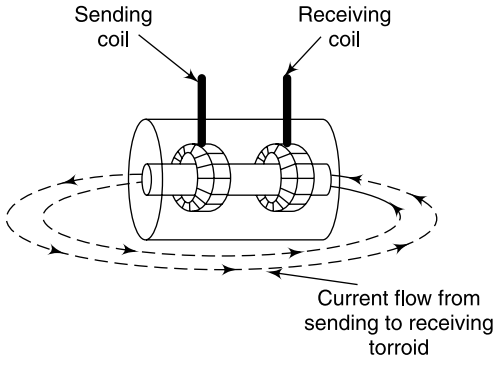


Figure 27.20 *Non-contact type conductivity metre*

and the conductivity $G = I/R$, then
G is determined as $G = I/R = I/V$

Typically AC signal is used as a voltage source to prevent ionisation of the electrodes.

An alternative technique is to use non-contacting type of cell, as shown in Figure 27.20. It uses a magnetic field to sense conductivity. A transmitting coil generates a magnetic alternating field that induces an electric voltage in a liquid. The ions present in the liquid enable a current flow that increases with increasing ion concentration. The ionic concentration is then proportional to the conductivity. The current in the liquid generates a magnetic alternating field in the receiving coil. The resulting current induced in the receiving coil is measured and used to determine the conductivity value of the solution. This type of cell offers complete galvanic separation of measurement from medium, reduced maintenance and resistance to chemical attack and there is no polarisation.

27.11.3 Dissolved Oxygen

The term dissolved oxygen (DO) is used to describe the amount of oxygen dissolved in a unit volume of water at a given temperature and a given atmospheric pressure. Since DO is critical for the entire biological community, it is one of the principle parameters use to measure water quality.

It is a measure of the ability of water to sustain aquatic life. The solubility of oxygen in water decreases with increase in temperature. DO is expressed in mg per litre (ppm). DO can be measured by a special sensor kept in an electrochemical cell by amperometric method. The cell comprises of a sensing electrode, a reference electrode and a supporting electrolyte, a semi permeable membrane, which serves dual function. It separates the water sample from the electrolyte, and at the same time permits only the DO to diffuse from the water sample through the membrane into the supporting electrolyte. The dissolved gas may subsequently react at the sensing electrode and thus causes a current flow. The range for this measurement is 0–20 ppm, with an accuracy of ±1 ppm. BOD of water could be determined from DO measurements.

Although DO is usually displayed as mg/L or ppm, DO sensors do not measure the actual amount of oxygen in water. Instead, it is the pressure of oxygen in water which is measured. Oxygen pressure is dependent on both salinity and temperature.

There are two techniques which are you used to measure DO: galvanic and polarographic. Both probes make use of an electrode system where the DO reacts with the cathode to produce a current.

DO metres consist of a DO probe, as shown in Figure 27.21, connected to a metre/analyser and are similar in construction to pH metres. The probe is comprised of two electrodes suspended in a potassium chloride (KCl) electrolyte solution, all of which is enclosed with glass and/or a semi permeable membrane. The electrodes are connected to the metre, which provides a small DC current to the electrodes. When the sensor is submerged in a liquid, oxygen from

the liquid crosses the membrane and reacts with the cathode, causing a measurable current change; this change is converted into a millivolt output and is finally displayed by the metre.

This current is passed through a thermistor and therefore, the actual output from the sensor is in millivolts. The thermistor connects for membrane permeability errors due to temperature change. Similarly, atmospheric pressure affects the saturation of oxygen. DO probes must be calibrated for the barometric pressure when reading in mg/l.

Like pH instruments, DO metres typically measure more than DO. Multi-function metres may also measure pH, oxygen reduction potential (ORP), temperature, conductivity and other liquid quality parameters.

The methods for cleaning and maintaining a DO probe are also similar to those for maintaining a pH probe. Ensuring proper levels of electrolyte solution, eliminating membrane leakage and routine calibration are key factors in maintaining accurate metre output.

Because DO levels change rapidly when water is removed from its source, DO metres are commonly portable, handheld devices suitable for repeated field measurements. Figure 27.22 shows a typical handheld DO metre.

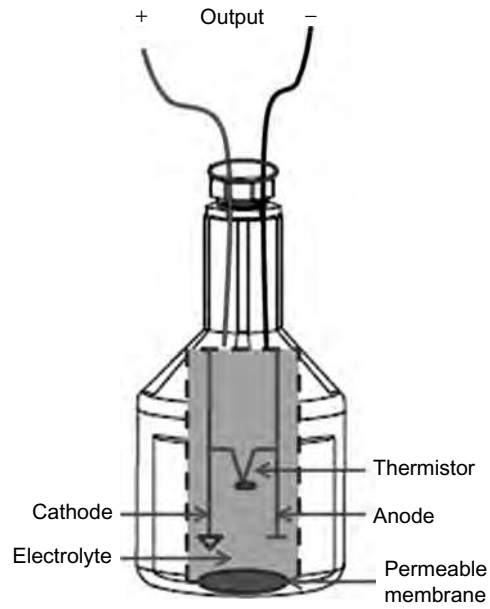


Figure 27.21 *A typical DO probe (Courtesy: M/s National Instruments)*

Figure 27.22 *Portable dissolved oxygen analyser based on the use of galvanic probe (Courtesy: M/s Hanna Limited)*

27.11.4 pH Measurement

pH is the logarithm of active hydrogen ion concentration in moles per litre. The pH of a neutral solution is 7. Values lower than 7 are considered acidic and higher than 7 are basic. Many important chemical and biological reactions are strongly affected by pH. In turn, chemical reactions and biological process (e.g. photosynthesis and respiration) can affect pH. Natural water shows only slight fluctuations in pH. Rapid changes in pH are generally due to pollution, such as dumps of acids and alkalies. The pH of the water sample is measured with the combined glass-calomel electrode immersed in the water sample. Instrument manufacturers guidelines should be followed for calibrating the pH metre.

27.11.5 Oxidation-reduction Potential

Oxidation-reduction potential (ORP) is an electrochemical potential developed by oxidising or reducing materials in water. Industrial wastes usually contain strong oxidising and reducing agents. Oxidants and reductants present in neutral waters tend to balance out to give zero ORP. A platinum electrode is used in conjunction with calomel electrode for ORP measurements.

27.11.6 Temperature

Temperature affects the solubility of oxygen and other chemical contents of water. The changes in water temperature are normally slow. They are seasonal unless pollution is experienced. Temperature of water can be measured by using a forward biased silicon diode as sensor. The diode forms part of a Wheatstone bridge and the unbalanced voltage is amplified and given to the metre for display. The metre is calibrated in terms of temperature in °C. The range is 0–50°C with a reading accuracy of ±0.5°C.

27.11.7 Turbidity

Turbidity is a commonly accepted criterion of water quality in water treatment for industrial or potable purpose. It is an expression of optical property of a sample which causes light to be scattered and absorbed rather than transmitted in a straight line through the sample. It is a measure of the undissolved solids in water including slit, clay, algae, rust, bacteria and other micro-organisms. Turbidity is expressed in Jackson Turbidity units.

Size, shape and refractive index of the particles affect the transparency of water. It is, therefore, not possible to correlate turbidity and quantity of these particles by weight. Instruments for measuring turbidity work on the principle of measuring the intensity of scattered light at an angle from a strong light beam. Two methods are commonly used.

Turbiditimetry is the measurement of the degree of attenuation of a radiant beam incident on particles suspended in a medium, the measurement being made in the directly transmitted beam.

Nephelometry is based upon the measurement of light scattered by a suspension. This is usually done by measuring the scattered radiation at right angles to the collimated beam.

The choice between nephelometric and turbidimetric measurement depends upon the fraction of light scattered. When scattering is extensive, owing to the presence of many particles, the turbidimetric measurement is more satisfactory. If the suspension is less dense and diminution in power of the incident beam is small, nephelometric method provides a more satisfactory method. In dilute suspensions, the attenuation of a parallel beam of radiation by scattering is given by the following formula:

$$P = P_o\, e^{-jb}$$

where P_o and P are the power of the beam before and after passing through the length b of the turbid medium. The quantity J is called the turbidity coefficient and is linearly related to the concentration C of the scattering particles. So, the following results:

$$\log_{10} P_o / P = kbc$$

where $k = 2.3\ J/c$

The relationship between $1\log_{10} P_o/P$ and C is established with standard samples, the solvent being used as a reference to determine P_o. The resulting calibration curve is used to determine the concentration of samples from turbidimetric measurements.

27.11.7.1 Single-beam design

Turbidimetric measurements are carried out with a filter photometer and a typical optical path is shown in Figure 27.23. The basic turbiditimeter instrument contains a light source, sample container or cell and photodetectors to sense the scattered light. The most common light source used is the tungsten filament lamp.

As light passes through a sample containing suspended solids, the particles absorb the light energy and re-radiate the energy in all directions. Particle shape, size, colour and refractive index determine the spatial distribution of the scattered light by the particle. Particles smaller than the wavelength of light (e.g. bacteria) scatter light in equal intensities in all directions, while particles larger than the wavelength of light result in a greater forward scattering.

Besides tungsten incandescent lamps, modern turbiditimeter designs utilise monochromatic light sources, such as light emitting diodes (LEDs), lasers, mercury lamps and various lamp filter combinations. Monochromatic light has a very narrow band of light wavelengths, only a few colours. By selecting light wavelengths that are not normally absorbed by organic matter, the monochromatic light source can be less susceptible to interference by sample colour. However, some of these alternate light sources respond differently to particle size and are not as sensitive to small-sized particles as the tungsten filament lamp.

In turbiditimeters, photodetectors detect the light produced from the interaction of the incident light and the sample volume and produce an electronic signal that is then converted to a turbidity value. The four types of detectors commonly used include PMTs, vacuum photodiodes, silicon photodiodes and cadmium sulphide photoconductors (Sadar, 1992).

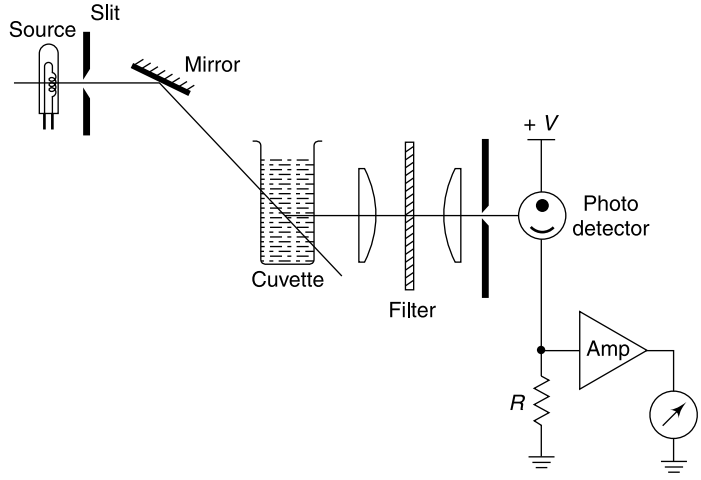


Figure 27.23 *Optical arrangement for measurement of turbidity of water*

Turbidity readings are affected by suspended-sediment particle size, entrained air bubbles, floating debris and other particles in the water that may collect on or near the optic sensor during data collection. Modern instruments are microprocessor-based, in which the software provides for temperature compensation.

Field calibration of the turbidity sensor is done by using Formazin or other approved primary standards and following the manufacturer's calibration instructions.

The concentration of a variety of ions can be determined by this technique, by the use of suitable precipitating agents, so as to form a stable colloidal suspension. Surface-active agents such as gelatine are frequently added to the sample to prevent coagulation of the colloid. A computer controlled laser nephelometer has been described by Koyuncu (1986) to determine the concentrations of liquid solutions.

The single-beam design does, however, have limited accuracy at higher turbidities. As turbidity increases and the amount of scattered light increases, multiple scattering can occur when light strikes more than one particle as it reacts with the sample fluid. The resulting scattered light intensity reaching the 90° detector can diminish as the instrument effectively 'goes blind'. For this reason, a single-beam design does not typically demonstrate stable measurement capability at high turbidities and is generally only applicable for turbidity readings from 0 to 40 NTU (EPA, 1999).

The design of the single-beam instrument is also limited by the need for frequent recalibration of the instrument due to the decay of the incandescent light source. Because of the polychromatic nature of the light source, these instruments also can demonstrate poor performance with samples containing natural colour. Since most treated water samples have low or no colour, use of the single-beam design is considered appropriate.

As the light scattered in the forward direction is variable depending upon the particle size, the measurement of light transmitted through the sample will yield variable results. This problem is eliminated when turbidity is measured at right angles to the incident light beam - an angle considered to be very sensitive to light scatter by particles in the sample. This arrangement is shown in Figure 27.24.

By using additional photodetectors located at other angles than 90° from the incident light (forward scatter detector, a transmitted light detector and a backscatter detector), the signals from each of these detectors can be mathematically combined to calculate the turbidity of the sample with much better performance with coloured samples. The transmitted light and the 90° scattered light are affected almost equally by the colour of the sample because they travel nearly the same

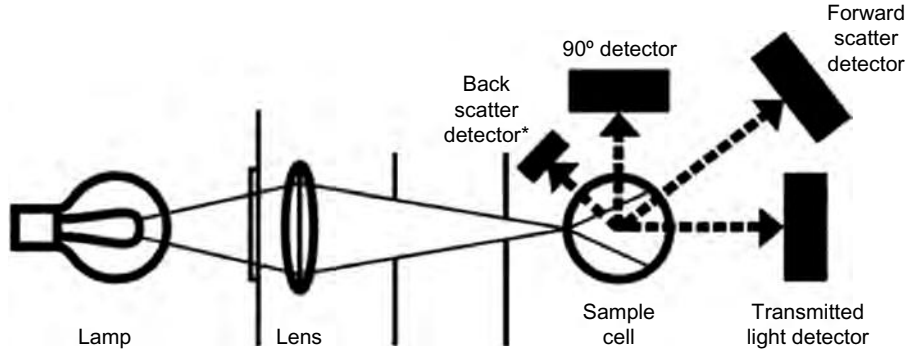


Figure 27.24 *Single-beam ratio turbidimeter (Sadar, 1996)*

distance through the sample volume. When the ratio of the two readings is taken, the effects of colour absorption on the two readings tend to cancel mathematically.

27.11.7.2 Surface scatter design

When particle concentration exceeds a certain point, the amount of transmitted and scattered light decreases significantly due to multiple scattering and absorption. This point is known as the optical limit of an instrument. In such a case, a surface scattered design is used in the turbiditimeters.

The surface scatter design utilises a light beam focused on the sample surface at an acute angle. As shown in Figure 27.25, light strikes particles in the sample and is scattered towards a photodetector that is also located above the sample surface. As turbidity increases, the light beam penetrates less of the sample, thus shortening the light path and compensating for interference from multiple scattering. The reported range of surface scatter instruments is about 0 to 9999 NTU, although these instruments are best suited for measuring high turbidities such as are present in raw water and recycle streams.

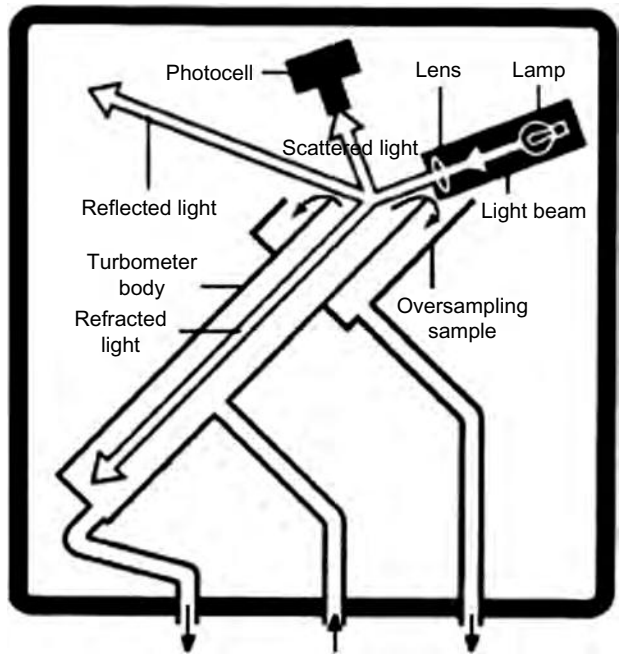


Figure 27.25 *Surface scatter turbidimeter (Hach Corporation, 1995)*

Figure 27.26 shows the block diagram of the laser-based nephelometer. It uses a 5 mW He – Ne laser as a light source. The laser has the advantage of that it gives a coherent monochromatic beam (λ = 632.8 nm) of high intensity. D_1 and D_2 are the two diaphragms which provide a Gaussian intensity distribution across the beam profile. The sample cell is placed in front of the beam at an angle. A lens 'L' focuses the scattered light on a photodiode. The main exit beam is then directed into an optically absorbent medium to avoid secondary reflections. The output of the photodiode is proportional to the incident intensity of the scattered light. This in turn is directly related to the

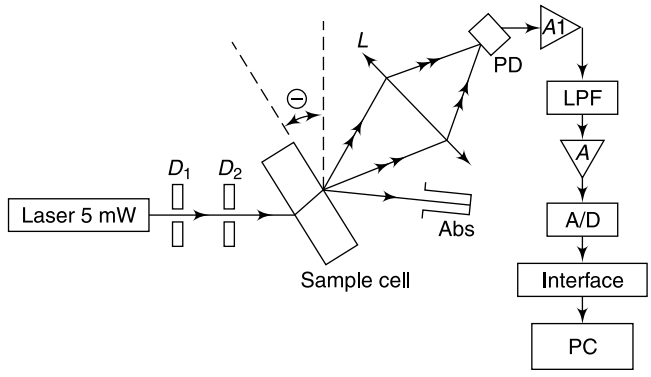


Figure 27.26 *Block diagram of the nephelometer system (Redrawn after Koyuncu, 1986)*

concentration of the liquid in the sample cell. The electronic circuitry eliminates my high frequency noise effects and produces compatible analog voltage levels from a 12-bit analog-to-digital (A/D) converter. A/D output is interfaced via buffers to a personal computer. Suitable software enables to display the liquid concentrations.

Pollution of water is also determined to a large extent by the presence of anions such as halides, nitrate, sulphate, cyanide, carbonate, etc., and cations such as lead, zinc, chromium, arsenic, copper, etc. Inorganic anions are generally estimated by spectrophotometric and electrochemical techniques. With the availability of ISEs, the task of estimating both anions and cations has become easy and at the same time, accurate and reliable. ISEs for anions such as fluoride, chloride, carbonate, cyanide, etc. and for cations such as sodium and potassium are now available. Polarography is useful in the analysis of metal ions in water, because of its high sensitivity, ability to analyse mixtures and to tolerate large quantities of dissolved solids.

Bacteriological and radioactive measurements are limited only to very special cases.

Highly monochrometric light is not required. It can be done by directing a light beam from a light-emitting diode into the water sample and measuring the light that scatters off the suspended particles present in the water. The nephelometric measurement method is used in most commercially available instruments with a sensor range of 0–1,000 nephelometric turbidity units (NTU) and an accuracy of ±5 percent of 2 NTU, whichever is greater.

Table 27.1 gives the performance requirements for typical automatic water pollution monitoring instruments.

Table 27.1 *Performance requirements of water pollution monitoring instruments*

Parameter	Transducer	Range	Full-scale accuracy
Conductivity	Potentiometric	0–60,000 mohs	1%
pH	Glass and calomel electrode	2–12	1%
Dissolved oxygen	Polarographic	0–24 mg/I	1%
Temperature	Silicon diode thermistor or thermocouple	0–50°C	0.5°C

Parameter	Transducer	Range	Full-scale accuracy
Chloride	Ion-selective electrode	0–240 mg/I	5%
		0–2400 mg/I	
Turbidity	Optical	0–120 JTu	2%
		0–1200 JTu	
		0–2400 JTu	

The instrument must have automatic temperature compensations and should be stable at least up to 2 weeks. It is not always possible to fix up the water sample from the site thoroughly and expect the results not to vary in the laboratory measurements. Therefore, portable instruments have been designed, which can be conveniently taken to site and the results obtained almost immediately.

27.12 IN SITU MEASUREMENTS

If we need to monitor an analyte's concentration over time, it may not be possible to physically remove samples for analysis. This is often the case, for example, when monitoring industrial production lines or waste lines, when monitoring a patient's blood, or when monitoring environmental systems. With a fibre optic probe we can analyse samples in situ. An example of a remote sensing fibre optic probe is shown in Figure 2.39. Fibre optic probes that show chemical selectivity are called optrodes.

27.13 OIL IN WATER APPLICATIONS

Environmental regulations specify composition limits for effluent water; for this reason, accurate monitoring of effluent water in wastewater, oil and gas, and manufacturing industries is an important requirement. In addition, to environmental concerns, high oil content in process water is often an indicator of equipment failure (particularly in the case of a leaking heat exchanger), or that the wastewater under measurement is unfit for re-use.

In general, oil in water monitors are useful in any application which requires oil monitoring within a treated or untreated water system, including wastewater, groundwater, produced water, process water and natural run-off.

The detection of oil in water is method-dependent, meaning that there must be some standardisation of test methods used to establish baseline measurements for which to compare data from disparate sources and formulate discharge limits and other legislation. By rule, reference methods are more time-consuming and involved than field methods, but are absolutely essential for the accuracy of subsequent monitoring. Following three types of reference methods are generally used (Yang, 2011).

Infrared Absorption: This method begins by extracting an oily water sample using a solvent. The sample is then purified, dried and placed into an infrared instrument, which transmits infrared radiation through the sample. By comparing the absorbance and transmittance of this radiation to known standards, the oil content can be determined.

Infrared absorption may involve the transmission of one or three separate wavelengths. The most common active single-wavelength standard, specifies emission at 2,930 cm^{-1}. While single-wavelength testing is capable of measuring only the total hydrocarbons within a sample, the three-wavelength method allows the calculation of aromatic and aliphatic compounds. It typically involves emission at 3,030, 2,960 and 2,930 cm^{-1}.

It is a fact that both infrared methods are well-established and fairly simple to employ using portable instruments.

Gravimetric method: Gravimetric methods rely on the weight difference between oils, water and solvents. A sample is first removed using a solvent and the solvent/oil mixture is separated from the water. The solvent is then evaporated, leaving only the residual oil, which is dried and weighed.

While gravimetric methods are simple and inexpensive to perform, they lack compositional analysis and can fall victim to volatile compound loss through evaporation.

GC-FID: In a GC-FID process, the sample is extracted by a solvent as in the two other methods. The sample is dried and purified before injecting into a GC instrument. A carrier gas is used to move the sample through a column, while different hydrocarbons leave the column at different times, at which point they are measured and burned off by a FID.

GC-FID methods are fast becoming the preferred reference method due to their lack of CFC solvents and volatile emission, as well as their detailed compositional analysis. Their drawback, however, is that their sophisticated instrumentation is expensive and typically requires skilled operators.

While reference methods are effective and essential for continuous monitoring, comparing results, and developing legislation and guidelines, they may be too involved or complicated for processes such as process optimisation, which requires repeated, quick results. For this reason, inexpensive bench-top or online devices are available for taking field measurements.

28

COMPUTER-BASED ANALYTICAL INSTRUMENTS

28.1 COMPUTERS IN ANALYTICAL LABORATORIES

It is well recognised that computers have much to offer in analytical field, both in routine applications as well as in research. This can be in the way of automatic calculation of the calibration curve, automatic drift correction, processing the data from the interfaced instruments into directly readable signals and increased productivity. The primary benefit, however, is in reduction of time and labour of the technician, which is usually required for conversion of raw results to proper functional scales. This reduces the associated errors, which inadvertently creep in with manual methods of computation. Computers, in fact, have become basic laboratory tools that aid the chemist in carrying out the required analyses in a more efficient manner, particularly their applications as components of analytical instrument systems (Dessy, 1984). New measurement methods have been developed which are possible only through the use of computerised instrumentation and high-speed data processing techniques.

A computer is a combination of devices, which serves to make mathematical calculations and thus find solutions to various types of problems, which are otherwise difficult and time consuming. This is possible because the computers do two things with extraordinary speed, reliability and patience (Friedman, 1984). They 'crunch' numbers and shuffle data. When we say a computer can 'crunch numbers', we mean it will allow you to feed in seemingly endless numerical data and mathematically process the data until it arrives at the solution to your problem.

When we say a computer can shuffle data, it is meant that a computer will accept any kind of information – alpha (letter and punctuation), numeric (numerals) and alpha-numeric, store it, relate it to other data, extract specific data, re-arrange the data in a desired order and display to the user in any preferred order. Depending on the kind of data, the sophistication of the computer processing power and the way in which the information is displayed to the user, the software that shuffles data is called a data base. In other words, all data handling software is referred to as the data base.

There are two main classes of electronic computers, namely analog and digital. In general, digital computers have a much higher degree of speed and accuracy than the analog computers and they have almost replaced the latter.

28.2 DIGITAL COMPUTER

The block diagram (Figure 28.1) shows the two major functional parts of a typical digital computer. They are as follows:

- The central processor
  - Internal storage or memory
  - Arithmetic unit
  - Control unit
- The peripheral unit
  - Input devices
  - Output devices
  - Auxiliary memory or back-up store

For a solution of any problem, all essential information (data) is fed into the input. This information consists of the numerical data involved in the problem and the coded instructions of what should be done with the data. This combined data are passed on to the memory unit in the central processor, where each part of the information is stored in separate locations in the form of magnetic storage or in solid-state memory.

The memory unit stores the information received from the input and from other functional parts, and delivers this information at designated time during the computing process. The control unit gets instructions from numerical codes, which are stored in the memory and interprets these codes in the appropriate sequence and directs the operations of the entire machine. The function of the control unit can be illustrated by considering a simple problem of summation. The control unit shall send a signal to the memory unit to pass the stored numbers to the arithmetic unit, where they are temporarily stored in the registers. As a next step, it signals the arithmetic unit to add these numbers and send the output back to the memory unit from where it is passed to the output under instructions from the control unit. In practice, however, the problems are not so simple and they involve a series of arithmetic operations to be carried out before the results can be passed to the output. If the usual memory unit is not sufficient to accommodate all the data involved in a certain problem, it can be supplemented with an auxiliary or a backup storage unit.

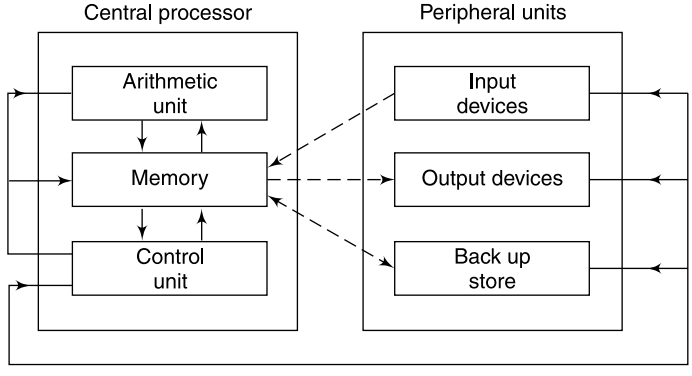


Figure 28.1 *Block diagram of a digital computer*

The function of an input device is to present the basic symbols, 0, 1, 2, ..., 9; A, B, C, ..., Z; +, –,; %, x, etc., in a coded form, so that they can be held within the computer's internal storage. This is achieved by using a keyboard similar to that of a typewriter.

The solution of a problem by a digital computer is ultimately transferred to the output section. The output can be in the form of a display on the LCD Monitor. A graph plotter incorporated in the system can also be operated with programmed instructions, so that graphs can be drawn from a pattern of discrete digits held in internal storage. The output data may also be obtained in the printed form from printers.

A simplified block diagram of a computer with interconnections of its major parts is shown in Figure 28.2. It consists of the following parts:

CPU Module: Contains the central processing (CPU) system, timing and interface circuitry to memory and I/O devices.

Memory: Contains read-only-memory (ROM or PROM) for program storage and random access memory (RAM) for data storage.

Input/output (I/O) ports: Contains circuitry that allows communications with devices outside of the system. (e.g. keyboard, cassette tape, video display unit).

The CPU and its memory and peripheral devices are connected together by buses. Each of the bus is made up of a number of transmission lines. There are three buses that interconnect the blocks.

Data Bus: A bi-directional path on which data can flow between the CPU and memory or I/O. It carries the actual data being manipulated.

Address Bus: A unidirectional group of lines that identify a particular memory location or I/O device.

Control Bus: It carries all the control and timing signals. It is a unidirectional set of signals that indicate the type of activity in current process. The types of activities could be memory read, memory write, I/O read, input/output write and interrupt acknowledge.

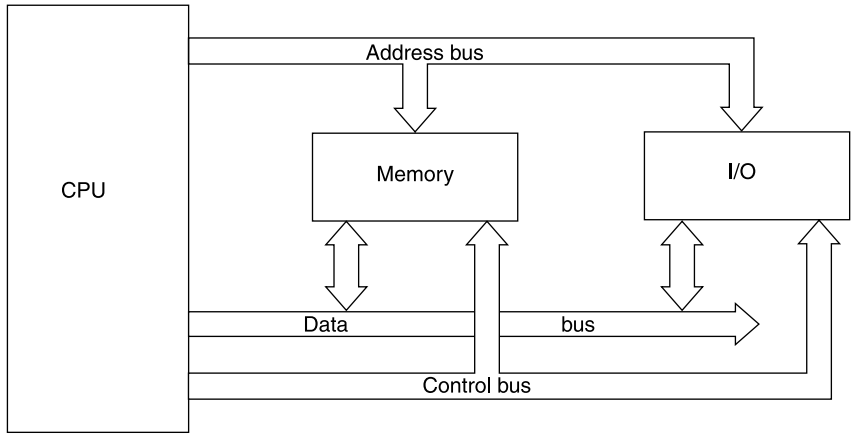


Figure 28.2 *Building blocks of a computer system*

For rapid communication among the various parts of a computer, all of the digital signals making up a word are generally transmitted simultaneously by the parallel lines of the bus. The number of lines in the internal buses of the CPU is equal to the size of the word processed by the computer.

CPU is the most important component of any computer system – big or small. In a computer, it is the microprocessor chip which acts as the CPU. The microprocessor can be termed as the heart of the computer, because its capabilities determine the capabilities of the computers. In such a system, the CPU requests instructions prepared by the programmer, asks for data and makes decisions related to the instructions. Based on the data, the processor determines appropriate actions to be performed by other parts of the system. Since there are many peripherals associated with the given system, the microprocessor must be capable of selecting a particular device. It identifies each device by means of a unique address code. A typical microprocessor has 16 binary address lines providing 65,536 addressing codes. Data to and from the processor is carried across a bi-directional 8- or 16-bit wide data bus. Many processors also provide a serial data path. Several microprocessors use a multiplexed address/data bus on which both address and data are transmitted on the same signal paths. In this case, the first portion of the bus cycle transmits the address while data transfer takes place later in the cycle. This architecture is popular for microprocessors with an 8-bit data bus.

The CPU unifies the system by controlling the functions performed by the other components. The CPU must be able to fetch instructions from memory, decode their binary contents and execute them. It must also be able to reference memory and I/O ports, as necessary in the execution of instructions. In addition, the CPU should be able to recognise and respond to certain external control signal, such as INTERRUPT requests.

28.2.1 Input-Output Systems

The computer will be of no use unless it is able to communicate with the outside world. I/O devices are required for users to communicate with the computer. In simple terms, input devices bring information INTO the computer and output devices bring information OUT of a computer system. These I/O devices are also known as peripherals since they surround the CPU and memory of a computer system.

The I/O interfaces are digital ports through which programs and control commands may be loaded and from which digital data may be transmitted to peripherals. In the same way, the computer also requires one or more output ports that permit the CPU to communicate the result of its processing to the attached equipment.

Some commonly used input devices are keyboard, mouse, joystick, scanner, light pen, touch screen, digital cameras, CD ROMS, USB flash memory and transduced signals from analytical instruments.

Similarly the generally used output devices are LCD monitor, printer, plotter, CD ROMS, USB flash memory and speakers.

28.2.2 Storage Memory Systems

Computers are getting more and more powerful and are being employed in applications requiring large memories. The semi conductor memories are still quite expensive and it is economical to use other storage devices to serve as a back-up for storing bulk information. Therefore, the following two levels of memories are used in the computers:

(a) The memory unit which communicates directly with the CPU. This is called the main memory.
(b) Back-up storage or auxiliary memory or bulk storage devices to store program and data not currently needed by the processor. The information is transferred to the main memory on a demand basis. Most commonly used auxiliary memory device in computers are compact discs (CD) and hard discs.

The speeds of these two levels of memory are matched by using a buffer memory.

Magnetic disk medium, at present, is the best computer peripheral for large systems, which are of the following two types:

- Magnetic disks (hard disk drives)
- Optical disc (CD/ DVDs)

Floppy disks were once a common means of storage but have now become obsolete. Removable disks, such as ZIP disks are widely available with several 100 megabyte capacity. The USB flash drive is a semi conductor memory device that plugs into a USB port and contains from 32 MB to 1 GB or more of non-volatile memory. These devices are comparable to a ball point pen in size and can be readily carry in a pocket. They are appropriately called pen drives.

Hard Disk: Hard disk (Winchester) systems are high density storage units and are faster than floppy disk systems. Hard disks are available having enormous storage capacity, of 100 GB.

Compact Disk: Another powerful medium for storing digital data is the CD. It is non-volatile optical data storage medium and was original invented for digital audio. The CD-ROM (abbreviation for Compact Disc Read-Only-Memory) reading devices are frequently included as a component in the personal computers (PCs). A CD is a flat, plastic disc with digital information encoded on it in a spiral from the centre to the limit, the outside edge. CDs can either be burned or pressed. Small amounts are burned, larger amounts pressed. The break-even point seems to be somewhere between 100 and 500 copies.

CDs are available in a range of sizes but the most commonly available is 120 mm in diameter. A 120 mm disc can store about 74 min of music or about 650 MB of data. Discs that can store about 700 MB (80 min of music) have become more common. For drives installed in computers, all current CD-ROM and DVD-ROM drives can read and write CD-ROM and CD-RW discs. CD-ROM drives may connect to an IDE (ATA) interface, a SCSI interface or a proprietary interface, such as the Panasonic CD interface. Most CD-ROM drives can also play audio CDs.

The memory stores the data to be manipulated by the CPU, as well as the program that directs that manipulation. A program is a group of logically related instructions. The CPU reads each instruction from memory in a logically determined sequence, and uses it to initiate processing actions. The CPU can rapidly access any data stored in memory. Also, the CPU can address one or more output ports, added to receive information from external equipment and input the data contained there.

Hardware alone does not make a computer. Before any computer, and for that matter any computer, can be put to work, it must be given a set of instructions (program). It is the program that states the procedure the computer is to follow in solving the program at hand. By changing the program, the same hardware can perform many different functions.

28.2.3 Offline/Online Computers

An offline computer accepts the experimental data from input devices such as CD or magnetic tape or pen drive. It does not take data directly from the analytical instrument, nor does it process in real time.

The offline configuration is generally implemented with large computers in situations requiring complex calculations and manipulation of sizable amounts of data. The computer running in an offline configuration is not required to respond to the instantaneous needs of a specific instrument. Offline applications of computers are generally referred to as passive applications.

On the other hand, an online computer takes data directly from the instrument or process, and analyses it to take decisions based on it. The instrument and the computer in this case are linked through an electronic interface to perform tasks such as acquisition and processing of data as well as instrument control functions. The computer responds instantaneously to the data acquired, carry out compilations provides output information rapidly enough to improve the dynamic operation of the process and the instrument examples of requirement of rapid execution of complex mathematical transformations functions are found in Fourier transform of nuclear magnetic resonance and infrared spectroscopy which would not have been possible without the help of online computers. The applications involving online operations of the computers are called active applications.

It is thus obvious that with the offline computer, only the past history of an experiment can be studied, whereas with the online computer, it is possible to determine results in real time to investigate new and unusual occurrences during the course of an experiment.

28.2.4 Dedicated Computers

A computer dedicated to a specific function in a laboratory has been largely responsible for a revolution in the methodology, economy, quantity and quality of experiments performed and data analysed. In combination with an analytical instrument, the computer becomes an element in a total analytical instrumentation system and is capable of acquiring and analysing data, and controlling the instrument and experiment based on the data received. This has enabled to design considerably more sophisticated instrumentation systems that were not possible earlier by having computers as internal components. Dedicated computer may be a micro-controller, an embedded system or a PC.

Laboratory systems built around the dedicated computers are already in use in a variety of analytical fields, such as mass spectroscopy, gas chromatography, nuclear magnetic resonance and clinical chemistry. Programmed data processing systems and associated software are available as black box devices for applications.

Dedicated computers are of low initial cost and provide much more computer capability for a particular problem, than when it is interfaced to a timed-sharing system. In addition, a dedicated computer system does not need programming staff.

28.3 TYPES OF COMPUTERS

Computers are generally classified as super, mainframes, minis and micros. Though these categories are now well established, understood and talked about by everyone, but how computers are actually defined for these groups continues to change. This is because computer technology

changes so rapidly that such definitions quickly become obsolete. For example, what is called a minicomputer today might have been considered a main-frame computer 10 years ago. This implies that almost any criteria accepted today, quickly becomes invalid or obsolete.

The generally accepted basis of classification of computers is according to word size, memory capacity and processing speed. It may be remembered that the greater the word size, memory capacity and processing speed, the larger the computer. For example, a minicomputer has a greater word size, memory capacity and processing speed than a microcomputer. Similarly, a super computer would process data faster than a mainframe, which is faster than a mini, which is again faster than a micro. Let us first understand something about word size, processing speed and memory capacity. It should be noted that the distinction between the various categories is not sharp, and the differences are only matters of degree rather than kind.

Word Size: A word' is a group of bits that the computer treats as a single unit of information. The number of bits in a word varies. Small computers may use words of four or eight bits, large computers may use words of 32, 64 or more bits. Figure 28.3 illustrates words consisting of 4-, 8- and 16-bits. The larger the word size, the larger the computer category that the computer fits into.

Memory Capacity: Memory capacity is determined by the number of memory locations available in a system. Memory size is generally measured in thousands of words or kilowords. One kiloword (k) is actually equivalent to 1,024 words. For example, if a computer has 64 k memory, it means a capacity of 65,536 locations ($64 \times 1{,}024$), if the measurement unit is bits. If the memory is expressed in bytes, the same 64 would represent 524,288 ($64 \times 1{,}024 \times 8$) memory locations. The memory of large computers is expressed in gigabytes.

Processing Speed: Processing speed implies number of instructions (spelled out in computer program) executed per unit time. Obviously, super computers can process data faster than mainframes, which can process faster than a mini, which can process faster than micros.

Microcomputers are more likely to be used as components of electronic systems, whereas mini, mainframe and supers are used as general-purpose computers (i.e. they are likely to be used for a wide range of data processing activities, many times for offline applications). Larger (mini, mainframe, super) computers generally have far more extensive software support than microcomputers.

It may be noted that PCs are general-purpose microcomputers. They are now used with analytical instruments, many times as attachments, for data processing and control. In the book, the term computer has been used to include microcomputers or PCs (both desktop as well as laptop) or any such device which performs the function of a computer.

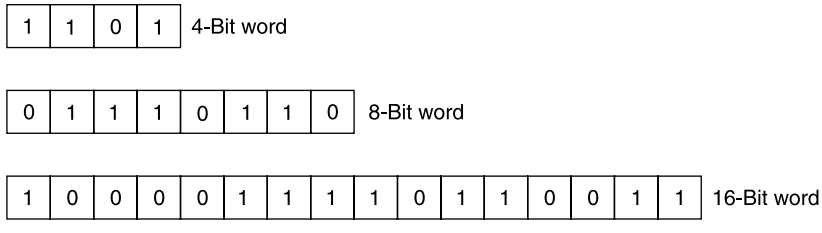


Figure 28.3 *Word sizes – 4, 8 and 16-bit*

28.4 MODEMS

The transfer of relatively small amounts of data from one computer to another can be done via a physical storage medium such as magnetic disk or CD. But for transferring larger amounts of data requires wiring the computers together to enable direct exchange. This can be done by making use of telephone lines, but they are analog while computers are digital. The translation of data from one signal form to another for transmission over telephone lines is achieved by using a modem. The working of a modem is illustrated in Figure 28.4

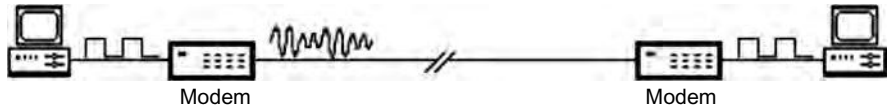


Figure 28.4 *Principle of computer communication using modems*

The term modem combines two functions: modulation and demodulation. It consists of a transmitter that converts digital (base-band) signals from the computer into analog (passband) signals suitable for transmission over telephone lines. The modern receiver demodulates the received passband signals into base-band signal. The working of the modem is transparent to the computing task and the operator is not concerned with it. The computer and remote terminal operate as if connected directly. Modems operating on telephone lines are termed voice band or voice grade modems.

Each application makes different demands on a modem. The parameters for selecting a modem are the data transmission rate, communication modes, synchronisation modulation techniques, types of lines and industry standards. The most important parameter is the data transmission rate and the modems can be categorised as: low speed (bit rate up to 600 bps), medium speed (1,200–2,400 bps) and high speed (4,800 bps and up). Data transmission rates are specified as bit rate or baud rate. The bit rate is the actual transfer rate of transmitted data (bits of data per unit time), whereas the baud is the unit of signalling. In order to achieve higher data transmission rates, special modulation techniques are used, which enable transmission of multiple numbers of bits per baud. High speed modems require two, three or four bits per baud to implement bit rates of 2,400, 4,800 or 9,600 bps. In low speed modems, bit and baud rates are the same.

The applications determine whether one- or two-way communication is needed, whether two locations are required to be connected point-to-point or a multiple location network is required.

28.5 COMPUTER SOFTWARE

In computer science, computer software refers to the information processed by the computer system. It means a program or a set of programs which is needed to get computer to perform its intended function. In general, the term software is used to refer to all instructions, routines and programs and their associated techniques. It is because they are changeable, they are called software. This is in contrast to hardware which is the term used for computer equipment and their logic circuits of physical components. The program stored in the ROM is often referred to as Firmware to indicate they are something between hard logic and soft programs.

Software is typically often divided into the following two major categories:

- System software
- Application software

28.5.1 System Software

System software provides the basic system-specific functions of the computer. It is responsible for controlling, integrating and managing the individual hardware components of a computer system, so that other software and the users of the system see it as a functional unit without having to be concerned with the low-level details such as transferring data from memory to disk, or rendering text onto a display. Generally, system software consists of an operating system and some fundamental utilities such as disk formatters, file managers, display managers, text editors, user authentication (login) and management tools, and networking and device control software.

Operating Systems: An operating system is essentially an agent between the hardware, the software and the user (Figure 28.5). Every general-purpose computer must have operating system to run other programmes. Operating systems perform basic tasks, such as recognising input from the keyboard, sending output to the display screen, keeping track of files and directories on the disk, and controlling peripheral devices such as disk drives and printers. It is a set of programs that manage collectively all of the resources available to the computer, including the CPU, the peripheral devices and the software. For maximum speed, most successful operating systems are written in machine language.

The operating system makes sure that different programmes and users running at the same time do not interfere with each other. The operating system is also responsible for security, ensuring that unauthorised users do not access the system. Operating systems can be classified as follows:

- Multi-user: Allows two or more users to run programs at the same time. Some operating systems permit hundreds or even thousands of concurrent users.
- Multiprocessing: Supports running a program on more than one CPU.

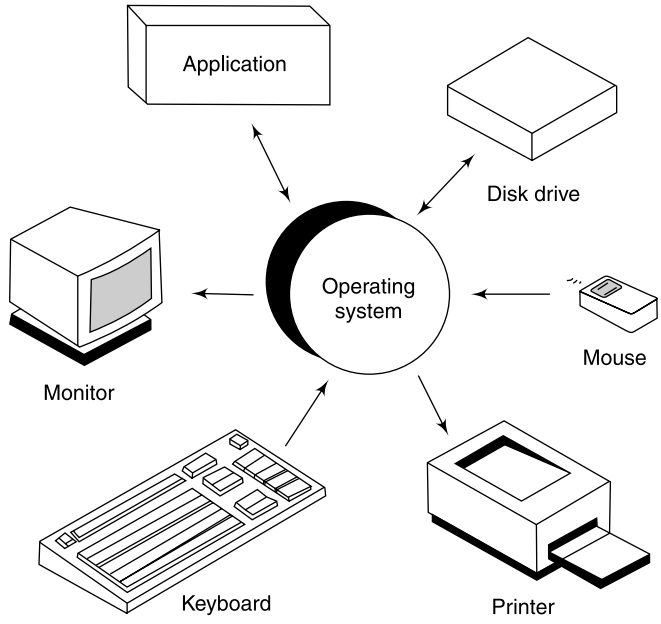


Figure 28.5 *Function of operating system in computers*

- Multi-tasking: Allows more than one program to run concurrently.
- Multi-threading: Allows different parts of a single program to run concurrently.
- Real time: Responds to input instantly. General-purpose operating systems, such as DOS and UNIX, are not real time.

Operating systems provide a software platform on top of which other programs, called application programs, can run. The application programs must be written to run on top of a particular operating system. Your choice of operating system, therefore, determines to a great extent the applications you can run. For PCs, the most popular operating systems are Windows, but others are also available, such as Linux.

A user normally interacts with the operating system through a set of commands. For example, the Windows operating system contains commands such as Copy, Paste, Cut, Delete, etc. and changing the names of files, respectively. The commands are accepted and executed by a part of the operating system called the command processor or command line interpreter. Graphical user interfaces allow you to enter commands by pointing and clicking at objects that appear on the screen.

At present, the major operating systems in widespread use on PCs have consolidated into two families: the Microsoft Windows family and the UNIX-style family, which includes various definitions of UNIX, Linux and Mac OS X.

UNIX is widely used in academic institutions and back-end implementations, while Windows is popular among home users as well as businesses for front-end use. In general, Windows is by far the most widely used operating system with studies variously placing Microsoft's market share anywhere from 90 to 98%. Linux is widely used in web servers, and is making inroads into home and business environments. Mac OS X, which incorporated major parts of UNIX, and its predecessors are popular with multimedia designers. Mainframe computers and embedded systems use a variety of different operating systems, many with no direct connection to Windows or UNIX.

28.5.2 Application Software

Application software is used to accomplish specific tasks other than just running the computer system. Application software may consist of a:

- Single programme, such as an image viewer
- Small collection of programmes (often called a software package) that work closely together to accomplish a task, such as a spreadsheet or text processing system
- Larger collection (often called a software suite) of related but independent programmes and packages that have a common user interface or shared data format, such as Microsoft Office, which consists of closely integrates word processor, spreadsheet, database management system, etc.
- Software system, such as a database management system, which is a collection of fundamental programmes that may provide some service to a variety of other independent applications.

In above types of embedded systems, the application software and the operating system software may be indistinguishable, such as in the case of the software used to control a microwave oven, a VCR or DVD player.

28.5.3 Software Creation

Software is created with programming languages and related utilities. Therefore, it is of special importance that the users understand the computer language(s) used by his or her equipment. A programming language or computer language is a standardised communication technique for expressing instructions to a computer. It is a set of syntactic and semantic rules used to define computer programmes. A language enables a programmer to precisely specify what data a computer will act upon, how these data will be stored/transmitted and precisely what actions to take under various circumstances.

The most basic computer language is the machine language, which is a rudimentary method of communicating directly to the computer through a system of binary digits. The programming with a machine language or assembly language (based on use of mnemonic to each instruction) is a long and tedious process. That is why various high-level languages have been developed. These languages allow the user to work in a language that is closer to English, thereby improving efficiency and simplifying communication.

High level languages make computer programmes less dependent on particular machines or environments (Figure 28.6). This is because programming languages are converted into specific machine code for a particular machine rather than being executed directly by the machine. The concept that easier to understand languages could lead to less error prone and more rapid development was the basis for Fortran II way back in 1958, and the foundation for the high-level languages of today. Since the 1950s, however, numerous high-level languages have propped up. You have heard and worked with most of these. Fortran is still used in engineering, and general programming problems are often solved with C, C++, Java and Perl, which are all considered high level.

There are two mechanisms used to translate a programme written in a programming language into the specific machine code of the computer being used.

If the translation mechanism used is one that translates the programme text as a whole and then runs the internal format, this mechanism is spoken of as compilation. The compiler is therefore a programme which takes the human-readable programme text (called source code) as data input and supplies object code as output. The resulting object code may be machine code which will be executed directly by the computer's CPU, or it may be code matching the specification of a virtual machine.

If the programme code is translated at runtime, with each translated step being executed immediately, the translation mechanism is spoken of as an interpreter. Interpreted programmes run usually more slowly than compiled programmes, but have more flexibility because they are able to interact with the execution environment. Although the definition may not be identical, these typical fall into the category of scripting programming languages.

Most languages can be either compiled or interpreted, but most are better suited for one

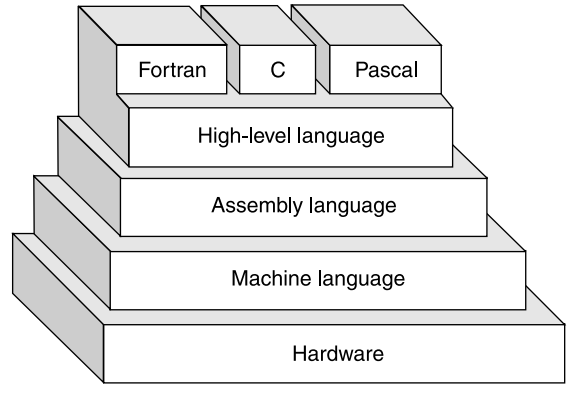


Figure 28.6 *Combination of hardware-software vis-à-vis languages*

than the other. In some programming systems, programmes are compiled in multiple stages, into a variety of intermediate representations. Typically, later stages of compilation are closer to machine code than earlier stages. Popular high-level languages include BASIC, Pascal and FORTAN.

Choosing a language is a purely subjective matter. The choice of a language is, however, usually based on user's experience in the language and on how well established the language is (Owen, 1984).

Software can be recorded on the printed page or on disk. Reading the data off a printed page and feeding it manually to the computer via keyboard is the slowest process. Feeding the data into the computer's memory from a pre-recorded floppy disk or CD is a much more efficient and currently most widely used process.

28.5.4 Popular Software Packages

28.5.4.1 Spreadsheet

A spreadsheet is a computer application program that simulates a physical spreadsheet by capturing, displaying and manipulating data arranged in rows and columns. The spreadsheet is one of the most popular uses of the PC.

In a spreadsheet, spaces that hold items of data are called cells. Each cell is labelled according to its placement (e.g. A1, A2, A3...) and may have an absolute or relative reference to the cells around it. A spreadsheet is generally designed to hold numerical data and short text strings and usually provide the ability to portray data relationships graphically. Spreadsheets generally do not offer the ability to structure and label data items as fully as a database and usually do not offer the ability to query the database. In general, a spreadsheet is a much simpler program than a database program.

Microsoft Excel, the most popular spreadsheet contains many functions that can be used to save steps when developing complex statistical or engineering analyses.

28.5.4.2 LabVIEW

LabVIEW, short for Laboratory Virtual Instrument Engineering Workbench, is a programming environment in which you create programs using a graphical notation. In this regard, it differs from traditional programming languages like C, C++, or Java, in which you program with text. However, LabVIEW is much more than a programming language. It is an interactive program development and execution system. The LabVIEW development environment works on computers running Windows, Mac OS X, or Linux. LabVIEW can create programs that run on those platforms, as well as Microsoft Pocket PC, Microsoft Windows CE, Palm OS and a variety of embedded platforms, including Field Programmable Gate Arrays (FPGAs), Digital Signal Processors (DSPs) and microprocessors.

Using the very powerful graphical programming language that many LabVIEW users often call 'G' (for graphical), LabVIEW is specifically designed to take measurements, analyse data and present results to the user.

LabVIEW has extensive libraries of functions and sub-routines to help the user with most programming tasks, without the fuss of pointers, memory allocation and other arcane programming problems found in conventional programming languages. LabVIEW also contains application-specific libraries of code for data acquisition (DAQ), General Purpose Interface Bus (GPIB), serial instrument control, data analysis, data presentation, data storage and communication over the

Internet. The Analysis Library contains a multitude of useful functions, including signal generation, signal processing, filters, windows, statistics, regression, linear algebra and array arithmetic.

Because of LabVIEW's graphical nature, it is inherently a data presentation package. Output appears in any form you desire. Charts, graphs and user-defined graphics comprise just a fraction of available output options. LabVIEW applications include improving operations in any number of industries, from every kind of engineering and process control to biology, farming, psychology, chemistry, physics, teaching and many others.

Figures 28.7 show a simple LabVIEW user interface for a DAQ system which allows the user to choose parameters such as wavelength calibration, unit conversion, and peak and bandwidth reading of the spectrum curve. It can conveniently redisplay and analyse previously saved data. Users can also inspect each dataset using cursor move, data refresh, figure enlarge and diminish, and graph recover tools. A File Dialog function will prompt the user to upload a data file. The Read From Spreadsheet File and Index Array function will convert the wavelength and intensity data to X and Y arrays, respectively. Wavelength unit conversion is realised by the case structures, and then the processed data is sent to the XY graph for display. Spectrum graph printing is achieved by using the relevant report generation functions.

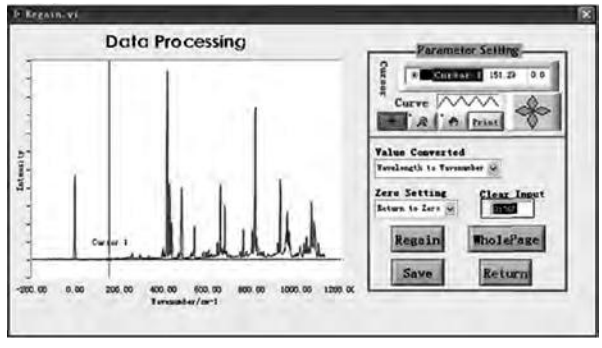


Figure 28.7 *LabVIEW front panel for data processing*

The data analysis part of LabVIEW includes tools such as curve fitting, signal generation, peak analysis, deconvolution, smoothing and various mathematical operations. The programme also integrates with standard mathematics software such as MATLAB.

LabVIEW uses terminology, icons and ideas familiar to scientists and engineers. It relies on graphical symbols rather than textual language to define a program's actions. Its execution is based on the principle of dataflow, in which functions execute only after receiving the necessary data. Because of these features, LabVIEW can be learnt even if you have little or no programming experience.

28.5.4.3 Mathematics software

There are several types of mathematics tools which are of interest to the chemist and are available commercially. These programmes help to quickly solve mathematical equations which are found in the study of multiple equilibria. Some of the common mathematics tools available are:

Excel: It is an electronic spreadsheet program that can be used for storing, organising and manipulating data.

MINITAB: Minitab, originally intended as a tool for teaching statistics, is a general-purpose statistical software package designed for easy interactive use. Minitab is well suited for instructional applications, but is also powerful enough to be used as a primary tool for analysing research data.

Mathcad: It is computer software primarily intended for the verification, validation, documentation and re-use of engineering calculations. First introduced in 1986, it was the first to introduce live editing of typeset mathematical notation, combined with its automatic computations.

MATLAB (*matrix laboratory*): It is a multi-paradigm numerical computing environment and fourth-generation programming language. Developed by MathWorks, MATLAB allows matrix manipulations, plotting of functions and data, implementation of algorithms, creation of user interfaces, and interfacing with programs written in other languages, including C, C++, Java, and Fortran.

TK Solver: It is a mathematical modelling and problem-solving software system based on a declarative, rule-based language, commercialised by Universal Technical Systems, Inc.

28.6 INTERCONNECTING LABORATORY INSTRUMENTS TO COMPUTERS

For real-time processing of data, it is required to connect the instrument to a computer for which a variety of interfacing possibilities are available. The purpose of an interface is to accept experimental data as presented by the instrument and transmit that data to the computer in a form it can deal with. The computer carries out data analysis and long-term data storage by using a variety of software packages. The operator controls the overall process through interactions with the computer. In this way any instrument is experiment can be interface with a computer for efficient analysis and display of instrumental data. The interface configuration depends on (Liscouski, 1982) the following aspects:

- Characteristics of the data to be collected, which would in turn depend on the needs of the experiment.
- Capabilities of the computer (i.e. whether it can accept the data rates and perform the real-time processing required).

The interface between the instrument and the computer consists of hardware and software resident in the computer or in the form of embedded intelligence in the instrument itself. The proportions of interfacing functions that are provided by hardware and software depend on the type of interface (Dessy, 1986b).

28.6.1 Types of Interfaces

Computer communications refers to the wide range of hardware and software products and protocols used to communicate between standard computer platforms (PC, Macintosh or workstation) and devices used in automation applications. There are four common types of interfaces for getting information from an instrument into a computer.

- *Analog I/O*, which requires the computer to read directly an analog signal such as voltage. Here the user determines where to pick up the instrument signal and how to get it to the computer.

- *Digital or parallel I/O*, in which the two voltage levels are used to represent logical 0 and 1.
- *Serial IO*, in which the instrument generates a bit stream of alphanumeric characters as eight data bits.
- *IEEE-488 standard interface* defines the 16-line bus, linking the instrument and computer; the shape, size and number of pins in the connections; all voltages; the handshaking procedure including addressing; and the method of data transfer.

Irrespective of the type of interface selected, application software needs to be written for the computer to handle the data and process the same. Depending on the requirements of the experiment, the development of the software may be a trivial or a major task. Many data reduction and analysis sub-routines are available in commercial packages for the most popular computers.

For data collection from instruments under external control, all three of the communication protocols-RS 232 C, binary and IEEE 488 can be employed. The fundamental control element is a binary output representing an ON/OFF request. The TTL signals generated by the basic parallel output boards are given to solid-state relays to allow the user to control 8–16 solid-state switches with TTL inputs. These switches are optically isolated and can control 110–220 V AC up to 5 A.

Control of external devices is an inherent part of the IEEE 488 standard, whereas commercial modules are available that interpret a pre-selected set of ASCII characters transmitted over a serial line as the command to activate or deactivate a target device.

A timing element is usually needed if external control of a device is required. It is common to incorporate clock-timer modules into the computer's option slots to provide controllable real-time functions. These devices have programmable counters, in which externally provided pulses cause them to count down towards zero. When time-out occurs, a system interrupt is generated, indicating that a real-time period has elapsed and some service action is required. Although it is possible to program complex situations with one clock, multiple overlapping asynchronous events are best handled with multiple real-time clocks.

28.6.2 Analog Interfaces

Instruments that lack a digital output require an analog interface to convert voltage or other analog output of an instrument to a binary form that can be handled by the digital computer.

Figure 28.8 shows the functions of an analog interface, which converts the analog DC signals from a primary measuring instrument into digital signal. A pre-amplifier may be necessary to obtain satisfactory resolution from the Analog-to-digital (A/D) converter. A/D converters are characterised by their resolution, dynamic range and maximum throughput (i.e. total samples per second

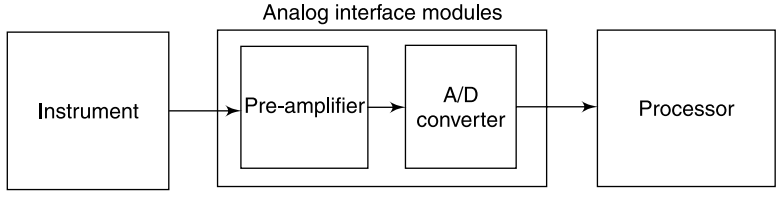


Figure 28.8 *An analog interface. It converts the DC signal from the instrument into binary numbers. A pre-amplifier may be necessary to obtain a better resolution from the A-D converter*

that can be handled). Since software costs generally far exceed hardware costs, the analog – digital interface structure must permit software effective transfers of data command and status signals to realise the full capability of the host computer. At the same time, it must acquire and digitise data, often from hostile industrial environments, with no degradation in the resolution or accuracy of the signal.

28.6.3 Digital I/O Interfaces

Digital I/O was the first type of computer interface developed for the discrete signal outputs of such instruments as pH metres, balances and spectrophotometers, etc. In such interfaces the data are handled in digital form (1 and 0). Binary-coded decimal (BCD) was the first such method and it is still the most common method of digital data notation. Each digit may be transmitted individually and successively (bit parallel, digital serial). Alternatively, all of the digits for a datum may be transmitted at one time (bit parallel, digital parallel). In the former only four bits of data need to be collected at one time, while in the latter format the bit string length is determined by the dynamic range and resolution of the internal BCD converter.

If the BCD instrument output uses TTL, it may be connected directly to a parallel port of a computer. This parallel port must have a sufficient number of input bits to handle the data string. In addition, it must have at least one output bit that can control the update line of the data latch associated with BCD output devices. Figure 28.9 shows a typical parallel BCD interface. The A/D converter in these systems feeds BCD output to a set of data flip-flops used to latch the information. An update line prevents the system from changing the latch contents, while the computer is reading the data.

It may be noted that incoming data are in BCD and most computer programs (and languages) implement straight binary arithmetic. A BCD-to-binary conversion is generally required.

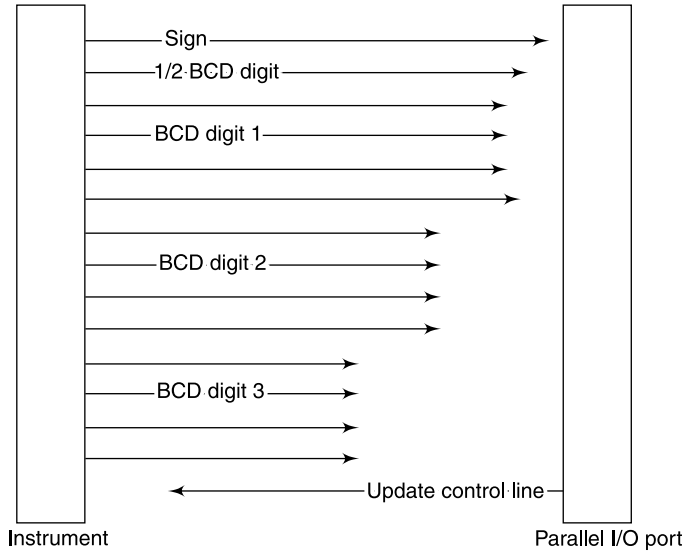


Figure 28.9 *Parallel BCD interface*

Data transfers through digital I/O interfaces are customarily asynchronous. They take place whenever the instrument and the computer are prepared to accept it. Coordination of instrument and computer is provided by handshaking software on the computer. In handshaking, the procedure is that the instrument sends the computer a signal asking whether it is ready to receive data, receives an acknowledgement in the affirmative from the computer on a separate REPLY line and then sends the data. A representative data word architecture used in transmitting experimental data from an instrument to a computer through a digital I/O interface is shown in Figure 28.10. From the software, it is known as to what the bit positions represent in the data word. The 1 and 0 levels are represented in both input and output voltages by 3.5 and 0 V DC, respectively.

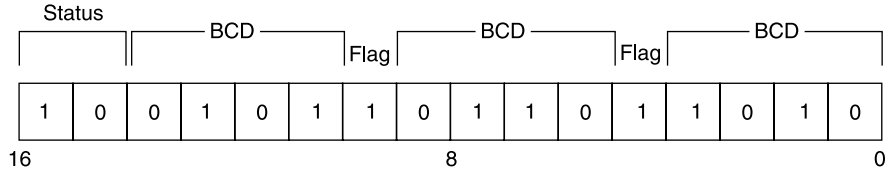


Figure 28.10 *Digital I/O interface for transmitting experimental data from an instrument to a computer*

28.6.4 Serial Interface

28.6.4.1 Serial ASCII interface

A serial ASCII instrument generates a bit stream of alphanumeric characters as defined by ASCII code by American Standard Committee for Information Interchange. This code is a 7- or 8-bit code consisting of 1's and 0's that represent letters, numbers and control characters. The 7-bits can encode 128 possible values. The 26 uppercase and 26 lowercase letters, 10 Arabic digits, and a variety of grammatical symbols can be expressed in the code. Computer keyboards generate most of these codes by using combinations of the regular alphanumeric (7-bit ASCII code is not generally used for instrument applications). Thus data and control information are sent along the two data channels in ASCII code.

There are two types of arrangements for transmitting bits between the instruments and interface modules: 2OmA serial ASCII interface and RS 232 C interface.

20 mA Serial Interface: Figure 28.11 shows the 20 mA transmission interface, which is based on a two-wire closed current loop between the instrument and computer. The instrument generates and instantaneous current of 20 mA to represent a '1' bit and zero current to represent a '0' bit. Obviously, there cannot be two active devices on the same 20 mA loop. This is done by inserting an optical isolator between the instrument and interface.

The 20 mA bit transmission interface permits long distances between the instrument and computer, which could be as much as 1,000 ft. The main disadvantage is that, there are no extra lines for handshaking or modem control, and therefore 20 mA transmission is limited to local wiring only. Without handshaking lines, synchronisation of instrument and computer is provided by software on both sides.

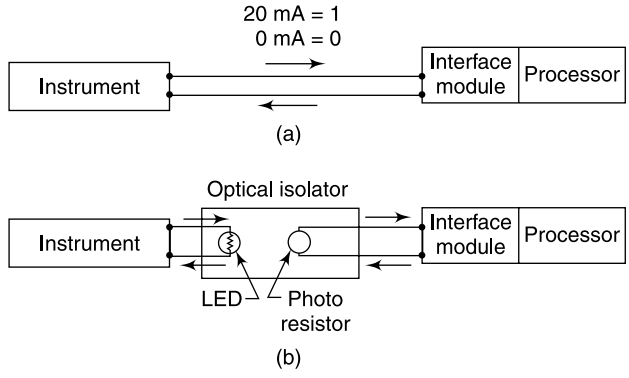


Figure 28.11 *(a) 20 mA serial ASCII interface (b) Use of an optical isolator inserted in a 20-mA loop to avoid two active devices transmitting signals over the same loop. For two-way communications between instrument and computer, two independent 20 mA loops with isolators are used.*

28.6.4.2 RSXXX serial interface

In analytical instruments, the most common serial interface standards include EIA standards RS-232, RS-422 or RS-485 to connect to computers and to one another.

RS 232 C Interface: The most prevalent digital connection found on IBM compatible PCs between instruments and computers is the RS232C standard recommended by EIA (Electronic Industry Association), in which data are represented by voltage level rather than current as in a 20 mA loop. The '1' state is represented by any voltage more negative than 3V DC to ground, and the '0' state by any voltage more positive than + 3V DC to ground (Dessy, 1986a).

Figure 28.12 shows a typical RS 232C interconnect, which makes use of the pins on the *25-pin* output connector on the instrument. Pin '2' is the transmit pin and pin '3' is the receive pin, whereas pin '7' is the ground connection. In addition to these, there are a number of specifically assigned control and handshake pins that may or may not be utilised by the instrument.

In order to coordinate exchange, the data bit string is encapsulated between START and STOP bits. This is the only synchronising element in the communication link. After the START bit, the computer samples the incoming data stream at a rate determined by its own internal clock independent of the instruments clock. The communication is essentially asynchronous. A parity bit is also transmitted to enable the computer to ascertain whether the character received is valid.

In order to coordinate information exchange, RS 232C protocol provides hardware 'handshake', which are shown in Figure 28.13. Referring to a modem (modulator-demodulator), one end is called the data terminal and the other the data set. According to convention, pin 20 (data terminal ready) and pin 6 (data set ready) are used to communicate that the units are physically present, power has been applied and they are operational. Pin 4 (request to send) is used to demand the attention of the computer, while pin 5 (clear to send) acknowledges this request. Hardware handshake lines can be replaced by software handshakes involving the exchange of control characters along the pin 2 and pin 3 data lines.

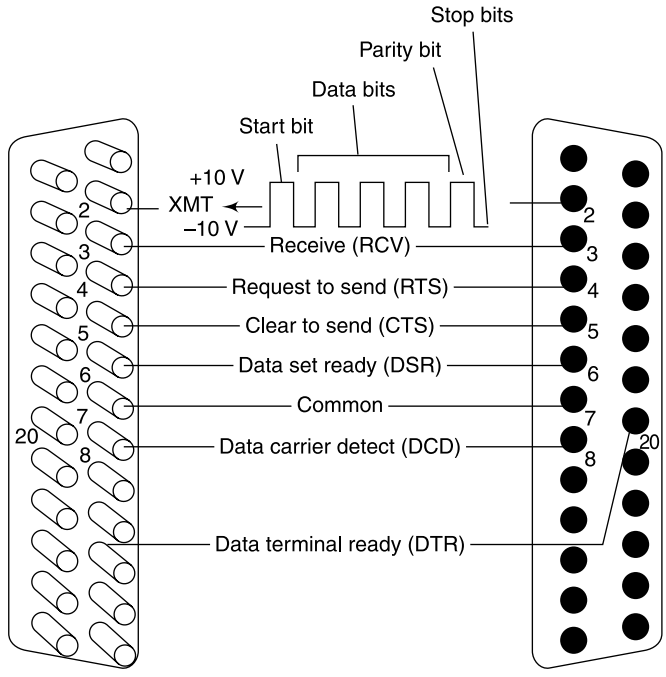


Figure 28.12 *RS-232C interface – use of 25 pin connectors*

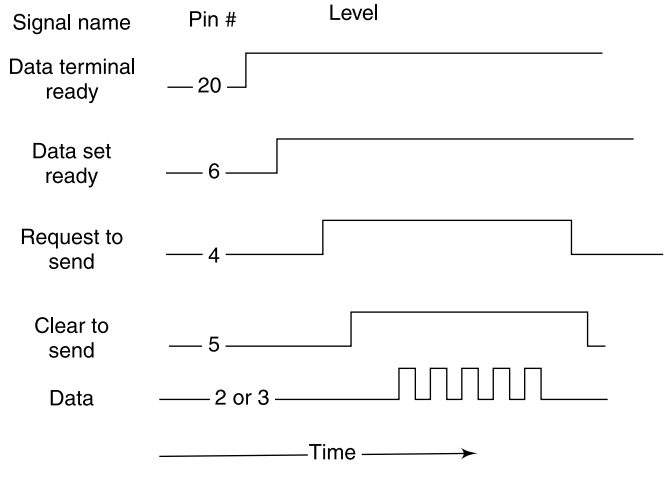


Figure 28.13 *RS-232C protocol. The figure shows relative timing of the handshake signals*

The computer must expect bits to arrive at the same rate that the instrument places them on the line. This is expressed in bits per second, often referred to as baud rate. Common values of baud rate vary from are 150 to 19,200 baud. At 9,600 baud, it takes about 1 ms for a character to transit the wire. Directly connected RS 232C protocols will usually support communication at 2,400 baud for

a kilometre. For longer distances, modems can be used. Due to improvements in line drivers and cables, applications often increase the performance of RS-232 beyond the distance and speed listed in the standard. RS-232 is limited to point-to-point connections between PC serial ports and devices.

RS 232C circuit's noise susceptibility rises with increasing baud rate and distance. RS 232C interface lines are therefore, usually limited to a short distance between instrument and computer. About 20 m is maximum for some instruments. Nevertheless, RS 232C transmission is far more popular than other interfacing techniques.

RS-422 (EIA RS-422-A Standard) is the serial connection used on Apple Macintosh computers. RS-422 uses a differential electrical signal, as opposed to the unbalanced signals referenced to ground with RS-232. Differential transmission, which uses two lines each for transmit and receive signals, results in greater noise immunity and longer distances as compared to RS-232. The greater noise immunity and distance are big advantages in industrial environments.

RS-485 (EIA-485 Standard) is an improvement over RS-422 because it increases the number of devices from 10 to 32, and defines the electrical characteristics necessary to ensure adequate signal voltages under maximum load. With this enhanced multi-drop capability, you can create networks of devices connected to a single RS-485 serial port. The noise immunity and multi-drop capability make RS-485 the serial connection of choice in industrial applications, requiring many distributed devices networked to a PC or other controller for data collection and other operations.

A common misconception about these specifications is that they define specific software protocols. The ANSI/EIA RS-xxx standard specifies only the electrical characteristics – not the software protocol. Table 28.1 summarises the main features of these three-serial interfaces as defined in their respective standards documents.

28.6.4.3 IEEE-488 standard interface (HPIB, GPIB)

Institute of Electric and Electronic Engineers (IEEE) 488 standard defines the interface between instrument and computer in a digital DAQ system. It basically defines the 16-line bus (physical cable) linking the instrument and computer (called the controller); the shape, size and number of pins in the connectors; and all voltages, the handshaking procedure and the method of data transfer. In essence, the standard provides for a cable of eight data lines, eight parallel control and handshake lines and eight ground lines that interconnect a family of instruments (Figure 28.14). A 24-pin ribbon connector is used, with either tristate or open-collector TTL negative logic levels for the signal lines. Up to 15 instruments can be multi-dropped along IEEE 488 lines, which can reach 20 m lengths and communication speeds of 500 KB/s (4,000 k bits/s). Interface cards and software support programs for this popular interface are available from a number of vendors (Harman, 1986).

Table 28.1 *Features of RS-232, RS-422 and RS-485*

	RS-232	RS-422	RS-485
Type of transmission lines	Unbalanced	Differential	Differential
Maximum number of drivers	1	1	32
Maximum number of receivers	1	10	32
Maximum cable length meters (feet)	15.2 (50)	1.2 (4000)	1.2 (4000)
Maximum data rate	20 kb/s	10 Mb/s	10 Mb/s

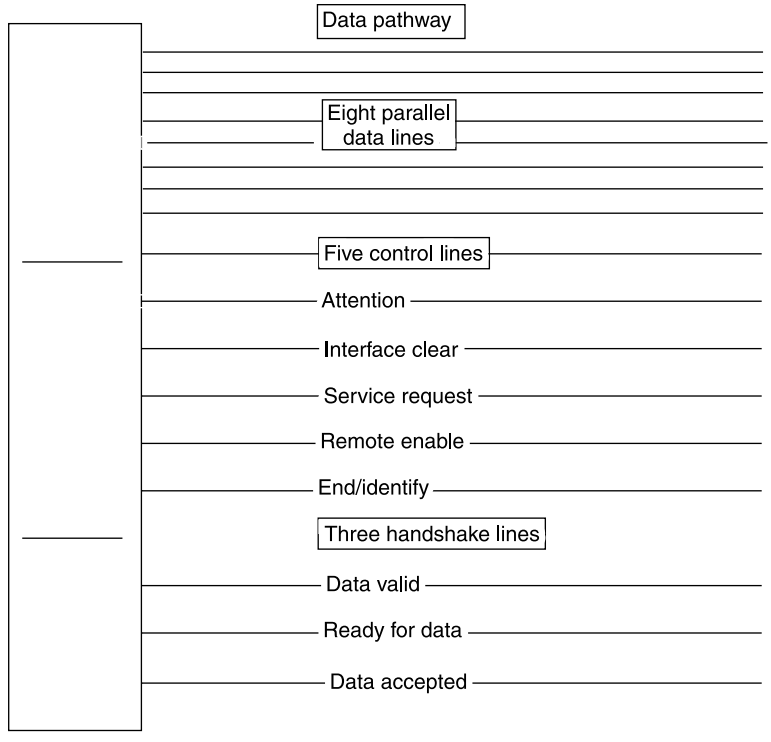


Figure 28.14 *IEEE-488 standard*

IEEE-defined communications software in the instruments and the computers interface modules provides for exchange of messages on the bus between what are called Talkers and Listeners. As bus controller, the computer observes activity on the bus and issues commands, designating which instruments talk and which listen at any given time. Each instrument on the bus is assigned an address between zero and 30 that the controller uses to identify that instrument when instructing it to talk or listen. Each instrument's address can be set with switches located on the instrument itself.

The IEEE 488 standard describes a number of message routines, through which instruments communicate over the bus. The contents of each message string and the vocabulary of characters that are meaningful are unique to the particular instrument and this information is provided by its user's guide.

All communications using GPIB including commands and data, use a hardware handshake for every byte. All devices connected to the bus participate in that handshake. As a consequence, every device on the bus can influence the ongoing communication or cause severe communication problems such as bus hanging or data corruption. The reason for that can be firmware error or hardware failure in one of the participating devices such as a printer. Powering an idle GPIB device 'on' or 'off' during ongoing communication also can cause such problems (Winter and Huber, 2000)

The IEEE 488 specification elegantly defines the three elements of an active system that can most readily be standardised, namely mechanical interface, electrical interface and data transfer. It precisely defines connector types, the number of connecting tunes, tune driver and receiver circuit parameters, and the exact protocol of message transfer between devices (Jensen, 1981).

Outstanding advantages of the standard IEEE-488 bus include byte serial, bit parallel digital data handling, synchronised communication among devices at varying data, rates and hardware interchangeability and interconnections (Williams, 1980). However, it requires highly complex logic protocol and time consuming design analysis. This has been overcome with the availability of long-scale integrated (LSI) chip Intel 8292 controller chip which has built-in IEEE standard 488 logic controls.

In modern analytical instruments, the current trend is to use devices that connect to the computer via a USB port. The USB standard allows connection of up to 127 different devices to the computer. The devices include printers, plotters, recorders, scanners, disk drives, web cams, networking components and DAQ systems.

28.7 COMPUTER NETWORKS

28.7.1 Local Area Network

Most computer users have come to recognise the benefits of networking, both as a solution to system growth and expansion problems and as a flexible method of computer resource integration (Barrett, 1986). The success of networking, however, depends on the ability to equip a broad range of equipment with a common interface. Ethernet has established itself as industry-standard networking medium. Ethernet defines the electrical specifications for the physical connection of equipment to the network, but it does not specify any communications protocol standards for traffic wishing to use it.

Local area network (LAN) connectivity is mostly required to connect and facilitate diagnostic data exchange between various analytical instrument systems inside an industry or hospital. The links provide very high-speed data exchange capability between two systems. This link requires a hardware commonly known as Network Interface Card (NIC), which is either mounted on the motherboard or is available as a PC add-on card.

The LAN consists of a dedicated transmission network. However, in this configuration multiple sending and review stations are available. Thus, images/data can be sent from several different locations within a building or cluster of nearby buildings to a review station. The arrangement is shown in Figure 28.15.

It may be noted that in both the point-to-point and LAN configurations, only those devices which are connected to the dedicated transmission network, such as sending and receiving stations can communicate with each other.

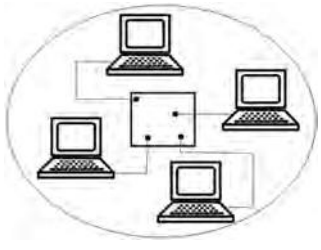


Figure 28.15 *LAN transmission network*

A typical example of a LAN is the transmission of reports of an ICU admitted patient from the clinical laboratory to the ICU for review by the doctors responsible for the patients care. The reports can also be viewed by other physicians or other health care professionals at other sites in the hospital.

LANs are characterised by comparatively high-speed communication. A LAN typically spans a single building or campus and is usually wired through a 'hub' so that any workstation can connect

to any other workstation or device located on the network. The high speeds are possible because of usage of one type of cable and limited distance, which is generally 10 kms or less.

The development of standard networking protocols and media has resulted in worldwide proliferation of LANs throughout business, health facilities, educational organisations and industry.

28.7.2 LAN Communication Using TCP/IP

Of the various protocols available on Ethernet, only transmission control protocol/internet protocol (TCP/IP) is truly vendor-independent (Wilson and Martin, 1986). LAN communication using the TCP/IP enables devices to exchange information over a network. TCP/IP is often referred to as the 'Language of the Internet'. This technique involves breaking of information into pieces or packets. The packets are specifically structured to allow error detection and correction by using redundancy mechanisms like checksums. In principle, checksums are a running total of all transmitted bytes attached to the packet and are used by the recipient to back-calculate and compare with the original checksum provided by the sender. If a mismatch is detected, a re-transmission is requested. This technique guarantees error-free data transport and enables implementation of 'device checks' and system checks (Arneh, 1994).

Communication in a TCP/IP environment is unaffected by addition or removal of idle devices in the network and supports the safety procedures of analytical laboratories that require turning off instruments not currently in use.

28.7.3 Wide Area Network (WAN)

Wide area network (WAN) refers to the technologies which interconnect computers over large distances, which may be over tens to hundreds of kilometres. Usually, the system provides for multiple LAN or LAN-to-WAN interconnections that are geographically separate.

The easiest way to visualise a WAN is that it consists of many LANs interconnected to create a 'super' network as shown in Figure 28.16.

A typical example of a WAN is when a physician from one medical facility sends reports of a patient to a specialist in a distant location for consultation or second opinion.

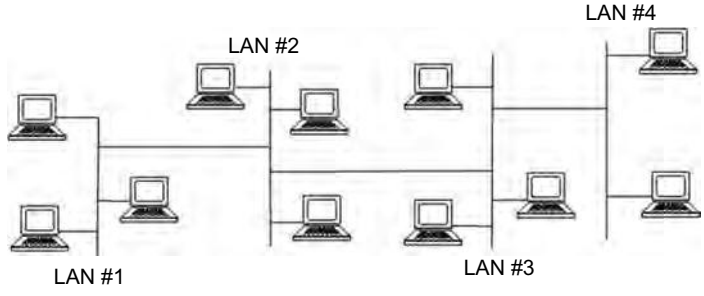


Figure 28.16 *Wide area network configuration*

28.8 LABORATORY INFORMATION MANAGEMENT SYSTEM

Laboratory information management system (LIMS) are collections of software, communication devices and computers that acquire, store, analyse, and present data and information on laboratory samples and their processing. LIMS are used to coordinate workflow and the movement of samples and information through different laboratory processes. These systems centralise data storage, automate data analysis and provide quality assurance reports for process monitoring. The central component of a modern LIMS is a relational database management system (RDBMS) running on a computer with one or more software interfaces allowing users to enter, view and process data. LIMS usually has the following four functional areas:

- Data and information capture
- Data analysis and reports
- Laboratory management
- System management

The basic objective of LIMS is to increase laboratory productivity, improve data accuracy and increase the laboratory's overall effectiveness. A LIMS can organise all the information that is pertinent to the laboratory and allows for rapid data retrieval and reporting. It also allows data to be accessible to other authorised persons and, promoting collaboration among different departments of an establishment. In addition, LIMS can enable various laboratories to utilise either a local or WAN to share network printers and information. Most LIMS are set up in client/server configuration. In this configuration, the database tables reside on the server and the graphical user interface resides on the client machines. The advantage of this configuration is that data processing occurs on the system server.

The core features and their functionality in a typical LIMS are as follows (Paszko and Pugsley, 2000):

Sample Tracking	Allows laboratories to track their samples through different departments in the laboratory with a computer-generated unique sample identification number and provides a complete chain of custody.
Data Entry	Allows analysts to enter results into the LIMS and to assign QC run batches. Reporting to clients via fax, e-mail, or a hard copy.
Sample Scheduling	Automatically logs in samples, receives them into the laboratory, prints labels and assigns the tests for projects on a routine basis.
QA/AC	Allows users to generate control charts and view trend analysis graphs. Control charts can encompass blanks, duplicates, spikes, surrogates, standards, etc.
Electronic Data Transfer	Allows automatic transfer of data from analytical instrumentation into the LIMS. It Increases productivity and greatly decreases the potential for transcription errors.
Chemical and Reagent Inventory	Functionality that tracks the purchase and usage of supplies in the laboratory and manages lot and order numbers, shelf life, costs, etc., assisting in supply management.

Personnel and Equipment Management	Allows users to track employee training records for ISO or other similar purposes and also track instrument calibration, repairs, costs, monitor trends, etc.
Maintenance	A function that allows the database administrator to manage the database, keeping track of client lists, employees, tests, methods, parameters, permissions, priorities, etc.

Figure 28.17 shows the role of LIMS in an analytical laboratory. Creating the right LIMS environment means that you consider all the other systems in the lab that must interface with the LIMS. This includes other applications such as scientific data management systems (SDMS), chromatography data system (CDS) and electronic laboratory notebook (ELN), as well as various related data systems that may be interfaced to any of these systems or that operate independently. It also includes analytical instruments, chromatographs and other sources of laboratory observations.

Data can be transferred by a variety of means such as the following:

- Direct data capture from an instrument interfaced to the LIMS
- Data capture from an instrument with analysis and interpretation by the attached data system and only a result is transferred to the LIMS

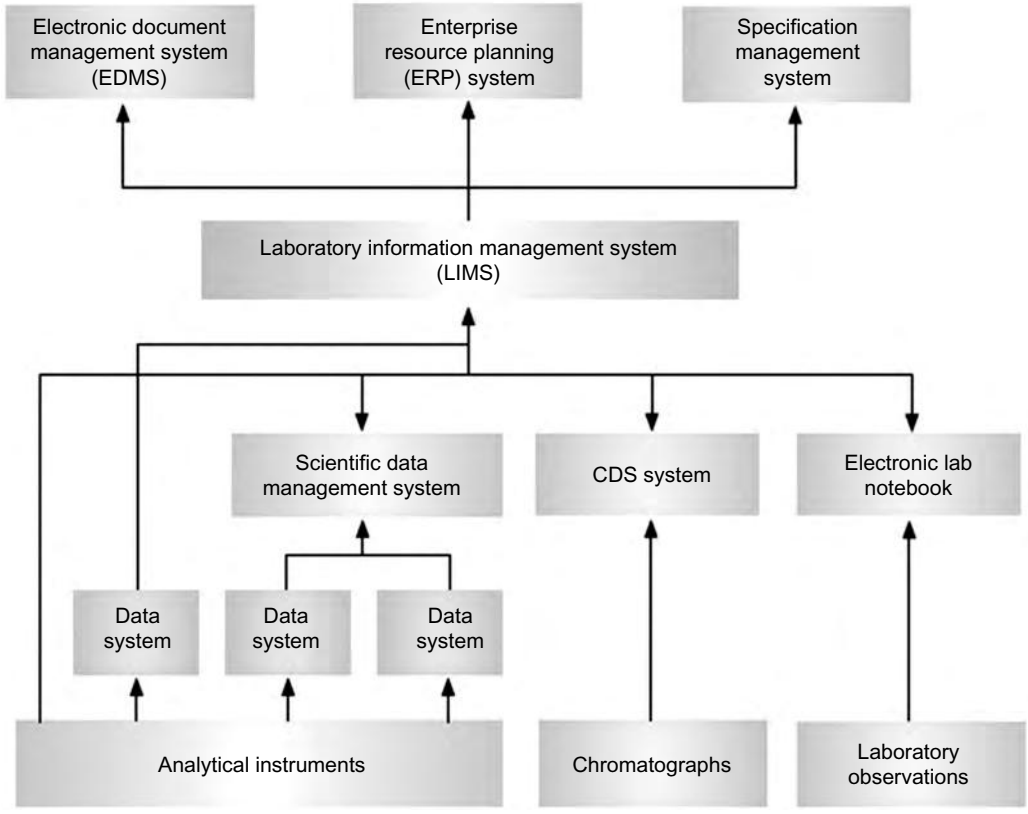


Figure 28.17 *Diagram of a LIMS environment (Courtesy: M/s PerkinElmer)*

- As above, but the results or electronic records are transferred to the LIMS via an SDMS
- Laboratory observations can be written into a notebook then entered manually into the LIMS or captured electronically via an ELN and transferred electronically to LIMS.

In this connection, the following terms need to be well understood:

SDMS: An SDMS is a system used to manage electronic records generated by laboratory instruments. Typically, an SDMS will provide facilities for long-term data preservation, accessibility and retrieval.

ELN: An ELN can be considered to be a direct replacement for the paper lab notebook. It can provide the generic functionality to support 'broad' scientific documentation processes required for patent evidence creation, cross-discipline collaboration and general record keeping.

Most general-purpose LIMS do not provide an interface to automatically collect data from an analytical instrument to store in a database. Data are still needed to be transferred manually between instruments and LIMS, which results in the increasing need for integrating instruments and LIMS.

A typical example of integrating inductively coupled plasma mass spectrometer with a LIMS is from M/s Agilent which greatly simplifies the task of managing large amounts of data while eliminating the necessity for manual data entry and transcription of results.

The Agilent ChemStation Series software is most widely sold data base and popular system in the analytical industry. With the ability to handle a wide range of chromatographic applications such as LC, LC/MS, GC, GC/MS, A/D, CE and CE/MS, ChemStation has an easy-to-use graphical user interface and built-in standard report templates to reduce the amount of time spent on routine tasks. Such integration eliminates the need for redundant sample-related data entry, with the associated possibility for error, and also maintains an electronic chain of custody for each sample, from receipt through to final report. Figure 28.18 shows Screen shot of Instrument Control Window in Standby Mode in ICP-MS ChemStation (M/s Agilent Technologies)

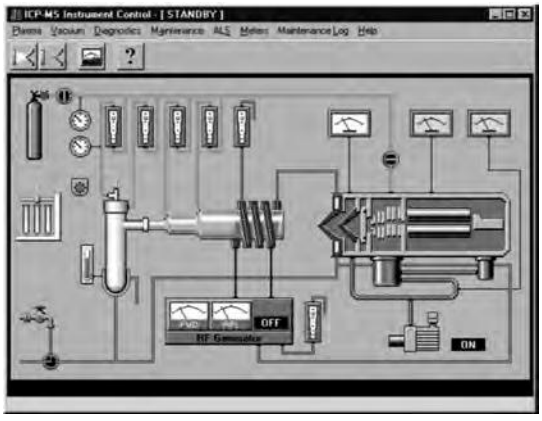


Figure 28.18 *Screen shot of instrument control window in standby mode in ICP-MS ChemStation (Courtesy: M/s Agilent Technologies)*

Sample information such as sample name, source, date received and analysis requested is entered into the LIMS at the time of sample receipt. This can be accomplished either manually, by barcode scanning, or a combination of both. Samples for common analyses with similar turnaround time requirements can be automatically batched into sample analysis groups by the LIMS. After sample analysis, the data is reviewed for QA/QC compliance and the approved data batch is uploaded to the LIMS for archival and final reporting.

For these steps to function, the ICP-MS instrument computer must be capable of bi-directional communication with the LIMS computer(s). Most commonly, this is accomplished by networking the ICPMS computer with the LIMS computer(s) through either a

LAN or a WAN. Serial communication via a modem and telephone is also possible, though typically much slower and less robust. A number of industry-standard network strategies are available, some client-server based, some peer to peer and most can be interconnected as necessary. In this example, it is assumed that the LIMS utilises Microsoft networking running on a Windows-based server. A UNIX-based network server would behave almost identically. The client in either case is the ICP-MS computer (ChemStation) which operates under Microsoft Windows and uses Microsoft networking to communicate with the server (Wilbur, 2002).

In order for information to flow through the ICP-MS ChemStation, the ChemStation must import sample and batch information directly from the LIMS, correctly analyse the associated samples and supply the results to the LIMS in a format which is easily processed by the LIMS. There should also be a mechanism for data review by the ICP-MS analyst to ensure that only data of acceptable quality is reported. This review can occur either at the level of the ChemStation or at the level of the LIMS.

This time reduction is realised through elimination of manual data entry into the LIMS and through elimination of the tedious task of manually checking for transcription errors. Furthermore, the capability for the analyst to quickly review QC compliance before uploading data reduces the need to re-upload out-of-control data, saving even more time.

While every LIMS implementation is different, the fact that the Agilent ICP-MS ChemStation uses built-in, industry-standard networking hardware and software minimises difficulties in network connectivity. In addition, powerful and flexible connectivity tools simplify both the import of sample information and export of sample and quality assurance data to the LIMS.

28.9 SMART LABORATORY

The use of information technology into all aspects of laboratory operations has resulted in fundamental changes in laboratory work. Traditionally, laboratory information has been managed on paper, typically in the form of the paper laboratory notebook, worksheets and reports. This provided a simple means of recording results, observation, laboratory procedure, laboratory apparatus and hypotheses and conclusions. However, the introduction of digital technologies to the laboratory has brought about a revolution in the way we work in the laboratory, now known as Smart Laboratory. A smart laboratory seeks to deploy modern tools and technologies to improve the efficiency of the scientific method by providing seamless integration of systems, searchable repositories of data of proven integrity, authenticity and reliability, and the elimination of mindless and unproductive paper-based processes.

The two primary areas of technology that apply to a smart laboratory are broadly categorised as laboratory automation and laboratory informatics. In general, laboratory automation refers to the use of technology to substitute manual manipulation of equipment and processes. It comprises different automated laboratory instruments, devices, software algorithms and methodologies used to enable, expedite, and increase the efficiency and effectiveness of routine operations and scientific research in the laboratories. The evolution of sophisticated lab instrumentation, data and information management systems, and electronic record keeping has brought about a revolution in the process of acquiring and managing laboratory data and information.

Laboratory informatics generally refers to the application of information technology to the handling of laboratory data and information, and optimising laboratory operations. It encompasses multi-user laboratory systems that provide sample and experiment management, reporting, and

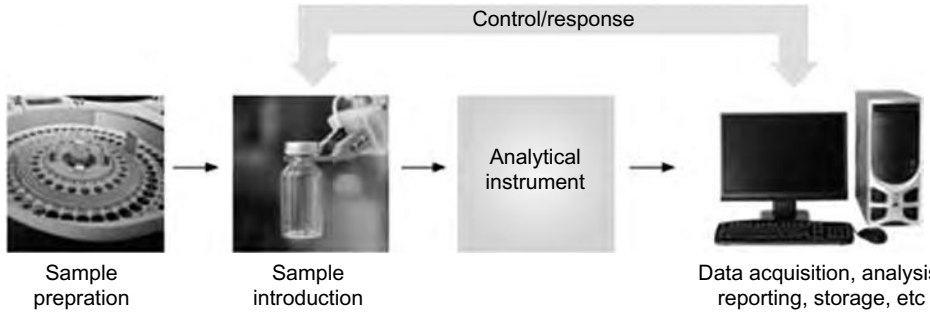


Figure 28.19 *Automated sample processing system (Adapted from Segalstad et al., 2013)*

scientific data management. Laboratory informatics encompasses four major multi-user systems: LIMS, ELNs, laboratory execution systems (LES) and SDMSs.

The underlying purpose of laboratory automation and laboratory informatics is to increase productivity, improve data quality, to reduce laboratory process cycle times, and to facilitate laboratory DAQ and data processing techniques that otherwise would be impossible.

One key to improving laboratory productivity is to develop an automated process for sample preparation, introducing the sample into the instrument, making measurements and then forwarding that data into systems for storage, management and use (Figure 28.19). Understanding the elements and options for these systems is the basis for engineering systems that meet the needs of current and future laboratory work.

The control/response link is needed to synchronise sample introduction and DAQ. Depending on the nature of the work, that link can extend to sample preparation. The end result is a system that not only provides higher productivity than manual methods, but does so with reduced operating costs.

The introduction of a feedback facility would significantly improve productivity. At the end of the analysis, any results that are outside the expected limits would have been checked and the system's integrity verified. Making this happen depends on connectivity and the ability to integrate the components.

The problem of integration, streamlining operations and improving productivity has been addressed through automation in the manufacturing sector and in clinical laboratories. In the clinical laboratories, the programme came under the title 'Total laboratory automation' and resulted in a series of standards that allowed instrument data systems to connect with Laboratory Information Systems (similar to LIMS) and hospital administrative systems.

For clinical work, the standards have been integrated under HL7 (www.hl7.org), which provides both message and data formatting. While hospital and clinical systems have the advantages of a limited range of testing and sample types, making standardisation easier, there is nothing in their structure that prevents them from being applied to a wider range of instruments, such as mass spectrometry.

The connection between the instrument and computer system may be as simple as an RS-232 connection or USB. Direct Ethernet connections or connections through serial-to-Ethernet converters can offer more flexibility by permitting access to the device from different software systems and users. The inclusion of smart technologies in instrumentation significantly improves both their utility and the lab's workflow.

29

ELECTRONIC DEVICES AND CIRCUITS

Analytical instruments are used to obtain qualitative and quantitative information about the composition of a sample of matter. For doing so, the chemical or physical quantity to be measured undergoes a series of transformations from the input to the output stage. Modern analytical instruments depend to a large extent, on the use of various electronic devices, components and circuits to carry out these transformations. In order to understand the principle of working of various analytical instruments, a good level of understanding is required about the various electronic devices and circuits which form the basic building constituents of these instruments.

29.1 ELECTRONIC COMPONENTS

An electronic component is any device that handles electricity. They come in many different shapes and sizes and perform different electrical functions depending upon the purpose for which they are used. Accordingly, electronic equipment make use of a variety of components.

29.1.1 Active vs Passive Components

There are broadly two types (Figure 29.1) of components: Passive components and Active components.

Passive Components: A passive device is one that contributes no power gain (amplification) to a circuit or system. It has no control action and does not require any input other than a signal to perform its function. They always have a gain less than one, thus, they cannot oscillate or

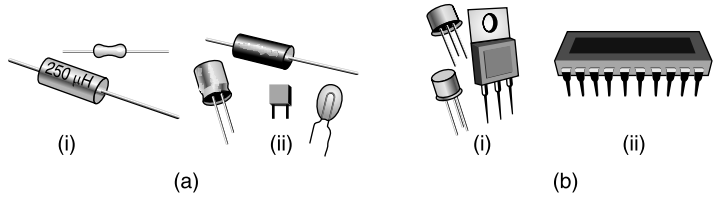


Figure 29.1 *Types of components (a) passive components (i) resistors (ii) capacitors (b) active components (i) transistors (ii) integrated circuits*

amplify a signal. A combination of passive components can multiply a signal by values less than one; they can shift the phase of a signal, they can reject a signal because it is not made up of the correct frequencies, they can control complex circuits, but they cannot multiply by more than one because they basically lack gain. Passive devices include resistors, capacitors and inductors.

Active Components: Active components are devices that are capable of controlling voltages or currents and can create a switching action in the circuit. They can amplify or interpret a signal. They include diodes, transistors and integrated circuits. They are usually semiconductor devices.

29.1.2 Discrete vs Integrated Circuits

When a component is packaged with one or two functional elements, it is known as a discrete component. For example, a resistor used to limit the current passing through it functions as a discrete component. On the other hand, an integrated circuit is a combination of several interconnected discrete components packaged in a single case to perform multiple functions. A typical example of an integrated circuit is that of a microprocessor which can be used for a variety of applications.

29.2 PASSIVE COMPONENTS

Resistance: Resistance is the opposition to the flow of current offered by a conductor, device or circuit. It is related to current as follows:

Resistance = voltage/current (Ohm's Law)

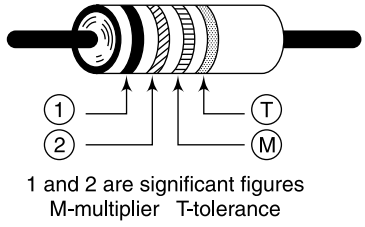


Figure 29.2 *Colour code of resistors*

The resistance is expressed in ohms (abbreviated Ω). The value of resistance of a metal is temperature dependent and it increases with the rise in temperature. Most commonly encountered resistors in electronic circuitry are carbon resistors. Their values are either printed in numbers or put in the form of colour coded bands around the body. Each number from 0 to 9 has been assigned a colour according to the following table (Figure 29.2):

Black	0	Blue	6
Brown	1	Violet	7
Red	2	Grey	8
Orange	3	White	9
Yellow	4	Gold	±5% tolerance
Green	5	Sliver	±10% tolerance

The first band closest to the end of the resistor represents the first digit of the resistance value, the second band gives the second digit and the third band gives the number of zeros to be added to the first two digits to get the total value of the resistor. If there is a fourth band, it indicates either ±5% or a ±10% tolerance. If it is absent, the tolerance is ±20%.

High stability resistors are made by depositing a layer of cracked carbon on a ceramic rod and completely insulating the resistive element, so that it can withstand arduous operating conditions. Still superior in stability and for low-noise applications are metal-oxide film resistors.

Some resistors are made to give an adjustable or variable resistance. They are three-terminal devices (Figure 29.3), in which the central terminal is connected to the moveable contact or wiper. A variable resistance is obtained between the wiper arm and any one end of the resistor track. Such a device is called a rheostat.

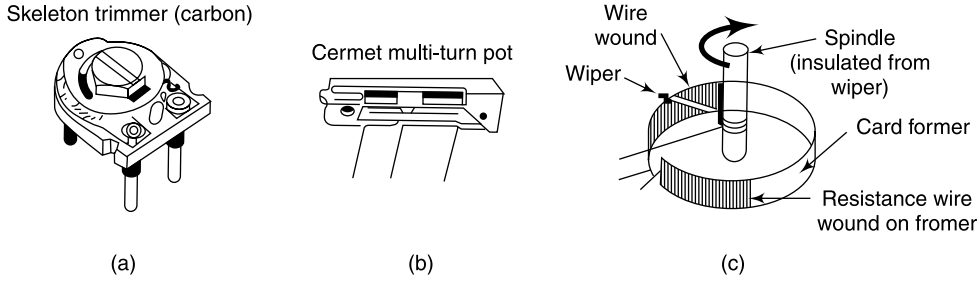


Figure 29.3 *Types of variable resistors (a) carbon composition (b) multi-turn cermet (c) wire wound*

If several resistances (R_1, R_2, R_3, R_4) are connected in series, then total value (R_T) is given as follows:

$$R_T = R_1 + R_2 + R_3 + R_4$$

When these resistances are connected in parallel, the value R_T is given below:

$$\frac{1}{R_T} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} + \frac{1}{R_4}$$

Capacitor: A capacitor (also called condenser) consists of two conductors separated by a dielectric or an insulator. The dielectric can be paper, mica, ceramic or plastic film or foil. High value capacitors are usually of electrolyte type. They are made of a metal foil with a surface that has an anodic formation of metal-oxide film. The anodised foil is in an electrolytic solution. The oxide film is the dielectric between the metal and the solution. High value of capacity of electrolytic capacitors in a small space is due to the presence of a very thin dielectric layer. An electrical charge can be placed on the plates by connecting a voltage source to the capacitor. Figure 29.4 shows various types of capacitors used in electronic circuits

Capacitance is measured in farads. A capacitor has a capacitance of one farad when one coulomb charges it to one volt. The farad is too large a unit. Usual sub-units used are microfarad (μF) and the picofarad (10^{-12} F).

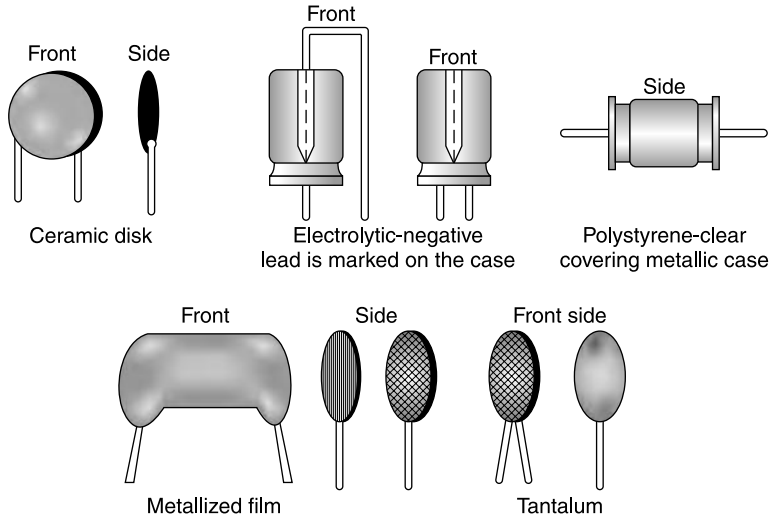


Figure 29.4 *Various types of capacitors*

Trimmer capacitor is a variable capacitor. Spacing between the metal plates which are separated by a dielectric can be adjusted to give a variable capacitance.

Inductance: Inductance is the characteristic of a device which resists a change in the current through the device. Inductors work on the principle that when a current flows in a foil of wire, magnetic field is produced, which collapses when the current is stopped. The collapsing magnetic field

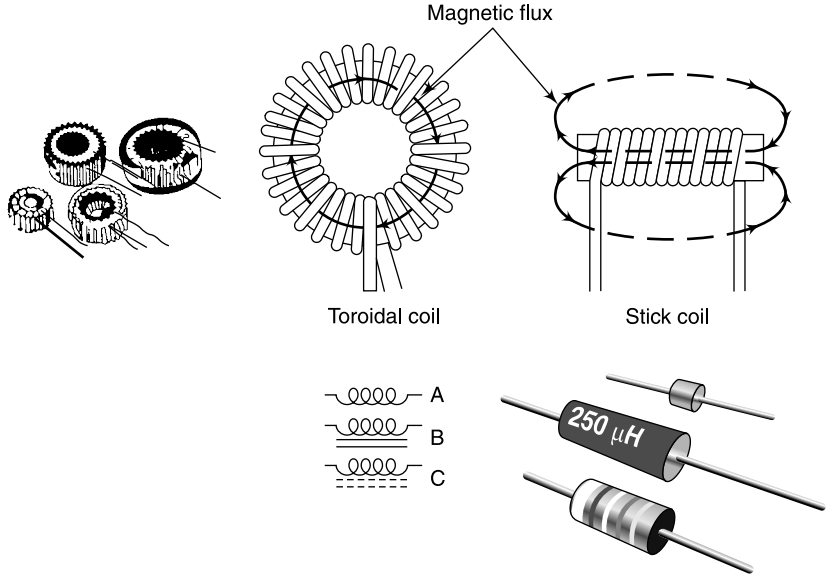


Figure 29.5 *Shapes of commonly used inductors*

produces an electromotive force, which tries to maintain the current. When the coil current is switched, the induced EMF would be produced in such a direction, as to oppose the build-up of the current.

$$e = -L\, di/dt$$

The unit of inductance is henry. An inductance of one henry will induce a counter EMF of one volt, when the current through it is changing at the rate of one ampere per second. Inductances of several henries are used in power supplies as smoothing chokes, whereas smaller values (in the milli or microhenry ranges) are used in audio and radiofrequency circuits. Figure 29.5 shows the shapes of commonly used inductors.

29.3 SEMICONDUCTOR DEVICES

Unlike other electron devices, which depend for their functioning on the flow of electrons through a vacuum or a gas, semiconductor devices make use of the flow of current in a solid. These solids, called the semiconductors, have poorer conductivity than a conductor, but better conductivity than an insulator. These elements belong to the fourth group of the periodic table. The materials most often used in semiconductor devices are germanium and silicon. The atoms of these elements have a crystal structure and the outer or valence electrons of individual atoms are tightly bound to the electrons of adjacent atoms in electron pair bonds or the covalent bonds. In order to obtain free electrons from such structures, very small amounts of other elements having different atomic structure can be added as impurities. These impurities can thus modify and control the basic electrical characteristics of the semiconductor materials.

When the impurity atom has one more valence electron than the semiconductor atom, this extra electron cannot be bound in the electron pair and is held loosely by the atom. This free electron requires very little excitation to break away from the atom and the material becomes a better conductor. The presence of this extra free electron makes the material to have a negative charge and the resulting material is called *N*-type.

A different effect is produced when the impurity added is the atom having one less valence electron than the semiconductor atom. This means that one of the bonds in the resulting crystal structure cannot be completed, because the impurity atom lacks one valence electron. A vacancy or hole thus exists in the lattice structure, which encourages the flow of electrons in the semiconductor material, consequently increasing its conductivity. The vacancy or hole in the crystal structure is considered to have a positive electrical charge, because it represents the absence of an electron. Semiconductor material which contains these holes or positive charges is called *P*-type material.

Semiconductor devices are considerably small in size and are of light weight. They have no filaments or heaters for electron emission and therefore require no heating power or warm-up time. They are solid in construction, extremely rugged and are free from micro-phonics and therefore can withstand severe environmental conditions. Because of these advantages, semiconductor devices have almost displaced vacuum tubes from many applications, in which it was formerly pre-eminent. The emphasis in the following text will therefore be on circuits making use of semiconductor devices in discrete and integrated forms.

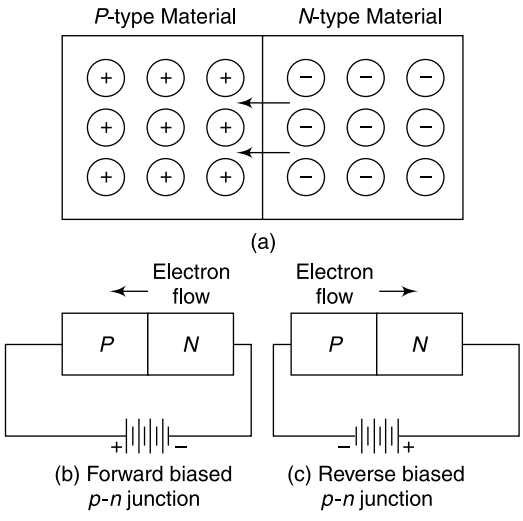


Figure 29.6 *(a) P-N junction. (b) Forward biased P-N junction. (c) Reverse biased P-N junction*

29.3.1 *P-N* Junction

When *N*-type and *P*-type materials are joined together (Figure 29.6a), a very important phenomenon takes place at the junction of the two materials. The free electrons from the *N*-type material diffuse across the junction and recombine with holes in the *P*-type material. This is called diffusion current.

Let us consider the condition when an external battery is connected across a *P-N* junction (Figure 29.6b) with its positive terminal connected to the *P*-type material and the negative terminal to the *N*-type material. Under these circumstances, the junction is said to be forward-biased, as the polarity of the external battery facilitates the movement of the charge carriers across the junction and the junction offers a very low resistance to the current flow. The *P-N* junction under this condition is called forward-biased.

If the polarity of the externally applied battery is reversed (Figure 29.6c), the charge carriers, under the influence of the external battery, move towards the adjacent sides of the junction and form a barrier layer, with the result that the electron flow in the external circuit is very low. The *P-N* junction under this condition is said to be reverse-biased.

29.3.2 Semiconductor Diode

The simplest type of semiconductor device is the diode, which is basically a *P-N* junction (Figure 29.7). The *N*-type material which serves as the negative electrode is called cathode and the *P*-type material which serves as the positive electrode is referred to as the anode. The arrow symbol shows the direction of the flow of conventional current, which is opposite to the flow of electrons.

The semiconductor diode conducts current more easily in one direction than in the other, so that it is an effective rectifying device. The semiconductor diodes are available in a wide range of current capabilities, suitable practically for almost all applications.

Conventional diodes normally show a low value of forward resistance and a very high value of reverse resistance. The variation in resistance is due to the non-linear voltage/current characteristics of the diode. Figure 29.8 shows voltage current diagram for a typical semiconductor diode.

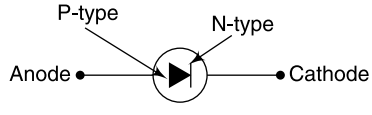


Figure 29.7 *Semiconductor diode*

Signal Diodes: They are general purpose diodes, which find applications involving low currents and a wide range of voltages, sometimes extending up to 50 kV.

Switching Diodes: They change their state from conducting to non-conducting state and vice-versa in a very short time when the voltage is reversed.

Rectifiers: They are similar to signal diodes, but are more suitable for high currents.

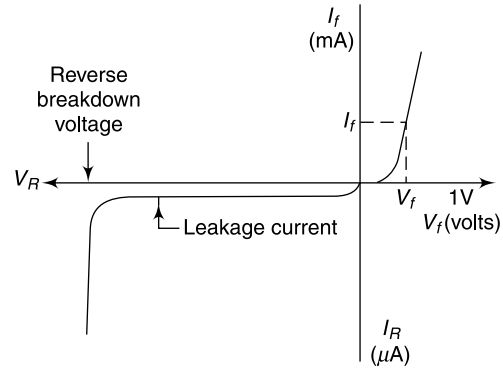


Figure 29.8 *Voltage current characteristics of semiconductor diodes*

Low- and medium-power diodes are usually available in axial packages where as high power diodes are available in a large variety of packages of a vast range of shapes and sizes. Very high power diodes have a thread for mounting on to a PCB or a heat sink. Figure 29.9 shows various shapes of diodes, which are commercially available. Diode arrays or networks, containing up to 48 devices are also available in packages similar to integrated circuits.

A single diode when used for rectification gives half wave rectification. When 4 diodes are combined, full wave rectification can be obtained. Devices containing 4 diodes in one package are called diode bridges. Diode bridges with large current capacities require a heat sink. Typically, they are screwed to a piece of metal or the chassis of equipment in which they are used. The heat sink allows the device to radiate excessive heat.

Zener Diode: A silicon diode has a very low reverse current, say 1 μA at an ambient temperature of 25°C. However, at some specific value of reverse voltage, a very rapid increase occurs in reverse current. This potential is called breakdown avalanche or the Zener voltage and may be as low as 1 V or as high as several hundred volts, depending upon their construction.

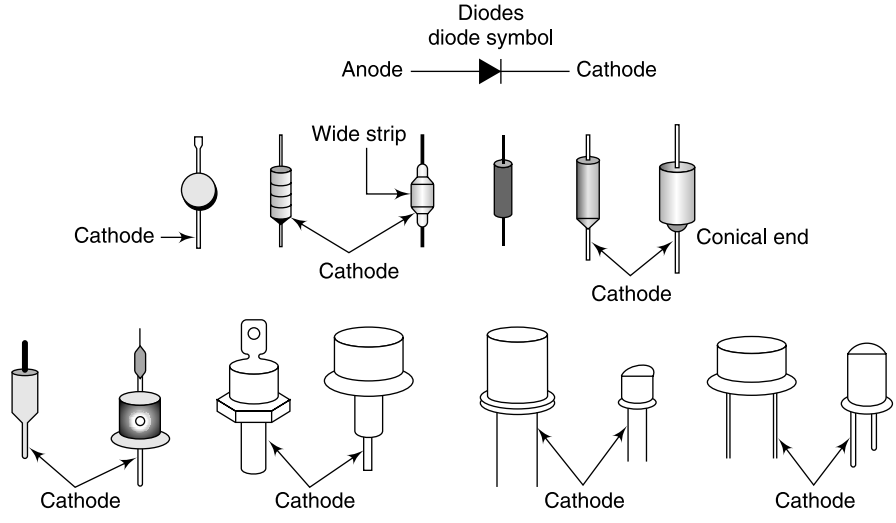


Figure 29.9 *Various shapes of diodes*

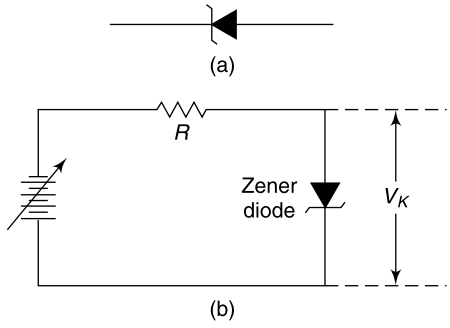


Figure 29.10 *Zener diode (a) symbol (b) use as a constant voltage source*

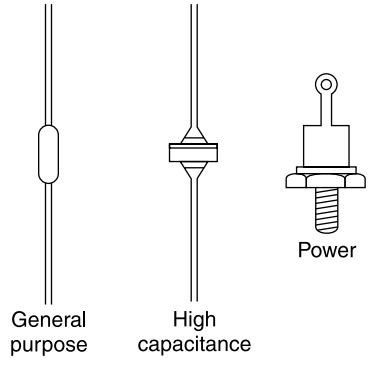


Figure 29.11 *Varactor diode-different types*

A Zener diode has very high resistance at bias potentials below the Zener voltage. This resistance could be several megohms. At Zener voltage, the Zener diode suddenly shows a very low resistance, say between 5 and 100 Ω.

A Zener diode behaves as a constant voltage source in the Zener region of operation, as its internal resistance is very low. The current through the Zener diode (Figure 29.10) is then limited only by the series resistance *R*. The value of series resistance is such that the maximum rated power rating of the Zener diode is not exceeded.

To aid in distinguishing the Zener diode from a general purpose diode, the former is usually labelled with its specified breakdown voltage. Since this voltage is required in the circuit design, the value is generally indicated on the diode. For example, some common values are 6.8, 7.2, 9.6 V, etc.

Varactor Diode: A varactor diode is a silicon diode that works as a variable capacitor in response to a range of reverse voltage values. Varactors are available with nominal capacitance values ranging from 1 to 500 pF, and with maximum rated operating voltages extending from 10 to 100 V. They mostly find applications in automatic frequency control circuits. In a typical case, a varactor shows 10 pF capacitance at reverse voltage of 5 V and 5 pF at 30 V. Figure 29.11 shows different shapes of varactor diodes.

Varistor: A varistor is a semiconductor device that has a voltage dependent non-linear resistance which drops as the applied voltage is increased. A forward-biased germanium diode shows such type of characteristics and is often used in varistor applications, such as in bias-stabilisation circuits.

Light Emitting Diodes (LED): A light emitting diode is basically a *P-N* junction that emits light when forward-biased. LEDs are available in various types (Figure 29.12) and colours like red, yellow and green. They are used mostly in displays employing seven segments that are individually energised to form alphanumeric characters.

LED displays are encountered in test equipment, calculators and digital thermometers whereas LED arrays are used for specific applications such as light sources, scanners, position readers, etc.

Electrically, LEDs behave like ordinary diodes except that their forward voltage drop is higher. For example, the typical values are; IR (infrared): 1.2 V, Red: 1.85 V, Yellow: 2 V,

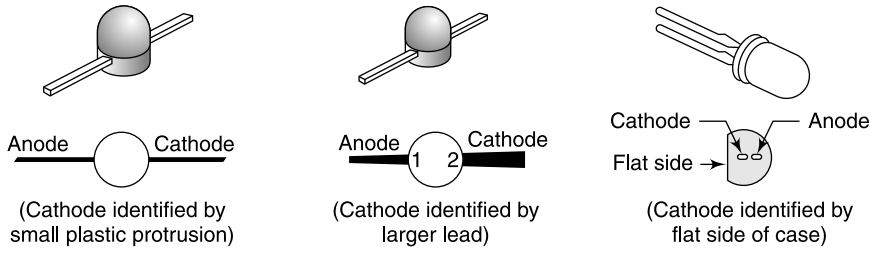


Figure 29.12 *Light emitting diodes*

Green: 2.15 V. Further, the actual voltages may vary depending upon the actual technology used in the LED.

Photodiode: A photodiode is a solid-state device, similar to a conventional diode, except that when light falls on it (*P-N* junction), it causes the device to conduct. It is practically an open circuit in darkness, but conducts a substantial amount of current when exposed to light.

29.4 TRANSISTORS

29.4.1 Bipolar Transistors

The most commonly used semiconductor device is the transistor having characteristic to control voltage and current gain in an electronic circuit. These properties enable the transistor to provide amplification, rectification, switching, detection and almost any desired function in the circuit. It is the basic device of all solid-state electronics, both as a single component or as an element of integrated circuit.

A transistor is a three-terminal device. The terminals are called base (*B*), collector (*C*) and emitter (*E*). Basically, it is made up of two diodes: a base-emitter diode and a base-collector diode. In normal amplifier operation, the base-emitter diode is forward-biased and the base-collector diode is reverse-biased.

Depending upon the direction of current flow, different symbols (Figure 29.13) are used for *N-P-N* and *P-N-P* transistors. The first two letters of the *N-P-N* and *P-N-P* designations indicate the respective polarities of the voltages applied to the emitter and the collector in normal operation. In an *N-P-N* transistor, the emitter is made negative with respect to both the collector and the base, and the collector is made positive with respect to both the emitter and the base. In a *P-N-P* transistor, the emitter is made positive with respect to both the collector and the base, and the collector is made negative with respect to both emitter and the base.

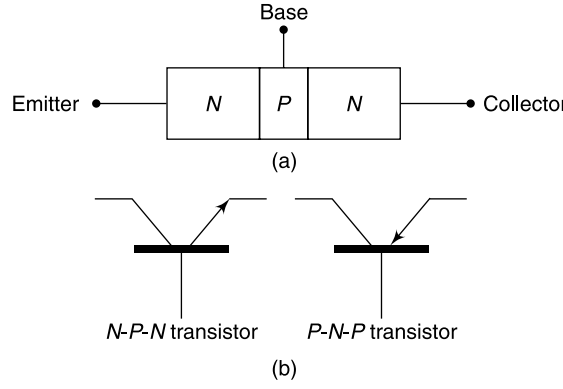


Figure 29.13 *(a) Functional diagram of a transistor and (b) Symbols used for transistors.*

More than 500 packages are listed in the component manufacturers' catalogues. However, only about 100 types are in common use. Metallic packages (TO-3, TO-5 and TO-18) have been in use for a long time. However, they have been mostly replaced in low- and medium-power applications by cheap plastic packages due to their low cost. For high power applications, however, metallic packages, both stud or bolt type are still common, although flat type packages are being replaced by plastic versions, with a metallic tab to improve heat dissipation. Figure 29.14 shows commonly used transistor packages and their terminals.

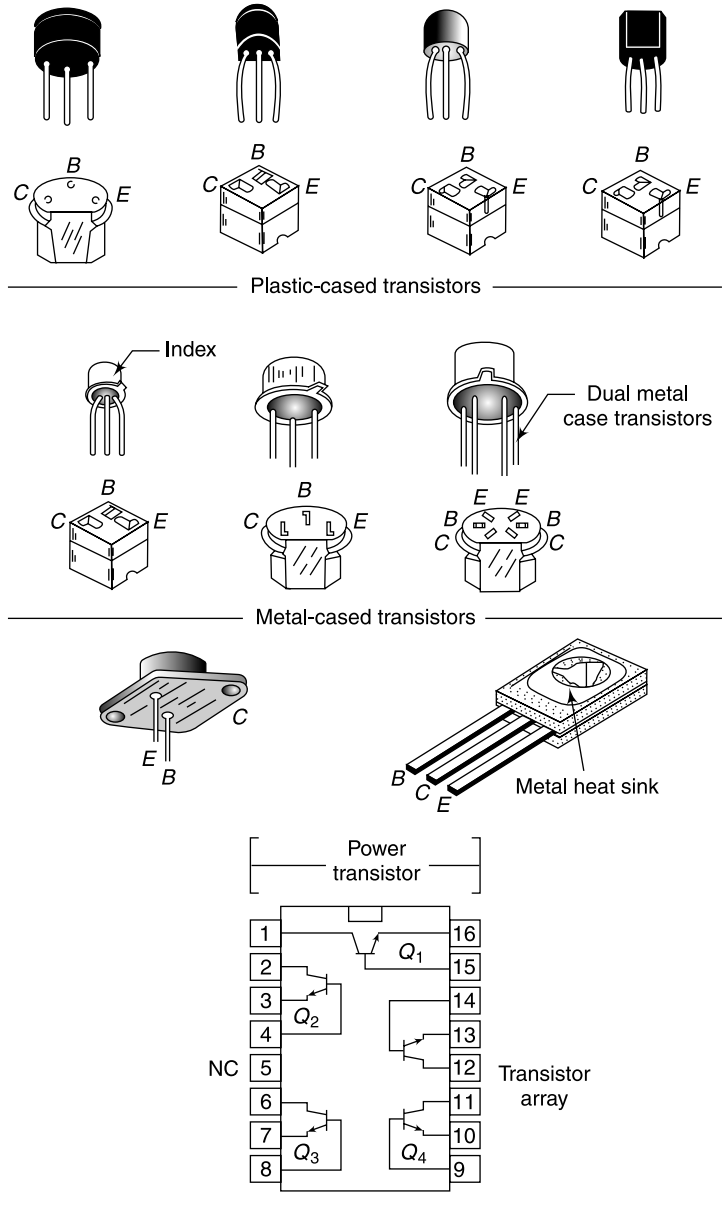


Figure 29.14 *Transistor symbols and terminals*

Figure 29.15 indicates the bias polarity required to forward-bias the base-emitter junction. The arrow head distinguishes between the emitter and the collector and shows the direction of a 'conventional' current flow. Electron flow is opposite from the direction of the arrow points. The figure also compares the biasing required to cause conduction and cut-off in *N-P-N* and *P-N-P* transistors. If the transistor's base-emitter junction is forward-biased, the transistor conducts. However, if the base-emitter junction is reverse-biased, the transistor is cut-off.

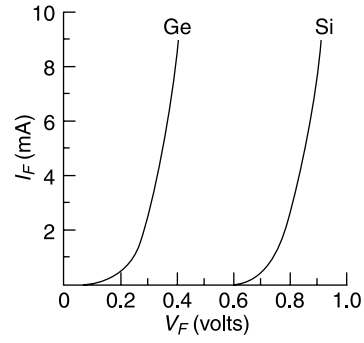


Figure 29.15 *Forward voltage of base-emitter junction in* Ge *and* Si *transistors*

The voltage drop across a forward-biased emitter-base junction varies with transistor's collector current. Figure 29.16 shows the relationship between voltage and current for base-emitter junction in silicon transistors.

A common problem in transistors is the leakage, which can shunt signals or change bias voltages thereby upsetting circuit operation. This problem is particularly serious in direct-coupled or high-frequency stages. Leakage current is the reverse current that flows in a junction of a transistor when specified voltage is applied across it, the third terminal being left open. For example, I_{CEO} is the DC collector current that flows when a specified voltage is applied from collector to emitter, the base being left open (unconnected). The polarity of the applied voltage is such that the collector-base junction is reverse-based. Obviously, in a transistor, six leakage paths are present (with the third electrode open), as shown in Figure 29.17 Leakage current increases with temperature and doubles about every 10°C.

Figure 29.18 shows typical bipolar transistor junction resistance readings. The polarity of the ohmmeter to be applied on the various transistor leads is also indicated on the figure.

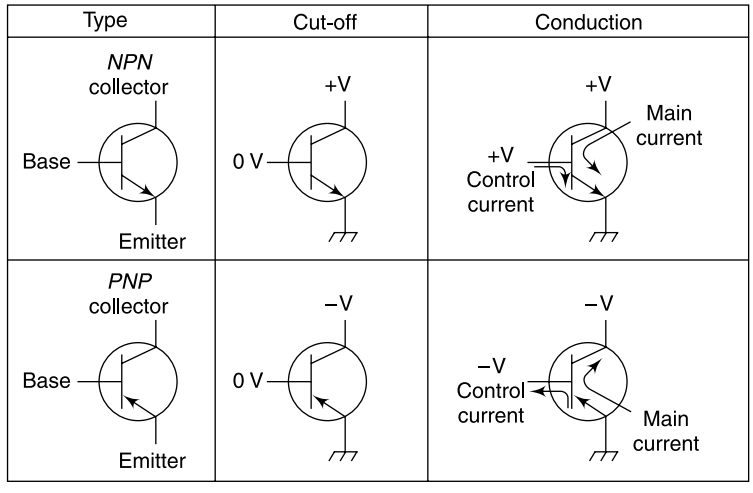


Figure 29.16 *Three basic biasing arrangements in transistors*

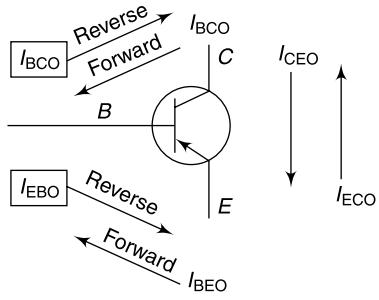


Figure 29.17 *Leakage paths in a transistor*

Power Transistors: The junctions of the power transistors have comparatively larger areas than small signal transistors and have the following characteristics:

- Forward-resistance values are generally lower than those for small signal silicon transistors.
- Similarly, they have lower reverse-resistance values. The test results with an ohmmeter on a silicon power transistor are shown in Figure 29.19.

Power transistors are usually mounted on the heat sinks or heat radiators (Figure 29.20). They are sometimes mounted on the chassis using silicone grease to increase heat transfer.

Darlington Transistors: A Darlington is a special type of configuration usually consisting of two transistors fabricated on the same chip or at least mounted in the same package. Darlington pairs

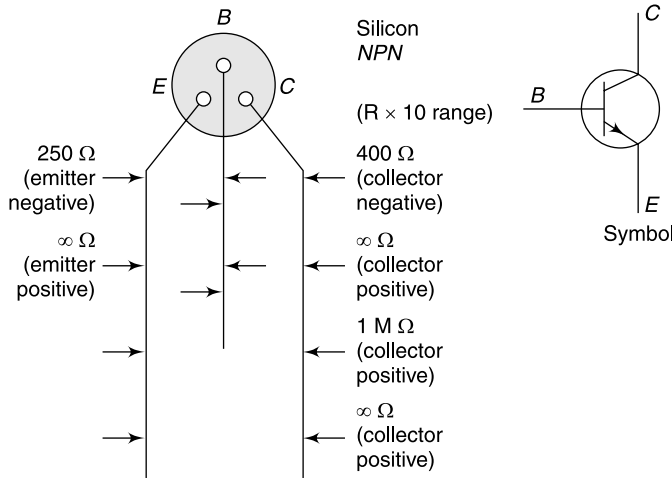


Figure 29.18 *Bipolar transistor junction resistance values*

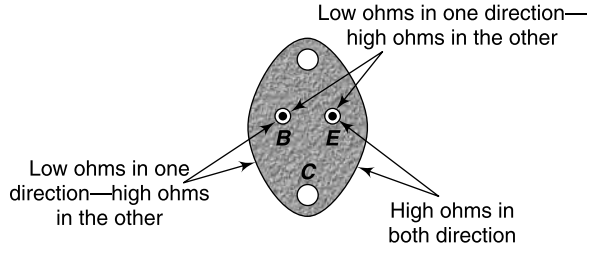


Figure 29.19 *Power transistor: junction resistance values*

are often used as amplifiers in input circuits to provide a high-input impedance. Darlingtons are used where drive is limited and high gain typically over 1,000 is needed. In this configuration, (Figure 29.21) the emitter-base junctions are connected in series and the collector terminals are connected in parallel. A Darlington configuration behaves like a single transistor where the current gains (h_{fe}) of the individual transistors it is composed of are multiplied together and the base-emitter voltage drop of the individual transistors are added together.

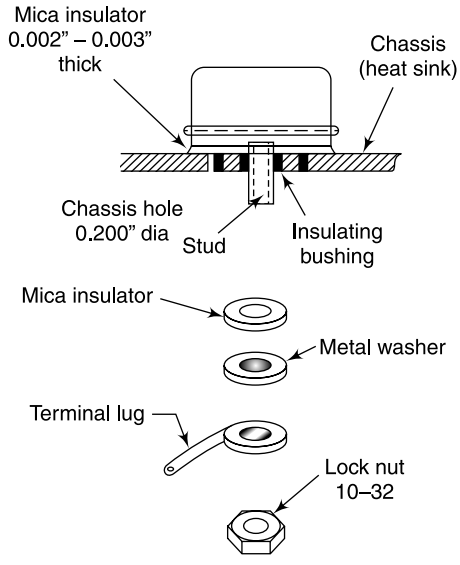


Figure 29.20 *Power transistor: mounting arrangement*

29.4.2 Field-Effect Transistor

The field-effect transistor (FET) is basically a three-terminal semiconductor device. It is a voltage-operated device and is biased by a voltage. No input current normally flows and hence its input resistance is virtually infinite.

Field-effect transistors are of two types: the junction FET (JFET) and the metal-oxide semiconductor FET (MOSFET). The MOSFET is also called insulated-gate FET (IGFET).

In the JFET, a thin conducting channel (Figure 29.22) of finite conductance is established between two *P*-*N* junctions. The current from the source to the drain, for a given voltage, depends on the dimensions of the channel. If the *P*-*N* junctions are reverse-biased by applying a voltage to the gate, a depletion region containing no mobile carriers is formed and the width of the conducting channel is reduced. Thus, the magnitude of current between source and the drain can be controlled by the reverse bias applied to the gate electrode. This provides a means of controlling the amplification in the FET.

Depending upon the type of material of the channel, the FET may be *N*-channel or *P*-channel.

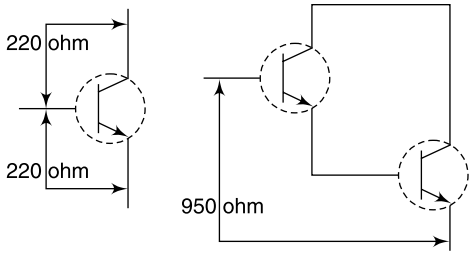


Figure 29.21 *Darlington pair: forward resistance values*

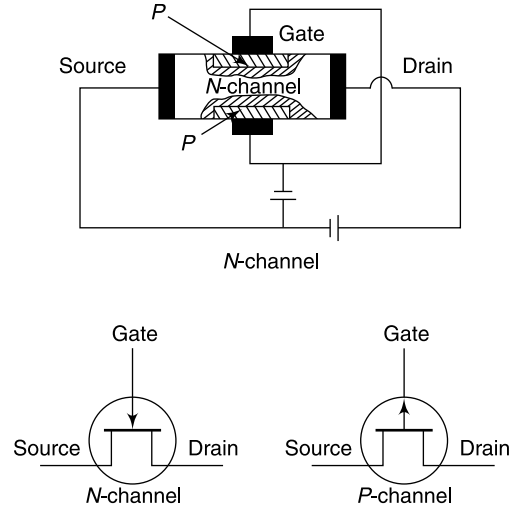


Figure 29.22 *Field-effect transistor*

29.4.3 MOSFET

In the MOSFET, a thin layer of silicon oxide insulates the gate contact from the channel. There are two types of MOSFET: depletion (normally on at zero bias) and enhancement (normally off at zero bias). Figure 29.23 shows depletion type *N* and *P* channels and enhancement type *N* and *P* channels. Also there is a fourth lead. This is the substrate or body of the MOSFET and is usually connected to the source or ground. The substrate is not always shown in an illustration of a MOSFET and for all intents and purposes, can be disregarded in considering the FET operation. We can identify depletion MOSFET by the solid channel and enhancement FET by the broken channel.

The *P*-channel enhancement type transistor is mostly used. The MOSFET exhibits an extremely high-input resistance, which may be in the range of 10^{12}–10^{15} Ω. Unlike the JFET, the MOS maintains a high-input resistance without regard to the magnitude or polarity of the input gate voltage.

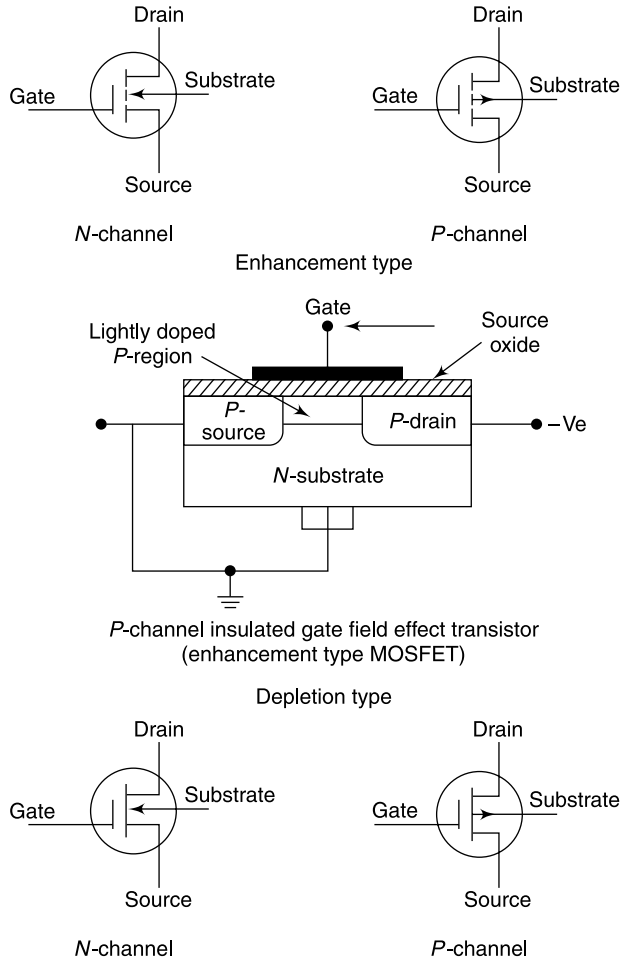


Figure 29.23 *MOSFET (metal-oxide insulated gate field-effect transistor)*

The thickness of gate insulating material in a MOSFET is typically below 2×10^{-5} cm. Consequently, gate to substrate voltage of the order of 50 V or so will cause breakdown of the insulation and the MOSFET is ruined. Any static charge on a person's finger or some tool can destroy a MOSFET. To prevent destruction, most MOSFETS are supplied by the manufacturers with their leads twisted, so that the gate is shorted to the substrate. Shorting leads should be clipped to the gate when soldering or installing MOSFET.

As in the bipolar transistor, the MOSFET is capable of both switching and amplification. The MOSFET gain is measured in terms of a voltage ratio while the bipolar gain is represented as a current ratio. While the bipolar transistors have a much higher speed than the MOS transistors, the MOS transistors offer a packing density roughly four times, as compared to bipolar transistors due to the difference in their fabrication techniques.

29.5 INTEGRATED CIRCUITS

Integrated circuits eliminate the use of individual electronic parts, such as resistors, capacitors, transistors, etc., as the building blocks of electronic circuits. In their place, we have tiny chips of semiconductor material, whose functions are not those of single components but of scores of transistors, resistors and capacitors and other electronic elements, which are all interconnected to perform the function of a complete and complex circuit. So, instead of usual semiconductor devices, resistances and capacitances, complete multi-stage amplifiers, complex flip-flops and dozens of other functional circuits have become the basic components of electronic equipment.

Integrated circuits are placed in a protective housing and connections to the outside world are provided through pins. The common packages are the TO-5 metal can, the ceramic flat pack and the dual-in-line ceramic or plastic flat packs known as dual-in-line packages (DIPs). The popular packages have 14, 16, 18, 24 or 40 connecting pins. Figure 29.24 shows standard IC packages.

Circuits requiring close matching and tracking of components can be more advantageously adapted for integrated circuits. Also, it is less expensive to fabricate integrated transistors than passive elements, such as resistors and capacitors. Large values of resistances and capacitors are to

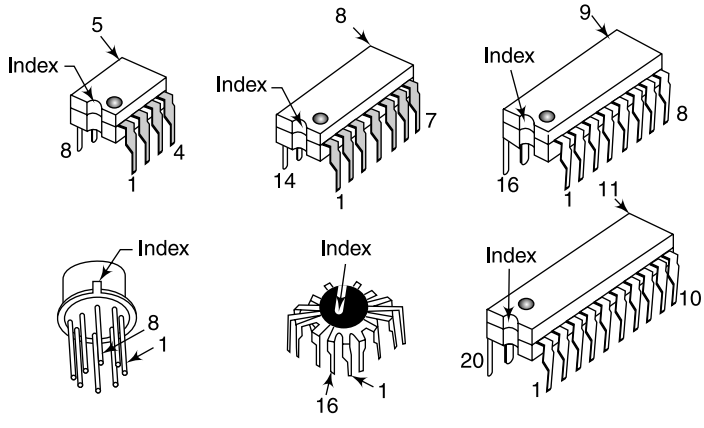


Figure 29.24 *Commonly available IC packages*

be avoided, as they are not economical. Also, the circuit design has to be such that it should be non-critical as to component tolerances. For example, 20% tolerances of resistances and capacitances shall have much lower cost than 10% tolerance values. Another severe limitation of integrated circuit technology for linear applications is the non-availability of inductances as integrative circuit elements. So, either the inductances are usually eliminated while designing a particular integrated circuit or hybrid circuit would have to be used. In digital electronics, standard circuits are available for virtually every application.

Linear integrated circuits are characterised by an output that is proportional to its inputs. Such circuits are designed as DC amplifiers, audio amplifiers, rf amplifiers, if amplifiers, power amplifiers, differential amplifiers, etc.

An important class of linear integrated circuits is operational amplifiers (op-amps). These amplifiers were originally utilised in analog computers to perform various mathematical operations such as addition, subtraction, integration and differentiation. Op-amps are now used to perform precise circuit functions, some of which will be discussed in this chapter.

29.6 OPERATIONAL AMPLIFIERS (OP-AMPS)

An op-amp is a high gain DC amplifier originally intended to be used for doing mathematical operations. But the versatility of this amplifier owning to the provision for external selectable feedback has made it possible for adaption to many applications in the field of analytical instruments. It is used for the construction of AC and DC amplifiers, active filters, phase inverters, multi-vibrators, comparators, etc. The op-amps are available in the integrated form and thus simplify the design of equipment by offering a high-quality amplifier in one package and result in considerable size reduction. They are popularly known as op-amps.

Block diagram of a typical op-amp is shown in Figure 29.25. The input stage is a conventional differential amplifier with a constant current source placed in the emitters of the two transistors. It is desirable to have a high gain in this section, so that any imbalances or imperfections in the succeeding stages have little or no effect on the output signal.

The signal output from the first stage is fed differentially to the second-stage differential amplifier. Because common-mode rejection capability is not as stringent in this stage, this stage does not require a constant current source in the emitters. Normally, the second stage is needed only to provide some additional gain. Its input resistance should be high enough to prevent excessive loading of the first stage. Therefore, an emitter follower circuit is often employed.

The output of the second stage is normally taken to be single-ended. Here, the amplified signal is associated with a certain amount of DC voltage at its output. Some means of level translation

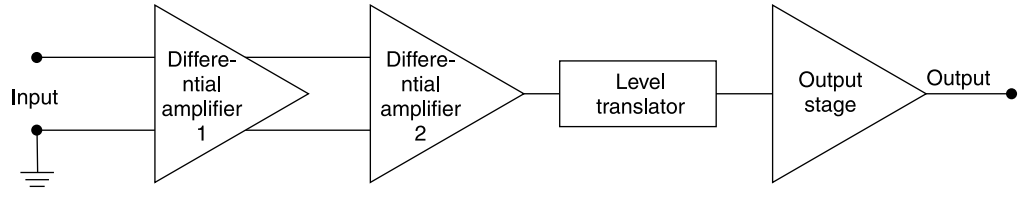


Figure 29.25 *Block diagram of an operational amplifier*

is thus necessary between the second and the final stages. By eliminating the DC level at the final stage, the output voltage will vary about a zero reference level, thus preventing any undesired DC current in the load and also enhancing the permissible output voltage swing.

29.6.1 Symbolic Representation

The op-amp is symbolically represented as a triangle (Figure.29.26) on its side. In digital circuit symbols, the inverter is represented as a triangle, but the op-amp symbol is much larger. The triangle indicates the direction of signal flow. It is associated with three horizontal lines, two of which (*A* and *B*) indicate signal input and the third (*C*), the output signal connections.

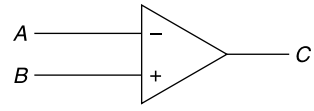


Figure 29.26 *Symbolic representation of an op-amp*

The signal input terminals are described by minus (–) and plus (+) signs inside the triangle. The (–) input is called the Inverting input, because the output voltage is 180° out of phase with the voltage to this input. On the other hand, the (+) input is called the non-inverting input, because the output voltage is in phase with a voltage applied to this terminal. The names inverting and non-inverting terminals have been given to indicate the phase of output signal, in comparison to the voltage applied at the inputs. Figure 29.27 shows the operation of the op-amp as inverting and non-inverting amplifier.

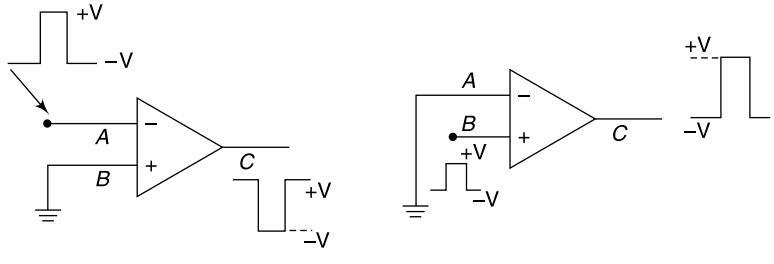


Figure 29.27 *(a) Inverting operation of an op-amp (b) Non-inverting operation of an op-amp*

29.6.2 Power Supply Requirements for Op-Amps

Op-amps need to be powered by DC power supply, like any other transistor amplifier. The power supply should be of proper voltage regulation and filtering for correct operation of the op-amp.

The power supply leads on the op-amp are usually marked +V and –V, to which positive and negative supply voltages should respectively be connected, with reference to ground. The positive and negative supply voltages are usually symmetrical (i.e. the two voltages are equal but opposite in sign). The most commonly used voltage to power op-amps are +15 V and –15 V. However, this is not always the case. Therefore, it is advisable to consult manufacturer's data manuals on the op-amp of interest to determine the power-supply requirements.

It may be noted that mostly on the circuit schematics, the power-supply leads are not shown on the op-amps. It is assumed that the reader is aware that DC voltage is necessary for operation of the op-amp.

29.6.3 Output Voltage Swing

Just as standard transistor amplifiers are limited in their output voltage swing, so also are the op-amps. The limitations on the output voltage swing are generally dependent upon the magnitude of the positive and negative power-supply voltages of the op-amp. Usually, the output of an op-amp can go to a voltage value no more positive than the positive power-supply and no more negative than the negative power-supply can provide. When the output of an op-amp goes to +V and –V, it is said to be in positive or negative voltage saturation.

29.6.4 Output Current

Op-amps are designed to provide a limited current in the output, which is usually a few milliamperes, less than 10 mA in most standard op-amps. If more current is drawn, the output signal begins to change, because of the current limiting provisions built into the op-amp output circuit, which limit their own output current to a safe operating region.

Some op-amps are designed to deliver larger currents, of the order of amperes, at the output pin, but they can only be characterised as special devices and not standard op-amps.

29.6.5 Characteristics of Op-Amps

An ideal op-amp would have the following characteristics:

1. Infinite open-loop voltage gain *(A)*
2. Infinite bandwidth
3. Infinite input impedance
4. Zero output impedance
5. Zero offset (voltage at output when input is zero)
6. Maximum output voltage equals +V and minimum – V

By carefully examining these characteristics, the following implications are obvious:

- The voltage across the input terminals of an amplifier with infinite gain must be zero or negligibly small (input voltage = output voltage/gain of amplifier)
- No (zero) current can flow between the input terminals of the amplifier, because of the infinite input impedance (input current = input voltage/input impedance). If one input terminal is at ground reference potential, then the other must be at ground potential. The terminal is thus called a virtual ground and the input is called virtual short circuit (in virtual short circuit, no potential can exist across it, and no current can flow through it)
- With the infinite voltage gain, we can expect to get a very very large voltage output from a very small voltage input. In fact, with a small voltage across the input terminals, the amplifier output is driven into positive or negative saturation very easily. An op-amp is said to be operating properly when the output is in the linear region of operation, unless the op-amp circuit is designed to operate to perform non-linear function.

29.6.6 Performance Characteristics of Op-Amps

The performance characteristics of an op-amp are defined as:

Gain: The voltage gain of an amplifier consisting of n similar stages is equal to the gain of a single stage raised to the power n.

Linearity: It defines the relationship between input and output. It is generally desirable that a plot of output signal against input signal level shows a linear relationship that passes through zero on both axes.

Offset: If the plot between input and output does not pass through the origin, the amplifier is said to exhibit offset.

Noise: Noise is a spurious signal that is superimposed on the amplified signal. The noise signal generally has components at all frequencies within the bandpass of the amplifier.

Drift: Very low frequency noise is termed as drift.

Reliability: The ability of an amplifier to provide a reproducible output for a given input over an extended period of time is called reliability.

Stability: It refers to the ability of the amplifier to avoid uncontrolled oscillation.

It is obvious that in order to provide accurate performance, an amplifier must be linear, reliable and free from offset. However, in practice, the components used in amplifiers are sensitive to environmental changes, especially temperature, and are affected by ageing. To achieve desirable standards of accuracy, the design of the instrument is made in such a way that is not critically dependent as a whole, on individual amplifier behaviour.

29.6.7 Typical Op-Amp Circuits

Typically, an op-amp has two input terminals and one output terminal. It is basically a differential amplifier and is normally used with external feedback networks that determine the function performed. Several examples of the use of op-amps appear at different places in the text. Some of the basic application circuits are given below:

29.6.7.1 Basic inverting circuits

Many circuits require a signal to be inverted as well as amplified. Op-amps are easily configured for such applications. In the circuit of Figure 29.28, the input signal is applied to the inverting (negative) terminal and the non-inverting (positive) terminal is grounded. The input voltage E_i is applied in series with input resistance R_1. The feedback resistance R_2 is connected between the input and the output terminals. The closed-loop gain of the amplifier is given by the following formula:

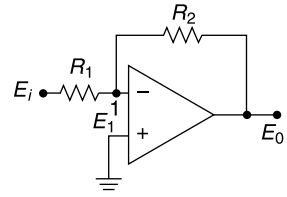


Figure 29.28 *Basic inverting circuit*

$$A = E_0 / E_i = -R_2 / R_1$$

This shows that the gain of the inverting op-amp depends only on feedback resistor R_2 and resistor R_1. The minus sign indicates that the output signal is 180° out of phase with the input signal. Also, the gain is unaffected by changes in temperature, device parameters or frequency, as the gain depends primarily on the components in the external feedback circuit.

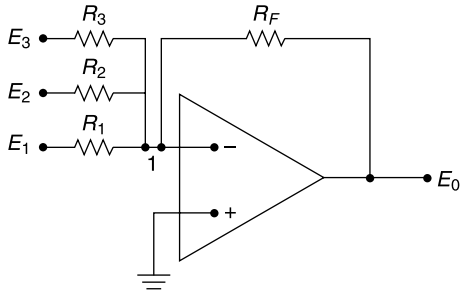


Figure 29.29 *Summing circuit*

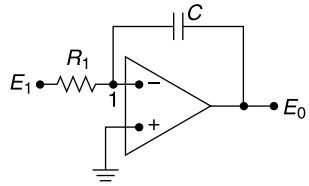


Figure 29.30 *Integrating circuit*

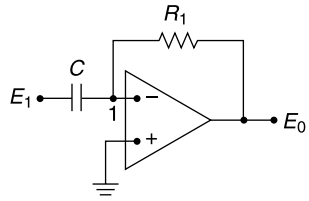


Figure 29.31 *Differentiating circuit*

29.6.7.2 Summing circuit

A useful and practical extension to the simple inverter circuit is obtained by providing a number of inputs, so that the voltage signals can be added to each other. The various input signals can be summed up in different proportions by suitably adjusting the values of the input resistors. Referring to Figure 29.29,

$$E_0 = R_F[E_l/R_1 + E_2/R_2 + E_3/R_3]$$

29.6.7.3 Integrating circuit

Figure 29.30 shows a simple running integrator (no reset or hold logic) that can be used within a stable feedback loop. Here the feedback path is provided by a capacitor. Hence, sum of the currents at point 1 is given as follows:

$$E_1/R_1 + C\,dE_0/dt = 0$$

Integrating with respect to time

$$E_0 = -1/R_1\,C \int E_1\,dt$$

Integrating circuits are required for modelling dynamic systems and solving differential equations.

29.6.7.4 Differentiating circuit

The circuit shown in Figure 29.31 is arranged to generate an output voltage which is proportional to the differential with respect to time of the input voltage. The circuit can be analysed by considering sum of the currents at point 1.

$$C.\frac{dE_1}{dt} + \frac{E_0}{R} = 0$$

$$E_0 = -RC.\frac{dE_1}{dt}$$

This shows that the output voltage is proportional to the derivative of the input. Differentiating circuits are susceptible to noise and instability and are, to be used with care. They are usually followed by a filter to limit the effective bandwidth.

29.6.7.5 Voltage follower

lf the output of the op-amp is connected back to the input at the inverting and, while the input is given at the non-inverting terminal, the circuit functions as a voltage follower (Figure 29.32). In this case, the voltage gain of the amplifier is almost unity and the output voltage follows changes in the

input voltage. The circuit provides a very high-input impedance and low output impedance. Therefore, this configuration is ideal for isolating and driving other circuits.

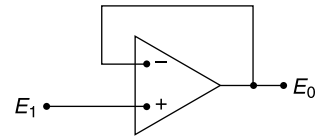


Figure 29.32 *Voltage followers*

29.6.7.6 Unity gain inverter

A unity-gain voltage inverter is formed by using identical resistances in the input and feedback paths (Figure 29.33). Inverters are used wherever sign changes are necessary. It can also be used simply to lower the impedance level or raise the power level of a signal. The circuit is capable of functioning well over a wide range of signal levels, frequencies and impedances.

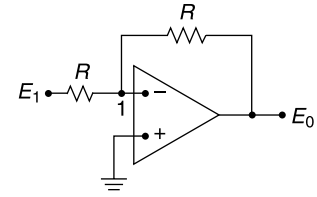


Figure 29.33 *Unity gain voltage inverter*

29.6.7.7 Integrator

The integrator is shown in Figure 29.34 and performs the mathematical operation of integration. This circuit is essentially a low-pass filter with a frequency response decreasing at 6 dB per octave. An amplitude-frequency plot is shown in Figure 29.35.

The circuit must be provided with an external method of establishing initial conditions. This is shown in the figure as S_1. When S_1 is in position 1, the amplifier is connected in unity gain and capacitor $C1$ is discharged, setting an initial condition of zero volts. When S_1 is in position 2, the amplifier is connected as an integrator and its output will change in accordance with a constant times the time integral of the input voltage.

For integration operation, the amplifier used should generally be stabilised for unity-gain operation and R_2 must equal R_1 for minimum error due to bias current.

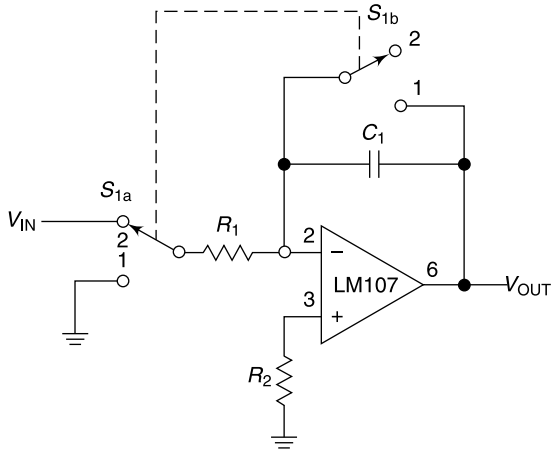


Figure 29.34 *Integrator*

29.6.7.8 Current to voltage converter

Some transducers produce an output voltage, others an output current. Current transducers include photodiodes, some temperature sensors and a variety of biological probes. Often the currents produced are very small of the order of nano-amps or less. Such currents

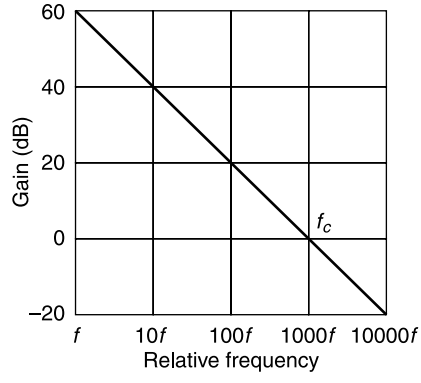


Figure 29.35 *Integrator frequency response*

require amplification before they can be used in a system and the first stage of such amplification is usually a current-to-voltage converter.

Current may be measured in two ways with an op-amp. The current may be converted into a voltage with a resistor and then amplified or the current may be injected directly into a summing node. Converting into voltage is undesirable for two reasons: first, an impedance is inserted into the measuring line causing an error; second, amplifier offset voltage is also amplified with a subsequent loss of accuracy. The use of a current-to-voltage converter using an op-amp and a feedback resistor avoids both of these problems.

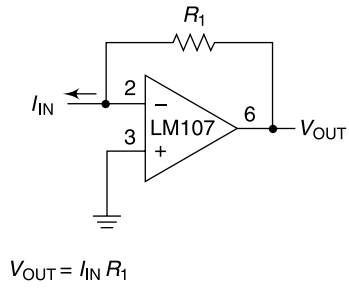


Figure 29.36 *Current-to-voltage converter*

The current-to-voltage converter is shown in Figure 29.36. The input current is fed directly into the summing node and the amplifier output voltage changes to extract the same current from the summing node through R_1. The scale factor of this circuit is R_1 volts per amp. The only conversion error in this circuit is I_{bias} which is summed algebraically with $I_{IN.}$

29.6.7.9 Photocell amplifier

Amplifiers for photoconductive, photodiode and photovoltaic cells are shown in Figure 29.37 respectively.

The feedback resistance, R_1, is dependent on cell sensitivity and should be chosen for either maximum dynamic range or for a desired scale factor. R_2 is elective: in the case of photovoltaic cells or of photodiodes, it is not required in the case of photoconductive cells, it should be chosen to minimise bias current error over the operating range.

Photodetector is optimised by operating into a fixed low load impedance. Currently available photovoltaic detectors show response times in the micro-second range at zero load impedance and photoconductors, even though slow, are materially faster at low-load resistances.

All photogenerators display some voltage dependence of both speed and linearity. It is obvious that the current through a photoconductive cell will not display strict proportionality to incident light if the cell terminal voltage is allowed to vary with cell conductance. Somewhat less obvious is the fact that photodiode leakage and photovoltaic cell internal losses are also functions of terminal voltage. The current-to-voltage converter neatly sidesteps gross linearity problems by fixing a constant terminal voltage, zero in the case of photovoltaic cells and a fixed bias voltage in the case of photoconductors or photodiodes.

29.6.7.10 Log converter

A number of instrumentation applications can benefit from the use of logarithmic or exponential signal processing techniques. The principle used for making log converters is based on the relationship between collector current and emitter-base voltage in a transistor which is precisely logarithmic from currents below one picoamp to currents above one milliamp. Using a matched pair of transistors and integrated circuit op-amps, a linear to logarithmic converter can be constructed with a dynamic range in excess of five decades.

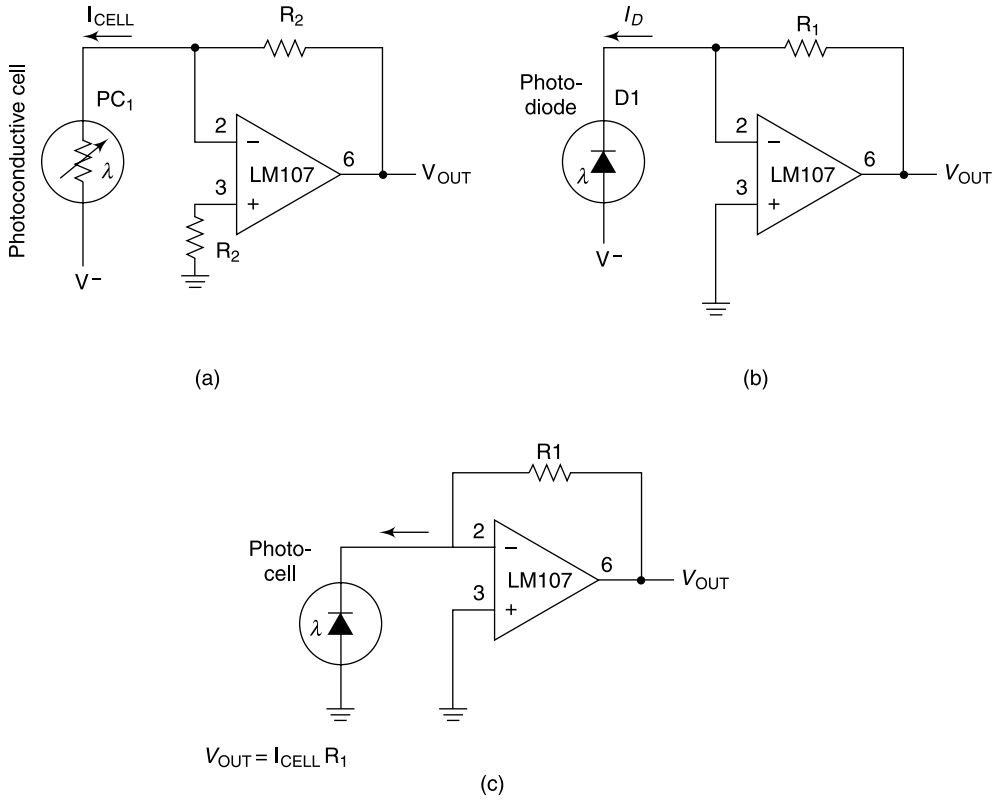


Figure 29.37 *Amplifiers for (a) photoconductive cell (b) Photodiode (c) Photocell*

The circuit in Figure 29.38 generates a logarithmic output voltage for a linear input current. Transistor Q_1 is used as the non-linear feedback element around an op-amp U_1. Negative feedback is applied to the emitter of Q_1 through divided R_1 and R_2, and the emitter-base junction of Q_2. This forces the collector current of Q_1 to be exactly equal to the current through the input resistor. Transistor Q_2 is used as the feedback element of an U_2 op-amp. Negative feedback forces the collector current of Q_2 to equal the current through R_3. For the values shown, this current is 10 μA. Since the collector current of Q_2 remains constant, the emitter-base voltage also remains constant. Therefore, only the V_{BE} of Q_1 varies with a change of input current. However, the output voltage is a function of the difference in emitter-base voltages of Q_1 and Q_2.

$$E_{out} = \frac{R_1 + R_2}{R_2}\left(V_{BE2} - V_{BE1}\right)$$

For matched transistors operating at different collector currents, the emitter-base differential is given by

$$\Delta V_{BE} = \frac{kT}{q}\log_e \frac{I_{C1}}{I_{C2}},$$

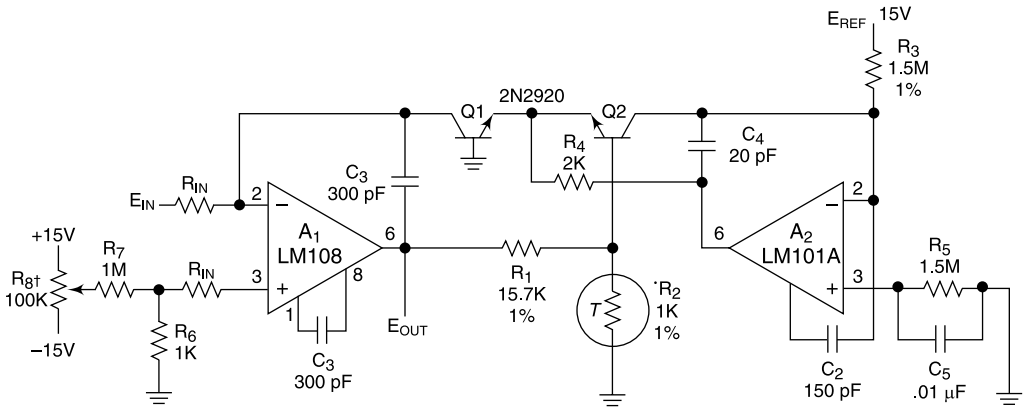


Figure 29.38 *Log converter*

where k is Boltzmann's constant, T is temperature in degrees Kelvin and q is the charge of an electron. Combining these two equations and writing the expression for the output voltage gives.

$$E_{OUT} = \frac{-kT}{q}\left(\frac{R_1 + R_2}{R_2}\right)\log_e\left(\frac{E_{IN}R_3}{E_{REF}R_{IN}}\right)$$

for $E_{IN} > 0$.

This shows that the output is proportional to logarithm of the input voltage.

29.7 SOURCES OF NOISE IN ELECTRONIC CIRCUITS

Noise enters the measurement system from two sources: external interference and the inherent noise of the circuit itself.

External Interference is noise which originates from sources not related to the actual circuit. Such noise sources include ground and power-supply noise created by other circuitry in a system, stray electromagnetic pickup of line frequency energy (and the harmonics thereof) and radio and radar transmissions, contact arcing in mechanical switches and relays, and transients due to switching in reactive circuits. External interference can often be eliminated once the interfering source is identified and appropriate action taken.

The second type of noise is the inherent noise of the circuit itself and it appears as a result of fundamental, intrinsic properties of the system. Unlike external interference, it cannot be totally eliminated since it is caused by components in the actual circuit such as resistors and sources within the electronic circuitry. The best that can be accomplished is to minimise the noise in a specific bandwidth of interest. Since the fundamental noise arises from the discontinuous nature of matter and energy, it ultimately limits the sensitivity of every instrument measurement. The main types of fundamental noise associated with solid-state electronic devices are thermal, shot and flicker noise.

29.7.1 Thermal Noise or Johnson Noise

Thermal excitation of the electrons in conductors causes random movement of charge. In a resistance this random current causes a noise voltage, known as Johnson Noise, whose amplitude is given by the formula:

$$E_N(\text{rms}) = \sqrt{4kTRB}$$

where: k = Boltzmann's Constant (1.38×10^{-23} J/°K)
T = Temperature °K
R = Resistance in Ohms
B =Bandwidth in Hertz

At room temperature (25°C), this may be simplified to

$$E_N(\text{rms}) \approx 1/8\sqrt{RB} \text{ or } e_n \approx 4\sqrt{R}$$

Where E_N = Total Noise in μV rms
e_n = Spectral Density in $nV\sqrt{\text{Hz}}$
R = Resistance in Kilohms
R = Resistance in Kilohms
B = Bandwidth in Kilohertz

Johnson noise is a fundamental property of resistances and is always considered when designing low noise circuitry. It is also called 'White Noise' because it is independent of the absolute values of frequencies. Johnson noise can be reduced by three ways: by reducing the temperature, the resistance itself or the working bandwidth.

Reducing the temperature is generally impractical. Since the function is a square root, cooling a resistor from room temperature (25°C/298K) to liquid nitrogen temperature (–196°C/77K) will only reduce the noise by 42%. Reducing the resistance itself or the working bandwidth are generally more useful. Again, reducing the bandwidth is recommended only if the frequencies important to the measurement are not excluded. As a reference point it is useful to remember that at room temperature a 1K resistor has $4\,\text{nV}/\sqrt{\text{Hz}}$ white noise. This is equivalent to 128 nV rms noise in a 1 kHz bandwidth.

29.7.2 Shot Noise

This kind of noise originates from the movement of charge carriers as they cross *p-n* junctions in semiconductor devices. There are statistical variations in the rate of electron flow and these manifest themselves as a noise current, given by the formula:

$$I_N = 5.7 \times 10^{-4}\sqrt{I_J B}$$

where I_N is noise current in picoamps
I_J is junction current in picoamps
B is bandwidth of interest (in Hertz)

Since the electronic charge is extremely small, the noise current is very small as well and is only significant when the bandwidth is very large or when the noise current is an appreciable fraction of the total current. Since noise current is proportional to the square root of the current, the second case occurs only at very low currents. Thus shot noise is important at high frequencies and in amplifiers with very low bias currents but rarely elsewhere.

Like thermal noise, shot noise is proportional to the square root of the measurement bandwidth, and is also termed as 'white noise'. Thus shot noise is also minimised by reducing the bandwidth. The magnitude of shot noise is usually small and therefore can often be ignored.

29.7.3 Flicker Noise

Flicker noise is the dominant noise at low frequencies. It has a power spectral density which is inversely proportional to frequency (hence the term '1/f Noise'). The noise voltage spectral density is therefore inversely proportional to the square root of the frequency and is represented by the following equation:

$$V_{av} = \sqrt{KI^2/f}$$

where K is a constant depending on factors such as resistor materials and geometry, I is the DC current and f is the frequency. Flicker noise predominates in measurements from 0 Hz (DC) up to about 300 Hz; it is due primarily to the contribution of the $1/f$ term. Flicker noise in amplifier systems is commonly referred to as 'Drift'. In sensitive measurements flicker noise may be eliminated by avoiding the use of low frequencies (including DC).

Noise testing is slow and time-consuming and therefore expensive. So it is more normal for manufacturers to quote typical specifications rather than absolute maxima.

29.7.4 Environmental Noise

Low-level signals which are usually encountered in analytical instruments are quite sensitive to external contaminations. This is especially troublesome where the signal source impedance is very high. The spurious signal or noise is an unwanted signal caused by the stray capacitance, inductance or resistance, which accidentally couple various parts of the circuit or its surroundings. It can produce errors in measurements and completely obscure useful data. The ratio of the wanted signal to the unwanted or noise signal is expressed as the signal-to-noise ratio.

The most common and omnipresent stray signals are those derived from the 50 Hz line voltage and they are readily identified on the recordings. They are caused by numerous reasons, but are picked up more if the instrument has poor connections.

A major consideration in combating stray signals in all low-level measurements and recording systems is properly grounding the circuit. Its primary function is to assure that electronic enclosures and chassis are maintained at zero potential. Modern laboratories have a third copper conductor in all electrical circuits, which is non-current carrying and is connected to the electric power ground or the cold water mains pipeline. This will usually provide a satisfactory system ground.

Where it is not practical to connect the signal source to the system ground, then it is imperative that a second low-impedance grounding point be established. It is called signal ground. It is

generally undesirable to connect the signal ground to the system ground. Moreover, the signal circuit should be grounded at one point and at only one point.

Interference is sometimes caused, when the ground current is returned by more than one path. Two separate grounds are seldom, if ever, at the same absolute voltage. Their potential difference creates an unwanted current in series with one of the signal leads, and causes a noise signal to be combined with useful signal. To prevent noise pick-up from electrostatic fields, low-level signal conductors must be surrounded by an effective shield. A woven metal braid under an outside layer of insulation is adequate for many applications. However, for transmission of micro-volt-level signals, very low leakage capacitance is essential. Specially designed cables having lapped foil shields plus a low resistance drain wire in place of the braided wire shield are used for this purpose. This design reduces leakage capacity from about 0.1 μμf per foot to 0.01 μμf per foot for a typical cable.

The signal-cable shield is grounded at the signal source. This prevents signal-cable capacity from shunting the amplifier's impedance to ground. It also preserves the high common-mode rejection of the amplifier. The shield is connected to the low side of the signal source.

To sum up:

- Every low-level recording or display system should have a stable system ground and a good signal ground.
- The signal-cable shield should not be attached to more than one ground, and this ground should' be at the signal source.
- More than one accidental or intentional ground on either the signal circuit or the signal-cable shield will produce excessive electrical noise in any low-level circuit. Therefore, the signal circuit should be grounded at only one point and never at more than one point.
- Always ground a floating signal circuit and the signal-cable shield only at the signal source.

Electromagnetic radiation that adversely affects circuit performance is generally termed electromagnetic interference (EMI). Normally, electronic equipments are well shielded to avoid EMI. *Shielding* is the use of conductive materials to reduce radiated EMI by reflection and/or absorption. Shielding can be applied to different areas of the electronic package, from equipment enclosures to individual circuit boards or devices. However, some interference may still get into the system, for one reason or other. A very common effect is that the mains and supply cables are infected with very high-frequency pulse type noise due to electromechanical switches, commutators, motors, etc. This pulse type noise is carried into the casing by mains cable and disturbs the whole system, influencing both the power supply and signal conductors. These problems can be solved by providing EMI filter at mains supply side. Robinson (1990) explains the types of shielding required to protect electronic equipment against electromagnetic and radio frequency interference (RFI).

29.8 SOURCES OF NOISE IN LOW-LEVEL MEASUREMENTS

29.8.1 Electrostatic and Electromagnetic Coupling to AC Signals

The distributed capacitance between signal conductors and from signal conductors to ground provides a low-impedance AC path, resulting in signal contamination from external sources like power lines and transformers. Similarly, the alternating magnetic flux from the adjacent power line wires induces a voltage in the signal loop which is proportional to the rate of change of the disturbing

current, the magnitude of the disturbing current and the areas enclosed by the signal loop, is inversely proportional to the distance from the disturbing wire to the signal circuit. Unequal distances of the two signal carrying conductors from the disturbing current wire result in unequal mutual inductances which cause the magnetic field to produce a noise voltage across the amplifier input terminals.

Low-level signals are sensitive to external contamination especially in the case of high source impedance. Referring to Figure 29.39, it is obvious that the currents generated by various noise signals will flow through the signal source impedance Z and result in an unwanted addition to the transducer signal. This may include electromagnetic noise pick-up, electrostatic pick-up and the unwanted current generated by a ground loop between two separate grounds on the same signal circuit. The magnitude of these unwanted signals will be directly proportional to signal source impedance as shown by the relationship given below:

$$\text{Amplifier input signal} = E + I\,Z$$

where E = normal signal amplitude
Z = impedance of signal source
I = current generated by noise

It is obvious that as the signal source impedance approaches zero, so will the noise input to the amplifier. In fact low-source impedance effectively shunts out the noise.

To prevent noise pick-up from electrostatic fields, low-level signal conductors are surrounded by an effective shield. This usually is a woven metal braid around the signal pair, which is placed under an outside layer of insulation. A more effective shielding is provided by a special type of signal cable, which has lapped foil shields, plus a low resistance drain wire instead of the conventional braided wire shield.

The easiest and generally the best way to protect a signal cable against external electromagnetic disturbances is to twist the circuit conductors closely together to electrically cancel the effect of an external magnetic field. The shorter the lay of the twist, the greater the noise rejection. Thus, electromagnetic coupling is reduced by shielding, wire twisting and proper grounding which provide a balanced signal pair with satisfactory noise rejection characteristics.

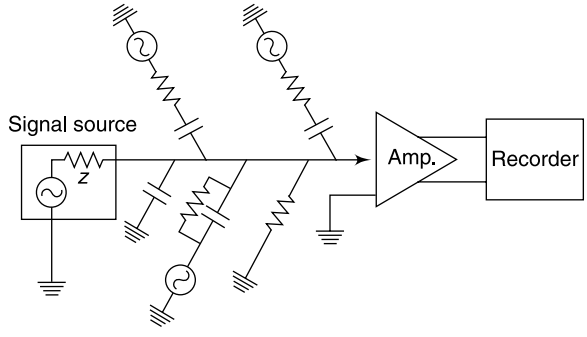


Figure 29.39 *Currents produced by various forms of noise flow through the signal source impedance and become an unwanted addition to the useful signal. The noise amplitude is directly proportional to signal source impedance (Courtesy: M/s Gould Inc., USA)*

29.8.2 Proper Grounding (Common Impedance Coupling)

Placing more than one ground on a signal circuit produces a ground loop which may generate so much noise that it may completely obscure the useful signal. The term 'grounding' means a low-impedance metallic connection to a properly designed ground grid, located in the earth. Stable grounding is necessary to attain effective shielding of low-level circuits to provide a stable reference for making voltage measurements and to establish a solid base for the rejection of unwanted common-mode signals. There are generally two grounding systems-a system ground and a signal ground. All low-level measurements and recording systems should be provided with a stable system ground to assure that electronic enclosures and chassis operating in an electromagnetic environment are maintained at zero potential. In most instances, the third copper conductor in all electrical circuits, which is firmly tied to electric power ground, the building ground and the water system, will provide a satisfactory system ground. The signal ground, on the other hand, is necessary to ensure a low noise signal reference to ground. This ground should be a low-impedance path to wet earth to minimise the introduction of spurious voltages into the signal circuitry. It is important to note that a signal circuit should be grounded at one point only.

Two separate grounds are seldom at the same absolute voltage. If we connect more than one ground to the same signal circuit, an unwanted current will flow in the ground loop thus created. This current combines itself with the useful signal (Figure 29.40). Also, there is a second ground loop through the signal-cable shield from the signal source to the amplifier. The current in the shield is coupled to the signal pair through the distributed capacitance in the signal cable. This current then flows through the output impedance of the signal source and back to ground, thus adding a second source of noise to the useful signal. Either one of these ground loops generates a noise signal that is larger than a typical millivolt useful signal. Ground loops are eliminated by floating lower input terminal of the amplifier. The amplifier enclosure is still solidly grounded to earth ground No. 2 but this will not create a ground loop, since the amplifier enclosure is insulated from the signal circuit. The ground loop through the signal cable is removed by grounding the shield only at the signal source which is the proper configuration for minimum noise pick-up (Figure 29.41).

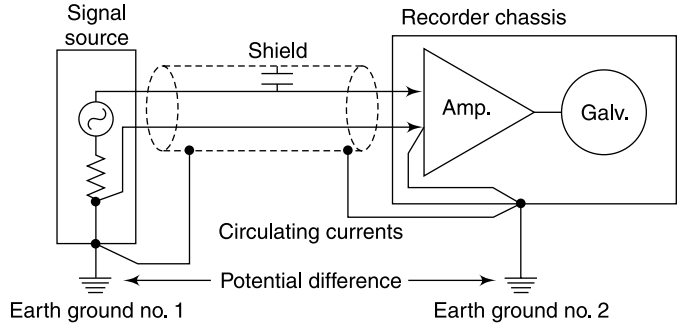


Figure 29.40 *Ground loop created by more than one ground on a signal circuit. The potential difference between earth ground No.1 and earth ground No.2 causes current to circulate in the signal-cable shield and also in the lower signal conductor, producing two separate ground loops.*

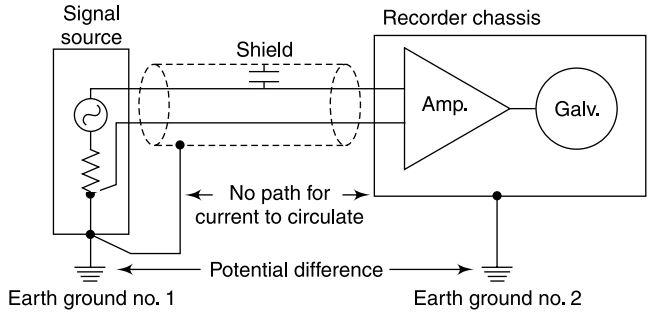


Figure 29.41 *Eliminating multiple grounds. The ground loop in the lower signal lead has been broken by removing the jumper wire to earth ground No.2. The ground loop in the cable shield has been broken by removing its connection to earth ground No.2 (Courtesy: M/s Gould Inc., USA)*

29.9 NOISE REDUCTION TECHNIQUES

29.9.1 Hardware Techniques

In a measurement system, it is often possible to ignore noise if the magnitude of the desired signal is relatively large as compared to the undesirable (noise) signal. However, many situations require the extraction of useful signal from the noisy background. Noise reductions have traditionally been carried out with electronic hardware circuits. This is mostly achieved with frequency as the most commonly used property. It was mentioned in the previous section that 'white noise' can be reduced by narrowing the range of measured frequencies (i.e. limiting the frequency range but ensuring the proper signal fidelity). The methods commonly used for frequency selection are filters, integrators and modulators. Modulations are used in chopper amplifiers. The modulation/DC modulation techniques are used to process a signal in a region of minimum noise and to discriminate between signal and noise on the basis of the signals unique modulation configuration relative to the random pattern of noise.

Commonly used noise reduction techniques are as follows:

29.9.1.1 Differential amplifier

Amplifiers designed for use in the input stage (pre-amplifiers) of analytical instruments are mostly of the differential type. That is they have three input terminals out of which one is arranged at the reference potential and the other two are live terminals. The differential amplifier is employed when it is necessary to measure the voltage difference between 2 points, both of them varying in amplitude at different rates and in different patterns.

The differential amplifier is an excellent device for use in the recording systems. Its excellence lies in its ability to reject common-mode interference signals which are invariably picked up by electrodes from the body along with the useful bioelectric signals. Also, as a direct-coupled amplifier, it has a good stability and versatility. High stability is achieved because it can be insensitive to temperature changes which is often the source of excessive drift in other configurations. It is versatile in that it may be adapted for a large number of applications, e.g., applications

requiring floating inputs and outputs or for applications wherein the grounded inputs and/or outputs are desirable.

The working of a differential amplifier can be explained with the help of Figure 29.42 where the two transistors with their respective collector resistances form a bridge circuit. If the two resistors and the characteristics of the two transistors are identical, the bridge is perfectly balanced and the potential difference across the output terminals is zero.

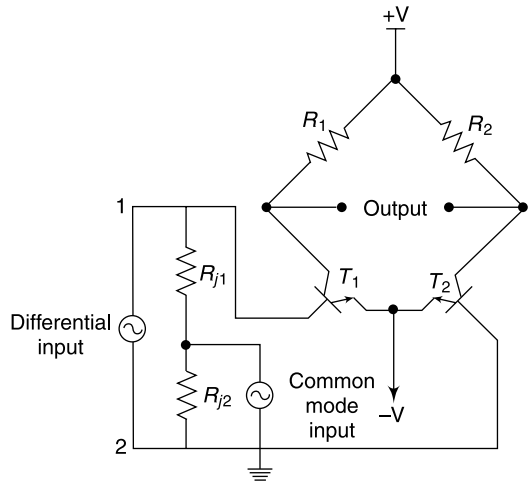


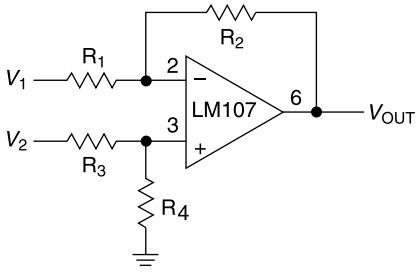
Figure 29.42 *Single op-amp differential amplifier*

Let us now apply a signal at the input terminals 1 and 2 of this circuit. The signal is to be such that at each input terminal, it is equal in amplitude but opposite in phase with reference to the ground. This signal is known as the differential mode signal. Because of this signal, if the collector current of T_1 increases, the collector current of T_2 will decrease by the same amount and the collector voltage of T_1 will decrease while that of T_2 increases. This results in a difference voltage between the two output terminals that is proportional to the gain of the transistors.

On the other hand, if the signal applied to each input terminal is equal in amplitude and is in the same phase (called the common-mode input signal), the change in current flow through both transistors will be identical, the bridge will remain balanced and the voltage between the output terminals will remain zero. Thus, the circuit provides high gain for differential mode signals and no output at all for common-mode signals.

The ability of the amplifier to reject these common voltages on its two input leads is known as common-mode rejection and is specified as the ratio of common-mode input to differential input to elicit the same response. It is abbreviated as CMRR (Common mode rejection ratio). CMRR is an important specification referred to the differential amplifier and is normally expressed as decibels. CMRR of the pre-amplifiers should be as high as possible so that only the wanted signals find a way through the amplifier and all unwanted signals get rejected in the pre-amplifier stage. A high rejection ratio is usually achieved by the use of a matched pair of transistors in the input stage of the pre-amplifier and a large 'tail' resistance in the long-tailed pair to provide maximum negative feedback for inphase signals. The technique of long-tailing (a technique used to current drive an active device) improves the CMRR in differential amplifiers without upsetting the gain for the desired signal. Very high CMRR can be achieved with the use of an active long-tail. Also, a high-input impedance is very necessary in order to obtain a high CMRR.

The design of a good differential amplifier essentially implies the use of closely matched components which has been best achieved in the integrated circuit form. High gain integrated DC amplifiers, with differential input connections and a provision for external feedback have been given the name op-amps because of their ability to perform mathematical operations. These amplifiers are applied for the construction of AC or DC amplifiers, active filters, phase inverters, multi-vibrators and comparators, etc., by suitable feedback arrangement, and therefore find a large number of applications in analytical instrumentation field.



$$v_{out} = \left(\frac{R_1 + R_2}{R_3 + R_4}\right)\frac{R_4}{R_1}v_2 - \frac{R_2}{R_1}v_1$$

For $R_1 = R_3$ and $R_2 = R_4$

$$v_{out} = \frac{R_2}{R_1}(v_2 - v_1)$$

Figure 29.43 *Differential amplifier*

Figure 29.43 shows a single op-amp in a differential configuration. The common-mode rejection for most op-amps is typically between 60 and 90 dB. This may not be sufficient to reject common-mode noise generally encountered in bio-medical measurements. Also, the input impedance is not very high to handle signals from high impedance sources. One method to increase the input impedance of the op-amp is to use FET in the input differential stage. A more common approach is to use an instrumentation amplifier in the pre-amplifier stage.

29.9.1.2 Instrumentation amplifier

The differential amplifier is well suited for most of the applications in analytical instrumentation. However, it has the following limitations:

- The amplifier has a limited input impedance and therefore, draws some current from the signal source and loads them to some extent.
- The CMRR of the amplifier may not exceed 60 dB in most of the cases, which is usually inadequate in modern biomedical instrumentation systems.

These limitations have been overcome with the availability of an improved version of the differential amplifier, whose configuration is shown in Figure 29.44. An instrumentation amplifier is a precision differential voltage gain device that is optimised for operation in an environment hostile to precision measurement. It basically consists of three op-amps and seven resistors. Basically, connecting a buffered amplifier to a basic differential amplifier makes an instrumentation amplifier.

In the Figure 29.44, op-amp A_3 and its four equal resistors R form a differential amplifier with a gain of 1. Only A_3 resistors have to be matched. The variable resistance R_{var} is varied to balance out any common-mode voltage. Another resistor R_g, is used to set the gain using the formula

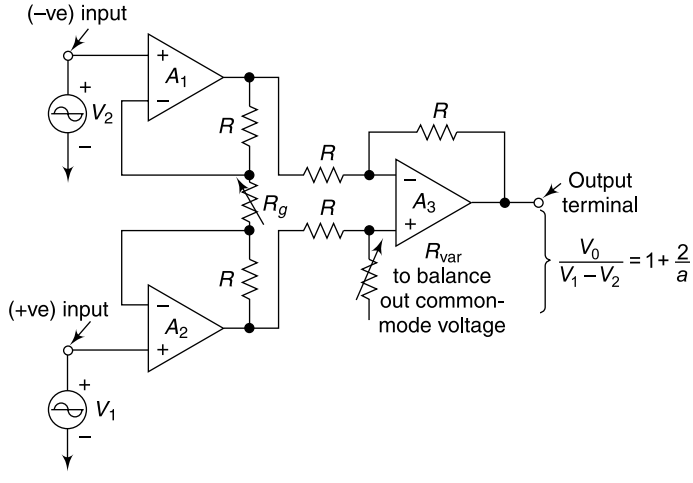


Figure 29.44 *Instrumentation amplifier*

$$\frac{V_o}{V_1 - V_2} = 1 + \frac{2}{a}$$

Where $a = R_g / R$

V_1 is applied to the +ve input terminal and V_2 to the –ve input terminal. V_o is proportional to the difference between the two input voltages.

The important characteristics of the instrumentation amplifier are:

- Voltage gain from differential input (V_1–V_2) to single-ended output, is set by one resistor.
- The input resistance of both inputs is very high and does not change as the gain is varied.
- V_o does not depend on common-mode voltage, but only on their difference.

If the inputs are prone to high voltage spikes or fast swings, which the op-amps cannot cope, they may be protected using back-to-back connected diodes at their inputs. However, this reduces the input impedance value substantially and also limits the bandwidth.

The instrumentation amplifier offers the following advantages for its applications in the biomedical field:

- Extremely high-input impedance
- Low bias and offset currents
- Less performance deterioration if source impedance changes
- Possibility of independent reference levels for source and amplifier
- Very high CMRR
- High slew rate
- Low power consumption

Good quality instrumentation amplifiers have become available in single IC form such as μA725, ICL7605, LH0036 etc.

29.9.1.3 Chopper amplifiers

The chopper amplifier is a useful device in the field of analytical instrumentation, as it gives a good solution to the problem of achieving adequate low frequency response, while avoiding the drift problem inherent in direct-coupled amplifiers.

This type of amplifier makes use of a chopping device, which converts a slowly varying direct current to an alternating form, with amplitude proportional to the input direct current and with phase dependent on the polarity of the original signal. The alternating voltage is then amplified by a conventional AC amplifier, whose output is rectified back to get amplified direct current. A chopper amplifier is an excellent device for signals of narrow bandwidth and reduces the drift problem to zero.

There are two types of choppers, mechanical chopper and transistor chopper. A mechanical chopper is simply an electronic switch driven by an alternating current. It is so designed that, the flux saturates a magnetic circuit, such that switching operation occurs only near the cycle zero points. For mechanical choppers, 50 Hz mains frequency is usually used as the chopping frequency. Choppers which operate at higher frequencies, say about 400 Hz are also available.

The use of a transistor as a chopper increases the possible rate of switching, and therefore can be useful for signals of wider bandwidths. Several typical transistor chopper circuit configurations

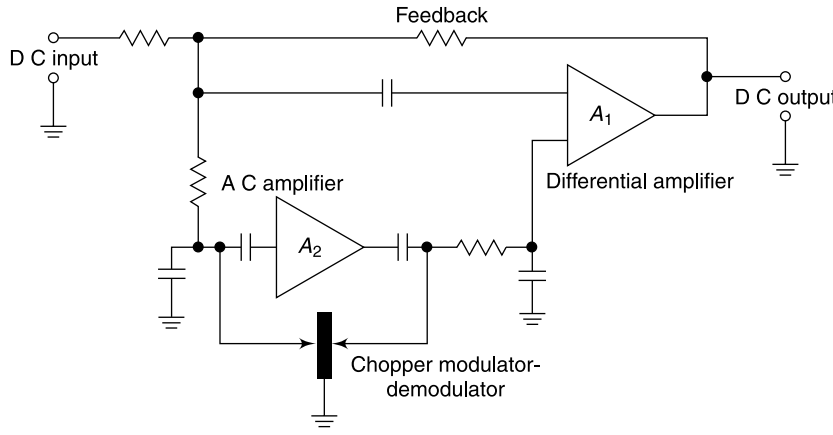


Figure 29.45 *Chopper amplifier*

are available in the literature. The action of the transistor as chopper is based on its low saturation resistance of ON mode and high resistance for OFF mode. The transistor can be used as a signal chopper at rates conveniently up to 100 kHz. Greater stability against drift can be achieved in the chopper stabilised DC amplifier, shown in the Figure 29.45.

Since drift is a low frequency phenomenon, the signal can be applied simultaneously to a chopper modulated AC amplifier of the narrow bandwidth and through an AC coupling to a differential output DC amplifier of wide bandwidth. The low frequency components are given high gain over the restricted bandwidth and the drift output is fed back for drift stabilisation. For higher frequency components, the gain is provided by the DC amplifier. In this arrangement, medium and higher frequencies pass through the DC amplifier with the gain say as A_1. The drift and very low frequency signals pass through both amplifiers in series with the gain A_1/A_2. The drift factor is reduced by A_1/A_2 and it is possible to obtain drift figures of only a few micro-volts.

Chopper amplifiers find application in analytical instrumentation in the amplification of small DC signals of a few micro-volts. Such order of amplitudes are obtainable in atomic absorption spectrophotometers, pH metres, etc. The frequency response of a chopper amplifier depends upon the value of the chopping frequency. The input impedance can be made high by using a sub-miniature electrometer tube or IGFET, at the input stage.

29.9.1.4 Carrier amplifiers

If the signal and noise cannot be separated by simple filtering, modulation/de-modulation techniques can be used to process a signal in a region of minimum noise. The signal and noise are discriminated on the basis of the unique modulation configuration of the signal relative to the random pattern of the noise. An amplifier based on this technique is called 'carrier amplifier'. This type of amplifier enables to obtain zero frequency response of the DC amplifier and the inherent stability of the capacitance coupled amplifier, a carrier type of amplifier is generally used.

The carrier amplifier consists of an oscillator and a capacitance coupled amplifier. The oscillator is used to energise the transducer with an alternating carrier voltage. The transducers, which require AC excitation, are those whose impedance is not purely resistive. Example can

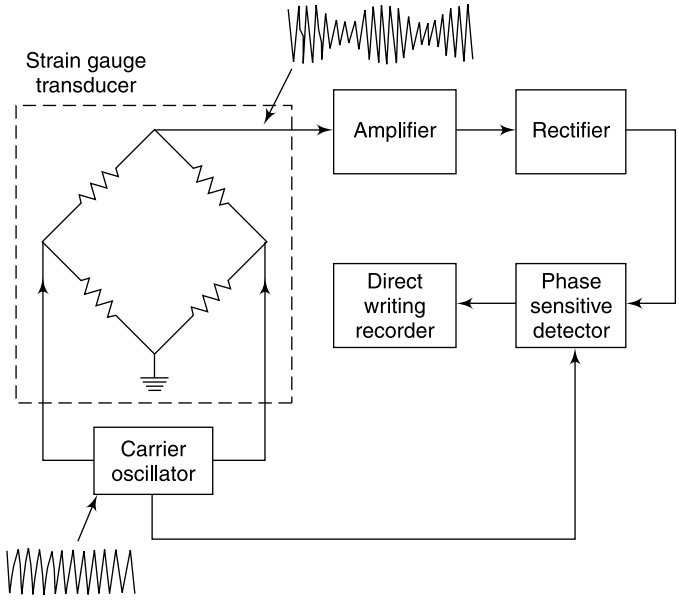


Figure 29.46 *Carrier amplifier*

be of a capacitance based pressure transducer whose impedance is mainly capacitative with a small resistive component. The frequency of the excitation voltage is usually around 2.5 kHz. The transducer shall change the amplitude of the carrier voltage in relation to the changes in the physiological variable being measured. The output of the transducer therefore, would be an amplitude modulated (AM) signal (Figure 29.46). The modulated AC signal can then be fed to a multi-stage capacitance coupled amplifier. The first stage produces amplification of the AM signal. The second stage is so constructed that it can respond only to signal frequency of the carrier. It can be further amplified in the following stage. After amplification, the signal is demodulated in a phase-sensitive demodulator circuit. This helps to extract amplified signal voltage after the filter circuit. The voltage produced by the demodulator can then be applied to the driver stage of the display system.

29.9.1.5 Lock-in amplifier

A lock-in amplifier is a useful version of the carrier technique designed for measurement of low-level signals buried in noise. This type of amplifier, by having an extremely narrow-width output band in which the signal is carried, reduces wideband noise and increases the signal-to-noise ratio. Thus, the difference between carrier amplifier and lock-in amplifier is that the former is a general purpose instrument amplifier while the latter is designed to measure signals in a noisy background.

In principle, the lock-in-amplifier works by synchronising on a single frequency, called the reference frequency. This frequency is made to contain the signal of interest. The signal is modulated by the reference frequency in such a way that all the desired data is at the single reference frequency whereas the inevitable noise, being broadband, is at all frequencies. This permits the signal to be recovered from its noisy background.

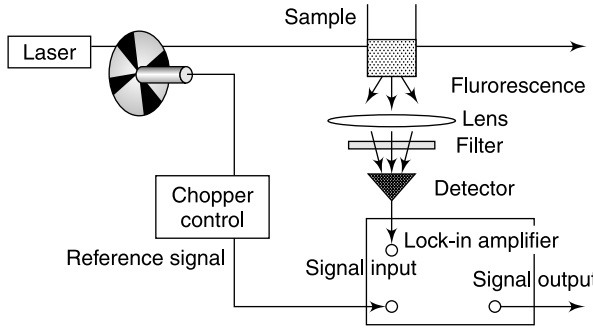


Figure 29.47 *Lock-in amplifier*

Lock-in amplifier primarily works on the principle of phase-sensitive detection to improve the signal-to-noise ratio in continuous wave experiments. A typical example of application of a lock-in amplifier is shown in Figure 29.47 wherein the analytical signal (the fluorescence) is modulated by chopping the optical excitation source (the laser) at the reference frequency.

29.9.1.6 Box car integrator

Integration of DC signals over a limited time period provides another method for reducing white noise. The signal with its coherent characteristics gets added with respect to the integration time, while the random noise adds as the square root of the integration time. Thus, the signal/noise ratio is enhanced as the square root of integration time. Although a simple RC circuit can be used to integrate signals, using an op-amp with a capacitor in the feedback circuit offers a more stable and precise integrating circuit.

An improvement over the simple integrator is the box car integrator in which the same portion of the signal is periodically sampled for a fixed period of time and then averaging the sample using a low-pass RC filter. The scheme is shown in Figure 29.48. The integrator is triggerable and gated and provides an S/N enhancement for the portion of the signal that is sampled, which is equal to

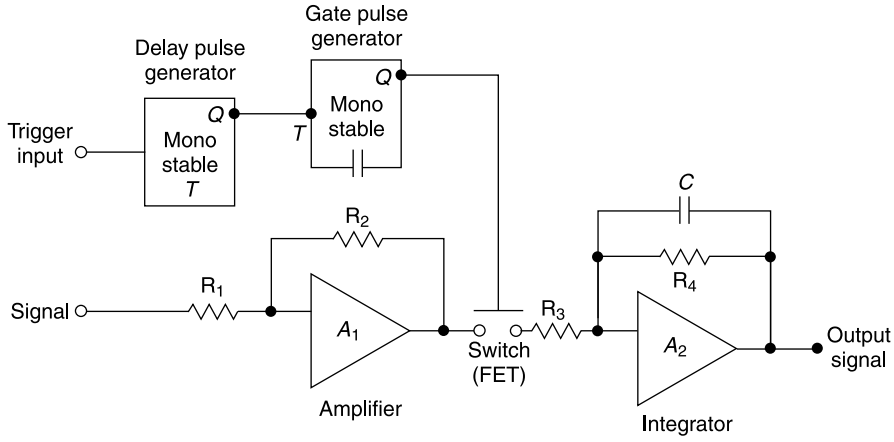


Figure 29.48 *Box car integrator (Redrawn after willard, et al., 1988)*

the square root of the number of pulses integrated. The technique is particularly useful in applications requiring pulsed signal detection.

29.9.1.7 Filters

In order to achieve reduction of noise, it is a common practice to narrow the range of measured frequencies by the use of electronic filters.

A filter is a circuit which amplifies some of the frequencies applied to its input and attenuates others. There are four common types of filters:

High-Pass: which only amplifies frequencies above a certain value

Low-Pass: which only amplifies frequencies below a certain value

Bandpass: which only amplifies frequencies within a certain band

Band stop: which amplifies all frequencies except in those in a certain band.

Filters may be produced using many different methods. These include *passive filters* which use only passive components such as resistors, capacitors and inductors, *digital filters* which use analog-to-digital converters to convert a signal to digital form and then use high-speed digital computing techniques to filter it and active filters which use amplifiers in addition to resistors, capacitors and inductors in order to obtain performance impossible with passive filters. op-amps are frequently used as the gain blocks in active filters.

The simple low-pass filter is shown in Figure 29.49 This circuit has a 6 dB per octave roll-off after a closed-loop 2 dB point defined by f_c. Gain below this corner frequency is defined by the ratio of R_3 to R_1. The circuit may be considered as an AC integrator at frequencies well above f_c, however, the time-domain response is that of a single *RC* rather than an integral.

A gain frequency plot of circuit response is shown in Figure 29.50 to illustrate the difference between this circuit and the true integrator.

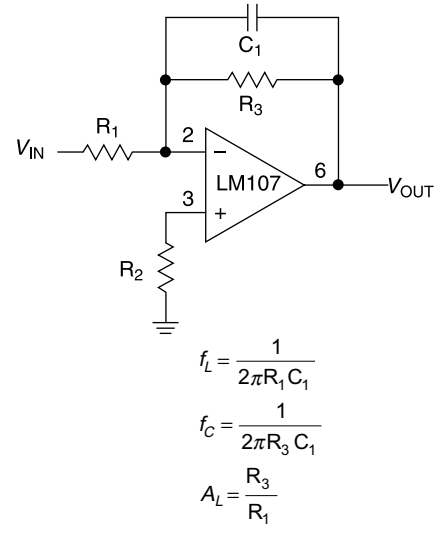


Figure 29.49 *Simple low-pass filter*

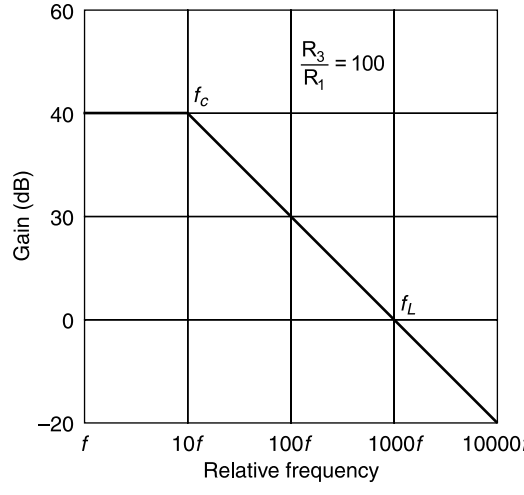


Figure 29.50 *Lowpass filter response*

29.9.2 Software Techniques

29.9.2.1 Fourier transform

In analytical instrumentation, sensors/transducers pick up signals from sources of chemical information.

Signal processing employs sophisticated mathematical analysis tools and algorithm to extract information buried in these signals received from various sensors and transducers. Signal processing algorithms attempt to capture signal features and components that are of diagnostic value. Since most signals of analytical interests are time varying, there is a need to capture transient phenomena when studying the behaviour of such signals.

As illustrated in Section 3.5 (Chapter 3), there are two different presentations of the same experimental data, known as domains. These are the *time domain,* in which the data are recorded as a series of measurements at successive time intervals and the *frequency domain*, in which the data are represented by the amplitudes of its sine and cosine components at different frequencies. For example, for recording and display purposes, the signals are represented in the time domain, that is, the signals are represented in the time domain, which means the signal is represented by means of its value (Y-axis) on the time axis (X-axis). In the frequency domain, any signal may be described as consisting of sine waves and having different amplitudes and phases (Y-axis) as a function of frequency (X-axis). The transformation between the two representations is given by the Fourier Transformation (FT).

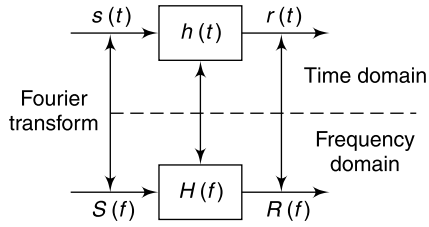


Figure 29.51 *A system transfer function, h(t) and H(f) characterises its response r(t) in the time domain and R(f) in the frequency domain to receive stimuli, s(t) and S(f). The two domains are related by the Fourier Transforms*

The basic motivation for developing the frequency analysis tools is to provide a mathematical and pictorial representation for the frequency components that are contained in any given signal. The term spectrum is used when referring to the frequency content of a signal. The process of obtaining the spectrum of a given signal using the basic mathematical tools is known as frequency or spectral analysis. Most biomedical signals of practical interest can be decomposed into a sum of sinusoidal signal components. For the class of periodic signals, such a decomposition is called a Fourier series. For the class of finite energy signals, the decomposition is called the Fourier transform.

Referring to Figure 29.51 a system's response to a varying input signal $s(t)$, with a frequency spectrum $S(f)$, can be described essentially interchangeably, by the response $r(t)$ in the time domain (as a time history) or $R(f)$ in the frequency domain (as a frequency spectrum).

The key system characteristics operate on the signal to produce the response to the stimulus. In the frequency domain, the operation can be expressed as a simple product, and the ratio of response to stimulus is called the transfer function, $H(f)$:

$$H(f) = R(f) / S(f)$$

The time domain and frequency domains are closely connected via FT.

29.9.2.2 Fast fourier transform (FFT)

Using FT can become tedious and time-consuming even when using computers and especially when a large number of points have to be considered. The situation has considerably been eased

with the introduction of fast Fourier transform (FFT) algorithm, which expands the signal into sine and cosine functions. The frequency spectrum is computed for each discrete segment of the signal. The output of the FFT algorithm is a set of coefficients, two for each frequency component in the signal's spectrum. One coefficient (A) is multiplied by the cosine, or amplitude portion of the component. The other (B) is multiplied by the sine, or phase portion of the component. Each component in the FFT series can then be represented as:

$$A \cos(\omega t) + i\, B \sin(\phi)$$

where ω = Angular frequency of the component

A = An FFT coefficient

B = An FFT coefficient

ϕ = The phase angle of the component

i = The imaginary number, $\sqrt{-1}$

The number of frequency components and pairs of FFT coefficients necessary to represent a given waveform is a function of the highest frequency to be resolved and the sample rate used.

Figure 29.52 illustrate the decomposition of a typical time varying signal into its basic frequency components and then displays them as a frequency spectrum. The diagram shows frequency components along with the amplitude present in each frequencies. Once the frequency spectrum of a given segment of the signal has been calculated, a number of techniques are available to display the information.

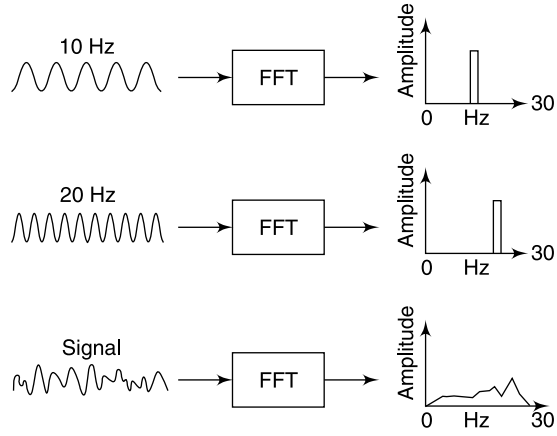


Figure 29.52 *Time varying signal decomposed into basic frequency components*

The FFT method assumes the signal to be stationary and is therefore insensitive to its varying features. However, most signals are non-stationary and have highly complex time frequency characteristics. The stationary condition for the non-stationary signal can be satisfied by dividing the signals into blocks of short segments, in which the signal segment can be assumed to be stationary. This method, is called the *short-time Fourier transform* (STFT). However, the problem with this method is the length of the desired segment. Choosing a short analysis window may cause poor frequency resolution. On the other hand, a long analysis window may improve the frequency resolution but compromises the assumption of stationary within the window.

The Fourier theorem states that any waveform can be duplicated by the superposition of a series of sine and cosine waves. As an example, the following Fourier expansion of sine waves provides an approximation of a square wave as shown in Figure 3.17. As more terms are added the superposition of sine waves better matches a square wave.

FT uses the above concept to convert between two different descriptions of a physical system.

$$f(t) = 1/2\ \pi F(\omega) e^{-i\omega t}\, d\omega$$

$$F(\omega) = f(t)^{i\omega t}\, dt$$

In these equations, ω is angular frequency (2 π x frequency), t is time and

$$Ei\omega t = \cos(\omega t) + I \sin(\omega t)$$

where $I = \sqrt{-1}$

The $F(\omega)$ function gives the frequencies at which the signal is non-zero and the $f(t)$ function gives the times at which the signal is non-zero. Both of these functions are suitable descriptions of a waveform or physical system

Given a function in time, $f(t)$, we can transform it to an equivalent function in frequency, $F(\omega)$. We can look at the second expression in detail to understand what is happening.

$$F(\omega) = \int f(t)^{i\omega t}\, dt$$

In order to do the transform, we multiply $f(t)$ times [cos (ωt) + I sin (ωt)]. We do this at all times between 00 and –00.

29.9.2.3 DFT and FFT

Experimental data usually consists of discrete data points rather than a continuous function as used in the equations above. The Discrete Fourier-Transform (DFT) is an algorithm for doing the transform with discrete data. The DFT is an order N^2 calculation, meaning that the number of multiplications is equal to the square of the number of data points. This algorithm has been supplanted by FFT algorithms, which reduce redundancies and take much less computer time. The order of this calculation is $N/\log N$.

A key experimental parameter is the sampling frequency, which must be at least twice as large as the highest frequency component that is present in the data. This sampling rate is called the Nyquist critical frequency. Sampling at less than the Nyquist frequency results in aliasing of the result. Aliasing can be prevented by filtering out all frequencies above the Nyquist frequency so that they do not create artefacts in the transformed spectrum.

FFT algorithms are used in NMR, infrared, Raman and mass spectrometry instrumentation. The following are the advantages:

- Multiplex (or Fellgett) advantage
- Throughput (or Jaquinto) advantage
- Rapid signal averaging of data to greatly improve the signal-to-noise ratio of the data.

The multiplex advantage arises from recording all signal frequencies simultaneously. The throughput advantage arises because no physical slit is necessary to obtain resolution in the resulting spectra.

29.9.2.4 Wavelet transform

An emerging method to analyse non-stationary signals is the *wavelet transform*. The wavelet method acts as a mathematical microscope in which we can observe different parts of the signal by just adjusting the focus. In practice, it is not necessary for the wavelet transform to have continuous frequency (scale) parameters to allow fast numerical implementations; the scale can be varied as we move along the sequence. Therefore, the wavelet transform has very good time resolution at the

high frequencies, and good frequency resolution at the low frequencies. In analytical instrumentation field, wavelet transform have been widely used in many research areas including spatial filtering, edge detection, feature extraction, data compression, pattern recognition, image compression and texture analysis.

Wavelets constitute a relatively new signal processing method. A wavelet transform is almost always implemented as a bank of filters that decompose a signal into multiple signal bands. It separates and retains the signal features in one or a few of these bands. Thus, one of the biggest advantages of using the wavelet transform is that signal features can be easily extracted. In many cases, a wavelet transform outperforms the conventional FFT when it comes to feature extraction and noise reduction.

Adaptive Filter: Another method of signal analysis is that of adaptive filter which can continuously adjust itself to optimally perform under the changing circumstances. This is achieved by correcting the signal according to the specific application. The correction may be enhancement or some reshaping, for which a correction algorithm is required. This can be best implemented digitally. Most adaptive filters, therefore, are implemented by means of computers or special digital signal processing chips.

29.10 POWER SUPPLIES

Power supply requirements for analytical instrumentation are as varied as the applications themselves. Power supply voltages ranging from 3 to 3,00,000 V can be found within a given instrument. Although in some equipment, the power may be derived from a battery, but more usually, it is obtained from the single-phase AC power mains. Since most of the modern solid-state equipment operates on low DC voltages, the purpose of the power supply is to convert the 230 V, 50 Hz AC mains supply into a form necessary for operating the internal circuitry of the equipment, which is usually a regulated DC voltage. Since all parts of an equipment need power to run, a failure in the power supply can often lead to a complete failure of the equipment.

Besides DC-regulated power-supply, electronic circuits also sometimes contain converters and inverters. An *inverter* is a power unit that converts DC power into AC power output. The DC source is usually a battery. The frequency of the AC output may be 50 Hz or higher like 400 Hz. A *converter* is basically an inverter followed by rectification (i.e. it converts AC into DC). A typical example of a DC to DC converter is that of generating high voltage, say 2 kV, from 9 V battery supply.

Most of the modern analytical equipment may need one or more of the voltages in three ranges, ±5 V for operation of digital and logic circuits, ±12 V or ±15 V for operation of linear integrated circuits and transistors, higher voltages for operation of specialised parts of the circuit, say 24 V for operation of certain types of solenoids, 100 V for driving deflection coils on a video monitor, 50 kV for scanning electron microscope.

29.10.1 Types of Regulators

Two techniques are used to provide regulated DC voltages:

(a) Linear series regulator
(b) Switched-mode power supply

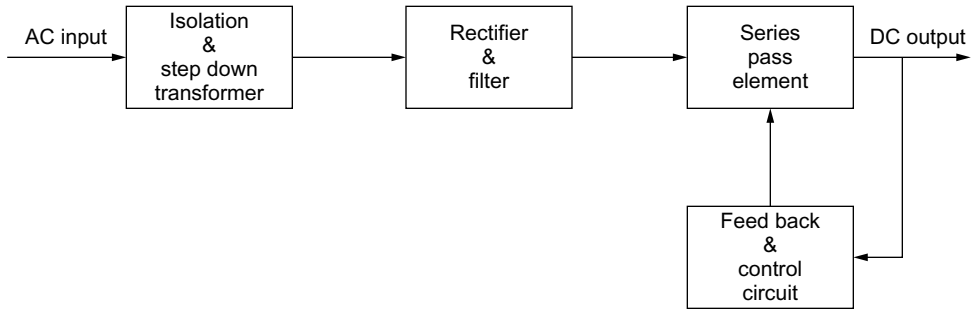


Figure 29.53 *Block diagram of a linear power-supply*

29.10.1.1 The linear power supply

A linear power-supply essentially operates on line frequency and has the following blocks as shown in Figure 29.53.

(a) Isolation and step down power transformer
(b) Rectifier and filter
(c) Series-pass element
(d) Feedback and control

The isolation transformer provides the necessary isolation of power-supply circuit from the input mains AC, and steps down the voltage to the required level. This secondary voltage is then rectified and filtered to give unregulated DC which is fed to the series-pass element (active device such as a transistor) operating in the active region.

By sampling a portion of the output voltage and comparing it to a fixed reference voltage, the series-pass element is used in the form of 'variable resistor' to control and regulate the output voltage. Since the series-pass transistor operates in the active region, it dissipates lot of power as heat, necessitating use of heavy heat sinks and resulting in low efficiency between 30 and 50%.

29.10.1.2 Linear regulators

A typical example of a linear regulator is shown in Figure 29.54. Basically, it is a high gain control circuit that continuously monitors the DC output voltage and automatically corrects the output so that it remains constant irrespective of changes in load current and input AC voltage. In the circuit shown, the regulated output voltage is 10 V from a 15 V unregulated supply. The control element in the circuit is formed by the Darlington pair (Q_2 and Q_3). The base current for the Q_3 is provided by Q_2 which gets adjusted by the error amplifier Q_1. The emitter of Q_1 is held at the Zener voltage (5.6 V) and under normal conditions, the base voltage of Q_1 will be 0.6 V higher than its emitter voltage. In case of a drop of voltage across the output, a portion of this drop appears across base of Q_1 resulting in a decrease in bias of Q_2 which then tends to increase base/emitter voltage. This will raise the collector voltage of $Q_{1,}$ increasing forward-bias of Q_2 and Q_3 which then tends to increase or correct the output voltage, restoring it to the set value.

Several features much as over voltage protection and current limiting currents are provided to protect the power supply from overload currents.

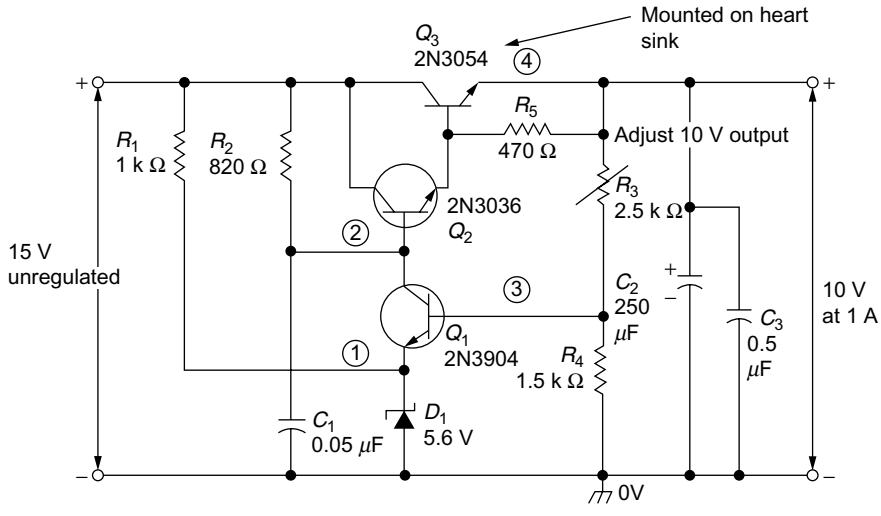


Figure 29.54 *Linear regulator power-supply circuit*

Linear power supplies have tight regulation band, low output noise and ripple. There are obvious disadvantages of low efficiency, bulky and heavy heat sinks, large power transformer, cooling fan, etc. All this makes them almost unfit for today's compact electronic systems.

29.10.2 IC Regulators

Monolithic IC regulators are used in most of the modern power supplies. The most common IC regulator in use has been the uA 723, although several other ICs are also available. The pin configuration of this IC is shown in Figure 29.55. This IC contains, internally, a reference source, error amplifier, series transistor and a current limiting resistor. The maximum output current and capability of the IC is 150 mA. However, the output current of the regulator can be increased by using an external power transistor in the output circuit.

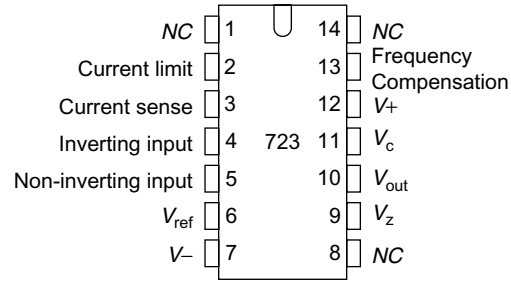


Figure 29.55 *μA723-pin configuration*

29.10.3 Three-pin Voltage Regulators

These are three pin devices (Figure 29.56) in which input is applied between the centre leg and the input terminal and the output is taken between the output terminal and the centre leg. They are available for fixed voltages like 5, 9, 12 V, etc. Most of the modern equipment makes use of these regulators.

Figure 29.56 *3 pin voltage regulator-L7805 (5 V)*

29.10.4 Switched Mode Power Supplies (SMPS)

Switched-mode power supply is also called switching power-supply or sometimes chopper controlled power supply. They use high-frequency switching devices such as bipolar junction transistors (BJTs), MOSFETS, insulated gate bipolar transistors (IGBTs) or thyristors (SCRs or Triacs) to take directly rectified line voltage and convert it to a pulsed waveform. Most small SMPS use BJTs or MOSFETS whereas IGBTs are generally found in large systems. SCRs or Triacs are used where their advantages of latching in the on state and high power capability are required.

Switching-mode power supply regulators are preferred over their linear counterparts for the following benefits:

Size and weight: Since the transformer and the filter run at a high frequency (in the range of 20 KHz to 1 MHz or more), they can be much smaller and lighter than the big bulky components needed for 50 Hz operation. Power density for SMPS compared to linear power supplies may easily exceed 20: 1.

Efficiency: Since the switching devices are ideally fully 'on' or fully 'off', there is relatively little power lost. So the efficiency can be much higher for SMPS than for linear power supplies, especially near full load. Efficiencies of SMPS may exceed even 85% whereas it is 50–60% for typical linear supplies.

With the advent of portable analytical instruments, the importance of optimising power utilisation has increased dramatically. There are now many ICs which are commercially available for controlling and implementing SMPS with relatively few external components.

29.10.4.1 Functional block diagram

The functional block diagram of a typical SMPS is shown in Figure 29.57. The four major blocks are as follows:

(a) The input rectifier and filter section
(b) High-frequency inverter section
(c) Output rectifier and filter section
(d) Feedback and control circuit.

The switched-mode power supply, as can be seen from the block diagram, is a relatively complex circuit. The AC mains are rectified and filtered. The high voltage DC is then fed to the high-frequency inverter. The operating frequency range is from 20 KHz to 1 MHz.

The high-frequency square wave so generated is stepped down by the high-frequency transformer and then rectified and filtered to produce the required DC output. The output is sensed, compared with a reference and pulse width modulated to get the desired regulation by the control circuit. The regulation of the output voltage is achieved by varying the duty cycle of the square wave. As the load is removed or input increases, the slight rise in the output voltage will signal the control circuit to deliver shorter pulses to the inverter. Conversely as the load is increased or input voltage decreases, wider pulses will be fed to the inverter. The voltage control circuit can be incorporated either in the primary or secondary side of the transformer.

In the inverter, since the switch (i.e. the transistor) is either 'on' or 'off', it dissipates very little energy resulting in a very high overall efficiency in the range of 70 to 80%. Also, the power

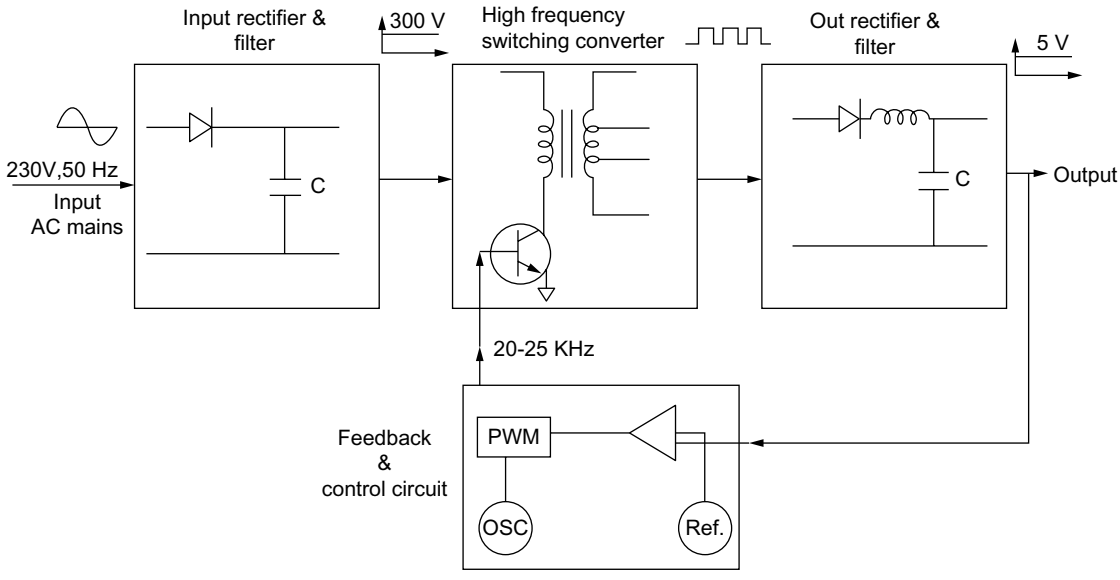


Figure 29.57 *Functional block diagram of a switched-mode power supply*

transformer size is reduced because of high-frequency operation. Hold-up time is greater for switched mode power supplies because it is easier to store energy in high voltage capacitors (200–400 V) than in lower voltage (20–40 V) filter capacitors common to linear power supplies. This is due to the fact that the physical size of the capacitor is dependent on its CV (capacitor × voltage) product while energy stored is proportional to CV^2. Hence, switched-mode power supply offers an irresistible combination of high efficiency, smaller size, wider input voltage range and good hold-up time.

The input section of a SMPS has some special circuit components which require consideration. A block diagram of the input circuit is shown in Figure 29.58.

The fuse provides safety from over current and voltage conditions. In addition to the fuse, flame proof or fusable resistors are also employed to provide an over voltage protection function.

This is following by the input transient voltage protection circuit. High voltage spikes caused by inductive switching or the natural causes like electrical storms or lightning, although of short

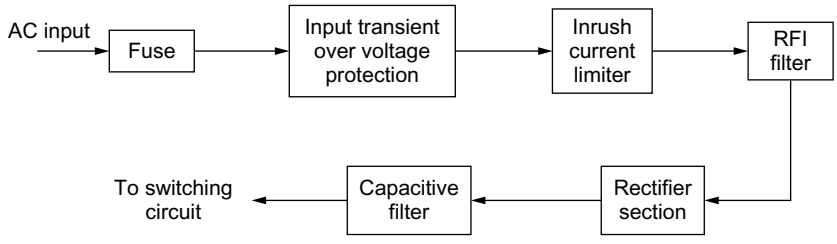


Figure 29.58 *Input section of a typical switched-mode power supply circuit*

duration, sometimes carry energy to prove fatal to the different devices operating on AC mains, unless they are successfully suppressed.

The most common suppression device in use is the MOV, metal-oxide varistor. MOV is used across the AC line input. Varistors are symmetrical non-linear voltage dependent resistors. At normal operating voltages, varistors are nearly open circuited. Upon application of a high voltage pulse, such as lightning charge, they conduct a large current, thereby absorbing the pulse energy in the bulk of the material with only a relatively small increase in voltage, thus protecting the circuit.

An off-the-line switching power supply may develop high peak inrush currents during turn-on because of the filter capacitors, which at turn-on present very low impedance to the AC lines, generally only their ESR (effective series resistance).

One method can be used to limit to inrush current is the use of a negative temperature coefficient thermistor placed in series with the AC input. Thermistors offer high resistance when the power supply is switched 'on', thus limiting the inrush current. As the, current starts to flow through the thermistor, it heats up. Because of their negative temperature coefficient, the thermistor resistance drops. The most common method of noise suppression at switching power supply AC mains is the utilisation of an LC filter for differential and common-mode RFI suppression.

29.11 HIGH VOLTAGE DC POWER SUPPLIES

While most voltage requirements in an analytical instrument can be satisfied with 'off the shelf' products, the high voltage requirements are usually addressed by a custom design for a specific application. Custom designed high voltage power supplies can be found in instruments for spectroscopy, capillary electrophoresis, mass spectrometry, electrospray, lasers, spectrometers, X-ray diffraction, X-ray fluorescence and many other analytical imaging and process applications.

Each application of High Voltage Power will require careful attention to critical variables. Voltage ripple, long and short term stability, repeatability and accuracy are important factors in the consideration of reliable scientific data. Also, as analytical instrumentation finds its way into production process control, reliability and quality are equally important in the considerations for high-voltage power-supply specification.

Although high voltage power supplies can be made by using an AC power line transformer with a secondary wound with the necessary number of turns, the units tend to be bulky. Therefore, in most of these equipments, a preferred method to produce high voltage DC is to use a DC to DC converter (often unregulated). In this technique, a low voltage DC is switched across the primary winding of a ferrite core transformer. The switching frequency could be 10–25 kHz. The transformer secondary has a large number of turns, and it produces high voltage AC. Voltage multiplier rectifiers then convert this secondary AC into a high value DC.

Voltage multiplier circuits (Figure 29.59) can provide only a very limited amount of current. Also, the regulator is poor because of the high values of output impedance which increases approximately with the number of stages used. Voltage doubler, tripler and quadrupler circuits are quite common.

Figure 29.60 shows the basic building blocks of most high voltage power supplies. The Power Input stage provides conditioning of the input power source. The input power source may have a wide range of input voltage characteristics. AC sources of 50–400 Hz at <24Vac to 480Vac are common. DC sources ranging from 5V DC to 300V DC can also be found. The power stage can

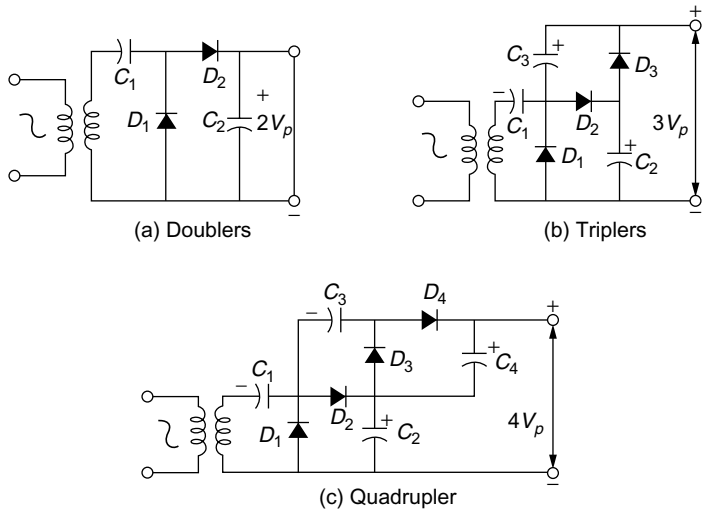


Figure 29.59 *Voltage multiplier circuits for high voltage power supplies*

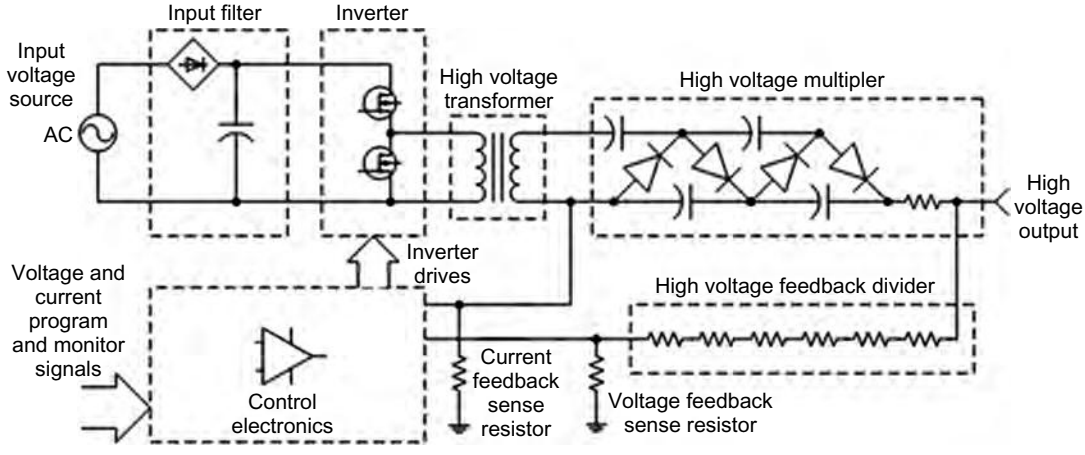


Figure 29.60 *Basic high-voltage power supply circuit (Courtesy: M/s Spellman High Voltage Electronics Corporation, USA)*

provide rectification and filtering for AC signals, filtering for DC signals and circuit protection. Also, auxiliary power sources to power the high-voltage power supply control circuits are typically part of the power input stage responsibilities.

The output of the power input conditioning stage is typically a DC voltage source. This DC voltage provides the energy source for the inverter stage. The inverter stage converts the DC source to a high-frequency AC signal. Many different inverter topologies exist for power supplies. The inverter is followed by a specially wound high voltage transformer.

The high voltage output stage is responsible for rectification and filtering of the high-frequency AC signal supplied by the high voltage transformer secondary. This rectification and filtering process invariably utilises high voltage diodes and high voltage capacitors. The high voltage output stage also provides feedback and monitoring signals which will be processed by the power-supply control circuits.

Control circuits are microprocessor controlled. They monitor all aspects of the high voltage power. However, the basic requirement which every control circuit must meet is to precisely regulate the output voltage and current as load, input power, and command requirements dictate. This is accomplished by a feedback control loop.

The safety aspects of high voltage use require careful attention. High voltage sources can be lethal. The novice user of high voltage should be educated on the dangers involved. A general guideline for safety practices is found in IEEE standard 510-1983 'Recommended Practices for Safety in High Voltage and High Power Testing'.

High voltages should be measured very carefully. Some moving coil metres enable you to measure voltages up to 3 kV. But they require about 50 uA current for full scale deflection which may be quite high for some high voltage supplies. Electrostatic voltmeters are preferred for measuring high voltages up to 50 kV with negligible loading on the supply voltage. The electrostatic metre has a non-linear scale, which makes it difficult to measure lower voltages accurately.

30

DIGITAL CIRCUITS

30.1 DIGITAL CIRCUITS

In analytical instrumentation, most circuitry is concerned with the amplification and processing of signals, which are available in an analogue form. However, the introduction of compact digital computers has made possible the digital manipulation of the analogue signals after they have been converted into a digital form. To do this, an analogue-to-digital converter is employed, which basically samples the analogue signal at a predetermined rate to get their digital equivalents. Using combinations of pulse circuits such as logic gates and flip-flops, it is possible to carry out arithmetical manipulations upon the series of digital values. Logic circuits are used to test whether a predetermined set of conditions has been obeyed and can route the path of signals accordingly. Flip-flops are used to store information in the form of noughts and ones, corresponding to a transistor being cut off, or switched on, respectively. They can be cascaded to get an electronic counter. Measuring frequency and voltage are two important applications of electronic counters. The availability of integrated circuit logic modules at low costs has led to an universal use of digital techniques. These modules are used in large quantities in digital computers, because of their small size, low power consumption and high reliability. The small size of MOS elements has enabled to have large-scale integration (LSI), in which thousands of elements are created on a single chip. The digital ICs are available in a big range as simple logic gates, memory units and even as complete data processing units like microprocessors.

There does not seem to be any area in modem analytical instrumentation where digital circuits in some form are not or will not be used. The basic reason for this is that digital circuits operate from defined voltage levels, and this reduces any uncertainty about the resulting output and behaviour of a circuit. Many circuits operate with voltages that can only be on or off (e.g. a light can be on or off, a motor can be running or stopped or a valve can be open or shut). All these are digital operations and would need digital circuit elements for their operation and control.

30.1.1 Binary Number System

The binary number system is used practically exclusively in digital computers. This is because of its most remarkable feature of simplicity – just two digits, namely 0 and 1. The binary system has a base of 2 and any number can be expressed in the binary number with powers of 2. For example, the number 10 in the binary number is 1010.

$$10 = 1.2^3 + 0.2^2 + 1.2^1 + 0.2^0$$
$$= 8 + 0 + 2 + 0$$
$$10_{10} = 1010_2$$

The subscripts 2 and 10 are used to indicate the base in which the particular number is expressed. The binary system is also a positional value system, in which each digit has its value expressed as powers of 2.

The zeros and ones in the binary notation are commonly called bits. This is an abbreviated form of binary digits. Most frequently, we deal with 8-bit combinations. An 8-bit unit is known as byte. Thus a byte represents numbers in the range 0–255. 4-bit units are often referred to as nibbles. Thus, a byte consists of two nibbles. 16-bit unit is generally known as word. A word thus, consists of two bytes and four nibbles.

Binary numbers consisting of many digits are often tedious to handle by the human operators. The digits of binary numbers are therefore bunched together in groups of three or four, octal or hexadecimal notation respectively. Their relationship is shown in Table 30.1.

To provide a shorthand notation for the system of logic based on a single valued function with two discrete possible states, Boolean algebra is used. This type of algebra, based on logical statements that are either true or false, is a very useful tool in design and troubleshooting of digital logic circuits. The validity of a Boolean statement can be verified by drawing a truth table.

Table 30.1 *Binary numbers and their equivalents*

Decimal	Binary	Octal	Hexadecimal
1	1	1	1
2	10	2	2
3	11	3	3
7	111	7	7
8	1000	10	8
9	1001	11	9
10	1010	12	A
11	1011	13	B
12	1100	14	C
13	1101	15	D
14	1110	16	E
15	1111	17	F
16	10000	20	10
17	10001	21	11
27	11011	33	1B

30.1.2 Truth Tables

Truth tables provide a tabular means of presenting the output side of logic devices for any set of inputs. Truth tables contain one column for each of the inputs and a column for the output. In basic truth tables, the column notations are usually H or L (for high and low) or, for binary notation, 1 or 0. For example in a logic circuit, the truth table can be represented as:

Input states		Output states
A	**B**	
0	0	0
0	1	0
1	0	0
1	1	1

30.1.3 Logic Circuits

Logic circuits are decision-making elements in electronic instruments. They are the basic building blocks of the circuits that control data flow and processing of signals. In most systems which use logic, the output function represents a voltage level, either high or low:

There are several ways to represent two-state 'yes' and 'no' decisions. Some of these are given below.

Yes	**No**
Open	Closed
1	0
Positive	Negative
True	False
High	Low
On	Off

30.1.4 Logic Convention

In digital circuits, 0 and 1 are represented by two different voltage levels, often called HIGH and LOW. The logic convention usually employed to relate these two entities is as follows (Figure 30.1):

In the positive logic convention, logic 1 is assigned to the most positive (HIGH) level of the voltage and logic '0' to the least positive (LOW) level.

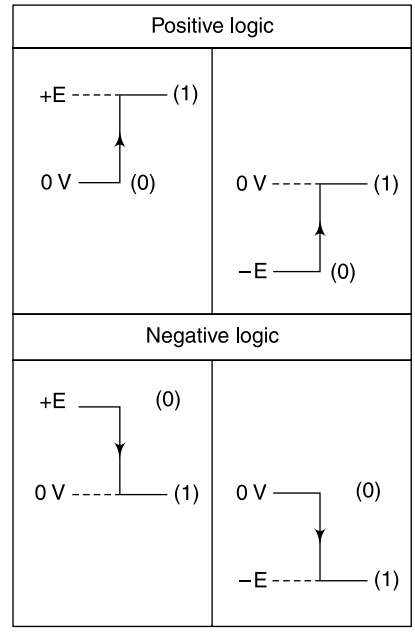


Figure 30.1 *Logic convention usually employed to represent two levels, high and low in digital circuits*

In the negative logic convention, logic 1 is assigned to the most negative (LOW) level and logic 0 to the least negative (HIGH) level.

This convention is important to understand the interpretation of digital data. For example, suppose 1001 (binary) data is presented on a set of binary coded decimal output lines. In positive logic, this would mean 1001 (binary) data is presented on a set of binary coded decimal output lines. In positive logic, this would mean 1001 (binary) 9 (decimal), while in negative logic, the same would mean 0110 (binary) = 6 (decimal).

30.2 TYPES OF LOGIC CIRCUITS

30.2.1 The AND Gate

When the presence of two or more factors are necessary to produce a desired result, the AND gate is employed. This implies that the output of the AND gate will stand at its defined '1' state if and only if, all of the inputs stand at their defined '1' states.

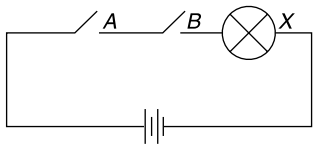
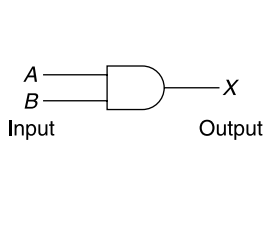


Figure 30.2 *AND gate equivalent circuit*



A	B	X
1	0	0
0	1	0
1	1	1
0	0	0

(b)

Figure 30.3 *AND gate (a) graphical representation (b) truth table*

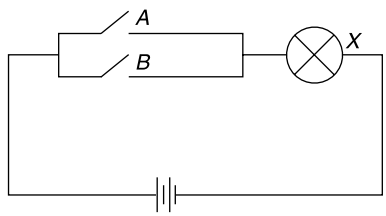


Figure 30.4 *OR gate equivalent circuit*

Figure 30.2 shows the functioning of an AND gate, which is like that of a set of switches in series. Only when they are all closed simultaneously, can there be an output (i.e. when the power is applied, both switches *(A* and *B)* must be closed before the lamp *X* will light). The logical notation is expressed in Boolean terms as $AB = X$ *(A* and *B* equal *X*). The graphical representation and the truth table of the AND gate is shown in Figure 30.3.

The AND gate is used primarily as a control element with one input regulating the traffic through others. If a word is to be allowed to pass through the gate, a '1' at the control input will open the gate. The '0's in the word are maintained in the right position at the output, because the gate is closed whenever there is at least one '0' input.

30.2.2 The OR Gate

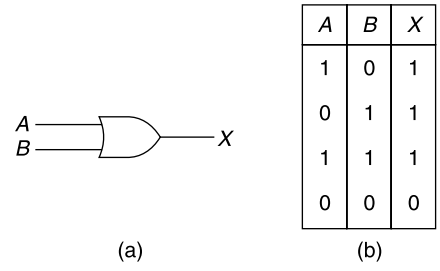
The OR gate provides the means of achieving a desired result with a choice of two or more inputs. This means that the output of the OR gate will stand at its defined '1' state if and only if one or more of its inputs stand at their defined '1' state.

As shown in Figure 30.4, the functioning of the OR gate is similar to a set of switches

connected in parallel; If either or both of the switches *(A* and *B)* is/are closed, power will be applied to the lamp *X* causing it to glow. The logical notation is expressed in Boolean terms as $A + B = X$ *(A* or *B* equals *X*). The truth table and graphical representation of the OR gate is shown in Figure 30.5.

The OR gate differs from the AND gate, in that a '1' at one input or the other input will give a '1' out-put: hence the OR gate. However, two '0' inputs give a '0' output and two '1' inputs give a '1' output.

The OR gate is designed to prevent interaction or feedback between inputs.



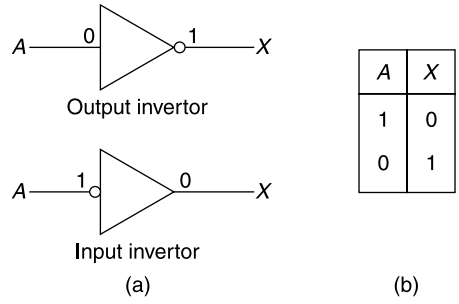
A	B	X
1	0	1
0	1	1
1	1	1
0	0	0

Figure 30.5 *OR gate (a) graphical representation (b) truth table*

30.2.3 The INVERTER (NOT) Gate

An INVERTER is used if it is necessary to change the state of information before it is used. Therefore, the output of an inverter is always the complement of the input (i.e. '0' becoming '1' and '1' becoming '0'). Figure 30.6 shows the symbol and truth table of an INVERTER circuit.

The INVERTER is never used by itself, but always in conjunction with another logic element. It is then represented by a small circle directly connected to the other logic element.



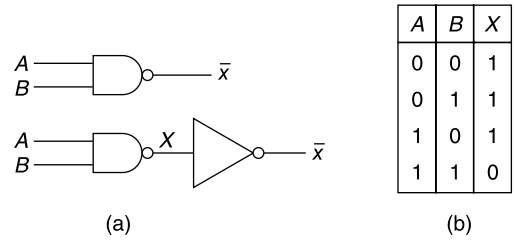
A	X
1	0
0	1

Figure 30.6 *Inverter (a) graphical representation (b) truth table*

30.2.4 The NAND (NOT-AND) Gate

When an AND gate has an inverter at the output, the combined circuit is called a NAND gate. This is in effect the opposite of the AND gate. When all inputs are '1' the output is '0'. Typical NAND gate circuitry is shown in Figure 30.7.

The functioning of this gate is equivalent to a number of switches in series, in parallel with a lamp (Figure 30.8) At least one switch must be open in order to make the lamp 'ON'.



A	B	X
0	0	1
0	1	1
1	0	1
1	1	0

Figure 30.7 *(a) Typical NAND gate configuration (b) Truth table of NAND gate*

30.2.5 The NOR Gate

The NOR gate is an OR gate with an inverter in its output circuit, which produces a NOT OR. Thus, when neither one input NOR the other is

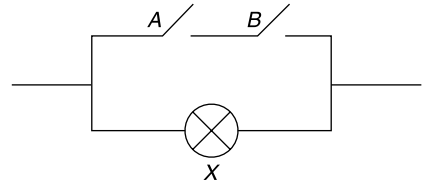
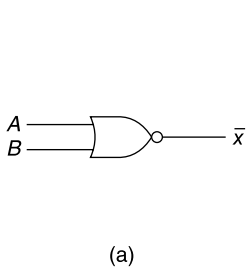


Figure 30.8 *Equivalent circuit of NAND gate*



A	B	X
0	0	1
1	0	0
0	1	0
1	1	0

Figure 30.9 *NOR gate (a) symbol (b) truth table*

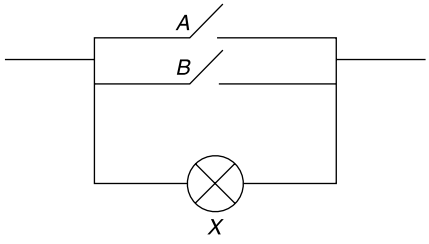
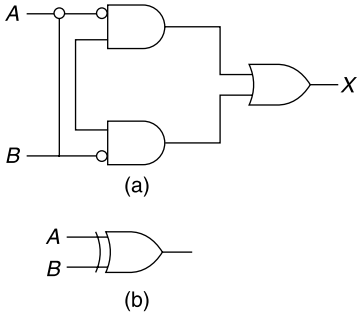


Figure 30.10 *Equivalent circuit of NOR gate*



A	B	X
0	0	0
0	1	1
1	0	1
1	1	0

(c)

Figure 30.11 *EX-OR gate (a) configuration, (b) symbol, (c) truth table*

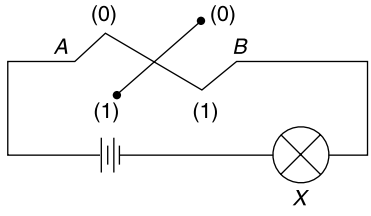


Figure 30.12 *EX-OR equivalent circuit*

a '1', the output is '1'; hence the name NOR gate. The symbol and truth table of NOR gate is shown in Figure 30.9.

The functioning of NOR gate is similar to that of a number of switches in parallel with a lamp. All switches must be open if the lamp is to glow (Figure 30.10).

30.2.6 The EXCLUSIVE-OR (EX-OR) Gate

The EX-OR gate may be regarded as a combination of AND and OR gates. It produces a 1 output only when the two inputs are at different logic levels.

A (two-input) EX-OR gate as shown in Figure 30.11 can be regarded as the combination of two AND gates and an OR gate.

An EX-OR gate has always two inputs, and its output expression is

$$X = A+B$$

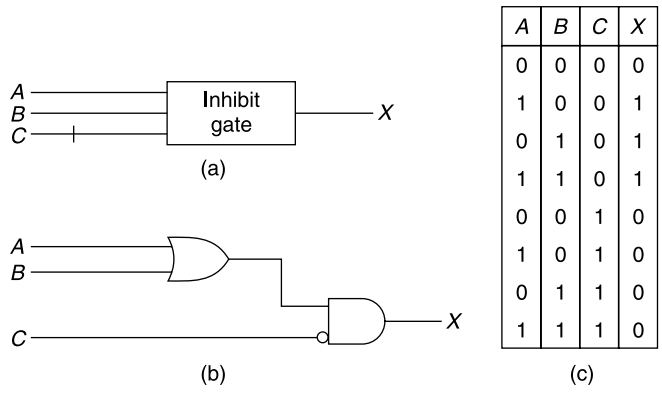
The equivalent circuit of an EX-OR gate is given in Figure 30.12.

30.2.7 The INHIBIT Gate

The INHIBIT gate is an OR gate with an inhibiting input. In this gate, the output will stand at its '1' state if, and only if, the inhibit input stands at its defined '0' state, AND one or more of the normal OR inputs stand at their defined '1' state.

The symbol and truth table of the INHIBIT gate is shown in Figure 30.13. The equivalent circuit of an INHIBIT gate is shown in Figure 30.14.

This gate is very useful for controlling inputs (A and B) by means of the inhibiting signal C. When the inhibiting signal is present ($C = 1$), the output is always OFF ($X = 0$), but when the inhibiting signal is absent ($C = 0$), the signals A and B can pass to the output X.



A	B	C	X
0	0	0	0
1	0	0	1
0	1	0	1
1	1	0	1
0	0	1	0
1	0	1	0
0	1	1	0
1	1	1	0

(c)

Figure 30.13 *INHIBIT gate: (a) symbol, (b) configuration (C) INHIBIT gate truth table*

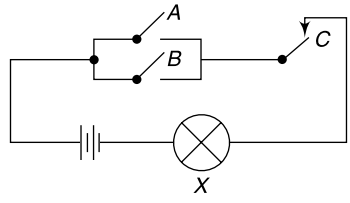


Figure 30.14 *Equivalent circuit of INHIBIT gate*

30.3 LOGIC FAMILIES

The nature of the basic logic elements depends on the properties of the electrical components used to realise them. In the early days of digital techniques, when diodes were largely used in the circuitry, it was natural to take the AND or OR gates as the basic elements. Later, when transistors came to the fore, it became natural to take basic logic circuits on the NAND and NOR gates. This is because the output signal of the transistor is opposite to the sign of its input. The most popular and most widely used circuits in modern digital equipment are the transistor-transistor logic and complementary metal-oxide semiconductor (CMOS) logic families. The logic circuits have become increasingly complex and bulky. The introduction of integrated circuits and in particular the development of planar technology have solved the problem of bulk along with the possibilities of obtaining several functions on one chip.

30.3.1 Transistor-Transistor Logic

The most popular and the most widely employed logic family is the transistor-transistor family. The various logic gates are manufactured in the integrated circuit form by most manufacturers of semiconductors.

The basic function of the Transistor-Transistor Logic (TTL) gate is the NAND function. This is shown in Figure 30.15. The input transistor has a number of emitters equal to the desired fan-in of the

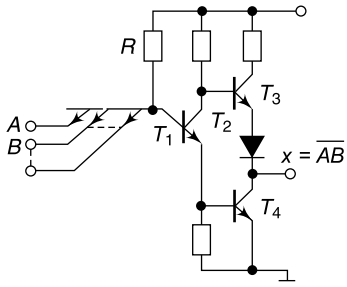


Figure 30.15 *Basic transistor-transistor logic (TTL) NAND gate*

circuit. The multi-emitter input has the advantage, that less space is required on the semiconductor chip for a given number of inputs.

If one or more inputs are at logic '0', current will flow through input resistance R. Consequently, the collector of the input transistor will be LOW. Only when all inputs are HIGH will the collector be HIGH too. The input circuit in fact gives normal AND operation. The next stage acts as a kind of phase splitter for driving the totempole' output. When T_2 is cut-off, T_4 remains cut-off too. However, if T_3 is on, T_4 will be on and T_3 off, resulting in a LOW output, which is a NAND function. The diode in the output chain ensures that T_3 is cut-off when T_4 goes on.

The popularity of TTL family rests on its good fan-in and fan-out capability, high speed (particularly Schottky TTL version), easy interconnection to other digital circuits and relatively low cost. The main characteristics of TTL logic are: propagation delay 10 ns, flip-flop rate 20 MHz, fan-out 10, noise margin 0.4 V, dissipation per gate 10 mW. The standard TTL gates are marketed as 74 series which can operate up to 70°C. However, 54 series are operatable up to a temperature of 125°C. Most IC packages contain more than one gate. For example IC 7400 is a quad 2-input NAND gates whereas 7420 is a dual 4-input NAND gates. There are various types of TTL families mostly differing only in speed and power dissipation.

The propagation time of 10 ns for most TTL circuits is too high for certain applications. This can be reduced by keeping out the transistor off saturation region by clamping the base collector junction to a voltage below the saturation level. This is achieved in Schottky TTL logic family. Schottky TTL logic gates are available in the integrated form as 74S/54S series. A low power Schottky TTL series is also commercially available as 54LS/74LS. The gates in the family are faster than standard TTL and consumes much less power. The internal power dissipation is minimised by increasing resistance values.

30.3.2 Emitter-Coupled Logic (ECL)

The ECL family provides another means of achieving higher speed of the gate. This differs completely from the other types of logic families, in that the transistors when conducting are not saturated, with the result that logic swings are reduced. For example, if the ECL gate is operated from 5 V, the logic '0' is represented by 0.9 V and logic '1' by 1.75 V.

30.3.3 CMOS Logic Families

The CMOS logic families offer significant advantages over bipolar transistor based logic circuits, particularly they feature very low power dissipation and good noise immunity.

The function of a MOS logic gate can be explained with the MOS inverter shown in Figure 30.16. When the input is '0', the driver MOST Q_1 (metal-oxide semiconductor transistor) is OFF and Q_2 (lower) pulls the output to the –V (minus the threshold voltage) level (i.e. logic '1'). The threshold voltage for MOSTs is defined as the gate voltage at which conduction between source and drain

first starts. When the input is '1', Q_1 is conducting and thus has a low impedance. The output voltage thus drops to logic '0'. When two or more driver MOSTs are connected in parallel to the inverter, a NOR gate is obtained. Replacing the driver MOST Q_1 in series gives, in negative logic, a NAND gate.

The main disadvantage associated with MOS gates is their limited switching speeds as compared to bipolar circuits. This is due to the very high impedance of the MOST devices, because of which stray capacitances cannot be charged quickly. Typical switching times are of the order of 1 μs. One way of improving switching times is to make use of CMOS techniques, in which both *p*-channel and *n*-channel MOSTs are employed. Figure 30.17 shows a typical configuration of CMOS inverter. With this arrangement, only one device is ON (low impedance) at a time, the other device being OFF (high impedance), resulting in a lower power consumption. With a '0' at the input, the *n*-channel MOST will be OFF and the p channel will be ON, which gives an output at logic '1'. With a '1' at the input, the conditions are reverse, with an output voltage at logic '0'. The advantage of the circuit is that, when the impedance of one device is decreasing, the impedance of the other is increasing, giving a push-pull effect, which narrows the transition region, giving sharper transfer characteristics and thus increasing the speed.

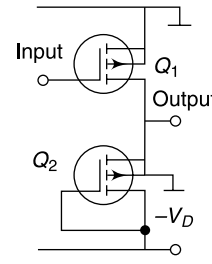


Figure 30.16 *MOS inverter logic gate configuration*

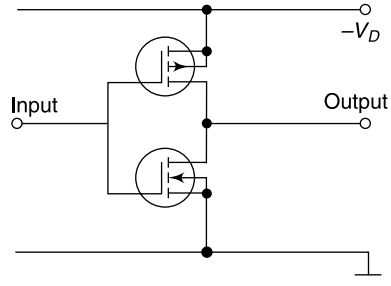


Figure 30.17 *Circuit configuration of CMOS inverter*

To construct NOR gates, a number of *p*-channel gates connected in parallel are used. Similarly, a NOR gate is made using a number of such devices in series.

The great advantage of MOS technique is the possibility of high density packing of a large number of devices. The technique is most suitable for construction of large-scale integrated circuits, rather than simple gates and flip-flops. Commercially available CMOS gates are available as 4000 series. For example, Quad 2-input AND gate in CMOS comes as 4081 (7408 TTL), while Quad 2-input NOR gate as 4001 (7402 TTL).

30.3.4 Characteristics of Integrated Circuit Logic Gates

The various families of logic gates are associated with different characteristics. This means that one of them may prove to be best suited for a particular application. The important characteristics are as follows:

Speed of Operation: The speed of operation of a logic gate is the time required by it to pass from one state to another. This is generally expressed in terms of the propagation delay. The propagation delay of a gate takes place on account of the switching time of a transistor and rise time of the switching input voltage. The rate at which a flip-flop can switch from one state to the other is called its clock rate.

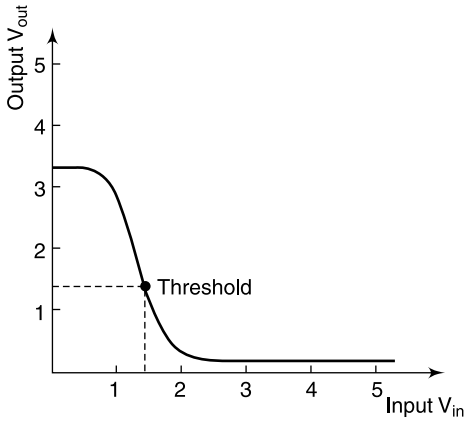


Figure 30.18 *Positive logic threshold level in TTL gate*

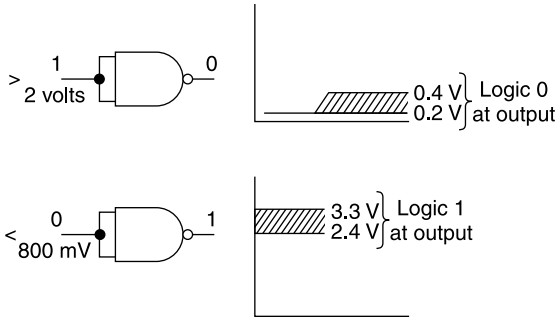


Figure 30.19 *Logic levels at gate outputs*

Threshold Value: The threshold voltage of a gate circuit is defined as the input voltage at which the gate just switches from one state to the other. For TTL logic family, the threshold voltage is 1.4 V (Figure 30.18). However, the maximum input voltage that will definitely give logic '0' is 0.8 V, where as the minimum input voltage giving a definite logic '1' is 2.0 V.

For correct operation of a gate, specific voltage levels must be applied. For example, for a TTL gate, logic '1' has a typical voltage of 3.3 V and a minimum value of 2.4 V (Figure 30.19). On the other hand, logic '0' is typically 0.2 to 0.4 V.

Noise Margin: In order to avoid errors in a logic system due to parasitic voltages like spikes, logic devices should have a wide voltage swing between the two states (i.e. logic devices should have wide noise margin). Figure 30.20 shows noise margins in high and low states.

Power Dissipation: Power dissipation generally implies the power required for operation of the logic device. As circuit complexity increases, the power dissipation per gate must decrease, so as to limit the amount of heat which may be dissipated in the semiconductor junctions.

Fan-in and Fan-out: The *fan-in* of a gate is the number of inputs which can be connected to the gate without seriously affecting its performance. Similarly, the *fan-out* is defined as the maximum number of circuits that can be connected to its output terminals without the output falling outside the specified logic levels.

30.4 CATEGORIES OF ICS BASED ON PACKING DENSITY

Small-scale integration (SSI) means integration levels typically having about 12 equivalent gates on chip. Available in 14 or 16 pin DIP or Flat packs.

Medium-scale integration (MSI) means integration typically between 12 and 100 equivalent gates per IC package. Available in 24-pin DIP or Flat pack or 28-pin ceramic chip carrier package.

Large-scale integration (LSI) implies integration typically up to 1,000 equivalent gates per IC package. Includes memories and some microprocessor circuits.

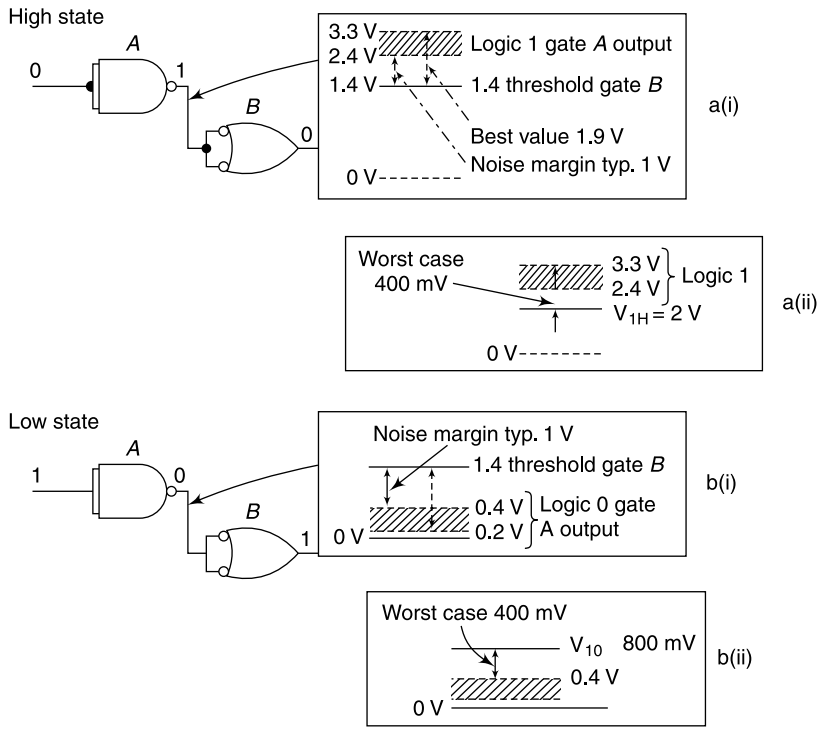


Figure 30.20 *Noise margins in TTL gate (a) high state (i) typical 1 V (ii) worst case 400mV (b) low state (i) typical 1 V (ii) worst case 400 mV*

Very large-scale integration (VLSI) means integration levels with extra high number of gates, say up to 1,00,000 gates per chip. For example, a RAM may have more than 4,000 gates in a single chip, and thus comes under the category of VLSI device.

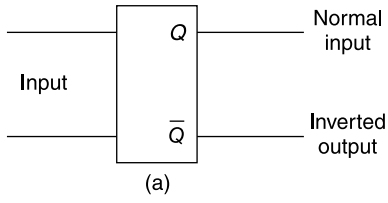
30.5 TYPICAL DIGITAL INTEGRATED CIRCUITS

30.5.1 Flip-Flops

A flip-flop is a two-state electronic device, which can be either turned on or turned off when commanded to do so. It is a bistable logic element with one or more inputs and two complementary outputs. A flip-flop essentially remains in its last state, until a specific input signal causes it to change state. Because of the ability of the flip-flop to store bits of information (1 or 0), it has memory characteristics. It is thus one of the most important basic building blocks in digital circuitry.

Nearly all flip-flops have two output levels (Figure 30.21a) designated as Q and $\bar{Q}$ on which the true state (Q) and the complement ($\bar{Q}$) of the stored function is available. The input terminals may receive either discrete level or pulse signals depending on the circuit. A flip-flop is known by several other names; the most common being bistable multi-vibrator, binary or latch.

The truth table (Figure 30.21b) indicates the two possible output conditions for a flip-flop and the corresponding definition for the state. There are many forms of flip-flops, each of which has its



Outputs		Flip-flop
1	0	State
High	Low	1
Low	High	0

(b)

Figure 30.21 *(a) Basic flip-flop configuration (b) Truth table of a basic flip-flop circuit*

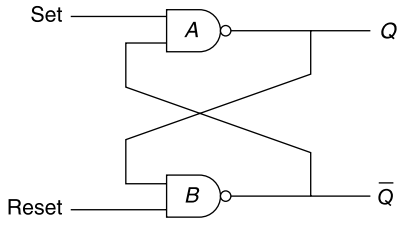


Figure 30.22 *R-S flip-flop configuration*

specific features. However, all those various forms of flip-flops contain essentially the same bistable element, the RS flip-flop.

Reset-set Flip-flop or the R-S Flip-flop: A basic flip-flop can be constructed using two NAND gates (Figure 30.22) cross-coupled to the inputs. When power is applied, opposite states will appear on the outputs of gates *A* and *B*. If the *Q* output of gate *A* is '1', this '1' will be applied to the input of gate *B* whose output ($\overline{Q}$) will then become '0'. When this '0' is applied to the input of gate *A*, a '1' will remain on the *Q* output of gate *A*. Thus, the gates are latched into a stable state. Since output *Q* is high, the flip-flop is in the high or '1' state. Any additional pulse at the SET input will have no effect on the output. However, when a pulse is applied to the RESET input, the output reverses or flips. Further, pulses at the RESET input have no effect on the outputs. Switching the inputs again causes the output to flop back to their original condition.

The flip-flop is like a toggle switch, either in one position or the other; and once the change-over has been made, repeating the action has no further effect. The condition is stable, either way.

The R-S flip-flop is commonly used when there are no possibilities of simultaneous set and reset inputs. If both the inputs are simultaneously enabled with a low pulse, both outputs will go high for the duration of, the pulse. Removing the enabling pulses will make the flip-flop to go over to an indeterminate state (i.e. it could latch in either the '1' or '0' condition). The stable state to which it will finally race depends upon the relative time delay of the two NOR gates used in the circuit. Though R-S flip-flop in itself has limited applications, it is the basic building block of flip-flop chains in integrated circuit form. They normally utilise a clock input to synchronise the changes from one state to another.

Clocked Flip-Flops: The problem of races can be avoided by using a synchronous or a clocked flip-flop, in which the inputs can only be applied in coincidence with a clock signal. It is convenient to enable or condition the flip-flop by applying the appropriate levels to the inputs first and then arrange for the flip-flop to change state on receipt of a pulse from another source. The pulse is called clock, which may be from an oscillator circuit. Thus, the clock signal is a signal that initiates action at regular spaced intervals. Figure 30.23 shows a typical clock signal. The operations in the system take place at the time when the transition occurs from 1 to 0 (falling edge) or 0 to 1 (rising edge).

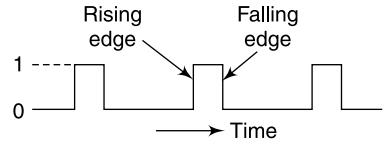


Figure 30.23 *Typical clock signal*

Clocked flip-flops are designed to change states at the appropriate clock transition and will rest between successive clock pulses. Figure 30.24 shows the circuit configuration of a clocked R-S flip-flop. The flip-flop section (gates *C* and *D*) is identical to the R-S flip-flop. In addition, there is a circuit arrangement which applies clock pulses to either the SET or RESET input terminal of the flip-flop. If the SET input line is enabled with a high signal, gate *A* will be enabled when a high pulse is presented at the clock input. When gate *A* is enabled, it will provide a low SET signal to the R-S flip-flop, which will go to the '1' condition.

If the SET input line has a low level and the RESET line is enabled with a high level, gate *B* will be enabled when a high clock pulse occurs. When gate *B* is enabled, a low RESET signal will be presented to the flip-flop and it will go to the '0' condition. It may be noted that the flip-flop will only change state when a clock pulse is applied.

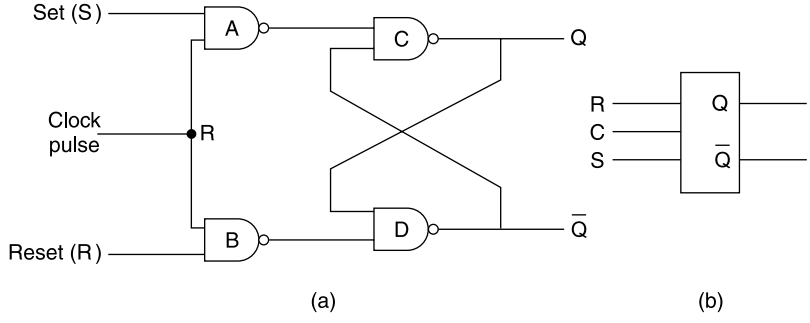
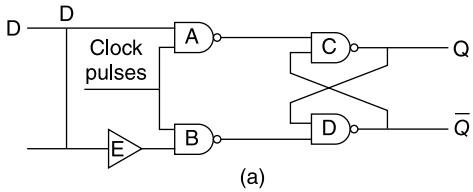


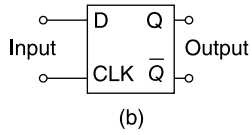
Figure 30.24 *Clocked R-S flip-flop: (a) circuit configuration, (b) symbol*

D Flip-flop: One way of avoiding the intermediate state found in the operation of the simple R-S flip-flop is to provide only one input, which can be either high or low. This input is called the *D* input or data input and the flip-flop thus constructed is called *D* flip-flop. Figure 30.25 shows the circuit arrangement of a *D*-type flip-flop. A '1' or a '0' applied to this input is passed directly to one of the inputs of the flip-flop proper and inverted to the other input.

From Figure 30.26, it is obvious that whatever information is present at the *D* input prior to and during the clock pulse is propagated to the *Q* output when the clock pulse is applied, while the inverse of that information appears at the $\overline{Q}$ output. The flip-flop is thus set in the '1' state if the *D* input is made '1' and in the '0' state if the *D* input is made '0'. This type of circuit is known as edge-triggered *D* flip-flop and is one of the most commonly used in computers. In *D*-type latch, which is similar to the *D* flip-flop except that it can change states during the HIGH portion of the clock signal (i.e. as long as clock is HIGH), *Q* will follow the *D* input even if it changes when the clock goes LOW, *Q* Will store (or latch) the last value it had and the *D* input has no further effect.

J-K Flip-flop: One of the most useful members of the flip-flop family is the *J-K* flip-flop. A unique feature of the *J-K* flip-flop is that it has no ambiguous state. It is the most widely used flip-flop type in logic circuitry and is the ideal memory element to use.





D	Q
1	Goes to 1 on positive logic of clock
0	Goes to 0 on positive edge of clock

Figure 30.25 *D flip-flop: (a) circuit configuration (b) representative symbol, (c) truth table for D flip-flop*

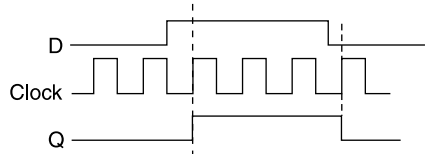


Figure 30.26 *Positive edge triggering in a D flip-flop*

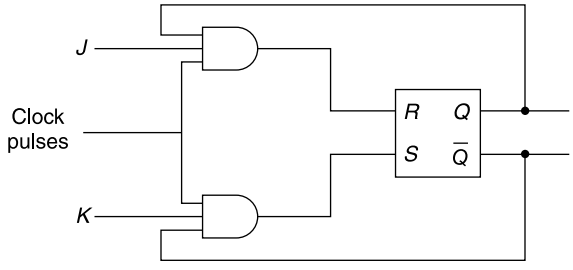


Figure 30.27 *J-K flip-flop circuit arrangement*

A *J-K* flip-flop often has more than one *J* input and more than one *K* input. In this case, one *J* input and one *K* input are generally connected together for use as input for clock pulses. This kind of circuit arrangement is shown in Figure 30.27. The operation of the circuit is controlled as follows:

(a) A clock pulse will not cause any changes in the state of the flip-flop if neither the *J* nor the *K* input is activated.
(b) If both the *J* and *K* inputs are activated, the flip-flop will change state when the next clock pulse is received.
(c) The *J* and clock pulse inputs used together set the flip-flop in the set position, while the *K* and clock pulse inputs reset it.

It may be observed that propagation delay time prevents the *J-K* flip-flop from racing (toggling more than once during a positive clock edge). This is because the outputs change after the positive clock edge has struck. By then, new Q and $\bar{Q}$ signals are too late to coincide with positive spikes driving the AND gates. Commercially available *J-K* flips-flops also give the facility of a synchronously setting the output to 1 (pre-set) or 0 (clear). Integrated circuit DM 74733 has two *J-K* flip-flops with clear facility. Dual *J-K* with separate pre-set and clear facilities are available in DM 74766 and with common pre-set and clear in DM 7478.

It may be noted that the output of *J-K* flip-flop becomes stable only when the clock pulse goes to zero. To avoid this problem, a special form of the '*J-K* flip-flop known as the '*J-K* master-slave flip-flop' is used. As the name implies, it comprises two flip-flops – a master and a slave – being triggered at positive and negative edge of the clock respectively. This is advantageous, because a sequence of such master-slave flip-flops could then be triggered simultaneously by a clock pulse derived from the same source, without ambiguity. Figure 30.28 shows master-slave *J-K* flip-flop.

If *J* and *C* inputs of the *J-K* flip-flop are connected together it becomes a toggle or a *T* flip-flop, whose output toggles whenever a '1' is applied at the input.

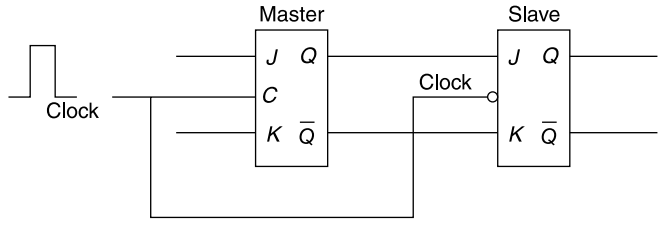


Figure 30.28 *Circuit configuration of master-slave J-K flip-flop*

Flip-flops are the basic blocks, using which, it is possible to build many other useful sequential circuits, some of which are described in the subsequent section.

30.5.2 Counters

A counter is a sequential circuit consisting of a series of flip-flops, which go through a sequence of states on the application of pulses at its input. Counters are constructed out of *T* or *J-K* flip-flops.

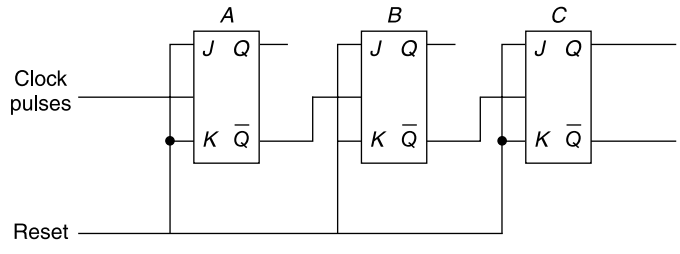
In Figure 30.29 it may be seen that three *J-K* flip-flops are combined to give a three-bit counter. One output gives the information at each stage and the other is to carry to the next stage. Let us assume that all outputs at *Q* are initially cleared to '0'. A pulse applied to the input of the first stage switches its counting output *Q (A)* to '1', while the other output $\bar{Q}$ *(A)* becomes '0' and thus has no effect on the second stage. The circuit has now counted the first pulse (binary 1). On the receipt of the second pulse, the count output $\bar{Q}$ (A) of the first stage switches back to '0', while the carry output *Q (A)* flips to '1'. This switches the second stage *(B)* to count '1'. The circuit has now counted two, which in binary notation is 10. The third pulse switches stage one to count '1', *Q (A)*, but the second stage is not switched. The count is 11, binary notation for three. The counting continues in this fashion until the circuit output is one-one-one, which as we know is 7 in binary notation. The eighth count resets the circuit to zero. The pulse diagram of a 3-bit binary counter is shown in Figure 30.29a. Figure 30.29b gives truth table of this counter.

Decimal counters are binary counters which employ four flip-flops and are so constructed that they count up to 9 (instead of 15) and reset to 0000 on receipt of the tenth input pulse. By cascading more flip-flops to the chain, we can build a ripple counter of any length. Eight flip-flops give an 8-bit ripple counter, 12 flip-flops result in a 12-bit ripple counter and so on.

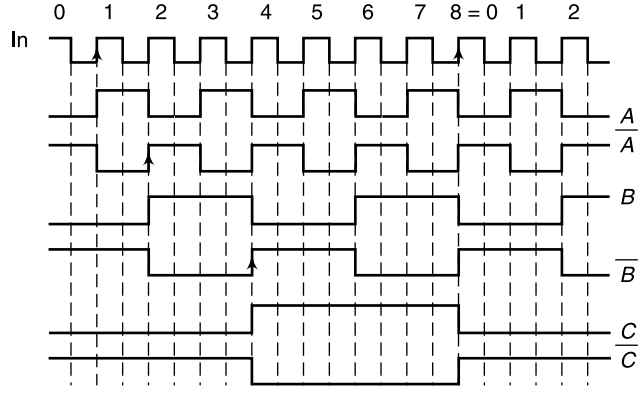
The binary counters as shown in Figure 30.29 are also called ripple counters, because the carry moves through the flip-flops like a ripple on water. Because of this, the most significant flip-flop cannot change states until three propagation delay times have elapsed. Because the carry has to propagate from the least to the most significant flip-flop, ripple counters are too slow for some application. This problem is overcome by using synchronous counters, in which all flip-flops clock simultaneously. Because of this, the correct binary word appears after one propagation delay time rather than three, thus offering high speed of operation. For an 8-bit counter, the maximum binary number is 1111 1111, which is equivalent to decimal 255. Similarly, for 16-bit counter, the binary number and its decimal equivalent are

1111 1111 1111 1111 = 65,535

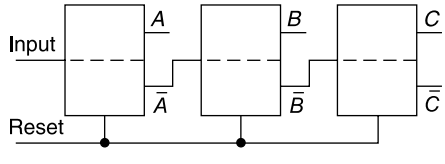
To design a counter, one has to choose the kind of flip-flop to be used and decide the number of flip-flops required.



(a)



(b)



In	A	B	C
0	0	0	0
1	1	0	0
2	0	1	0
3	1	1	0
4	0	0	1
5	1	0	1
6	0	1	1
7	1	1	1
8 = 0	0	0	0

(c)

Figure 30.29 *(a) Logic diagram of a 3-bit counter (b) Timing diagram of a 3-bit counter, (c) Truth table of a 3-bit counter*

These counters are capable of counting only upwards from zero to some maximum count and then reset to zero. Counters have also been designed to count in either direction, which are called up/down counters. These counters have an up/down input, which is used to control the counting direction, that is, one type of logic level causes the counter to count up from zero and the other logic level applied to it causes the counter to count down, say from the 1111 to 0000 (in 4-bit counters). In some up/down counters, two separate clock inputs are employed, one for counting up and the

other for counting downwards. Up/down counters fall in the class of controlled counters, that is, where the counting sequence can be changed by changing the value of a control signal.

A wide variety of counters are available commercially as standard integrated circuit packages. They avoid necessity of constructing counters using individual flip-flops. Among the popular counters are DM 7492, which is a divide by 12 counter, and a binary counter. DM 74191, DM 74192 are synchronous up-down counters.

30.5.3 Registers

A register is a group of memory elements employed to store binary information. Registers have an important place in digital computers, because the very operation of computers is based on transferring binary information from one register to another and carrying on certain operations before it is again transferred. The simplest register is a flip-flop.

In digital circuitry, a register usually consists of parallel latches. It can represent a number in the range from 0 to 2^n–1, where n is the number of latches in parallel. The register works under the control of a clock, which signals when the register should record the input, the data at the last latch always appears at the output. Registers are internal to the microprocessor and are very important because of the rather lengthy process involved in accessing data-in memory. For example, intermediate results can be temporarily kept in registers, rather than returning them to main memory repeatedly. Hence, the number of programmable registers in the microprocessor (central processing unit) is very important.

Shift Registers: A shift register is a group of serially connected flip-flops that is used for the temporary storage of information. They can also be used for shifting of the information stored in the register either one position to the right or left with each clock pulse. This is accomplished by gating the outputs of the flip-flops to the appropriate inputs for performing either a left or right shift. This shift direction is controlled by a mode input.

A shift register can be built using series connected *R-S*, *J-K* or *D*-type flip-flops. They are so connected that the output of each flip-flop becomes the input to the next flip-flop. As the register is clocked, the data is shifted one position to the left or right for each clock pulse. They are, however, constructed using a number of integrated flip-flops of TTL or CMOS families. The capacity of an integrated shift register ranges from 4 bits in the TTL family to 2048 bits in the CMOS family. CMOS shift registers are usually only of the serial-in-serial-out type, because of the many stages involved. There are not enough pins for parallel-input or output connections.

Methods of Using Shift Registers: Shift registers can be used in any one of the four ways:

(a) Serial-in/parallel-out
(b) Parallel-in/serial-out
(c) Serial-in/serial-out
(d) Parallel-in/parallel-out

With a serial-in/parallel-out shift register (Figure 30.30a) data are fed in the serial form, and when the complete word is stored, the bits are read off simultaneously from the output of each stage.

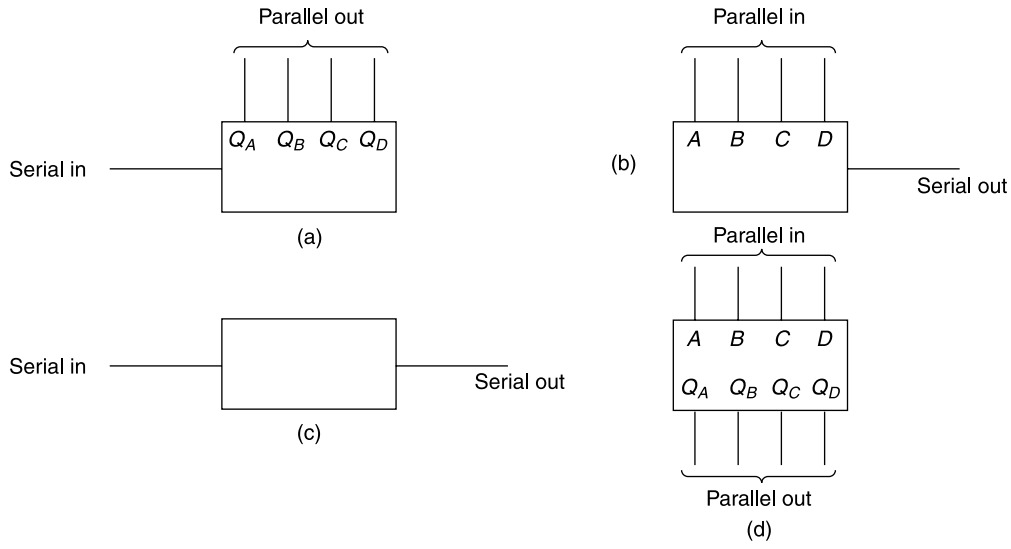


Figure 30.30 *Methods of using a shift register (a) serial-in/parallel-output, (b) parallel-in/serial-out, (c) serial-in/serial-out, (d) parallel-in/parallel-out*

Figure 30.30b shows parallel-in/serial-out shift register in which the data is stored, after clearing all the stages, in each flip-flop. The data are then read out serially (i.e. one bit at a time under the control of the clock).

The serial-in/serial-out shift register (Figure 30.30c) merely acts as a temporary delay circuit. The data are read out in the same order in which it has been stored.

Similarly parallel-in/parallel-out shift registers (Figure 30.30d) act as a temporary storage.

30.5.4 Multiplexer

A multiplexer is a logic circuit which accepts several data inputs and outputs only one of them at a time. In essence, it behaves as a multi-position switch which operates under the control of SELECT or ADDRESS inputs. Figure 30.31 shows the representation of a digital multiplexer.

Multiplexers are available in the form of integrated circuits. For example, 7415 lA is an eight input multiplexer which gives out complementary outputs.

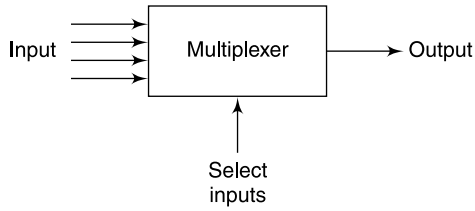


Figure 30.31 *Symbolic representation of a multiplexer*

Other popular multiplexers are 74153, which is dual 4:1 multiplexer and 74150, which is 16:1 multiplexer.

30.5.5 Demultiplexer

A demultiplexer performs the reverse operation of multiplexer. It receives a single input and distributes it over several outputs. The SELECT input code determines to which output the data

input will be transmitted. Figure 30.32 shows a schematic diagram of a demultiplexer.

A typical example of demultiplexer is 74155, which is a dual 1: 4 demultiplexer. It converts line inputs to 4-line outputs.

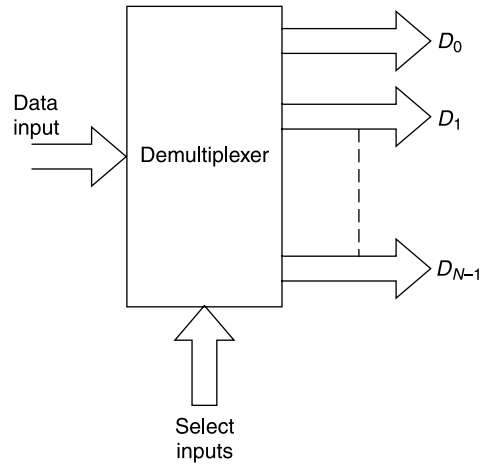


Figure 30.32 *Schematic representation of a demultiplexer*

30.5.6 Encoders

In digital circuits including microcomputers, the data are handled in the binary form, whereas the most common language of communication is decimal numbers and alphabetic characters. Therefore, there is a need to devise interface circuits between digital systems and human operators. Several binary codes have been developed which carry out the function of code conversion. The process of generating binary codes is known as encoding.

Some of the commonly used codes are decimal-to-BCD encoder, octal-to-binary encoder and hexadecimal-to-binary encoder. 74147 IC is a priority encoder which can be used for decimal-to-BCD conversion. The block diagram of 74147 is given in Figure 30.33. Similarly 74148 IC provides octal-to-binary encoding.

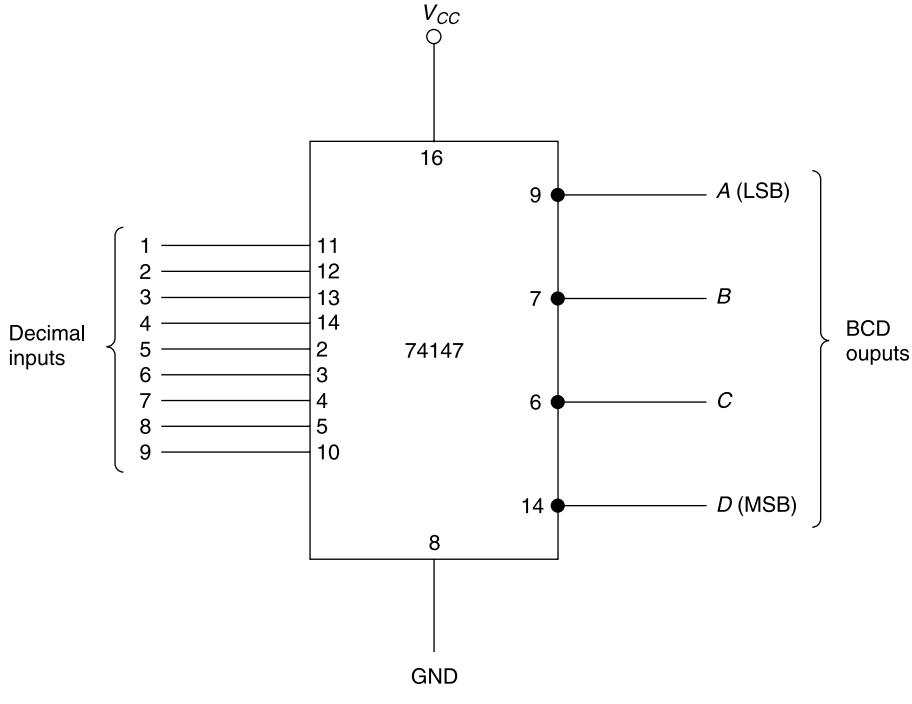


Figure 30.33 *Block diagram of a 74147 decimal-to-BCD encoder*

Hexadecimal code is commonly used in microcomputers, especially when long binary words are handled. Hexadecimal-to-binary encoder can be realised by using two 74148 octal-to-binary encoders and a data selector.

30.5.7 Decoders

A decoder is a logic circuit which converts the input bit binary code into an appropriate output signal, to identify which of the possible 2^n combinations is present. The most commonly used decoder is BCD-to decimal decoder, which provides decoding from 4 line-to-10 line decoding function. The input is 4-bit binary information, out of which only 10 BCD input codes are used. Correspondingly, there are 10 output pins.

In the digital display systems, the digits are displayed on seven-segment light emitting diodes (LEDs). Therefore, it is more convenient if the BCD code is decoded into seven-segments. Thus, BCD-to-seven-segment decoder is the most popular display device used in digital systems. Figure 30.34 shows the block diagram of BCD-to-seven segment decoder along with seven-segment LED (Figure 30.35) display unit. The decoder circuit has four input lines for BCD data and seven output lines to drive a seven-segment display, *a* through *g* outputs of decoder are to be connected to *a* through *g* inputs of the display, respectively. The outputs of the decoder can be active-low or active-high and the seven segments of the display may be seven cathodes, with a common anode or seven anodes with a common cathode. The decoders normally include drivers in the chip itself. The typical examples of BCD-to-seven-segment decoder driver ICs are 7446A and 7447A (active-low, open collector) and 7449 (active-high, open collector).

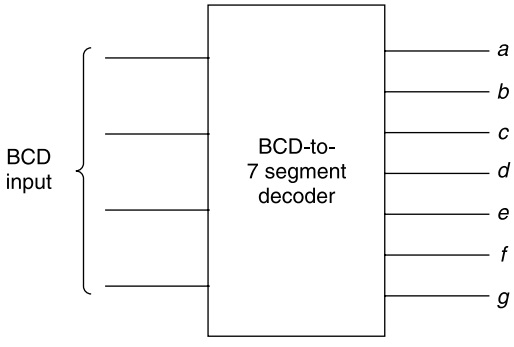


Figure 30.34 *Block diagram of BCD-to-7 segment decoder*

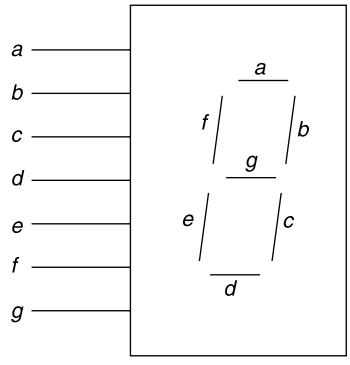


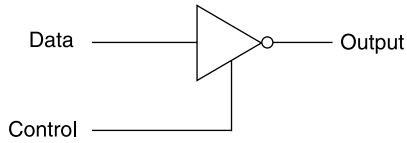
Figure 30.35 *7-segment display arrangement*

30.5.8 Tristate Logic

Tristate gates are designed so as to give output in three distinct states. Besides the normal two states of logic '1' (high) and logic '0' (low), a third state having a very high output impedance is available in tristate gates.

Figure 30.36 shows two possible arrangements of tri-state buffers. When the control signal is high, the switch is off and no signals flow through the device. When the control line is low, the switch is on, and the input signals are passed through to the output.

Tristate buffers are usually used at each outlet of data and address bus. This is one of the first ICs a signal encounters as it comes into the computer.



(a)

Data	Control	Output
1	1	0
0	1	1
0	0	High *z*
1	0	High *z*

(b)

Figure 30.36 *TTL tristate inverter: (a) symbol, (b) truth table*

30.6 SEMICONDUCTOR MEMORIES

A digital memory is an array of binary storage elements arranged in a manner that it can be externally accessed. The memory array is organised as a set of memory words. Each word consists of a number of single bit storage elements called memory cells. The word length of a memory word is typically one, four, or eight memory cells. Therefore 1-bit, 4-bits or 8-bits (byte) of information can be stored by the memory word respectively. The memory capacity is the product of the number of memory words and the number of memory cells in each word. It is measured in bits and frequently expressed in kilobits where 1 kilobit = 2^{10} = 1024.

30.6.1 Random Access Memory

Random access memory (RAM) is used in a microprocessing system to store variable information. The CPU under programme control can read or change the contents of a RAM location as desired. RAMs are a generic category that encompasses all memory devices in which the contents of any address can be accessed at random in essentially the same time as any other address.

There are two types of RAMs: static and dynamic. Both dynamic and static MOS random access memories are popular; the dynamic ones for their high circuit density per chip and low fabrication costs and the static RAMs for single power supply operation, lack of refresh requirements and low power dissipation. Bipolar RAMs are used for very high speed scratch-pad memories.

Dynamic RAM: In the dynamic RAM, information is stored as electrical charge on the gate capacitance of MOS transistors. Since these capacitors are not perfect, the charge will leak away and the information is likely to be lost with time if the charge is not periodically refreshed. This can be done in several ways and depends upon the type of device in use.

Static RAM: Static RAM does not need to be refreshed, as the memory cells are bistable and similar in design to conventional flip-flops. In general, a static RAM consumes more power than its dynamic counterpart.

However, it requires less support circuitry. Also, there are no problems of synchronising the memory refresh cycles with normal CPU read and write operations.

When the information is stored in the memory, it is written into the memory. When information is retrieved from a semiconductor memory, it is read from the memory. These are the only two functions that are done to static memories. Writing information into a memory is done in a write cycle. Reading information from a memory is done in a read cycle. The term cycle means a fixed period of time required to perform the function of writing into or reading from a memory. In fact, the electrical data or information is stored as a level of DC voltage. One DC voltage

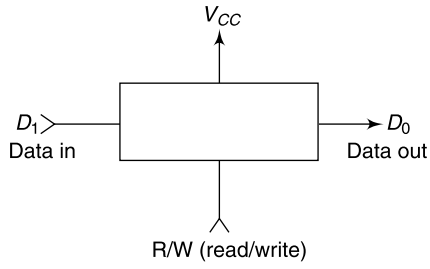


Figure 30.37 *Block diagram of one-bit memory*

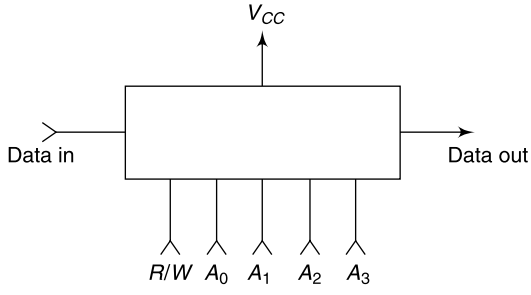


Figure 30.38 *Block diagram of a 16-bit memory*

level corresponds to a '1' stored in the memory. A different DC voltage level corresponds to a '0' being stored in the memory.

In a semiconductor memory (Figure 30.37), data are entered on an input pin on the physical device labelled 'data in'. Data being read from a memory, are read on a device output pin labelled 'data out'. Therefore, one-bit memory device will have four major physical connections: power input (V_{CC}), data input (D_1), data output (D_0) and read cycle or write cycle (*R/W*). If we want 16 bits of information storage, four address pins will be required ($2^4 = 16$) in the memory device (Figure 30.38). Under those conditions, no particular sequence will be needed to read or write information in the memory.

Most semiconductor RAM is volatile (i.e. the stored information is lost when the power supply is removed). The problem is avoided by using a battery-maintained power supply.

30.6.2 Read-Only Memory (ROM)

In a microprocessor based system, ROMs are normally used to hold the program of instructions and data constants such as look-up tables. Unlike the RAM, the ROM is non-volatile (i.e. the contents of the memory are not lost when the power supply is removed). Data stored in these chips is either unchangeable or requires a special operation to change. This means that removing the power source from the chip will not cause it to lose any data.

There are five basic ROM types:

- ROM
- PROM
- EPROM
- EEPROM
- Flash Memory

(a) *Mask programmed ROMs* which are programmed by the manufacturer to the user's requirements. ROM chips contain a grid of columns and rows and use a diode to connect the lines if the value is a (1). If the value is (O) then the lines are not connected at all. This type of ROM is only used if fairly large number of units are required, because the cost of preparation of creating the bit pattern on the chip is quite high. The contents of these ROMs cannot be altered after manufacture. Once the chip is made, the actual chip can cost very little money. They use very little power, are extremely reliable and, in the case of most small electronic devices, contain all the necessary programming to control the device.

(b) *Programmable Read-Only Memory (PROM)* is programmed by the user. Selectively fusing (open-circuiting) the metal or polysilicon links in each memory cell sets that cell to a fixed state. The process is irreversible. In one form of PROM, the information is stored as a charge in a MOSFET cell. Blank PROM chips can be coded with anyone with their programmer.

PROM chips have a grid of columns and rows just as ordinary ROMs do. The difference is that every intersection of a column and row in a PROM chip has a fuse connecting them. A charge sent through a column will pass through the fuse in a cell to a grounded row indicating a value of '1'. Since all the cells have fuse, the initial, or blank, state of a PROM chip is all '1's. To change the value of a cell, to '0', the programmer is used, which sends a specific amount of current to the cell. The higher voltage breaks the connection between the column and the row by burning out the fuse. The process is known as burning the PROM.

The contents of a PROM can be erased by flooding the chip with ultraviolet radiation. Following this process, a fresh pattern can be entered. PROMs are used in the microprocessor based systems during the system development phase and on the production system when the total production run is not high enough to justify the use of mask-programmed ROMs.

PROMs can only be programmed once. They are more fragile than ROMs. A jolt of static electricity can easily cause damage to the chip. But blank PROMs are inexpensive and are great for prototyping the data for a ROM before committing to the costly ROM fabrication process.

(c) *Erasable Programmable Read-Only Memories (EPROM)*. These devices provide the facility of re-writing the chips several times. EPROMs are configured using an EPROM programmer that provides voltage at specified levels, depending upon the type of EPROM used.

For erasing the chips of its previous contents, an EPROM requires a special tool that emits a certain frequency of ultraviolet (UV) light. Because the UV light will not penetrate most plastics or glasses, each EPROM chip has a quartz window on top of the chip. The EPROM is kept very close to the eraser's light source, within an inch or two, to work properly. An EPROM eraser is not selective, it will erase the entire EPROM. The EPROM must be removed from the device it is in and placed under the UV light of the EPROM eraser for several minutes. An EPROM that is left under UV light too long can become over-erased. In such a case, the chip cannot be programmed.

(d) *Electrically Erasable Programmable Read-Only Memories (EEPROM) or Read-Mostly Memories (RMM)* are designed such that the contents of these memories can be altered electrically. However, this is a fairly slow process. It often requires voltages and circuit techniques that are not commonly found in normal logic circuitry.

EEPROMs remove the following drawbacks of EPROMs:

- The chip does not have to be removed to be rewritten.
- The entire chip does not have to be completely erased to change a specific portion of it.
- Changing the contents does not require additional dedicated equipment.

(e) *Flash Memory* is a type of EEPROM that uses in-circuit wiring to erase by applying an electrical field to the entire chip or predetermined sections of it called blocks. Flash memory works much faster than traditional EEPROM because it writes data in chunks, usually 512 bytes in size, instead of a byte at a time.

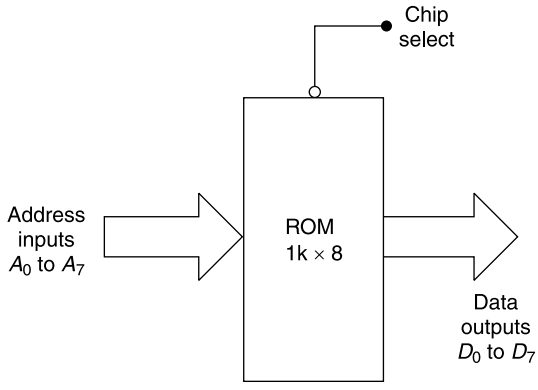


Figure 30.39 *Typical symbol of ROM*

Figure 30.39 shows a typical symbol of ROM, for storing 1024 8-bit words. This is also called a 1 K × 8 ROM where 1 K represents 1024. Similarly, a 2048 × 8 can be written as a 2 K × 8 and so on. Since 1 K ROM stores 1024 different words, it needs 10 address inputs ($2^{10} = 1024$). The word size is 8 bits, so there are eight output lines. The memory chip is enabled or disabled through the chip select (CS) input. ROMs do not provide for data input or read/write control because they do not normally have write operation. Some ROMs do provide for special input facilities for initially writing the data into the ROM which is generally shown on the symbol. The Intel 2716 is a popular 16,384 (2 K × 8) bit UV erasable and electrically programmable ROM.

30.7 MICROPROCESSOR

The microprocessor, in essence, consists of basic circuit elements such as transistors, resistors and diodes which when combined form the basic logical elements, namely AND, OR and INVERTERS. In principle, the complete operation of the microprocessor could be described by a combination of these devices. More complex circuit elements such as flip-flop, counters, registers and arithmetic logic unit are formed from these gates and go to make the complete microprocessor. Microprocessor is a single integrated circuit with 40 or even 64 are even higher connection pins.

Microprocessors are usually classified depending upon their word length. The word length of a microprocessor defines the basic resolution and memory addressing capability. For example, an 8-bit microprocessor will perform all calculations on binary numbers with 8 digits. 8 binary digits give a decimal number between 0 and 255.

The arithmetic and logic unit (ALU) and control section of a computer have traditionally been constructed together, and form the core of the microprocessor (Figure 30.40). The ALU is that portion of the hardware which performs the arithmetic and logical operations on the data. It essentially contains an adder which is capable of combining the contents of the registers in accordance with the logic of the binary arithmetic. This enables the processor to perform the arithmetic manipulations on the data, it obtains from memory and from its other inputs. Using only the basic adder, a programmer can achieve complete arithmetic operations.

The control unit constitutes the brain of the microprocessor. IT coordinates all the parts of the microprocessor, so that events happen in a logical sequence and at the right time. Numerically encoded instructions which are stored in the memory are sent to the control unit to direct it to carry out certain basic algorithmic steps. The control unit is also capable of responding to external signals, such as an interrupt or wait request.

The microprocessor contains registers which are temporary storage devices. Some registers, such as the program counter and instruction register have dedicated uses, whereas most other registers, such as accumulator are more for general-purpose use.

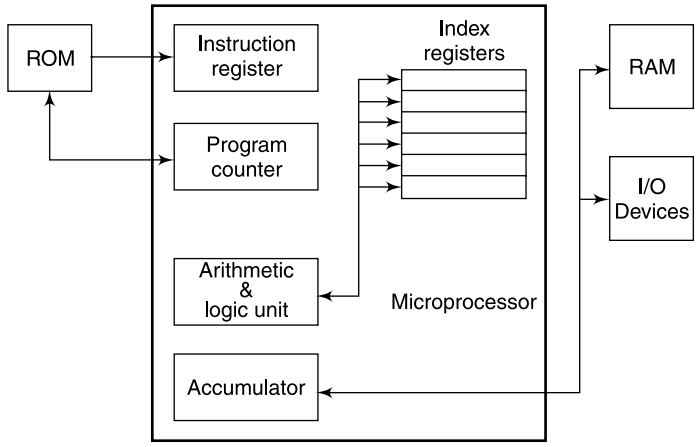


Figure 30.40 *Microprocessor – basic parts*

The operation of the microprocessor and synchronisation of various activities under its control is maintained by a crystal controlled clock or oscillator, which is usually at a fixed frequency, generally greater than 5 MHz.

The microprocessor's most powerful asset is its enormous speed of operation. This is possible because the microprocessor can store all the necessary instructions and data, until required, in the memory. Two types of memories are used in the microprocessor, namely ROM and RAM. The ROM generally stores the programs, whereas the RAM stores the data. The microprocessor can rapidly access any data stored in memory, but often the memory is not large enough to store the entire data bank required for a particular application. This problem can be solved using external storage equipment, such as floppy disk or hard disk system. A microprocessor also requires input/output ports, through which it can communicate its results with the outside world, like a display or peripheral device or provide control signals that may direct another system.

As already mentioned above, the function of the CPU is to manipulate data in accordance with the instructions stored in the memory. For this, the CPU transfers data and internal state information via an 8-bit bidirectional 3-state data bus Memory and peripheral device addresses are transmitted over a separate 16-bit 3-state address bus. Timing and control outputs are given out for synchronisation and control purposes, whereas control inputs like reset, hold and ready and interrupt are used to perform specific functions.

Data Lines: Data-in lines are used to transfer instructions or data from memory or data from input device to the CPU. On the other hand, data-out lines are used to transfer results from CPU to memory or output devices. The number of lines in data-in or data-out is related to the word length of the computer. Normally, a n-bit processor has n data-in lines and n data-out lines. In an 8-bit CPU, one bus of eight lines can be used to carry all the data signals, whether for reading or writing. To ensure that this operation is correctly carried out, a read/write control is provided in the microprocessor package. A bus which carries signals in both directions is called a bidirectional bus and the data bus is the only one which is bidirectional.

The CPU's internal data bus is isolated from the external data bus by an 8-bit bidirectional three-state buffer. In the output mode, the internal bus content is loaded into an 8-bit latch that,

in turn, drives the data bus output buffers. The output buffers are switched-off during input or non-transfer operations.

During the output mode, data from the external data bus is transferred to the internal bus. The internal bus is pre-charged at the beginning of each internal state, except for the transfer state.

Address Lines: These are employed to address the memory to fetch instructions or data from it. These are also used to address and connect I/O devices to the CPU. The number of address lines determine the size of the memory a particular CPU can handle, or maximum number of I/O devices that can be connected to the CPU.

A typical microprocessor has 16 pins as address outputs, which are labelled as A_0 to A_{15}. They are used to select particular locations in the memory. For example, the first signal on the address lines will comprise of 16 logic '0', address '0'. As the microprocessor starts operating, A_0 will change to 1 and when the read/write control signal is used for reading, a byte will be fetched from that particular memory location corresponding to this address. After this, the address will step up at the end of first instruction and call for the next instruction.

The 16 address lines can carry up to 2^{16} (65,536) bits of information, which is equivalent to 64 k memory. Microprocessors in general will, however, need much smaller memory for small machines. Thus, there will always be unused address lines, which can be connected to video display unit or output signals to other circuits, under the control of the program.

Multiplexing is a useful technique employed with microprocessors for obtaining additional address and data lines For example, by multiplexing it is possible to handle 16-bit data signals, through an eight-line data bus. Similarly, the address lines and data lines are also multiplexed and we have address and data available on the same physical lines at different instants of time. However, the result of any time-multiplexing is slowing down the system.

As microprocessor systems are based on the binary numbering system, it is necessary to use multiple connections generally 8, 16 or 32 between each of the integrated circuits (chips). These interconnections are usually referred to as buses. There are three buses in a microprocessor system.

The assembly language of a microprocessor enables to extract the greatest run-time performance because it provides for direct manipulation of the architecture of the processor. However, it is also the most difficult language for writing programs, so it falls far from the optimal language line.

The C language which is used to develop modern versions of the Unix operating system provides a significant improvement over assembly language for implementing most applications, it is the language of choice for real-time programming. It is an excellent compromise between a low level assembly language and a high level language. C is standardised and structured. C programs are based on functions that can be evolved independently of one another and put together to implement an application. These functions are for software but black boxes are for hardware. C programs are transportable. By design, a program developed in C on one type of processor can be relatively easily transported to another.

Historically, the first microprocessor was introduced by Intel in November 1971. It was 4004, the 4-bit CPU having about 2200 transistors on it. Shortly afterwards, Intel introduced an 8-bit microprocessor chip, the 8008. It had more computing power and flexibility than 4004, had 45 instructions with an average execution time of 30 μs. It was better suited for applications of data handling and control. The 8008 remained the sole 8-bit microprocessor for 2 years till Intel announced an upgraded version, the 8080. Simultaneously, National Semiconductor and Rockwell among others announced their own entries.

In mid-1974, Motorola came out with 6800 8-bit microprocessor. Unlike other microprocessors that required multiple power supplies (for example, 8080 required three), the 6800 was the first +5 V single power supply microprocessor. 1974 saw the introduction of the 1802, the first CMOS processor by RCA. Two major introductions in 1976 were the Intel 8085 and the Zilog Z80. The 8085 offered additional features to the 8080 and required a single +5V power supply. The Z80 contained improvements in architecture over the 8080 and incorporated all the 8080 instructions in its instruction set.

The era of 16-bit microprocessors began in 1974 with the introduction of the PACE chip by National Semiconductor. The Texas Instruments TMS 9900 was introduced 2 years later. Subsequently, the Intel 8086 became commercially available in 1978, the Zilog Z800 in 1979 and the Motorola MC 68000 in 1980. Several higher performance versions of the original chips are now available.

Microprocessors with 32-bit internal paths and 16-bit external paths have been in existence since 1980. However, the era of true 32-bit microprocessors began in 1981 with the commercial introduction of the Intel iAPX 432, followed by Motorola 68020, Zilog Z80000 and Intel iAPX 386. All these chips reflect a sustained and conscious attempt towards the integration of an enormously large numbers of transistors.

30.8 MICRO-CONTROLLERS

A micro-controller contains a CPU, clock circuitry, ROM, RAM and I/O circuitry on a single integrated circuit package. The micro-controller is therefore, a self-contained device, which does not require a host of associated support chips for its operation as conventional microprocessors do (Simpson, 1980). The block diagram of a typical micro-controller is shown in Figure 30.41. It offers several advantages over conventional multi-chip systems. There is a cost and space advantage as extra chip costs and printed circuit board and connectors required to support multi-chip systems are eliminated. The other advantages include cheaper maintenance, decreased hardware design effort and decreased board density, which is relevant in portable analytical instruments.

Micro-controllers have traditionally been characterised by low cost high volume products requiring a relatively simple and cheap computer controller. The design optimisation parameters require careful consideration of architectural trade-offs, memory design factors, instruction size, memory addressing techniques and other design constraints with respect to area and performance. Micro-controllers functionality, however, has been tremendously increased in the recent years. Today, one gets micro-controllers, which are stand alone for applications in data acquisition system and control. They have analog-to-digital (A/D) converters on chip, which enable them direct use in instrumentation. Another type of micro-controller has on-chip communication controller, which is designed for applications requiring local intelligence at remote nodes and communication capability among these distributed nodes. Advanced versions of the micro-controllers in 16-bit configuration have been introduced for high-performance requirements particularly in applications where good arithmetical capabilities are required.

Micro-controllers are controlled by programs stored in the ROM. These programs (firmware) are placed in the computer by the manufacturer and cannot be altered by the instrument user. The use of computers as integral components of instruments, the configuration is termed as in-line. In-line computers offer several advantages in terms of increased reliability (decreased maintenance)

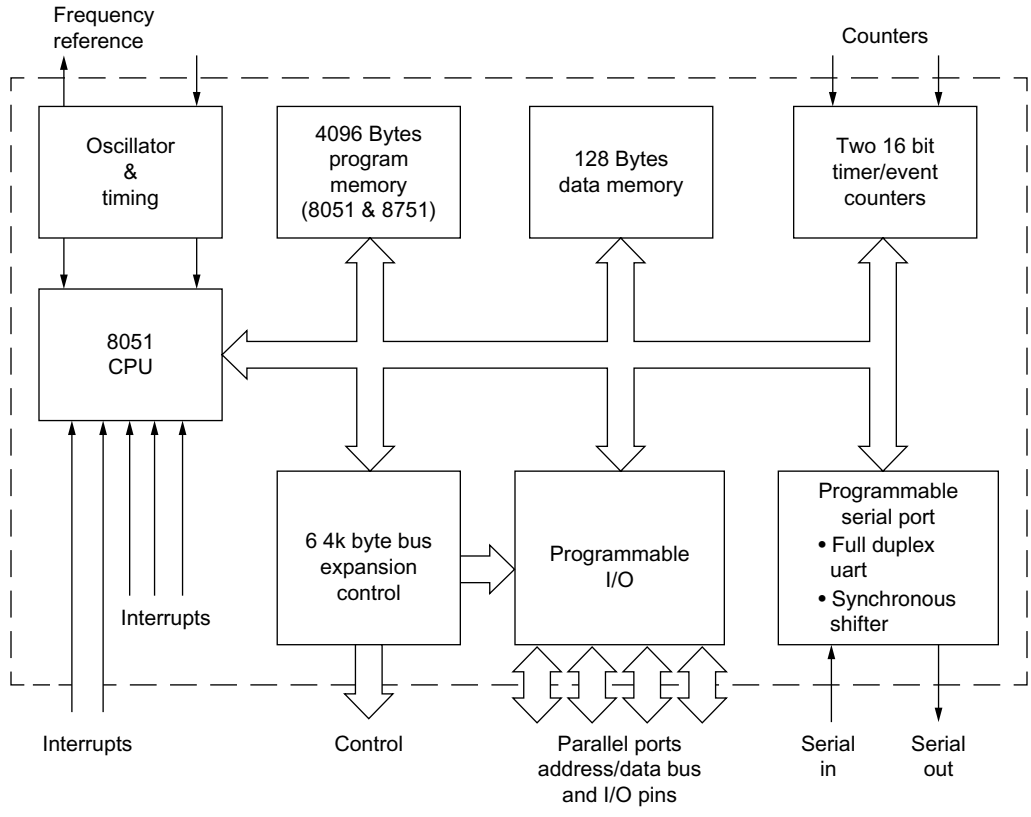


Figure 30.41 *Block diagram of intel 8051 micro-controllers (Courtesy: M/s Intel Corp. USA)*

due to substitution of discrete components, improved accuracy of results with automatic periodic instrument calibration, higher precision of results using digital signal processing and ease of communication with other devices external to the instrument.

30.9 EMBEDDED SYSTEMS

Embedded systems – computers lodged in other devices where the presence of the computers is not immediately obvious – are the fastest-growing portion of the electronic industry and/or often found in portable analytical instruments. An embedded system is a computer or processor based system that has been designed for a specific purpose. The system gains its name from the fact that the software is embedded into it for a particular application.

The basic characteristics of an embedded system are:

- Embedded systems are designed for a specific task. Although they use computer techniques, they cannot be used as a general-purpose computer using a variety of different programmes for different task. In this way their function can be focused onto what they need to do, and they can accordingly be made cheaper and more efficiently.

- The software for embedded systems is normally referred to as firmware. Rather than being stored on a disc, where many programmes can be stored, the single programme for an embedded system is normally stored on chip referred to as firmware.

Primarily, embedded systems contain two main elements:

- *Embedded system hardware*: As with any electronic system, an embedded system requires a hardware platform on which to run. The hardware will be based around a microprocessor or micro-controller. The embedded system hardware will also contain other elements including memory, input output (I/O) interfaces as well as the user interface, and the display.
- *Embedded system software*: The embedded system software is written to perform a particular function. It is typically written in a high level format and then compiled down to provide code that can be lodged within a non-volatile memory within the hardware.

The hardware required for an embedded system varies greatly depending on its intended use. Some tiny systems have barely 1K data space and 16K of instruction space, while high-performance systems might run a 1 GHz 64-bit processing engine with 32 MB Compact Flash and 128 MB DRAM.

Figure 30.42 represents a block diagram of a typical embedded system. All embedded systems use some kind of non-volatile storage (flash memory, EPROM, ROM) and some form of RAM. Most have some channel they can use to communicate with a development host (a serial port, Ethernet port, or JTAG port.).

Embedded systems are designed around either a micro-controller or a microprocessor. Microcontrollers, however dominate the embedded systems market. They are even becoming available as logic cores designed for integration into very large programmable devices called field programmable gate arrays (FPGA).

The embedded system also has memory, often several different types in one system. The memory is used to store the software that the processor will run. It also provides storage for data such as program variables, intermediate results, status information and any other data generated throughout the operation. The memory is an important part of any embedded system because it may dictate how the software is designed, written, and developed.

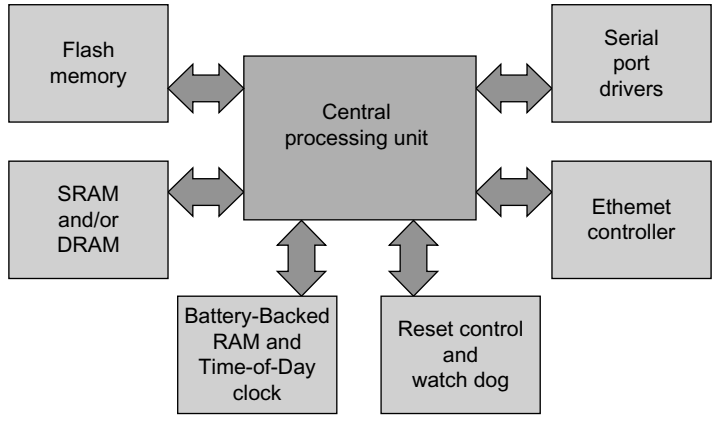


Figure 30.42 *Block diagram of a typical embedded system (Adapted from Sutter, 2002)*

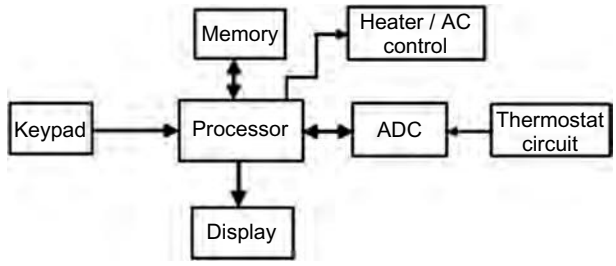


Figure 30.43 *A typical example of a an embedded system used in a digital thermostat*

An embedded system communicates with the outside world through peripherals. The main types of peripherals that are used include digital inputs and outputs and serial interfaces.

While processors operate on the digital data, the surrounding world is analog in nature. Therefore, interfaces between the system and the external world requires A/D conversions and vice versa. Displays are used by the processor to display the status information, error messages, and output results. They could be simple LEDs, seven-segment displays, or character LCD panels. Keypads are used by the end user to provide inputs to the embedded system. The inputs could be anything such as entering the password, changing functional settings, switching between menu items, etc.

Figure 30.43 shows a block diagram of a typical example of the use of an embedded system inside a digital thermostat.

The surrounding temperature is sensed by the thermostat (a temperature sensor) and is converted to a proportional analog voltage. The analog signal is converted to an equivalent digital number through an A/D conversion. The processor then compares this temperature reading with the high and low temperatures settings defined by the user, and turns the heater/AC on or off if required. The user uses Keypad to enter the temperature settings, which are saved into the memory. The processor displays the temperature settings and the current temperature on the LCD screen. The software to perform the entire function of monitoring and controlling the temperature is stored in the memory. The processor reads instructions from the memory and executes them.

30.10 DATA CONVERTERS

In the world of analytical equipment, most electric circuitry is concerned with acquisition, amplification and processing of signals which are available in an analog form. However, the availability of low cost and highly programmable microprocessors and compact digital computers have made possible the digital manipulation of the analog signals after they have been converted into a digital form. On the other hand, the display systems are often of analog type, requiring the digital output to be converted it into an analog form. The class of devices for converting analog signals to digital form and digital signals to analog form are called Data Converters. They are obviously of two types:

Analog-to-Digital (A/D) Converter is a device which has an analog signal as its input and a digital representation of that input as its output.

Digital-to-Analog (D/A) Converter is a device which receives a digital input and outputs an analog quantity.

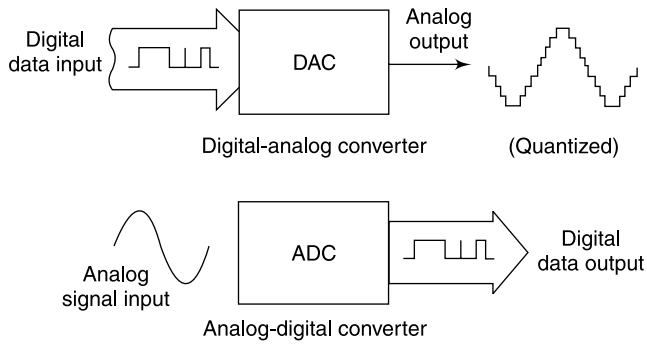


Figure 30.44 *Principle of data converter*

Figure 30.44 shows the principle of data converters. It may be seen that output of a D/A converter is not purely analog, but it is quantised and made up of discrete steps. This is because the input to D/A is digital. There can be only a finite number of input states (i.e. there can be only a finite number of output states). The size of the discrete steps depends on the resolution and conversion rate of the system.

Resolution of an A/D conversion is defined as the smallest analog change that can be discriminated. From a digital point of view, resolution is the number of discrete steps into which the full analog input-signal range of the converter is divided. It is usually expressed as a number of bits (binary) or a number of digits. For example, in a 12-bit A/D conversion, if the input voltage range is ±5V, the smallest analog change that can be resolved is 10 / 4096 (2^{12}) = 2.44 mV DC.

Conversion Time is the time elapsed between application of a convert command and the availability of data at its output.

The working of a data converter is usually checked by using an oscilloscope and observing the analog/digital waveform at the input/output terminals.

30.10.1 A/D Converters

A/D conversion can be divided into two basic groups: open loop types and feedback types. The open loop converter generates a digital code directly upon application of an input voltage and is generally an asynchronous operation. The feedback type, on the other hand, generates a sequence of digital codes, reconverts (D/A) each one in turn back to an analog value and compares it to the input. The resulting digital output will be the closest value of the reconstructed analog voltage compared to the real analog voltage. The feedback method is more popular. Under this technique, we shall consider only successive approximation A/D converter and dual-slope A/D converter.

30.10.1.1 Successive approximation A/D converters

The most direct approach to A/D conversion is using a D/A converter in digital feedback loop, as shown in Figure 30.45. The loop is closed at the analog comparator, which compares the current output of the D/A converter against the input current developed by the analog input voltage. This comparison is made one bit at a time and the method is, therefore, known as successive approximation technique.

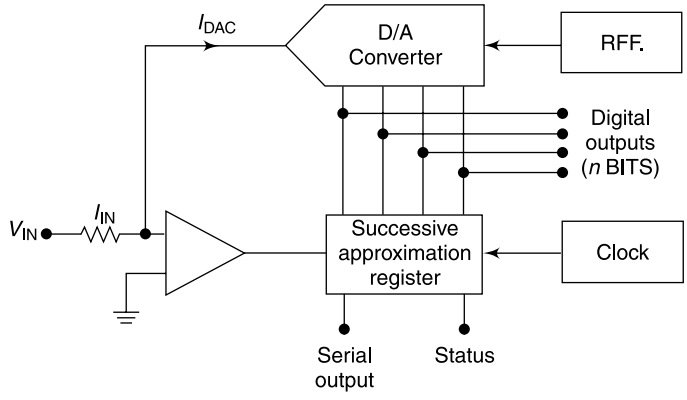


Figure 30.45 *Successive approximation A-D converter*

A clock circuit steps the converter through n comparison steps, where n is the resolution of the converter in bits. In the first clock period, the D/A converter's MSB (most significant bit) output which is one-half of full scale, is compared against the input. If it is smaller than the input, the next largest bit is turned on. If the MSB current is larger than the input, in the next clock period it is turned off when the next bit current is turned on. The D/A converter output at any given time is the cumulative total of all the previous bit currents which have been left on.

The comparison process is continued one bit at a time from the MSB down to LSB (least significant bit). After the last clock period, the output of the successive approximation register contains the digital word representing the analog input. The converter also puts an end to conversion or status pulse, indicating that conversion is complete. In addition to the parallel data output on n digital lines, there is also a useful serial output from most converters derived from the comparison process.

This class of A/D converters represents an excellent compromise between circuit complexity, speed and ability to produce high accuracy codes. This type of converter can have resolution of from 8 to 16 bits. This is the commonest of A/D converters and there are numerous areas of application.

For this type of conversion, the digital output corresponds to some previous value of the analog input during the conversion. Thus, the aperture is equal to the total conversion time, which vary with these devices from 1 to 220 μs.

30.10.1.2 Dual-slope A/D converter

The dual-slope technique operates on a voltage-to-time principle. The basic idea involved is to integrate the unknown input and the known reference and compare their slopes. Figure 30.46 shows a block diagram of the dual-slope A/D converter.

The conversion cycle begins by switching the operational integrator to the input voltage, which is then integrated for a fixed time period. After this time, the integrator is switched to a reference voltage of opposite polarity and the integrator output integrates back to zero for a period of time, which is measured by the counter. The resultant count is then the digital value of the input voltage.

The advantage of the dual-slope technique is that, the accuracy and stability are dependent only on the reference, and not on other components in the circuit. This assumes, of course, that the operational integrator is linear. Some devices also incorporate automatic zeroing circuitry to

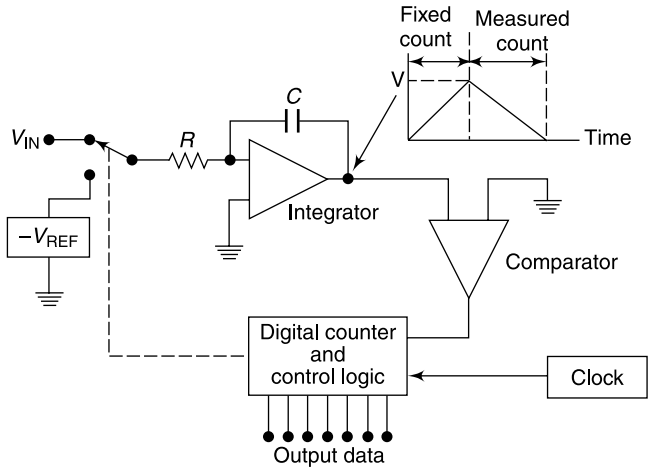


Figure 30.46 *Principle of dual-slope integration method of A/D conversion*

reduce the effect of offset drift with time and temperature. The technique offers simplicity, accuracy and noise immunity due to integration of the input signal. The chief drawback is relatively slow conversion time.

30.10.1.3 Charge balancing A/D converter

Another integrating conversion technique which is quite popular is the charge-balancing A/D converter, illustrated in Figure 30.47. In this arrangement, an operational integrator is enclosed in a digital feedback loop consisting of a comparator, pulse timer circuit and a switched reference.

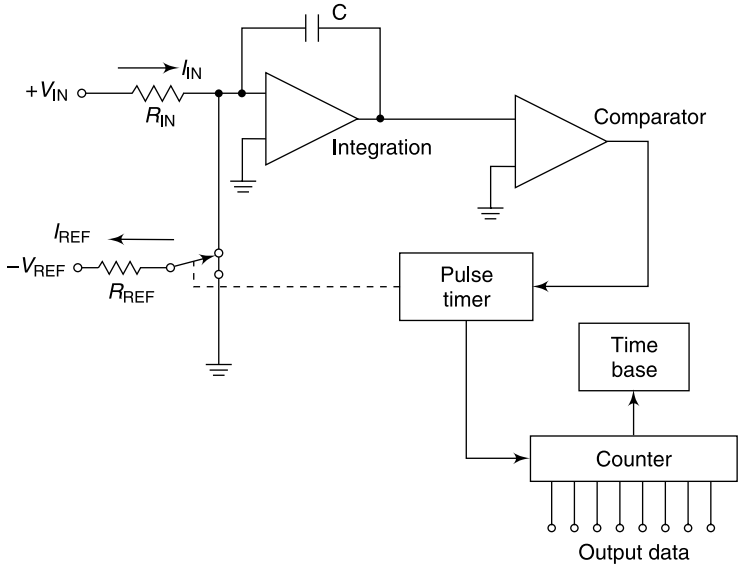


Figure 30.47 *Charge-balancing A-D converter*

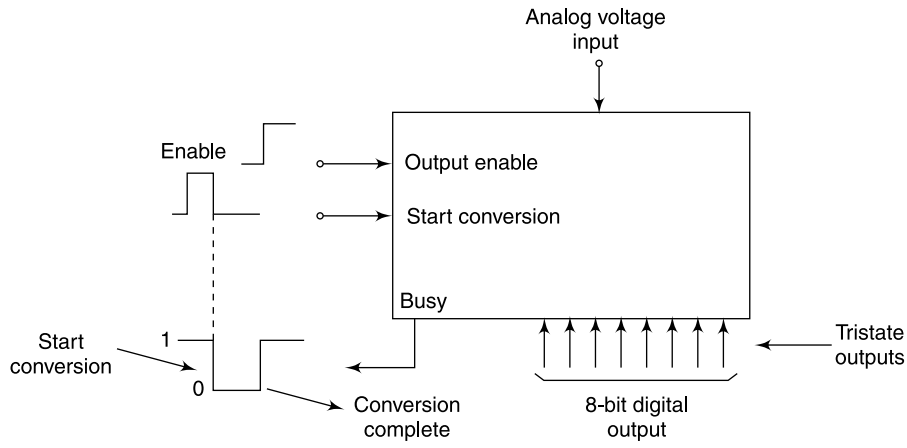


Figure 30.48 *A-D converter for μP-based applications*

A positive input voltage causes the integrator output voltage to cross zero volts, which is detected by the comparator and triggers the pulse timer circuit. The output pulse from the timer switches a negative reference current to the integrator output to increase in the positive direction. Therefore, every time the integrator output crosses zero, another pulse is generated and integrated.

A state of equilibrium exists when the average current developed by the pulses just equals the input current. Since each current pulse is a fixed amount of charge, the name charge-balancing is given to this technique.

Figure 30.48 shows a typical A/D converter for microprocessor based applications. The digital output lines D_7 through Do come from tristate latches, which are part of the converter. A HIGH on the ENABLE input will enable these outputs, so that the digital representation of the analog input is present on these lines. A LOW on the ENABLE input puts these output lines in their high-z state. Normally, the ENABLE input is pulsed HIGH only after the BUSY output has indicated that the conversion is complete. If the ENABLE input is made HIGH during the conversion time interval, the output lines will indicate the results of the previous A/D conversion.

A typical example of an integrating A/D converter is that of ICL 7109 from Intersil. It is monolothic, CMOS, 12 bit A/D converter and is not intended for very fast applications. The data sheet specifications are quoted at 7.5 conversions per second, corresponding to an internal clock frequency of about 61.5 kHz, or a clock period of 16.3 μs. The separate tristate byte wide outputs of the ICL 7109 make it ideal for interfacing to 8-bit microprocessor buses.

30.10.1.4 Delta sigma A/D converters (ΔΣ ADC)

Delta sigma analoge-to-digital converters are ideal for converting analog signals to digital format over a wide range of frequencies, from DC to several megahertz. Basically, these converters consist of an oversampling modulator followed by a digital/decimation filter that together produces a high-resolution data-stream output. The design of delta-sigma A/D converters is approximately three quarters digital and one-quarter analog.

The hardware architecture of a delta-sigma A/D converter consists of an integrator, a comparator, and a 1-bit digital-to-analog (D/A) converter arranged in a negative-feedback loop, as shown in Figure 30.49. An integrator circuit is fed the sum of the input signal and the negated output of the D/A converter. The output of the integrator is a ramp signal whose slope is proportional to its

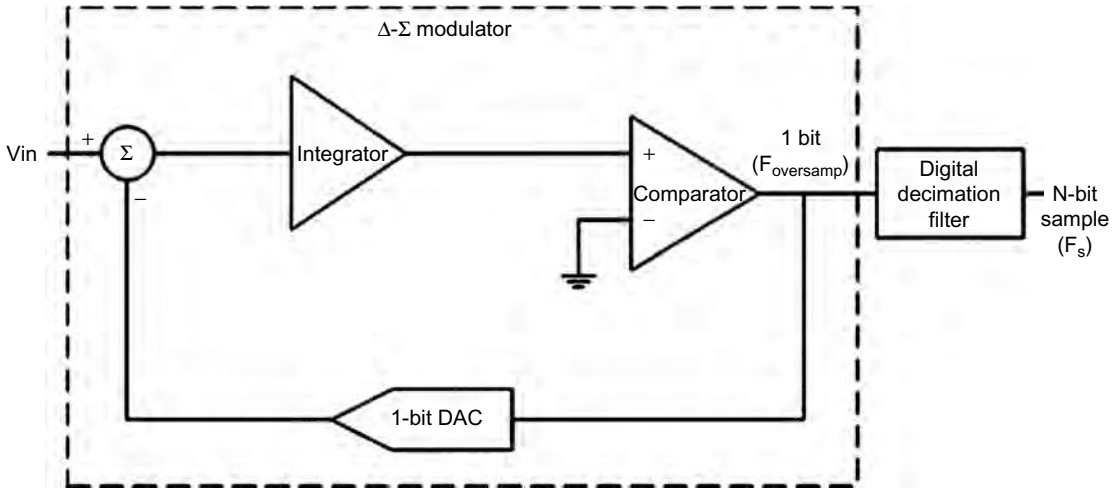


Figure 30.49 *Basic delta-sigma (Δ–∑) A/D converter consists of an integrator, a comparator, and a 1-bit D/A converter*

input. The integrator output is compared against the comparator reference signal to generate a 0 or 1. The binary output of the comparator is clocked into the digital decimation filter on every edge of the A/D clock F (oversample). Each bit represents the direction of the ramp output of the integrator with respect to the comparator reference and, after multiple iterations, the bit stream resembles the quantised value of the input signal. Essentially, the feedback loop works such that the average output of the D/A converter matches the input signal. The digital decimation filter averages the bit stream to output an n-bit sample at the desired sample rate F_s.

The ΔΣ converter's primary internal cells are the ΔΣ modulator and the digital/decimation filter. The internal ΔΣ modulator coarsely samples the input signal at a very high rate into a 1-bit stream. An analog signal applied to the input of the converter needs to be relatively slow so the converter can sample it multiple times, a technique known as oversampling. The sampling rate is hundreds of times faster than the digital results at the output ports. Each individual sample is accumulated over time and 'averaged' with the other input-signal samples through the digital/decimation filter (Baker, 2011).

The digital/decimation filter then takes this sampled data and converts it into a high-resolution, slower digital code. While most converters have one sample rate, the ΔΣ converter has two – the input sampling rate and the output data rate.

The ΔΣ modulator is the heart of the ΔΣ A/D. It is responsible for digitising the analog input signal and reducing noise at lower frequencies. In this stage, the architecture implements a function called noise shaping that pushes low-frequency noise up to higher frequencies where it is outside the band of interest. Noise shaping is one of the reasons that ΔΣ converters are well-suited for low-frequency, high accuracy measurements.

30.10.2 Key Parameters in A/D Converters and their Selection

The choice of an A/D converter and associated front end signal-conditioning hardware demands a careful analysis. Choosing an A/D converter sub-system would usually depend upon the following considerations (Lockhart, 1990):

30.10.2.1 Resolution

Resolution is defined as the smallest analog change that can be discriminated. From a digital point of view, resolution is the number of discrete steps into which the full analog input-signal range of the converter is divided. It is usually expressed as a number of bits (binary) or as number of digits (BCD).

Successive approximation A/D converters are 12-bit devices, and so divide a voltage range into 4096 steps. For an A/D converter whose analog input voltage range is ±5V DC, the smallest analog change that can be discriminated is 10 ÷ 4096 = 2.44 mV DC. Integrating converters are frequently 16-bit devices and therefore have a wide dynamic range; for example they can discriminate the same analog voltage change of 2.44 mV at a full-scale input voltage 16 times as large. The resolution can be improved by using an amplifier at the input, and the effective resolution is increased by the magnitude of the amplifier gain.

30.10.2.2 Sampling rate

The sampling rate on a particular experiment depends on how much information is required from the analog signal (such as intensity as a function of time), and on what kind of signal processing is planned for the collected data. For example, if the peak is 1s long and it is decided that the system should collect at 10 points over the length of that peak, the minimum data collection rate would be 10 points/s or 10 Hz. if the peak is 0.1s long, the sampling rate would have to be at least 100 Hz.

The signal processing usually requires a higher sampling rate. For example, according to the Nyquist frequency criterion, the fast Fourier transform (FFT) analysis requires that the sampling rate be more than twice the highest frequency component of the input.

It is important to know as to whether the computer memory can handle the sample throughput rate. For example, if throughput is 10,000 Hz (points per second) and lasts only for a burst of 1 s, the computer should have enough main memory to handle the 10,000 samples of raw data. But if this rate is sustained for 10s, the computer may not have enough main memory to handle 1,00,000 samples. This problem of handling volumes of data that exceed the capacity of main memory is tackled by periodically transferring the current block of data to mass storage disc units.

Throughput in data collection alone and with real-time processing can be substantially improved, by upgrading the computer to a more powerful model having a larger main memory. Another approach is to use parallel computers. One machine is dedicated to collecting and storing data on a shared mass storage device and the second computer devotes all its capabilities to processing the data and controlling the experiment.

30.10.2.3 Speed of an A/D converter or conversion rate

Speed of an A/D converter is generally expressed as its conversion time (i.e. the time elapsed between application of a convert command and the availability of data at its outputs). A/D converters can operate in three different ways.

Wait loops: Here a program starts the converter and then waits for the Status line to show an end of conversion. The computer cannot perform other tasks during this operation. If the program is written in assembler or machine code, each pass through the loop might take 20 µs. Also, it may take about 30 µs to store the digital values in memory, increment a pointer to memory, preparing for the storage of the next data point and decrement a counter used by the program to detect if all the desired points have been taken. All these may take about 30 µs. Therefore, even if the

conversion time of the A/D converter were infinitely fast, the A/D converter cannot take data faster than 20 kHz.

Interrupts: The A/D converter is started and the CPU continues executing other programs. When the conversion is over, it demands a program interrupt that temporarily stops execution of the current program. Next operation automatically attends to the service routine for the A/D converter. These operations may take 200–300 μs. Even if the A/D converter is infinitely fast, data collection would be limited to 3–5 kHz.

Direct Memory Access (DMA): In DMA, the A/D converter takes control of the computer's bus during transfer of a data word into memory. The transfer is completed in a few hundred nanoseconds, thereby offering a great stealing advantage. The actual transfer takes place by stealing time from the CPU.

30.10.2.4 Single-ended vs differential mode

Laboratory instruments are often connected to a microcomputer through a suitable interface. There are two choices of wiring between the instrument and interface.

Single-ended: In which the signal (V_0) received by the interface (Figure 30.50) is the difference between the potential at an input terminal and ground. Though only one input terminal is needed per channel, the differences in ground potential at the instrument and interface result in common mode noise pickup, in which the received signal:

$$V_0 = V_s + V_n, \text{ where } V_n \text{ is the ground loop noise component.}$$

Differential Wiring: In which the signal of interest (V_0) is the instantaneous potential difference between the input signal $(V_1 + V_n)$ and the return signal $(V_2 + V_n)$, so that common mode noise is not present in V_0 (Figure 30.51). In addition, electrostatic noise may be eliminated by grounded

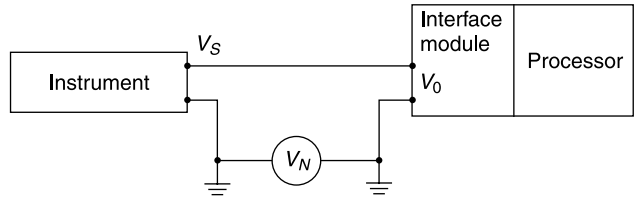


Figure 30.50 *Single-ended wiring arrangement-here the signal to be processed is between input terminal of the interface module and ground. Common mode signal pick up limits the data collection rate and resolution*

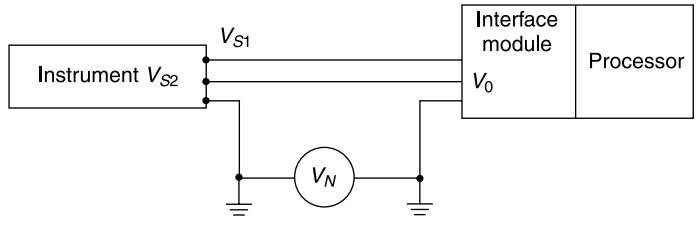


Figure 30.51 *Differential wiring arrangement, in which the common mode signals are effectively cancelled*

cable shielding and magnetically induced noise eliminated by using twisted pair. Any reasonable noise condition that cannot be handled with shielded, twisted differential wiring can usually be suppressed satisfactorily with signal averaging and other digital filtering.

Many A/D converter sub-systems give the user the option of choosing single-ended or differential input. Obviously, the latter will cut the available number of multiplexed channels in half.

When extreme noise is encountered, it may be necessary to transmit the signal from a source as a variable current or frequency, and the information converted back to a potential at the A/D converter end. Voltage-to-frequency converters can generate a pulse train proportional to voltage at the source end of a transmission cable, and a frequency-to-voltage converter regenerates a voltage at the A/D converter end. Like FM radio, frequency transmission is quite noise immune.

30.10.3 D/A Converters

Digital output signals often have to be converted into analog form so that they can be used and acted upon by external circuits (e.g. chart recorders, printers, display devices, etc.). The relationship between analog and digital circuit is usually defined in terms of the number of bits used to specify a given range of signal values. For example, an 8-bit D/A converter can specify voltages in the range 0 to +5V with resolution of 5×10^{-8} so that

00 00 00 00 = 0 V
00 00 00 01 = 0.01953 V
11 11 11 11 = 4.96095 V

A D/A converter essentially is a circuit which when presented with a binary number at its input, gives a an output analog voltage proportional to that number.

The resistive network is the classical D/A converter. The simplest system is the weighted-resistor (Figure 30.52) converter, requiring one resistor per bit. Currents of magnitude 1/2, 1/4, 1/8 etc are generated in resistors R, 2R, 4R etc, by switching the resistors to a reference voltage. The currents are summed up and converted to a voltage by means of an additional resistor R_L. The disadvantage of this method is that it is difficult to match the temperature coefficients of resistors with widely varying values. The switches shown in the diagram can be CMOS transistors.

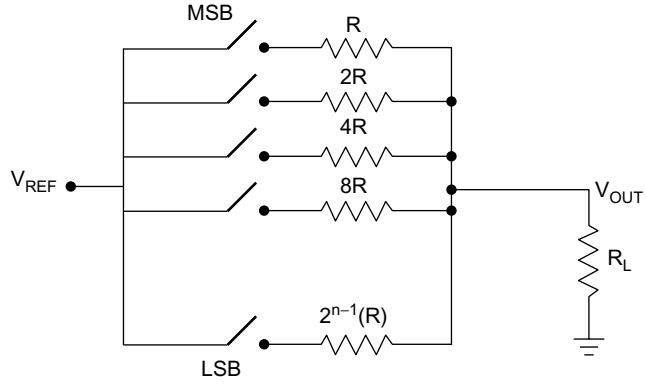


Figure 30.52 *Weighted-resistor type D/A converter*

An improvement over the basic weighted-resistor network is the ladder network which when combined with CMOS technology is available as a monolithic circuit.

30.11 DIGITAL SIGNAL PROCESSING

When we pass a signal through a device that performs an operation, as in filtering, we say we have processed the signal. The type of operation performed may be linear or non-linear. Such operations are usually referred to as signal processing.

The operations can be performed with a physical device or software. For example a digital computer can be programmed to perform digital filtering. In case of digital hardware operations (logic circuits), we have a physical device that performs a specified operation. In contrast, in digital processing of signals on a digital computer, the operations are performed on a signal consist of number of mathematical operations as specified by the software.

Most of the signals encountered in analytical instrumentation are analog in nature (i.e. the signals are functions of a continuous variable such as time or space). Such signals may be processed by analog systems such as filters or frequency analysers or frequency multipliers. Until about two decades ago, most signal processing was performed using specialised analog processors. As digital systems became available and digital processing algorithms were developed, the digital processing became more popular. Initially, digital processing was performed on general-purpose microprocessors. However, for more sophisticated signal analysis, these devices were quite slow and not found suitable for real-time applications. Specialised designs of microprocessors have resulted in the development of digital signal processors, which although perform a fairly limited number of functions, but do so at very high speeds.

Basically, DSPs are processors or microcomputers whose hardware, software, and instruction sets are optimised for high-speed numeric processing applications, an essential for processing digital data representing analog signals in real time. What a DSP does is straightforward. When acting as a digital filter, for example, the DSP receives digital values based on samples of a signal, calculates the results of a filter function operating on these values, and provides digital values that represent the filter output; it can also provide system control signals based on properties of these values. The DSP's high-speed arithmetic and logical hardware is programmed to rapidly execute algorithms modelling the filter transformation. Digital signal processing offers several advantages such as Programmability, Upgradability and Stability.

A digital signal processor (popularly known as DSP) requires an interface (Figure 30.53) between the analog signal and digital processor, which is commonly provided by an A/D converter. Once the signal is digitised, the DSP can be programmed to perform the desired operations on the input signal. The programming facility provides the flexibility to change the signal processing operations through a change in the software, whereas hardwired machines are difficult to configure. Hence programmable signal processors are common in practice. On the other hand when the signal

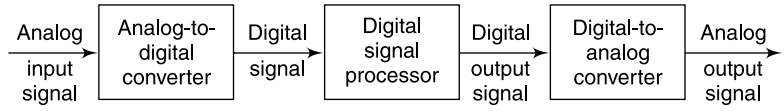


Figure 30.53 *Basic elements of a digital signal processor (DSP) system*

processing operations are well defined, as in some applications, a hardwired implementation of the operations can be optimised so that it results in cheaper and faster signal processors. In cases when digital output from processor is to be given to user in analog form, a D/A converter is required.

DSPs are available as single chip devices and are commercially available. The most widely used DSP family is the TMS320 from Texas Instruments. Another range of processors is available from Motorola as DSP56001. For sake of comparison of speed, the 16-bit Motorola 68,000 microprocessor can handle 2,70,000 multiplications per second while the DSP56001 is capable of 10,000,000 multiplications per second, thus giving an increase in speed of 37 times. Because of the flexibility to reconfigure the DSP operations, they are used in most of the modern analytical instruments for signal processing applications like transformation to the frequency domain, averaging and a variety of filtering techniques.

30.12 DATA ACQUISITION SYSTEMS FOR ANALYTICAL INSTRUMENTS

Most analytical signals are in the form of analog voltages. Data acquisition is simply the gathering of information about such signals present in a system or process. It is a core tool to the understanding, control and management of such systems or processes. Parameter information such as pH, temperature or pressure is gathered by transducers that convert the information into electrical signals. Depending upon the number of parameters to be measured, the signal from the transducers are transferred by wire, optical fibre or wireless link to an instrument which conditions, amplifies, measures, scales, processes, displays and stores the sensor signal. This constitutes the Data Acquisition system. Today's data acquisition systems make use of powerful microprocessors and computers which do this job more accurately, more flexibly, with more sensors, more complex data processing, and elaborate presentation of the final information.

For interfacing analog signals to microprocessors/microcomputers, use is made of some kind of data acquisition system. The function of this system is to acquire and digitise data, often from hostile clinical environments, without any degradation in the resolution or accuracy of the signal. The functional block diagram of a data acquisition system with multiple inputs is shown in Figure 30.54. Since software costs generally far exceed the hardware costs, the analog/digital interface structure must permit software effective transfers of data and command and status signals to avail of the full capability of the microprocessor.

The analog interface system, in general, handles signals in the form of voltages. The physical parameters such as temperature, flow, pressure, etc. are converted to voltages by means of

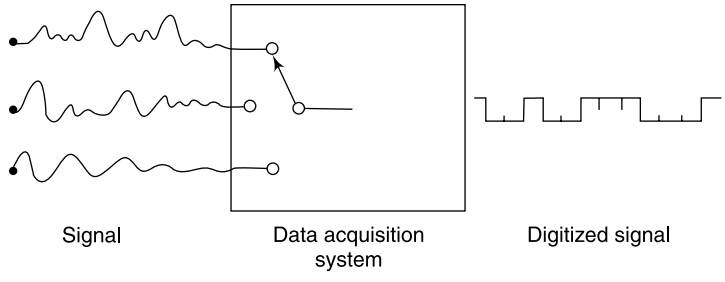


Figure 30.54 *Basic data acquisition system*

transducers. The choice and selection of appropriate transducers is very important, since the data can only be as accurate as the transducer.

Figure 30.55 shows a block diagram of a universal interface circuit for connecting analog signals to microprocessors. It basically comprises a multiplexer, instrumentation (buffer) amplifier, a sample-and-hold circuit, A/D converter, tristate drivers and control logic. These components operate under the control of interface logic that automatically maintains the correct order of events.

Multiplexer: The function of the multiplexer is to select under address control, an analog input channel and connected to the buffer amplifier. The number of channels is usually 8 or 16. Depending on its input configuration, the multiplexer will handle either single-ended or differential signals.

The address logic of most multiplexers can perform both random and sequential channel selection. For real-time systems, the random mode permits the multiplexer to select any channel when the program responds to a peripheral service request. Sequential channel selection, as the name imploys involves addressing each channel in order.

Buffer amplifier: The buffer amplifier conditions the selected input signal to a suitable level for application to the A/D converter. Driven by the multiplexer, the buffer amplifier, which is usually an instrumentation amplifier, provides impedance buffering, signal gain and common mode rejection. It has a high input impedance, 100 Mohms are more to reduce the effects of any signal distortion caused by the multiplexer. The high input impedance also minimises errors due to the finite on-resistance of the multiplexer channel switches.

To improve system sensitivity, the amplifier boosts the input signal. If it is required to have analog signals of differing ranges, connected to the multiplexer input, then a programmable gain amplifier would be preferable where the gain would be set in accordance with the multiplexer selection address. The use of programmable gain amplifiers removes the necessity to standardise on the analog input ranges.

Sample and hold circuit: The A/D converter requires a finite time for the conversion process, during which time the analog signal will still be hanging according to its frequency components. It is therefore necessary to sample the amplitude of the input signal, and hold this value on the input to the A/D converter during the conversion process. The sample-and-hold circuit freezes its output on receipt of a command from the control circuit, thereby providing an essentially constant voltage to the A/D converter throughout the conversion cycle.

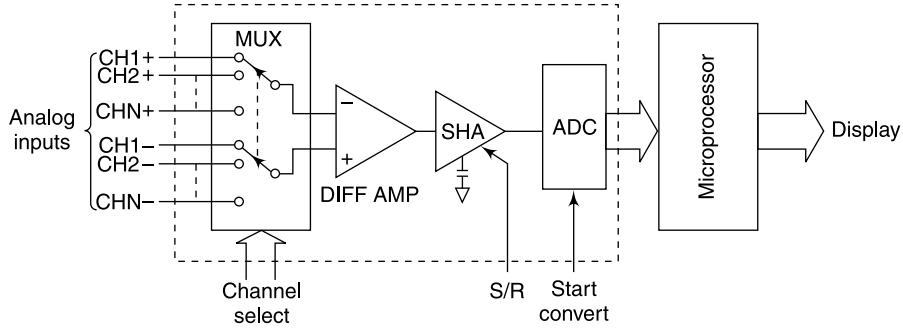


Figure 30.55 *Interfacing analog signals to microprocessors*

The sample hold is essentially important in systems having resolution of 12-bits or greater, or in applications in which real-time inputs are changing rapidly during a conversion of the sampled value. On the other hand, a sample hold may not be required in applications where input variation is low compared to the conversion time.

A/D Converter: The A/D converter carries out the process of the A/D conversion. It is a member of the family of action/status devices which have two control lines – the start conversion or action input line and the end of conversion or status output line.

An A/D converter is a single chip integrated circuit having a single input connection for the analog signal and multiple pins for digital output. It may have 8, 12, 16 or even more output pins, each representing an output bit. The higher the number of bits, the higher the precision of conversion. Each step represents a change in the analog signal: 8-bits gives 256 steps, 12-bits provides 4096 steps and we get 32768 steps with 16 output bits.

Tri-state Drivers: The tri-state drivers provide the necessary isolation of the A/D converter output data from the microprocessor data bus and are available as 8-line units. Thus, for the 10 or 12 bit converters, two drivers would be required which would be enabled by two different read addresses derived from the address decoder.

Some A/D converters have in-built tri-state drivers. However, because of their limited drive capability, they can be used only on lightly loaded buses. For heavily loaded systems, as in microcomputers, the built-in drivers are permanently enabled and separate tri-state drivers employed for the data bus isolation.

Control logic: The control logic provides the necessary interface between the microprocessor system but and the elements of the acquisition unit in providing the necessary timing control. It is to ensure that the correct analog signal is selected, sampled at the correct time, initiate the A/D conversion process and signals to the microprocessors on completion of conversion.

Output Interface: Digital output signals often have to be converted into analog form so that they can be used and acted upon by external circuits (e.g. oscilloscope, chart recorder, etc.). Therefore, D/A converters are used for converting a signal in a digital format into an analog form. The output of the D/A converter is either current or voltage when presented with a binary signal at the input.

The input coding for the D/A converter is similar to the output coding of the A/D converter, while full-scale outputs are jumper-selectable for 0 to 1, ±5 and ±10 V. D/A converters generally deliver the standard 4 to 20 mA output and loading can range from 50 Ω to 4 kΩ. The important parameters which govern the choice of an A/D converter or D/A converter are resolution, measurement frequency, input characteristics, offset error, noise, microprocessor compatibility and linearity, etc.

Data Acquisition can be divided into two broad classifications – real-time data acquisition and data logging. Real time data acquisition is when data acquired from sensors is used either immediately or within a short period of time, such as when controlling a process. Data logging on the other hand is when data acquired from sensors is stored for later use. IN reality, there is a continuum of devices between real-time data acquisition and data logging that share the attributes of both of these classifications.

A Data Logger on the other hand is an electronic instrument which connects to real world devices for the purpose of collecting information. For example, data logger can be pictured as a black box recorder in aeroplanes, which record voice and the plane status data information.

Building a data acquisition system requiring a hardy cross-breed of both analog design and digital design no longer requires elaborate design efforts involving design of individual circuit blocks. The design problem of data acquisition systems have been greatly simplified with the introduction of single chip systems. Included on-chip is an 8-bit A/D computer with bus oriented outputs, a 16-channel multiplexer, provisions for external signal conditioning and logic control for systems interface.

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