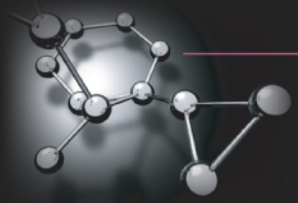


Nanotechnology Science and Technology Series

CARBON NANOTUBES

A NEW ALTERNATIVE FOR ELECTROCHEMICAL SENSORS

Gustavo A. Rivas
Mariá D. Rubianes
Maria L. Pedano
Nancy F. Ferreyra
Guillermina Luque
Silvia A. Miscoria

NOVA

NANOTECHNOLOGY SCIENCE AND TECHNOLOGY SERIES

CARBON NANOTUBES: A NEW ALTERNATIVE FOR ELECTROCHEMICAL SENSORS

NANOTECHNOLOGY SCIENCE AND TECHNOLOGY SERIES

Safe Nanotechnology
Arthur J. Cornwelle
2009. ISBN**:** 978-1-60692-662-8

National Nanotechnology Initiative: Assessment and Recommendations
Jerrod W. Kleike (Editor)
2009. ISBN 978-1-60692-727-4

Nanotechnology Research Collection - 2009/2010. DVD edition
James N. Ling (Editor)
2009. ISBN 978-1-60741-293-9

Nanotechnology Research Collection - 2009/2010. PDF edition
James N. Ling (Editor)
2009. ISBN 978-1-60741-292-2

Safe Nanotechnology in the Workplace
Nathan I. Bialor (Editor)
2009. ISBN 978-1-60692-679-6

Strategic Plan for NIOSH Nanotechnology Research and Guidance
Martin W. Lang (Author)
2009. ISBN: 978-1-60692-678-9

Nanotechnology in the USA: Developments, Policies and Issues
Carl H. Jennings (Editor)
2009. ISBN: 978-1-60692-800-4

New Nanotechnology Developments
Armando Barrañón (Editor)
2009. ISBN: 978-1-60741-028-7

Electrospun Nanofibers and Nanotubes Research Advances
A. K. Haghi (Editor)
2009. ISBN: 978-1-60741-220-5

Nanostructured Materials for Electrochemical Biosensors
Umasankar Yogeswaran, S. Ashok Kuma and Shen-Ming Chen
2009. ISBN: 978-1-60741-706-4

Magnetic Properties
and Applications of Ferromagnetic Microwires with Amorpheous
and Nanocrystalline Structure
Arcady Zhukov and Valentina Zhukova
2009. ISBN 978-1-60741-770-5

Electrospun Nanofibers Research: Recent Developments
A.K. Haghi (Editor)
2009. ISBN 978-1-60741-834-4

Nanotechnology: Environmental Health and Safety Aspects
Phillip S. Terrazas (Editor)
2009. ISBN: 978-1-60692-808-0

Nanofibers: Fabrication, Performance, and Applications
W. N. Chang (Editor)
2009. ISBN**:** 978-1-60741-947-1

Carbon Nanotubes: A New Alternative for Electrochemical Sensors
Gustavo A. Rivas, María D. Rubianes, María L. Pedano,
Nancy F. Ferreyra, Guillermina Luque and Silvia A. Miscoria
2009. ISBN: 978-1-60741-314-1

NANOTECHNOLOGY SCIENCE AND TECHNOLOGY SERIES

CARBON NANOTUBES: A NEW ALTERNATIVE FOR ELECTROCHEMICAL SENSORS

GUSTAVO A. RIVAS,
MARÍA D. RUBIANES,
MARÍA L. PEDANO,
NANCY F. FERREYRA,
GUILLERMINA LUQUE
AND
SILVIA A. MISCORIA

Nova Science Publishers, Inc.
New York

LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA

Carbon nanotubes : a new alternative for electrochemical sensors / Gustavo A. Rivas ... [et al.].
p. cm.
Includes bibliographical references and index.
ISBN 9781607413141
1. Nanotubes. 2. Electrochemical sensors--materials. I. Rivas, Gustavo A.
TA418.9.N35C348 2009
681.2--dc22 2009008016

Published by Nova Science Publishers, Inc. ✣ New York

CONTENTS

ACKNOWLEDGEMENTS

The authors are grateful to Fundación Antorchas, CONICET, ANPCyT, SECyT-UNC and ABC for the financial support of the research activities.

Chapter 1

INTRODUCTION

The goal of this book is to summarize the recent advances in carbon nanotubes as a new material for electrochemical sensors. Since their discovery in 1991, carbon nanotubes have received considerable attention in different fields. Their special geometry and unique electronic, mechanical, chemical and thermal properties make them a very attractive material for the design of electrochemical biosensors.

The first application of carbon nanotubes in the preparation of a sensor was reported by Britto in 1996. Since then, an increasing number of publications involving sensors based on carbon nanotubes (either single or multi-wall) for substrates like glucose, lactate, alcohols, phenols, neurotransmitters, aminoacids, proteins, carbohydrates among others, have been reported. This fact demonstrates the usefulness of carbon nanotubes for the development of electrochemical sensors.

The advantages of carbon nanotubes for promoting electron transfer reactions -with special emphasis in those involving biomolecules-, the different methodologies for incorporating carbon nanotubes in sensors (either suspended in solutions, in polymeric films or in composite matrices), the analytical performance of the resulting biosensors as well as future prospects are discussed in this book.

Chapter 2

GENERAL ASPECTS OF CARBON NANOTUBES

In the last years there has been an increasing interest in nanoscience, basically to understand the behavior of structures with sizes close to atomic dimensions. Even when many nanostructures are currently under investigation, the area of nanotubes is one of the most active. Carbon nanotubes (CNTs) present one of the simplest chemical composition and atomic bonding configuration, at the same time that show the most extreme diversity and richness among nanomaterials referred to structures and associated properties [1]. Since their discovering in 1991 by Iijima [2], carbon nanotubes have been the target of numerous investigations due to their unique properties [1,3-6]. The outstanding structural, electronic and mechanical properties make them a very unique material attractive for a wide range of applications [7].

It is important to mention the crucial aspect of carbon hybridization in the properties of the resulting material. While in diamond the sp^3 hybridization originates a rigid and almost isotropic structure, the sp^2 of graphite shows planar bonds, three-fold coordinated in the planes with weak bonding between planes and anisotropic physical properties [4, 7]. Carbon nanotubes are built from sp^2 carbon units and present a seamless structure with hexagonal honeycomb lattices, being several nanometers in diameter and many microns in length [3, 6]. CNTs are closed structures that present two well defined regions with properties clearly different, the tube and the cap [8]. Each end of the nanotubes is capped with half of a fullerene-like molecule that is responsible for the diameter of the tube [9]. To obtain the convex structure, it is necessary to introduce a positive curvature into the planar hexagonal graphite lattice and it is done by creating topological defects, which in this case are pentagons [6]. As it is discussed in the following sections,

these caps can be opened by using different treatments providing new alternatives for many interesting applications.

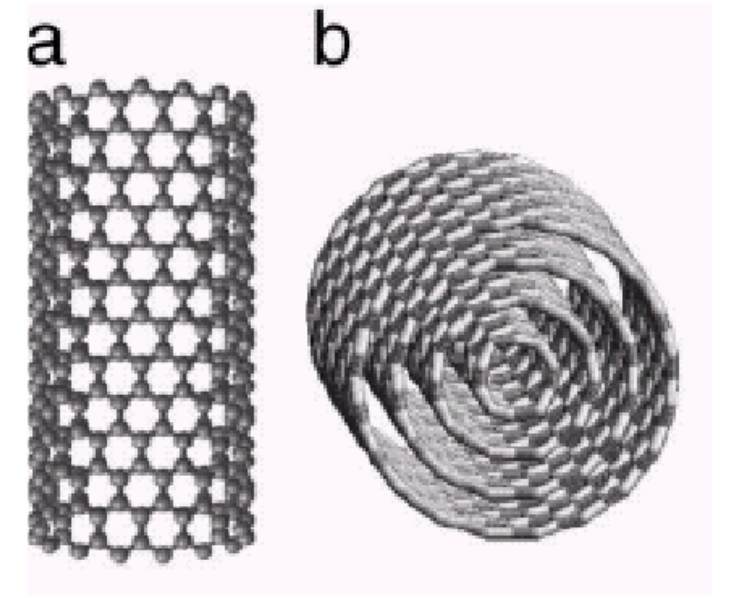


Figure 1. Schematic diagram of a single-wall carbon nanotube (SWNT) (a) and a multi-wall carbon nanotube (MWNT) (b). Adapted from Reference 10.

There are two groups of carbon nanotubes, multi-wall (MWCNTs) and single-wall (SWCNTs) carbon nanotubes [3,6,10,11] (Figure 1). MWCNTs can be visualized as concentric and closed graphite tubules with multiple layers of graphite sheet defining a hole typically from 2 to 25 nm separated by a distance of approximately 0.34 nm [2-4]. SWCNTs consist of a single graphite sheet rolled seamlessly, defining a cylinder of 1-2 nm diameter. MWCNTs can be considered as a mesoscale graphite system while SWCNTs are real single large molecules [7].

It is important to define the chiral vector of the nanotube C_h, which is given by $C_h = n\bar{a}_1 + m\,\bar{a}_2$ where a_1 and a_2 are unit vectors in the two-dimensional hexagonal lattice, and n and m are integers as shown in Figure 2 [12]. The diameter, d_t, is the length of the chiral vector divided by ¼. Another important parameter is the chiral angle θ, which is the angle between C_h and a_1.

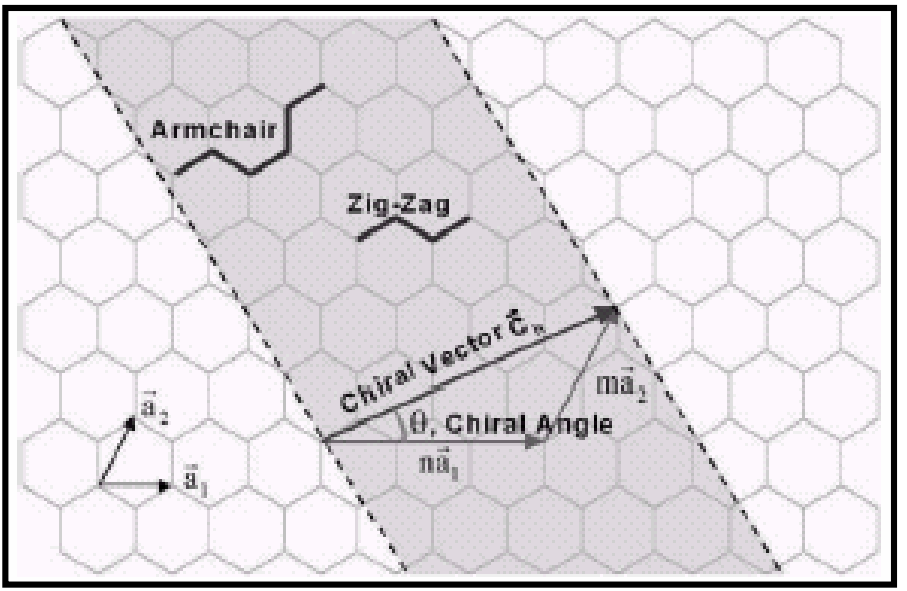


Figure 2. Schematic diagram of the hexagonal sheet of graphite. Carbon atoms are at the vertices. The parameters that define the nanotube structure when the sheet is 'rolled' (chiral angle, chiral vector, basis vectors a_1 and a_2) are indicated in the figure. From reference 12.

The ends of the chiral vector meet each other when the graphene sheet is rolled up to form the cylinder. According to this, tubes of different diameters and helical arrangements of hexagons can arise by changing the values of n and m. In other words, depending on the values of n and m it is possible to have different nanotube structures [4,9,10]. In fact, depending on how the two-dimensional graphene sheet is rolled up, there are three types of carbon nanotubes, armchair, zigzag and chiral. A schematic representation of these structures is given in Figures 3 and 4. Armchair nanotubes are formed when n = m and the chiral angle is 30^o. Zigzag nanotubes are formed when either n or m is zero and the chiral angle is 0^o. All other nanotubes are known as chiral nanotubes and present chiral angles intermediate between 0^o and 30^o [1,6,9,12].

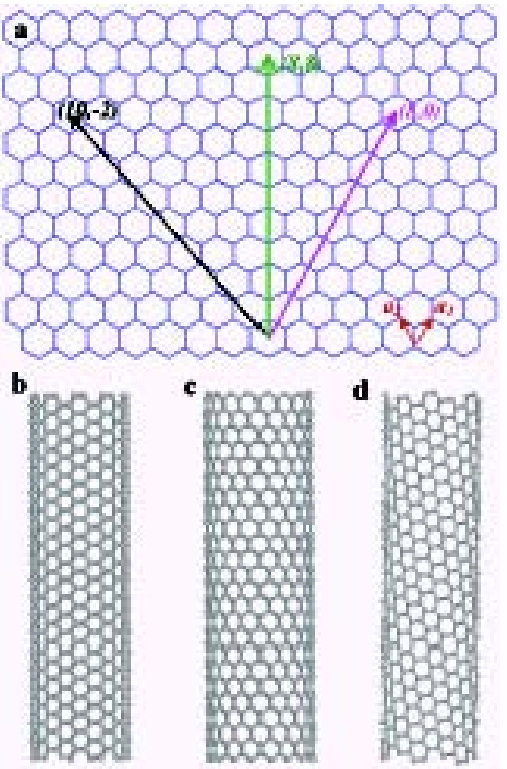


Figure 3. (a) Schematic honeycomb structure of a graphene sheet. SWCNTs can be formed by folding the sheet along lattice vectors. The vectors a_1 and a_2 are shown. Folding of the (8,8), (8,0), and (10,-2) vectors lead to armchair (b), zigzag (c), and chiral (d) tubes, respectively. From reference 1.

The electrical properties of CNTs depend sensitively on the (n,m) indices and, therefore, on the diameter and chirality [4, 9 ,12]. According to the m,n structural parameters values, SWCNTs can be either a metal, semiconductor or small-gap semiconductor [1,4,9,12]. When n=m, the CNTs are metallic. If n – m = 3 x integer, the CNTs present an extremely small band gap and at room temperature they have metallic behavior. For other intermediate values of n – m the behavior is that of a semiconductor with a given band gap [4,9]. This extreme sensitivity of electronic properties on structural parameters is one of the most important aspects of nanotubes that make them very unique. Calculations have predicted that all the armchair tubes are metallic while the zigzag and chiral tubes are either metallic or semiconductor depending on their diameter and chiral angle [6,13].

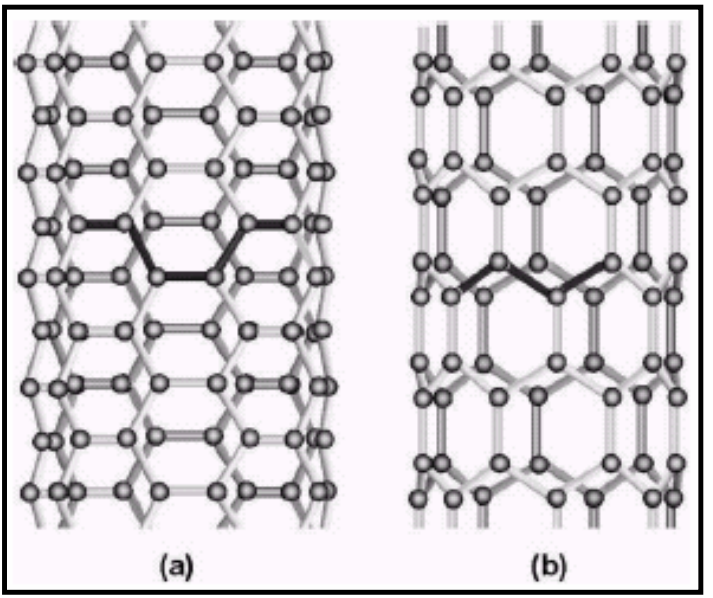


Figure 4. Schematic representation of the atomic structure of an armchair (a) and a ziz-zag (b) nanotube. From reference 12.

The combination of size, structure and topology give nanotubes important mechanical properties such as high stability, strength and stiffness, low density and elastic deformability with interesting surface properties (selectivity, surface chemistry). The helicity as well as the diameter introduce important changes in the electronic density of states, given to nanotubes unique electronic characteristics. These electronic properties open the doors to a wide range of fascinating electronic devices applications [7,9]. Topological defects in nanotubes result in local perturbations to their electronic structure. In this sense, the caps are more metallic than cylinders due to the pentagonal defects. These defects also enhance the chemical reactivity of the ends giving the possibility to open the tubes, fill them with foreign substances and functionalize the ends [14-19].

The strength of carbon bond determines the fascinating mechanical characteristics of this material that are superior to other known materials [20, 21]. CNTs are extremely flexible. In fact, they can be twisted, flattened and bent into small circles without breaking. They can also be compressed without fracture [6,7,9].

CNTs also possess interesting electrochemical properties. Several works have demonstrated the electroactivity of CNTs due to the presence of reactive groups on the surface [10, 22-24]. The small dimensions produce high current densities

on the surface of the electrodes allowing the study of heterogeneous process with excellent results. Ab-initio calculations demonstrated that the improvement in the electron transfer is due to the curvature of the tubes that originate changes in the energy bands close to the Fermi level. The presence of pentagonal defects produced regions with charge density higher than those observed in the region of hexagonal graphite, either in planar or in tubular structures (3-4 times higher) demonstrating the connection between topological defects and electroactivity of CNTs [23]. As it is discussed in the next sections, lower peak potential separations and higher peak currents are observed in the voltammetric behavior of several molecules in the presence of CNTs. These results suggest an interesting electrocatalytic activity, associated with the carbon nanotubes dimensions, the electronic structure and the topological defects present on the tube surface [7, 11, 22-24].

Chapter 3

SYNTHESIS OF CARBON NANOTUBES

The synthesis of CNTs is receiving considerable interest and the main goal is to obtain large scale production of highly pure CNTs. There are three basic methods for synthesis of SWCNTs and MWCNTs: electrical arch discharge, laser ablation (laser vaporization) and chemical vapor deposition (CVD) (or catalytic decomposition of hydrocarbons) [1,7, 9,10, 25, 26].

3.1. ELECTRIC ARC DISCHARGE

It was the first method used for fabricating CNTs and it consists of establishing an electric arc discharge between two graphite electrodes (cathode of around 8-12 mm and anode of 6-8 mm) separated approximately 1 mm under an inert helium atmosphere. A bias of around 10-35 V is applied to the electrodes to establish currents of 60-100 A. The most important factor is to get stable discharge plasma. Since elevated temperatures are achieved, the anode material is sublimated and deposited on the cathode and surrounding walls. The inner deposit contains mainly MWCNTs mixed with polycrystalline graphite nanoparticles, while the outer shell is composed mainly of fused graphite powders, nanoparticles and amorphous carbon [27, 28]. It was reported that the use of single or bi-metal mixtures of Co, Ni, Y and Fe favored the production of SWCNT [29, 30], the most efficient being the combination Co/Y and Ni/Y.

Several are the parameters that affect the arch-discharge nanotubes production such as gas type, pressure and flow rate, electric field strength, electrode materials and dimensions, in addition to unquantified variables such as

apparatus size and geometry, and thermal gradients [25]. The typical production rates are around 20-100 mg/min.

Lee et al [31] proposed the use of plasma rotating electrode process to generate a more stable discharge plasma. The goal was to distribute the micro-discharges more regularly between the two electrodes, stabilizing, therefore, the plasma. On the other hand, the rapid rotation produces a centrifugal turbulence that allows the vaporized material to move it outwards and deposit on the chamber walls or close collectors rather than on the cathode surface, producing CNTs of smaller diameters. Another avenue [32] consisted of the discharge of the electrodes submerged in liquid nitrogen providing, in this way, an inert environment and temperature control.

3.2. LASER ABLATION

The solid graphite target was mounted in a quartz tube and placed in a temperature-controlled oven. After vaporization of this target with a pulsed Nd:YAG laser, a carbon-based soot was collected from the inside of the apparatus. To obtain SWCNTs, it is necessary to dope the graphite target with transition metal catalysts. The system was improved by the incorporation of a second laser Nd:YAG delayed slightly behind the first [33]. The incorporation of equal parts (0.5-1.0 atom %) of cobalt and nickel powders in graphite have demonstrated to be highly efficient for the production of SWCNTs. Eklund et al [34] have produced SWCNTs at rates around 1.5 g/h using a 1.7 kW sub-picosecond free electron laser (FEL). As in the case of arch-discharge, a careful evaluation of the different variables has to be made in order to optimize the production.

3.3. CHEMICAL VAPOR DEPOSITION

This procedure involves the pyrolysis of gas molecules with high content in carbon at elevated temperatures in the presence of catalyst [10, 25]. There are two basic protocols, in one of them, called supported growth process (the most used), the catalyst is prepared and deposited on a support medium, which is inserted into a flow apparatus (a tube at atmospheric pressure in a temperature controlled furnace) and exposed to elevated temperatures, usually 500-1100 ^{o}C for a given time. In the other protocol, called floating-catalyst growth, the catalyst and the

carbon source are injected into the system simultaneously, either in gas phase or in aerosol. Therefore, the decomposition and reaction can occur suspended in the gas flow or following self-deposition on a surface in the reactor. The most common support media are metallic Si, Si wafers or different SiO_2 based materials. Fe, Co and Ni have demonstrated to be the most successful catalysts. It is important to mention that the diameter of the carbon nanotubes is proportional to the particle size, making it possible to tune the nanotube diameter by controlling the catalyst deposition. Even when the most widely used hydrocarbon is acetylene; methane, ethylene, propylene and few aromatic compounds have been also employed. The use of templates for growing the nanotubes inside porous materials has been also described. The advantage of catalyst incorporation into the gas flow is to circumvent the effects of catalyst deactivation by coating with pyrolized hydrocarbons. Floating catalyst methods contribute to the production of SWCNTs, while most of other CVD-based methods produced mainly MWCNTs.

Chapter 4

USE OF CARBON NANOTUBES AS ELECTRODE MATERIAL

Due to their peculiar properties, carbon nanotubes have received enormous attention for the preparation of electrochemical sensors, as it was reviewed by Zhao et al [3], Wang [35] and Li et al. [36]. Different procedures for immobilizing the nanotubes onto electrochemical transducers have been described and the most representative are presented in the following sections.

4.1. PRETREATMENTS OF CARBON NANOTUBES

As in the case of other carbon materials [37], some pretreatment of CNTs is necessary to improve the electron transfer properties and/or to allow further functionalization. The protocols are based, in general, on the oxidation of CNTs. Depending on how drastic is the treatment, it is possible not only break the tubes but also shorten them. In all cases the ends and side walls are modified with a high density of diverse oxygenated functions, mainly carboxyl groups. Different schemes have been proposed and in order to facilitate the understanding, they were agrupated in chemical and electrochemical. Spectroscopic techniques like Raman and IR; microscopies like AFM, SEM and TEM; and electrochemical techniques indicated that the physical structure of CNTs remains the same, although the ends were opened and oxidized to give carboxylic groups.

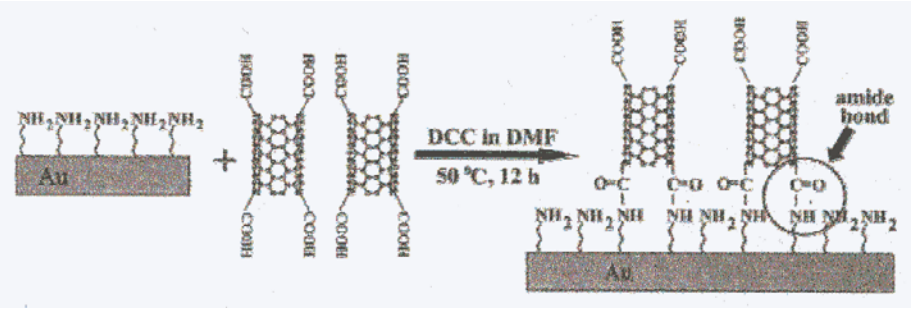
4.1.1. Chemical Pretreatments

Oxidation in air is one of the methods for purifying CNTs. However, at the same time that purified them, this oxidative scheme produces the activation of CNTs. Palleschi et al. [38] proposed the oxidation at 400 ^{o}C using an air flow of 12 mL/min for 1 hour. Sotiropoulou and Chaniotakis [39] proposed two mechanisms for activating MWCNTs grown by CVD on Pt substrate. One of them was performed by air oxidation at 600 ^{o}C for 5 min under air flow.

There are a large number of protocols that use acidic solutions for activating carbon nanotubes. Solutions of sulfuric, nitric and hydrochloric acids either concentrated or diluted, or mixtures of them have been used at room temperature or under refluxing with or without sonication for different times. In a general scheme, once the surface was oxidized, the next step was the careful rinsing of the oxidized nanotubes with ultrapure water, followed by the drying step either at room temperature by exposure to air or under IR lamp, or in vacuum at a given temperature.

Compton et al. [40] proposed the activation of MWCNTs in concentrated nitric acid at 60 ^{o}C for 20 hours. Mao et al. [41] have reported the pretreatment of MWCNT by refluxing in 3 M nitric acid for 12 h.

A more drastic scheme proposed the acidic oxidation using a mixture of concentrated sulfuric (98 %) and nitric acid (65 %) in a ratio 3:1 for 8 hours at 40 ^{o}C [39]. Under these conditions the tube caps are opened and the tubules are shortened in fragments of different length. After that, the CNTs were washed with pure water and dried at 100 ^{o}C overnight.



Scheme 1. Schematic representation of the formation of SWNT assemblies. From reference 68.

In another protocol [42], the MWCNTs-modified electrode was prepared by using MWCNTs (obtained by CVD) previously treated by refluxing in

concentrated nitric acid for about 5 hours, filtered and washed with pure water until neutral pH and then dried under vacuum.

Palleschi et al. [38] proposed an additional oxidation of CNTs previously oxidized in air flow by dispersing in 6.0 M HCl for 4 hours under ultrasonic agitation followed by washing until neutral pH and drying. The same group also presented another treatment by dispersing CNTs in 2.2 M nitric acid for 20 hours at room temperature (under ultrasonic agitation the first 30 min), then washing with distilled water to neutrality and drying in oven at 37 ^{o}C.

Luo et al. [43] proposed the dispersion of CNTs in 4.0 M HCl for 4 hours under ultrasonic agitation to eliminate metal oxide catalysts. After rinsing carefully with water until neutral pH and drying, the CNTs were dispersed in 60 mL mixture of concentrated nitric acid plus sulfuric acid (1:3) with ultrasonic agitation for 4 hours in a water bath followed by washing until neutral pH and finally drying.

Hu et al. [44] performed pretreatments by soaking the CNTs in 5 M nitric acid, ultrasonically dispersing for 6 min and drying under IR lamp oven for 4 h at 45 ^{o}C. Then, the CNTs were diluted with a large amount of water and a little Triton X-100 to increase solubility, sonicated until getting a black solution which after filtering gave the nanotubes. The picture displays in Figure 5 clearly shows the absence of the caps in CNTs after performing the pretreatment.

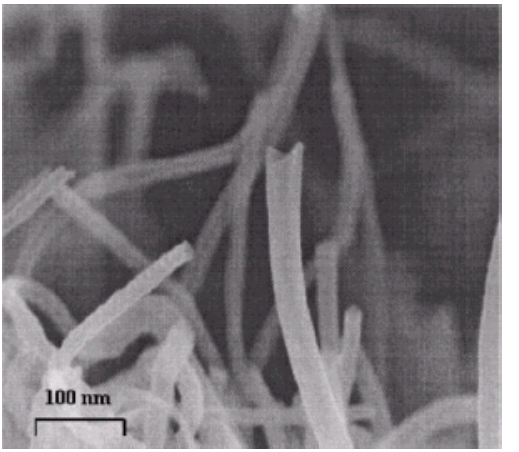


Figure 5. SEM micrograph of the carboxyl-modified CNTs. From reference 44.

Hu et al. evaluated the functionalization of CNTs using different chemical pretreatments [45]. They oxidized the CNTs in two ways, by using mixed acids for 10 min at 65 ^{o}C in a water bath, and by soaking them in nitric acid under

sonication for 10 or 20 min. An increase in the resistivity was found for longer times in the acidic solution and by using mixture of acids.

4.1.2. Electrochemical

Different schemes of electrochemical pretreatments have been proposed. In general, they depend on the system under investigation. For instance, for the oxidation of dopamine, 18 cycles between -1.00 V and 1.50 V at 1.0 V/s in a 0.050 M phosphate buffer pH 7.40 demonstrated to be the optimum pretreatment for obtaining the best voltammetric behavior of dopamine [46]. However, for the oxidation of amitrole, 75 cycles between -1.0 and 1.5 V at 1.0 V/s in 0.050 M phosphate buffer solution pH 7.4 were necessary to obtain the best analytical signal [47].

In some cases a combination of several pretreatments was used to improve the electron transfer. For instance, Palleschi et al. [48] proposed a treatment consisting of an oxidation step at 400 ^{o}C in the presence of O_2 for 1 h, then dispersion in 6.0 M HCl for 4 hours under ultrasonic agitation. Another pretreatment proposed by the authors was the oxidation of CNTs in a 2.2 M HNO_3 solution for 20 h at room temperature with sonication the first 30 min. After that, once the CNTs were immobilized on the electrode electrochemical pretreatments were performed by preanodization and precatodization at 1.70 V and -1.50 V for 3 min each in a phosphate buffer solution (0.2 M pH 7.0). Another scheme also proposed by Palleschi's group [49] was the combination of the typical chemical oxidation in oxygen flow or in the presence of nitric acid and electrochemical pretreatment by cycling with increasing potentials range in 0.5 M sodium sulfate between 0.2 y 0.1 V or between 0.5 and –0.1 V at 100 mV/s for 5 min, depending on the compound under investigation, followed by a succesive cycling in a wider potencial range up to 1.8 V y -0.4 V several cycles.

4.2. STRATEGIES FOR THE PREPARATION OF CNTS-MODIFIED ELECTRODES

One of the problems for the preparation of sensors based on the use of carbon nanotubes is their insolubility in usual solvents. Therefore, it is necessary to disperse them in an adequate medium. Several strategies have been proposed for

the immobilization of carbon nanotubes on electrochemical transducers, the most significant are summarized below.

4.2.1. Dispersion in Different Solutions

4.2.1.a. Acidic Solutions

CNTs have to be dispersed in an adequate solution before immobilizing on a given electrode. In general, the procedures are based on casting the electrode, usually glassy carbon or gold, with a drop of the given dispersion and drying under different conditions.

In one case [50] the electrode was prepared by casting a polished and clean glassy carbon electrode GCE with 10 μL of a solution obtained by dispersion of MWCNTs in concentrated sulfuric acid (1 mg/mL) followed by drying at 200 ^{o}C for 3h and careful rinsing.

Wang et al. [51] proposed the preparation of MWCNT-GCE by casting a polished GCE with 20 μL of a concentrated nitric acid solution containing 2 mg/mL MWCNTs followed by a drying step at room temperature for 30 min.

4.2.1.b. N,N-Dimethylformamide

In general, the immobilization of CNTs on electrodes by dispersing in N,N-dimethyl formamide (DMF) consists of polishing the electrode, casting it with the CNTs suspension in DMF and drying to evaporate the solvent.

Li et al. [52, 53] have reported the preparation of CNTs-modified GCE by casting the GCE with 15 μL of a SWCNTs suspension (armchair structure, prepared by arch-discharge and purified by oxidation in air) in DMF (0.1mg/mL). The drying step was performed under an IR heat lamp.

Pang et al. [54] proposed the use of a SWCNT-modified GCE prepared by casting the polished GCE with 2 μL of the black suspension of SWCNT in DMF (1mg/mL), followed by heating under an infrared lamp to remove the solvent.

4.2.1.c. Nafion

Wang et al. [55] have reported on the ability of the perfluorosulfonated polymer Nafion to solubilize SWCNTs and MWCNTs. They proposed that a homogeneous, well-distributed solution of Nafion/CNTs was obtained with 0.5 % v/v (in 0.05 M phosphate buffer pH 7.4) and 5% v/v polymer solution for SWCNTs and MWCNTs, respectively. A polished GCE was modified with 20 μL of a 2 mg/mL CNTs solution in 0.5 % w/w Nafion. The coating was allowed to

dry at room temperature for 2 hours. The dispersion was prepared by agitation in an ultrasonic bath for 10 min and in this way, it was stable for 3-4 days.

Mao et al. [41] reported the use of a CNT-modified GCE prepared by casting the GCE with 5 μL of a CNTs dispersion prepared in 0.5 % methanolic Nafion solution and followed by the evaporation of the solvent for 10 min.

4.2.1.d. Chitosan

Multiwalled carbon nanotubes were solubilized in an aqueous solution of the biopolymer chitosan (CHIT) [56]. A 0.50 w/v % CHIT stock solution was prepared by dissolving chitosan flakes in hot (80-90 °C) aqueous 0.05 M HCl. The solution was cooled at room temperature, and its pH adjusted to 3.5-5.0 using a concentrated NaOH solution. CNTs were solubilized in CHIT solutions (0.50-3.00 mg of CNT mL^{-1}) by ultrasonic agitation for 15 minutes. An aliquot of 30 μL of the colloidal solution of CNT-CHIT was placed on the surface of GCE and dried for 2 hours at room temperature.

4.2.1.e. Other Media

Compton et al. [57] proposed the immobilization of CNT on basal plane pyrolitic graphite electrodes using different approaches. One of them was done by dispersing the powder in acetonitrile and then casting the electrode. The solvent was eliminated by evaporation.

Other methodology proposed the preparation of MWCNTs-modified electrode by casting a gold surface previously cycled between 0.0 and 1.5 V in 0.5 M sulfuric acid with 5 μL of a black suspension of CNTs prepared by dispersing the oxidized MWCNTs in double distilled water (0.5 mg/mL) [42]. The electrode was then dried under vacuum at about 50 ^{o}C.

Hu et al [58] have proposed an amperometric sensor by casting a GCE with 10 μL of the dispersion of MWCNTs in water in the presence of dihexadecylhydrogenphosphate (DHP) (1mg of CNTs in 5 mg DHP and 5 mL water, under sonication). The solvent was allowed to evaporate at room temperature in air.

4.2.2. Incorporation in Composite Matrices Using Different Binders

4.2.2.a. Teflon

The advantages of using an electrode based on the dispersion of CNTs in a Teflon binder was reported by Wang et al. [59]. The CNT/Teflon composite was

prepared by hand-mixing (with spatula) CNTs and granular Teflon for 10 min and then packed the paste into the electrode cavity.

A MWCNT/Teflon electrode was prepared by hand-mixing the desired amounts of the CNT with granular Teflon for ten minutes [60]. The portions were packed in a Teflon tube (2 x 2 mm cavity), smoothed on a weighing paper and rinsed with distilled water.

4.2.2.b. Bromoform

Hill et al. [61] have reported on the feasibility to use carbon nanotubes as electrode material, by packing oxidized CNTs into a glass capillary in bromoform, nujol, deionised water or mineral oil. When necessary, bromoform was eliminated by backing the packed nanotubes in an oven at 80-100 ^{o}C. Britto and co-workers [62] have proposed the preparation of an electrode based on the dispersion of CNTs with bromoform and packing inside a glass tube.

4.2.2.c. Mineral Oil

Rivas and Rubianes [46] reported for the first time the advantages of a new composite material prepared in an easy, fast and very effective way by dispersing MWCNTs within mineral oil as binder (60.0/40.0 % w/w) . The resulting carbon nanotube paste electrode (CNTPE) retained the properties of the classical graphite carbon paste electrode (CPE) such as the feasibility to incorporate different substances, the low background currents the easy renewal of the surface and composite nature. Electrodes with oil content smaller than 40.0 % w/w were difficult to pack into the Teflon tube. Pastes with 50.0 % w/w showed similar behavior. CNTPE prepared with short (1-5 microns length) and long MWCNTs (5-20 microns length) of 20-50 nm diameter demonstrated to be highly useful as detectors in flow systems [63]. The content of mineral oil was an important variable in the preparation of these carbon nanotubes composites and even when no substantial differences were observed between the electrodes, those prepared with long carbon nanotubes (55.0 % w/w) and mineral oil (45.0 % w/w) allowed to obtain less noisy and more reproducible signals.

Palleschi et al. [38] reported on a composite electrode by mixing SWCNTs and mineral oil, 60/40 % w/w. SEM pictures showed a surface topography more uniform than in the case of CPE with the nanostructures embedded inside the oil binder.

Wang et al. [64] proposed the use of CNTPE for the detection of homocysteine. The composite was prepared by mixing MWCNTs of 5-20 nm length with oil in a ratio 3:2 (CNT/oil) and then placed in a plastic pipette tip (0.5 mm diameter).

Magno et al. [65] reported an amperometric biosensor prepared by mixing MWCNTs with mineral oil (60/40 % w/w) followed by the covering with a polymer obtained by electropolymerization of a 1.0 mM 3,4-dihydroxybenzaldehyde (3,4-DHB) (in 0.1 M phosphate buffer solution pH 7.0) at 0.3 V for 1 min.

Wang and coworkers [66] reported on the use of CNTPE containing Cu as a detector for Capillary Electrophoresis for the determination of carbohydrates compounds. The electrode was prepared by hand mixing mineral oil, MWCNT and copper powder in a weight ratio 1:1:2 (carbon/oil/Cu).

4.2.2.d. Inks

Another interesting strategy proposed by Wang et al. [67] was the preparation of a composite material based on the dispersion of CNTs in ink, in a mode similar to that for preparing graphite screen printed electrodes (SPE). The electrodes were fabricated following two strategies. In one case they were prepared using the same ink as that for preparing the SPE, obtaining a very efficient combination of the advantages of thick-films sensors with the excellent electrochemical properties and analytical performance of CNTs. The other way for preparing these screen printed electrodes consisted of mixing 60 mg CNTs with 500 μL of isophorone solution containing 2 % (w/v) PVC, 2 % (v/v) DBE-4 and 2% (v/v) DBE-5 (DBE-4: dibasic ester containing 98.4 % dimethyl succinate and 0.3 % dimethyl glutarate; DBE-5: dibasic ester containing 99 % dimethyl glutarate and 0.4 % dimethyl succinate) until homogeneous aspect. The ink was printed on alumina ceramic plates. After that, the resulting electrodes were cured for 1 h at 150 ^{o} C and then allowed to cool down at room temperature. SEM pictures showed a microporous structure of flake-shaped particles non-uniformly distributed.

4.2.3. Immobilization on Pyrolitic Graphite Electrodes

Compton et al. [40] proposed the immobilization of MWCNTs on basal plane pyrolitic graphite electrodes by abrasively attaching CNTs on the electrode surface by gently rubbing a polished electrode on a filter paper containing 2 mg MWCNTs for 1 min.

Luo et al. [43] proposed an electrode prepared by intercalation of CNTs in a pyrolitic graphite electrode previously polished with emery paper and alumina slurries and sonicated with water. It was performed by grounding the dry graphite electrode on a weighing paper containing a suitable amount of CNTs powder to intercalate them on the graphite surface by mechanical force and adsorption.

4.2.4. Using other Methodologies

Liu et al. [68] proposed the use of SWCNTs previously cut by oxidation in a mixture of acidic solutions under sonication. Once the carboxyl groups were generated, the assemblies were prepared on the top of the gold surface modified with monolayers of 11-amino-N-undecylmercaptan. The covalent attachment between the carboxyl residues and the amino groups was performed by using dicyclohexylcarbodiimide as condensing agent.

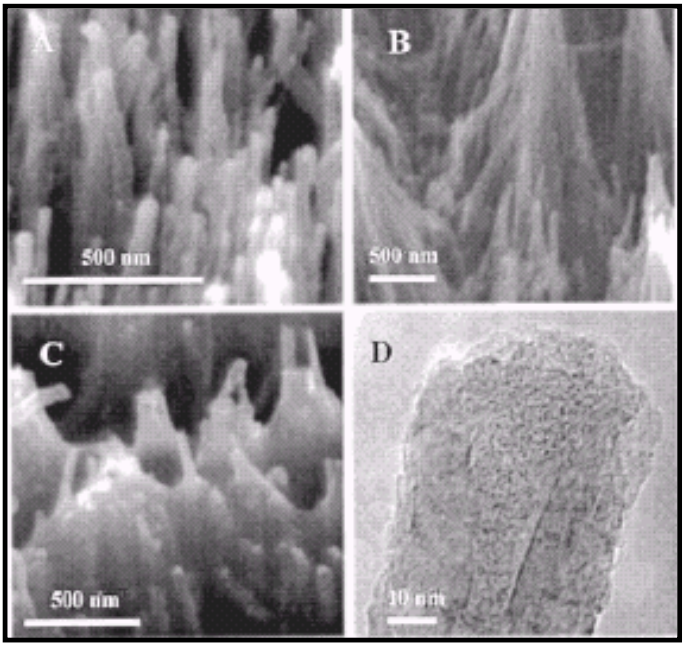
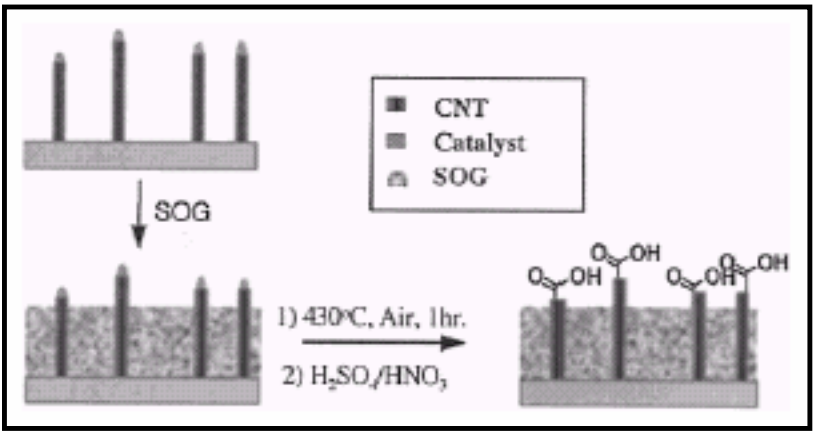


Figure 6. Electron microscope images. (A) Vertically aligned multiwalled CNT arrays with length about 1 μm. (B) Collapsed CNT arrays after purification process. (C) CNT arrays with SOG after purification and tip opening process. (D) High-resolution transmission electron microscope image of an opened CNT end. From reference 69.

Another interesting avenue was the use of MWCNTs arrays grown by CVD using Fe as catalyst and C_2H_4. SEM and TEM showed that CNTs were relatively straight with ends closed and metal-encapsulated catalyst [69]. After an extensive oxidative pretreatment by heating in air and acid oxidation to open the nanotubes and generate carboxylic groups, the CNTs collapse in most cases. Therefore, the authors proposed the use of a film of spin-on glass (SOG) to fill the gaps between the individual CNTs. The spin-on glass film with 15 % w/w methyl groups bond

to Si atoms of Si—O backbone was deposited at a spin speed of 3000 rpm, followed by curing at 400 ^{o}C for 4 hs under positive pressure of argon (Scheme 2). Therefore, the SOG film provides the structural support to the carbon nanotubes and serves as a dielectric material insulating the individual CNTs. Figure 6 shows SEM images of the vertically aligned CNTs array(A), collapsed CNTs array before depositing SOG film (B), CNTs array with SOG after oxidative treatment (C) and TEM image of an open-ended CNTs.



Scheme 2. Fabrication and oxidative pretreatment of carbon nanoelectrode arrays for functionalization. From reference 69.

Lin et al [70] proposed a similar methodology by preparing the electrodes modified with CNTs. The nanotubes were obtained by CVD using Ni as catalyst and once they were grown, an Epson 828 epoxy-based polymer with an MPDA curing agent was spin-coated on the substrate covering the half of the CNTs. The protruding part of them was eliminated by polishing. The oxidation of the CNTs was performed by electrochemical oxidation in 1.0 M NaOH at 1.5 V for 90 s. Once the carboxylic residues were obtained, they were activated in the presence of carbodiimide (EDC) and N-hydroxysuccinimide (NHS).

The self assembled layer-by-layer of polyelectrolytes on CNTs previously functionalized was also proposed [71]. CNTs were synthesized on a grid for TEM by CVD using metallic catalysts. The negative charges were originated onto the surface of carbon nanotubes by adsorbing a pyrene derivative.

Poly(diallyldimethylammonium (PDDA) and polystyrene sulfonate (PSS) were alternatively adsorbed (starting with the polycation) from 30 mM aqueous solutions for 1 hour (first bilayer) or for 18 min for the subsequent layers. The

studies were performed by TEM and elemental analysis. A uniform coverage was obtained even for nanotubes of diameter as small as 2 nm. The pyrene derivatives do not modify the morphology of CNTs, they just provide them a negative charge density. Some studies with Confocal Fluorescence Microscopy were also performed using colorants of opposite charge to the layer under investigation. It was found that after every adsorption step a charge inversion was obtained despite the interpenetration of the layers.

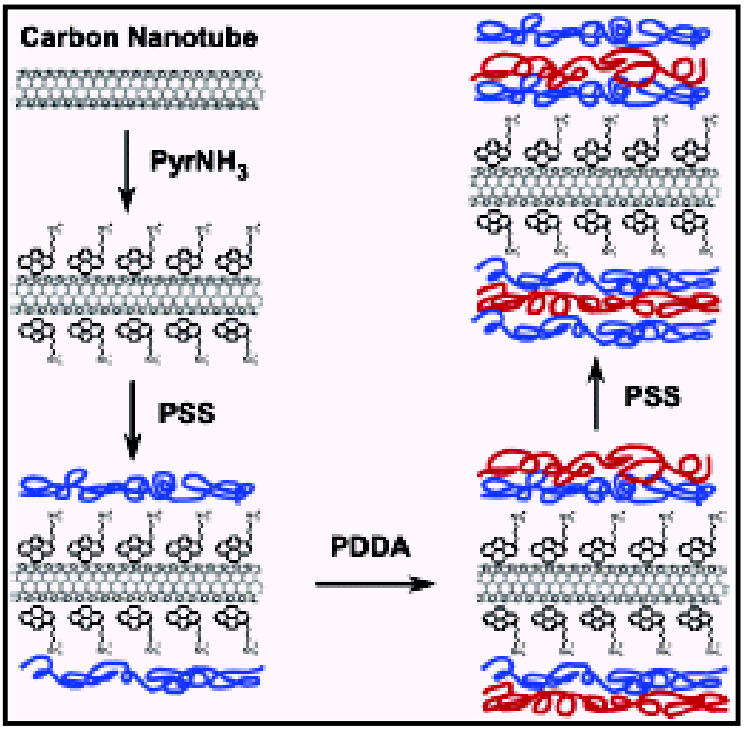


Figure 7. Scheme of the CNTs modification using layer-by-layer electrostatic self-assembly of polyelectrolytes. From reference 71.

Guo et al. [72] reported on the non-covalent modification of SWCNTs by using an interesting scheme. The SWCNTs were previously refluxed in concentrated nitric acid, washed with water and dried. The SWCNTs were first converted to acylchloride in $SOCl_2$ and then they reacted with didecylamine at 90-100 ^{o}C for 96 hours. After extranting the nanotubes and removing the solvent they were ready to use. In a typical experiment, 3 mg of the interlinker 17-(1-pyrenyl)-13-oxo-heptadecanethiol (PHT) was incubated with 10 mL solution of modified

SWCNT in toluene (0.5 mg/mL). The PHT binds on the surface of SWCNT mainly through π-π interactions between the pyrenyl units of PHT and the side-wall of modified SWCNT. Since the PHT presents a thiolated residue, the gold nanoparticles can be bond to the architecture and obtain MWCNT densely coated with gold nanoparticles, converting this architecture in a good platform for further biosensors designs. Figure 8 shows a TEM image of the gold nanoparticles immobilized at MWCNTs through PHT linker.

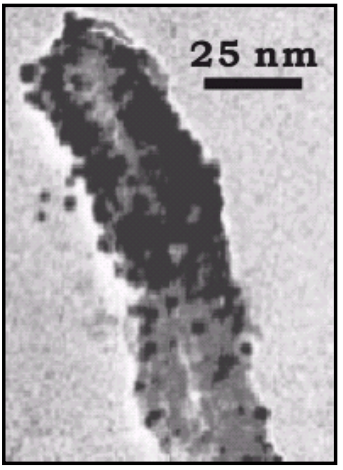


Figure 8. TEM image of gold nanoparticles self-assembled on the surface of MWNTs through 17-(1-pyrenyl)-13-oxo-heptadecanethiol. From reference 72.

Another avenue for immobilizing CNTs was the dispersion of CNTs in cyclodextrin [73]. The electrode was prepared in the following way, 1 mg of CNTs was dispersed in 10 mL β-cyclodextrin (CD) (2% aqueous solution) to give a 0.1 mg/mL solution. An aliquot of 7 μL was dropped on the previously polished, sonicated and dried under IR lamp graphite electrode. The electrode modified in this way was dried under IR lamp. As it is discussed below, the presence of cyclodextrin is not only useful for immobilizing the CNTs but also for improving the molecular recognition of the resulting structure.

Wang et al. [74] have made an interesting comparison of the electrochemical response of glassy carbon electrodes modified with MWCNTs prepared by arc-discharge (ARC) and chemical vapor deposition (CVD). The MWCNTs were dispersed in different media: Nafion, concentrated nitric acid and DMF. The electrochemical performance of the resulting electrodes was evaluated using potassium ferricyanide, NADH and hydrogen peroxide. They found that the

electrocatalytic activity, the background current and the overall electroanalytical performance are highly dependent on both, the method used for preparing the CNT and the dispersing agent. The lowest capacitances were obtained with CNTs prepared by ARC while the best amperometric detection of the redox markers was obtained with nanotubes prepared by CVD and using a DMF as dispersing agent. It is very important to evaluate the connection between the preparation conditions of carbon nanotubes and the performance of the resulting electrode material.

Chapter 5

ELECTROCHEMICAL BEHAVIOR OF DIFFERENT COMPOUNDS AT CNTS-BASED ELECTRODES. SENSING PROPERTIES

5.1. CYTOCHROME C AND AZURIN

Hill et al. [61] have reported the electrochemical behavior of proteins at CNTs mixed with mineral oil, deionised water, nujol or bromoform and packed within a glass capillary. They studied the electrochemical response of cytochrome c and azurin and found that these proteins can be immobilized on and within the packed opened nanotubes without denaturation.

Similar study was performed with cytochrome c using bare GCE, GCE modified with untreated CNTs and GCE modified with treated CNTs [53]. While no response was observed at the GCE and an irreversible behavior was obtained at the unactivated SWCNT-modified GCE; a quasi reversible bevahior was observed at the activated SWCNT-modified GCE, with a peak potential separation of 73.7 mV. These results point out the advantages of CNTs in the electron transfer reaction. A linear relationship between peak current and cytochrome c concentration was found between 3.0×10^{-5} M and 7.0×10^{-4} M with a detection limit of 1.0×10^{-5} M.

5.2. DOPAMINE AND RELATED COMPOUNDS

Britto and co-workers [62] have reported a dramatic improvement in the electrochemical behavior of dopamine using a composite prepared by dispersion of CNTs in bromoform. Cyclic voltammograms at this electrode showed a ΔEp of 30 mV, demonstrating the reversibility of the dopamine/dopaminequinone redox couple at this material. The electrode was also challenged by immersing it in brain tissue homogenates and no change in the voltammetric response for dopamine was observed under these conditions.

Rubianes and Rivas [46] demonstrated the advantages of a composite material prepared by mixing MWCNTs and mineral oil (CNTPE) on the electrochemical behavior of different biomolecules. The voltammetric signal for dopamine, ascorbic acid, dopac and uric acid largely improved at the composite containing CNTs. Figure 9 shows cyclic voltammograms obtained at 0.100 V/s for 1.0 x 10^{-3} M ascorbic acid (A), uric acid (B), dopamine (C) and dopac (D) at composite carbon electrodes containing only graphite (40.0 % w/w), graphite (30.0 % w/w) and 10.0 % w/w MWCNTs and only MWCNTs (40/60 carbon/oil). In all cases, larger voltammetric peak currents and lower overvoltages for the oxidation of the different compounds was obtained. For instance, the peak potential separation for dopamine and dopac decreased 133 and 313 mV at CNTPE compared to CPE, while the overvoltages for the oxidation of ascorbic acid and uric acid decreased 230 and 160 mV, respectively.

Palleschi et al. [38] reported the use of a composite electrode obtained by mixing SWCNTs and mineral oil in a ratio 60/40 % w/w (carbon/oil). Comparable background currents were obtained with CPE and CNTPE when CNTs were not pretreated, increasing in a factor of 100 after CNTs pretreatment. The authors reported a significant improvement on the electrochemistry of dopamine, serotonin, 5-Hydroxytryptamine and other compounds like caffeic acid, ferricyanide, sodium hexachloroiridate (III) and catechol.

Palleschi et al. [49] also compared the voltammetric behavior of Pt, GCE and CNTPE (60/40 % w/w ratio) using inorganic and organic redox couples. The capacitances were 30.7 and 5.6 μF/cm^2 for CNTPE and CPE, respectively. Both, untreated and pretreated CNTPEs showed resistances similar to those of Pt and CPE, while GCE exhibited the largest one. The redox behavior of dopamine and other compounds such as hexacyanoferrate, hexachloroiodate (III), hexaminruthenium (III), p-methylaminophenol sulfate and ferrocenemonocarboxylic acid were evaluated at the different electrodes. CNTPE showed good electroactivity with all molecules, especially using CNTs pretreated

with nitric acid. The oxidation of CNTs produced an increase in the capacitive current making even worst the electrochemical response. After 15 days exposed to air the signal decreased just 30 %.

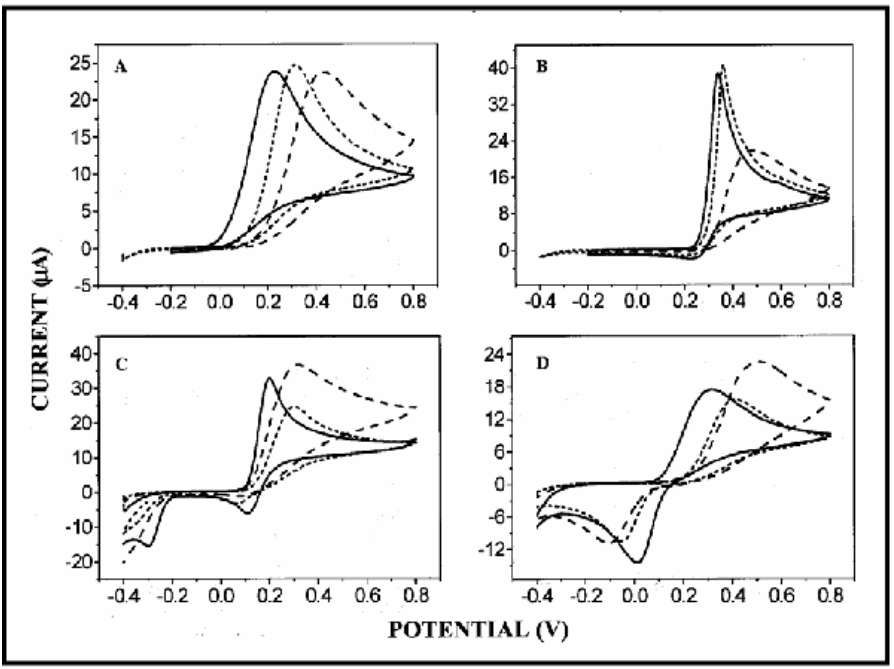


Figure 9. Cyclic voltamograms for 1.0 x 10^{-3} M ascorbic acid (A), uric acid (B), dopamine (C) and dopac (D) at diferent electrodes: (- - -) CPE; (. . .) CPE containing 10% w/w MWCNTs; (———) CNTPE (40/60 % w/w). Supporting electrolyte: 0.050 M phosphate buffer solution, pH 7.40. Scan rate: 0.100 V s^{-1}. From reference 46.

Luo et al. [43] described an electrode based on the incorporation of CNTs for the simultaneous determination of dopamine, ascorbic acid and serotonin. By using differential pulse voltammetry, dopamine could be detected in the range of 0.5 to 10 μM in the presence of 5 μM serotonin and 0.5 mM ascorbic acid, with a detection limit of 0.1 μM. For serotonin, the signal was linear between 1.0 and 15 μM in the presence of 5 μM dopamine and 0.5 mM ascorbic acid, with a detection limit of 0.2 μM. No interference was reported on the determination of 5 μM dopamine and 5 μM 5-hydroxytryptamine in the presence of 10-fold excess dopac, 5-fold excess uric acid, 200-fold excess oxalate and 500-fold excess glucose. Excellent reproducibility was also reported. No interference was observed in the determination of dopamine and serotonin in brain of rabbit.

The study of the groups involved in the redox behavior of CNTs as well as the electrochemical behavior of dopamine and related compounds was also reported [75]. A GCE was modified by casting a suspension of nitric acid containing SWCNTs (prepared by arc-discharge). The studies were performed by XPS and IR, demonstrating the participation of the carboxylic groups in the redox behavior of the electrode, which were reduced to CH_2OH coupled with four electrons. At a scan rate of 0.1 Vs^{-1}, the E_{pc} and E_{pa} were -0.126 and -0.024 V vs SCE in a Britton-Robinson solution pH 6.9. The resulting electrode demonstrated to have highly catalytic activity towards several biomolecules such as dopamine, epinephrine and ascorbic acid. At the SWCNT-film modified GCE, there is an important shifting of the dopamine oxidation peak potential. The current was linear from 1.0 x 10^{-6} M to 2.0 x 10^{-4} M dopamine.

Bai et al. [76] studied the electrochemistry of empty nanotubes and nanotubes filled with toluene after casting NTs in a gold electrode. MWCNTs were shortened and then filled with toluene. The empty nanotubes and toluene-filled nanotubes were dispersed with ultrasonic agitation in ethanol forming a 0.1 mg/mL solution. 15 μL of this solution were dropped on a polished gold surface and the solvent was then allowed to evaporate. The authors proposed that the filling of CNTs with toluene may affect the electronic properties of nanotubes, improving the charge transfer of dopamine and epinephrine. A linear relationship between peak current and dopamine concentration was obtained between 5.0 x 10^{-6} and 3.0 x 10^{-4} M while the detection limit was 3.0 x 10^{-7} M. In the case of epinephfrine, an improvement in the voltammetric behavior was also obtained.

Li. N. et al [52] reported a study about the voltammetric behavior of dopac at GCE modified with SWCNTs. The behavior of dopac drastically changes at the CNTs-modified GCE, with important increase in the peak current in comparison with GCE, indicating a noticeable increment in the heterogeneous rate constant. A linear relationship between peak current and dopac concentration was found between 1.0 x 10^{-6} and 1.2 x 10^{-4} M, while the detection limit was 4.0 x 10^{-7} M. Dopac can be determined at CNTs-modified GCE in the presence of 3-methoxy-4-hydroxyphenylacetic acid (HVA). Dopac can be also detected in the presence of 5-hydroxy-tryptamine since both oxidation peaks are clearly distinguishable.

Pang et al. [54] studied the electrochemical behavior of L-dopa at SWCNT-modified GCE. Before starting, the electrode was immersed for 120 s in the L-dopa solution. L-dopa showed an irreversible behavior at bare GCE with peak potential separation of 161 mV. On the contrary, a quasi reversible behavior with peak potential separation of 55 mV was obtained at the SWCNTs-modified electrode. Experiments performed by differential pulse voltammetry showed a

linear range between 5.0 x 10^{-7} and 2.0 x 10^{-5} M L-dopa and a detection limit of 3.0 x 10^{-7} M.

Li et al. [77] reported the electrocatalytic oxidation of norepinephrine at a GCE modified with SWCNTs. The electrode showed a very good reproducibility and stability. A linear relationship was obtained between the oxidation peak current and norepinephrine concentration between 1.0 x 10^{-5} and 1.1 x 10^{-3} M and the detection limit was 6.0 x 10^{-6} M. The electrocatalytic activity of the SWCNTs-modified-GCE was also demonstrated with dopamine, epinephrine and ascorbic acid.

Another approach to determine epinephrine was proposed by Compton et al. [40]. They used MWCNTs abrasively attached to the basal plane pyrolytic graphite (bppg). Despite an important increase in the background currents was observed in the presence of CNTs, the electrode demonstrated a good performance. A decrease of 300 mV in the oxidation overvoltage for epinephrine and a significant increase in the associated peak current was obtained with the CNT-bppg in comparison with the bppg electrode. Amperometric detection of epinephrine performed with a rotating disk electrode at 0.25 V showed a linear range from 0.1 μM to 0.1 mM, with a detection limit of 0.02 μM. The electrode demonstrated to be highly stable since after 20 min at 0.25 V in a 40 μM epinephrine solution the response remained almost constant. Another interesting fact was the very good resolution of ascorbic acid and epinephrine oxidation peaks, with a difference of 220 mV in the oxidation peak potentials, at variance with the response at bare graphite electrode where just one wave was obtained as a result of the overlapping of the two processes. The electrode showed very good short and long term stability.

5.3. HOMOCYSTEINE AND RELATED COMPOUNDS

Wang et al. [64] proposed the use of CNTPE for the detection of homocysteine. Voltammetric experiments of 160 μM homocysteine at CNTPE showed a well defined signal at 0.28 V that reached a maximum at 0.64 V. This peak current depended linearly with the square root of the scan rate. On the contrary, at CPE only a slight increase in the oxidation current was obtained at 0.40 V, with no peak current definition. A linear relationship between the voltammetric current and homocystein concentration at 0.64 V was observed between 20 and 180 μM, with a detection limit of 17.3 μM. Amperometric experiments were also performed at a potential of 0.70 V and a linear relationship between steady-state currents and homocystein concentration was obtained

between 5 and 50 μM with a detection limit of 4.6 μM. The authors also evaluated the response of different thiolated compounds like cysteine, glutathione and N-acetylcysteine and the results revealed that in all cases the oxidation at CNTPE occurs at lower potentials than at the classical CPE with substantially higher currents.

Another electrochemical method for the sensitive determination of homocystein was reported by Mao et al. [41]. They employed a CNT-nafion-modified GCE. Two processes were observed at this electrode in the presence of 1.0 mM homocystein, one at 0.0 V and the other at around 0.35 V. The first one was attributed to the catalytic oxidation of homocystein mediated by the oxygen-containing molecules present at the oxidized CNTs and the other one to the direct oxidation of homocystein at the CNT-modified GCEs facilitated by the CNTs. The amperometric response at 0.0 V was very fast, reaching the steady state in 10 s, with a detection limit of 6.0×10^{-2} μM. The flow injection response was more stable at the CNT-Nafion-GCE than at GCE with a very small decrease of the signal after 50 min. Standars deviations of 2.3 % were obtained after 20 injections of 0.80 μM homocystein. The response was very reproducible, with negligible changes electrode-to-electrode, low charge currents and good conductivity.

5.4. CARBOHYDRATES

The direct oxidation of glucose in alkaline solutions by using MWCNTs attached to the surface of a GCE by using conductive silver paint was also proposed [78]. The electrode, of an effective area of 0.0474 cm^2, was used without any pretreatment. A substantial decrease in the oxidation overvoltage for glucose was observed in a highly alkaline medium, demonstrating the catalytic activity of CNTs towards the oxidation of glucose. A linear relationship was observed between the steady-state current at 0.20 V and glucose concentration between 2.0 and 11.0 mM, with a sensitivity of 4.36 $\mu A cm^{-2} mM^{-1}$ and a detection limit of 1.0 μM. The sensitivity remained without changes even in the presence of chloride, indicating that under these conditions there was not poisoning of the electrode. The CNTs-modified GCE resulted very stable and useful as electrochemical detectors, although, the selectivity needs to be improved for further application.

The use of MWCNT composite electrodes containing Cu as a detector for capillary electrophoresis determination of carbohydrates compounds was also reported [66]. The oxidation of sucrose, galactose and fructose at Cu-CNTPE started at potentials around 0.2 V lower than at Cu electrodes allowing in this way

a highly sensitive detection of different sugars using NaOH solution pH 12.5 for the amperometric detection. Compared to Cu or CNTs alone, this new material displayed a substantially greater promotion of the oxidation of carbohydrates and, consequently, significatively higher sensitivity with detection limits of 20 μM glucose and 25 μM gluconic acid.

5.5. NADH

Wang et al. [50] have reported on the highly catalytic activity of a GCE coated with MWCNTs towards NADH oxidation. A decrease of 490 mV in the oxidation overvoltage compared to GCE was obtained, allowing the detection of NADH at low potentials. The response was very stable, since after 60 min at 0.60 V the signal for 5 x 10^{-3} M decreased just a 10 %. A fast response (8-10 s) was obtained under conditions that at bare GCE would have been impossible to get.

Another interesting work proposed by Wang et al. [67] showed the advantages of CNTs-SPE. Cyclic voltammograms for NADH among other compounds such as hydrogen peroxide, potassium ferricyanide and catechol showed higher electrochemical activity at CNT-SPEs than at SPEs, especially evident for the first two compounds.

Rubianes and Rivas [79] reported that the oxidation of NADH at CNTPE started at -0.100 V, that is, 0.300 V less positive than at CPE due to the catalytic effect of carbon nanotubes. The CNTPE demonstrated an effective short term-stability since even after 15 min at 0.400 V, the oxidation signal of 1.0 x 10^{-5} M NADH decreased less than 20 %. In similar experiments at CPE the signal decreased more than 80 %.

5.6 AMINOACIDS

Wang et al. [80] proposed the amperometric detection of non-electroactive aminoacids at CNTs-GCE and at Ni-CNTs-GCE. The CNTs previously treated with nitric acid were immobilized onto the GCE using a 1 % v/v Nafion as dispersing agent. The detection was performed either in a stirred NaOH solution or in flow system (1.0 mL/min) by applying a potential of 0.55 V. While no response was observed at unmodified GCE for several non electroactive aminoacids like arginine, histidine, lysine, asparagine, methionine and phenylalanine, an excellent response was obtained at GCE modified with CNTs at

potentials higher than 0.3 V. Figure 10 shows amperometric recordings at 0.55 V in 0.1 M NaOH for successive additions of histidine (A), asparagines (B) and methionine (C) at unmodified (a) and SWCNT-modified GCE (b). The advantages of the presence of CNTs are clear, since no response is observed at the bare GCE, while a fast and well defined signal is obtained at the SWCNT-modified GCE. Some differences were found using SWCNTs or MWCNTs and even with MWCNTs prepared by ARC or CVD. CNTs prepared by CVD demonstrated to be more active. At electrodes prepared with SWCNTs the oxidation started a lower potentials compared to the ones prepared with MWCNTs. In the case of Ni-CNTs-GCE, the nickel was deposited at -2.0 V for 3 min from a 5 mM nickel sulfate solution prepared in an acetate buffer pH 4.5. Then the electrode was transferred to a 0.1 M NaOH solution and the potential was cycled 20 times from -0.9 to 0.9 V at 100 mV/s to ensure complete formation of the nickel hydroxide layer. The presence of nickel hydroxide improved even more the amperometric response of different aminoacids like arginine, hystidine, lysine, asparagines, methionine and phenylalanine by complex formation, with detection limits in the order of 10^{-5} M. An electrocatalytic process is produced at the Ni-CNTs as a consequence of the reduction of the newly formed NiO(OH) in the presence of aminoacids.

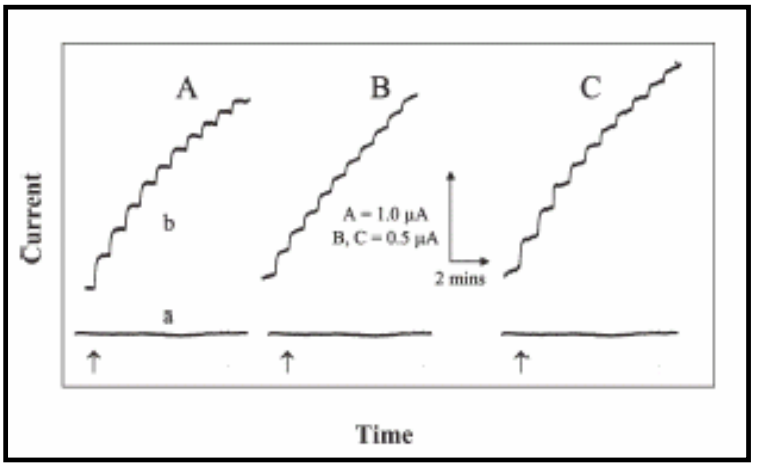


Figure 10. Current–time response for successive 10 μM additions of histidine (A), asparagine (B), and methionine (C) at unmodified (a) and SWCNT-modified (b) glassy carbon electrodes. Operating potential: + 0.55 V; supporting electrolyte: sodium hydroxide (0.1M, pH 13); stirring rate: 300 rpm. From reference 80.

5.7. URIC ACID

A selective response for uric acid was obtained at β-CD-CNT-GE depending on the nature of the cyclodextrin used [73]. The oxidation current for uric acid at β-CD-CNT-GE was several times higher than the corresponding at α-CD-CNT-GE, indicating that the structure of β-CD facilitates the capture of uric acid. On the contrary, α-cyclodextrin interacts better with ascorbic acid than with uric acid. Using a β-CD-CNT-GE it was possible to detect ascorbic acid in the presence of uric acid due to the significant peak potentials separation (about 360 mV). The resulting electrode was stable for 4 days and the reproducibility was 1.5 %. A linear relationship between peak current (from differential pulse voltammograms in 0.2 M acetate buffer pH 4.5) and uric acid concentration was obtained from 5 x 10^{-5} to 5 x 10^{-7} M UA. The detection was 0.2 μM. The oxidation of CNTs did not show any advantage in uric acid quantification.

5.8 OTHER COMPOUNDS

Liu et al. [68] proposed the fabrication and characterization of chemically assembled SWCNTs on gold surfaces and reported the advantages of using this electrode to study the electron transfer of some reactions. While no response for $[Ru(NH_3)_6]Cl_3$ was observed at the thiolated gold electrode, a response similar to that at bare gold was obtained in the presence of CNT evidencing the important improvement in the electron transfer originated by the presence of CNTs.

Hu et al. [58] reported a well defined peak at 0.68 V for 5.0 x 10^{-6} M indole-3-acetic acid (an important hormone present in plants) in pH 2.0 phosphate buffer at GCE modified with MWCNT dispersed in DHP [58]. The oxidation peak current of the indole-3-acetic acid increased gradually with the amount of MWCNT-DHP dispersed at the GCE up to a volume of 15 μL. For higher volumes the thickness of the layer blocked the electron transfer. The oxidation peak current presented a linear relationship with the concentration of the hormone from 1 x 10^{-7} M to 5 x 10^{-5} M, with a detection limit of 2 x 10^{-8} M and a reproducibility of 4.3 % for 36 measurements of 5 x 10^{-6} M. The authors extended the use of this sensor to the determination of the hormone in gladiola, apple and phoenix leaves showing a very good agreement with HPLC determinations.

The detection of the anthracycline daunomycin by accumulation at MWCNTs-modified GCE was also reported [81]. Daunomycin was accumulated at open circuit for 3 min and then it was reduced by differential pulse voltammetry

from -0.10 to -0.90 V. The reduction peak current at -0.526 V was linear with the concentration of daunomycin in the range from 2 x 10^{-8} to 1 x 10^{-5} M, the detection limit being 8 x 10^{-9} M. The relative standard deviation for 2 x 10^{-7} M daunomycin was about 6%. The method was successfully used for the determination of the analyte in urine samples of cancer patients.

The MWNTs-GCE was also used to study the electrochemical behavior of brucine by cyclic voltammetry and square wave voltammetry [82]. The current for brucine at the modified electrode increased linearly with the concentration in the range between 1 x 10^{-6} to 1 x 10^{-4} M with a detection limit of 2.0 10^{-7} M.

The adsorption of molecular oxygen on CNTs was studied by Collins et al [83] and they found that the electrical properties of CNTs are very sensitive to oxygen adsorption. Kong et al proposed the use of nanotubes for developing miniaturized sensors for the detection of gas molecules at room temperature [84]. The mechanism for the NO_2 was a physisorption close to chemisorption, the oxidizing NO_2 molecule takes 1/10 of an electron charge from the nanotube, increasing the hole carriers and enhancing the conductance for the p-type nanotubes. In the case of NH_3, there is a physisorption, the Lewis base donates a small amount of electrons to nanotubes and reduce the hole-carriers, decreasing the conductance [84].

Chapter 6

USE OF CNTS FOR THE DEVELOPMENT OF ELECTROCHEMICAL BIOSENSORS

A biosensor is basically a chemical sensor with two main components, a biorecognition layer encharged of the biomolecular recognition of the analyte and a transducer that is responsible for the conversion of the biorecognition event into an useful electrical signal [85].

Enzymes, antigens, antibodies, nucleic acids, receptors and tissues have been used as biorecognition elements. According to this element, it is possible to separate in enzymatic (involving a biocatalytic event) and affinity (involving an affinity event) biosensors [86]. Enzymatic biosensors are connected with the use of enzymes as biorecognition element, while affinity biosensors involve the use of nucleic acids, antibodies, antigens or receptors. Concerning transducers, they can be optical in their different modes, piezoelectric, thermal and electrochemical. In the case of electrochemical biosensors, the electrical signal obtained as a consequence of the interaction analyte/biosensing layer can be displayed as a given signal depending on the electrochemical transduction mode. The nature of the electrode is very important for the transduction process. In this sense, CNTs represent an important alternative for the transduction event due to their excellent electronic properties [10].

6.1. ENZYMATIC ELECTROCHEMICAL BIOSENSORS

The first enzymatic electrode was proposed by Clark and Lyons more than 40 years ago [87]. Since then, electrochemical biosensors based on the use of enzymes as biorecognition layer have received considerable attention due to the

advantages they possesses as a result of the very efficient combination of the biocatalytic activity of enzymes with the highly sensitive electrochemical transduction.

Several strategies for immobilizing proteins on CNTs modified electrodes have been proposed. The step of immobilization is critical, since the enzyme has to remain as much active as possible in order to perform an efficient biorecognition of the substrate. The other aspect to consider is that the transducer where the protein will be immobilized has to allow a fast charge transfer to ensure a rapid and sensitive response. Therefore, it is important to take into account that the noncovalent functionalization of the sidewalls of SWCNTs is the best way to preserve the sp^2 nanotube structure and their electronic characteristics.

6.1.1. Immobilization of Proteins on Carbon Nanotubes

A very interesting discussion about the adsorption of proteins on CNTs was reported by Sun et al. [88]. The authors used ferritin as a model and evaluated the natural affinity of purified SWCNTs towards this protein. CNTs were dispersed first in phosphate buffer solution pH 6-7 by sonication for 1 h. The protein was then added to the solution and allowed to interact for 24 h while stirring. The authors suggest that the presence of defects on the surface of CNTs may play a very important role in the interaction with ferritin and that the nonspecific interactions between the protein and CNTs include hydrogen bonding and electrostatic and hydrophobic interactions. One important aspect to consider for the development of biosensors is that the functionalization of CNTs with hydrophilic polymers or with oligomeric polyethylenglycol moieties made the protein adsorption more difficult. The authors also discussed about the affinity of SWCNTs by amine groups. Considering that proteins are very rich in these groups, they could play a very important role in the interaction protein-SWCNT. When the interaction is performed in the presence of carbodiimide the immobilization of ferritin became more favorable.

Davis et al. [89] reported an important work regarding the immobilization of metalloproteins and enzymes on oxidized, purified and vacuum-annealed SWCNTs in aqueous solution. AFM experiments showed that the immobilization is mainly physical, without need for covalent activation or electrostatic interaction. In fact, cytochrome c at pHs below the isoelectric point and ferritin at pHs above the isoelectric point showed an important adsorption obtained just by stirring the nanotubes dispersion (0.03 mg/mL) in dilute protein solutions (50-100 μg/mL) for a given time (2-20 h). GOx could be also adsorbed in a very efficient

way, as the picture showed in Figure 11 indicates. The enzyme immobilized remained active since in the presence of 0.5 mM ferrocenemonocarboxyic acid and glucose, an important catalytic current was obtained (Figure 12).

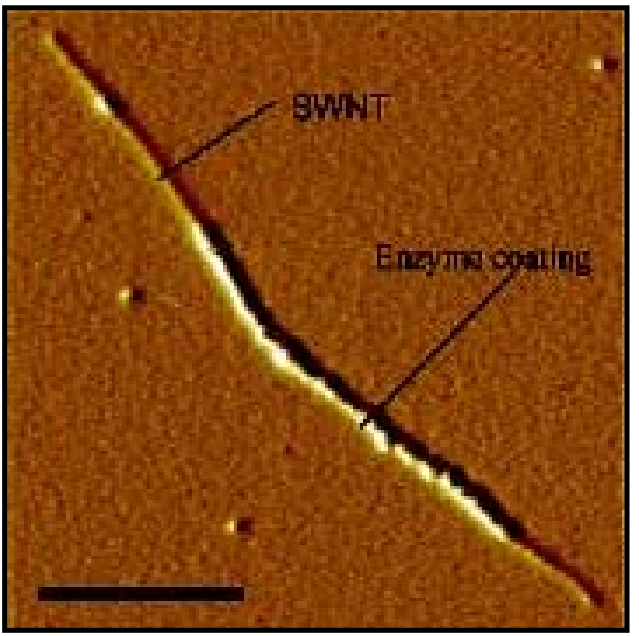


Figure 11. AFM image of a glucose oxidase-modified SWCNT. From reference 89.

Dai et al. [90] reported a very interesting work introducing a simple and general approach for noncovalent functionalization of the sidewalls of CNTs for further immobilization of ferritin, streptavidin and biotinyl-3,6-dioxaoctanediamine in a very efficient way. The first step was the noncovalent functionalization of SWCNTs by irreversible adsorption of a bifunctional molecule, 1-pyrenebutanoic acid, succinimidyl ester onto the hydrophobic surfaces of SWCNTs dispersed in DMF or methanol.

This molecule interacts in a very stable way in aqueous solution through the aromatic rings with the basal plane of graphite via π-stacking with the sidewalls of SWCNTs. The succinimidyl residues are highly reactive to nucleophilic substitution by primary and seconday amines of proteins or other molecules. SWCNTs were incubated in a pyrenebutanoic acid, succinimidyl ester solution (6 mM in DMF or 1 mM in methanol) for 1 h at room temperature followed by careful rinsing in pure DMF or methanol. The proteins were then immobilized by

incubation in aqueous solution for 18 hours at room temperature, rinsed thoroughly in pure water for 6 hours and then dried.

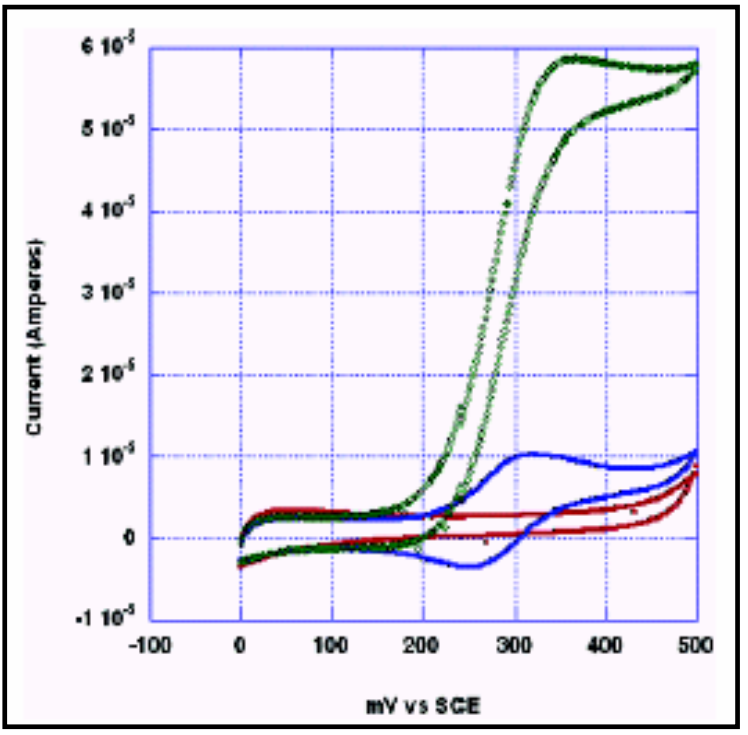


Figure 12. Voltammetric response of a GOx-SWCNT-modified glassy carbon electrode in the absence (red) and presence (blue) of 0.5 mM ferrocene monocarboxylic acid. The catalytic response (green) after the addition of 50 mM glucose is also shown. From reference 89.

The enzymatic activity of α-chymotrypsin was evaluated in composites of poly(methyl metacrilate) with different carbon materials [91] demonstrating that the incorporation of SWCNTs into enzyme-polymer composites results in active and stable polymeric films. The release of the protein from the composite was evaluated measuring the enzymatic activity in the supernatant in contact with the composite. The results showed that in the case of SWCNTs the leaching of the protein from the composite was lower. This fact was attributed to the union of the protein to the CNTs. The effect of other polymers such as polystyrene and poly(lactic acid) was also analyzed and the leaching of the protein was significant in the absence of SWCNTs. Only the hydrophobic ones (poly(methyl metacrilate)

and polystyrene) promoted the protein adsorption. The increase of CNTs percentage in the composite also produces higher α-chymotrypsin retention.

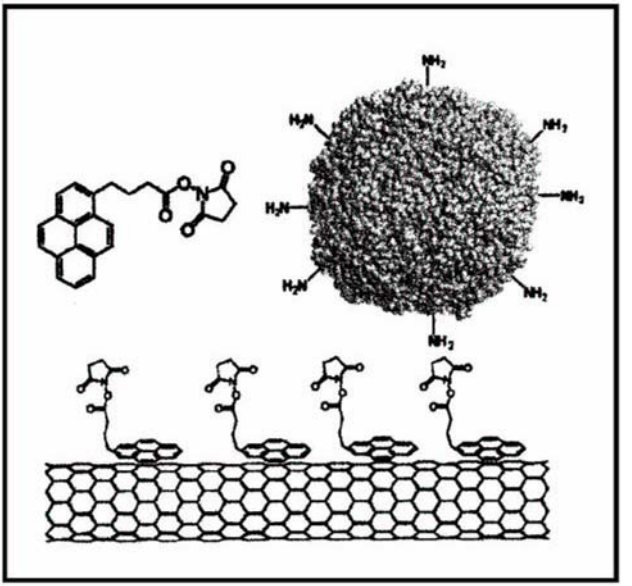


Figure 13. 1-Pyrenebutanoic Acid, Succinimidyl Ester 1 irreversibly adsorbed onto the sidewall of a SWCNT. From reference 90.

Gooding et al. [92] presented a strategy for studying the electron transfer properties of redox enzymes like microperoxidase 11 attached to the end of aligned SWCNTs. A polycrystalline gold electrode previously cleaned in 0.05 M sulfuric acid was derivatized with cysteamine by interacting for 5 hours with a 1 mM cysteamine ethanolic solution. Subsequently, this modified-gold surface was immersed for 4 hours in a dispersion of oxidatively shortened SWCNTs in 1 mL of DMF containing 0.5 mg of dicyclohexylcarbodiimide to convert the carboxyl group located at the end of shortened CNTs into active carbodiimide esters. The SWCNTs were aligned normal to the electrode surface. Then, microperoxidase was attached to the free ends of the tubes by incubation in a 0.5 mg/mL microperoxidase in HEPES solution pH 7.5 at 4^{o}C overnight. The schematic representation of the protocol is displayed in Figure 14.

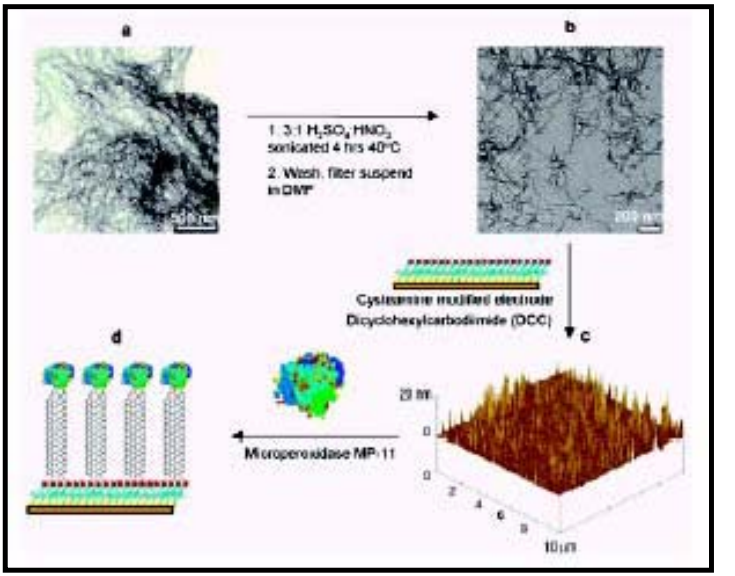


Figure 14. Scheme of the different steps involved in the fabrication of aligned shortened SWCNT arrays for direct electron transfer of enzymes such as microperoxidase MP-11. From reference 92.

Since the iron center of the protein was not shielding, the electron transfer occurred and cyclic voltammograms showed a voltammogram with a redox couple with $E_{1/2}$ at -390 mV. The coverage obtained from the reduction peak area was 35 pmolcm^{-2}, well correlated with the the value of 32 pmolcm^{-2} estimated from the AFM images. In summary, the SWCNT normal aligned can act as molecular wires to allow the electrical communication between the electrode and redox proteins covalently attached to the ends of SWCNTs.

Fructose Biosensor

Magno et al. [65] reported an amperometric biosensor for fructose using an electrode prepared by dispersion of MWCNT within mineral oil (60/40 % w/w) and covered by a polymer obtained from the electropolimerization of dihydroxybenzaldehyde. The fructose dehydrogenase was immobilized on different membranes placed on the top of CNTPEs and then covered with an additional polycarbonate membrane (0.03 μm pore size) to prevent fouling and microbial attack.

Glucose Biosensor

Glucose biosensors have received a lot of attention due to the importance that the fast, sensitive and selective glucose determination presents in the diagnostic and control of diabetes, one of the most important diseases of this century. Different strategies involving CNTs have been proposed for the development of glucose biosensors, and the most representative are included below.

The suitability of CNTPE for developing highly sensitive glucose enzymatic biosensors by incorporation of glucose oxidase (GOx) within the composite matrix was illustrated by Rubianes and Rivas [46]. The resulting enzymatic electrode allowed the highly sensitive and selective determination of glucose even without redox mediators, metals or anti-interferents layers due to the important electrocatalytic effect of carbon nanotubes on the reduction of hydrogen peroxide. Figure 15A shows the amperometric recordings obtained at -0.100 V at CPE-GOx (a) and CNTPE-GOx (b) for successive additions of 2.0 mM glucose. Almost no response is observed at the graphite composite electrode. On the contrary, a fast and sensitive response was observed at CNTPE-GOx due to the catalytic activity of CNTs towards hydrogen peroxide. Figure 15B shows the corresponding calibration plots. The sensitivity obtained with CNTPE-GOx was 43 times higher than that obtained with the CPE-GOx. A linear range was obtained from 2.0 mM to 25.0 mM glucose. A negligible interference was observed even for large excess of ascorbic acid, uric acid and acetaminophen.

Glucose oxidase [55] was also immobilized at a GCE modified with CNTs dispersed in Nafion by dipping the electrode in a solution containing 19.5 mg/mL GOx and 5 mg/mL glutaraldehyde for 4 hours at room temperature, followed by 6 immersions in 0.5 % v/v Nafion solution for 1 hour and a final one in a 5.0 % v/v Nafion solution for 1 hour. The important electrocatalytic activity of this CNTs towards the oxidation and reduction of hydrogen peroxide allowed the very sensitive glucose quantification at -0.050 V where no interference of ascorbic acid, uric acid and acetaminophen was found. The linear range was between 2 and 20 x 10^{-3} M glucose.

GOx was also immobilized on Pt modified with chemically oxidized SWCNTs through covalent attachment using EDC [93]. The amperometric experiments at 0.40 V gave a sensitivity of 18.7 $mAM^{-1}cm^{-2}$, a linear range up to 12 mM glucose and an apparent K_m of 13.1 mM. The biosensor showed a good stability, keeping a 90 % of the activity after 4 months.

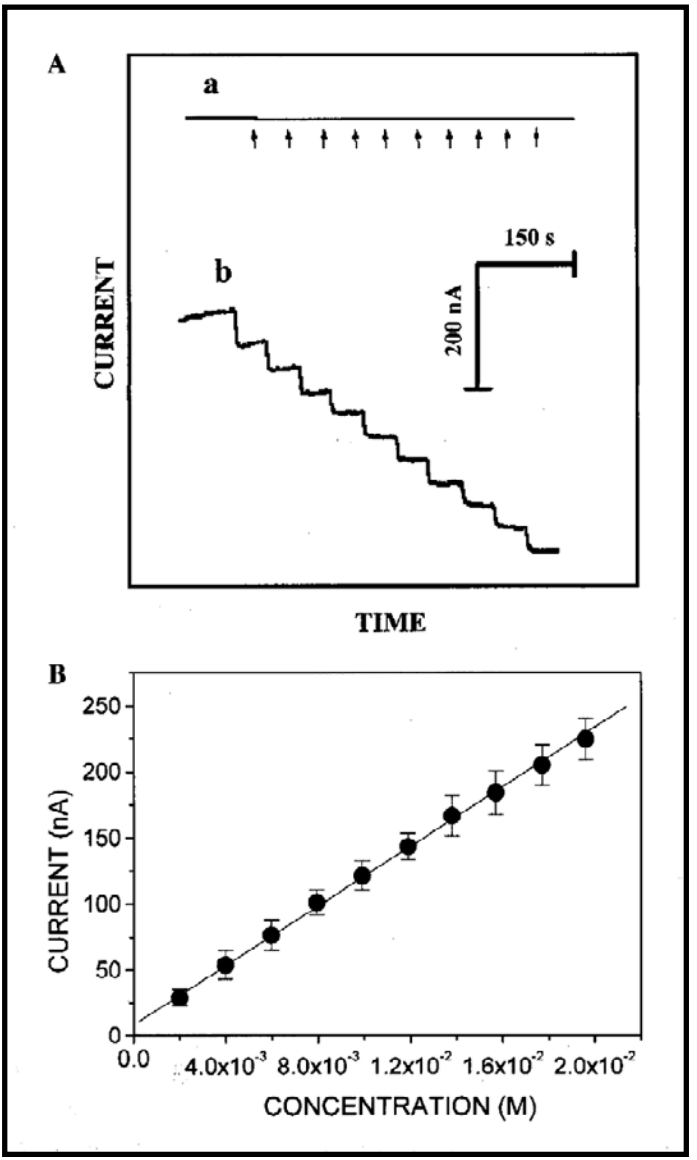


Figure 15. (A) Amperometric recordings obtained at CPE-GOx (a) and at CNTPE-GOx (b) for successive additions of 5 mM glucose. The content of GOx was 10.0% w/w in both electrodes. (B) Calibration plot obtained from amperometric recordings for successive additions of 2 mM glucose. Working potential: - 0.100 V. Supporting electrolyte: 0.050 M phosphate buffer solution, pH 7.40. From reference 46.

Dekker et al. [90] demonstrated that semiconductcing SWCNTs can be used for the development of biosensors. GOx was immobilized on SWCNTs by using a linking molecule, which binds on one side to the SWCNTs through Van der Waals interaction and on the other side to the enzyme through an amide bond. To do so SWCNTs deposited on a silicon wafer were left in 2.3 mg/mL 1-pyrenebutanoic acid succinimidyl ester in DMF for 2 h while stirring, washed with clean DMF, left in 10 mg/mL GOx for 18 h, and washed in pure water for 6 hours. From AFM images they concluded that GOx is inmobilized specifically on the modified SWCNTs. The conductance of the semiconducting SWCNT was measured as a function of the liquid-gate voltage. The attachment of GOx significantly decreased the conductance of the semiconducting SWCNT as a result of the change in capacitance of the tube because GOx immobilized on the surface of a SWCNT inhibits the movement of ions close to the tube. GOx-coated semiconducting SWNTs show strong pH dependence, indicating the possibility to use these sensors to measure pH changes down to 0.1 units. After addition of glucose the conductance increases. Therefore, it can be used as an excellent nanosize pH sensor and glucose.

Wang et al. [94] reported the use of CNTs-composite for continuous measurement of glucose with excellent selectivity, high sensitivity, wide linear range, fast response, long-term and thermal stability and oxygen independence. The glucose microsensor was prepared by dispersing GOx directly within CNTs and graphite and packing the resulting biocomposite into a 21-gauge needle. The CNT-GOx biocomposite was prepared by mixing 2.5 mg CNTs and 7.5 mg graphite powder for 5 min, followed by the addition of 2 mg GOx and mixing for additional 5 min. The resulting mixture was packed into a 300-μm polyimide tubing that was inserted into a 21-gauge needle. Once the surface was smoothed, it was coated with 1 % Nafion solution by three 10 s dipping. The CNTs demonstrated to have an important effect upon the sensing behavior. In fact, the response increased rapidly up to 25 % w/w CNT and then more slowly. The selected biocomposite composition was 21% w/w CNT, 17% w/w GOx and 62% w/w graphite. The thickness of the Nafion layer demonstrated to have an important effect on the sensitivity, a thicker Nafion film was accompanied with lower sensitivity and wider linear range. After 80 days the response decreased just 20 %. To assess the CNT-induced enzyme stabilization, the thermal stability was examined during storage at 90º C. It was found that when GOx was incorporated within CNTs, the thermoresistance dramatically increased. In fact, the response decreased just 20 % and 25 % after 3 and 24 hours at 90 ^{o}C, respectively. Another interesting advantage is that packing the CNT/GOx biocomposite within the needle facilitated the monitoring of glucose under severe oxygen deprivation.

The effect of the incorporation of Pt nanoparticles in SWCNTs-Nafion film on GC and on a carbon fiber electrodes (CFE) for developing a sensible glucose biosensor was reported by Luong et al. [95]. GCE was polished, electrochemically activated and then modified with an aliquot of a solution containing the CNTs (2 mg SWCNTs in a mixture of 100 μL of Nafion and 900 μL of Pt nanoparticles). Platinum nanoparticles were in electrical contact with GCE and CFE through the SWCNTs, enabling the composite structure to be used as an electrode. The enzymatic electrode was prepared by dropping 3 μL GOx solution in 50 mM phosphate buffer solution pH 7.2 (20 mg/mL). Subsequently, glutaraldehyde (3.0 uL 2.5 %) was applied on the resulting electrode for the cross-linking. Under these conditions the detection limit was 0.5 μM and the response time was 3 s. A linear range was observed from 0.5 μM to 5mM glucose with a sensitivity of 2.11 μAmM^{-1}. A similar response was observed using CFE as substrate. Maximum levels of AA, UA and acetaminophen did not show any interference.

Another glucose enzymatic electrode was prepared by covalent attachment of glucose dehydrogenase (GDH) to the CNT-CHIT films using glutaraldehyde (GDI) to cross-link the enzyme [56]. Due to the electrocatalytic properties of CNTs, NADH could be oxidized at potentials 0.300 V less positive than at the GCE, allowing a very sensitive and selective determination of glucose and a linear range up to 300 μM with a sensitivity of 80 mA M^{-1} (in 0.05 M phosphate buffer solutions pH 7.4). The performance of the bioelectrode has allowed the successful determination of glucose in urine.

Palleschi et al [96] presented a Prussian Blue (PB) modified with SWCNTs as an efficient platform for developing enzymatic electrodes. The PB was synthesized in the presence of SWCNTs, starting from $K_3Fe(CN)_6$ and $FeCl_3$. The paste was prepared 60/40 w/w CNTs/mineral oil by hand mixing in a mortar and packed in a Teflon tube. Voltammetric and amperometric parameters were compared between PB-graphite and PB-CNTs. PB-CNTPEs showed a slower kinetics and did not improve the analytical performance of the sensors towards hydrogen peroxide. PB-CNTPEs showed a linear range for glucose between 0.1 and 50 mM glucose. The advantage of the PB-CNTPE was the stability at very basic pH, attributed to the peculiar structure of SWCNTs.

The authors investigated the use of aligned carbon nanotubes as platform for the production of a conducting polymer-glucose oxidase based biosensor [97]. The aligned CNT films were prepared by pyrolizing Fe(II)-phthalocyanine under Ar/H_2 at 900ºC. GOx immobilization was performed during pyrrole polymerization in a solution containing $NaClO_4$ and GOx. The resulting material showed excellent electrocatatalytic properties towards the oxidation of hydrogen

peroxide. SEM pictures showed that the polymer was not only located in the intertube spacing, but also on the top of the mat.

Wallace et al. [98] developed a new material consisting of an aligned, highly orientated carbon nanotubes array in three dimensions, coated by a layer of polypyrrole, which allowed the immobilization of enzymes, during its polymerization onto the nanotubes array. The immobilization of GOx was performed by electrochemical oxidation of pyrrole (0.10 M) in a buffer solution pH 7.45, containing 2 mg/mL GOx and 0.10 M $NaClO_4$ at 1 V for 1 min at 10 ºC. The presence of the enzymes was confirmed by the increase in the N/C and O/C ration in the XPS spectra of the Ppy/CNTs array before and after the GOx immobilization. The glucose response of this bioelectrode was 10-20 times higher than that for a corresponding flat gold electrode. The 3-D structure of CNTs provides a good template for a large enzyme loading in an ultra thin polymer layer (<10nm).

In another work, MWCNTs were vertically adhered to a gold film and the glucose biosensor was obtained by immersing in a 0.1 M phosphate buffer solution containing GOx to obtain a Au-MWNTs-GOx [99]. Figure 16 shows an scheme of the biosensor. The MWCNT-based biosensor exhibited a fast and sensitive response. The effect of opening the tubes on the response of the biosensor was also evaluated. The nanotube caps were opened by treating them with a mixture of HNO_3 and HF. Once the tubes were opened, GOx could enter into the hollow of MWNTs, increasing in this way the amount of enzyme immobilized. On the other hand, when the MWCNTs were treated with the acidic solution the generated carboxylic groups give a hydrophilic environment that allows the adsorption and insertion of the enzyme into the cavity of the CNTs while preserving its functionality. As a consequence of that, the stability of the biosensor largely increased. In fact, after 4 months storage at 4^oC, the activity remained in a 86.7 % of the original value.

Willner's group proposed an interesting approach for the construction of a glucose biosensor based on the use of CNTs [100]. Oxidized SWCNTs were covalently attached to a thiolated gold surface through the generated carboxylic functions. The other end of the SWCNT was covalently attached to FAD. Therefore, when the apo-GOx was immobilized, it could be reconstituted on the edge of SWCNTs, and in this way the electrons could be transported long distances.The length of the CNTs was the responsible for controlling the rate of the process.

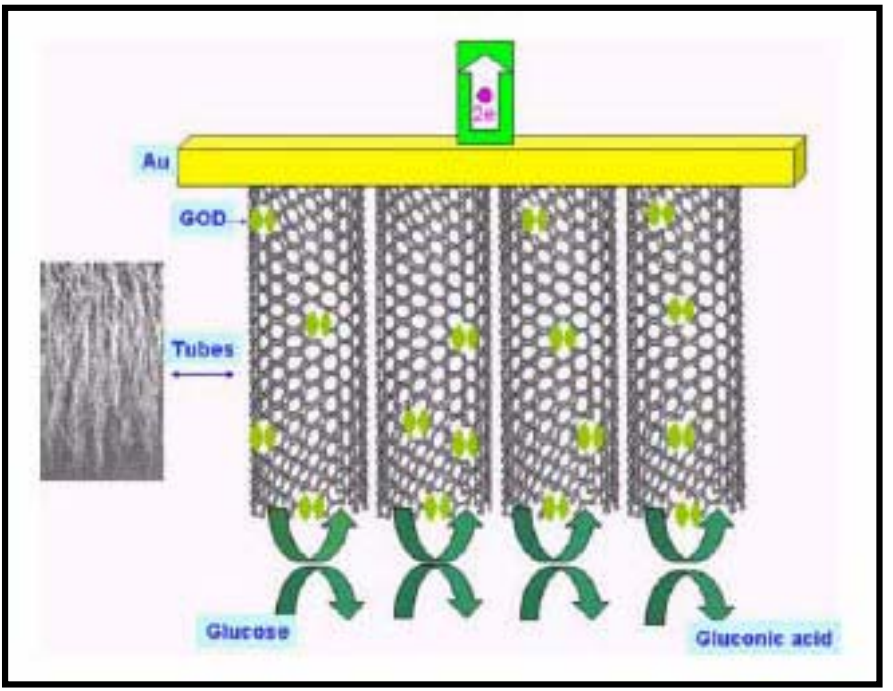


Figure 16. Schematic illustration of the MWNT-based biosensor for glucose detection. From reference 99.

Lactate Biosensor

Rubianes and Rivas [79] proposed the immobilization of lactate oxidase (LOx) into the CNTPE. The amperometric response to lactate was linear up to 7.0 x 10^{-3} M with a detection limit of 3.0 x 10^{-4} M.

The increase in the activity of lactate dehydrogenase (LDH) on a GCE modified with a SWNTs film, compared to a bare GCE was also proposed [101], by using chronoamperometry in a convective system. The LDH-SWNT-modified GCE was more active than the plain GCE. The irreversible catalytic oxidation of NADH was demonstrated by the 150 mV shifting of the anodic peak potential towards more negative values, compared to the plain GCE. The authors evaluated the SWNTs-modified electrode performance in the reduction of pyruvate using NADH as coenzyme in 50 mM Tris-HCl buffer solution pH 7.5, by chronoamperometry in a forced convection system.

Phenols and Catechols Biosensor

Since the product of the oxidation of phenols and catechols catalyzed by polyphenol oxidase (PPO) is the corresponding quinone, Rubianes and Rivas evaluated the influence of MWCNTs on the electrochemical behavior of hydroquinone at carbon composite electrodes with different content of CNTs [79]. It was found that as the percentage of MWCNTs increases, the response becomes more reversible, decreasing the peak potential separation and increasing the associated currents. Consequently, the response for dopamine was 12 times more sensitive at CNTPE-PPO than at CPE-PPO and the detection limits for dopamine and phenol were 1.0×10^{-6} M and 1.0×10^{-7} M, respectively. The study of the electrochemical behavior as well as the application of the resulting biolectrodes for the determination of phenols, catechols and alcohols in real samples was also illustrated.

Hydrogen Peroxide Biosensor

Shi et al [102] have studied the catalytic activity of MWCNTs-horseradish peroxidase(HRP)-GCE towards hydrogen peroxide. Three microliters of a solution of 2.0 U/mL HRP and 0.5 % w/w BSA in PBS were dropped on the MWCNTs-GCE followed by cross-linking in a closed vessel contained 25 % glutaraldehyde and water vapor for 20 min and dried at room temperature for 1 h. The presence of CNTs had allowed the sensitive and selective determination of hydrogen peroxide. The latter immobilization of oxidases like GOx and LOx made it possible the detection of glucose and lactate from the hydrogen peroxide enzymatically generated. The working potential was -300 mV and the flow rate of 2.0 μL/min.

Zhao et al. [103] proposed the immobilization of myoglobin on the surface of MWCNT-modified GCE. They found that the protein immobilized can catalyze the reduction of hydrogen peroxide. The pH selected for the analysis was 4.0 and under these conditions, the response of the biosensor was very fast, reaching the 90% in 6 s. The relationship between current and hydrogen peroxide concentration was linear up to 330 μM, the reproducibility was 5.9 % and the detection limit was 4.2 μM under anaerobic conditions. The voltammetric response did not change significantly after 1 month at 4 ^{o}C or 1 week stored in air.

Zhao et al. [104] reported the electrochemical behavior of myoglobin on MWCNT-GCE and the potential application for the development of a nitric oxide biosensor. Myoglobin was immobilized on the acid-treated-MWCNTs-GCE by dipping the electrode into 0.24 mM myoglobin solution (in acetate buffer pH 5.6) over 72 hours. After that, the electrode was removed, washed with water and stored at 4 ^{o}C. Electrochemical impedance spectroscopy experiments

demonstrated that the charge transfer resistance for a redox marker couple (potassium ferro/ferricyanide) increased for electrodes prepared by longer immersion times of the protein. Cyclic voltammetry of the resulting electrode containing myoglobin showed a couple of reversible peaks due to the reduction of the Fe(III)-myoglobin and the reoxidation in the reverse scan. In the presence of NO, a new cathodic peak appears at around -0.8 V in phosphate buffer pH 7.0 due to the reduction of NO that could be assisted by the electroactive myoglobin. Amperometric experiments performed at -0.8 V show a linear relationship between steady-state current and NO concentration up to 18 μM. Zhao et al [105] have also proposed the direct electron transfer between the strongly adsorbed cytochrome c and MWCNTs-GCE. Based on these results, it was possible to detect hydrogen peroxide in the range 2-420 μM and a detection limit of 1.02 μM.

Another work [106] reported the covalent attachment of enzymes onto the ends of vertically oriented SWCNTs. The ends of orthogonally arrays of shortened SWCNTs can be linked to electrochemically active heme proteins through traditional bioconjugate chemistry. Proteins were attached to the end of SWCNTs by using the known chemistry of EDC to promote amide linkages between carboxyl nanotubes and lysine residues of the proteins. The authors showed that myoglobin (Mb) and horseradish peroxidase (HRP) covalently linked to SWNT exhibited quasi-reversible Fe^{III}/Fe^{II} voltammetry and sensitive response to H_2O_2. Monolayers of vertically aligned, shortened SWNTs were assembled on ordinary pyrolitic graphite electrodes from DMF dispersions onto an underlying composite bed of Nafion ionomer and Fe^{3+} precipitated hydroxide. Voltammetric peaks of the enzymes attached onto SWCNTs forests were stable, and did not decay during repetitive multiple scans. This behavior is congruent with covalent attachment of proteins to the carboxylate-bearing ends of SWNTs. The nanotubes forest behaves electrically similar to a metal, conducting electrons from the external circuit to the enzymes. The oriented SWCNTs forests have the potential of being fabricated as ultamicroelectrodes on the nanometer scale, offering the future possibility of multielement nonobiosensor arrays. Detection limits of 70 nM and 50 nM for hydrogen peroxide were found at Mb- and HRP-modified electrodes.

A new electrode material based on the use of CNTs for studying the direct electron transfer of hemoglobin [107] was also proposed. The electrode was fabricated in the following way, a 100 μm diameter Pt microelectrode was first chemically etched to obtain a cavity of tens of μm depth and then ground the etched tip on a flat plate with CNTs until the microcavity was filled. In the case of the material containing hemoglobin, the CNT powder was mixed with 100 μL 0.1M acetate buffer pH 5.4 containing 50 mg/mL hemoglobin and 0.1 M KCl and

then dried at 1°C under N_2 flow. In the presence of hydrogen peroxide a well defined peak appeared between -0.5 and -0.8 V, indicating the catalytic activity of the hemoglobin immobilized. Therefore, it was possible to perform the detection of hydrogen peroxide at -0.8 V. The response was fast, with stable value after 7 seconds. The current was linear with the hydrogen peroxide concentration from 2.1 x 10^{-4}M to 9 x 10^{-4} M and a detection limit was 9 x 10^{-6} M.

Alcohol Biosensor

[59] A composite biosensor was prepared by adding the desired amount of alcohol dehydrogenase (ADH) and NAD^+ cofactor to a 50/50 % w/w MWCNT/Teflon composite. The electrocatalytic properties of MWCNT towards NADH and the biocatalytic properties of ADH are not impaired because of the presence of Teflon. A marked decrease in the overvoltage for the oxidation of the liberated NADH was shown.

Rubianes and Rivas also proposed the immobilization of ADH (12.0 % w/w) into a CNTPE in the presence of NAD^+ (12.0 % w/w) [79]. Based on the excellent electrocatalytic properties of MWCNTs towards the oxidation of NADH, a very fast and sensitive response for ethanol was obtained at CPE-ADH-NAD^+.

6.2. ELECTROCHEMICAL DNA BIOSENSORS

Electrochemical DNA biosensors are based on the use of nucleic acids or analogues as biorecognition element and electrochemical techniques for the transduction of the physical chemical signal. Two aspects are essential in the development of hybridization biosensors, sensitivity and selectivity. Traditional methods for detecting the hybridization event are too slow and require special preparation. Therefore, there is an enormous interest in developing new hybridization biosensors, and the electrochemical represent a very good alternative [108].

An electrochemical DNA hybridization biosensor basically consists of an electrode modified with a single stranded DNA called probe [109]. Usually the probes are short oligonucleotides (or analogues such as peptide nucleic acids). The first and most critical step in the preparation of an electrochemical DNA biosensor is the immobilization of the probe sequence on the electrode. The second step is the hybrid formation under selected conditions of pH, ionic strength and temperature. The next step involves the detection of the double helix

formation by using a methodology able to obtain an electrical signal that clearly demonstrates the specific recognition of the complementary DNA among others.

Another interesting aspect of sensors containing DNA as biorecognition layer is the detection of chemical and physical damage produced on DNA. In this case, it is necessary to immobilize double stranded DNA at the electrode surface to build the probe. The second step consist of the interaction of the immobilized DNA layer with the given damage agent under controlled conditions and the last step is the transduction of the signal, either from the nucleobases signal or from the redox signal of the damage agent [109].

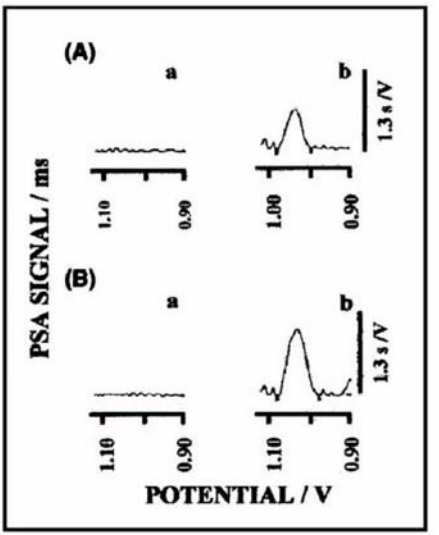


Figure 17. Chronopotentiometric signals obtained in a 0.200 M acetate buffer solution pH 5.00 after 5 min accumulation at 0.200 V in a 0.200 M acetate buffer solution pH 5.00 containing 5.0 mg/L ssDNA (A) and 2.00 mg/L $oligo_X$ (B) at CPE (60.0% w/w graphite powder and 40.0% w/w mineral oil) (A, a; B, a), and at untreated CNTPE (60.0% w/w carbon nanotubes and 40.0% w/w mineral oil) (A, b; B, b). Stripping current: 8.00 μA. Initial potential: 0.500 V. From reference 110.

Pedano and Rivas [110] reported the adsorption and electrooxidation of oligo and polinucleotides at a CNTPE. Figure 17 shows the chronopotentiometric signals obtained in acetate buffer solution (0.200 M pH 5.00) after 5 min accumulation at 0.200 V in 5.0 ppm ssDNA (A) and 2.00 ppm oligonucleotide (21 bases) (B) solutions at untreated CPE (A,a: B,a) and untreated CNTPE (A,b; B,b). Almost no signal was obtained at the classical graphite paste electrode. On the

contrary, a very well-defined signal at 1.06 V was obtained at the CNT-based composite (29 and 61 times larger for the ssDNA and the oligonucleotide, respectively), demonstrating the advantages of this material. No shifting in the peak potential was obtained, indicating that the main effect of CNTs occurred in the adsorption of the nucleic acids. The state of the surface demonstrated is very important for the adsorption and further electrooxidation of nucleic acids. A pretreatment of 1.3 V for 20 seconds in a 0.200 M acetate buffer solution pH 5.0 demonstrated to be the most effective. An interaction mainly hydrophobic was reported for the interaction DNA-CNTPE.

Hamers et al. [111] reported the covalent attachment of DNA to CNTs previously oxidized and derivatized with amino residues through a linker that promotes the attachment of nucleic acids and a more efficient dispersion of CNTs in solution.

The immobilization of calf-thymus DNA molecules on multi-walled CNTs activated with EDC and NHS by immersion in a phosphate buffer solution containing 2 mg/mL of ssDNA for several hours was also reported [42]. Cyclic voltammograms showed that the gold electrode containing NTs present a large background current, apparently capacitive. In the presence of DNA, the peaks due to the carboxylic groups on CNTs disappeared and the background current drastically decreased. The authors also performed impedance experiments to demonstrate the presence of the nucleic acid at the electrode surface. The measurements were performed at open circuit using 10 mM potassium ferricyanide/ferrocyanide plus 0.1 M NaCl, for frequency ranges between 0.1 Hz to 100 kHz and amplitude of 5 mV. The presence of the DNA layer (either single or double stranded) made more difficult the access of the redox couple to the electrode due to the charge repulsion, therefore, decreasing the currents, and increasing the peak potential separation and charge transfer resistance (Figures 18 and 19). EDC/NHS demonstrated to be necessary to obtain an effective immobilization of DNA, three hours interaction being the optimum interaction time. For instance, the changes in the charge transfer resistances were very small when the NA was immobilized in the absence of EDC and NHS. The electrode was also used for studying the interaction with ethidium bromide (EB), a known intercalator of DNA. Therefore, the immobilized DNA keeps their recognition properties and the EB effectively can intercalate. The impedance experiments showed an increase in the resistance from 16.02 to 56.19 kΩ in the absence and after 18 min EB interaction, respectively.

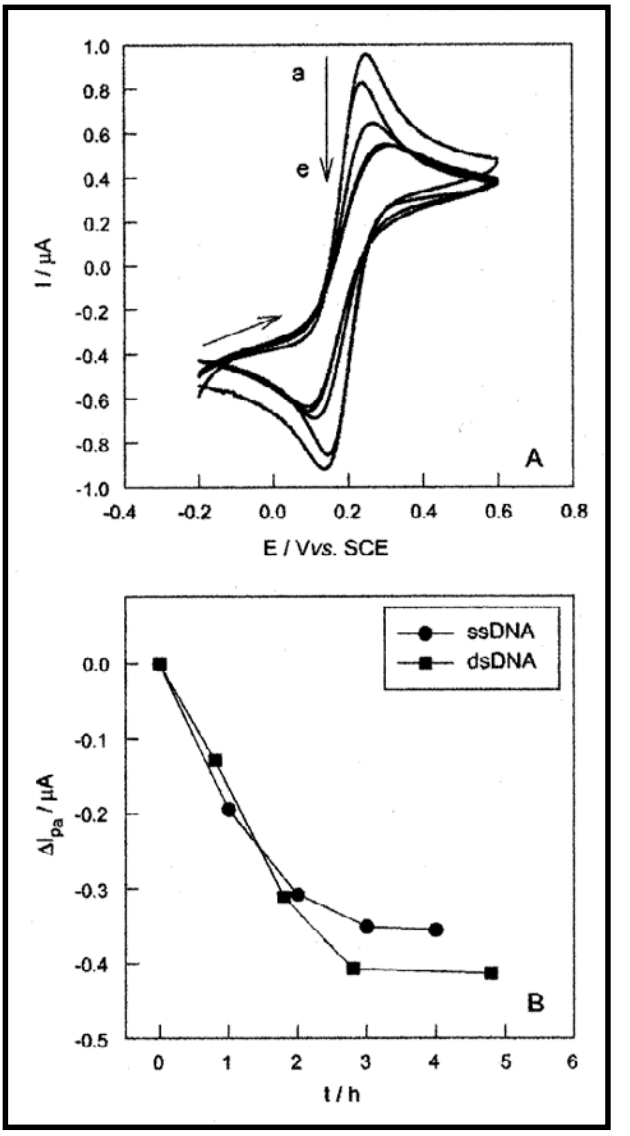


Figure 18. (A) Cyclic voltammograms for MWCNTs-modified gold electrode treated with 2 mg/mL dsDNA with EDC/NHS activation at different times in the presence of 10 mM $K_4Fe(CN)_6$ and 10 mM $K_3Fe(CN)_6$ containing 0.1 M NaCl. (a) 0 h; (b) 0.8 h; (c) 1.8 h; (d) 2.8 h; (e) 4.8 h. (B) Changes of the anodic peak current of $Fe(CN)_6^{-3}$ / $Fe(CN)_6^{-4}$ redox couple at MWCNTs-modified electrode immobilized with dsDNA (■) and ssDNA (●) for different time intervals. From reference 42.

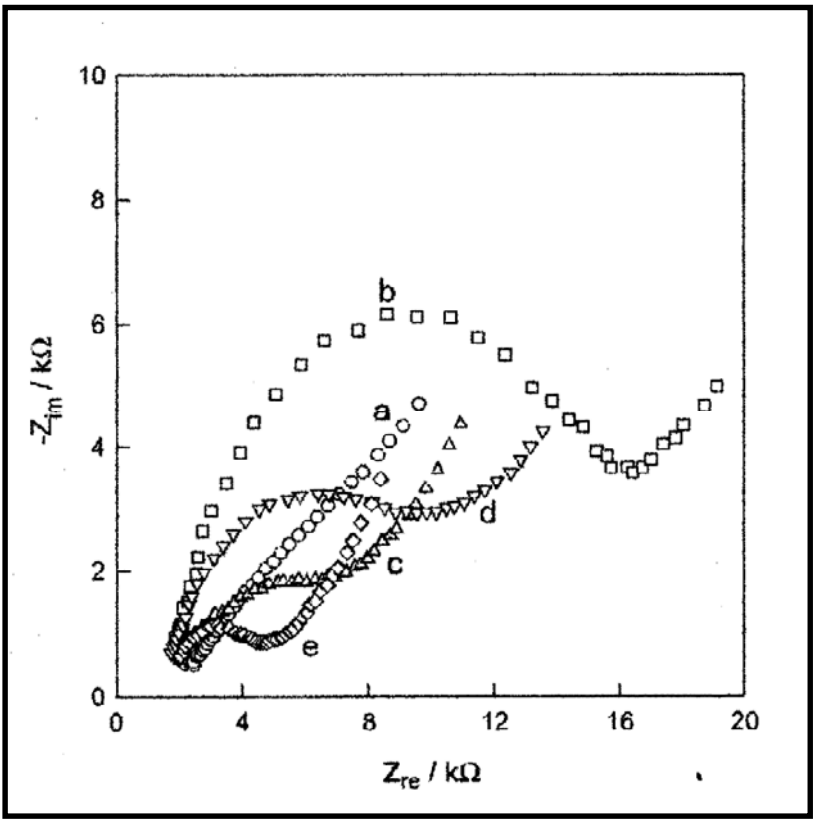


Figure 19. Nyquist plots (Z_{im} vs. Z_{re}) for different electrodes in 10 mM $K_4Fe(CN)_6$ +10 mM $K_3Fe(CN)_6$ + 0.1 M NaCl aqueous solutions. (a) MWCNTs-modified gold electrode; (b,c) MWCNTs-modified gold electrode treated with 2 mg/mL dsDNA with and without EDC/NHS activation, respectively; (d,e) MWNTs-modified gold electrode treated with 2 mg/mL ssDNA with and without EDC/NHS activation, respectively. From reference 42.

Wang et al. [112] reported a significantly higher sensitivity for DNA oxidation at CNTs-GCE compared to GCE, although the oxidation occurs at more elevated potentials, indicating that CNTs promotes the interfacial accumulation more than the electron transfer. After 3-min accumulation, the guanine oxidation signal was 17-fold higher than that obtained at the bare GCE. The CNT-GCE was also used as a detector for the hybridization event performed using the two-surface hybridization scheme. In this way, the selected biotinylated probe was immobilized at the magnetic beads covered with streptavidin, followed by the hybrid formation and transduction. The analytical signal was obtained from the guanine residues after digestion. The presence of CNTs allowed an efficient way

to amplify the label-free electrochemical detection of DNA hybridization. This amplified guanine response was used to develop a new hybridization scheme for detecting a sequence of BRCA1 breast cancer gene. Performing the digestion of the DNA sample in the presence of copper, another interesting alternative, well defined hybridization signals were obtained in the range 50-250 μgL^{-1} after 20 min hybridization. The detection limit was 40 ng/mL, 2 ng or 100 fmol in the 50 μL hybridization solution.

Wang et al. [113] reported the ultrasensitive electrical biosensing of DNA by using CNTs. Carbon nanotubes were used in two directions, for the recognition and for the transduction event. For the biorecognition event, alkaline phosphatase was bond to CNTs through a carbodiimide linker, with coverage of around 9600 enzyme molecules per CNT. Once the hybrids are formed, the alkaline phosphatase bond to CNTs catalyzes the formation of the enzymatic product α-naphtol that can be electrochemically detected. The sensitivity, improved almost 10^4 times in the presence of CNT modified with alkaline phosphatase. If CNTs are also used in the transduction step by modifying the glassy carbon used as transducer, a second amplification was obtained (30 fold), in connection with the accumulation of the enzymatic product α-naphtol in the presence of nanotubes. A detection limit of 1 fg/mL, or 54 aM, or 820 copies or 1.3 zmol in the 25 μL sample was obtained after 20 min accumulation.

Wang et al. [114] also proposed an attractive alternative for the amplification of DNA hybridization based on the ultrasensitive stripping-voltammetric detection of the dissolved CdS tags coming from the SWCNTs (Figure 20). The nanotubes were previously treated with acetone in an ultrasonic bath for 1 h and dried in air for 15 h to eliminate the free acetone. The activated nanotubes were dispersed in toluene and then mixed with the oligonucleotides modified with CdS for 4 hours with shaking. The biotinylated probe 1 was added to the streptavidin assay plate at room temperature and the hybridization took place (in a 750 mM NaCl/150 mM sodium citrate) after addition of the probe 2-coated-SWCNT-CdS-streptavidin previously obtained by mixing streptavidin and the biotinylated oligonucleotide called probe 2. After 1 h interaction the CdS was dissolved by addition of 1 M nitric acid. The electrochemical determination was performed in 0.2 M acetate buffer solution pH 5.2 using a mercury film-glassy carbon electrode in connection with square wave voltammetry to measure the cadmium obtained. Under these conditions the detection limit at -0.60 V was 40 pg/mL or 330 amol in the 50 μL solution.

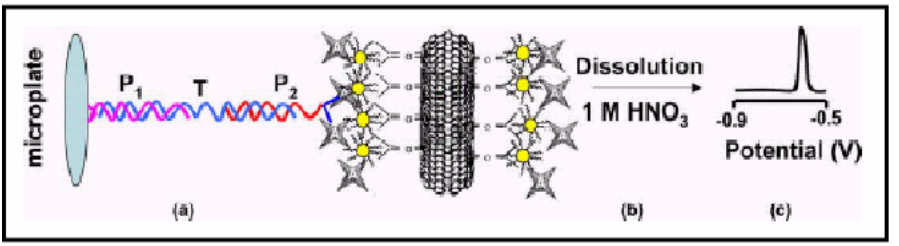
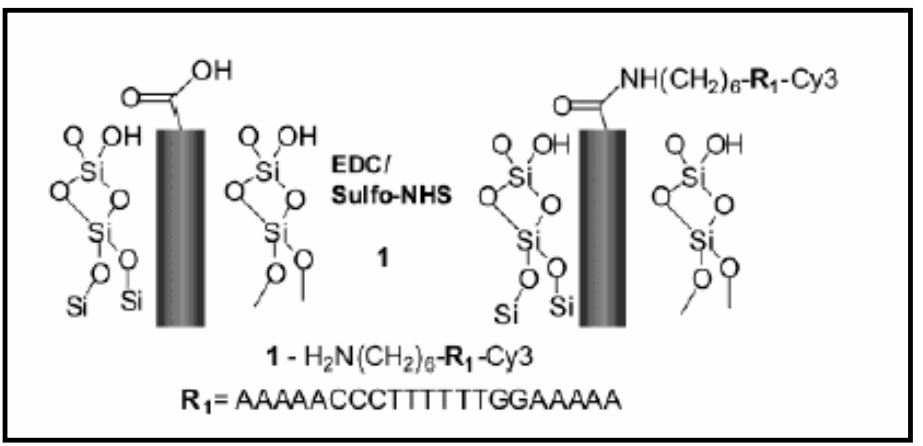


Figure 20. Schematic representation of the analytical protocol of the biosensor: (a) Dual hybridization event of the sandwich hybridization assay, leading to the capture of the CdS-loaded CNT tags in the microwell; (b) dissolution of the CdS tracer; (c) stripping voltammetric detection of cadmium at a mercury-coated glassy carbon electrode. P1, DNA probe 1; T, DNA target; P2, DNA probe 2. From reference 114.

Another work reported the immobilization of nucleic acids on CNTs previously opened by oxidative treatment and protected by a spin-on-glass film [69]. Once the carboxyl residues were obtained they were activated with EDC and NHS and the nucleic acids were immobilized (Scheme 3). The hybridization event in this case was evaluated by using a peptide nucleic acid sequence as probe and a target containing a fluorescent residue.



Scheme 3. Schematic representation of the immobilization of nucleic acid to the functionalized CNT array. From Reference 69.

Dai and He [115] proposed a new way for detecting the hybridization event by using aligned CNT-DNA probes immobilized on the tip and wall of plasma aligned CNTs. The CNTs were treated in acetic acid-plasma, followed by grafting the probe DNA sequence through the carboxyl functions generated during the pretreatment and the amino terminal of the DNA located at 5´-phosphate position in the presence of EDC. The detection of the hybridization event was performed through the amperometric response ferrocene-carboxaldehyde used as a label of the target DNA.

Fang et al. [116] described the direct detection of the hybridization event by combining the advantages of CNTs electron transfer properties with the incorporation of nucleic acids as dopants during the polypyrrole formation. The sensing layer was obtained by immobilization of oxidized MWCNTs in DMF on GCE, followed by the incorporation of DNA within the polypyrrole film during the electropolymerization process. The hybridization was performed at 42 ^{o}C for 30 min and the analytical signal was obtained from the decrease of impedance, especially in the region of high frequencies. The authors attributed this behavior to the higher conductivity of dsDNA compared to ssDNA. The detection limit was 1.0×10^{-8} M.

Yao et al. [117] reported the electrostatic assembly of calf thymus DNA on oxidized-MWCNTs-modified gold electrode using a cationic polyelectrolyte as linker. The nanotubes films were prepared by dropping the suspension of MWCNTs in water (0.1 mg/mL) and then evaporated under vacuum at 50 ^{o}C. The electrodes were dipped in 1.0 mg/mL PDDA for 1 hour and then in DNA for around 30 min. The multilayer film was formed by alternate immersion in the polycation and DNA. Cyclic voltammetry in 1/15 M phosphate buffer pH 4.5 containing 0.1 M KCl showed a couple at 0.035 and 0.128 V (cathodic and anodic peaks) related to carboxylic acid groups, responsible for the negative charges. An interesting study of the multilayer formation using Quartz Crystal Microbalance (QCM) and Electrochemical Impedance Spectroscopy is described. The amount of DNA immobilized at the PDDA-MWCNT-modified gold electrode was calculated from QCM experiments, being 517.6 ng. A much smaller amount of DNA was immobilized at bare gold electrode or at gold electrode modified with MWCNTs, demonstrating the importance of the presence of PDDA. Electrochemical Impedance was performed using 10 mM potassium ferricianyde and potassium ferrocyanide, a frequency range from 0.1 Hz to 100 kHz and potential amplitude of 5 mV. The resistances for charge transfer decreased when the gold electrode was covered with MWCNs, increasing after the immobilization of PDDA and DNA due to the non-conductivity of these molecules. On the other hand, the negative charge of DNA can also prevent the access of the marker to the electrode

surface. The resistance increased with the concentration of DNA and also with the number of bilayers. The authors used the electrode for detecting the interaction of DNA with chlorpromazine, which interacts with dsDNA through the intercalative association of the planar tricyclic phenothiazine ring system into the double helix. By QCM it was possible to calculate the binding constant, which is 8.41×10^4 M^{-1} and the binding site equal to 4.70.

6.3. IMMUNOSENSING SCHEMES

Immunosensors are based on high affinity reactions antigen/antibody. Several strategies can be used to immobilize the recognition element, either the antigen or the antibody, depending on the protocol. The detection of the recognition event uses the same principle as the enzymatic immunoassay. In general, an enzyme is coupled to the recognition layer (the antigen or antibody) and, to quantify the antigen/antibody interaction, the enzymatic reaction is developed in the presence of the substrate and electrochemically detected [118].

An amperometric biosensor based on the adsorption of antibodies onto perpendicularly oriented assemblies of single wall CNTs called forest was proposed [119]. The SWCNTs were functionalized with carboxyl residues and shortened by sonication in a mixture of sulfuric and nitric acids for 4 hours at 70 ^oC. The graphite electrode was dipped in 1mg/mL Nafion solution for 15 min and then immersed in a 5 mg/mL $FeCl_3.6H_2O$ for 15 min. After washing, the electrode was placed in a sonicated suspension of SWCNTs in DMF for 30 min, washed with methanol and dried. The immobilization of the anti-biotin antibody was performed by incubation for 3 hours on the surface of SWCNTs. After adequate washing, the electrode was blocked with 2 % bovine serum albumine in PBS. The detection of horseradish peroxidase bound to biotin was evaluated by using a rotating disk electrode at -0.3 V, 2000 rpm with 1 mM hydroquinone and 400 μM hydrogen peroxide. The detection limit was 2.5 nM and the linear range was up to 25 nM. Unlabelled biotin was detected in a competitive approach with a detection limit of 16 μM. The platform could be stored in a humidic aerobic chamber at 4^oC for one week without significant changes (16.7 vs 17.3 μA).

Tzeng et al. [121] reported a work dealing with the study of the influence of different chemical treatments of CNTs vertically aligned on a Si substrate on the adsorption of antibodies. Further application for the interaction with bacteria was also proposed.

Bianco et al. [122] presented the functionalization of CNTs with two polypeptides employing two different strategies. In both cases, the CNTs were

previously modified to expose amine groups. In the first methodology, a model peptide was condensate on the CNTs surface through a liker in DMF. In the second case, the peptide was the B-cell epitope from the foot and mouse disease virus (FMVD). The immobilization was performed employing succinimide. ^{1}H NMR experiments showed that in both cases the peptides keep its conformational structure. Immunological assays coupled to Surface Plasmon Resonance (SPR) methodology showed that the adsorbed peptide was recognized by its polyclonal antibodies being this an indication that the adsorption process did not produce significant conformational changes in the secondary structure of the peptide. Preliminary experiments of immnunogenicity employing FMDV-CNTs indicated that the conjugate produces strong anti-peptide antibody immunization in mice. This peptide modification could be applied for immunologic test offering several advantages such as higher accessibility to the peptide and the possibility to adsorb different epitopes, allowing in this way higher diagnostic accuracy.

Chapter 7

FINAL CONSIDERATIONS

In summary, CNTs have demonstrated to be an excellent material for the development of electrochemical sensors. The state of CNTs surface is a very important aspect and several pretreatments have been proposed. They possess the main goal to oxidize CNTs improving the electron transfer and making them more accessible for further derivatization. In general, CNTs are broken and converted into open tubules rich in oxygenated functions, process that can be detected by different spectroscopic and electrochemical techniques.

A large number of protocols for the preparation of electrodes based on the use of CNTs have been proposed. As in the case of pretreatments, in general, the selection of a given scheme will depend on the system under investigation. For instance, composites are easier to obtain from the point of view of the preparation time, while some others like the modification of GCE with CNTs dispersed in Nafion, are very convenient because in one step it is possible not only to immobilize the CNTs but also to have a barrier for interferents or a platform for further modifications.

The combination of the uniqueness of CNTs with the powerful recognition properties of enzymes, nucleic acids and antibodies, and the known advantages of the electrochemical techniques, represents a very good alternative for the development of biosensors able to address the new biosensing challenges of the future.

REFERENCES

[1] Dai, H. *Acc. Chem. Res.* 2002, 35, 1035-1044.

[2] Iijima, S. *Nature* 1001, 354, 56-57.

[3] Zhao, Q.; Gan, Z.; Zhuang, Q. *Electroanal.* 2002, 2002,14, 23 and references therein.

[4] Cohen, M. L. *Materials Science and Engineering C* 2001, 15, 1-11.

[5] Baughman, R. H.; Zakhidov, A.; de Heer, W. A. *Science*, 2002, 297, 787.

[6] Ajayan, P. M. *Chem. Rev*., 1999, 99, 1787-1799.

[7] P. M Ajayan, Zhou, O. Z. *Carbon nanotubes, Topics Appl. Phys.*, 80, 391-425, 2001, Dresselhaus, G. Dresselhaus, Ph. Avouris (Eds):Springer-Verlag Berlin Heldelberg, 2001.

[8] Ajayan, P. M.; Ebbesen, T. W. *Rep. Prog. Phys. 1997,* 60,1025-1062.

[9] Carbon Nanotubes, *Physics web* , 1998.

[10] Dresselhaus, M. S.; Lin, Y. M.; Rabin, O.; Jorio, A.; Souza Filho, A. G.; Pimenta, M. A.; Saito, R.; Ge., G. ; Samsonidze, G. ; Dresselhaus, G. *Material Science and Engineering C* 2003, 23, 129-140.

[11] Balasubramamian, Burghard, M. *Small* 2005, 1, 180-192.

[12] Thostenson, E. T. ; Ren, Z. ; Chen, T.- W. *Composites Science and Technology* 2001, 61,1899-1912.

[13] Dai, H. *Surface Science*, 2002, 500, 218-241.

[14] Zhou, C., Kong, J., Dai, H. *Phys Rev. Letter* 2000, 84, 5604-5607.

[15] Hamada, N., Sawada, S. I., Oshiyama, A. *Phys. Rev. Lett*, 1992, 68, 1579-1581.

[16] Blasé, X., Benedict, l. X., Shirley, E. L., Louie, S. G. *Phys. Rev.Letters* 1994, 72, 1878-1881.

[17] Ajayan, P. M., Ebbesen, T. W., Ichihashi, T., Iijima, S., Tanigaki, K., Hiura, H. *Nature* 1993, 362, 522-525.

[18] Tsang, S. C., Chen, Y. K., Harris, P. J. F., Green, M. L. H. *Nature* 372, 1994, 159.
[19] Nigoyi, S., Hamon, M. A., Hu., H., Zhao, B., Bhowmik, P., Sen, R., Itkis, M. E., Haddon, R. C. *Accounts of Chemical Research* 35, 2002, 12.
[20] Falvo, M. R., Taylor, R. M., Helser, A., Chi, V., Brooks, F. P., Washburn, S. Superfine, R. *Nature* 1999, 397, 236.
[21] Treacy, M. M. J., Ebbesen, T. W., Gibson, T. M. *Nature* 1996, 381, 680-687.
[22] Hiura, H., Ebbesen, T. W., Tanigaki, K. *Adv. Mater.* 7, 1995, 275-276.
[23] Britto, P. J., Santhanam, K. S. V., Alonso, V., Rubio, A., Ajayan, P. M. *Adv. Mater.* 1999, 11, 154.
[24] Negent, J N.; Santhanam, S. V.; Rubio, S. V.; Ajayan, P. M. *NanoLetters* 2002, 1, 87-89.
[25] C. T. Kingston, B. Simard, *Anal. Letters* 2003, 15, 3119-3145.
[26] Zhou, O.; Shimoda, H.; Gao, B.; Oh, S.; Fleming, L.; Yue, G. *Acc. Chem. Res.* 2002, 35, 1045-1053.
[27] Ebbesen, T. W., Ajayan, P. M.. Nature, 1992, 358, 220-222.
[28] Ebbesen, T. W. *Ann. Rev. Mater. Sci.* 1994, 24, 235-264.
[29] Bethund, D. S., Kiang, C. H., de Vries, M. S., Gorman, G., Savoy, R., Vazquez, J., Beyers, R. *Nature* 1993, 363, 605-607.
[30] Journet, C., Maser, W. K., Bernier, P., Loiseau, A., Lamy de la Chapelle, M., Lefrant, S., Deniard, P., Lee, R., Fischer, J. E. *Nature* 1997, 388, 756-758.
[31] Lee, S. J., Bailk, H. K., Yoo, J. Han, J. H *Diam. Rel. Mater.* 2002, 11, 914-917.
[32] Jung, S. H., Kim, M. R., Jeong, S. H., Kim, S. U., Lee, O. J., Lee, K. H., Suh, J. H., Park, C. K. *Appl. Phys. A.* 2003, 76, 285-286..
[33] Thess, A., Lee, R., Nikolaev, P., Dai, H., Petit, P., Robert, J., Xu, C., Lee, Y. H., Kim, S. G., Rinzler, A. G., Colbert, D. ., Scuseria, G. E., Tomanek, D., Fischer, J. E. *Science*, 1996, 273, 483-487.
[34] Eklund, P. C., Pradhan, B. K., Kim, U. J., Xiong, Q., Fischer, J. E., Friedman, A. D., Holloway, B. C., Jordan, K., Smith, M. W. *NanoLett.* 2002, 2, 561-566.
[35] Wang, J. *Electroanal.* 2005, 17, 7-14.
[36] Li, N.; Wang, J.; Li, M. *Reviews in Anal. Chem.* 2003, 22, 19-33.
[37] Mc. Creery, R. L.; Cline, K. K., Chapter 10, in *Laboratory Techniques in Electroanalytical Chemistry, 2nd Edition*, Marcel Dekker, NY, 1996. Peter Kissinger, Williams R. Heineman (Eds).

[38] Valentini, F.; Amine, A; Orlanducci, S.; Terranova, M. L.; Palleschi, G. *Anal. Chem.* 75, 2003, 5413.
[39] Sotiropoulou, S.; Chaniotakis, N. A. *Anal. Bioanal. Chem.* 375, 2003, 103-105.
[40] Salimi, A.; Banks, C. E.; Compton, R. G. *Analyst* 2004, 225-228.
[41] Gong, K.; Dong Y.; Xiong, S.; Chen, Y.; Mao, L.; *Biosensors and Bioelectronics* 2004, 20, 253-259.
[42] Guo, M.; Chen, J; Liu, D.; Nie, L.; Yao, S. *Bioelectrochem*. 2004, 62, 29-35.
[43] Wang, Z. H.; Liang, Q- L. ; Wang, Y.- M. ; Luo, G.- A. *J. Electroanal. Chem.* 2003, 540, 129-134.
[44] Hu, C. G.; Wang, W. L.; Wang, S. X.; Zhu, W.; Li. Y. *Diamond and Related Materials* 12, 2003, 1295- 1299.
[45] Hu, C. G.; Wang, W. L.; Liao, K. J.; Liu, G. B.; Wang, Y. T. *J. of Physics and Chemistry of Solids* 65, 2004, 1731-1736.
[46] Rubianes, M. D.; Rivas, G. A. *Electrochem. Comm* 2003, 5, 689-694.
[47] M. Chicharro, E. Bermejo, M. Moreno, A. Sanchez, A. Zapardiel, G. Rivas, Electroanal. 2005, 17, 476-482.
[48] Valentín, F.; Orlanducci, S.; Terranova, M. L.; Amine, A.; Palleschi, G. *Sensors and Actuators B* 2004, 100, 117-125.
[49] Antiochia, R. ; Lavagnini, I. ; Magno, F. ; Valentini, F. ; Palleschi, G. *Electroanalysis* 2004, 16, 1451-1458.
[50] Musameh, M.; Wang, J.; Merkoci, A.; Lin, Y. *Electrochem. Comm.* 2002, 4, 743-746.
[51] Wang, J.; Kawde, A.- N.; Musameh, M. *Analyst* 2003, 128, 912-916.
[52] Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. *Anal. Chem.* 2002, 74, 1993-1997.
[53] Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. *Electrochim. Acta* 2001, 47, 651-657.
[54] Yan, X. X.; Pang, D.- W.; Lu, Z.- X.; Li, J.-Q. ; Tong, H. *J. Electroanal. Chem*. 2004, 1,47-52.
[55] Wang, J.; Musameh, M.; Lin, Y.; *J. Am. Chem. Soc.* 2003, 125, 2408-2409.
[56] Zhang, M.; Smith, A.; Gorski, W. *Anal. Chem.* 2004, 76, 5045-5050.
[57] Moore, R. R.; Banks, C. E.; Compton, R. *Anal. Chem*. 2004, 76, 2677-2682.
[58] Wu, K.; Sun, Y.; Hu, S. *Sensors and Actuators B* 2003, 96, 658-662.
[59] Wang, J.; Musameh, M. *Anal. Chem*. 2003, 75, 2075.
[60] Wang, J.; Musameh, M. *Anal. Letters* 2003, 36, 2041-2048.
[61] Davis, J. J.; Coles, R. J.; Hill, H. A. O. *J. Electroanal. Chem.* 1997, 440, 279-282.

[62] Britto, P. J.; Santhanam, K. S. V.; Ajayan, P. M. *Bioelectrochem. and Bioenergetics* 1996, 41, 121.
[63] Chicharro, M.; Sanchez, A.; Bermejo, E.; Zapardiel, A.; Rubianes, M. D.; Rivas, G. A. *Anal. Chim. Acta* 2005, in press.
[64] Lawrence, N. S.; Deo, R. P.; Wang, J. *Talanta* 2004, 63, 443-449.
[65] Antiochia, R.; Lavagnini, I. ; Magno, F. *Anal. Letters* 2004, 37, 1657.
[66] Wang, J.; Chen, G.; Wang, M.; Chatrathi, M. P. *Analyst* 2004, 129, 512-515.
[67] Wang, J.; Musameh, M. *Analyst* 2004, 129, 1-2.
[68] Diao, X. P.; Liu, Z.; Wu, B.; Nan, X.; Zhang, J.; Wei, Z. *Chem. Phys. Chem*. 2002, 10, 898-901.
[69] Nguyen, C. V.; Delzeit, L.; Carsell, A. M.; Li, J.; Han, J.; Meyyappan, M.; *NanoLetters* 2002, 2, 1079-1081.
[70] Lin, Y.; Lu, F.; Tu, Y.; Ren, Z. *NanoLetters* 2004, 4, 191-195.
[71] Artyukhin, A. B.; Bakajin, O.; Stroeve, P.; Noy, A. *Langmuir* 2004, 20, 1442-1448.
[72] Liu, L.; Wang, T.; Li, J.; Guo, Z.-X.; Dai, L.; Zhang, D.; Zhu., D. *Chem. Phys. Lett.* 367, 2003, 747-752.
[73] Wang, Z.; Wang, Y.; Luo, G. *Analyst* 127, 1353-1358.
[74] Lawrence, N. S.; Deo, R. P.; Wang, J. *Electroanalysis* 2005, 17, 65-72.
[75] Luo, H.; Shi, Z.; Gu, Z.; Zhuang, Q. Z. *Anal. Chem*. 2001, 73, 915-920.
[76] Zang, H.; Wang, X.; Wan, L.; Liu, Y.; Bai, C. *Electrochim. Acta* 2004, 49, 715-719.
[77] Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. *Electroanalysis* 2002, 14, 225-230.
[78] Ye, J.- S.; Wen, Y.; Zhang, W. D.; Gan, L. M., Xu, G. Q.; Sheu, F.- S. *Electrochem. Comm*. 2004, 6, 66-70.
[79] Rubianes, M. D.; Rivas, G. A. *Electroanal.* 2005,17, 73-787.
[80] Deo, R. P.; Lawrence, N. S.; Wang, J. *Analyst* 2004, 129, 1076-1081.
[81] Lü, S. *Anal. Letters* 2003, 36, 2597-2608.
[82] Wang, S.- F., Xu, Q. *Anal. Letters* 2005, 38, 657-671.
[83] Collins, G.; Bradley, K.; Ishigami, M.; Zettl, A. *Science* 2000, 287, 1801-1804.
[84] Kong, J.; Franklin, N.; Zhou, C.; Chapline, M.; Peng, S.; Cho, K.; Dai, H. *Science* 2000, 287, 622-625.
[85] Cunningham, J. *Introduction to Bioanalytical Sensors*, J. Wiley & Sons, Inc., NY, 1998.
[86] Ramsay, G. *Commercial Biosensors. Applications to Clinical, Bioprocess, and Environmental Samples,* John Wiley & Sons, Inc., (1998).
[87] Clark, L. C.; Lyons, C. *Am. NY. Acad. Sci* 1962, 102, 29.

[88] Lin, Y.; Allard, L. F.; Sun, Y.-P. *J. Phys. Chem.* B 2004, 108, 3760-3764.
[89] Azamian, B. R.; Davis, J. J.; Coleman, K. S.; Bagshaw, C. B.; Green, M. L. H. *J. Am. Chem. Soc.* 2002,124, 12664-12665.
[90] Chen, R. J.; Zhang, Y. ; Wang, D.; Dai, H. *J. Am. Chem. Soc.* 2001, 123, 3838-3839.
[91] Besteman, K.; Lee, J.- O; Wiertz, F. G. M.; Heering, H. A.; Dekker, C.; *NanoLetters* 2003, 3, 727-730.
[92] Gooding, J. J.; Wibons, R.; Liu, J.; Yang, W.; Losic, D.; orbons, S.; Mearns, F. J.; Shapter, J. G.; Hibbert, D. B. *J. Am. Chem. Soc.* 2003, 125, 9006-9007.
[93] Xue, H.; Sun, W.; He, B.; Sheu, Z. *Synthetic Metals* 2003, 135-136, 831-832.
[94] Wang, J.; Musameh, M. *Analyst* 2003, 128, 1382-1385.
[95] Hrapovic, S.; Liu, Y.; Male, K. B.; Luong, J. H. T. *Anal. Chem.* 2004, 76, 1083-1088.
[96] Ricci, F.; Amine, A.; Moscone, D.; Palleschi, G. *Anal. Letters* 2003, 36, 1921-1938.
[97] Gao, M.; Dai, L.; Wallace, G. G. *Electroanal.* 2003, 15, 1089-1094.
[98] Gao, M.; Dai, L.; Wallace, G. G. *Synthetic Metals* 2003, 137, 1393-1394.
[99] Wang, S. G.; Zhang, Q.; Wang, R., Yoon, S. F. *Biochem. and Biophysical Research Comm.* 2003, 311, 572-576.
[100] Patolsky, F.; Weizmann, Y.; Willner, I. *Angew. Chem. Int. Ed.* 2004, 43, 2113.
[101] Gan, Z.-H.; Zhao, Q.; Gu, Z.- N.; Zhuang, Q.-K. *Anal. Chim. Acta* 2004, 516, 29-34.
[102] Yamamoto, K.; Shi, G.; Zhou, T. ; Xu, F.; Xu, J.; Kato, T.; Jin, J.-Y.; Jiu, L. *Analyst* 2003, 128, 249-254.
[103] Zhao, G.- G.; Zhang, L. ; Wei, X.- W. *Anal. Biochem.* 2004, 329, 169-161.
[104] Zhao, G. C.; Zhang, L.; Wei, X.- W.; Yang, Z.-S. *Electrochem. Comm.* 2003, 5, 825-829.
[105] Zhao, G. C.; Yin, Z.-Z.; Zhang, L.; Wei, X.-W. *Electrochem. Comm.* 2005, 7, 256-260.
[106] Yu, X.; Chattopadhyay, D.; Galeska, I.; Papadimitrakopoulos, F. ; Rusling, J. F. *Electrochem. Comm.* 2003, 5, 408-411.
[107] Zhao, Y.-D.; Bi, Y.-H.; Zhang, W.-D.; Luo, Q.-M. *Talanta* 2005, 65, 489-494.
[108] Drummond, T. G.; Hill, M. G.; Barton, J. *Nature Biotechnology* 2003, 21, 1192.
[109] Palecek, E. Talanta 2002, 56, 809.

[110] Pedano, M. L.; Rivas, G. A. Electrochem. Comm., 2004, 6, 10-16.
[111] Baker, S. E.; Cai, W.; Lasseter, T. L.; Weidkamp, K. P.; Hamers, R. J. *NanoLetters* 2002, 2, 1413-1417.
[112] Wang, J.; Kawde, A.-N.; Musameh, M. *Analyst*, 2003, 128, 912-916.
[113] Wang, J.; Liu, G.; Jan, M. R. *J. Am. Chem. Soc.* 2004, 126, 3010-3011
[114] Wang, J.; Liu, G.; Jan, M. R.; Zhu, Q. *Electrochem. Comm.* 5, 2003, 1000-1004]
[115] He P.; Dai, L. *Chem. Comm*, 2004, 348.
[116] Cai, H.; Xu, Y.; He,P.-G.; Fang, Y.-Z. *Electroanal.* 2003, 15, 1864-1870.
[117] Guo, M.; Chen, J.; Nie, L.; Yao, S. *Electrochim. Acta* 2004, 49, 2637-2643.
[118] Wang, J. *Analytical Electrochemistry, Second Edition*, Wiley-VCH (2000).
[119] O´Connoer, M.; Kim, S. N.; Killard, A. J.; Foster, R. J.; Smyth, M. R.; Papadimitrakopoulos, F.; Rusling, J. F. *Analyst* 2004, 129, 1176-1180.
[120] Huang, T. S.; Tzeng, Y.; Liu, Y. K.; Walker, K. R.; Guntupalli, R.; Liu, C. *Diamond and related materials* 2004, 13, 1098.
[121] Pantarotto, D.; Partidos, C. D.; Graff, R.; Hoebeke, J.; Briand, J.-P.; Prato, M.; Bianco, A.; *J. Am. Chem. Soc.* 2003, 125, 6160-6164.

INDEX

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X