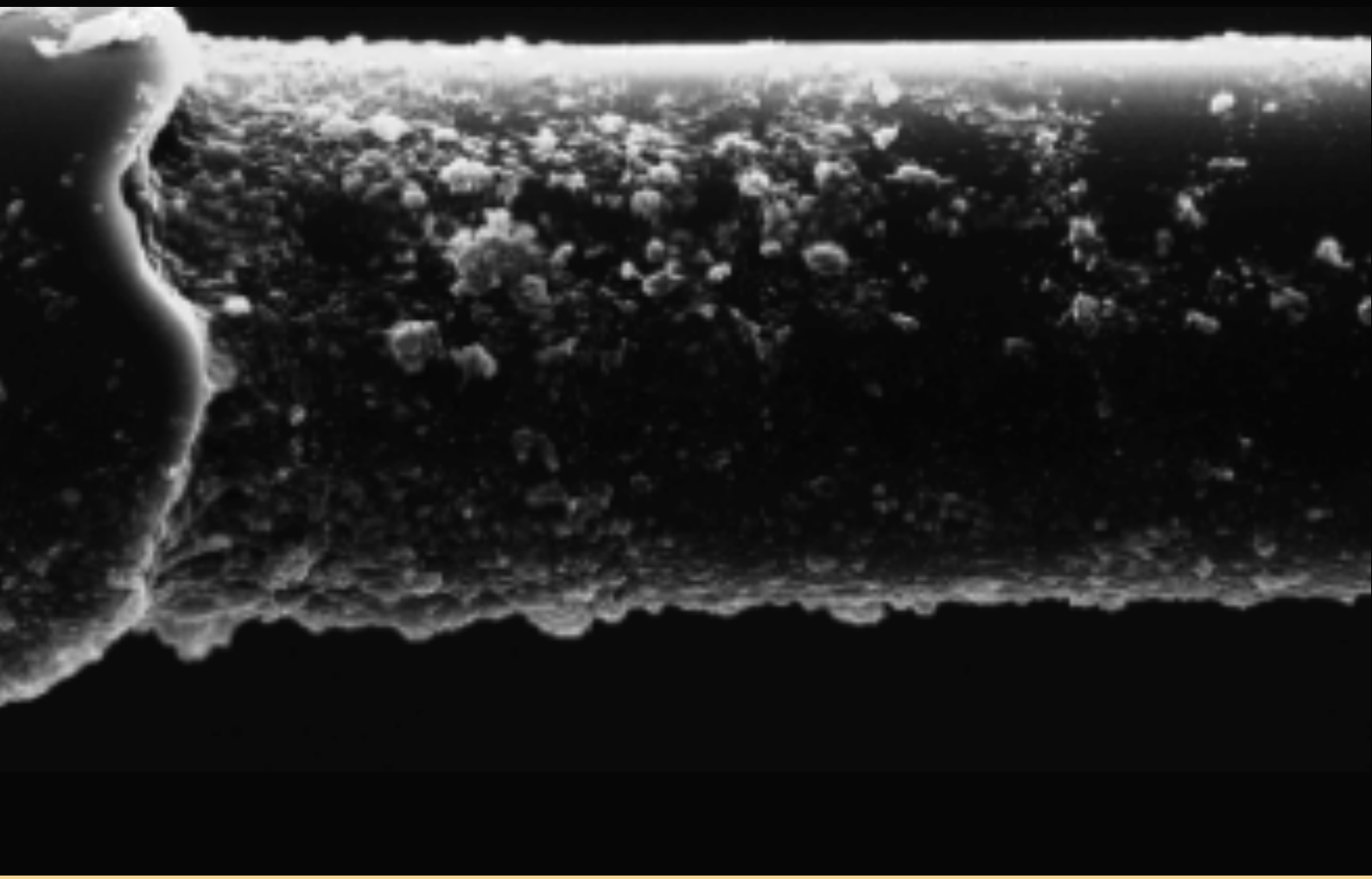

Sérgio Manuel Tavares da Costa

Carbon-based nanomaterials for electrochemical sensing and biosensing

Monograph on carbon-based nanomaterials for electrochemical sensing and biosensing, guided by Professor Rui M. Barbosa, Faculty of Pharmacy, under the Master in Pharmaceutical Sciences, Faculty of Pharmacy, University of Coimbra

September 2015

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Universidade de Coimbra

Faculty of Pharmacy,
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September, 2015

UNIVERSIDADE DE COIMBRA

"The true method of knowledge is experiment"

William Blake

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Coimbra, 09 de Setembro de 2015.

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The teacher and adviser

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(Prof. Rui M. Barbosa)

The oriented student

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(Sérgio Manuel Tavares da Costa)

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Coimbra, 09 September 2015

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To my family for all the effort and support during all moments.

To all my friends that make everything possible to accomplish.

To all of those who crossed my path and make every moment special.

A very special thank you

Abbreviations

E – Electric potential

E^0 – Standard cell potential (at a given temperature)

R – Universal gas constant

T – Absolute Temperature

n – Number of electrons transferred

F – Faraday constant

[Ox] – Concentration of oxidized specie

[Red] – Concentration of reduced specie

WE – Working electrode

RE – Reference electrode

CE – Counter electrode

CFM – Carbon Fiber Microelectrode

FCV – Fast Cyclic Voltammetry

SWV – Square Wave Voltammetry

ms – Milliseconds

nm – Nanometers

µm – Micrometers

LOD – Limit of Detection

GRPH – Graphene

CNTs – Carbon Nanotubes

DNA – Deoxyribonucleic acid

E.coli – Escherichia coli

GCE – Glassy Carbon Electrode

GOX – Glucose Oxidase

SWCNT – Single-Wall Carbon nanotubes

MWCNT – Multi-Wall Carbon nanotubes

°C – Celsius degrees

DET – Direct Electron Transference

MEA – Microelectrode Array

AA – Ascorbic Acid

DA – Dopamine

Abstract

This work was developed with the objective of showing the importance of carbon nanomaterials for sensing and biosensing.

Nowadays, carbon nanomaterials are considered one of the most exciting materials due to its chemical and physical properties which improve, significantly, the analytical signals obtained by electrochemical techniques.

However, there is a need for an element that does the connection between the electrochemical techniques and the nanomaterials - the electrode. The electrode is essential, because it can take advantages from the presence of nanomaterials. Moreover, it is the element that allows the electrochemical technique to generate measurable signals.

Throughout this monograph a number of different aspects regarding the nanomaterials and electrochemical techniques, will be made including the preparation and use, in particular in the brain. The use in other areas will be also addressed.

Finally, the appendix includes some theoretical knowledge for a better understanding of this monograph.

Resumo

Este trabalho foi desenvolvido com objetivo de demonstrar a importância dos nano materiais de carbono, para a monitorização dos diferentes analitos no organismo humano.

Os nano materiais de carbono, atualmente, são uma das ferramentas mais interessantes, tanto de um ponto de vista químico, como físico. As suas propriedades conseguem melhorar significativamente os sinais obtidos através das diversas técnicas eletroquímicas.

No entanto, estes intervenientes necessitam de um elemento de ligação, também chamado de elétrodo. Este é um elemento fundamental, na medida em que vai tirar partido das propriedades dos nano materiais para melhorar as suas características. Além disto, é através dele que as técnicas eletroquímicas podem ser aplicadas, tendo em vista a aquisição de dados.

Ao longo deste trabalho serão feitas referências a todos estes intervenientes, à melhor maneira de os preparar e utilizar. Serão também apresentadas algumas aplicações importantes que este tipo de sensores podem ter para outras áreas.

Por fim, mas não menus importante, será apresentado um apêndice com algumas informações e dados que sustentam o fundamento teórico apresentado durante este trabalho.

Basics on Electrochemistry

In its essence, electrochemistry is the science that study the processes that cause electrons to move. This movement of electrons, from one chemical specie to another generates electricity in a "redox" reaction.(*Electrochemistry Basics - Chemwiki*, [s.d.])

In this type of reaction there are two species: one that suffers oxidation (loss of electrons) – reducing agent, and another called the oxidizing agent, that suffers reduction (gain of electrons).(*Electrochemistry Basics - Chemwiki*, [s.d.])

Electrochemistry is a major field in analytical chemistry and has some interesting features when compared to other techniques, such as:

- Specific measurements due, for example, to its ability to distinguish between two states of oxidation;
- Low cost instrumentation;
- Analytical information of ionic activity as well as concentration which is of great importance in physiological processes.

Even though it is a vast field, we can group these techniques in a fairly manner, as depicted in Figure 1. On one side we have the techniques that are able to measure the analyte in the bulk solution, while on the other side we have the interfacial techniques. As our main goal is to focus on bio-sensing based on electrochemical transducers, we will address, in more detail the interfacial techniques. (Skoog, Holler e Crouch, 2007)

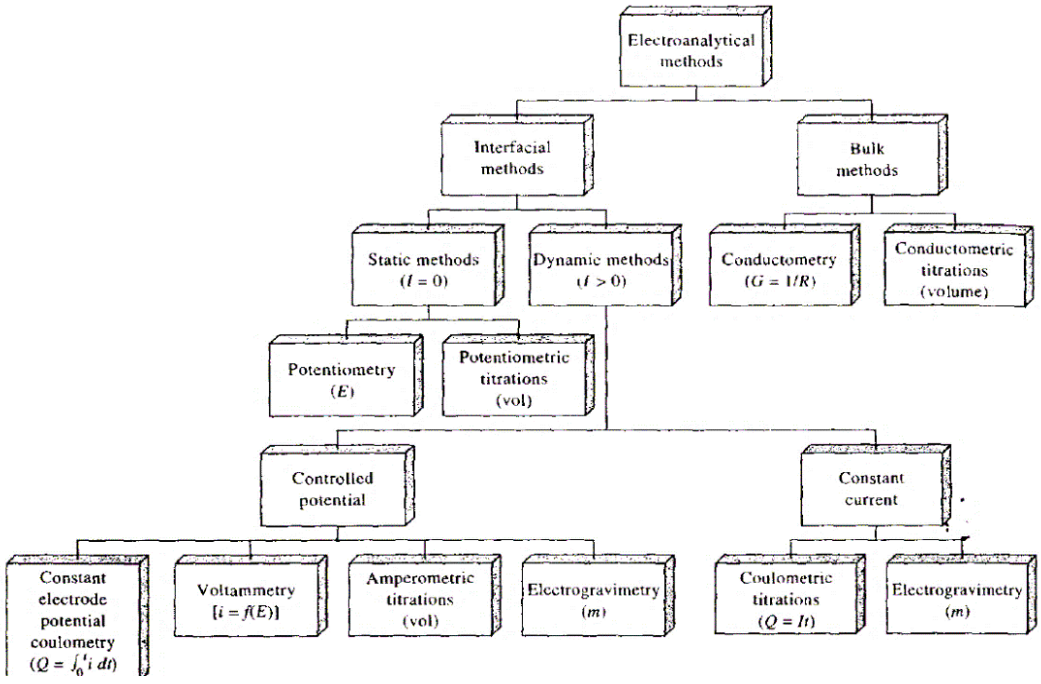


Figure1- Summary of commun electroanalytical method. (Skoog, Holler e Crouch, 2007)

However, general statements about electrochemistry should be pointed out:

- The analyte can suffer other reactions beyond oxidation and reduction (as the formation of a complex, which may affect the outcome as a result of varying the quantities of free analyte);
- The concentration at the surface of the electrode may not be the same as in the solution (The concentration is calculated by the Nernst equation - see figure 2 - which establishes a relationship between the oxidized and reduced form of the analyte. This ratio is different at the surface of the electrode because of its potential);
- The current generated is the measure of the extension rate of the reaction (a reduction is characterized by the consumption of electrons and thus the need to be replaced. This flow of electrons generates a current, which in turn allows us to see the rate of a reaction. We can also see when the reaction reaches equilibrium, as at this point the current is null.). (Skoog, Holler e Crouch, 2007)

$$E = E^0 + \frac{RT}{nF} \ln \frac{[\mathrm{Ox}]}{[\mathrm{Red}]}$$

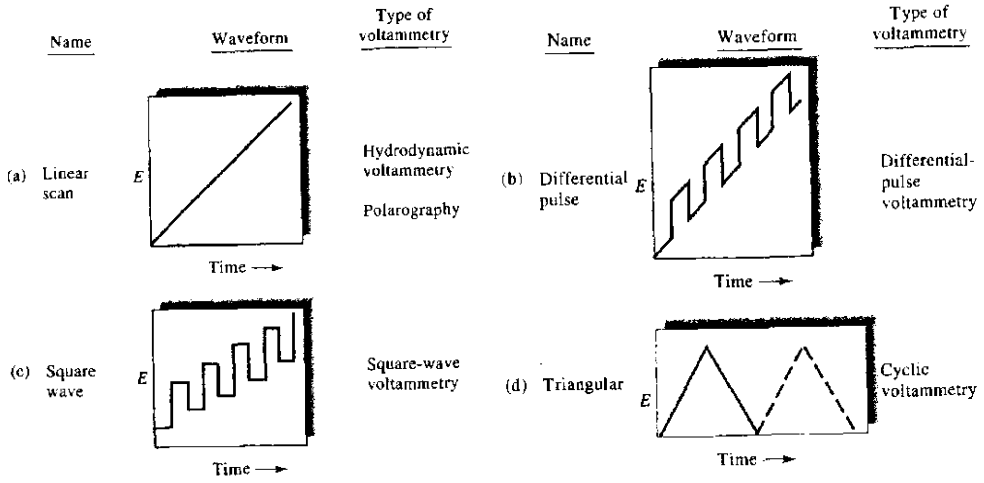
Figure 2 - Nernst equation. (Skoog, Holler e Crouch, 2007)

Electrochemical Techniques

Voltammetry

Since 1960's, critical improvements were made to electrochemical techniques, namely selectivity and sensitivity that boosted their relevance in analytical chemistry area. (Mirzaei e Sawan, 2014)

These techniques are based on the measurement of current depending on the electrode potential (Working Electrode). This potential is then used to promote a bidirectional charge transference, resulting in a current proportional to the concentration of the electroactive species. (Mirzaei e Sawan, 2014) Depending on how this potential is applied there can be different electrochemical signals generated. These signals are summarized in Figure 3. (Skoog, Holler e Crouch, 2007)



Picture 3 - Voltage vs Time excitation used in voltammetry. (Skoog, Holler e Crouch, 2007)

We will be focus on these techniques because they are the most important and widely used in this field of (bio) analysis. They are characterized by having an excellent spatial and temporal resolution, as well as great sensitivity. These characteristics allow to study concentration dynamics both in vivo, ex vivo and in vitro. (Mirzaei e Sawan, 2014)

Like other electrochemical techniques, voltammetry requires an electrochemical cell comprising of:

- Working electrode (WE) - where the reaction takes place;
- Reference electrode (RE) - usually a silver/silver chloride electrode in which the potential remains constant;
- Counter or auxiliary electrode (CE) - usually a platinum wire that allows the current to flow through the system.

In particular for the in vivo experiments the use of a microelectrode such as the carbon fiber microelectrode (CFM) has significant advantages due to the micrometer dimensions of the surface area, high current densities, low IR drop and biocompatibility (Liu *et al.*, 2009; Silva *et al.*, 2014). Despite their advantages, the selectivity is always a concern and by this reason efforts should made to improve this analytical figure. (Liu *et al.*, 2009; Silva *et al.*, 2014)

The voltammogram, which is a plot of current vs. potential, gives quantitative and qualitative information about the analyte (identification and concentration, for example). (*Electrochemistry Basics - Chemwiki*, [s.d.]; Liu *et al.*, 2009)

Voltammetry is a non-destructive technique, with a relatively easy analytical procedures (due to the simple preparation of the samples), high sensitivity and moderate selectivity. (Sanghavi *et al.*, 2014)

There is a wide variety of electrochemical techniques that can be used in order to acquire a meaningful signal, the most frequently used for in vivo recordings being the amperommetry, cronoamperometry as well as the fast cyclic voltammetry (FCV). And in a less extend the Square Wave Voltammetry (SWV).

Amperommetry

The amperommetry method is a technique in which the potential is hold constant while the current is measured continuously. It is very sensitive to the concentration of the analyte, and allows us to do very fast detection in a millisecond time scale. (Skoog, Holler e Crouch, 2007)

There are some precautions we need to take when using this technique, because the selectivity is poor implying the use of coatings of the electrode surface. Nonetheless, it is very useful for rapid measurements requiring simple instrumentation. (Mirzaei e Sawan, 2014)

Fast Cyclic Voltammetry

Regarding the FCV, this technique is able to perform a measurement every 100ms (mili-seconds) with an increase in selectivity. Because each cyclic voltamgramm acts as an electrochemical fingerprint of that analyte. With this technique not only are we able to detect changes of analytic concentration, but also to monitor them as a result of its fast acquisition speed (more than 100V/s). Although it needing a special equipment. (Mirzaei e Sawan, 2014)

Amperometry and FCV are included in a wide range of bio-sensing platforms with a wide range of materials and construction materials. (Yáñez-Sedeño *et al.*, 2010)

Square Wave Voltammetry

Concerning the SWV, it is a technique based on pulsed potential according to a square waveform and by sampling the current. It offers speed of acquisition and also high sensitivity. With this technique it's possible to obtain a voltammogram (plotting the difference of currents vs the potential) in less than 1s. This technique has the look of a staircase and the magnitude of the pulse is enough that a forward pulse results in a cathodic current, while the reverse pulse results in an anodic current. Usually the difference between the currents is proportional to the concentration, thus allowing us to quantify the analyte. (Skoog, Holler e Crouch, 2007)

Nanotechnology and nano-materials

Nanotechnology has become a very interesting discipline, not only as a science but also for the technology it uses. It is a field that includes many branches, ranging from the simplest of things to highly complex matters. (Holzinger, Goff, Le e Cosnier, 2014)

The strength of this field arises from the fact that it is multi-disciplinary hence the importance of the information and know how´s exchange between engineers, physicists and chemists, among others. (Holzinger, Goff, Le e Cosnier, 2014)

In the last few years, it became common to hear about nanomaterials as chemical entities smaller then 100nm, (or 500 μm) as part of this nanotechnology field. (He *et al.*, 2014; Sanghavi *et al.*, 2014)

These materials can be of different types and can be obtained through a "*controlled assembly of nanoscale building blocks*" or by a *"controlled elimination of starting materials and biomaterials to the nanoscale*". (Holzinger, Goff, Le e Cosnier, 2014)

The increasing interest in these materials derives from some of their properties such as:

- Small size, shape and high surface to volume ratio;
- Signal amplification (greater sensibility, selectivity and lower LOD);
- Physical proprieties that can be chemically shaped (better biocompatibility);
- Target binding proprieties (immobilization support like activity);
- Structural robustness.

Due to these characteristics, nanomaterials are highly useful in the sensing and bio-sensing filed. (Holzinger, Goff, Le e Cosnier, 2014; Sanghavi *et al.*, 2014; Tian, Prestgard e Tiwari, 2014)

They can either be used in conjunction with biomolecules or to target analytes through various interactions like covalent, physical adsorption or electrostatic. The nano-materials is a vast field where we can find diverse materials such as:

- Gold nanoparticles;
- Quantum dots;
- Magnetic nanoparticles;
- Carbon nanoparticles and nanostructures.

(Hayat, Catanante e Marty, 2014; Holzinger, Goff, Le e Cosnier, 2014; Wang e Dai, 2015)

In this work we will be focusing our attention on the carbon nanostructures, due to their interest in their application in bio-sensing.

In recent years there has been great progress in the application of these materials to biosensors. A sensor is a device that incorporates a sensing element intimately connected or integrated with the transducer. In the case of a biosensor this element is a biological one, which has the particularity of recognition based on affinity or reaction specificity. This property makes it possible to obtain a concentration-proportional signals. (Wang e Dai, 2015)

Biosensors have emerged in the recent years as a good alternative to classic methods like chromatography, mainly due to their ability to convert a biologic response into a signal that can be detected and measured. (Wang e Dai, 2015)

One of the main advantages of this devices is the direct contact between the element that does the recognition and the surface area of the electrode. (Wang e Dai, 2015) Incorporating nanostructures in a biosensor brings a lot of advantages, all of them related to the nanoscale, such as better transducing ability at the molecular level, improved sensibility, lower LOD, better response time and also health monitoring. (Holzinger, Goff, Le e Cosnier, 2014; Merkoåi, 2007; Wang e Dai, 2015)

In order to fabricate an efficient sensor we need to choose the substrate where we disperse the sensing material carefully, because it will determine the sensor performance. Equally important is the efficiency of the bio functionalization of nanomaterials, for which there are two major approaches: non covalent and covalent binding. The non-covalent approach is represented by electrostatic interactions, preserving the specific properties of both nanomaterials and biomolecules. While covalent binding is more stable and reproducible, a major drawback is the uncontrolled anchoring of the biomolecule, which can affect the recognition site and interfere with the signal obtained. (Holzinger, Goff, Le e Cosnier, 2014; Krishnamoorthy, 2015; Merkoåi, 2007; Wang e Dai, 2015)

Both graphene (GRPH) and carbon nanotubes (CNTs) are the leading structures in the nanomaterials for bio-sensing application, and despite being different, each one has its own advantages. Although there is a lot of different materials to choose there's is no doubt that the combination of these materials will result in a significant improvement in the performance of the biosensor. It is anticipated that the combination into a single composite will open new avenues in many research fields. (Hayat, Catanante e Marty, 2014; Krishnamoorthy, 2015)

Carbon Nano-Materials

There are a few different types of carbon materials with novel properties and different applications. However, as we seen before, we will focus on the two that are consider to be the most relevant (Zhang, Guo and Cui, 2009):

-Carbon nanotubes (CNT)

-Graphene (GRPH)

Carbon Nanotubes

By the 1970's, ***Morinobu Endo*** prepared filaments of carbon that were thought to be carbon nanotubes. These failed to meet the measurement criteria for width and thus were denied their identity as CNT. (*The History of Carbon Nanotubes – Who Invented The Nanotube?,* [s.d.])

It was only 20 years later, in 1991, that ***Sumio Iijima*** made the first contact with Multi-walled nanotubes. (He *et al.*, 2013) He was then credited with the discovery even tough at that point, he didn`t realize the importance of his discovery.(*The History of Carbon Nanotubes – Who Invented The Nanotube?*, [s.d.])

Three years later ***Lijima and Donald Bethune,*** made the first contact with Single-walled nanotubes, and at this point the scientific community started to understand the importance that these would have in the future. (*The History of Carbon Nanotubes – Who Invented The Nanotube?,* [s.d.])

Ever since their discovery, CNTs have attracted a great deal of attention and interest due to their unique mechanical, physical and chemical proprieties. (Herbst, 2004; *The History of Carbon Nanotubes – Who Invented The Nanotube?*, [s.d.])

Among many different applications, they can be used to fabricate ultrasensitive electrochemical (bio) sensors that exhibit high electrical conductivity and good mechanical and chemical properties (for example: a composite between a polymer and CNT applied to a carbon fiber microelectrode). (Silva *et al.*, 2014; Zhang, Guo e Cui, 2009) In addition, nanowire morphology, biocompatibility and electronic properties greatly enhance the sensor performance. (Tian, Prestgard e Tiwari, 2014)

There are many applications including:

- Detection of glucose;
- Detection of DNA;
- Detection of Proteins;
- Detection of Pesticides (liposome based biosensor);
- Detection of E.coli (using bismuth nanofilm).

Not only do they have great importance in the sensing field, they also behave well in drug delivery systems, by stopping the drug from suffering any metabolism and reaching the cells safer and more effectively. (He *et al.*, 2013) They can also be very important in the pharmaceutical industry, as they allow for a better separation of chiral drugs. (He *et al.*, 2013)

Figure 4 illustrates the improvement of a sensor when combined with an enzyme and polymer, (in this case a hydrogel and glucose oxidase). (Zhang, Guo e Cui, 2009)

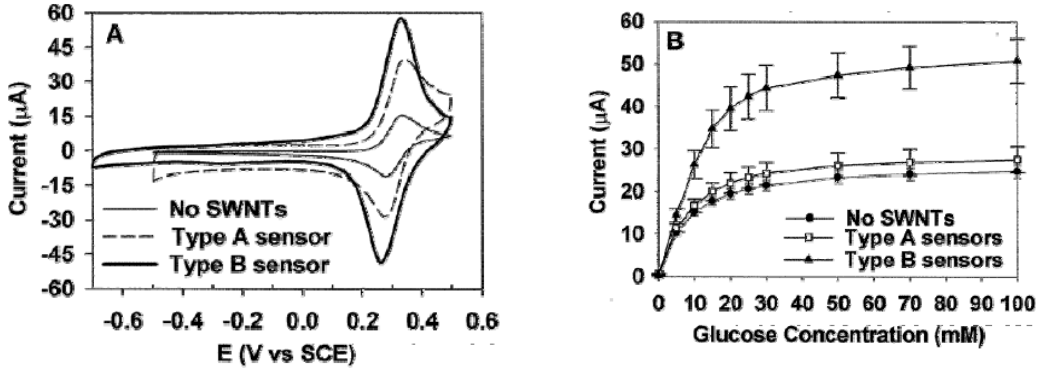
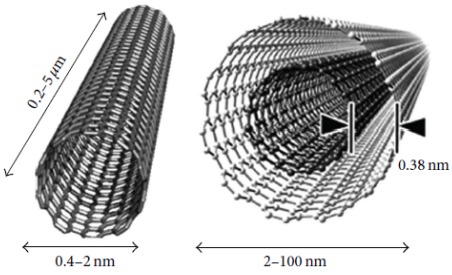


Figure4 - Electrochemical characterization of glucose oxidase sensors. (A) Cyclic voltammograms of a GCE modified with the redox hydrogel alone (-); a GCE modified first with a film of SWNT and then coated with the redox hydrogel (----) (type A sensor); (III) A GCE modified with a redox hydrogel containing GOX-treated SWNTs (-) (type B sensor). Scan rate 50 mV/s. (B) Glucose calibration curves for the three types of sensors described in (A). T = 25C, E = 0.5 V vs SCE. (Zhang, Guo e Cui, 2009)

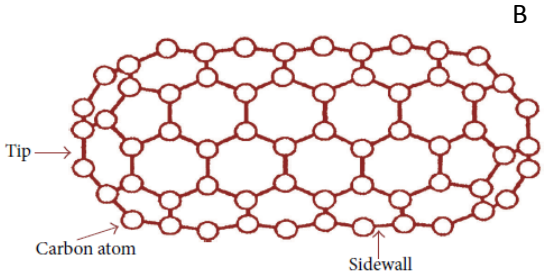
As stated before, there are two types of CNTs: Single-wall (SWCNT), with a diameter ranging from 0, 4 to 2, 5 nm; and Multi-wall (MWCNT), with diameters up to 100nm. (Tian, Prestgard e Tiwari, 2014) Both have a cylindrical shape with a few nanometers of diameter and several millimeters of length, however, MWCNT are composed of several sheets of graphite as opposed to SWCNT which are only composed of one single sheet. (Tian, Prestgard e Tiwari, 2014)

Despite this difference, they both show good mechanical strength, high electrical/thermal conductivity and high electroactive surface area, due to the highly porous three dimensional network, not to mention the hollow core in which some "guest" molecules can be stored, acting as a wire into the bulk electrode. (He *et al.*, 2013; Krishnamoorthy, 2015; Merkoåi, 2007; Tian, Prestgard e Tiwari, 2014; Wang, 2005)

The Figure 5 summarizes the main differences between MWCNT and SWCNT, and also shows the structure of both materials. As we can see they are composed of two main regions: tips and sidewalls. The tips are the most important area because it was demonstrated that they actively are involved in exchanging of electrons. (He *et al.*, 2013)



A



B

SWCNT	MWCNT
(1) Single layer of graphene	Multiple layer of graphene
(2) Catalyst is required for synthesis	Can be produced without catalyst
(3) Bulk synthesis is difficult	Bulk synthesis is easy
(4) More defection during functionalization	Less defection, but difficult to improve
(5) Purity is poor	Purity is high
(6) Less accumulation in body	More accumulation in body
(7) Easy characterization and evaluation	Difficult characterization and evaluation
(8) Easily twisted	Difficult to twist

C

Figure 5

A: Diagrams of SWCNT on the left and MWCNT on the right;

B: CNT with closed ends;

C: Comparison between SWCNT and MWCNT;

(He *et al.*, 2013)

It was demonstrated that surface of pre-treated CNT exhibited a higher electron transfer rate, as a result of the presence of oxygen moieties into the surface and to the removal of metallic impurities. (Rivas *et al.*, 2007; Silva *et al.*, 2014)

It is important to note that the pre-treatment protocol is able no only to oxygenate the surface but could possibly break the tubes or even shorten them (oxidation under air flow at 400C or at 600C are two ways of doing this) (Rivas *et al.*, 2007; Silva *et al.*, 2014).

Due to these unique properties, an interesting electrocatalytical activity is obtained, usually lower overvoltage's and higher peak currents are observed in the voltametric response of electrodes modified with CNTs. (Rivas *et al.*, 2007; Silva *et al.*, 2014)

The insolubility in the common solvents constitutes a major drawback and has led to some investigation, which in turn resulted in proposals of dispersion in different solvents, being nafion one of the most used due to his unique cationic ion-exchange properties and biocompatibility, or the incorporation into composites of different matrices using distinct binders. (Rivas *et al.*, 2007; Wang, 2005)

It was demonstrated that vertically aligned CNTs show a better electrocatalytical activity than those randomly aligned, due to the higher number of free tips available when vertically aligned, which contributes to a higher direct electron transference rate (DET). (Silva *et al.*, 2014)

Graphene

Graphene is a two dimensional single atomic carbon sheet packed into a honeycomb structure. (Brownson e Banks, 2010) This type of material was thought not to exist until the year of 2004, when **Andre Geim** published is first article on graphene – "*Electric field effect in atomically thin carbon films*". (*This Month in Physics History: October 2009*, [s.d.])

Graphene is a million times thinner than paper sheet, stronger than diamond and more conductive than copper. Ever since that moment, it has been one of the most cited paper.

The ultimate goal of the project came from the ideia that it was possible to fabricate a material like a carbon nanotube in an unfolding configuration. (*This Month in Physics History: October 2009*, [s.d.])

Graphene is available nowadays with some interesting properties such as a planar structure, which makes it possible to wrap into SWCNT or MWCNT depending on how many graphene sheets are present. (Sanghavi *et al.*, 2014)

There is also a vast and easily modified area, good mechanical strength and thermal stability alongside a chemical inertness and good electronic properties. (Hayat, Catanante e Marty, 2014) An image of graphene structure is shown in Figure 6

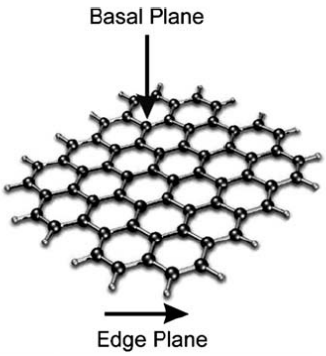


Figure 6 – Conceptual schematic of the structure of graphene. (Brownson e Banks, 2010)

Among others, the field of electrochemistry has a huge interest in this material due to its unique physical, chemical, electronic, optic, mechanical and thermal properties, better than other types of nanomaterials. (Brownson e Banks, 2010)

Regarding the synthesis of graphene, there is one common aspect that should be taking into account. Because graphene is a material that oxidize very easily in contact with air all the processes make a reduction of the product in the end. The most common reduction processes are chemical or electrochemical reductions. (Brownson e Banks, 2010; Sanghavi *et al.*, 2014)

Examples of the synthesis processes includes dry mechanical exfoliation, which is ideal to investigate the physical properties, chemical exfoliation, unzipping of CNT (electrochemical, chemical or physical methods) and sugar reduction (a new method that is

cheaper and allows for an industrial scale production). (Brownson e Banks, 2010; Sanghavi *et al.*, 2014)

When compared to CNTs, this material exhibits some advantages which can improve the potential for sensing and biosensing, even further, as well as the trace metal analysis, sensing of gaseous species, among others. Concerning the surface area, it is believed that the theoretical area of graphene exceeds that of the CNT by two times its value ($2630 m^2 g^{-1}$ vs $1315\ m^2 g^{-1}$). Even higher difference is found in the electrical conductivity, which can reach a value of sixty times the one calculated for the CNTs (64 mS cm^{-1} for the graphene). (Brownson e Banks, 2010; Sanghavi *et al.*, 2014)

The electrical conductivity is also more stable in a wide range of temperatures, which could be of a valuable property considering the number of applications. (Brownson e Banks, 2010). The presence of oxygen-containing groups on the edge and surface of the graphene is also important for the electrochemical performance. As we have seen before CNTs need to be modified in order to insert these oxygen molecules. (Brownson e Banks, 2010). Considering its unique properties, it is possible that graphene can transport higher currents when compared to CNTs. (Brownson e Banks, 2010)

In addition to these exceptional properties, they also have the ability to affect the microenvironment of molecules and act as a good immobilization support for enzymes. (Hayat, Catanante e Marty, 2014)

The Figure 7 shows us the electrocatalytic properties of graphene on the oxidation of paracetamol. (Brownson e Banks, 2010)

The application of graphene can include different types of sensors such as glucose, cholesterol biosensor and hydrogen peroxide sensor, as well as sensors for ascorbic acid, uric acid and dopamine. (Kuila *et al.*, 2011)

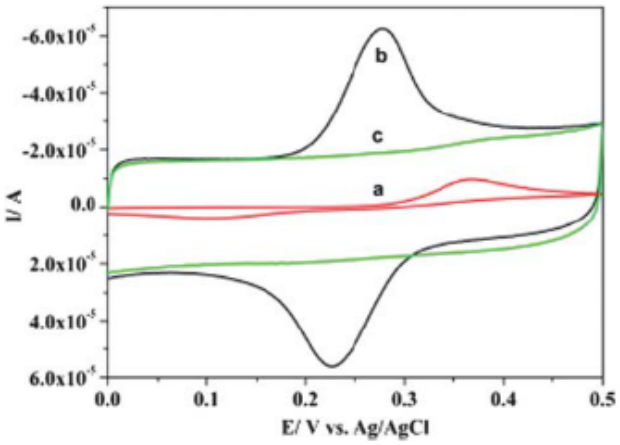


Figure 7 - Electrochemical sensing of 100uM paracetamol at a bare glassy carbon electrode(GCE) (a) and compared with graphene modified GCE with (b) 20uM paracetamol and without paracetamol (c) in the buffer of 0,1 M NH_3 H_2O-NH_4CL, pH=9,3, scan rate 50mV/s

(Brownson e Banks, 2010)

Sensor Performance

As mentioned before, CNTs have attracted a great attention due to their properties. More recently, graphene has been replacing CNTs, because it is not necessary to remove metallic impurities inherent to the process of fabrication of CNTs and it's expected to have a better sensitivity, selectivity, faster response from the electrode, better dynamic ranges and also lower limit of detection (Brownson e Banks, 2010; Sanghavi *et al.*, 2014). Furthermore, graphene is highly biocompatible, noncytotoxic and suitable for biomedical applications. (Kuila *et al.*, 2011)

Importance and application

Monitoring of molecules in vivo in real time such as neurotransmitters, metabolic markers and hormones is of major importance to achieve a better understanding of physiology and pathophysiology. (Marinesco e Dale, 2013).

According to IUPAC, a biosensor is "a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biologic recognition element which is in direct spatial contact with a transducer element". (Koyun, Ahlatcıoğlu e İpek, 2012)

The key factors for a biosensor are the analyte (what we want to measure), the biologic element (providing specific recognition properties), the transducer (does the conversion of the event into a signal that we can measure) and the immobilization matrix. Therefore there can be a wide variety of different biosensors depending on the different biologic elements that are used, like enzymes, antigens and nucleic acid, among others. Also according to the transducer element used they can be amperometric, potentiometric or conductimetric, just to quote a few. (Moura *et al.*, 2007)

We intend here to refer some examples of biosensors based on microelectrodes platforms namely, Carbon Fiber Microelectrodes (CFM), Microelectrodes Array`s (MEA).

Carbon Fiber Microelectrodes

Carbon fiber microelectrodes (CFMs) have been extensively used over the last three decades mainly for detecting catecholamine neurotransmitters (dopamine, noradrenaline) in the brain. (Liu *et al.*, 2009; Silva *et al.*, 2014) To obtain the desire selectivity, the active surface should be coated with perm selective membranes (e.g. Nafion) and nanomaterials. (Sanghavi *et al.*, 2014) The fouling, on the other hand, is caused by the blockage of the surface area that derives from macromolecules that slow down the electron transference kinetics. In order to try and solve this issue there are several coatings that can be applied, using different polymers film and nanomaterials. (Liu *et al.*, 2009; Silva *et al.*, 2014)
The fabrication process usually involves the insertion of a single carbon fiber usually 7 μm or 30 μm diameter in a glass capilar and pulling in a puller, creating a sealing zone near the tip that must always be inspected to check the quality of the glass-fiber seal.

A more detailed explanation about CFM and their procedure of fabricating and coating and results will be presented in the appendix 1.

MicroElectrode Arrays

Regarding MEA, they are fabricated by photolithography technique using a ceramic-based material and platinum active sites. (Talauliker *et al.*, 2011) This microelectrode array can be fabricated with multiple active sites from 4 to 16 recording sites in a different design geometrical configurations. (Talauliker *et al.*, 2011; *Welcome to CenMeT Service Center*, [s.d.]) Concerning this recording sites, atomic force microscopy showed that they are not regular. At the nano level, they are presented as a rugged surface, increasing the current per unit and thus better than the other types of sensors. (Talauliker *et al.*, 2011)

When comparing the production cost against CFM, there is no doubt that MEA are much more expensive. However, they can be produced at an industrial scale with much more precision and with much higher durability and reproducibility. (Talauliker *et al.*, 2011)

These devices are most suited for amperometric recording, providing a great spatial and temporal resolution. Despite less flexible, when compared to CFM, there are a few interesting properties regarding its physical structure: self-reference techniques are possible due to its organization and structure, which means that we can use one site, coated with specific components, to monitor our analyte, and simultaneously, with the nearest site, be able to subtract the background (as this site is not coated with the same elements as the monitoring site).

The possibility of using self-reference techniques is one of the most important properties associated with MEA as it can help us understand what a signal is truly and what is just a fluctuation on the baseline.

We need to be very thorough, however, when coating the sites, because the minimum contamination of the reference site can result in a misinterpretation of the results.

With CFM, this was never a possibility and, despite its smaller size and diameter, the tissue we are interested in analyzing will suffer more damage due to the need of using more than one sensor.

Although not perfect, one of the major drawbacks of the arrays is the fact that there is some cross-talk between sites as a result of their proximity. This cross-talk can either be physical or chemical.

Conclusion

The development of sensors and biosensors are a great way to take a look into the inside the organism. In the present monograph we aimed to address specific points like the electrochemical techniques and nano-materials used to manufacture different types of (bio)sensors.

The electrochemical techniques are fundamental to obtain analytical signals. Each technique with its own specificities. The nano-materials, as well as the enzymes and different polymers, make the sensor more robust by improving their analytical performance.

We have been pointing to a perspective of monitoring some specific analytes in the organism, especially in the brain. In the neuroscience field, sensors and biosensors are more frequently playing a role in the understanding brain function and disfunction, namely in the neurodegenerative and psicotic diseases.

Also, sensors and biosensors can be used in clinical diagnosis (routine blood tests for glucose, lactate and cholesterol, among others), therapeutic drug monitoring, in the food and beverages industry (determining the sugar or alcohol, for example) and in industrial process control (control of the fermentation process, for example). Furthermore, they can be applied in other areas suchs as pollution control and defense against attacks with chemical warfare agents.

Overall, the wide range of applications demonstrates how vast and interdisciplinary this area is, and why it is one of the major fields of study and research nowadays.

We strongly believe that the future of this area will pass through the vertically aligned deposition of nanomaterials, as it will greatly improve the coating procedure, making it much more reproducible and homogeneous.

With the great amount of attention that the nanotechnology field as received it is likely that new materials and techniques will be discovered opening horizons to an even brighter future.

Appendix I

With this appendix we intend to give support to some information that was provided in this work.

We will present some pictures of the different types of electrodes, some steps involved in their preparation as well as some data acquired with them.

CFM preparation: Materials and Equipment's

The preparation of the CFM follows the next steps:

- Insertion of the carbon fiber (7 or 30 µm) into a borosilicated capillary (1,16 mm id x 2,0 od, from Harvard Apparatus Ltd, UK);
- The capilar and the fiber are then pulled in a vertical puller (Harvard Apparatus Ltd, UK) – The strength and heat of this puller can be adjusted.
- Selection of the half-part of the capilar that contains the fiber;
- Observation of the sealing zone using an inverted optical microscope (Olympus CK2, Japan);
- After verified the sealing, proceed to cutting the fiber, aiming to a size of 200±50 µm (with the help of a chirurgical scissor);
- Insert a copper wire, into the capilar, in order to make contact with the fiber. This allows a connection from the equipment to the electrode.
- Glue the copper wire and the fiber with silver glue;
- Dry at room temperature;
- After properly dried, use the oscilloscope in FCV mode, to determine the profile of the CFM and also to activate the carbon fiber (procedure done with Tektronix TDS 220 Oscilloscope and the Ensman Potentiostat in phosphate buffer saline medium). (Santos *et al.*, 2008)

Figure 8 - The puller used in the fabrication of the cfm, on the left, and a finalized cfm on the right.

Coating procedure

This coating can be done with different types of materials, but as our focus is on carbon nanomaterials, we used CNTs and GRPH.

These types of coating follow the next general steps:

- Prepare the stock solution of the desired material, adjusting it to the ideal concentration;
- From the stock solution we proceed to the preparation of the composite (most frequently nafion and a nanomaterial) that will coat the CFM. Usually this is done in an Eppendorf tube in order to make it easy for the next steps;
- After the preparation of the composite we should take it to the ultrasounds for at least 20 minutes, in order to homogenize the dispersion;
- The next step involves dipping the tip of the CFM during 30 seconds into the Eppendorf tube. After the dipping we take the CFM to the oven, at 170°C, to dry for 5 minutes. (this cycle is repeated 5 times);
- Check under the microscope if the coating is relatively uniform;
- Calibration and evaluation of the sensor (regarding its sensibility, selectivity, linearity and limit of detection). (Santos *et al.*, 2013)

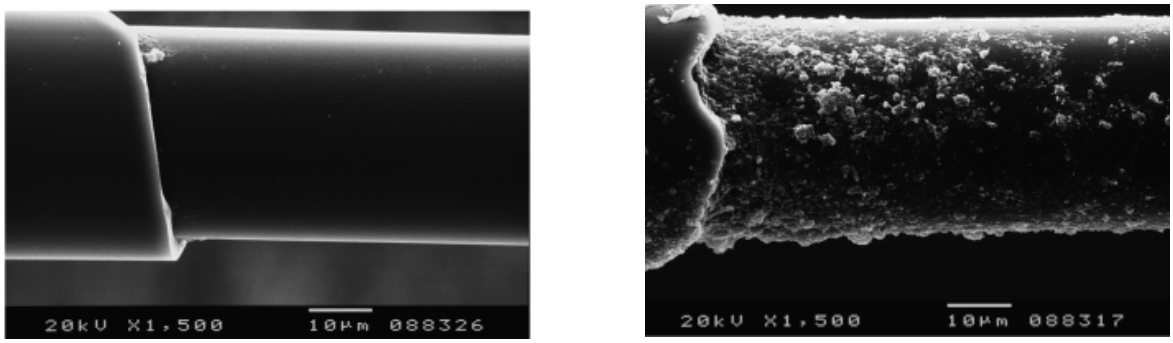


Figure 9 - Scanning electron microscopy of bare cfm on the left and coated (nafion and SWCNT) on the right. (Ferreira *et al.*, 2013)

The impact of CNT on the sensor performance

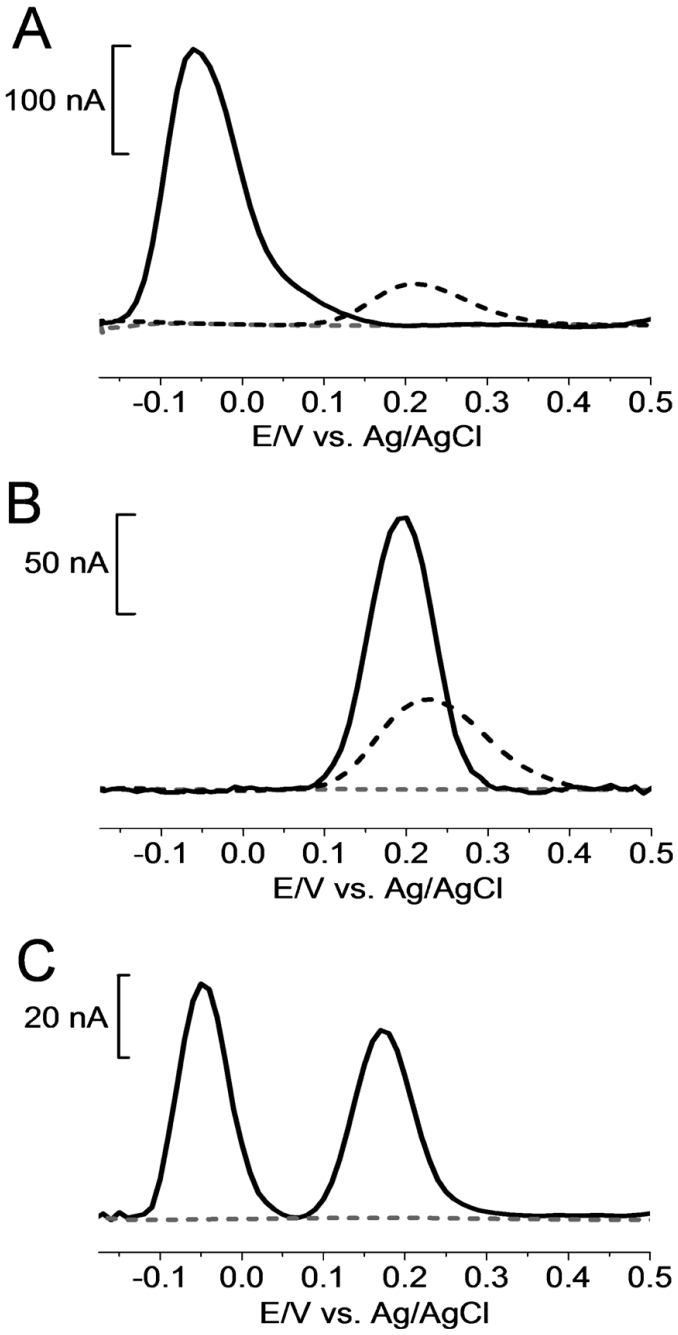


Figure 10

A – Change in the oxidation peak of AA for cfm bare (dashed line) vs cfm coated with CNT (solid line)

B – Change in the oxidation peak of DA for cfm bare (dashed line) vs cfm coated with CNT (solid line)

C – AA and DA oxidation peaks measured with coated cfm

(Ferreira *et al.*, 2013)

This previous figure is the perfect example of how the properties of CNTs can improve the sensors performance.

In order to a better understanding, we need to think that AA and DA have the oxidation peaks in, approximately, the same potential. However, after applying the CNT coating to the cfm we observe that there is a shift in the AA oxidation peak, as it doesn't overlaps with DA oxidation peak anymore.

Besides the difference in the oxidation peak, we also see that the sensitivity was greatly improved, as the peak becomes mode defined and evident.

Graphene coated cfm

At this point we only did some preliminary tests with graphene coated cfm. The fabrication protocol for the cfm, as well as the coating procedure, were the same as stated in this appendix, with the exception to the solution of graphene, which was purchased already prepared.

In this preliminary test, we used the graphene coated cfm to see if there was any change regarding the oxidation peak of DA and AA, and the results were intriguing.

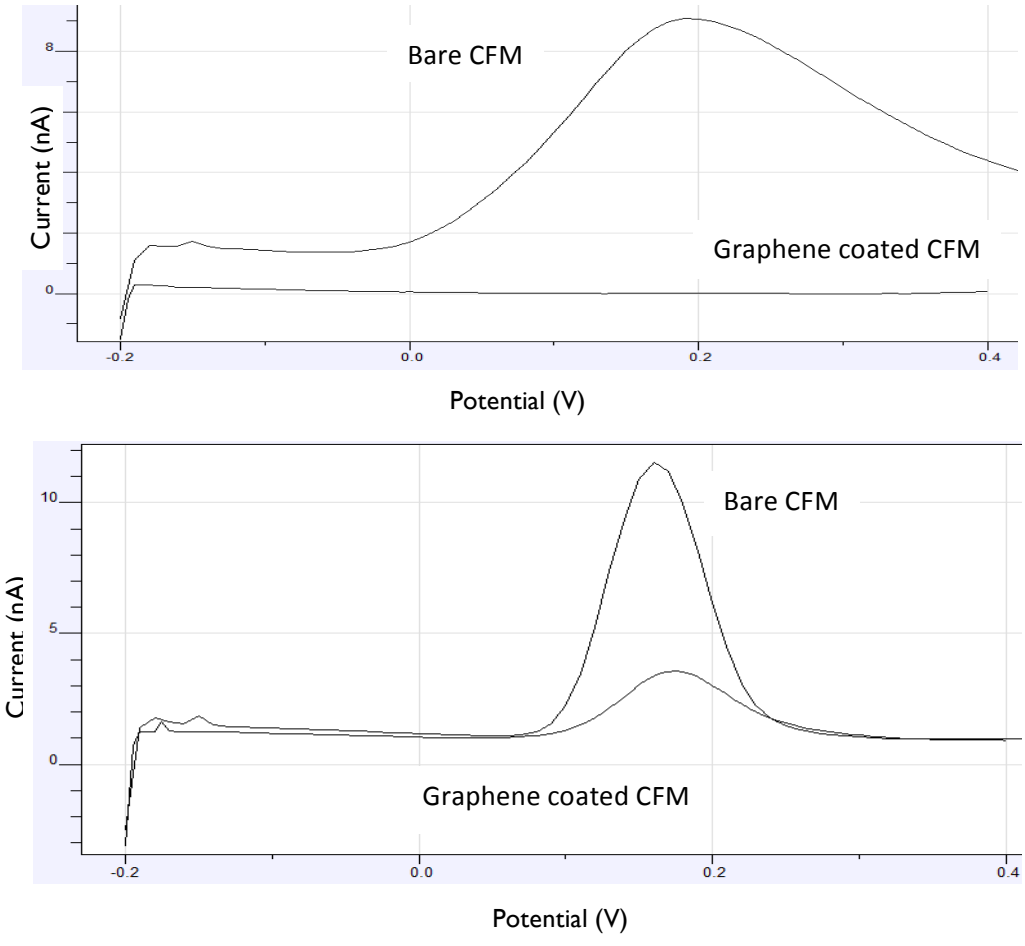


Figure 11 - AA Oxidation peak with graphene coated cfm (on top) and DA oxidation peak with graphene coated cmf (at bottom)

There was no change in the potential to which AA and DA undergo oxidation. Moreover, we see that there was a loss of sensitivity after coating the cfm, as the peak tend to be less evident.

Considering the literature this was not expected and after giving it some thoughts we reached the conclusion that, maybe, the coating procedure was not indicated for this material.

When we dry the sensor we promote the conversion of graphene to graphite, which blocks the electroactive surface thus the loss of sensitivity that we observed.

MicroElectrode Arrays

As already mentioned these type of sensors are purchased and can be coated with the same solution as the cfm. However, this coating can`t be done using the dipping technique, as we need to be much more precise because of the existence of multiple sites.

In the figure 12 I will show this type of device and its different configuration.

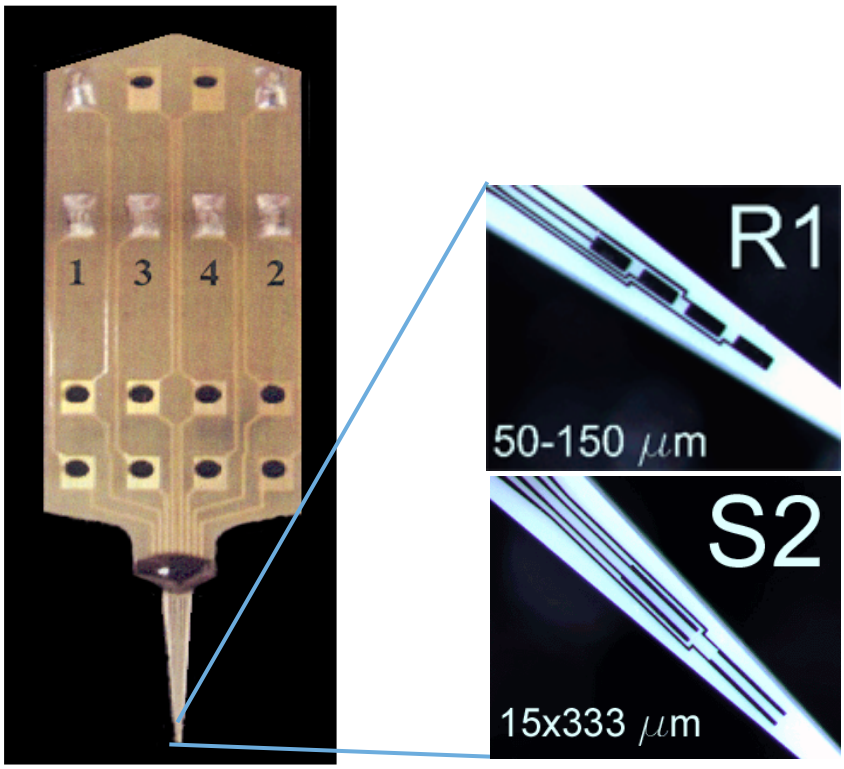


Figure 12 - MEA structure on the left and its different types of (tip) configuration.

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