DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HYDROGEN PEROXIDE DETERMINATION

Marta Borràs Brull

UNIVERSITAT ROVIRA i VIRGILI

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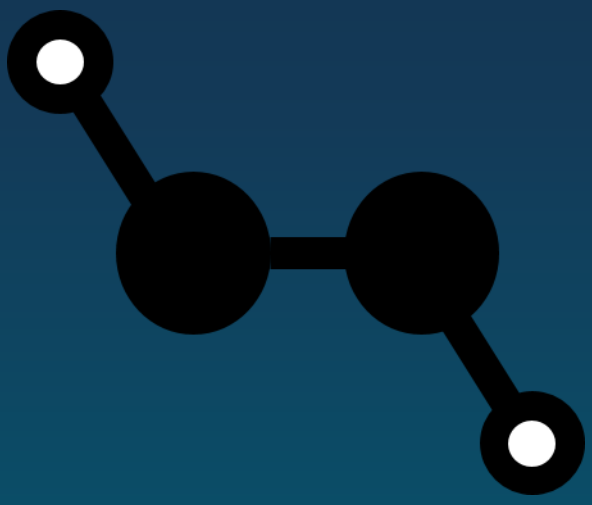
MARTA BORRÀS BRULL



DOCTORAL THESIS - 2020

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Development of electrochemical sensors for hydrogen peroxide determination

Doctoral Thesis

Supervised by

Dr. Jordi Riu Rusell and Dr. Pascal Blondeau

UNIVERSITAT ROVIRA i VIRGILI

Department of Analytical and Organic Chemistry

Tarragona, 2020

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Development of electrochemical sensors for hydrogen peroxide determination

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WE STATE that the present study, entitled "Development of electrochemical sensors for hydrogen peroxide determination", presented by *Marta Borràs Brull* for the award of the degree of Doctor, has been carried out under our supervision at the Department of Analytical and Organic Chemistry of this university. Furthermore, we state that the aforementioned thesis qualifies for the International Doctorate Mention.

Tarragona, February 10th 2020

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Dr. Jordi Riu Rusell

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AGRAÏMENTS

Primerament, m'agradaria donar les gràcies al grup en general per l'oportunitat de realitzar aquest doctorat durant estos 3 anys, en els quals he aprés tant a nivell acadèmic com personal. Agrair en especial al Dr. Jordi Riu la seua supervisió i ajuda rebuda durant tot este temps. D'ell he aprés a confiar i a afrontar-ho tot des d'una perspectiva optimista. No pot faltar als agraïments la menció a la seua manera d'amenitzar els *coffees* de bon matí amb los seus *xistes* tant dolents, que en realitat a mi m'encanten. Agradecer también al Dr. Pascal Blondeau por su supervisión e implicación a fondo con el trabajo, el suyo y el de todos. Con él se aprende a ir al grano y a priorizar y a optimizar en cada detalle. Agradecer también al Dr. Franciso Javier Andrade por acogerme como una alumna suya más y transmitirme sus conocimientos, tanto académicos en los *group meetings*, como personales, en los *coffees*. I al Dr. Santiago Macho, per la seua resolució en qualsevol dubte i problema tècnic, així com per fer dels *coffees* unes reunions més properes i familiars. Finalment, donar les gràcies també al Professor Francesc Xavier Rius per la seua càlida benvinguda i constant implicació amb el grup i tots els membres.

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SUMMARY AND CHAPTER OVERVIEW

The main motivation behind this doctoral thesis is the development of novel and low-cost (bio)chemical sensors that can set the basis to design robust, affordable, scalable and user-friendly sensing platforms. The first approach is based on the incorporation of conducting polymers to achieve enhanced analytical performance as well as desirable final device features, such as affordability and simple operation. The second part of the thesis is addressed on the exploration and development of biosensors for non-invasive diagnostics, with the aim of improving the quality of life of potential end-users.

The work begins by introducing the importance of the determination of hydrogen peroxide, including some of its several applications in different fields and a brief description of the thesis structure and objectives. It continues by presenting some of the most recent works on hydrogen peroxide detection using conducting polymers. A critical comparison of the analytical performance and some of the future challenges are described. Fruit of these challenges, the first part of the thesis is based on the development of new approaches for such detection in the form of experimental work. The frame of the second part of the thesis points out the development of electrochemical sensors based on enzymes in order to determine hydrogen peroxide as a byproduct of the main oxidase reaction. In addition, some background of scientific foundation, technological methods and principles on which the work stands is also provided. A more detailed information about each chapter is provided below:

CHAPTER 1 briefly illustrates the fundamentals of the electrochemical techniques used throughout the development of this thesis together with a comparison of their main features.

CHAPTER 2 displays a general view of current works and methodologies in hydrogen peroxide detection using conducting polymers. The chapter explains the importance of its detection, the attractive features of conducting polymers and the different strategies which are currently carried out when applied to sensing.

CHAPTER 3 & 4 depict two different approaches based on two different electrochemical techniques carried out as alternative ways to directly determine hydrogen peroxide through the use of conducting polymers. First CHAPTER 3 focusses on the conductometric approach, and CHAPTER 4 on the potentiometric one.

CHAPTER 5 & 6 present two different approaches based on two different electrochemical techniques performed in order to indirectly detect hydrogen peroxide. Both chapters are based on the use of enzymatic-based electrochemical sensors for the detection of glucose. CHAPTER 5 describes the construction and characterization of assembled macro- and micro-electrodes for glucose determination using amperometric sensors. CHAPTER 6 presents the characterization and validation of a potentiometric platinum paper-based sensor for glucose determination in saliva. It is presented as an alternative and noninvasive methodology for glucose quantification.

CHAPTER 7 outlines the main conclusions derived from the experimental work as well as details on next steps in order to continue with the improvement of these devices in the field.

Finally, some appendices have been added with additional information corresponding to:

- Appendix 1 presents the list of abbreviations.
- Appendix 2 shows the figure, scheme and table index.
- Appendix 3 points out the list of publications resulting from this thesis as well as the corresponding congress contributions.

RESUM I ÍNDEX DE CAPÍTOLS

La motivació principal darrera d'aquesta tesi és el desenvolupament de nous sensors (bio)químics de baix cost que puguin assentar les bases pel disseny de plataformes de detecció robustes, econòmicament assequibles, escalables i fàcils d'utilitzar. La primera estratègia es basa en la incorporació de polímers conductors per aconseguir un rendiment analític millorat i al mateix temps, unes característiques finals del dispositiu d'assequibilitat i senzillesa en el seu ús. La segona part aborda l'exploració i el desenvolupament de sensors per al diagnòstic no invasiu, amb l'objectiu de millorar la qualitat de vida dels usuaris finals.

El treball comença introduint la importància de la detecció de peròxid d'hidrogen, incloent algunes de les seves moltes aplicacions en diferents camps, i una breu descripció de l'estructura de la tesi i els objectius. A continuació es presenten alguns dels treballs més recents en la detecció de peròxid d'hidrogen utilitzant polímers conductors. La comparació del rendiment analític així com els reptes a afrontar en un futur també són descrits. Fruit d'aquests reptes, la primera part de la tesi es basa en el desenvolupament de noves estratègies per la determinació de peròxid d'hidrogen en forma de treball experimental. La segona part enfoca el desenvolupament de sensors electroquímics basats en l'ús enzims oxidasa per la detecció de peròxid d'hidrogen com a producte de la reacció principal. A més a més, s'expliquen també els fonaments científics de les tècniques utilitzades i els principis de detecció en que es basa la tesi. Tot seguit s'adjunta informació més detallada de cada capítol en concret:

El **CAPÍTOL 1** explica breument els fonaments de les tècniques electroquímiques utilitzades al llarg de la tesi juntament amb la comparació de les seves característiques principals.

El **CAPÍTOL 2** mostra una visió general dels treballs més recents i metodologies emprades en la detecció de peròxid d'hidrogen utilitzant polímers conductors. El capítol explica la importància de la seva detecció, les característiques principals dels polímers conductors, així com les diferents estratègies utilitzades actualment.

Els **CAPÍTOLS 3 & 4** descriuen dos estratègies diferents basades en dos tècniques electroquímiques diferents que s'han portat a terme com a mètodes alternatius per la detecció de peròxid d'hidrogen utilitzant polímers conductors. Primer, el CAPÍTOL 3 es basa en un mètode conductomètric, i el CAPÍTOL 4 en un mètode potenciomètric.

Els **CAPÍTOLS 5 & 6** mostren dos estratègies diferents basades també en dos tècniques electroquímiques diferents per la detecció indirecta de peròxid d'hidrogen. Ambdós capítols es basen en l'ús de sensors electroquímics basats en enzims per la detecció de glucosa. El CAPÍTOL 5 descriu la construcció i la caracterització de macro- i micro-elèctrodes per la determinació de glucosa utilitzant sensors amperomètrics. El CAPÍTOL 6 presenta la caracterització i la validació d'un sensor potenciomètric de paper per la detecció de glucosa en saliva. Aquest mètode es presenta com una alternativa no invasiva per la quantificació de glucosa.

El **CAPÍTOL 7** descriu les principals conclusions derivades de la part experimental d'aquesta tesi, així com les possibles estratègies a seguir per al futur desenvolupament d'aquests dispositius.

Finalment, la informació addicional s'ha afegit en forma d'apèndix, corresponent a:

- L' Apèndix 1 mostra la llista d'abreviatures.
- L'Apèndix 2 mostra la llista de figures, esquemes i taules.
- L'Apèndix 3 mostra la llista de publicacions derivades d'aquesta tesi, així com les corresponents participacions a congressos.

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Hydrogen peroxide (H_2O_2) is a simple molecule with relevant roles in several fields. For example, in the food industry H_2O_2 has been used as sterilizing compound in packaging and food manufacturing due to its antimicrobial and fungicidal properties and biological degradability [1]. It is also used to rate the quality and safety of cosmetic and pharmaceutical formulations [2]. In cosmetic and personal care it is used to form dyestuffs during oxidative hair dyeing (by eliminating black-brown melanins) or to oxygenate stains or teeth to increase whiteness. Hydrogen peroxide is also used in paper products and textiles bleaching, providing a high degree of brightness and preserving the mechanical properties of the fibers. In metallurgical processes, hydrogen peroxide is used for ore leaching in order to save eluents and acids application and to simplify the management of chemical and waste. Since it is an oxidizing agent, its use in the chemical synthesis of flame retardants, catechol or herbicide production, among others, has also been reported for industrial purposes. We can also find hydrogen peroxide acting as oxidizing agent on waste water treatment, soil remediation or in air pollution control.

It is considered an important analyte in clinical diagnostics due to its implication in several routes of aerobic metabolism, in which its level can be used as a biomarker of some metabolic disorders related to the oxidative stress (e.g. asthma, osteoporosis or cardiovascular disorders, among others) [3,4]. It is also involved in some cellular signal transduction, mediating some physiological responses such as cell proliferation, differentiation and migration [5]. It also creates intracellular thermogenesis, which is extremely important for life's processes [6] and it is an analyte

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[6] W.C. Douglas, Hydrogen peroxide: Medical miracle, Rhino Publishing S.A., Panama, **2003** pp.20.

under study for inducing apoptosis on cancer cells [7]. Within the clinical field, it is also important due to its nature as a side product generated from biochemical reactions catalyzed by enzymes, such as glucose oxidase, cholesterol oxidase, glutamate oxidase, lysine oxidase, *etc*.

It is of great significance then, to design and develop a reliable and cost-effective method for H_2O_2 determination, which can generate a great impact in all the above-mentioned fields. Although several methods have already been proposed such as spectrometry [8,9], titrimetry [10], chemiluminescence [11,12], fluorimetry [13] or chromatography [14], electrochemical approaches present simple and compact tools able to provide great performance (high sensitivities and selectivity and low limits of detection).

A large number of electrochemical sensors have been described for the determination of hydrogen peroxide, either by direct detection or by using enzyme reactions which generate H_2O_2 as a byproduct.

This thesis aims to contribute to the development of electrochemical analytical tools for H_2O_2 determination. The thesis is divided in two main blocks according to the strategy used for

[7] W.A. Wlassoff, C.D. Albright, M.S. Sivashinski, A. Ivanova, J.G. Appelbaum, R.I. Salganik, Hydrogen peroxide overproduced in breast cancer cells can serve as an anticancer prodrug generating apoptosis-stimulating hydroxyl radical under the effect of tamoxifen-ferrocene conjugate, *J. Pharm. Pharmacol.,* **2007** DOI:10.1211/jpp.59.11.0013.

[8] C. Matsubara, N. Kawamoto, K. Takamura, Oxo[5, 10, 15, 20-tetra(4-pyridyl)porphyrinato]titanium(IV): An ultra-high sensitivity spectrophotometric reagent for hydrogen peroxide, *Analyst*, **1992** DOI:10.1039/AN9921701781.

[9] R.F. Nogueira, M.C. Oliveira, W.C. Paterlini, Simple and fast spectrophotometric determination of H_2O_2 in poto-Fenton reactions using metavanadate, *Talanta*, **2005** DOI:10.1016/j.talanta.2004.10.001.

[10] E.C. Hurdis, H. Romeyn, Accuracy of determination of hydrogen peroxide by cerate oxidimetry, *Anal. Chem.,* **1954** DOI:10.1021/ac60086a016.

[11] W. Chen, B. Li, C. Xu, L. Wang, Chemiluminescence flow biosensor for hydrogen peroxide using DNAzyme immobilized on eggshell membrane as a thermally stable biocatalyst, *Biosens. Bioelectron.,* **2009** DOI:10.1016/j.bios.2009.01.010.

[12] S. Hanoka, J-M. Lin, M. Yamada, Chemiluminescent flow sensor for H_2O_2 based on the descomposition of H_2O_2 catalyzed by cobalt(II)-ethanolamine complex immobilized on resin, *Anal. Chim. Acta.,* **2001** DOI:10.1016/S0003-2670(00)01181-8.

[13] A. Mills, C. Tommons, R.T. Bailey, M.C. Tedford, P.J. Crilly, Reversible, fluorescence-based optical sensor for hydrogen peroxide, *Analyst,* **2007** DOI:10.1039/B618506A.

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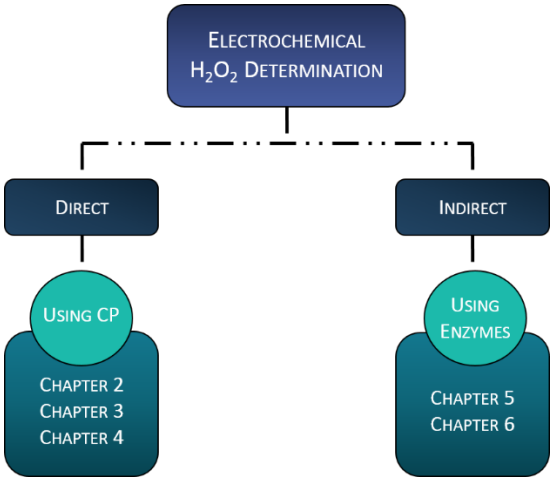
such determination. A schematic representation of the chapter's organization is shown in Scheme 0.1. The first part of the thesis addresses the direct electrochemical determination of hydrogen peroxide by the integration of conducting polymers into the proposed electrodes. The possibility of using alternative electrochemical techniques as the most reported ones is explored. The second part of the thesis aims at the indirect electrochemical determination of hydrogen peroxide, focused mainly on glucose detection through enzymatic reactions. In this case, two different electrochemical approaches are explored as well.



Scheme 0.1. Basic scheme of the main topics addressed in this thesis (classified into different chapters).

Overall, the general objective of the thesis is the development and optimization of novel and affordable electrochemical electrodes with robust analytical parameters for the hydrogen peroxide determination. This general goal can be divided in the following specific objectives:

- To explore the possibility to develop low-cost conducting polymer-based electrodes for H_2O_2 determination, taking advantage of the conducting polymer properties (CHAPTER 2, 3 and 4).
- To explore new strategies in order to understand the contribution of the conducting polymers in the sensing field (CHAPTER 3 and 4).
- To explore the determination of a physiological relevant target by using different techniques (CHAPTER 5 and 6).
- The validation of a paper-based potentiometric sensor based on enzymatic reaction to monitor glucose in a complex matrix (CHAPTER 6).

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CHAPTER 1

FUNDAMENTALS ON ELECTROCHEMICAL TECHNIQUES

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In this chapter we provide an overview of the foundational aspects of the different electrochemical techniques used in this thesis, namely conductometry, potentiometry and amperometry. For each detection technique, the working principle will be briefly described in order to rationalize the sensor response.

ELECTROCHEMICAL TECHNIQUES

Conductometric detection

Conductometry is conceptually the simplest electroanalytical technique, which is based on the detection of a change in the electrical conductivity (inverse of resistance) of a material due to a recognition event. There are several devices to measure the conductometric response of a sensor. The most used and simplest configuration is a chemiresistor, where the conducting layer is deposited between two electrodes separated by a narrow gap. Typically, the electrodes are interdigitated electrodes patterned on an insulator substrate, in order to increase the surface area of the sensing layer. A typical scheme of chemiresistor configuration is shown in Figure 1.1. The conductivity is measured by applying a constant current or voltage (DC or AC) between the two electrodes and measuring the resulting voltage or current [1]. There is another measurement based on four-point technique which measures the conductance of the bulk layer without the influence of the potential drop on the sensing layer-metal contacts. The combination of both techniques allows simultaneous measurements [2].

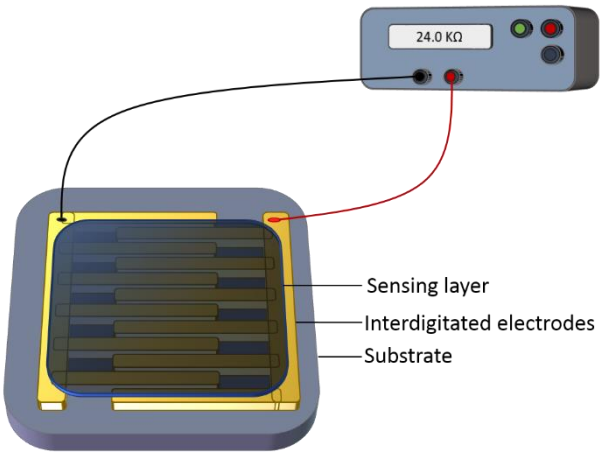

Figure 1.1. Schematic representation of a conductometric measurement with chemiresistors.

Chemiresistors are usually measured in DC mode and the capacitance in the equivalent circuit can be neglected. Thus, the analytical information is obtained from the modulation of the surface (R_S), contact (R_C) or bulk (R_B) resistances. Chemiresistors which use organic semiconductors usually depend on the chemical modulation of the bulk resistance, while the ones which use inorganic semiconductors operate on the principle of chemical modulation of the surface resistance.

Ohm's law states that the resistance of a material is given by:

$$R = \frac{E}{i} \qquad 1.1.$$

where R is the resistance of the material (Ω), E is the voltage applied to the material and i is the current of electrons flowing through the material. The resistance *R* depends on the dimensions of the conductor, then:

$$R = \frac{\rho\, L}{A} \qquad 1.2.$$

where ρ is the resistivity (Ω m), L is the length and A is the cross-sectional area. The resistance is then expressed in "ohm meter" (Ω m). Both resistance and resistivity indicate how difficult is to make the electrical current flow through a certain material.

If the measurements are done with AC current, conductance becomes frequency-dependent and resistance becomes impedance [3].

Potentiometric detection

Potentiometry is a well-stablished technique which revolutionized analytical chemistry in the last century [4] and since then, it has experienced a massive growth [5]. The principle of potentiometry is based on the measurement of the difference of potential between two electrodes (working (WE) and reference (RE)) in open circuit potential conditions (negligible current flowing through the system). The electrochemical cell is constructed by dipping these two electrodes into the solution to complete the circuit. A typical scheme of an electrochemical cell with potentiometric configuration is shown in Figure 1.2. The reference electrode has a well-stablished electrode potential, which is reached by the use of a redox system (for instance Ag/AgCl in saturated KCl) with constant concentration of the two redox components. The working

electrode potential varies according to the composition of the solution. To name two possible mechanisms studied in our group:

- If a recognition element is incorporated embedded in an electrode membrane, the electrode can be selective for a particular target. In this way if the membrane contains a given ionophore, the electrode will be selective for a certain ion. In this mechanism the potential is explained by the phase-boundary model [6] and the potentiometric response is Nernstian.
- If the electrode is modified by a bioreceptor, then targets can be biomolecules or even microorganisms. In this mechanism the potentiometric response is not Nernstian.

The electric potential (referred in terms of electromotive force (EMF)) is related to the analyte concentration when thermodynamic equilibrium is reached between the free analyte in solution and the analyte present at the recognition element.

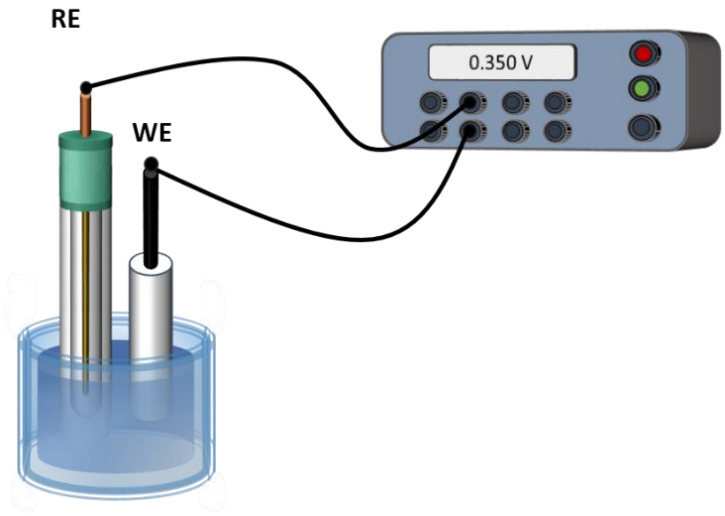

Figure 1.2. Schematic representation of an electrochemical potentiometric measurement.

Potentiometric response

The electrochemical potential is the combination of electrical and chemical potentials. The electric potential is the work required to bring a charge from infinity to a point in the electric field per unit of charge, and the chemical potential of a substance in a mixture is related to the Gibbs free energy of the mixture.

The electrochemical potential is the sum of potential of the working and the reference electrodes. Potentials generated across the circuit are also included at the potential measured sum, such as the phase boundary potential (potential at the interfaces electrode-membrane and membrane-solution) and the diffusion potential inside the membrane.

While the open circuit potential of ion-selective electrodes can be explained by the conventional redox approaches using the Nernst equation, the potentiometric response generated on H_2O_2 determination depends on different factors that makes the response non-Nernstian, and it is driven by the mixed potential mechanism. The concept of mixed potential was firstly introduced by Wagner and Traud back to 1938 [7] and was mainly used in corrosion field, reaction kinetics and catalysis. Electrochemically, the mixed potential theory encompasses the combination of simultaneous reactions occurring in parallel, balancing all the redox reactions and conditions interfering with the electrode potential, as the total potential of the system. Unlike in redox equilibrium, where the overpotential is zero, the mixed potential results in a non-zero overpotential net, due to the need to balance the Faradaic production of different kinetically controlled reactions on the surface of the electrode, generated by the absence of a well-defined redox couple in solution in the electrochemical cell. It has been recently reported by Baez et al. [8] that the mixed potentials are originated by the current exchange of different reactions involving the electrode material, the solvent and other solution components. Indeed, they reported the use of polyelectrolytes as a way to modulate the kinetic factors controlling the response on platinum-based electrodes, under the control of oxygen reduction reaction.

Therefore, and as part of these different reactions occurring simultaneously on the surface of the electrode, the mixed potential mechanism also includes the generation of redox potentials (caused by the energy transfer of an electron from a donor to acceptor species in the electrochemical cell), as well as the Donnan potential (caused when a different charged substance is unable to pass through the membrane due to differential mobility near the semi-permeable membrane, and thus, creates an electrical charge distribution).

All in all, the measured electrochemical potential in a two electrode electrochemical cell is the sum of all the potentials generated, taking into account all the boundaries from the electrode, membrane and solution, and the electroactive behavior of all the compounds in solution.

Amperometric detection

Amperometry is included in the group of voltammetric techniques which are based on the application of a potential that causes an electrochemical oxidation or reduction of an electroactive compound, by means of generating a measurable current. When the applied operating potential is constant, the voltammetric method is called amperometry (for instance, when the potential is cycled, the technique is called cyclic voltammetry, and when the potential is swept linearly in time, linear swept voltammetry).

Amperometry is based on a three-electrode electrochemical cell (Figure 1.3.); using the working and reference electrode (as in potentiometry) plus an auxiliary electrode (also called counter electrode (CE)). The current flowing between the working and the counter electrode is recorded as a function of its potential against the reference electrode.

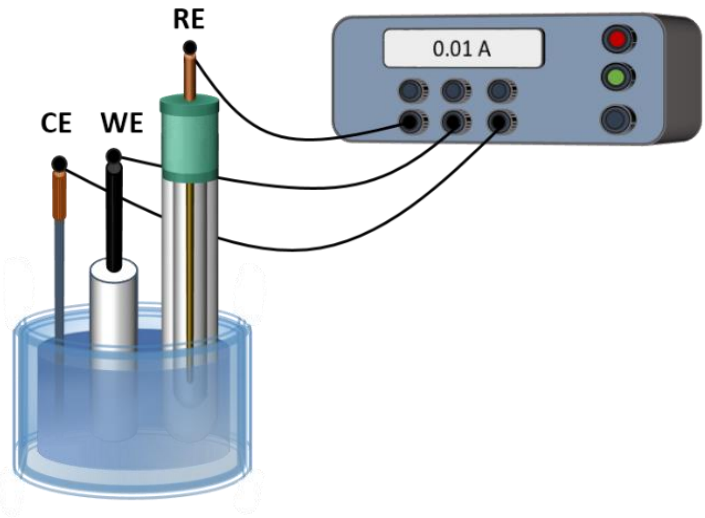

Figure 1.3. Schematic representation of an electrochemicall amperometric measurement.

The limiting current (I_l) measured at the electrochemical reduction of the analyte, T, under hydrodinamically controlled conditions, can be expressed as:

$$I_l = n\,F\,A\,m_T c_T \qquad 1.3.$$

where m_T is the mass transport coefficient, which is flow-rate dependent, c_T is the analyte concentration, n is the number of electrons in the redox reaction, F is the Faraday constant and A is the electrode surface area. If the mass transport coefficient is constant (which can be

maintained constant by ensuring a constant diffusion layer thickness), then the concentration variation of the analyte can be monitored by the current measurement [9].

In amperometry, as the solution is moving, the diffusion layer thickness is constant and thus a steady-state current is monitored, which is independent of time. The recorded current is, then, directly correlated to the bulk concentration of the electroactive species or their production-consumption rate within a contiguous catalytic layer. The generated current is stablished by the equilibrium in the diffusion of the analyte through the matrix onto the electroactive surface that undergoes oxidation or reduction of its species. Thus, the analyte concentration will be proportional to the equilibrium and the generated current.

In the case of amperometric biosensors which involve enzymes to achieve the signal-analyte concentration relationship, the kinetics of the enzyme reactions influences the monitored current [4]. In this thesis, we have used oxidase enzymes, which kinetic steps are:

$$S + E_{ox} \overset{k_{-1}/k_1}{\longleftrightarrow} E_{ox}S \overset{k_2}{\rightarrow} P + E_{red} \qquad 1.4.$$

$$E_{red} + O_2 \overset{k_3}{\rightarrow} E_{ox} + H_2O_2 \qquad 1.5.$$

Where S stands for the substrate (target analyte), E stands for the enzyme and P stands for the product of the reaction. Thus, taking into account that the steady-state current at an amperometric electrode under diffusion control (I_d) is:

$$I_d = \frac{n\,F\,D\,[S]}{d} \qquad 1.6.$$

where d is the diffusion layer thickness, D the diffusion coefficient of the measured species in the layer and [S] the substrate concentration. The rate of an enzyme-catalyzed reaction is given by:

$$\frac{d\,[S]}{dt} = \frac{k_2[E_0]\,[S]}{K_M + [S]} \qquad 1.7.$$

where K_M is the Michaelis-Menten constant and E_0 the total enzyme concentration. The current at an electrode under enzyme kinetic control can approximate to:

$$I_k = \frac{n\,F\,d\,k_2\,[E_0]\,[S]}{K_M + [S]} \qquad 1.8.$$

In order to evaluate and compare the characteristics of each electrochemical technique, Table 1.1. displays some of the features attributed to these different approaches, evidencing their advantages and limitations.

Table 1.1. Comparison of the characteristics of the three electrochemical techniques used in this thesis.

	CONDUCTOMETRY	**POTENTIOMETRY**	**AMPEROMETRY**
SIGNAL READOUT	conductivity or resistance through ions movement in solution	potential difference during redox process	difference on current intensity at an applied voltage
CELL	electrolytic	galvanostatic	electrolytic
SENSITIVITY	high sensitivity	Nernstian sensitivity in ISEs, high sensitivity for molecules	high sensitivity
LOD	low (ppb)	Moderate (µM-mM)	extremely low (nM- µM)
ROBUSTNESS	high	high	high
POWER CONSUMPTION	low	low	higher
COST	interdigitated electrodes use to be expensive due to the required techniques (e.g. nanolithography)	the advances in miniaturization procedures for massive production and low-cost substrates has decrease the final cost for both techniques	
INSTRUMENTATION SIMPLICITY	uses only one WE	uses WE and RE	uses WE, RE and CE

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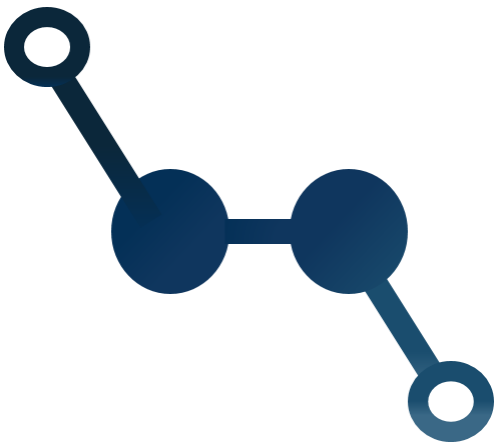
PART I:

DIRECT H_2O_2 DETERMINATION THROUGH CONDUCTING POLYMERS



UNIVERSITAT ROVIRA I VIRGILI
DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HYDROGEN PEROXIDE DETERMINATION
Marta Borràs Brull

CHAPTER 2

THE USE OF CONDUCTING POLYMERS FOR ENHANCED ELECTROCHEMICAL DETERMINATION OF H_2O_2

UNIVERSITAT ROVIRA I VIRGILI
DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HYDROGEN PEROXIDE DETERMINATION
Marta Borràs Brull

The role of hydrogen peroxide in a wide range of biological processes has led to a steady increase in research into hydrogen peroxide determination in recent years, and conducting polymers have attracted much interest in electrochemistry as promising materials in this area. We present an overview of electrochemical devices for hydrogen peroxide determination using conducting polymers, either as a target or as a byproduct of redox reactions. We describe different combinations of electrode modifications through the incorporation of conducting polymers as the main component along with other materials or nanomaterials. We critically compare the analytical performances cited and highlight some of the future challenges for the feasible application of such devices.

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INTRODUCTION

Hydrogen peroxide (H_2O_2) is a key marker in biological processes because it is involved in signaling paths such as cellular growth, senescence [1] and apoptosis [2], and can be generated by means of different stimuli [3]. It may be related to some neurological disorders such as Parkinson's, rheumatoid arthritis and Alzheimer's together with other reactive oxygen species [4]. In addition, it plays a crucial role in many other sectors including chemical, pharmaceutical and food manufacturing [5]. It is also important in wastewater treatment and wood and paper bleaching [6]. Moreover, it is a byproduct of many biochemical reactions involving oxidase enzymes such as glucose, cholesterol and lactate oxidase, among many others [7]; therefore, detecting H_2O_2 can indirectly detect those biomolecules in different fluids. For these reasons, and due to their wide range of applications, the development of H_2O_2 sensors has recently been the focus of extensive research.

Many different techniques have been developed to determine H_2O_2 including fluorimetry [8,9], chemiluminescence [10,11], chromatography [12,13] and spectrometry [14]. Nevertheless, the implementation of some of these techniques requires complex or expensive instrumentation as well as qualified personnel. Of all the techniques, electrochemical approaches measure H_2O_2 relatively simply and with better sensing parameters, including high sensitivity and fast response time [15]. The ease of miniaturization [16,17] and the broad range of electrode modification

possibilities make electrochemical devices suitable for *in situ* H_2O_2 determination. In spite of all the advantages, electrochemical devices have limitations, such as inefficient electron transfer from the recognition element to the substrate, only moderate selectivity with some real samples and lack of measurement reproducibility. The body of research into electrode modification has been continuously expanding in recent years in an effort to overcome these drawbacks while still encompassing essential electrode requirements: conductivity, chemical stability and appropriate surface area and properties.

Conducting polymers (CPs) are interesting candidates as sensing and transducer materials due to their electrical properties, and offer an alternative to metallic and inorganic semiconductors [18]. Conducting polymers are organic materials capable of conducting electricity along their backbones due to the conjugated π-electron or C=C conjugated bonds. Conducting polymers have been extensively studied in the thirty years since they were first discovered by Hegger, McDiarmid and Shikarawa [19]. Nevertheless, and in spite of reaching high conductivity values, early conducting polymers were unstable in air and were considered difficult to prepare [20]. Further research on the polymerization of polyanilines, polypyrroles and polythiophenes improved the preparation methods and led to a drastic increase in conductivity and stability under electrical and thermal conditions. The structures of the most commonly used conducting polymers in sensing applications, polyaniline (PANI), polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT), are shown in Figure 2.1.

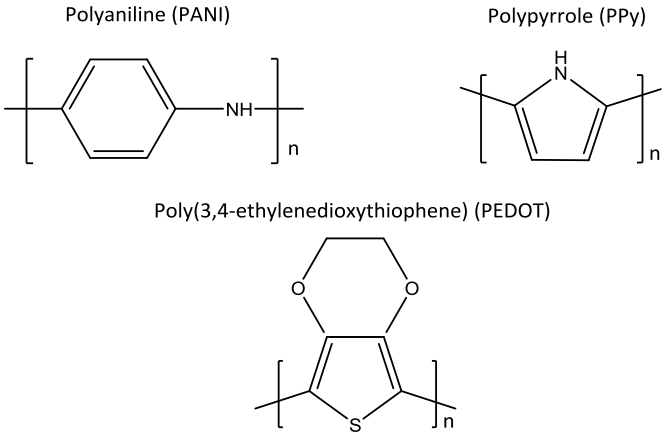


Figure 2.1. Structures of polyaniline, polypyrrole and poly(3, 4-ethylenedioxythiophene).

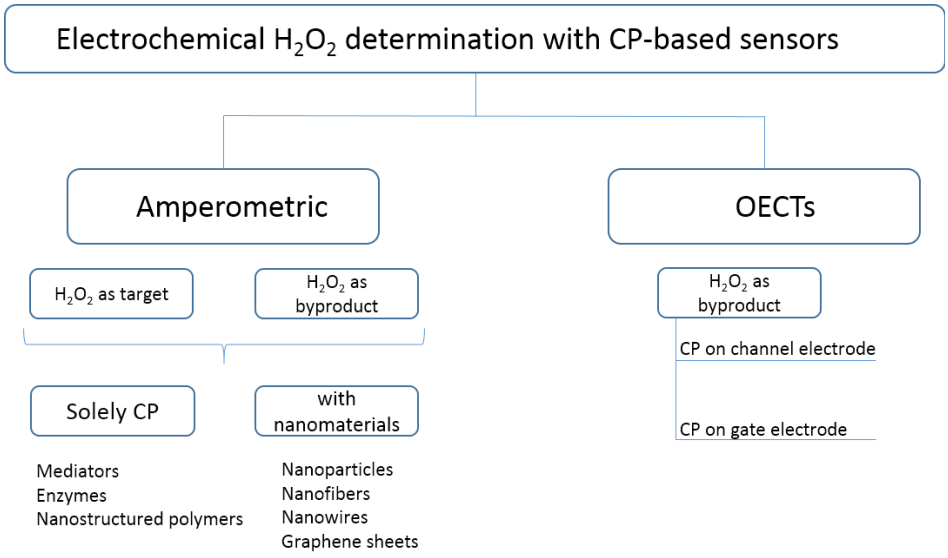
CPs offer unusual electronic properties such as low ionization potential and high electron affinity, and they have been successfully used as sensing elements, immobilization matrices and

even as semi-permeable membranes for electrodes [21]. The ability to change their electrical conductivity by several orders of magnitude and their electron affinity depending on their redox state make CPs especially attractive for enhancing sensing performance [22]. Many factors can influence the properties of conducting polymer film, such as the deposition method, the solution pH and temperature, the dopant and the extent of doping material used and the surface conditions of the electrode [20]. Controlling the polymerization method and how the polymer is deposited on the electrode surface are key factors in tailoring CP properties. Polymerization can be either chemical or electrochemical, and the thickness and conductivity of the film can vary depending on the technique used (e.g. potentiostatic or galvanostatic [23]). The deposition method defines the adhesion of the conducting polymer to the electrode surface, which is mainly established by weak physical interactions. Roughening the electrode surface and covalently attaching the polymer to the substrates are the most frequently used ways to improve adhesion and avoid delamination or cracking of the coating film when the electrodes are exposed to wet conditions or mechanical stress [24]. Doping is based on the insertion of molecules that modify the electronic structure via the formation of local excitations in the way of polarons and bipolarons as delocalized charge carriers that allow the electrons to move along the polymer backbone [25,26]. Depending on the nature of the dopant, the polymer can undergo oxidation (*p*-doping) and have a positive charge, or reduction (*n*-doping) and have a negative charge. The size of the dopant is also variable. The doping process not only modulates the final conductivity of the film, but also its bulk properties such as density, volume, porosity, solubility and color, and its ultimate electrochemical stability. In addition, the doping process is reversible and the polymer can switch from the insulator to the conducting redox state by means of the incorporation or release of the dopant by applying potential to the polymer [27]. The ability to tailor the characteristics of conducting polymers expands their range of application to include, for instance, field-effect transistors, supercapacitors, solar cells and biosensors [18]. However, the intrinsic limitations of conducting polymers can hinder their application in these fields. The over-oxidation mechanism of conducting polymer is not yet fully understood and it is a considerable barrier for advancing polymer-based devices towards real-world applications: It irreversibly changes the structure of the polymer by reducing the conjugation lengths and inducing chain scission, leading to a decrease in or loss of conductivity. Over-oxidation is influenced by pH, and the properties of conducting polymers are also affected and modulated by pH, temperature, humidity and sensitivity to O_2 and CO_2. To

overcome some of these inherent limitations, conducting polymers have been hybridized with other suitable materials.

Nanostructured and carbon-based materials such as metal nanoparticles, nanowires, carbon nanotubes and graphene sheets are very attractive components for use in sensor development. Their surface can be easily modified and they have a large surface-area-to-volume ratio, which enhances the transduction mechanism, thereby providing higher sensitivity towards the target analytes. The insertion of graphene or carbon nanotubes into the structure of conducting polymers by covalent functionalization and π-π or electrostatic interactions improves the order of the CP backbone and the delocalization of the charge carriers, leading to a higher conductivity [28]. Although the applications of hybrid composites combining nanomaterials and CPs have recently been reviewed for sensing and, especially, biosensing purposes [22,29,30], we focus on the use of CPs for the development of electrochemical sensors for the detection of H_2O_2.

This review includes reports from the last five years and highlights those which have addressed the analytical performance for H_2O_2 determination in real samples such as body fluids or beverages, among others. We have divided the review into two sections based on the electrochemical techniques used to determine H_2O_2: amperometry and organic electrochemical transistors (OECTs), as depicted in scheme 2.1. .



Scheme 2.1. The main electrochemical detection of H_2O_2 using CPs as a component of the device.

DETERMINATION OF HYDROGEN PEROXIDE

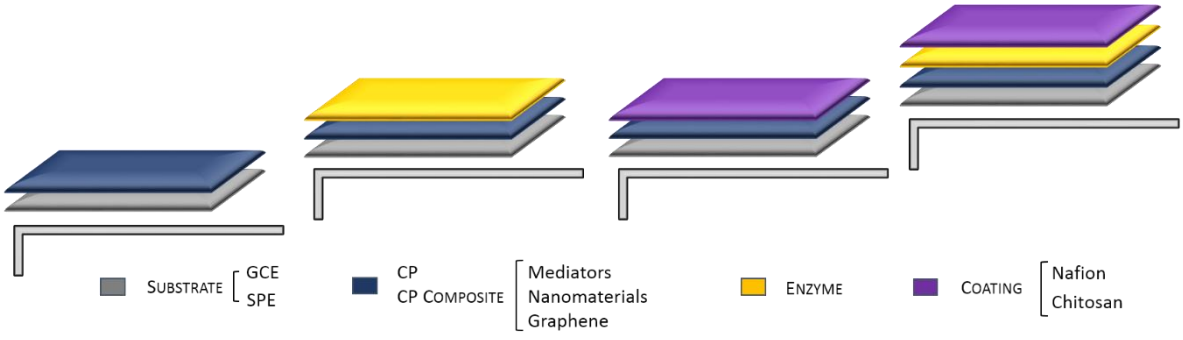
Amperometric H_2O_2 determination

In general, H_2O_2 can undergo electrochemical oxidation and reduction (*equations 2.1.* and *2.2.*) depending on a variety of factors, from the type of substrate to the experimental conditions [31]. Electrochemical H_2O_2 sensors turn chemical information into electrical information, enabling the specific quantification of the target analyte within a complex matrix.

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad 2.1.$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \qquad 2.2.$$

Most of the electrochemical sensors developed to detect H_2O_2 are voltammetric sensors, which take advantage of the redox reaction that occurs upon the application of a time-dependent potential to the working electrode (changing its potential relative to the fixed potential of the reference electrode), which produces a measurable current that flows between the working and auxiliary electrodes. Among the voltammetric techniques, amperometry, in which a constant potential is applied to the working electrode and the current is measured as a function of time, uses Faraday's law to calculate the amount of analyte, making this method the most commonly used tool for detecting H_2O_2 with high sensitivity and with low limits of detection. The following Scheme 2.2. shows the most frequently used combinations of sensor elements within the developed amperometric sensors, from the simplest, with only two components, to the most elaborate configuration combining up to four elements:



Scheme 2.2. The main combinations of components of amperometric sensors: substrate, CP-composite, enzyme and coating layer.

In this review, most of the sensors mentioned mainly use glassy carbon electrodes (GCE) and screen-printed electrodes (SPE) as their electrode substrates, while the CPs are basically those shown in Figure 2.1. . We have classified the sensors based on their H_2O_2 detection strategy: direct detection as the main target, or indirect detection as a byproduct of a redox reaction. We can also differentiate the sensors by the additional components they incorporate in their membranes to improve selectivity, and other analytical parameters such as polymeric coatings (e.g. Nafion), nanomaterials like nanoparticles or nanofibers, and enzymes, among others.

Direct amperometric H_2O_2 determination

Among the amperometric sensors that solely use CPs for the detection of H_2O_2, Agrisuelas et al. [32] have recently achieved direct H_2O_2 detection in commercial hair lighteners and antiseptics with recoveries of between 98.9 and 100.8% by electropolymerizing poly(azureA) on disposable screen-printed carbon electrodes (SPCEs), together with sodium dodecyl sulfate. The amperometric response to H_2O_2 was measured at 0.5 V with sensitivities of 72.4 nA μM^{-1} cm^{-2} in a concentration range from 5 µM to 3 mM. Ethanol, sodium citrate, glucose, caffeine and L-dehydroascorbic acid were tested as interfering species in order to assess the potential applicability of the sensor in real samples. None of these substances interfered with the H_2O_2 response.

Direct H_2O_2 determination can also be achieved using catalyzers or cofactors such as cytochrome C, hemoglobin and peroxidase enzymes. The most frequently used enzyme to decompose H_2O_2 is horseradish peroxidase (HRP) which can be easily isolated from plant radish, *Escherichia coli* or yeast, and is classified as oxidoreductase (1.11.1.7). HRP catalyzes the oxidation of a substrate using H_2O_2 as the oxidizing agent, allowing for direct electron transfer through the electrode. In these cases, the presence of the conducting polymer improves the performance of the sensor due to its intrinsic conductivity and its mechanical function as a matrix for the immobilization of the enzyme. For example, very low detection limits, 0.03 nM, were achieved by Zhang et al. [33] by preparing a hybrid composite via electrostatic interactions with PEDOT:PSS (poly (styrenesulfonate)) and chitosan micelles on top of a GCE surface. HRP was entrapped between the conducting polymer and a layer of Nafion by the drop-casting technique, and exhibited an excellent electrocatalytic activity towards H_2O_2, while the electron transfer was enhanced by the presence of the conducting polymer composite. Figure 2.2. shows the differential

normal pulse voltammograms and calibration curves, with a linear range of between 0.1 nM and 0.01 mM. The wide detection range was attributed to the large surface area of the hybrid film due to the ability to immobilize a large amount of enzyme. In addition, this large surface area facilitated the signal transduction from the enzyme to the electrode. The sensor was tested in real samples, testing the applicability to detect H_2O_2 in a commercial disinfectant and it was validated via the potassium permanganate titration method with satisfactory results and a relative standard deviation (RSD) from 3.1% to 4.8%.

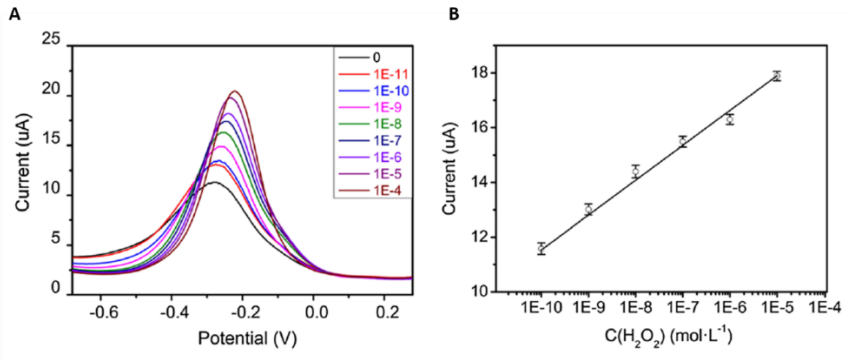


Figure 2.2. A) Differential normal pulse voltammograms for Nafion/HRP/PEDOT:PSS/CS micelle/GCE in 0.1 M PBS (pH 7) with different concentrations of H_2O_2. **B)** Calibration curves corresponding to the response recorded on the Nafion/HRP/PEDOT:PSS/CS micelle/GCE biosensor versus the concentration of H_2O_2. Reproduced with permission from ref. [33].

One-dimensional ordered conducting polymers (especially CP nanowires) have also been successfully used on H_2O_2 sensors. The use of these one-dimensional ordered conducting polymers enhances, for example, the electron transfer through the electrode, not only because of the intrinsic conductivity of the conducting polymer, but also because of the increased surface area, thus obtaining more effective modified electrodes. Rizarullah et al. [34] demonstrated this effect by characterizing carbon paste electrodes with and without PANI nanofibers. The final performance of these modified electrodes (HRP/glutaraldehyde/PANI nanofibers/carbon paste electrodes) resulted in a linear range between 0.1 and 0.3 mM and a sensitivity of 12104 nA μM^{-1} cm^{-2}. Nevertheless, the optimal conditions for these electrodes were pH 7 (in phosphate buffer saline (PBS)) and a working temperature of 50 °C. Although this temperature does not denature the immobilized enzyme, it can represent a substantial drawback when measuring real samples.

As mentioned above, nanomaterials have attracted much attention in the exploration of surface modifications due to the different physical properties in nanoscale compared to their respective properties in bulk. The incorporation of different nanostructures into a system that already contains CP films generally improves the interaction and the electrocatalytic activity with the analyte in a synergistic way, which leads to amplified output.

For example, the addition of Prussian Blue (PB) NPs on PEDOT [35] resulted in a modified GCE able to detect H_2O_2 with a limit of detection of 0.16 µM, within a linear range of 0.5 to 839 µM and a time response within 5 s. No interference was observed due to the presence of dopamine (DA), ascorbic acid (AA), uric acid (UA) or lactose, and real milk samples were evaluated by the standard addition method, with recoveries between 98% and 102.7% and RSD between 2.4% and 3.8%, demonstrating the viability of the sensor for use in real sample analyses.

Mercante et al. [36] achieved considerably high sensitivity (677 nA μM^{-1} cm^{-2}) using a ternary-graphene-based composite with reduced-graphene oxide (rGO), PEDOT:PSS and gold nanoparticles (AuNPs). HRP was immobilized on a screen-printed gold electrode by drop-casting and with the addition of a cross-linker to enhance the electron transfer between the *heme* group of HRP and the electrode surface. The electrocatalytic reduction of H_2O_2 was evaluated at -0.4 V and the obtained linear range, from 0.5 to 400 µM, was suitable for determining H_2O_2 in real tap water samples and bovine milk. Actually, the use of hydrogen peroxide as a pre-oxidant in municipal water treatment is well documented and has been practiced for over 20 years, and its use for the activation of the lactoperoxidase system in the preservation of milk has proven effective against both gram-positive and gram-negative bacteria [37,38]. Different electrode configurations were characterized to prove the synergistic effect between PEDOT:PSS and AuNPs, as well as the reduction in peak separation, which led to faster electron transfer with the whole electrode modification. However, all electrochemical measurements were performed under N_2-saturated solutions in order to avoid other reduced interferences. Thus, their real-world application in field measurements, for example, may be limited.

Kumat et al. [39] used a polymer nanocomposite to obtain synergistic effects from the mixture of polymer, nanoparticles and graphene, and achieved a composite with superior conducting properties. Silver nanoparticles (AgNPs) were incorporated into the previously synthesized PANI-rGO system by means of a self-assembly method. The final electrode was assembled onto a glassy carbon surface by drop-casting. Nafion solution was also deposited to

enhance the adherence of the complex. The quantitative determination of H_2O_2 was performed amperometrically at -0.4 V in phosphate buffer. Results showed a linear relationship in the range of 0.01 and 1000 µM with a limit of detection (LOD) of 50 nM and a sensitivity of 14.7 µA mM^{-1} cm^{-2}. The authors demonstrated the synergistic effect of the system, with PANI being responsible for the electron transfer during the catalytic reaction occurring between H_2O_2 and AgNPs. The electrode modified with the conducting polymer composite exhibited a greater electroactive surface area (resistance of charge transfer, R_{ct}=9 Ω with PANI versus R_{ct}=27.5 Ω without PANI) and facilitated the electron transport through the electrode. However, and in spite of having checked for interference species, similarly to the previous example of Mercante et al. [36], amperometric measurements were also done under N_2, which may represent a drawback for electrode implementation in terms of time and cost effectiveness.

Systems incorporating one-dimensional ordered conducting polymers and nanostructured materials exhibit better characteristics in terms of the individual performance of these components, demonstrating their synergistic effects. For instance, Yang et al. [40] presented a 3D-macroporous PEDOT prepared by electrodeposition on a GCE surface where PB NPs were incorporated into the system by immersing the electrode in an electrolyte solution containing PB, generating the spontaneous growth of NPs on top of the polymer film. In this case, the use of the conducting polymer had two functions: first, it reduced the iron to initiate PB NP growth; and second, it provided a larger surface area on which to capture more NPs. Chronoamperometric and CV experiments showed improved H_2O_2 catalysis. Amperometric measurements were taken under 0.11 V at pH 3 in a 0.1 KCl solution, yielding a linear range from 0.17 µM to 0.257 mM with an LOD of 80 nM for 3D-PEDOT-PB/GCE compared to the two linear ranges obtained from PB/GCE (from 12 µM to 0.57 mM and from 0.57 mM to 1.57 mM) with an LOD of 3 µM obtained based on the first linear range**.** Figure 2.3. shows the synergy between the components of the conducting polymer-modified electrode, which clearly shows an enhancement of the analytical parameters.

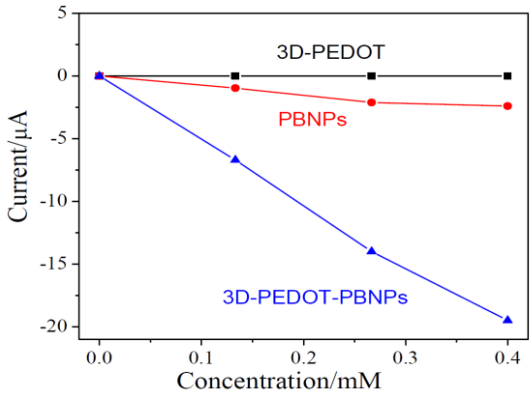


Figure 2.3. Peak current responses for 0, 0.13, 0.26 and 0.4 mM H_2O_2 on the indicated modified electrodes. Adapted from ref. [40].

Additionally, interference studies have also been conducted using UA, DA, glucose, highly concentrated sodium chloride (NaCl) and AA with negligible responses which did not interfere with H_2O_2 determination. 95% of the signal was also retained after a month, suggesting the potential of this hybrid composite for use in bioelectrochemical sensors and devices.

Baghayeri et al. [41] used a PPy nanocomposite made up of poly (styrene-alt-maleic anhydride) (PMSA) grafted with 4-aminobenzenesulfonate (4ABS) (PMSA-g-4ABS) with functional groups to improve the interaction with the redox protein hemoglobin (Hb). Hb has one electroactive iron *heme* group that allows for direct electron transfer from the protein to the electrode (GCE). The conducting polymer nanocomposite-based electrode demonstrated a fast response towards H_2O_2 detection of 4 s, a linear range of 0.8 to 460 µM and an LOD of 0.32 µM. With these characteristics, the sensor applicability was evaluated by detecting H_2O_2 in rainwater by means of the standard addition method, and human serum samples using 10-fold dilution and spiking known H_2O_2 concentrations. The overall recoveries of the sensor were between 97.8 and 103.5% [41].

Amperometric H_2O_2 detection as a byproduct of redox reactions

Apart from developing sensors to directly detect H_2O_2, sensors for the detection of H_2O_2 as a byproduct of the oxidation reaction are an essential component in the development of biosensors. The primary group of these biomolecules is made up of molecules such as glucose, cholesterol and xanthine, which undergo oxidation by an oxidase enzyme and produce H_2O_2 as a detectable byproduct. An electron cascade is generated from the oxidation of the main component to the final reduction of H_2O_2, which is detected by the electrode. Of all the electrochemical sensors detecting H_2O_2 as a byproduct, glucose sensors are undoubtedly the most frequently used and studied because of the ease of operation and the efficiency of their specific enzyme (glucose oxidase, GOx) and the global market coming from the diabetic population. General equations of the electrochemical glucose determination are given below:

$$glucose + O_2 \xrightarrow{Glucose\ Oxidase} gluconic\ acid + H_2O_2 \qquad 2.3.$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \qquad 2.4.$$

Kausaite-Minkstimiene et al. [42] demonstrated glucose detection in 10-fold diluted human serum samples using solely CP-based sensors. They described an environmentally friendly synthesis of poly (pyrrole-2-carboxylic acid) particles (PCPy) by means of chemical oxidative polymerization using H_2O_2 as the initiator of the polymerization. They took advantage of the carboxylic groups from the particles to attach the GOx, forming a covalent link between the conducting polymer and the enzyme. Thus, they achieved measurement recoveries in real samples of between 99.1 and 106%, with a linear range from 0.1 to 15 mM and an LOD of 39 µM.

Functionalization of the conducting polymer was also reported by Tekbaşoğlu et al. [43], who described direct glucose detection in commercial beverages (cola and juice samples) with recoveries of between 96.8% and 99.6% with 87% of the initial response retained after eight weeks. Their graphite electrode modification consisted of the oxidative polymerization of EDOT and bis(2-pyrdylimino) isoindolato-palladium complex, which had been shown to mimic catalytic enzymes which decompose H_2O_2 into O_2 and H_2O. Further immobilization of GOx was achieved by cross-linking the enzyme with the free amino functional groups of the polymeric composite. Despite attaining good analytical performance, real-world applications of this sensor for glucose detection may be diminished due to the narrow linear range capable of measuring glucose, which ranges from 0.25 to 2.5 mM.

Glucose content in diluted grape juice and honey was also determined by electrochemical synthesized PEDOT-modified electrodes with polyacrylic acid and poly(4-lithium styrenesulfonic acid) [44]. The amperometric glucose measurements were validated with a reference enzymatic method, and the sensor presented a linear range from 0.96 to 30 mM, with an LOD of 0.29 mM and proved stable for 30 days. Other recent examples involving the polymerization of the conducting polymer together with other electron mediator dopants are described, for instance, by Vagin et al. [45] and Wannapob et al. [46]. However, apart from testing some interference species such as AA and UA, none of them have reported glucose determination in real samples.

Hybrid composites using nanomaterials are also used to quantify glucose through H_2O_2 detection. Screen-printed carbon electrodes were modified with PEDOT microspheres and platinum nanoparticles (PtNPs) by Liu et al. [47]. Polymeric microspheres were obtained using calcium carbonate template-assisted polymerization by first preparing the $CaCO_3$ template particles, and then applying mild and advanced chemical oxidation. The colloid suspension was mixed with K_2PtCl_4 to obtain PtNPs on the surface of the conducting polymer microspheres. In this case, PtNPs worked as a catalyst for H_2O_2 oxidation, PEDOT-microspheres functioned as high-surface area support for the deposition of the NPs, while both together facilitated the fast electron transfer during the process. Absorption of GOx was achieved by incubation for 12h on an appropriate solution. The whole mixture was drop-casted onto the surface of screen-printed carbon electrodes followed by the drop-casting of a Nafion layer in order to protect the enzyme-hybrid composite. Amperometric measurements were taken at 0.6 V in a 10 mM PBS (pH 7.4) solution. Notably good results were obtained in terms of both linear range (from 0.1 to 10 mM, which includes main glucose blood levels in diabetic patients) and sensitivity (116.25 nA μM^{-1}), which is attributable to the morphology of the sensing layer, where a large inter-particle space helped the diffusion of the substances in between. Moreover, UA and AA were tested as interfering species at their corresponding concentrations in human serum with no interference in H_2O_2 measurements. Despite the good performance of this type of enzymatic sensor combining biocomponents, inorganic and organic materials (enzymes, metal NPs and conducting polymer), the authors did not present the analysis of glucose in any biological fluid, although they envisioned developing 'advanced functional bio-conductive inks' for future successful approaches to biosensor manufacturing [47].

Another hybrid composite using metal nanoparticles was characterized by Gokoglan et al. [48] using a distinct conducting polymer (poly(9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl) end capped with 2,5-diphenyl-1,2,4-oxadiazole (PFLO)). Glutaraldehyde cross-linking agent was used to immobilize the GOx-AuNPs solution on the polymer surface. The final graphene/PFLO/AuNPs-GOx electrode showed a sensitivity of 7.35 nA/μM^{-1} cm^{-2}, within a linear range of 0.1 to 1.5 mM and an LOD of 81 µM. Different mono- and disaccharides, such as fructose, galactose and mannose, as well as AA and urea, were tested as interference species with no significant response. Commercial lemon soda and milk glucose content were evaluated and compared to the glucose concentrations reported on the product label with relative errors below 3.6%, pointing to the real-world applicability of such a sensor. Moreover, the authors claim that this constitutes the development of a portable and cheap biosensor due to the use of graphene-paper as substrate. Although substrate materials and portable devices are beyond the scope of this review, it is worth highlighting the importance of developing simple, robust, portable and cheap devices for glucose detection for the point-of-care market [49,50].

More complex hybrid composites include the incorporation of ionic liquid into the system. Ionic liquids are characterized by their wide potential window, high ionic conductivity and electrochemical stability and high biocompatibility, which makes them suitable for use in biosensing. For instance, brominated 1-decyl-3-methyl imidazole ([Denim]Br) was used by Zhu et al. [51] in combination with a PANI-TNT composite (titanium oxide nanotubes) for an effective glucose amperometric determination with 177.16 nA μM^{-1} cm^{-2} sensitivity and a linear range from 0.01 to 2.5 mM. In spite of the insignificant response of the examined interference species (UA, AA and acetaminophen), experiments with real samples were not reported. Therefore, the real-world applicability of this hybrid composite has not been satisfactorily demonstrated. Conversely, Zhou et al. [52] prepared a hybrid composite with ionic liquid for the accurate determination of glucose levels in pre-treated and diluted animal serum and human urine and serum with recoveries of between 96.8 and 101.2%. The composite was made up of PEDOT, multi-wall carbon nanotubes functionalized with carboxyl groups (MWCNT-COOH) and the ionic liquid $BminPF_6$, which was obtained by on-step potentiostatical polymerization on GCE. GOx immobilization was done by COOH activation leading to covalent bonds between the enzyme and the polymer composite. After pH, temperature and working potential optimization, the electrodes showed a linear range of 0.6 µM to 2 mM with an LOD of 0.015 µM, with reproducibility RSD of 0.73% and repeatability

RSD of 1.01%. The electrodes maintained 98.3% of their response capability after 30 days within an RSD of 0.54%.

A comparative study of the analytical parameters reported in the articles reviewed that described amperometric sensors tested in real samples is listed in Table 2.1. Good performance was obtained in these reports particularly concerning stability, reproducibility and limits of detection. Most of them have tested the hydrogen peroxide/glucose conducting polymer-based amperometric sensor against other electroactive species that could interfere in sensor selectivity and sensitivity, such as ascorbic acid, glucose and uric acid, among others, which are mainly present in human body fluid and food samples. However, only a few of them really focused their work on testing the electrodes in real relevant samples, such as hair lighteners, disinfectants and milk, in which peroxide is used as a preservative and sterilizing additive [53,54], or in beverages and human body fluids, where glucose is present. The studies show characterization information to demonstrate the superiority of conducting polymers in terms of sensing parameters. Indeed, they demonstrate an increase in peak currents and a decrease in peak separation during cyclic voltammetries in different electrode configurations in order to highlight the role of the CPs, which finally accelerate the electron transfer between the analyte or mediators and the electrode surface. This information is also consistent with most of the electrochemical impedance spectroscopy spectra done to characterize the electrode surfaces as well. In general, the measured resistances are much lower when a CP is part of the system, indicating the higher conductivity achieved was due to the CP, which is related to the larger active surface area obtained when working with conducting polymers, and even larger when dealing with nanostructured conducting polymers. Nevertheless, only one of the mentioned articles [40] demonstrates and characterizes the contribution of the CP to the final analytical parameters of the sensor, such as sensitivity, linear range and limit of detection: noticeably, the LOD improved by two orders of magnitude to the respective control-modified electrode. Although characterization steps are crucial in electrode configuration, and in all examples, the use of CPs demonstrated better characteristics than in their respective controls, the quantification of the enhancement of H_2O_2 determination performance due to the incorporation of CPs still needs to be addressed.

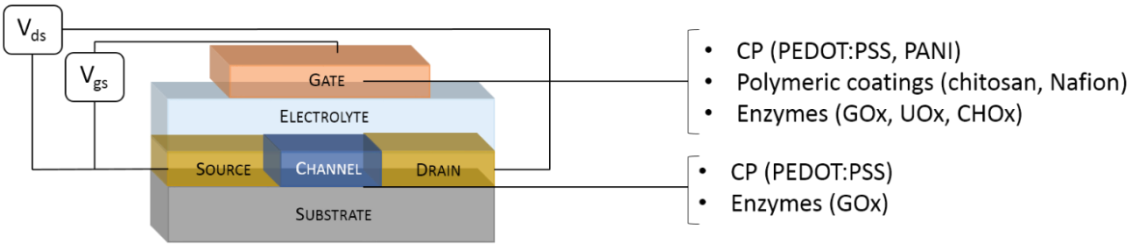
Table 2.1. Comparison of analytical performance of some H_2O_2 conducting polymer-based amperometric sensors used in real samples

WORKING ELECTRODE	LINEAR RANGE (mM)	SENSITIVITY (nA μM^{-1} cm^{-2})	LIMIT OF DETECTION (µM)	SAMPLE VALIDATION	ACCURACY	REF.
H_2O_2 as target						
SPCE/PAA(DS)	0.005 – 3	72.4	1.43	commercial hair lightener and antiseptic. R= 98-100 %	reproducibility RSD=6.2 %, repeatability RSD=3.4 %	[32]
GCE/Cs micelle/PEDOT:PSS/HRP/Nafion	0.0000001 – 0.01		0.00003	commercial disinfector diluted 10,000,000-fold	70 % signal after 4 weeks reproducibility RSD=3 %	[33]
GCE/PEDOT/PBNPs	0.0005 – 0.839		0.16	milk R=98-102.7 %	90.8 % signal after 4 weeks reproducibility RSD=4.5 %	[35]
SPGE/PEDOT:PSS/ rGO/AuNPs/HRP	0.0005 – 0.4	677	0.08	tap water and bovine milk R=99 %	94 % signal after 1 week reproducibility R=7.8 %	[36]
GCE/Ppy-PMSA-g-4ABS	0.0008 – 0.46		0.32	rainwater and diluted human serum R=97.8-103.5 %	92 % signal after 4 weeks repeatability RSD=3.2 % reproducibility RSD=4.34 %	[41]
H_2O_2 as byproduct						
Graphite/GA/PCPy-Gox	0.1 – 15		39	human serum 10-fold dilution R=99.17-106 %	95.3 % signal after 4 weeks reproducibility RSD=5.21 %, repeatability RSD=1.83 %	[42]
ITO/(PEDOT-PdBI-co-HKCN)/Gox	0.25 – 2.5		176	coke and juice R=96-99 %	87 % signal after 8 weeks	[43]
Pt/PEDOT/PAA/Gox	0.96 – 30		290	diluted grape juice and honey R=5 %	30 days stability	[44]
Graphene/PFLO/AuNPs-Gox	0.1 – 1.5	7.357	81	commercial lemon soda and milk relative errors 2.1 and 3.6	repeatability RSD=3.35 %	[48]
GCE/ILs/PEDOTM-MWCNT-COOH/GOx	0.00006 - 2	0.0895	0.015	diluted animal serum and human serum and urine R=96.8-101.2 %	98.3 % signal after 4 weeks reproducibility RSD=0.73 % repeatability RSD=1.01 %	[52]

(R = recovery; RSD=relative standard deviation).

Organic electrochemical transistors (OECTs)

Since OECTs based on polypyrrole were first reported by Wrighton et al. [55], organic electrochemical transistors have attracted much attention due to the low working voltages required and their stability to operate in aqueous media. The working principle of OECTs is based on a three-electrode system where the current flowing along the organic semiconductor connecting two of these electrodes (source and drain) changes as a function of the polarization of the third electrode (gate). Therefore, the potential is applied on the gate electrode in contact with the electrolyte and it modulates the ion motion in solution through the organic semiconductive channel, which leads to a change in the conductivity of the channel. Scheme 2.3. shows a schematic representation of an OECT electrode configuration and its possible functionalizations.



Scheme 2.3. A schematic of an OECT configuration.

The most often used configuration, as reported later in this review, consists of using the conducting polymer as the channel component, connecting the source and drain electrodes. Therefore, the conducting polymers act as the sensing and transducer material, since it changes its electrical properties as a function of the analyte concentration. Moreover, conducting polymers can also be placed on the gate electrode, either acting as the transducer or as an immobilization matrix where other biomolecules, such as enzymes or polymeric coatings, will be incorporated on the same gate electrode. Different configurations are also reported in this review, considering, for example, the incorporation of the recognition site in the conducting polymer along the channel between source and drain, or for instance, the manufacturing of the three electrodes of the OECT with the same conducting polymer.

PEDOT:PSS has been successfully used as the active layer bridging source and drain electrodes, and is the most used conducting polymer in OECTs due to its high conductivity, broad pH range of operation and electrochemical stability attributable to the bridging of the dioxyethylene group across the 3 and 4- positions of the heteroring. PEDOT:PSS goes from the

conductive (oxidized) $PEDOT^+$ state to the semiconducting (neutral) $PEDOT^0$ state due to the doping or de-doping caused by the oxidation/reduction process:

$$PEDOT^+ : PSS^- + M^+ + e^- \leftrightarrow PEDOT^0 + M^+ : PSS^- \qquad 2.5.$$

where M^+ is a cation in the electrolyte medium and e^- an electron from the source electrode. The migration of cations into the polymer causes compensation with the sulfonate groups of PSS, thus changing the electronic structure of the polymer, leading to a decrease in the current along the conductive channel.

Therefore, glucose sensors have been developed using OECTs based on the fact that the oxidation of glucose oxidase, and consequently, the production of H_2O_2, modifies the gate electrode voltage, which, in accordance with the working principles of OECTs, ultimately modifies the current along the channel as a function of glucose concentration. The working voltage on the gate is chosen depending on the gate modification materials as well as the change in conductivity it produces on the channel; the greater the difference in current before and after applying the corresponding voltage, the more sensitive the channel is.

The earliest CP-based transistors for glucose detection through GOx incorporation were based on the sensitivity of the polymers to pH changes, such as PANI [56] and the change in the redox state of the conducting polymer due to the oxidase reaction [57]. H_2O_2 involvement in such transistors was first demonstrated by Malliaras et al. [58,59] who used PEDOT:PSS as a sensing layer and Pt wire as gate electrodes where the H_2O_2 produced was oxidized. The mechanism behind the detection was suggested to be due to the reduction of the polymer after the H_2O_2 oxidation at the gate to maintain the charge balance, or due to the redistribution of the potential at the solution/conducting polymer interface after the H_2O_2 production. A better understanding of the sensing mechanism of those simple glucose sensors was reported afterwards [60], attributing it to the Faradaic current at the gate electrode generated by H_2O_2 production. Since then, different configurations and functionalizations of the channel and gate electrodes have been studied to improve sensing characteristics. Even transistors in which the channel, source, drain and gate electrodes were made from conducting polymer [61,62] were able to detect glucose down to micromolar concentrations. More sophisticated systems have emerged from the incorporation of nanomaterials or ionic liquid on gate electrode functionalization, which has greatly improved the sensitivity of such devices and has broadened the ranges of detection of different analytes determining the course for OECT implementation.

For example, Liao et al. [63] prepared gate electrodes by anodization of a Ti wire forming TiO_2 nanotube arrays (TiNTAs). PtNPs were then electrodeposited on the surface. The electrode was immersed in a GOx solution and then covered by a layer of Nafion. NP and enzyme loading was higher than in a conventional Pt gate electrode due to the porosity of the TiNTAs. PEDOT:PSS was used to bridge the source and drain electrodes. Figure 2.4. shows the current response of the OECT device to successive additions of a) H_2O_2 and b) glucose. The insets show the normalized current responses (NCR, which represents the drain current before and after the addition of H_2O_2/glucose at the concentration of interest) as a function of their respective concentrations. A linear range was obtained from 1 to 500 µM of H_2O_2 with a detection limit of 1 µM. The same trend was observed when GOx and Nafion layers were incorporated to use glucose as the target. A wider linear range was obtained (from 100 nM to 5 mM) with a sensitivity of 0.009 NCR decade^{-1}, with a lower detection limit of 100 nM. With the enzyme-Nafion configuration, selectivity tests were conducted by adding AA and UA as the main interferences in human body fluids without producing any significant interference.

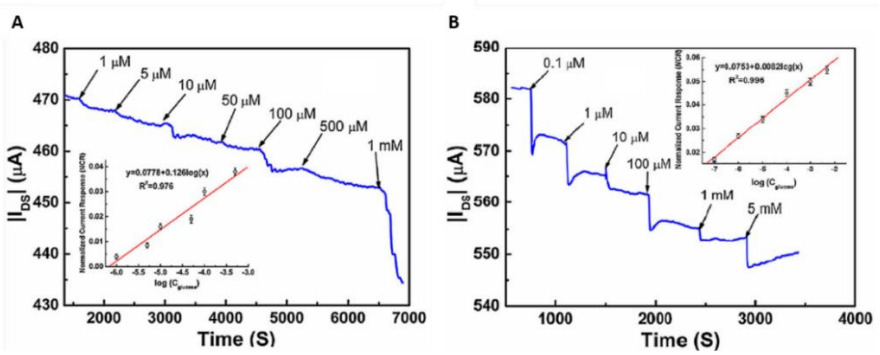


Figure 2.4. Current responses of the OECT to successive additions of **A)** H_2O_2 and **B)** glucose. Insets: NCR as a function of analyte concentration. Adapted with permission from ref. [63].

The same electrode configuration was tested amperometrically in order to compare the two different configurations. OECT resulted in better sensitivity and detection limits (100 µM amperometrically versus 100 nM with OECT). Real samples were also tested by diluting human serum and were validated against a hospital-used blood sugar instrument, achieving a relative error of less than 4%.

The simultaneous detection of glucose and lactate was achieved by Ji et al. [64] (Figure 2.5.), who combined two transistor systems with two different oxidase enzymes in one microfluidic chip with a dual channel. The H_2O_2 produced from each oxidase reaction did not diffuse and there was

no crosstalk between the two sensors. While PEDOT:PSS was spin-coated in both transistors channels, the gate modifications consisted of the deposition of poly (N-vinyl-2-pyrrolidone)-capped PtNPs with the subsequent drop-casting of a Nafion layer and enzyme immobilization using a chitosan matrix, both for GOx and lactate oxidase (LOx). The microfluidic chip had a detection time of around 1 min and an LOD of 1 µM for glucose and 10 µM for lactate. It was used to determine salivary glucose concentrations for both non-diabetic and diabetic patients in a 10-fold dilution. Glucose results were compared to those obtained by their portable prototype for real-time glucose determination, which was successfully validated. Although neither the validation of the lab results nor the prototype results compared to standard glucose determination methods were reported, the integration of the microfluidic transistor into a portable device linked to a smartphone via Bluetooth was considered to have great potential for real-time, non-invasive glucose sensing applications.

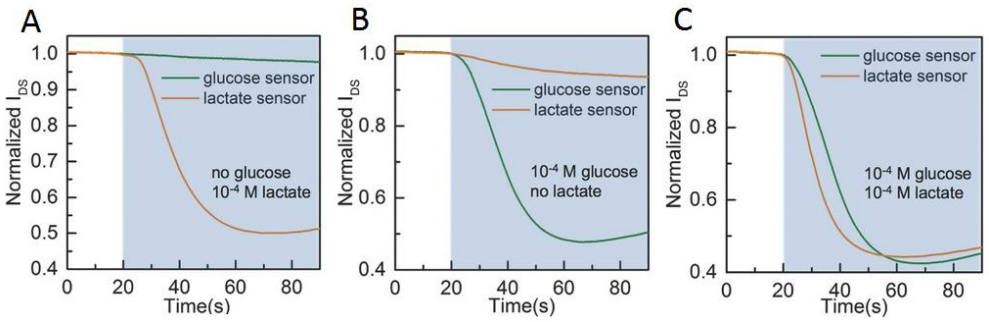


Figure 2.5. Normalized current response after addition of analyte **A)** 10^{-4} M lactate, **B)** 10^{-4} M glucose and **C)** 10^{-4} M lactate and 10^{-4} M glucose simultaneously, at V_{DS} -0.2 V and V_G 0.5 V. Adapted with permission from ref. [64].

Apart from being the key component on the bridging source and the drain channel, PEDOT:PSS can also be included in gate modification as the transducer element, as Pappa et al. showed [65]. A multianalyte biosensing platform was built by functionalizing the gate electrode by mixing PEDOT:PSS with polyvinyl alcohol (PVA) in order to introduce hydroxyl groups where the corresponding oxidase enzyme (either GOx, LOx or cholesterol oxidase (ChOx)) was covalently attached. A ferrocene-chitosan hybrid electron mediator was added to the gate electrode to improve the efficiency of the electron transfer. The final Au/PEDOT/oxidase/ferrocene-chitosan gate, together with the spin-cast PEDOT:PSS on the channel, provide LODs of 10 µM, 50 µM and 10 µM for glucose, lactate and cholesterol respectively, and linear ranges going from 0.02 to 1 mM for glucose, from 0.1 to 2 mM for lactate and from 10 to 700 µM for cholesterol. The multianalyte

platform consisted of a transistor microarray for the selective determination of the abovementioned three components, integrated together with a "finger-powered" microfluidic system, and was used to simultaneously determine glucose, lactate and cholesterol content in human saliva. As in the previous example, although the concentrations obtained were not validated against other conventional methods, the OECT array with finger-powered microfluidics resulted in a potential platform for use as a non-invasive, portable multianalyte device for point-of-care diagnostics (Figure 2.6.).

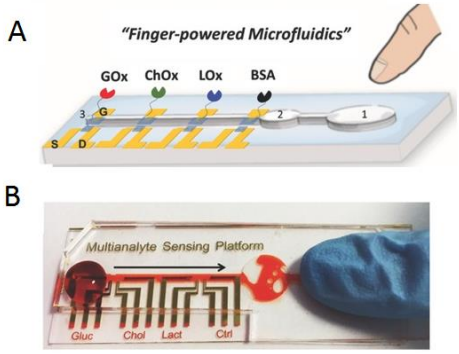


Figure 2.6. A) Schematic illustration of the embedded "finger-powered" microfluidic biosensing platform **B)** Photograph of the device, showing the red solution that was pressure-driven from the inlet through the sensing areas, as indicated by the arrow. Adapted with permission from ref. [65].

Another example in which PEDOT:PSS was used both for gate and channel modification is in the study conducted by Welch et al. [66]. PEDOT:PSS was spin-coated onto the Pt gate electrode and the surface activated by plasma oxidation. Poly(glycidyl methacrylate and poly(2-hydroxyethylmethacrylate) (PGMA:PHEMA) brushes were immediately polymerized on the PEDOT:PSS surface by means of the "grafting" method using an atom transfer radical polymerization technique. GOx was covalently attached to the brushes. Electrochemical measurements were taken using a phosphate buffer (0.12 M). The device exhibited a strong response at low concentrations and an even higher response at concentrations ranging from 3.84 to 100 mM. This range covers the physiological and pathological glucose levels in human blood, saliva and brain tissue.

Furthermore, Liao et al. [67] used two different conducting polymers in the same OECT device to detect uric acid, cholesterol and glucose through H_2O_2 production by their respective oxidase enzymes. PEDOT:PSS was deposited as the sensitive part of the channel and PANI worked as the transducer element included in the gate modification. The platinum gate electrode was

modified by a bilayer made of graphene oxide sheets (GO) and Nafion with graphene flakes. The gate modification was already useful for repelling positively charged molecules by the protonated conducting polymer and the anionic electroactive species by the acidic sulfonic groups of Nafion. In addition, selectivity towards the targets was achieved with the subsequent enzyme immobilization (either uricase (UOx), ChOx or GOx) by means of a glutaraldehyde cross-linker. UA sensors showed an LOD of 10 nM with a sensitivity of 147 mV decade^{-1} and a linear range of 100 nM to 500 µM. Comparatively, amperometric measurements with the same electrode configuration reached a limit of detection of around 3 µM, which was two orders of magnitude higher than the OECT configuration. The cholesterol sensors showed a limit of detection of 100 nM and glucose sensors showed an LOD of 30 nM. UA and glucose were also tested by adding saliva samples to PBS solution, and achieved consistent results for potential real-world applications.

A comparative study of the analytical parameters mentioned in the reported OECTs is listed in Table 2.2. In general, OECTs can achieve lower limits of detection than amperometric methods due to the fact that a small change in the gate voltage of an OECT can be reflected as a significant variation in the channel current, making these devices highly sensitive biosensors. However, as we have concluded from the studies reported in this review, most transistors are fabricated based on thermal evaporation and photolithographic techniques. Additionally, some of them require UV or plasma treatment, which must be taken into consideration in terms of a final application, as some of these techniques are expensive and time-consuming. The fabrication steps may therefore hamper the practicality of using OECT in low-cost, portable devices. This impracticality is also reflected in the number of studies that have tested their devices in real samples for real-world applications. Most of the devices listed in Table 2.2. were analyzed as proof-of-concept for the detection of glucose (or other molecules) by means of highly sensitive sensors using an H_2O_2 determination strategy, and wider applications have been suggested using other enzymes by modifying the gate electrode with the incorporation of other (nano)materials. Nevertheless, the demand for small portable sensor devices has driven the quest for other suitable substrates, such as fabrics or PET [68], and simpler fabrication techniques, such as screen printing or inkjet-printing [69] to allow for the low-cost mass fabrication of OECTs.

Table 2.2. Comparison of analytical performance of selected conducting polymer-based OECT based on H_2O_2 detection for different targets.

CP ON ELECTRODE	ADDITIONAL MATERIALS	LINEAR RANGE (μM)	LIMIT OF DETECTION (μM)	SAMPLE VALIDATION	OBSERVATIONS	REF.
PEDOT:PSS on channel	PtNPs/GOx/Nafion	0.1 to 5000	0.1	diluted human serum RSD <4%	90 % signal after 10 days. Reproducibility RSD=4.1 %	[63]
PEDOT:PSS on channel	PtNPs/Nafion/GOx-chitosan		1	simultaneous detection by 10-fold diluted human saliva	validated portable glucose prototype	[64]
	PtNPs/Nafion/LOx-chitosan		10			
PEDOT:PSS on channel	PEDOT:PSS/chitosan-ferrocene/GOx	20 to 1000	10	Simultaneous detection in human saliva	microfluidic device	[65]
	PEDOT:PSS/chitosan-ferrocene/LOx	100 to 2000	50			
	PEDOT:PSS/chitosan-ferrocene/ChOx	10 to 700	10			
PEDOT:PSS on channel and gate	PGMA:PHEMA brushes/GOx	--	0.95		100% signal after 100 days	[66]
PEDOT:PSS on channel. PANI on gate	UOx-GO/Nafion-graphene		0.01	Saliva in standard addition method		[67]
	ChOx-GO/Nafion-graphene		0.1			
	GOx-GO/Nafion-graphene		0.03	Saliva in standard addition method		

SUMMARY AND CONCLUSIONS

The main advantage of using conducting polymers as charge-transfer media is that they offer the possibility of tailoring their characteristics to adapt to the surrounding conditions. The chemical or electrochemical polymerization or the media in which they operate can help modulate the final performance of target detection and define the adhesion of the film to the electrode surface, even becoming a serious limitation for sensor development. In most of the works cited in this review, modified electrodes were compared to bare electrodes. The main electrochemical techniques used to characterize the electroactivity of the electrode surface were cyclic voltammetry and electrochemical impedance spectroscopy. In most cases, the incorporation of the conducting polymer improved the electrocatalytic activity towards hydrogen peroxide in comparison to bare electrodes, or in comparison to other modifications without the presence of the conducting polymer. Consequently, the main contribution found in the use of conducting

polymers in electrode modification is the increase in redox peak currents, leading to a faster electron transfer from the analyte to the electrode surface. The use of conducting polymers as a transducer material in hydrogen peroxide sensors provides an additional conduction path to the already conductive electrode surface, which theoretically results in enhanced H_2O_2 determination. Nevertheless, their use as a sensing material has not yet been demonstrated, since all the above mentioned examples report conducting polymers as the transducer part to facilitate or accelerate the electron transfer propagation in the devices, while the catalytic activity towards H_2O_2 is produced by the incorporated mediators or materials taking part in the blends and composites. The characteristics of conducting polymers increase the output signal in response to the chemical reaction taking place at the electrode: the determination of hydrogen peroxide is never due to the change in the polymer redox properties, but rather to the electrode redox properties. In most cases, the comparison of analytical parameters for H_2O_2 detection with and without conducting polymers has not been reported, although for OECTs this comparison would not be meaningful since it is the basic component of the channel. Thus, even if the increase in the sensitivities obtained for those sensors or the wider linear ranges reached have been demonstrated, the quantification of this improvement has yet to be addressed.

In general, although a great number of works have focused on the use of conducting polymers for sensing applications, their sensing mechanisms are not yet fully understood. Some potentiometric and chemiresistor studies have also been found in the literature [70–72], but the need to control the redox state as well as the acid-base and ionic equilibria simultaneously has paved the way for the progress of CP-based sensors towards amperometric devices or OECTs, since they operate by amplifying the signal due to the catalytic properties, or as immobilizing agents due to their biocompatibility [73]. Some of the intrinsic limitations of CPs, such as pH dependence, over-oxidation and influence of the electrolyte nature, must be addressed in order for CP-based sensors to become practical in real-world applications and to overcome the challenging issues of market access.

The applications of each conducting polymer also pose some limitations due to their intrinsic properties. For instance, PPy conductivity is irreversibly destroyed upon exposure to H_2O_2 [74,75], which hinders its use where hydrogen peroxide is present in the vicinity of the sensor. Conversely, PANI does not react with H_2O_2, which facilitates its application in the field, although its electrochemical activity decreases at pH above 5 [74]. However, some of these limitations have

been overcome over time by the use of conducting polymer derivatives and by tailoring their characteristics through the use of composite films [75] which extend, for instance, the operational pH range. It is not surprising, therefore, that the majority of the amperometric electrodes and organic electrochemical transistors, as mentioned in this review, incorporate PEDOT in their systems because of its environmental and thermal stability, its high electroconductivity and good film-forming properties. It is worth mentioning that PEDOT:PSS has also attracted attention due to its biocompatibility for the immobilization of biomolecules, as demonstrated by Richardon-Burn et al. [76]. It is for this reason that PEDOT is mainly used in combination with oxidase enzymes in most sensor configurations to achieve highly sensitive transistors.

Although hydrogen peroxide plays an important role in many different areas, as mentioned at the beginning of this review, most of the reported H_2O_2 sensors focus on healthcare applications, and especially on glucose determination. The increasing prevalence of diabetes mellitus, which an estimated 8.4% of the world's population currently suffers from [77], and the growing demand for means of monitoring and controlling glucose levels, has obviously driven the analytical field towards glucose detection in order to generate simple, low-cost, sensitive and reliable sensors. Although the advantages of the incorporation of CPs for the electrochemical detection of hydrogen peroxide have been significantly demonstrated, the implementation of CPs may require their integration in a portable, low-cost substrate such as paper [78] in order to make their contribution a real breakthrough in the field.

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CHAPTER 3

PEDOT:PSS PAPER-BASED CHEMIRESISTOR FOR H_2O_2 DETERMINATION

UNIVERSITAT ROVIRA I VIRGILI
DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HYDROGEN PEROXIDE DETERMINATION
Marta Borràs Brull

INTRODUCTION

Conductometric sensors, or chemiresistors, are chemical sensors made of materials that change their electrical resistance due to the presence of a chemical change in the nearby environment. They offer simplicity both in construction and signal measurement, since there is no need of a reference nor an auxiliary electrode) and do not require complex instrumentation (the output signal can be measured with a simple ohmmeter). Most common materials used in chemiresistors are metals and metal oxide semiconductors, such as SnO_2, TiO_2, MoO_3, In_2O_3, *etc*. which operate at high temperatures (200-500 °C) to achieve high sensitivities. One of their drawbacks is their poor processability. The use of conducting polymers as an alternative to those inorganic materials, provide some advantages to conductometric sensor fabrication: their synthesis is easy and tunable according the desired properties and they provide fast response and high sensitivity at room temperatures [1].

From the first incorporation of CP into conductometric devices back on 1983 by Nylabder et al. [2] for ammonia (NH_3) detection, many different conducting polymer-based chemiresistor formulations have been fabricated for many different compounds. The vast majority of conducting polymer-based chemiresisitive sensors are used to determine gaseous analytes [3,4], where the interaction of the electron donor/acceptor gas with the conducting polymer causes a change in the doping state of the polymer due to the oxidation/reduction or protonation/deprotonation reactions by means of a change in the measured conductivity [5]. For example, Kuberský et al. [6] reported NH_3 and humidity sensors based on polyaniline (PANI) and poly(3, 4-ethylenedioxythiophene) poly(styrene sulfonate) (PEDOT:PSS), respectively, which were fabricated onto flexible poly(ethylene terephthalate) (PET) foil substrates. Rañola et al. [7] developed an array of three different conducting polymers (PANI, polypyrrole (PPy) and poly-3-methylthiophene (P3MTp) electrodeposited on a Teflon substrate in between two gold electrical contacts. The array was able to distinguish among a variety of different coconut oils. Moreover, nanostructured conducting polymers have also attracted the interest of many studies due to the high sensitivity and fast responses provided due to the high surface-to-volume ratio given by the nanostructured material. For instance, Tang et al. [8] reported a conductometric gas sensor based on PEDOT:PSS nanowires which was sensitive to NH_3 and NO_2 at room temperature with a limit of detection (LOD) at the ppb range. Nevertheless, the authors pointed out the challenge of large-scale nanowire fabrication, which hampers the commercialization of devices based on such

nanostructures. NO_2 was also determined using PEDOT:PSS nanotubes by Shaik et al. [9] achieving ppb levels as well. In addition, chemiresistors based on conducting polymers have also taken advantages of the combination of two or more materials to obtain superior properties and characteristics. Thus, some conductometric sensors have been developed by the incorporation of nanomaterials such as metal nanoparticles or carbon based compounds (graphene, carbon nanotubes, *etc.*) [10]. NH_3 was also determined using PANI-functionalized multiwall carbon nanotubes by Abdulla et al. [11] showing enhanced analytical performances when compared to the conductometric configurations without the presence of the conducting polymer (i.e. response time with and without PANI was 6 s and 938 s, respectively). More recently, the real-time monitoring of pH during microbial fermentation was achieved by Chinnathambi et al. [12] using a Nafion-coated chemiresistor based on PANI-reduced graphene oxide (rGO) composite. In addition, another biological application of CP-based conductometric sensors was reported by Olean-Oliveira et al. [13] who also used rGO together with Azo-polymer to monitor oxygen consumption on biological processes involving mitochondria respiration.

Nevertheless, the use of conductometric sensors is not only restricted to gas analytes. The incorporation of molecular recognition elements into the sensing layer of the chemiresistive systems has broaden the variety of targets to detect, and consequently, it has allowed its application for detecting analytes in non-gaseous phase. Therefore, the ability to operate in liquid-phase allows conductometric probes to be used in body fluids or residual water, for instance, and provides the advantage of eliminating the need of controlling the humidity. The use of chemiresistors in aqueous environments has been demonstrated to detect proteins at low concentrations [14], to discriminate among four different species of bacteria in a bacterial culture [15] or to monitor human perspiration [16], among others [17-19]. The use conducting polymer in chemiresistive sensors together with the incorporation of receptors attached to the sensing channel has mainly attracted the attention of biomedical sciences. The most used configuration in this field has been using nanostructured nanowires or nanotubes conducting polymers. Their increased sensitivity to electrical changes allows the determination of different targets with low limits of detection. The biofunctionalization of PPy-nanowires with antibodies has been used to detect bacterial spores in artificial samples [20], viruses in real lake water samples [21] or cancer biomarkers in spiked human blood plasma [22].

Although the sensing mechanism of such conducting polymers is still not completely understood, there are different hypothesis supporting the detection mechanism of PEDOT:PSS in chemiresistors. The first one considers redox reactions taking place when the adsorbed analyte releases or accepts electrons to or from the polymer. Electrons from the conduction band and holes from the valence band of the conducting polymer recombine and as a result, the concentration of carriers decreases while the resistance increases. The second hypothesis aims at a direct charge transfer between the analyte and the polymer, which compensates the polymer charge lowering the final conductivity (increasing the resistance). And the third hypothesis do not imply a change on the redox state of the polymer, but a physical interaction involving absorbing or swelling of the target analyte what influences the polymer resistance. This third hypothesis is mainly adopted for volatile organic compound (VOCs) sensing devices [3]. Thus, the increase in resistance is due to the increase in the PEDOT interchain distance (a decrease of the electrical interconnections) on account to a swelling process that hinders the electron hopping along the polymer [23].

Taking this into account, we aimed at developing a simple and low-cost chemiresistive sensor based on commercial PEDOT:PSS able to determine H_2O_2 concentrations in aqueous environment. Our hypothesis held up the chemical interaction between the hydrogen peroxide and PEDOT:PSS, by an electron exchange due to the redox reaction occurred between the adsorbed hydrogen peroxide and the conducting polymer. Thus, a change on the redox state of the polymer by means of a change on the charge carriers from the polymer backbone, would cause a change in the polymer conductivity (or resistance). Therefore, the chemiresistor formulation was supported as transducing mechanism for turning such chemical change into an electrical detectable output. Hence this chapter describes the fabrication and analytical performance of a hand-made paper-based chemiresistor using PEDOT:PSS conducting polymer as the sensing layer for the determination of H_2O_2 concentration. In addition, the development of such conductometric sensor was carried out considering some breakthrough aspects on the implementation of devices in the sensing field; the low-cost of manufacturing techniques and materials and a final easy-to-use configuration.

EXPERIMENTAL

Materials and Reagents

Whatman® Grade 5 qualitative filter paper (GE Healthcare Life Sciences) was used as substrate of the chemiresistor and a carbon-based screen-printable electrically conductive ink (122-49, Creative materials, Inc., MA, USA) was used to build the contacts of the electrode. Poly(3, 4-ethylenedioxythiophene)-poly(styrenesulfonate) 1.3 wt % dispersion in H_2O conductive grade (Merck, KGaA, Damstadt, Germany) was used as the sensing layer of the chemiresistor. Plastic mask (ARcare® 8565, Adhesives Research Inc., Limerick, Ireland) and silicon rubber compound (RS Components, Ltd. Northants, UK) were used as additional materials on sensor fabrication. Hydrogen peroxide solution 30% (w/w) and methanol 99.8% were purchased from Merck (KGaA, Damstadt, Germany). All solutions were prepared using 18.2 MΩ cm^{-1} double deionized water (Milli-Q water systems, Merck Millipore). Nafion® perfluorinated resin solution (5 wt % in a mixture of lower aliphatic alcohols and water, 45% water), was also from Merck and in all cases it was used as received.

Electrochemical measurements and instrumentation

Resistance measurements were carried out using a Tenma 72-7720 digital multimeter (Tenma Corp.) and a Keithley 6514 electrometer from Keithley Instruments, Inc. (Ohio, USA) with the ExceLinx software for data acquisition, at room temperature.

Sensor fabrication

The first and simplest configuration of the chemiresistor was built based on the procedure described in Qin et al. [24] with some modifications. Briefly, the filter paper was cut into square pieces of 6 cm^2 and two carbon ink bands were manually drawn in order to form the electrical contacts with a gap in between of approximately 0.5 mm. The bands were dried at 90 °C for 15 min. and a second layer of carbon ink was placed onto the already drawn contacts to obtain a more homogenous surface with a stable and constant resistance value around 200 KΩ. A second drying process was done exactly as the first one. Both electrical contacts were then bridged by the as-received PEDOT:PSS 1.3 wt % by drop-casting. (Figure 3.1.)

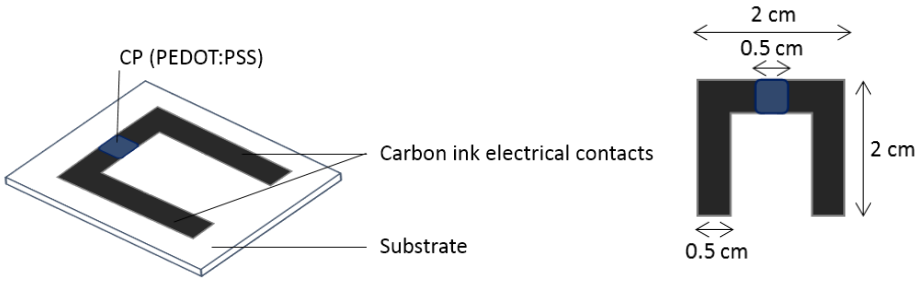

Figure 3.1. Scheme of chemiresistor configuration.

Further modifications of this first configuration were done in different levels to find an optimal device: the substrate material, the geometry of the electrical contacts, the incorporation of additional materials and the conducting polymer ink. Such modifications are described in the next section.

RESULTS & DISCUSSION

Substrate materials

The substrate of the chemiresistor was chosen out of four different low-cost materials with different properties. Hydrophobicity, porosity and robustness were the key differences among rubber, glass, laminated paper and filter paper materials tested. The carbon ink electrical contacts were drawn as previously described and the incorporation of the conducting polymer was done by drop-casting. The hydrophobicity and solubility of the commercial CP marked the adhesion of the film to the substrate: while on high hydrophobic substrates the PEDOT:PSS addition was not controlled and the CP was peeled off from the surface (e.g. in rubber and laminated paper), the porous nature of filter paper allows the CP seep through the paper fibers and remained embedded in it. In the case of glass substrates, and in spite of its hydrophobicity, the polymer film was held on the surface as well. Nevertheless, once the sensors were immersed for 24 h in MilliQ water, an obvious polymer delamination was observed on rubber, laminated paper and glass substrates, leading to sensors with an empty gap between electrical carbon ink contacts, leading a surface without CP. In the case of the paper, despite there was also some polymer detachment from the substrate, a great amount of polymer remained embedded into the paper fibers leading the gap between the electrical contacts still bridged with conducting polymer. Thus, further resistance measurements were viable.

Polymer adhesion

The previous water resistance experiments mentioned above, allowed us to dismiss the substrate materials which peeled off the CP from the surface, and continue working with filter paper. Although filter paper allowed a great amount of conducting polymer to remain in the gap between electrical contacts, the polymer adhesion was still an issue to be addressed in order to optimize the amount of polymer that takes part of the sensor. Polymer adhesion to the substrates depends on many factors, from the nature of the polymer and surface to the polymerization procedure (chemical or electrochemical [5]). As the adhesion of conducting polymer is basically determined by weak physical interactions, cracking or delamination of the coating can occur when, for example, ions or molecules from the electrolyte are incorporated or expulsed from the polymer backbone [25]. In addition, in the case of PEDOT:PSS, the addition of the water-soluble PSS surfactant provides the polymer the solubility it lacks, making it more soluble in aqueous media. However, the excess of the PSS chains causes a deterioration of the physical forces the polymer has with the substrate and the film becomes more prone to crack or detach from the surface [26]. Therefore, from the intrinsic challenge of conducting polymer adhesion, there arose many different strategies to improve polymer-substrate adhesion and avoid cracking and delamination. Surface chemistry provides different modifications to enhance polymer adhesion by strong physical interactions or by forming covalent bonds to anchor the polymer to the substrate. Roughening the surface prior to CP deposition [27,28], covalently attaching the polymer to the substrate by the addition of thiol groups to the polymer chain [29,30], the addition of adhesive or crosslinking agents [26] or the incorporation of carbon-based nanomaterials [31] or ionic liquids [32], are some of the reported strategies to improve the mechanical stability of the polymer, and thus, facilitate their implementation in devices.

In order to improve PEDOT:PSS adhesion to our surface and avoid delamination due to the polymer swelling when working in aqueous media, we tried two simple strategies found in literature. One was developed by Wagner et al. [33] and it was based on limiting the solubility of the polymer by adding cations that could stabilize the interaction of the positively charged PEDOT and the negatively charged PSS. The other was reported by Nakashima et al. [34] who could overcome ink-bleeding issues in conducting polymer deposition by mixing the polymer with methanol. The method was based on the hydrogen bond formation between the OH group of methanol and the sulfonate group of PSS [35]. It is supposed to induce screening effect of the charged particles of PEDOT:PSS, leading to gelation during the solvent evaporation. It consisted on

diluting the polymer solution with methanol at the following proportion: ink/methanol = 1/1 – 1/4 (v/v).

Delamination was not fully avoided with any of the two strategies tested and resistance stability measurements did not show any significant differences between both ink modifications. Therefore, since the method incorporating methanol [34] presented advantages over the addition of CTA^+ cations [33] by being simpler, cheaper and allowing a faster ink treatment, further experiments were performed using PEDOT:PSS/methanol blend.

In addition, resistance measurements were also performed during the *in situ* addition of water after chemiresistor fabrication with 25% PEDOT:PSS/ 75% methanol ink. As shown in Figure 3.2., the resistance remained constant after water evaporation on each addition, demonstrating the mechanical stability of the conducting polymer blend as well as confirming the absence of chain scission or polymer cracking which would interfere to resistance values.

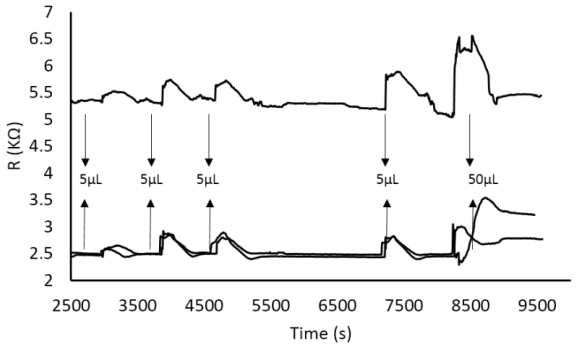

Figure 3.2. Time trace of resistance versus time during the addition of MilliQ water drops on three different devices. The arrows indicate the time and volume of each drop.

Thus, and taking into account the adhesion of the conducting polymer to the different tested substrates, 25% PEDOT:PPS/ 75% methanol ink was used to build chemiresistors and filter paper was chosen as the candidate substrate to perform all further experiments.

Practical considerations

In addition, other important aspects for practical use of the chemiresistor configuration, such as a delimited active area, the isolation of the active area from the rest of the sensor and the electrode geometry, were taken into account. Therefore, a home-made plastic template was used in order to draw the electrical contacts in a more reproducible way. In addition, a plastic mask with a 3 mm diameter circular window was added at the top of the conducting polymer channel to delimit the active area exposed to the medium and thus, the analyte, and the edges were sealed with glue. At the same time, silicon rubber compound was manually applied on both sides of the sensor to protect the carbon ink contacts from the aqueous medium. Moreover, the two different geometries shown in Figure 3.3. were also tested for different purposes.

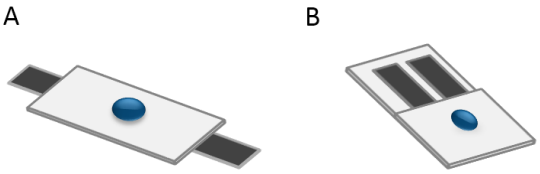

Figure 3.3. Scheme of chemiresistor configuration. **A)** Single electrode configuration for drop-casting experiments **B)** Chemiresistor configuration for experiments in solution.

The new configuration shown in Figure 3.3.A. was used for experiments were the analyte was drop casted onto the active area which is represented in the figure by the blue spot. In this case, the electrical contacts made of carbon ink were integrated in a single paper strip and the resistance was measured between the two extremes of the electrode. Regarding the configuration shown in Figure 3.3.B., the addition of the plastic mask was useful both for delimiting the active area and for protecting the electrical contacts from water media when working in solution.

PEDOT:PSS stability

Once chemiresistor configuration was well stablished, and conducting polymer delamination was overcome, we first tested the stability of the measured resistance depending on the amount of PEDOT:PSS placed on the channel bridging the two electrical contacts. Thus, a continuous resistance monitoring was performed with the Keithley electrometer while PEDOT:PSS was *in situ* drop-casted by drops of 5 µL in the gap of the electrode (Figure 3.4.).

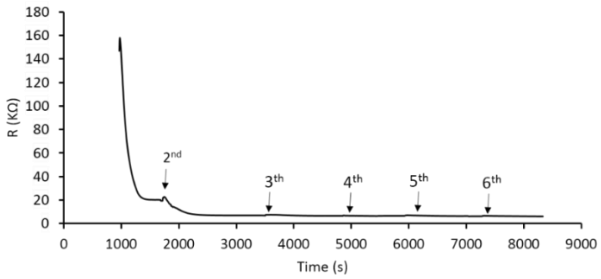

Figure 3.4. Continuous monitoring of chemiresistor construction: time trace of resistance versus time corresponding to the *in situ* additions of 5 µL of PEDOT:PSS on the channel area.

High and fluctuating resistance measurements were obtained from first and second drop additions. Thereafter, the resistance measurements stabilized to few KΩ and remained constant for the further conducting polymer additions. Thus, an amount of 15 µL of PEDOT:PSS was stablished as the minimum practical amount of conducting polymer required to achieve an stable resistance baseline.

H_2O_2 determination

Hydrogen peroxide experiments were conducted by monitoring the change in resistance with increasing H_2O_2 concentrations. As a first step, we tested the response of our chemiresistor to the hydrogen peroxide using the as-received H_2O_2 30% (w/w) which had an estimated concentration of 9.7 M. Figure 3.5. shows the time trace of resistance measurement versus time: first, the conducting polymer/methanol mixture was added *in situ* between the two electrical contacts as previously described, second, two drops of MilliQ water were added on top of the previously deposited PEDOT:PSS as a washing step and reference signal change for wet conditions, and third, the hydrogen peroxide was added at the same sensing region. The resistance signal increased drastically upon hydrogen peroxide addition with differences from the references values (ΔR) around 20,000 KΩ.

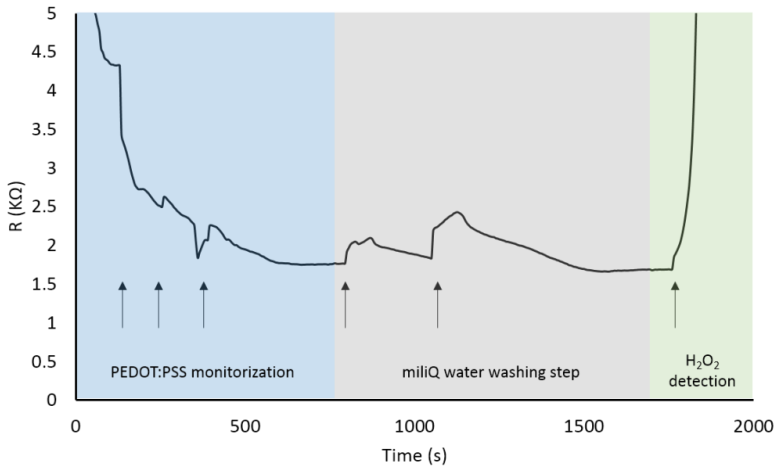

Figure 3.5. Magnification of the time-trace corresponding to the monitoring of the addition of 25% PEDOT:PSS/ 75% methanol, the washing procedure with additions of MilliQ water and the detection of H_2O_2 (additions of each compound are indicated by arrows).

Further H_2O_2 calibrations were done using H_2O_2 concentrations within a range from 10^{-6} to $10^{-1.5}$ M (0.001 to 32 mM). The calibration plots are shown in Figure 3.6., represented as absolute resistance values and relative resistance values calculated using the following formula:

$$relative\ resistance = \frac{(R_i - R_0)}{R_0}$$

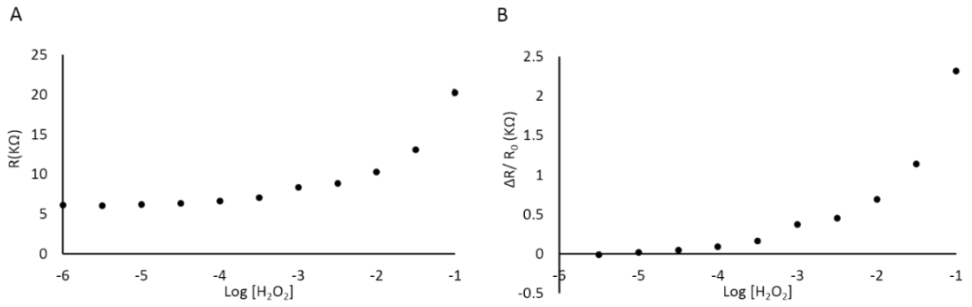

Figure 3.6. Calibration curves for increasing H_2O_2 concentrations represented as **A)** absolute resistance values versus logarithm of H_2O_2 concentration and **B)** relative resistance values versus logarithm of H_2O_2 concentrations.

As shown in both plots, the resistance of the conducting polymer measured in between the two electrical contacts increased with the increasing concentrations of H_2O_2. It suggested, thus, that the interaction between hydrogen peroxide and PEDOT:PSS lowered the amount of charge carriers available in the polymer backbone, an therefore, reduced the channel conductivity.

Nevertheless, we were not able to reproduce such results: the starting resistance was not reproducible as well as the resistance changes corresponding to each hydrogen peroxide addition. As shown in Figure 3.7., the differences between chemiresistor devices are clear and meant very high standard deviations for the average values.

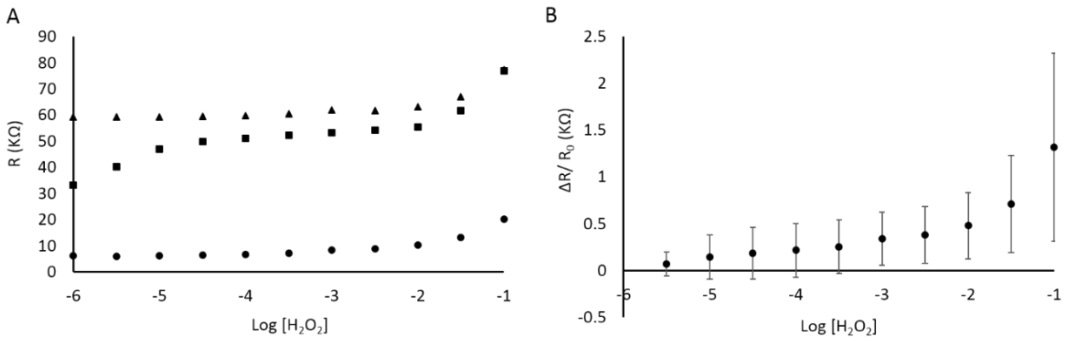

Figure 3.7. Calibration curves represented as resistance measurements versus logarithm of H_2O_2 of **A)** absolute resistance values from individual chemiresistor sensors (*N=3*) and **B)** average of the relative resistance values obtained from chemiresistors used in A with the corresponding standard deviation (*N=3*).

Limitations

Sensor irreproducibility lied on different parameters. Regarding the manufacturing procedure the first limitation is faced by the fact that a hand-made fabrication implies intrinsic variability between sensors. Although the fabrication procedure was always the same and was performed always by the same person, and although templates were used to improve reproducibility in the construction, it is understandable that all sensors were not identically the same. Although the criteria was fixed as achieving 200 KΩ on the carbon ink electrical contacts, neither the thickness nor the spreading through the paper fibers were controllable parameters. In addition, since resistance is a distance-dependent parameter, some of the differences in the measured resistance may come from the practical monitoring procedure. Second, the deposition of the conducting polymer was also a variable parameter. In spite of having stablished a fixed amount to achieve stable measurements and having a similar gap between electrical contacts

among all chemiresistors, the way the polymer spread through paper fibers and remained embedded in the paper was never a constant factor. In addition, the polymer spreading through the paper could overlap with the carbon ink electrical contacts which may also affect the transduction of the electrical signal. Third, the use of the plastic mask provided the advantage of ensuring that the area exposed to the analyte was always the same in each addition. Nervertheless, the role of the mask for such area delimitation may cast doubts since there was no way to demonstrate that the added analyte drop was only interacting with the exposed area through the mask, and did not interact with part of the polymer on the surface and embedded in the paper in the areas under the mask.

Different attempts on fabrication and practical optimizations in order to overcome some of these drawbacks were unsuccessfully performed. The use of glue on sensor edges, the addition of a Nafion-coating on the electroactive area or the different liquid ink deposition methods tested did neither depict any significant differences nor improved the already obtained first-time resistance results. Additional experiments in solution using Figure 3.3.B. configuration did not succeed either.

Regarding the analytical point of view, during an analyte drop-casting calibration every drop carried a different amount of target compound (from the lowest to the highest). Considering a physical interaction between polymer and target, the calibration may present an accumulative effect on the signal change, meaning that the observable resistance change from one point of the calibration could not be an absolute value of signal change, but a sum from all the changes from previous calibration points. Moreover, since the signal was expected to be directly related to the amount of target in contact with the polymer on the channel, a study of the signal dependence on the volume of analyte was also performed. Indeed, we saw higher resistance changes when higher target volumes were drop casted, even though target concentration was constant in all volumes tested. Thus discloses the need of the definition of a sample volume in order to dismiss any influence on the recorded signal.

CONCLUSIONS

In this section, the determination of hydrogen peroxide using conducting polymer-based conductometric electrode was described as a preliminary proof-of-concept. Although we demonstrated the sensitivity of paper-based conductometric sensor through hydrogen peroxide by the measured resistivity changes, we were not able to achieve a reproducible and reliable system in order to define the basic analytical parameters a sensor requires. Therefore, neither sensitivity, nor limits of detection or linear ranges, nor interference of different species were reported. Although the use of low-cost materials and processing techniques were successfully prioritized, the completely hand-made manufacturing procedure together with the unclear interaction between the PEDOT:PSS and the H_2O_2, hampered both analytical performance and optimization steps. Nevertheless, those preliminary results have revealed the need to define and control the redox state of the used conducting polymer and its stability over the time. Since long-term stability and the influence of some environmental conditions such as humidity and temperature is a current challenge in the field, the control over the redox state of the polymer could improve signal changes based on the oxidation-reduction process responsible of the output signal. In addition, further research should also address the reversibility of the process, considering the possibility of a change in the polymer conformation and backbone induced by the interaction of the hydrogen peroxide, which could cause the degradation of the polymer making the process irreversible.

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CHAPTER 4

DIRECT POTENTIOMETRIC DETERMINATION OF H_2O_2 BY CONDUCTING POLYMER-BASED SENSORS

UNIVERSITAT ROVIRA I VIRGILI
DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HYDROGEN PEROXIDE DETERMINATION
Marta Borràs Brull

INTRODUCTION

Potentiometric sensors are electrochemical sensors used to determine the concentration of an analyte in an electrochemical cell by measuring the potential difference between two electrodes (the working and the reference electrode) under zero current conditions (open circuit potential). As explained in CHAPTER 1, the electromotive force is related to the analyte concentration in solution when thermodynamic equilibrium is reached between the free analyte in solution and the analyte bound to the recognition element. Thus, analytes can be ions and small charged molecules selectively recognized by ionophores in the ion-selective membrane (ion-selective electrodes -ISE), can be biomolecules if the recognition element is modified with a biorecognition element (antibodies, enzymes, etc.), can be small organic molecules based on redox reactions with a particular metal. Indeed, in previous work from our research group, ion-selective electrodes were developed, for instance, for the determination of creatinine in urine samples [1] or for K^+ in blood [2]. The use of low-cost materials, such as paper [3,4], cotton yards [5], carbon fiber [6] and even skin tattoos [7] was also studied for their application to the detection in different biofluids (sweat *etc*.). The transduction mechanism of carbon nanotubes in ion-selective electrodes was also characterized in a work from the group [12]. Moreover, the incorporation of graphene-based materials set also the basis for the development of all-solid-state ion-selective potentiometric electrodes [13] and for the detection of living bacteria [14]. In addition, the integration of aptamers as biorecognition elements allowed the determination of ultra-low concentrations of a specific strain of *Salmonella* [8], as well as the detection of *Staphylococcus aureus* [9]. In combination with carbon nanotubes as transducer elements for such electrodes, *Escherichia coli* was also determined in real time measurements on complex matrices such as milk or apple juice [10] and glycoprotein from African Trypanosomes in diluted blood [11].

The application of conducting polymers to potentiometric sensors was firstly introduced by Dong et al. [15,16] in 1988. From then on, different studies have focused their attention to explain the transduction mechanism [17]. CP can act as cationic or anionic exchanger depending on the charge and the mobility of the dopant. Therefore if ions approach/move away from the CP layer, a redox reaction occurs and an ion-to-electron transduction process takes place [18]. In addition, conducting polymers in potentiometric sensors can play two different roles. First, the doping and de-doping process allows the polymer to become an ion-exchanger material and be used both as the sensing layer and as transducer, due to its intrinsic electrical conductivity. And second, it can

be used as a polymeric matrix for the immobilization of specific molecular receptors (such as complexation agents, enzymes or antibodies). For example, polypyrrole was used to determine lactate in real blood and sweat samples, with a limit of detection of 81 µM with the use of lactate-modified polypyrrole electrode [19], while in a different study, the same CP was used to immobilize the ionophore which conferred selectivity towards fluoxetine [20]. Besides, the use of CPs as transducer element can be combined with the addition of a conventional ion-selective membranes, which confers the selectivity to the electrode, while the CP would transduce the chemical event into a detectable signal [21,22]. In this case, the CP is mainly used as solid-contact due to its redox capacitance and reversible charge transfer properties, which allows to control the standard potential of the electrodes [23]. Many different studies have been developed on the use of CP in potentiometric sensors [13,24] and the way to overcome the main limitations (such as their poor stability due to undesired secondary redox reactions or the formation of a water layer between the CP and the electrode) [25–27]. For example, poly(*N*-methylpyrrole) was electropolymerized using potassium nitrate as electrolyte to obtain a selective film towards nitrate, which exhibited good selectivity and a strong preference for nitrate over other ion tested as interferences [28]. In another work, both PEDOT:PSS and polypyrrole were used as solid-contact layers together with a calcium selective membrane, and calcium determination was achieved with Nernstian responses in real tap water samples [29]. Another CP, POT (poly(3-octylthiophene)), was used by Vázquez et al. [30] together with additional membrane components containing silver ionophore, to develop a potentiometric sensor able to determine silver ions with Nernstian sensitivity and high selectivity. Moreover, the incorporation of nanostructured conducting polymer structures, such as nanoparticles, was also evaluated by Jaworska et al. who studied the electrochemical properties of polypyrrole nanoparticles for its use in potentiometric sensors [31]. They demonstrated the higher electroactivity of the nanostructured polypyrrole membrane compared to classical pyrrole films [32].

As above-mentioned, CPs were mainly reported as solid-contact transducer for ion-selective electrodes. The objective of this work was to develop a solid-contact potentiometric electrode based on CP to determine H_2O_2 in aqueous environment. The deposited CP would act as the transducer element, expecting that the interaction with the target analyte would induce a change in the chemical potential of the CP, which could be transduced in a detectable potential difference. Therefore, this chapter describes the fabrication of a PEDOT:PSS based potentiometric electrode, using both paper and glassy carbon electrodes as main substrates. In addition, the

analytical performance of such sensors and the tested optimizations, both regarding analytical and practical considerations, are described.

EXPERIMENTAL

Materials and Reagents

Whatman® Grade 5 qualitative filter paper (GE Healthcare Life Sciences) with carbon-based screen-printable electrically conductive ink (122-49, Creative materials, Inc., MA, USA) together with a plastic mask (ARcare® 8565, Adhesives Research Inc., Limerick, Ireland) and glassy carbon electrodes (GCE) with a Teflon® body were used as working electrodes. Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) 1.3 wt % dispersion in H_2O conductive grade, 2.8 wt % dispersion in H_2O, low-conductive grade and 3.0 -4.0% in H_2O, high conductivity grade (Merck, KGaA, Damstadt, Germany) were used as the sensing layer of the working electrode. The monomer 3,4-ethylenedioxythiophene 97% and sodium polystyrene sulfonate were purchased from Merck, and used for the electropolymerization of PEDOT:PSS films as sensing layers.

Hydrogen peroxide solution 30% (w/w) and methanol 99.8% were purchased from Merck. Nafion® perfluorinated resin solution (5 wt % in a mixture of lower aliphatic alcohols and water, 45% water), and polyvinyl butyral (PVB) were also from Merck.

Potassium chloride (KCl), sodium chloride (NaCl), disodium phosphate dibasic (Na_2HPO_4), potassium phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), sodium L-lactate ($C_3H_4NaO_3$), sodium acetate ($C_2H_3NaO_2$), acetic acid (CH_3COOH) were of analytical grade and were purchased from Merck. Urea, ascorbic acid (AA), uric acid (UA), dopamine and D-glucose (GLC) were also purchased from Merck. Potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$) and potassium hexacyanidoferrate (II) ($K_4Fe(CN)_6$), and 2-(N-morpholino)ethanesulfonic acid (MES), low moisture content 99% were purchased from Merck as well.

Solutions

All solutions were prepared using 18.2 MΩ cm^{-1} double deionized water (Milli-Q water systems, Merck Millipore). Phosphate buffered saline (PBS) was prepared with 0.1 M at pH 7.4 (100 mM Na_2HPO_4, 18 mM KH_2PO_4, 14 mM NaCl and 3 mM KCl). Artificial serum was prepared at pH 7.4 with 140 mM NaCl, 29 mM $NaHCO_3$, 2.2 mM KCl, 0.8 mM $MgCl_2$, 1 mM sodium lactate, 2.2

mM KH_2PO_4 and 2.5 mM urea. Acetate buffer was prepared with $C_2H_3NaO_2$ 0.01 M and CH_3COOH 0.05 M at pH 4.5. MES buffer was prepared at 0.01 M with 0.1 M KCl at pH 5. Ferricyanide/ferrocyanide characterization solution was prepared with ($K_3Fe(CN)_6$) 1 mM and ($K_4Fe(CN)_6$) 1 mM in MES buffer pH 5. Electropolymerization solution was prepared with 0.01 M EDOT and 0.1 M NaPSS and stirred overnight in dark conditions to ensure the proper dissolution of the monomer. The solution was bubbled with N_2 for 30 min before polymerization as described in Sjöberg et al. [33].

Electrochemical measurements and instrumentation

Electromotive force (EMF) was measured with a high input impedance (10^{15} Ω) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc. Malvern) at room temperature in a well stirred 4 mL cell. A double junction Ag/AgCl/KCl 3 M reference electrode (type 6.0726.100, Metrohm AG) containing a 1 M lithium acetate electrode bridge was used. The substrate of the working electrodes were either conductive paper or glassy carbon electrodes.

Cyclic voltammetry and electropolymerization experiments were carried out using a potentiostat/galvanostat Autolab PGSTAT128N with a frequency response analyzer electrochemical impedance module (FRA2) (AUTOLAB, Eco Chemie, B.V., Utrecht, The Netherlands) fitted with a three electrode electrochemical cell and NOVA software (v.1.11, The Netherlands) as a measuring interface. The corresponding paper or glassy carbon electrodes were used as working electrodes, the Ag/AgCl/KCl 3 M (type 6.0733.100, Metrohm AG) single junction electrode was used as the reference electrode, and a glassy carbon rod with a diameter of 3.0 mm was used as the counter electrode. All measurements were performed at room temperature.

Measurements of pH were made with a GLP 21 pH meter using Hamilton Polylite lab probe (reference 238403).

Absorbance measurements were taken in an UV-Vis spectrophotometer (Agilent Technologies, Spain) with a 10 mm light path plastic cuvette (BRAND GMBH+CO KG, Germany).

Sensor fabrication

Drop-casting on paper-based electrodes

For the fabrication of the paper-based electrode the filter paper was painted twice with carbon ink with a drying process at 90 °C for 15 min after each painting step. Then the paper was cut into strips of 0.5 cm x 2.0 cm leaving a non-painted region of around 0.5 cm in one extreme of

the strip. Commercial PEDOT:PSS (conductive, high-conductive or low-conductive grade) was drop casted in this region and then dried for at least 2 h at room temperature. Each strip was then sandwiched between two 1.0 cm x 1.5 cm plastic masks. The top mask had a 3.0 mm diameter circular window to expose the conducting polymer to the solution. In some cases, a layer of Nafion® was drop casted on top of the window area (Figure 4.1.).

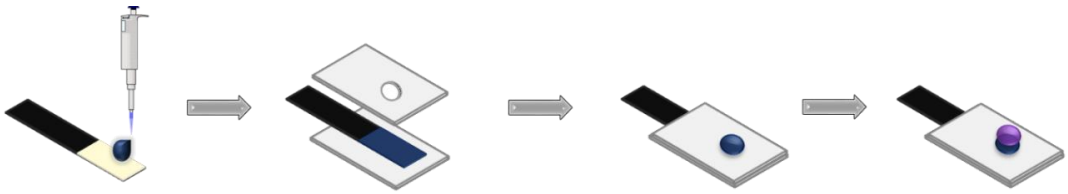

Figure 4.1. Hand-made paper-based electrode fabrication procedure.

Electropolymerized PEDOT:PSS

Prior to electropolymerization, the electrodes were cleaned with KCl 0.1 M by CV (potential from -2 to +2 V, 40 cycles and scan-rate of 100 mV/s) and the surface was stabilized electrochemically with MES buffer by CV (potential from -0.2 to +0.8 V, 10 cycles, and scan-rate of 50 mV/s). A first characterization of the electrode surface before electropolymerization was done with the ferricyanide/ferrocyanide solution by CV (potential from -0.5 to +0.7 V, 3 cycles, scan-rate 50 mV/s). The electropolymerization procedure was carried by CV (potential from -0.2 to +1.5 V, 15 cycles and scan rate 50 mV/s) with 0.01 M EDOT and 0.1 M NaPSS solution. A second characterization step with ferricyanide/ferrocyanide solution with the same conditions as already mentioned was done after the electropolymerization to ensure the proper conducting polymer film formation on the surface of the electrode.

The electropolymerization process was either applied to paper-based electrodes and GC electrodes. In the case of paper substrates, the strips were all covered by carbon ink and the polymerization was done with the window area exposed to the solution. In the case of glassy carbon electrodes, the Teflon body isolated the edges of the glassy carbon, thus, only the 3.0 mm surface exposed to the solution was polymerized (Figure 4.2.).

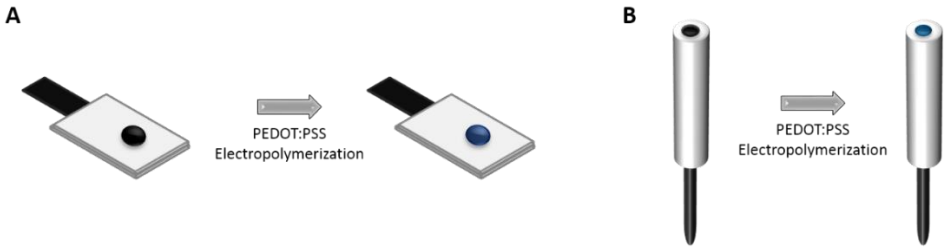

Figure 4.2. PEDOT:PSS electrodepositon on **A)** paper and **B)** GCE, by electropolymerization of EDOT and NaPSS.

RESULTS & DISCUSSION

Paper-based electrodes

First approaches were done using the sensor configuration shown in Figure 4.1. In order to avoid polymer delamination as described in the previous chapter, a Nafion-coating was used to retain the polymer on the substrate. The use of such polymeric layer did not show significant differences regarding the EMF_0 values nor the linear ranges obtained (both were from 10^{-3} to 10^{-1} M). Indeed, the electrodes with the Nafion-coating exhibited slightly higher sensitivity (56.1 ± 12 mV dec^{-1} versus 50.4 ± 13 mV dec^{-1}). Figure 4.3. shows the time traces of both types of electrodes (without and with Nafion, respectively) with the logarithm of the added concentrations indicated by arrows.

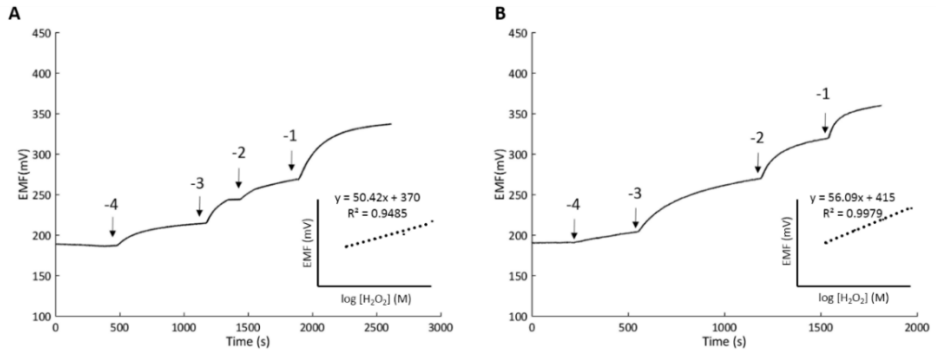

Figure 4.3. Time trace of EMF versus time in water media of H_2O_2 calibrations using paper-based electrodes **A)** without and **B)** with Nafion-coating. Insets: calibration curves with the corresponding sensitivities.

Nafion is a tetra-fluoroethylene polymer with negatively charged sulfonated chains which act as a permselective membrane. Therefore, apart from providing mechanical stability to the overall sensing area of the electrode and entrapping the PEDOT:PSS avoiding it to peel off the electrode surface, Nafion layer also acts as a proton exchange membrane blocking other negatively charged species to reach the electrode surface. Thus, Nafion is also useful to avoid possible interferences of other redox compounds that could interact with the conducting polymer and affect the final signal.

Regarding interference species, H_2O_2 calibrations under the presence of uric acid (UA) and ascorbic acid (AA) were performed in order to determine the degree of such interferences in our system. These compounds are commonly found in body fluids, especially in blood in a well-known concentration. Therefore, the determination of H_2O_2 produced by oxidase enzyme (e.g. glucose oxidase) in blood or any other body fluid could be interfered by such compounds.

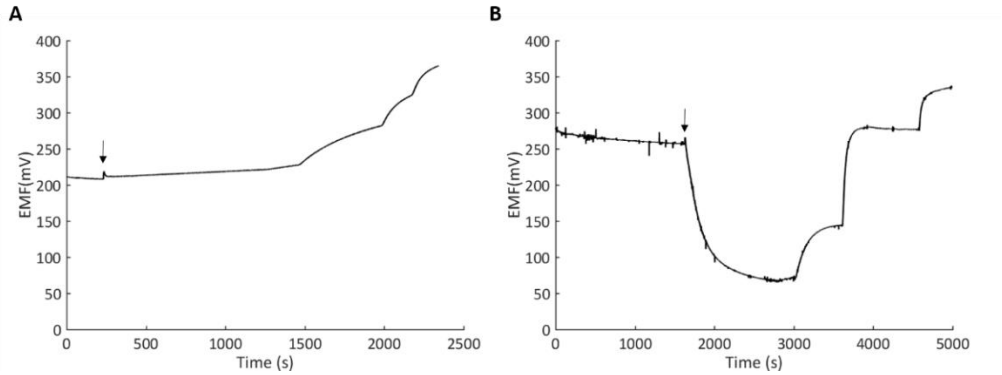

Figure 4.4. Time traces of EMF versus time in water media of two different H_2O_2 calibrations under the presence of **A)** 0.1 mM uric acid and **B)** 0.1 mM ascorbic acid. The addition of the interference compounds are indicated by arrows.

As depicted in Figure 4.4., the addition of uric acid did not interfere in the H_2O_2 calibration and it was performed following the same trend as shown in Figure 4.3. (without interference). On the contrary, the addition of ascorbic acid had a great impact on the formal potential of the electrode, which decreased about 200 mV. Therefore, the decrease of EMF generated a higher difference of potential between the initial potential and the final potential after H_2O_2 calibration, by means of an enhanced sensitivity towards H_2O_2. Thus, different ascorbic acid concentrations were tested to evaluate the effect on H_2O_2 determination. Table 4.1. depicts the sensitivities and

linear ranges obtained for H_2O_2 calibrations under the presence of the indicated concentrations of ascorbic acid.

Table 4.1. H_2O_2 sensitivity after the addition of ascorbic acid at different concentrations. The electrodes were tested in MilliQ water.

AA (M)	SENSITIVITY (mV dec^{-1})	LR ($log[H_2O_2]$ (M))
10^{-4}	103.5 ± 9.7	(-4 to -1)
10^{-3}	152.1 ± 31.7	(-3 to -1)
10^{-2}	34.7 ± 2.0	(-2.5 to -1)

As shown in the table, the effect of the addition of AA did not follow a linear tendency, thus, we were not able to conclude either there was a dependence or not between the electrodes sensitivities and AA concentration. However, in all cases the EMF_0 decreased 150-200 mV, and the sensitivity was significantly affected by the presence of AA. The greatest sensitivity was obtained when operating under 10^{-3} M of AA, while the widest linear range for H_2O_2 determination was achieved when using 10^{-4} M. With the exception of the 10^{-2} M AA, the tested concentrations resulted in better sensor characteristics. Nevertheless, we were able to observe the recovery of the initial potential to the original value once the ascorbic acid was removed from the cell, indicating that the influence of ascorbic acid is not permanent and stable in time, but reversible depending on its presence or absence. Since the presence of AA at a certain concentration improved the analytical performance of the electrode, experiments with the incorporation of AA as a component of the electrode modification was studied for further H_2O_2 calibrations, and it is described in the next section.

Redox state of the conducting polymer

From results described above and the ones obtained from CHAPTER 3, we considered the control over the redox state of the polymer to be a key factor regarding the performance and stability of the signal readout. Therefore, and taking into account the influence of ascorbic acid on the EMF and on the analytical performance, two different strategies were performed in order to reach a more reduced state of the polymer to achieve better analytical performances of the electrode.

The first approach consisted on mixing the AA with the commercial PEDOT:PSS and use it as an ink. Therefore, sensors were fabricated as abovementioned using PEDOT:PSS/AA instead of solely PEDOT:PSS. In general, potentiometric experiments did not show improved analytical performances of the electrodes. Table 4.2. depicts the sensitivities and linear ranges obtained for H_2O_2 calibrations with electrodes made of PEDOT:PSS together with the corresponding concentration of AA. Additionally, each sensor was covered by two different polymeric matrices; i.e. Nafion and PVB (1:10 in methanol) in order avoid the delamination of the ink from the substrate.

Table 4.2. Sensitivities and linear ranges for H_2O_2 calibrations with electrodes made of PEDOT:PSS blends with different AA concentrations, with either Nafion® or PVB coatings. The electrodes were tested in MilliQ water medium (N=4).

	NAFION® COATING		**PVB COATING**	
AA (M)	**10^{-3}**	**10^{-4}**	**10^{-3}**	**10^{-4}**
SENSITIVITY (mV dec^{-1})	35.2 ± 7.0	49.8 ± 3.2	50.6 ± 24.2	155.8 ± 2.4
LR (log[H_2O_2]) (M)	-3 to -1.5	-3 to -1.5	-3 to -1.5	-2.5 to -1.5

In almost all the cases, the linear range remained the same as in the previous section, and sensitivities were similar or even lower. Actually, only in one of the combinations the sensitivity towards H_2O_2 was improved almost three times, although the narrow linear range obtained and the lack of signal when tested in other media different from water, made us dismiss the electrode configuration for further experiments.

The second approach tested in order to change the redox state of the polymer was the use of different oxidizing and reducing agents mixed with the PEDOT:PSS and used as an ink as well. Massonet et al. [34] treated commercial PEDOT:PSS 1.3 wt % aqueous solution with different moderate reducing agents (Na_2SO_3 and $Na_2S_2O_3$) and strong reducing agents (TDAE and $NaBH_4$) in order to change the redox state of the CP and determined it by UV-Vis-NIR absorbance spectroscopy (Figure 4.5.). They demonstrated the improvement of the thermoelectric properties of PEDOT:PSS by reductive treatments.

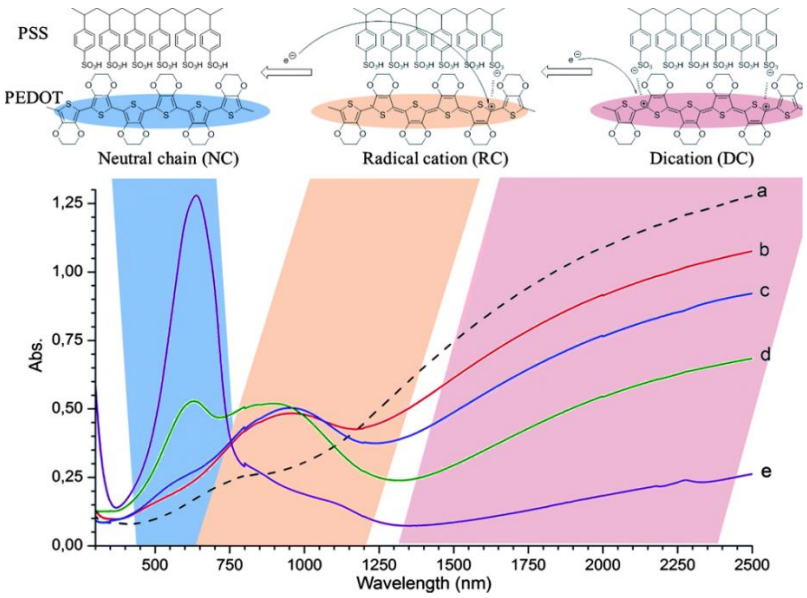

Figure 4.5. Top: chemical structures of PEDOT:PSS **(left)** neutral chain, **(center)** a radical cation charge carrier, **(right)** a dication charge carrier. Bottom: absorbance spectra of **a)** pristine PEDOT:PSS and thin films treated with **b)** $Na_2S_2O_3$, **c)** Na_2SO_3, **d)** $NaBH_4$, and **e)** TDAE (tetrakis (dimethylamino)ethylene. Reproduced with permission from ref. [34].

In our case, when using oxidizing agents such as NaClO, the ink turned to red, which suggested a possible degradation of the CP, since no significant differences were observed at the UV-Vis spectrum. Regarding reducing agents, although some variations in the UV-Vis spectra were observed (data not shown), the potentiometric experiments with such mixed inks (PEDOT:PSS with either Na_2SO_3, $Na_2S_2O_3$ or $NaBH_4$) were not carried out as expected since signal stabilization was never achieved. The incorporation of these compounds may turn the impedance of the deposited layer too high to allow potentiometric readouts.

Different strategies for depositing the layer of PEDOT:PSS

In order to explore other possibilities in the redox state of our conducting polymer, we also fabricated and tested the electrodes with two other commercial PEDOT:PSS (high conductive grade and low conductive grade). The difference relies on the amount of PSS added as a dopant, which actually makes the polymer more conducting (oxidized form) or less conductive (reduced form). Both types of PEDOT:PSS were drop-casted in paper-based electrodes in its pristine form. At the same time, all these ink combinations were also tested over a GCE to study a possible effect of the substrate on the potentiometric performances. In addition, the use of electropolymerized PEDOT:PSS on both substrates (paper and GC electrodes) was also considered for comparison.

In the case of the low conductive grade PEDOT:PSS, the delamination was clearly an issue when working both in paper and GC substrates. The addition of a polymeric coating such as Nafion or PVB prevented the delamination as abovementioned, but further potentiometric experiments could not be performed properly due to the instability of the signal readout. Stability was achieved only in some cases among the overall combinations, but no changes in the electromotive force were recorded during H_2O_2 calibrations. Thus, no further experiments were performed using low conductive grade PEDOT:PSS.

In the case of high conductive grade PEDOT:PSS, no delamination was observed when working either with or without coating layers. Although potentiometric experiments were performed as usual, the analytical performances of such electrodes did not improve the ones obtained with the commercial PEDOT:PSS 1.3 wt % in aqueous solution. Therefore, the use of this high conductive grade PEDOT:PSS did not contribute to improve any of the analytical parameters already achieved. Indeed, the obtained sensitivities towards H_2O_2 were lower compared to the firsts obtained with PEDOT:PSS 1.3 wt %.

Electropolymerization of PEDOT:PSS was successfully done both in paper and in GC electrodes. Figure 4.6. shows the characterization made by CV with ferricyanide/ferrocyanide solution before (grey) and after (blue) electrodeposition of the CP on each surface. Grey lines show the characteristic redox peaks Fe^{+2}/Fe^{+3} indicating the electroactivity of the cleaned surface allowing electron transfer between the electrode and the solution. Blue lines show the displacement of the same redox peaks and the increase of its intensity due to the polymer deposition on the surface. Usually, the blocking of the surface due to polymer deposition makes the intensity to decrease. However, since the deposited polymer is conductive, the more polymer is deposited the more current is recorded until reaching a saturation point.

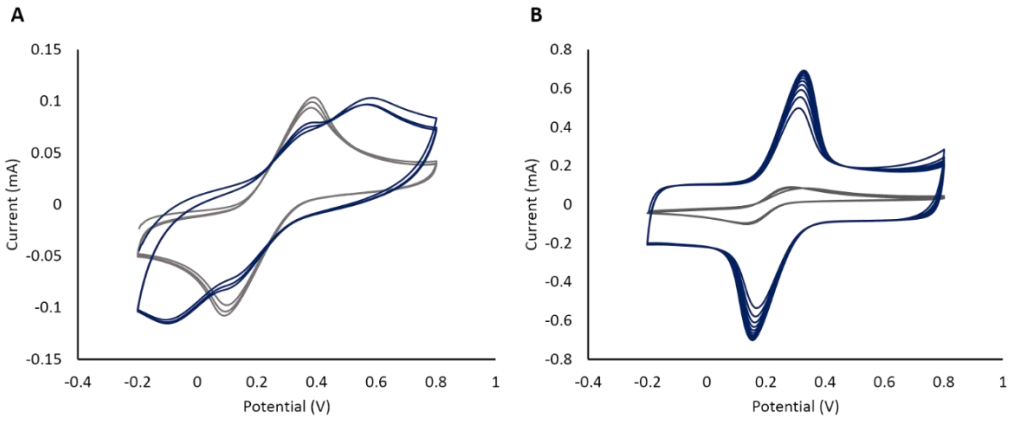

Figure 4.6. Surface characterization before and after PEDOT:PSS electropolymerization on **A)** carbon ink paper electrode and **B)** glassy carbon electrode.

Potentiometric measurements for H_2O_2 determination with paper-based electropolymerized PEDOT:PSS electrodes were performed. Nevertheless, the analytical performances did not provide any advantage over the drop-casted PEDOT:PSS paper-based electrodes.

In the case of glassy carbon electrodes with electropolymerized PEDOT:PSS, the potentiometric measurements showed improved results compared to the first obtained with drop-casted commercial PEDOT:PSS (Figure 4.7.). Both the sensitivities and linear ranges obtained with electropolymerized PEDOT:PSS were slightly higher (56.3 ± 5.2 mV dec^{-1} in a range between 10^{-4} and 10^{-1} M).

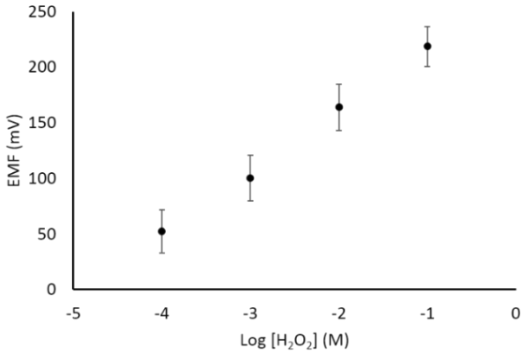

Figure 4.7. H_2O_2 calibration curve in water media of electropolymerized PEDOT:PSS on GCE (N=4).

In addition, electropolymerized electrodes provided an additional advantage over the drop-casted electrodes; no coating layers were needed to entrap the CP since electrodeposited films were already stable on the surface of the electrodes.

Different media

Since all experiments were carried out in MilliQ water, the electrodes were also tested in different media with different ionic strength or pH in order to evaluate the response to H_2O_2 under distinct conditions. GCE-based sensors were chosen to perform these experiments due to the higher stability they offer compared to paper-based sensors. Following the results obtained in the previous section, the response towards H_2O_2 in all these different media was tested with commercial PEDOT:PSS 1.3 wt %, high conductive grade PEDOT:PSS and electropolymerized films from EDOT and NaPSS.

NaCl 0.1 M was used to test the H_2O_2 response under a high ionic force medium. Commercial PEDOT:PSS formulations showed negligible responses towards H_2O_2 additions leading in poor analytical performances. On the contrary, electropolymerized PEDOT:PSS electrodes presented sensitivity values of 35.0 ± 9.0 mV dec^{-1} within a linear range from 10^{-2} to 10^{-1} M.

In acetate buffer 0.01 M at pH 4.5 the H_2O_2 calibration curves lead to sensitivities around 20 mV dec^{-1} in narrow ranges of 10^{-2} to 10^{-1} M in high conductive grade PEDOT:PSS (either pristine polymer, coated with Nafion or PVB). PEDOT:PSS 1.3 wt % was only stable and allowed signal recording when it was coated with PVB membranes, reaching sensitivity values of 28.0 ± 2.0 within a linear range from 10^{-3} to $10^{-1.5}$ M. Electropolymerized PEDOT:PSS electrode provided the widest linear ranges (10^{-4} to 10^{-1} M) with sensitivities of 49.0 ± 6.0 mV dec^{-1}.

Regarding PBS (0.1 M, pH 7.4) medium, potentiometric responses to H_2O_2 were almost negligible or null with drop-casted electrodes (both 1.3 wt % and high conductive grade PEDOT:PSS). Electropolymerized films reached sensitivity values of 30.9 ± 0.8 mV dec^{-1} but in a narrow linear range (10^{-2} to 10^{-1} M).

Artificial serum at pH 7.4 was also used to test the H_2O_2 potentiometric response. Commercial PEDOT:PSS 1.3 wt % undergo delamination immediately, while high conductive grade formulations were stable but with negligible responses to H_2O_2. In this medium, best results were obtained with electropolymerized electrodes achieving sensitivities of 52.9 ± 6.3 mV dec^{-1} within linear ranges from 10^{-4} to 10^{-1} M.

In conclusion, we observed that the solution pH, and thus, the ion concentration in solution, affects the reduction potential of hydrogen peroxide. Therefore, in some conditions above-mentioned, hydrogen peroxide may not have enough potential to undergo reduction and produce

a change in the redox state of the conducting polymer. Consequently, the electrochemical signal is hampered to be detected potentiometrically.

Taking into account these last results, electropolymerized PEDOT:PSS was clearly the most stable formulation under all the different media tested. Electrodeposited films did not undergo delamination and showed significant response corresponding to H_2O_2 calibrations in all conditions tested. Table 4.3. summarizes the analytical parameters obtained from H_2O_2 determinations performed with electropolymerized PEDOT:PSS films.

Table 4.3. Comparative table of different analytical parameters obtained from H_2O_2 calibration with electropolymerized PEDOT:PSS electrodes under different conditions.

	MILLIQ WATER	**NaCl 0.1 M**	**ACETATE pH 4.5**	**PBS pH 7.4**	**ARTIFICIAL SERUM pH 7.4**
LR (log [H_2O_2]) (M)	-3.5 to -1.5	-2 to -1	-4 to -1	-2 to -1	-4 to -1
SENSITIVITY (mV dec^{-1})	52.9 ± 6.3	30.9 ± 0.8	49.0 ± 6.0	35.0 ± 9.0	56.3 ± 5.2
LOD (log [H_2O_2]) (M)	-3.8	-2.2	-4.3	-2.3	-4.5
RESPONSE TIME (s)	100	400	250	200	200

Therefore, electropolymerized PEDOT:PSS on GCE was chosen as the electrode configuration to perform further experiments, in order to optimize sensor parameters.

Electropolymerized PEDOT:PSS on GCEs

Interference experiments were done with GCE/electropolyerized PEDOT:PSS to check which compound could compromise the performance of the sensor. From all the tested media, we chose artificial serum at pH 7.4 to perform all next experiments due to the complexity of its matrix and its similarity to human physiological serum.

Figure 4.8. shows the calibration curves corresponding to each interference. The addition of UA in the cell did not affect the initial EMF of the electrode, but decreased the sensitivity towards H_2O_2 in approximately the half (23.4 ± 6.7 mV dec^{-1} versus 56.3 ± 5.2 mV dec^{-1}) in the range of $10^{-3.5}$ to $10^{-2.5}$ M. The initial EMF decreased around 50 mV when dopamine was added in the cell, and

further H_2O_2 calibration was affected due to dopamine's presence, achieving sensitivity values no greater than 35.3 ± 6.9 mV dec^{-1} in the narrow range of $10^{-2.5}$ to 10^{-1} M. Under the presence of AA, the initial EMF decreased around 100 mV and further H_2O_2 calibration gave sensitivities of 60.9 ± 8.2 mV dec^{-1} in the range of 10^{-4} to $10^{-1.5}$ M. So far, the addition of AA improved the sensor performance towards H_2O_2, as occurred with paper based sensors previously.

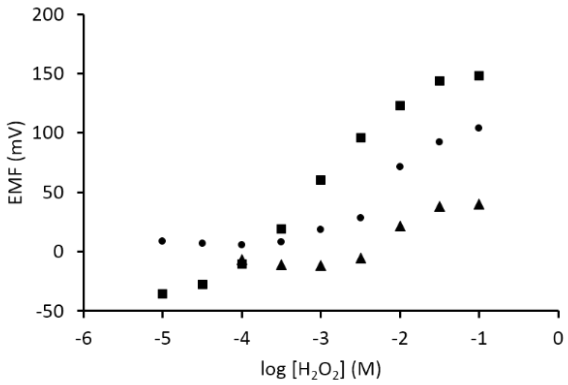

Figure 4.8. H_2O_2 calibration curves for electropolymerized PEDOT:PSS on GCE under the presence of ● 0.1 mM UA, ▲ 0.1 mM dopamine and ■ 0.1 mM AA.

Nevertheless, the rest of the tested substances also interfered to the signal response probably due to the electrochemical activity of the CP which results in an ionic and electronic equilibrium at the interface with the CP and the solution. The presence of such redox compounds hampered the development of further experiments considering further application in real samples with interference species.

CONCLUSIONS

In this chapter, the fabrication of a solid-contact conducting polymer-based potentiometric electrode has been described. Different formulations of the PEDOT:PSS, combined with different materials as substrates of the electrode have been developed and studied for the H_2O_2 determination in aqueous environments at different conditions. It was shown that in presence of AA, the detection of hydrogen peroxide was improved although the mechanism is not straightforward. Nevertheless, the detection is highly affected by the presence of other electroactive species in the media such as uric acid. Further optimizations did not improve the performance. Moreover, control experiments displayed sensitivity to H_2O_2 without the presence of

the conducting polymer. At that point, and due to the irreproducibility between CP-based GCEs, we could not ensure the reliability of the CP-based electrodes. Cracking or partial delamination of the conducting polymer in the surface of the electrode could be one of the reasons of the unreliable recorded signals. Such instability can also be caused by the formation of a thin aqueous layer between the electrode and the CP, which is usually responsible of potential drifts and irreproducibility of CP-based potentiometric sensors [17].

As we pointed out from results in the last chapter, we considered the control over the redox state of the conducting polymer a key factor for practical applications of such sensors. However, since the characterization of PEDOT:PSS was not fulfilled neither with UV-Vis spectroscopy nor FTIR spectroscopy, the redox state of the used polymer was not clearly determined. Therefore, the polymer could have been polymerized in one specific redox state but it could have varied and change its volume due to the absorption of small molecules after electropolymerization procedure. This uncontrolled parameter, could cause the irreproducibility and unrepeatability in the sensor response. In fact, Michalska et al [35] reported back to 1994 the influence of both the electrodeposition and conditioning steps on the open-circuit properties of CP films. While electropolymerzation is driven by the applied external electrical potential, in the conditioning process the chemical potential of the reactants in solution drives the reactions occurring at the electrochemical cell. Since electrodeposition conditions play an important role on the final properties of the CP film, (such as morphology, conductivity, etc.), the conditioning or soaking step also affects the CP characteristics, and therefore, the final analytical performance. Therefore, the lack of conditioning in our experimental part may be the reason behind non-reproducible results obtained due to a non-controlled re-distribution of charges within the polymer chain after the electropolymerization step [36].

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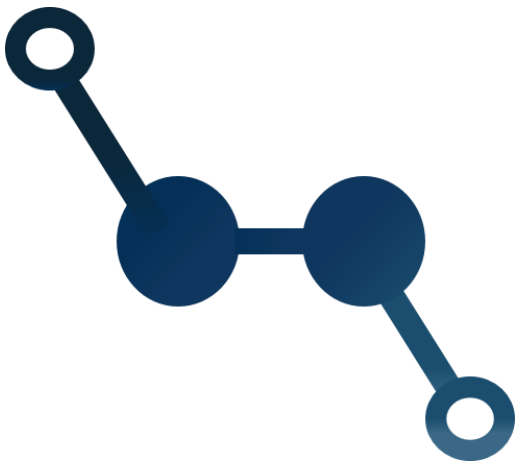
Marta Borràs Brull

PART II:

INDIRECT H_2O_2 DETERMINATION THROUGH ENZYMES



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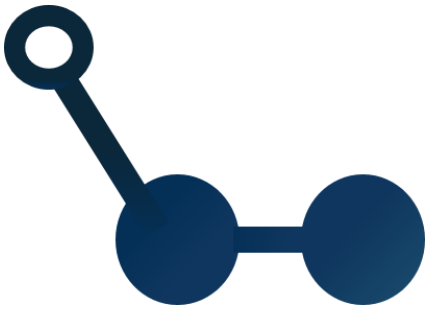
CHAPTER 5

GLUCOSE ENZYMATIC-CASCADE SENSOR ASSEMBLED ON MACRO- AND MICRO- ELECTRODES



UNIVERSITAT ROVIRA I VIRGILI
DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HYDROGEN PEROXIDE DETERMINATION
Marta Borràs Brull

The work described in this chapter was performed under the supervision of Dr. Elena Ferapontova, leader of the Electrochemical Biosensors and Bioelectrocatalysis Group, at the Interdisciplinary Nanoscience Center at Aarhus University (Denmark). The group is focused on the research on fundamental studies of electron transfer and interfacial properties of enzymes and nucleic acids and on the development of advanced technologies for biosensors, among other purposes.

The incorporation of enzymes on electrochemical biosensors provides selectivity towards a certain biomolecule. Detecting H_2O_2 from the reaction between a target and the corresponding oxidase enzyme provides a great opportunity to design platforms that may be then adapted for several targets. This work was focused on the assembly of enzymatic-based biosensors by studying their electron transfer properties in order to develop amperometric microsensors. Thus, the detection of H_2O_2 was performed to indirectly determine glucose.

INTRODUCTION

Although under certain stimuli lactate or ketone bodies can be used as energy substrates, it is known that glucose is the main energy source in mammalian brain. It is estimated than 25% of total glucose consumption from the human body is involved in cerebral functions [1]. Glucose metabolism provides the energy for physiological brain functions, the cellular maintenance and the generation of neurotransmitters [2]. There are also several intermediary metabolites, such as lactate, pyruvate and glutamate that are generated from glucose in the brain and can be oxidized for energy production. Disruptions on glucose pathways and metabolism may lead the brain to be sensible to many different diseases or brain disorders. Neuroglycopenia, cerebral ischemia phenomena, and Parkinson are considered to be related to the glutamate (and consequently to glucose) supply to the brain. Although it may not be the principal cause, energetic defects on pathophysiological mechanisms are important in neurodegenerative diseases [2].

The ability to measure glucose concentrations in human cerebral cortex has important implications in understanding the functions and signal transmission through the neural network in human brain. Several methods have been proposed for glucose monitoring, such as chromatographic techniques [3] and capillary electrophoresis [4]. However they are considered to be time-consuming and require sophisticated and expensive instrumentation. More recently,

different electrochemical biosensors based on enzymatic approaches have been reported [5]. These biosensors are usually based on the dispersion, adsorption or covalent cross-linking of the suitable enzyme onto an appropriate solid electrode. When using oxidase enzymes, the electrochemical measurement is based on the enzymatically generated hydrogen peroxide (H_2O_2) due to the oxidation of the substrate together with O_2 consumption. Amperometric oxidase-based biosensors are usually developed as bienzymatic sensors towards H_2O_2 detection. As explained in CHAPTER 1 (*equations 1.4. and 1.5.),* the oxidation of the substrate by enzyme 1 generates electrochemically active H_2O_2 that is subsequently oxidized by enzyme 2 (usually horseradish peroxidase (HRP)). Electrons from the last reaction are detected amperometrically and are directly proportional to the concentration of the original substrate. However, the application of a fixed potential for amperometric detection may become an issue due to the oxidation of other electroactive species present in the sample or surface fouling problems. The use of organic and inorganic mediators immobilized on the surface of the electrode has been developed to overcome this type of problems. In addition, the use of inorganic compounds to biomimic enzyme catalysis is currently studied as well. For instance, hemin is an iron-containing porphyrin derived from a haem group (a coordination complex consisting of an iron ion coordinated to a porphyrin acting as a tetradentate ligand, and to one or two axial ligands), responsible of using H_2O_2 as electron acceptor to catalyze different oxidative reactions. Hemin is stable in solution and is relatively inexpensive [6]. The role of hemin on the study of electron transfer processes in order to understand the kinetics and thermodynamics of biological redox processes lies on its ability to mimic peroxidase activity. Thus, hemin is used as mediator for amperometric detection of different species, as for example O_2, superoxides, tryptophan or H_2O_2 [7].

The aim of this work was to develop amperometric enzymatic sensors for glucose detection as an important neurochemical in bran. The use of micro-electrodes provides a less invasive and fast technique for the study of brain tissue and allows the study of what is known as *in vivo* electrochemistry [8]. Rapid measurements of this compound allow the study of the dynamics of the energy balance of the brain. In this context, we are willing to develop assays to be stable and be simple to use. While in macrosensors *eq. 1.4.* and *eq. 1.5.* from CHAPTER 1 occur as expected, in the case of micro-electrodes the determination of H_2O_2 generated by the first enzyme becomes an issue due to the diffusion of H_2O_2 in the solution before reaching the electrode surface. Therefore, the search for the fastest electron transfer mediator to avoid H_2O_2 diffusion was the first objective of the work. In this sense, the strategy was based on: first, using oxidase enzymes (glucose oxidase

(GOx)) as these enzymes are stable (being of extracellular origin) and faste and have stable covalently bounds mediators, and second, the use of hemin as mediator, taking advantage of its trend to be adsorbed on carbonaceous materials. The fact of using hemin instead of HRP could avoid any interaction between the electrode and the rest of the enzyme chain and provide a faster system for micro-electrode configuration.

EXPERIMENTAL

Materials and reagents

Graphite rods of spectro-grade ⅛ " (3 mm) x 12" (304 mm) (SGL Carbon AG Werk Rigsdorff, Bonn, Germany) type RW001, 3.05 mm diameter, fitted in Teflon holders, were used as working electrodes on macro-electrode approach and were polished on emery paper (Waterproof Silicon Carbinde paper, FEPA grade P1000). Micro-electrodes were fabricated using carbon fiber electrodes (Carbonstar-1, E10011-standard) and were purchased from Kation Scientific (Minneapolis, USA).

Glucose oxidase (GOx) from *Aspergillus niger* (type II-S, EC 1.1.3.4), hemin (chloroprotoporfhyrin IX iron-III, from bovine), glutaraldehyde (8% aqueous solution), D-glucose, polyethylenimine (PEI, 50% (m/v), M_w 750,000), sulfuric acid (H_2SO_4), and the buffer components, sodium chloride, mono- and di-basic sodium phosphate (NaH_2PO_4 and Na_2HPO_4, respectively) were obtained from Sigma Aldrich (Denmark). All reagents were of analytical grade and used without further purification.

Stock solutions of glucose were prepared and stored at 4 °C overnight before their use. H_2O_2 solutions were prepared immediately before measurements from 35 wt % H_2O_2 solution (Sigma Aldrich, Denmark). PBS buffer was prepared 20 mM of each sodium phosphate component, and NaCl 150 mM at pH 7. All solutions were prepared using 18.2 MΩ cm^{-1} double deionized water (Milli-Q water systems, Merck Millipore).

Electrochemical measurements and instrumentation

All electrochemical measurements were performed in a standard three-electrodes electrochemical glass cell with the spectroscopic graphite or carbon fiber corresponding working electrode, a platinum wire as counter electrode and a Ag/AgCl (3 M KCl) reference electrode

connected to a μAutolab potentiostat (Type III, Eco Chemie B.V., Utrecht, Netherlands) supported with NOVA (Type 1.8.17) software. Chronoamperometric measurements for glucose calibrations with micro-electrodes were supported with General Purpose Electrochemical System (GPES version 4.9.005). All measurements were performed at room temperature in 20 mM PBS/150 mM NaCl at pH 7. The reproducibility of the data was verified by measurements with at least three equivalently prepared electrodes.

Cyclic voltammetry was used to study electron transfer and it was mostly carried out within a range of -0.8V to +0.5V, using 1 cycle/experiment. Different scan rates (υ=V/s) were tested for each sample (20, 50, 100, 300, 500, 1000, 2000 and 5000 mV/s). All samples were tested under both aerobic and anaerobic conditions (using N_2 to de-aerate the cell).

Sensor fabrication

Macro-electrodes

Macro-electrode preparation was done by cutting the graphite rods into pieces of 2 cm approximately. Their disk surface was polished on emery paper grade P1000. Functionalization of the macro-electrodes was done by drop-casting 10 μL of hemin (10% DMSO) mixed with the corresponding amount of PEI (m/V) onto the polished electrode and left it dry under a plastic lid for 1.5 h. N_2 stream was also used to accelerate the drying process. Straightaway, 5 μL of 1 mg mL^{-1} of GOx solution was drop-cast on the graphite-hemin electrode and left at room temperature under a plastic lid for 2 h. After modification, the electrodes were rinsed with PBS buffer solution, inserted in the electrochemical cell. When not in use, the electrodes were stored with a drop of PBS buffer pH 7 on top, at 4 °C.

Micro-electrodes

Micro-electrodes consisted on 7 μm diameter carbon fibers with borosilicate glass as the insulating layer. The micro-electrode tip must be cleaned/rejuvenated by dipping it in a H_2SO_4 0.5 M solution for 15 min and after drying it has to be cleaned by dipping in MilliQ water for 15 more minutes. When not in use, the tip must be kept in distilled water or physiological saline (in case it dries, the forming microcrystals could destroy the tip's structure).

Functionalization of the micro-electrodes was done by dipping the protruding of the carbon fiber micro-electrode (Figure 5.1.) for 1 h at room temperature in a solution containing hemin mixed with PEI, followed by dipping in aqueous solution of glucose oxidase 0.5 U μL^{-1} . After the

coating, the electrodes were kept in a 5% glutaraldehyde chamber for 1 h for cross-linking. When not in use, micro-electrodes were kept at 4 °C.

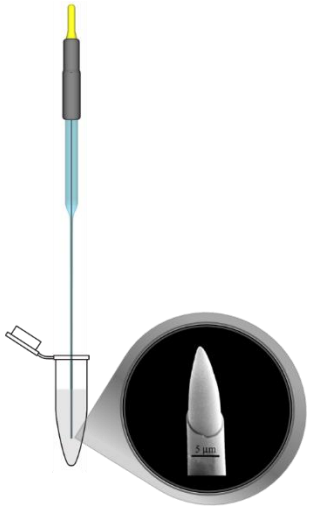


Figure 5.1. Micro-electrode functionalization by dipping. Inset: Tip magnification.

RESULTS & DISCUSSION

Electrode characterization

Characterization experiments were done to define and characterize the conditions of the system. CV was run at different scan rates with bare graphite electrodes under aerobic (Figure 5.2.A.) and anaerobic conditions (Figure 5.2.B.)

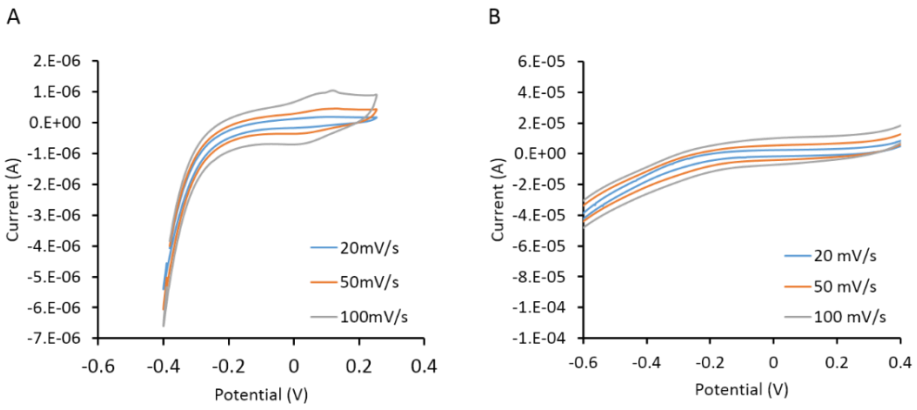


Figure 5.2. Representative CV recorded under **A)** aerobic conditions and **B)** anaerobic conditions, at -0.8 to +0.5 V, with 20 mM PBS/150 mM NaCl (pH=7) at different scan rates of bare graphite electrodes.

At higher scan rates, under the presence of oxygen we can observe the anodic and cathodic peaks corresponding to the oxidation and reduction processes (around +0.1 V) of hydroquinone-like groups formed on the surface of the graphite electrode, respectively [9]. However, working with N_2 atmosphere allows the elimination of the interference of oxygen during the experiments and therefore non-quinone reactions were observed. Same characterization experiments were also performed at different pHs (6, 7 and 8) and not significant differences on peaks nor on trends were found (data not shown).

Next, characterization experiments of modified graphite electrodes were done in order to check redox peaks of each component. Then CV under N_2 atmosphere was performed on bare graphite, graphite-hemin 1 mM and graphite-PEI (1, 5 and 10%). Well-defined and quasi-reversible redox peaks (around -0.2 V and -0.4 V) indicated the involvement of Fe^{3+}/Fe^{2+} redox couple coordinated in the porphyrinic ring, suggesting the favorable direct electron transfer between the electrode and the hemin molecules (Figure 5.3.). The oxidation of PEI was avoided by working at lower potential ranges than its redox potential (around +0.4 V), which allows its total integrity to immobilize and ensure hemin deposition on graphite electrodes.

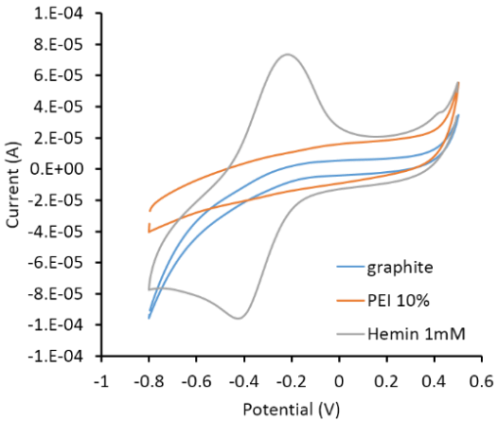


Figure 5.3. Representative CVs recorded under anaerobic conditions at -0.8 to +0.5V, with 20 mM PBS/150 mM NaCl (pH=7) at 50 mV/s of three different electrodes.

Henceforth, electrode modification was done by using different combinations of hemin/PEI looking for the configuration that conferred the fastest electron transfer to the system. Hemin concentrations were 0.01, 0.05, 0.1 and 0.5 mM, while PEI was tested with a mass/volume ratio of 1, 5 and 10%. Cyclic voltammetry was carried out to characterize the electron transfer rate of each configuration, and peak separation and rate constant (e^-/s) information was extracted from each

voltammogram by using Laviron's theory [10]. Figure 5.4. depicts an example of the shifting of potential on cathodic and anodic peaks of hemin redox activity at υ=300 V/s on two different hemin concentrations (mM) (0.5 black line, and 0.01 dotted line). Among all the tested combinations, electrodes modified with 0.5 mM hemin and 10% PEI were the ones which showed lower peak separation (14.7 mV versus 446 mV), indicating, at the same time, a higher rate constant (23.4 e^-/s versus 12.5 e^-/s), and thus, a faster electron transfer.

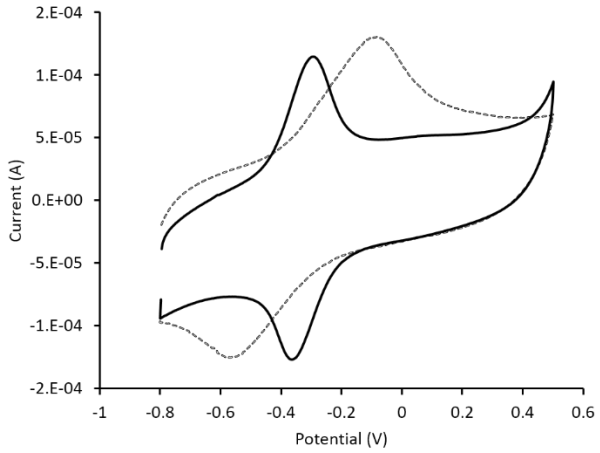


Figure 5.4. Representative CVs recorded under anaerobic conditions at -0.8 to +0.5V, with 20 mM PBS/150 mM NaCl (pH=7) at 300 mV/s of (—) graphite/0.5 mM hemin/PEI 10%, and (╍) graphite/0.01 mM hemin/PEI 10%.

H_2O_2 and glucose determination using macro-electrodes

Since graphite/0.5 mM hemin/PEI 10% was pointed out as the fastest configuration on electron transfer, hydrogen peroxide calibration were performed with macro-electrodes modified with such configuration in order to assess the viability of the system and define the electrochemical potential where hydrogen peroxide catalysis takes place under the experimental conditions. Figure 5.5. shows the cyclic voltammogram for the different hydrogen peroxide concentrations (0, 1, 3, 5, 7 and 10 mM).

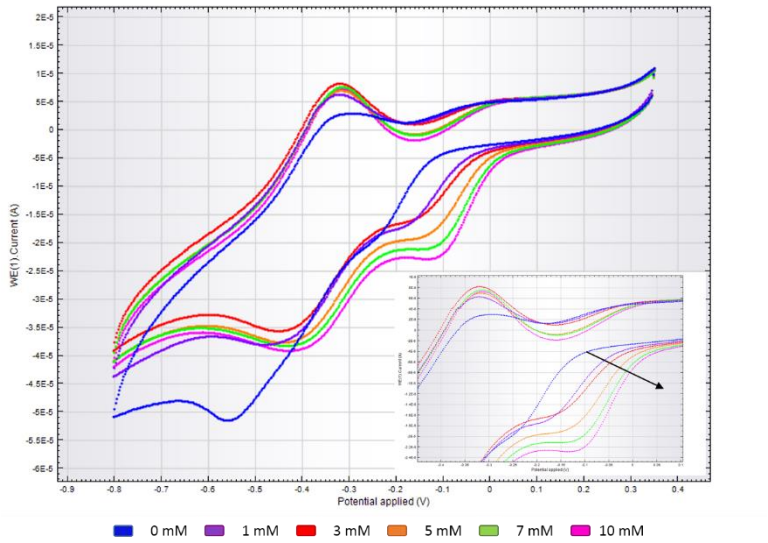


Figure 5.5. Cyclic voltammogram of increasing H_2O_2 additions on the electrochemical cell. Inset: magnification of the potential shifting indicated by an arrow.

As the concentration of hydrogen peroxide increased, so did the intensity of the cathodic current (ΔI was approximately 20 µA) by means of shifting the peak (around 50 mV) to more positive potentials. Nevertheless, the reproducibility of sensors was not completely achieved probably due to the polishing process of graphite, which was hand-made, and may affect the structure and organization at the top surface of the electrode that may provide more surface-volume ratio only in some electrodes, in which the signal was higher.

Glucose calibration was also performed with GOx-modified macro-electrodes to ensure the electron cascade was properly performed using GOx/hemin system. Since glucose oxidase requires the presence of oxygen to let the catalysis to take place, experiments were performed in aerobic conditions. Figure 5.6. shows the obtained voltammogram and the same trend as with H_2O_2 can be observed. Glucose additions were 0, 1, 3, 5, 7 and 10 mM following the same color legend as mentioned above.

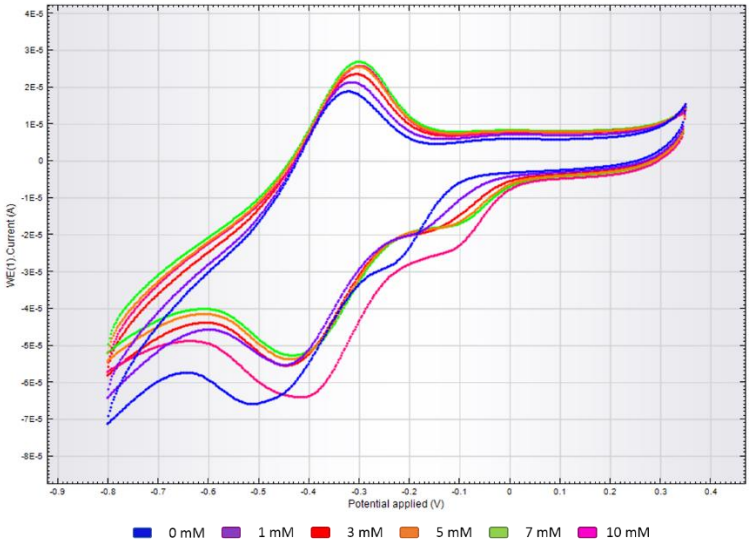


Figure 5.6. Cyclic voltammogram corresponding to increasing glucose concentrations.

From the characterization part with macro-electrodes we concluded on having a hemin based system that allowed a fast electron transfer from the reduced H_2O_2, which at the same time was generated by the GOx oxidation under increasing glucose concentrations. Actually, when working with HRP iron is stabilized on its inside in the form of Fe^{5+} showing its oxidation peak around 0.6 V. Since we worked with hemin, the catalytic part of HRP, the stabilization potential decreases and becomes Fe^{3+}, showing its peak around -0.2 V under the presence of oxygen. The addition of glucose in the solution, and the subsequent H_2O_2 production due to the oxidase reaction, makes the H_2O_2 reduction peak to shift to higher potentials until it reaches a saturation point. At the same time, O_2 reduction takes place around -0.4 V and the current signal decreases with increasing glucose concentration in the cell, due to the higher consumption of O_2 by the enzymatic reaction. On the contrary, the current decrease of the O_2 oxidation peak can be explained by the implication of hemin in the H_2O_2 and O_2 reduction, by means of having less hemin involved on the electron transfer at the electrode surface.

Glucose determination using micro-electrodes

After characterization on macro-electrodes, the system was extrapolated onto microelectrodes configuration. Microelectrodes were built as above-mentioned and chronoamperometry was used in order to determine the increasing glucose additions on calibration experiments. Since on H_2O_2 calibrations from macro-electrodes we were able to discern distinct H_2O_2 concentrations starting at 0 V, chronoamperometry was performed at a constant

potential of 0 V. In this technique, the mass transport is solely by diffusion, and the current-time monitored curve reflects the change in concentration gradient in the vicinity of the electrode surface. In the case of micro-electrodes, a time-independent current (proportional to the concentration) is obtained for times >0.1 s due to the large radial diffusion contribution.

Therefore, the glucose sensors were tested with different concentrations of glucose (0, 0.1, 0.5, 1, 2, 5 and 10 mM) at constant potential of 0 V while the glucose sensing current was recorded. Figure 5.7. shows the chronoamperograms (A) and calibration curves (B) for glucose determination by micro-electrodes.

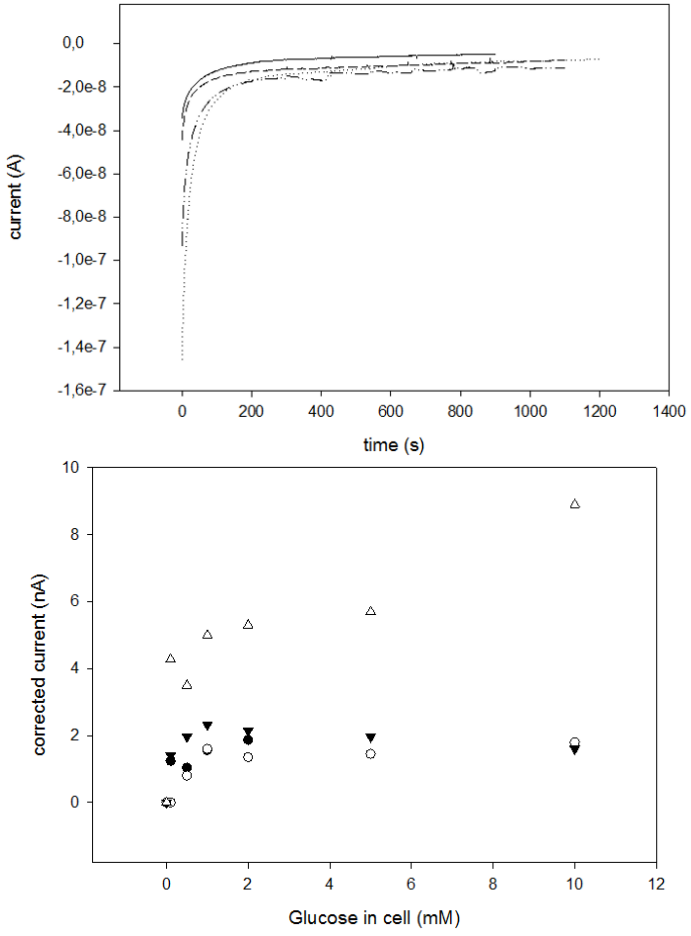


Figure 5.7. Glucose determination on 20 mM PBS/ 150 mM NaCl buffer at pH 7. **A)** Time trace of chronoamperometric sensing of glucose concentrations for four different modified carbon micro-electrodes. **B)** Corresponding calibration curves of the four different micro-electrodes.

Although the presented results were not conclusive, we were able to discern some correlation between current and glucose concentration at low concentrations (from 0 to 2 mM), but the electrodes reached saturation at higher glucose concentrations. More experiments are required to optimize the sensor response in order to reach significant analytical information (i.e. we were not able to characterize the sensor response in terms of sensitivity, linear range or limit of detection information, as basic analytical parameters). In addition, characterization experiments to ensure the presence of all the coatings (hemin and enzyme loading) on the microsensor tip should also be performed.

CONCLUSIONS

The electrochemical characterization of the graphite/hemin/PEI/GOx macro-electrodes was successfully performed and was encouraging to move the system to micro-electrodes. Although modified micro-electrodes seemed to allow glucose determination, the project was not completely fulfilled, and thus, we were not able to get the proper analytical information, such as sensitivity, linear range or limits of detection for such micro-electrodes. In addition, more characterization and optimization experiments are needed to achieve significant and valuable conclusions for the use of such glucose micro-electrodes.

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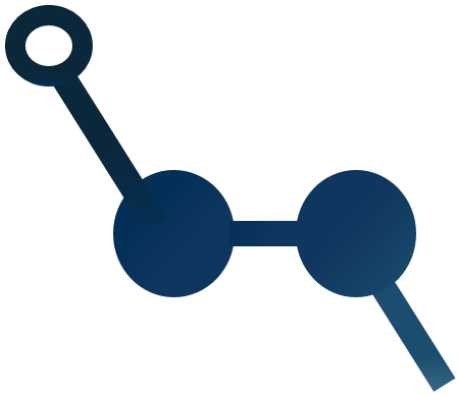
CHAPTER 6

CHARACTERIZATION AND VALIDATION OF A PLATINUM-BASED POTENTIOMETRIC SENSOR FOR GLUCOSE DETECTION IN SALIVA



UNIVERSITAT ROVIRA I VIRGILI
DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HYDROGEN PEROXIDE DETERMINATION
Marta Borràs Brull

INTRODUCTION

According to the International Diabetes Federation, the global prevalence of diabetes was estimated at 451 million cases in 2017, and following the continuous increasing trend over the last 40 years, it is expected to reach 693 million by 2045 [1]. Diabetes mellitus represents a group of metabolic disorders characterized by hyperglycemia. Uncontrolled blood glucose levels increase the risk of developing various serious vascular complications involving the heart, eyes, nerves and kidneys. Preventing these complications as well as improving patients' quality of life are key factors in diabetes management. Monitoring of blood glucose levels can help determine the most appropriate treatment in terms of dietary uptake or insulin dosage adjustment.

Blood glucose concentration is currently monitored by means of blood draw or finger-prick testing as a self-monitoring practice. Nevertheless, these invasive methods are painful and can generate anxiety or fear in the patients, who have to repeat the process from three to six times per day. This may lead them to forego the monitoring process, resulting in the inadequate control of glucose levels. Moreover, exposure to blood-borne pathogens such as hepatitis and HIV [2,3] poses a risk of infection to both patients and medical professionals. Therefore, non-invasive methods to monitor glucose levels have been studied to mitigate patient pain and discomfort. The correlation of blood glucose levels and body fluid glucose levels has been the focus of many studies in recent decades in attempts to develop non-invasive sensors that could replace phlebotomic techniques. For instance, numerous sensors have been developed to determine glucose concentrations in urine, tears, sweat or saliva [4–6].

Saliva is considered as advantageous biological fluid for use in the early diagnosis of many different cardiovascular, infectious and autoimmune diseases [7]. Although water is the main component of saliva, the solid content is based on inorganic ions, such as Na^+, K^+, Mg^{2+} or Cl^- among others, and organic substances such as proteins, carbohydrates or lipids. In addition, saliva also contains exfoliated epithelial cells, bacteria and bacterial metabolites which confer an additional complexity to the matrix. These molecules can be used as biomarkers for the early detection of some physiological and pathological changes in the human body, and have already been used in the detection of different cancers, malaria, HIV and the diagnosis of diabetes [7,8].

Since saliva is constantly produced, collecting and storing samples is a simple and low-cost process that is painless and safe both for patients and for medical personnel. At the same time,

saliva does not clot and is very stable. Therefore, salivary glucose determination provides a totally non-invasive and patient-friendly approach to monitoring glucose levels. However, some controversy remains regarding the correlation between glycaemia and salivary glucose [9–12] and some studies caution against using salivary glucose as diabetic diagnostic tool [13]. Although all studies confirm the fact that glucose concentration in saliva is higher in diabetic patient than in healthy ones, the differences on sample collection, glucose excretion rate and salivary flow hamper the correlation between salivary and blood glucose levels. These differences can be caused by multiple factors, such as medication, that can alter physiologic and metabolic regulation on diabetic patients. Nevertheless, many other studies have reported positive significant correlations between blood glucose levels and salivary glucose levels [14–19] with regression coefficient of 0.96, and thus, suggesting the determination of salivary glucose as reliable non-invasive method for predicting glucose concentrations in diabetics. The use of saliva as a diagnostic fluid requires highly sensitive sensors, since glucose concentrations in saliva are much lower than in blood (8 to 210 µM versus 3 to 30 mM, respectively). Many different techniques, such as liquid chromatography mass spectrometry, near and mid-infrared spectroscopy or fluorescence [20], for instance, have already been used to determine glucose in saliva matrices . Of all the techniques tested, electrochemical sensors [21] have been found to provide good sensitivity and selectivity, low operational costs and easy miniaturization and multiplexing for integration in portable devices. Within the electrochemical techniques, potentiometry has the advantages of simplicity of operation and instrumentation, low power consumption and the low-cost production of strips using, for instance, paper substrates, which facilitates miniaturization. Potentiometric devices can therefore be considered effective tools in the development of simple and affordable analytical platforms for use outside of the lab in keeping with the increasing trend towards self-monitoring in the field of health care and management. The combination of such instrumentation with the advantages provided by the use of paper-based substrates, as the accessibility and affordability, has made potentiometric paper-based analytical devices very attractive in the sensing community for the last decade [22–24]. Paper-based potentiometric sensors have been developed to determine multiple electrolyte concentrations of K^+, NH_4^+ and pH, [25] or Cl^-, Ca^{2+}, K^+ and Na^+ [26] among others. Indeed, our group has recently developed a fully integrated wireless electrochemical potentiometric platform to determine glucose in serum and whole blood based on the interaction of the hydrogen peroxide (H_2O_2) generated during the enzymatic redox reaction (using glucose oxidase (GOx)) with the Nafion-coated platinum paper-

based electrode [27]. The group has also reported on the use of the potentiometric enzyme-based electrode for the determination of glucose in fruit juices with high sensitivity and selectivity[28].

Taking advantage of the developed potentiometric electrodes and considering the advantages of using saliva as a means of non-invasive monitoring, this work aims to broaden the application of the paper-based potentiometric electrode with saliva determination as a new matrix of interest. Thus, the study presents the characterization and the analytical performance of the electrode for glucose detection in real human saliva. The results show good performance of the potentiometric electrode compared to a commercial enzymatic colorimetric assay, confirming the capability and versatility of the low-cost paper-based electrode to determine glucose levels in different human body fluids.

EXPERIMENTAL

Materials and methods

Whatman® Grade 5 qualitative filter paper was used for the fabrication of the working electrode. Nafion® perfluorinated resin solution (5 wt % in a mixture of lower aliphatic alcohols and water, 45% water), glucose oxidase (GOx) from *Aspergillus niger* type X-S, lyophilized powder, 100,000-250,000 units per g solid, hydrogen peroxide solution 30% (w/w) (H_2O_2), and D-glucose were purchased from Sigma-Aldrich. In all cases, Nafion solution was used as received. Analytical grade salts of potassium chloride, sodium chloride, calcium chloride, disodium phosphate, potassium phosphate and sulfuric acid were purchased from Sigma-Aldrich. All solutions were prepared using 18.2 MΩ cm^{-1} double deionized water (Milli-Q water systems, Merck Millipore).

Phosphate buffered saline (PBS) was prepared 0.1 M at pH 7.4 (100 mM Na_2HPO_4, 18 mM KH_2PO_4, 14 mM NaCl and 3 mM KCl) and used in all the experiments. Artificial saliva samples contained 10 mM KCl, 7.4 mM NaCl, 2 mM $CaCl_2$, 6.4 mM Na_2HPO_4, 2.5 mM $NaHCO_3$ at pH 7.4 [29].

Platinum sputtering was performed using a radiofrequency sputtering process (ATC Orion 8-HV, AJA International) operated at 3 mTorr for 65 s at 200 W on one side of a conventional filter paper to build the redox-sensitive electrode surface.

Electrochemical measurements

The electromotive force (EMF) was measured using a high input impedance (10^{15} Ω) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc., Malvern, PA, USA). A double junction Ag/AgCl/KCl 3 M reference electrode (type 6.0726.100, Metrohm AG) containing 1 M lithium acetate electrode bridge was used to study the working electrode. Laboratory measurements were taken using a 0.1 M PBS (pH 7.4) 4 mL cell at room temperature.

Fabrication of the enzymatic paper-based glucose sensor

The working electrode was built based on the procedure described in Cánovas et al. [27]. Briefly, the conducting platinum paper was cut into strips of 0.5 cm x 2.0 cm and then one strip was sandwiched between two 1.0 cm x 1.5 cm plastic masks (ARcare® 8565, Adhesives Research Inc., Limerick, Ireland). The top mask had a 3 mm diameter circular window to expose the electroactive platinized paper to cast the biosensing membrane and functionalize the electrode. A first layer of 7 µL Nafion solution was then drop cast and air-dried for at least 60 min at room temperature. Afterwards, 10 µL of a solution containing 20 mg mL^{-1} of glucose oxidase in distilled water was drop cast on top of the Nafion layer and left to dry for 24 h at 4 °C. Finally, a second 7 µL Nafion layer was drop cast on top in order to entrap the enzymatic layer and was also left to dry for 24 h at 4 °C. The electrodes (denoted as Pt/Nafion/GOx/Nafion) were kept at 4 °C when not in use. Figure 6.1. shows the schematic representation of the electrode.

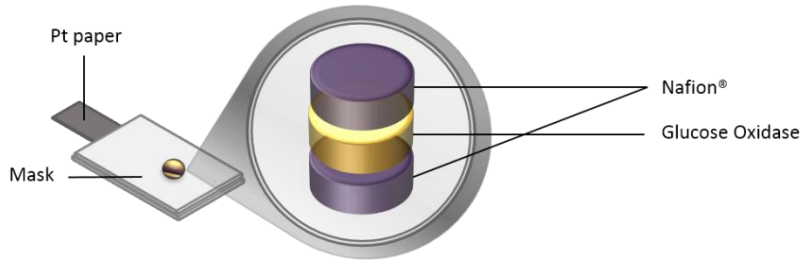


Figure 6.1. Schematic representation of Pt/Nafion/GOx/Nafion electrode.

Enzymatic assay

As a reference method, a commercial colorimetric glucose assay (glucose oxidase assay kit from Sigma-Aldrich) was used. Absorbance measurements were taken in an UV-Vis spectrophotometer (Agilent Technologies, Spain) with a 10 mm light path plastic cuvette (BRAND GMBH+CO KG, Germany). Real saliva was centrifuged and supernatant was collected to be used as

a control test, without reagents, to avoid great turbid differences between the control and test samples.

Analysis of real samples

Real saliva volumes were provided by different non-diabetic volunteers directly by spitting with no previous stimulation, and used as received without any treatment. Highly viscous saliva samples were dismissed to ensure precision in volume measurements. To simulate diabetic salivary glucose levels, D-glucose was artificially added to the samples at different concentrations (from 2 to 10 mM). The glucose oxidase colorimetric test was used as the standard method for the validation of the Pt/Nafion/GOx/Nafion potentiometric electrode.

RESULTS & DISCUSSION

Principle of detection and electrode response

The oxidation of D-glucose to gluconolactone uses oxygen as the electron acceptor and it is catalyzed by the enzyme glucose oxidase (GOx), generating hydrogen peroxide as a by-product of the reaction already mentioned in CHAPTER 2 (*eq. 2.3* and *2.4*). Since there is a direct relation between glucose consumption and hydrogen peroxide production, the glucose concentration can be calculated from the change in redox potential generated by the hydrogen peroxide production. However, most approaches, spectrophotometric or amperometric, for instance, require the use of a second enzyme or mediator (usually horseradish peroxidase) to break down hydrogen peroxide and produce a signal related to the glucose concentration, which increases the complexity of this method of indirect glucose detection. Nonetheless, since the potentiometric approach is based on monitoring the difference of potential generated at the boundary between the electrode and the solution, due to the generated gradient of electrochemical species, the hydrogen peroxide produced from the redox reaction can be directly detected potentiometrically without the need for a secondary enzyme. However, since platinum probes are sensitive to redox changes, they can also monitor any other redox-active substance present on the solution or sample, such as ascorbic acid, making the response non-selective due to the interference of the aforesaid substances. Therefore, in previous works, our group has demonstrated the improved performance of H_2O_2 detection based on platinum electrodes by using a Nafion coating [30]. The use of Nafion layers on platinum electrodes has proven to increase both sensitivity and selectivity parameters in

potentiometric H_2O_2 sensors. The increase in sensitivity is attributed to an enhanced potential due to the generation of a Donnan potential in the membrane interface, which is generated when there is a difference in the mobility of positive and negative charges. The use of Nafion increases the initial potential and the response to H_2O_2, which leads to an increased sensitivity dependent on the total ion concentration of the solution and the pH [30]. Selectivity is improved by Nafion by minimizing the interference of other negatively charged species. Moreover, the use of such a coating adds a level of complexity to the signal transduction, apart from the response dependence on the experimental conditions. Since the platinum surface is not homogeneous, the response to H_2O_2 varies depending on the Pt surface crystallinity, its interaction with the solution pH and composition and the different reactions involving H_2O_2 decomposition on the electrode surface, which may have parts acting simultaneously as anode or cathode due to the adsorption of oxygenated species on the electrode surface. Therefore, and taking into account that the detection principle is not fully understood yet, the mixed potential theory encompasses the combination from the balance of all the redox reactions and conditions interfering with the electrode potential, as the final potential of the system. Finally, the concentration of glucose is directly measured from the change in the mixed potential read-out generated by the hydrogen peroxide production near the platinum electrode.

Indeed, a detailed description and characterization of the H_2O_2 detection through Nafion layers is described in Parrilla et al. [30,31]. Recently, our group has reported the use of polyelectrolytes, such as Nafion, as a way to control the mixed potential of the platinum based electrode [32]. The open circuit potential of the Pt electrode is indeed shown to work under kinetic control of the oxygen reduction reaction.

Thus, experiments were conducted by monitoring the change in the electrochemical potential generated with increasing glucose concentrations. At an initial stage, the EMF was measured in a range from $10^{-4.5}$ to 10^{-2} M (0.03 to 10 mM) of H_2O_2 with sensors without enzyme (Pt/Nafion) to characterize the electrode response. Figure 6.2. shows the calibration plot of Pt/Nafion electrodes in 0.1 M PBS pH 7.4 and in artificial saliva medium for comparison, where the electrode potential decreases upon the addition of H_2O_2.

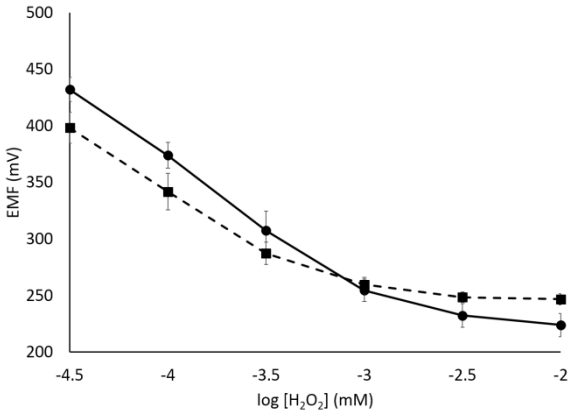


Figure 6.2. Calibration plot of Pt/Nafion electrodes with H_2O_2 additions in ●) 0.1 M PBS and ▪) artificial saliva medium. The error bars correspond to the standard deviation of 5 independent electrodes on each medium.

The Pt/Nafion electrodes showed a sensitivity to direct H_2O_2 additions of -119.3 ± 5.9 mV/dec in 0.1 M PBS pH 7.4 with a regression coefficient of 0.998, within a linear range from $10^{-4.5}$ to 10^{-3}. H_2O_2 sensitivity in artificial saliva was -98.6 ± 2.3 mV/dec with a regression coefficient of 0.979 (same linear range). The difference in the electrode performances is related to the mixed potential theory. As first described by Parrilla et al. [30], the electrode response depends on the pH of the solution and on the total concentration of the supporting electrolyte. Although in both PBS and artificial saliva pH is 7.4, the composition and thus, total ion concentration are different and affect the potential between the electrode and solution, demonstrating the need for strictly controlling the measurement conditions.

In the case of the Pt/Nafion/GOx/Nafion electrodes, the decrease in the electrochemical potential after glucose additions followed the same trend as when H_2O_2 was added, and the sensitivity to the H_2O_2 generated through the glucose oxidase reaction in 0.1 M PBS pH 7.4 was -93.2 ± 1.8 mV/dec with a regression coefficient of 0.985. The linear range in PBS measurements was from $10^{-3.5}$ to $10^{-2.5}$ M (0.3 to 3.2 mM), which is within the diabetic glucose saliva range values ($10^{-3.7}$ to $10^{-2.2}$ M or 0.2 to 6.3 mM) found in the literature [33–37]. Even though the thickness of the biosensing membrane is obviously higher in the Pt/Nafion/GOx/Nafion than in the bare Pt/Nafion electrode, the analytical performance is not compromised since the second layer of Nafion also helps in the immobilization of the enzyme by entrapment, as well as in the confinement of the produced H_2O_2 within the membrane, avoiding the leaching of both the enzyme and the by-product.

Taking into account that the diabetic salivary glucose range exceeds the linear range of our electrode and its complex matrix may influence analyte quantification, experiments with artificial saliva samples were done considering the dilution of the sample with 0.1 M PBS in order to, first, be able to detect the glucose in samples of saliva within the linear range of our potentiometric electrode, and second, study the matrix effect behavior of the final potential of the electrode. Artificial saliva containing 10 mM glucose was diluted with 0.1 M PBS pH 7.4 to different concentrations within the linear range of the potentiometric sensor ($10^{-3.5}$, 10^{-3} and $10^{-2.5}$ M or 0.32, 1 and 3.16 mM) in order to evaluate the analytical performance of Pt/Nafion/GOx/Nafion for glucose prediction in saliva matrix. An initial glucose calibration at 0.1 M PBS pH 7.4 was required to settle the calibration curve equation as the reference for further glucose predictions made with the artificial saliva samples (Figure 6.3.). Before the first glucose addition in Figure 6.3. we made sure that the signal was stable and the EMF was constant. Henceforth, the other glucose additions were done every 300 s. Electrodes were rinsed with double deionized water between each artificial saliva glucose prediction in order to clean the electrode surface.

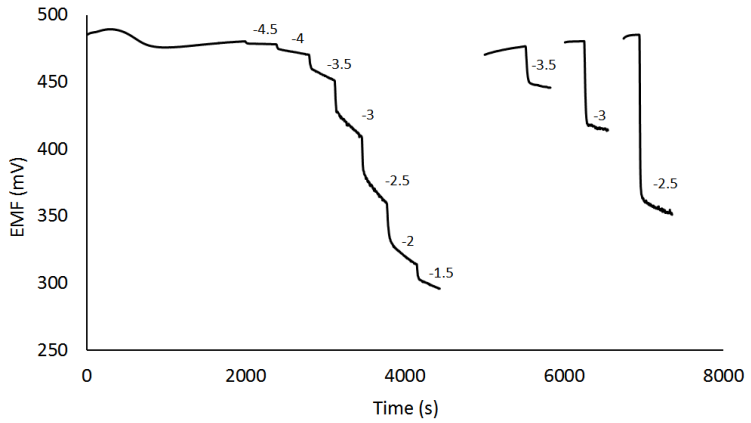


Figure 6.3. Time trace of glucose calibration in 0.1 M PBS pH 7.4 and following glucose predictions in artificial saliva pH 7.4 at $10^{-3.5}$, 10^{-3} and $10^{-2.5}$ M glucose.

Table 6.1. shows the comparison between the theoretical and experimental values of glucose concentrations from predictions shown in Figure 6.3., showing the recoveries and dilution factors needed in each case. Potentiometric experimental values are given as an average and their corresponding standard deviation from 23 different electrodes is also shown.

Table 6.1. Comparison between theoretical and potentiometric values for 0.32, 1 and 3.16 mM glucose concentrations in artificial saliva (N=23).

DILUTION FACTOR	THEORETICAL [GLUCOSE] (mM)	EXPERIMENTAL [GLUCOSE] (mM)	% RECOVERY
1:20	0.32	0.36 ± 0.05	113
1:10	1.00	0.99 ± 0.29	98
1:2	3.16	5.22 ± 2.97	165

These results confirm the influence of the matrix composition on the electrode potential. As the dilution factor decreases, the bias and deviation from the reference value increases due to the interference of other electroactive compounds from the matrix with the final potential of the system. In this case, the signal is enhanced, resulting in an erroneous final glucose quantification that compromises both precision and selective detection. In contrast, potentiometric predictions were more precise and accurate the higher the dilution factor. Higher dilutions imply less matrix load in the cell, allowing a more homogeneous medium with control over experimental conditions, such as pH or ionic strength of the solution. They minimize the effect of the interfering compounds from the complex saliva matrix on the final electrode potential by stabilizing it with the PBS buffer.

An intrinsic advantage of diluting the samples is reflected in the reproducibility and repeatability of the measurements, where the useful life of the sensors can be prolonged due to the reduced number of interfering species interacting with the sensing electrode surface. This is reflected by the low relative standard deviation (RSD) of initial EMF (EMF_0) between calibrations, which were less than 1.7% in all the cases. However, this does not represent a disadvantage since the electrodes are built to be disposable, in keeping with the increasing trend of single-use low-cost point-of-care devices for self-monitoring and management [23,38].

In addition, repeatability and reproducibility among sensors on glucose calibrations were also evaluated in 0.1 M PBS pH 7.4 medium. Figure 6.4.A. depicts the relative EMF in % of the different glucose additions compared to the logarithm of the glucose concentration, from three consecutive calibrations with four different sensors. Standard deviation is also represented and indicates the excellent repeatability of the measurements, and suggests the reusability of the electrodes for multiple measurements (at least three) while maintaining the same electrochemical response for each glucose concentration. Initial potential recoveries were 98.7% ± 1.2 for the

second and 94.9% ± 1.5 for the third calibration compared to the original EMF_0 from the first calibration, resulting in an average RSD of 1.4%.

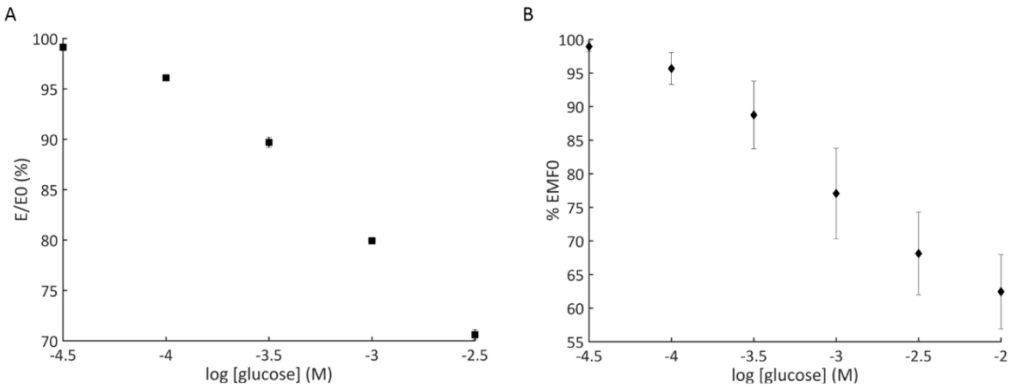


Figure 6.4. A) Measurement repeatability. Calibration plot of three glucose calibrations represented as % of the EMF_0. The error bars correspond to the standard deviation of 4 independent sensors **B)** Sensor precision at different days represented as the relative EMF compared to EMF_0 of each glucose concentrations from 80 different sensors.

Moreover, Figure 6.4.B. shows the corresponding average and standard deviation in % of the EMF at each glucose addition from glucose calibrations made with 80 individual sensors. The intermediate precision RSD from calibrations of 80 sensors on different days varies from an average of 2.9% for concentrations below $10^{-3.5}$ M to 8.8% for concentrations above $10^{-3.5}$ M.

Table 6.2. provides a comparison of the analytical performances of the potentiometric electrode described in this study with those of other recently reported electrochemical glucose sensors with different electrode configurations for glucose determination in real saliva matrices. Although the limit of detection is two orders of magnitude higher than the other examples, including amperometric ones (which usually give lower limits of detection than potentiometric sensors), the Pt/Nafion/GOx/Nafion provides the highest upper limit of the linear range. In this way, the sensor fits the purpose of determining glucose concentrations of diabetic people, which tends to be higher than healthy individuals. In comparison, the Pt/Nafion/GOx/Nafion electrode also exhibits good sensitivity for glucose detection in saliva and provides the intrinsic advantages of simplicity and low power consumption of the potentiometric devices.

Table 6.2. Comparison of analytical performances from different salivary glucose electrochemical based sensors.

WORKING ELECTRODE	TECHNIQUE	SENSITIVITY	LINEAR RANGE (μM)	LIMIT OF DETECTION (μM)	REF.
Pt/PAA/SWCNT/Cs/AuNPs/GOx	amperometric	61.4 μA mM^{-1} cm^2	17 - 810	5.60	[17]
Tin bronze	amperometric	77 μA mM^{-1} cm^2	20 - 320	4.70	[39]
GCE/IrO_2@NiO/Nafion	amperometric	1439.4 μA mM^{-1} cm^2	0.5 - 2500	0.31	[40]
SPCE/AuNPs/pTBA/MIP	potentiometric	76.6 mV/dec	0.32 - 1000	0.19	[41]
Pt/Nafion/GOx/Nafion	potentiometric	-93.2 ± 1.8 mV/dec	316 - 3160	180.00	This work

Pt – Platinum // PAA – Poly (allylamine) // SWCNT – Single wall carbon nanotubes // CS – Chitosan // AuNPs – Gold nanoparticles// GOx – Glucose oxidase // GCE – Glassy carbon electrode // IrO_2 – iridium oxide // NiO – Nickel oxide // SPCE – Screen printed carbon electrodes // pTBA - poly (2,2' :5'5"-terthiophene-3' –*p*-benzoic acid) // MIP – molecular imprinted polymer.

Analysis of real samples

The Pt/Nafion/GOx/Nafion potentiometric electrode was validated by comparing its results with the results from a commercial enzymatic assay for glucose determination. Five different saliva samples were obtained from non-diabetic volunteers, with no restrictions on sample collection. Saliva collection was not induced, and neither fasting conditions nor differences in salivary gland production were considered for fluid extraction. Since non-diabetic people have low glucose concentrations in saliva, D-glucose had to be added to reproduce diabetic glucose levels (from 2 mM to 10 mM). In the potentiometric approach, a two-point calibration curve with glucose standards corresponding to both limits of the linear range of the sensor was used to determine the concentration of glucose. Saliva samples (2, 4, 6, 8 and 10 mM) were diluted 1:2, 1:4, 1:7, 1:10 and 1:13, respectively, to fit in an intermediate detectable concentration (1 mM) of the linear range of the potentiometric sensor. The same procedure was carried out with the colorimetric approach, diluting each sample by factors of 1:8, 1:16, 1:24, 1:32 and 1:40, respectively, to reach a final glucose concentration of 0.25 mM which fit in the linear range of the commercial kit. Figure 6.5. shows the comparison between the potentiometric and the commercial enzymatic assay results. As expected from previous results obtained with artificial

saliva, the matrix effect is enhanced at lower dilution factors in the potentiometric electrodes leading to inaccurate concentration measurements where dilutions below 1:4 are required. Indeed, this effect was also evaluated by monitoring the pH and the conductivity of the solution during the potentiometric experiments in order to monitor possible changes in solution parameters that may affect the final potential read-out of the electrode. Since usual saliva pH ranges from 6 to 7.5 and the dilution buffer used was at pH 7.4, the pH of the solutions remained almost constant among all the different glucose concentrations tested (pH 7.40 ± 0.04). Meanwhile, the conductivity remained constant with a value of 23.4 ± 1.9 mS cm^{-1} in all cases except from dilution 1:2, which showed a decrease of 32% compared to the initial solution conductivity. It is not surprising then that changes in solution parameters due to the influence of the matrix compounds and characteristics may affect the charge distribution on the electrode membrane interfaces, resulting in an interfered change of potential, and thus, an erroneous glucose quantification.

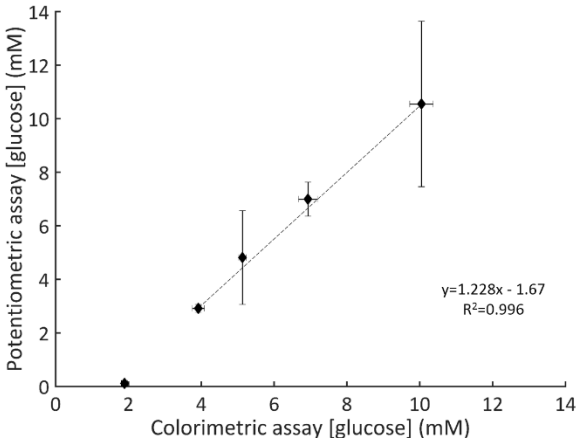


Figure 6.5. Comparison of glucose determination in five real saliva samples determined by potentiometric sensor (mean ± S.D. N=10) vs. a commercial enzymatic assay (mean ± S.D. N=3). Linear regression corresponds to four values (from 4 to 10 mM).

In contrast, the influence of saliva matrix was diminished when operating with dilutions of higher factors (above 1:4), which actually introduces fewer matrix components into the system. In these cases, neither pH nor conductivity changed significantly, and thus, glucose was properly quantified with the potentiometric Pt/Nafion/GOx/Nafion electrodes.

Our results show that the Pt/Nafion/GOx/Nafion electrode is able to accurately quantify glucose content in real saliva matrix with a dilution factor higher than 1:4. Changing the dilution

buffer to one that could maintain optimum solution conditions without compromising the simulated physiological conditions may be one way to overcome issues in samples with low dilution factors. However, since salivary glucose levels in diabetic patients are usually high (reaching maximum concentrations of around 6.3 mM), the Pt/Nafion/GOx/Nafion electrode could be used to monitor glucose in saliva with the proper dilution factor without much inconvenience.

Therefore, and taking into account the results from 4 to 10 mM (corresponding to dilutions higher than 1:4), we performed a statistical study to validate our results. To check if the potentiometric and the commercial enzymatic results are comparable over the tested linear range (4 to 10 mM), one has to check if the coefficients of the regression line would be comparable to the coefficients of the theoretical regression line obtained if the results in comparison were identical (intercept=0 and slope=1). The joint confidence interval for the intercept and the slope of the regression line [42] consisting of verifying the presence of the theoretical point (0,1) within the limits of the joint confidence region of the experimental intercept and slope was used to compare the results of the two methods. As Figure 6.6. shows, since the theoretical point (0,1) is within the limits of the joint confidence region for an α significance value of 5% we can conclude that the potentiometric and the commercial enzymatic results are comparable for the interval tested (4 to 10 mM).

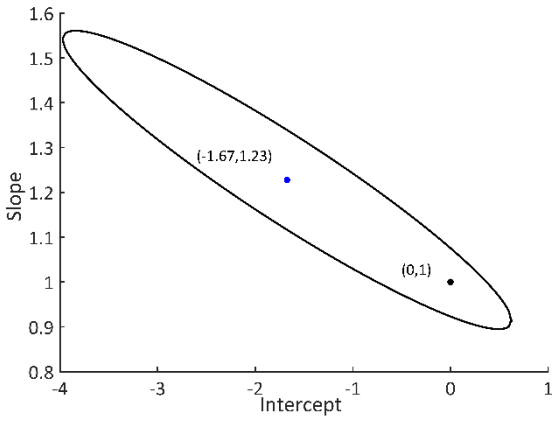


Figure 6.6. Joint confidence region plot comparing the slope of the regression line from validation process with enzymatic and potentiometric methods against the theoretical one

CONCLUSIONS

We have described the characterization and validation of a potentiometric enzyme-based electrode for the determination of glucose in human saliva. The combination of the potentiometric approach with a paper-based sensor, together with the use of Nafion to improve the analytical parameters, represents a simple and low-cost alternative for glucose detection in human saliva. Since saliva has been the focus of many studies into early diagnosis and glucose monitoring for decentralized and self-monitored health, the potentiometric sensor may be an effective alternative tool for that purpose. Results showed the potentiometric approach to be comparable to a conventional enzymatic commercial assay within an interval of glucose concentrations. The definition of this interval comes from the matrix effect that can somehow be modulated by diluting the sample. We have demonstrated accurate glucose quantification with dilutions higher than a factor of 1:4. Nevertheless, it is worth mentioning that real saliva samples were used as received without any pretreatment, which may have helped to broaden the interval of operation by decreasing the matrix effect.

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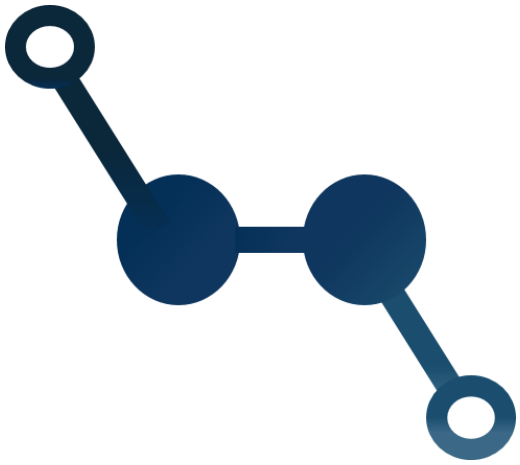
CHAPTER 7

CONCLUSIONS AND FUTURE PROSPECTS



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CONCLUSIONS AND FUTURE PROSPECTS

This doctoral thesis was designed to explore the development of low-cost sensors for hydrogen peroxide determination due to its important implications in nature and its use in various fields. While low-cost feature was ensured by the use of non-expensive substrates and techniques requiring low power consumption, reliable hydrogen peroxide determination was not always reached. In fact, we can divide the conclusions following the same main blocks of the thesis:

- On one hand, we addressed the direct detection by taking advantage of the electrical properties of conducting polymers. According to the conducting polymer features, we based our research aiming at causing a variation in the conducting polymer backbone that would lead in a detectable change in the corresponding signal readout. Although having reached hydrogen peroxide detection in both tested strategies, namely conductometric (CHAPTER 3) and potentiometric (CHAPTER 4) approaches, the exact mechanism of the conducting polymer-hydrogen peroxide interaction is still beyond our reach, since in practical terms, reliable and reproducible sensors were not achieved.

- On the other hand, the indirect hydrogen peroxide determination was successfully done by detecting glucose through different strategies; amperometric (CHAPTER 5) and potentiometric (CHAPTER 6) approaches. Despite the first project was not completed, the glucose detection using micro-electrodes configuration was achieved. Regarding the potentiometric approach, we have actually broaden the application of the purposed platinum paper-based electrode for glucose determination in saliva, introducing an alternative methodology for the non-invasive diagnostics.

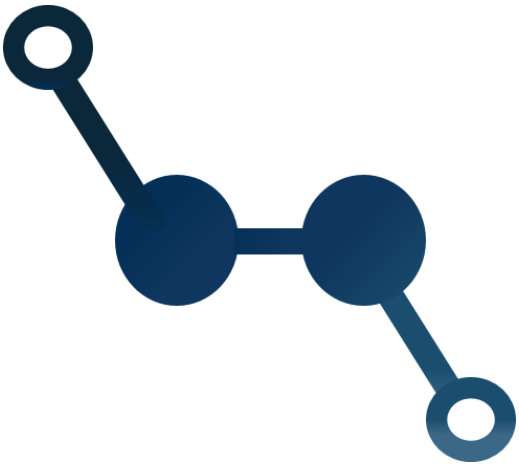
Nevertheless, there are many challenges beyond this thesis in order to improve the analytical performance of the developed electrodes:

- Regarding conducting polymer incorporation into electrodes, an exploratory path towards conducting polymer interaction with hydrogen peroxide is required to a better understanding of the mechanism. This would allow adapting sensor construction and consequently modifying the resulting analytical parameters. Future options to keep workin on could be based on fisrt, the development of a methodology that would ensure polymer adhesion on the substrate to facilitate the experimental procedures as well as the conducting polymer characterization in order to tailor

its characteristics by controlling its synthesis. The specific redox state in which the polymer is synthetized is also an important parameter to control and tune depending on the desired sensors characteristics. In addition, the characterization of the recognition event in different conditions is also an essential consideration that can pave the way of the final purpose. Although having some intrinsic limitation that can hamper conducting polymer application in sensing field, the combination of controlled conducting polymer characteristics together with the use of suitable materials for sensor assembly, can be improved to finally achieve a robust approach for an enhanced hydrogen peroxide determination.

- Concerning glucose detection, the incorporation of enzymes provides attractive analytical features such as the exceptional selectivity in complex matrix, good reproducibility and low limits of detection. Nevertheless, and in order to achieve a sensing platform able to contribute in the advancement of non-invasive monitoring platforms, optimizations on the analytical parameters of the developed sensors should be performed. Future works could entail non-diluted glucose detection by tuning the operational range of the sensor, the consideration of more complex matrices that could hamper reliable glucose detection in real samples, or even the use of other body fluids to broaden the applicability of the developed electrode.

APPENDICES



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APPENDIX 1

LIST OF ABBREVIATIONS

AA – Ascorbic acid

AC – Alternating current

Ag – Silver

AgCl – Silver chloride

AgNPs – Silver nanoparticles

Au – Gold

AuNPs – Gold nanoparticles

$BminPF_6$ – 1-Butyl-3-methylimidazolium hexafluorophosphate ionic liquid

C – Carbon

$C_2H_3NaO_2$ – Sodium acetate

$C_3H_4NaO_3$ – Sodium L-lactate

Ca^{2+} – Calcium cation

$CaCl_2$ – Calcium chloride

$CaCO_3$ – Calcium carbonate

CE – Counter/auxiliary electrode

CH_3COOH – Acetic acid

ChOx – Cholesterol oxidase

Cl^- – Chloride anion

CO_2 – Carbon dioxide

COOH – Carboxilic acid

CPs – Conducting polymers

Cs – Chitosan

CTA^+ – Hexadecyltrimethylammonium cation

CV – Cyclic voltammetry

DA – Dopamine

DC – Direct current

DOI – Digital Object Identifier

EMF – Electromotive force

EMF_0 – Initial potential

etc. – "Et cetera"

FTIR – Fourier-transform infrared spectroscopy

GA – glutaraldehyde

GCE – Glassy carbon electrode

GLC – D-glucose

GO – Graphene oxide

GOx – Glucose oxidase

H_2O – Water

H_2O_2 – Hydrogen peroxide

H_2SO_4 – Sulfuric acid

Hb – Hemoglobin

HIV – Human immunodeficiency virus

HRP – Horseradish peroxidase

IL – Ionic liquid

In_2O_3 – Indium (III) oxide

IrO_2 – Iridium oxide

ISE's – Ion-selective electrodes

ITO – Indium tin oxide

K^+ – Potassium cation

APPENDICES

K_2PtCl_4 – Potassium tetrachloroplatinate (II)

$K_3Fe(CN)_6$ – Potassium hexacyanoferrate (III)

$K_4Fe(CN)_6$ – Potassium hexacyanidoferrate (II)

KCl – Potassium chloride

KH_2PO_4 – Potassium phosphate

LOD – Limit of detection

LOx – Lactate oxidase

MES – 2-(N-morpholino)ethanesulfonic acid

Mg^{2+} – Magnesium cation

$MgCl_2$ – Magnesium chloride

MIP – Molecular imprinted polymer

MoO_3 – Molybdenum trioxide

MWCNT-COOH – Multi-wall carbon nanotubes functionalized with carboxyl groups

N – Number of electrodes

N_2 – Nitrogen

Na^+ – Sodium cation

Na_2HPO_4 – Disodium phosphate dibasic

$Na_2S_2O_3$ – Sodium thiosulfate

Na_2SO_3 – Sodium sulfite

$NaBH_4$ – Sodium borohydride

NaCl – Sodium chloride

NaClO – Sodium hypochlorite

$NaHCO_3$ – Sodium bicarbonate

NaPSS– Sodium polystyrene sulfonate

NCR – Normalized current response

n-doping – Negative doping

NH_3 – Ammonia

NH_4^+ – Ammonium

NiO – Nickel oxide

NIR – Near-infrared spectroscopy

NO_2 – Nitrogen dioxide

NP – Nanoparticles

O_2 – Oxygen

OECTs – Organic electrochemical transistors

P3MTp – Poly-3-methylthiophene

PAA – Poly (allylamine)

PANI – Polyaniline

PB – Prussian Blue

PBS – Phosphate buffered saline

PCPy – Poly (pyrrole-2-carnpxylic acid)

PdBI-co-HKCN – Bis(2-pyrdylimino) isoindolato-palladium complex

p-doping – Positive doping

PEDOT – Poly(3,4-ethylenedioxythiophene)

$PEDOT^+$ – Oxidized form of PEDOT

$PEDOT^0$ – Neutral form of PEDOT

PEI – Polyethylenimine

PET – Polyethylene terephthalate

PFLO – Poly(9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl) end capped with 2,5-diphenyl-1,2,4-oxadiazole

PGMA – Poly(glycidyl methavrylate)

PHEMA – Poly(2-hydroxyethylmethacrylate)

PMSA – Poly (styrene-alt-maleic anhydride)

POT – Poly-(octylthiophene)

PPy – Polypyrrole

PSS – Poly (styrene sulfonate)

Pt – Platinum

pTBA – poly (2,2' :5'5"-terthiophene-3' –*p*-benzoic acid)

PtNPs – Platinum nanoparticles

PVA – Polyvinyl alcohol

PVB – Polyvinyl butiral

R – Recovery

R_B – Bulk resistance

R_C – Contact resistance

R_{ct}– Resistance of charge transfer

RE – Reference electrode

rGO – Reduced-graphene oxide

R_S – Surface resistance

RSD – Relative standard deviation

SnO_2 – Tin (IV) oxide

SPCEs – Screen-printed carbon electrodes

SPE – Screen-printed electrode

SPGE – Screen-printed gold electrodes

SWCNT – Single wall carbon nanotubes

TDAE – Tetrakis(dimethylamino)ethylene

TiNTAs – Titanium oxide nanotubes arrays

TiO_2 – Titanium oxide

TNT – Titanium oxide nanotubes

UA – Uric acid

UOx – Uricase

UV-Vis – Ultraviolet and visible

V – Volts

VOCs – Volatile organic compounds

WE – Working electrode

A – cross-sectional area

c_T – Analyte concentration

D – Diffusion coefficient

d – Diffusion layer thickness

e^-– Electron

E – Potential / voltage

E_{ox} – Oxidized form of the enzyme

E_{red} – Reduced form of the enzyme

$[E_0]$ – Total enzyme concentration

F – Faraday constant

H^+ – Hydrogen proton

I_d – Steady-state current under diffusion control

$I_l \; or \; i$ – Current

K_M – Michaelis Menten constant

k_{-1} – Equilibrium constant inverse of reaction 1

k_1 – Equilibrium constant reaction 1

k_2 – Equilibrium constant reaction 2

L – Length

M^+ – Cation in the electrolyte medium

m_T– Mass transport

n – Number of electrons

R – Resistance

APPENDICES

ΔR – Resistance difference

R_0 – Initial resistance

R_i – Resistance at a certain point

P – Product of the reaction

$[S]$ – Substrate concentration

[Denim]Br – Brominated 1-decyl-3-methyl imidazole

3D – Three dimensional

4ABS – 4-aminobenzenesulfonate

°C – Degree Celsius

ρ – Resistivity

υ – Scan rate

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APPENDIX 3

LIST OF PUBLICATIONS

Journal publications:

1. Borràs-Brull, M., Blondeau, P., Riu, J., The use of conducting polymers for enhanced electrochemical determination of hydrogen peroxide*, Critical Reviews in Analytical Chemistry,* Published online on January 28th **2020***.* DOI:10.1080/10408347.2020.1718482.
2. Borràs-Brull, M., Blondeau, P., Riu, J., Characterization and validation of a platinum paper-based potentiometric sensor for glucose detection in saliva. Submitted to *Analytical and Bioanalytical Chemistry,* Pending of acceptance, **2020.**

Poster and Oral presentations:

1. Poster - Mátrafüred **2017**, International Conference on Electrochemical Sensors, Visegrád, Hungary. *PEDOT:PSS paper-based chemiresistor towards hydrogen peroxide detection*.
2. Poster - VIII International Congress on Analytcal Nanoscience and Nanotechnology **2017**, Barcelona, Spain. *PEDOT:PSS paper-based chemiresistor towards hydrogen peroxide detection.*
3. Poster – 3rd prize poster contribution 2nd year PhD students. 15th Doctoral day of the Doctoral Programme in Nanosciene, Materials and Chemical Engineering, URV, **2018**. *Direct potentiometric detection of H_2O_2 by conducting polymer-based sensors.*
4. Oral - 11th Ibero-American Congress on Sensors **2018**, Barcelona, Spain. *Direct potentiometric detection of H_2O_2 using conducting polymer-based sensors.*

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