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«Electrochemical detection of dopamine using platinum- and palladium- based catalysts»

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«Ηλεκτροχημική ανίχνευση ντοπαμίνης με τη χρήση καταλυτών με βάση λευκόχρυσο και παλλάδιο»

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ABSTRACT

Dopamine is an important neurotransmitter that plays a significant role in maintaining the functional activities of central nervous, cardiovascular, and hormonal systems. The detection of the change of the concentration levels of dopamine worths of research because it has been proved that numerous mental and physical disorders are associated with it. As an immediate result, reliable, rapid, smart, flexible and low-cost sensors need to be developed for dopamine detection. For this objective, the interest of the scientific community concentrates on developing electrochemical sensors, which convert electrochemical information into exploitable analytically signal. Various studies of metal-catalysts, such as Pt, Pd, Au, Ru and Ti demonstrate promising potential in electroanalysis and indicate that these materials can be used for sensor designing. Especially, Pt and Pd type catalysts have shown remarkable catalytic activity towards dopamine electrooxidation reaction.

In the current thesis, the catalytic effect of Pt(20%wt)/graphitized carbon, Pd(20wt%)/Vulcan-XC72 commercial and Pd(20wt%)/Vulcan-XC72 homemade for dopamine oxidation is investigated. Electrochemical techniques, such as cyclic voltammetry, chronoamperometry and differential pulse voltammetry are employed for the catalytic effect. The structure of the catalysts was analyzed with the transmission electron microscope and X-Ray diffraction techniques. It is found that Pt(20%wt)/graphitized carbon, Pd(20wt%)/Vulcan-XC72 commercial as well as homemade exhibit electrochemical activity towards dopamine electrooxidation reaction with peak values of the current density being $1403.7 \mu A\ cm^{-2}$, $1091 \mu A\ cm^{-2}$ and $780 \mu A\ cm^{-2}$ in 0.5mM dopamine in a 0.15M PBS at a temperature of $26^{o}C$ respectively for the two catalysts. The durability of the Pt(20%wt)/graphitized carbon, Pd(20wt%)/Vulcan-XC72 commercial and homemade catalysts were poor and the stability of all catalysts was satisfying. Finally, it is found that the increase in temperature causes a visible increase in the anodic peak current of the catalysts. Concluding, the catalyst formed with Pt(20%wt)/graphitized carbon exhibits better results, concerning the effective detection of dopamine and the durability, than the catalysts of Pd(20wt%)/Vulcan-XC72.

ΠΕΡΙΛΗΨΗ

Η ντοπαμίνη είναι ένας σημαντικός νευροδιαβιβαστής που παίζει σημαντικό ρόλο στη διατήρηση των λειτουργικών δραστηριοτήτων των κεντρικών νευρικών, καρδιαγγειακών και ορμονικών συστημάτων. Η ανίχνευση της μεταβολής των επιπέδων συγκέντρωσης της ντοπαμίνης χρήζει περαιτέρω έρευνας διότι έχει αποδειχθεί ότι πολλές ψυχικές και σωματικές διαταραχές συνδέονται με αυτήν. Ως άμεσο αποτέλεσμα, αξιόπιστοι, γρήγοροι, εφαρμόσιμοι και χαμηλού κόστους αισθητήρες χρειάζεται να αναπτυχθούν για την ανίχνευση ντοπαμίνης. Για τον σκοπό αυτό, το ενδιαφέρον της επιστημονικής κοινότητας συγκεντρώνεται στην ανάπτυξη ηλεκτροχημικών αισθητήρων, οι οποίοι μετατρέπουν την ηλεκτροχημική πληροφορία σε εκμεταλλεύσιμο αναλυτικό σήμα. Εκτεταμένες έρευνες σε μεταλλικούς καταλύτες, όπως Pt, Pd, Au, Ru και Ti επιδεικνύουν πολλά υποσχόμενες δυνατότητες στην ηλεκτροανάλυση και ενδεχομένως αποτελούν χρήσιμα υλικά για τον σχεδιασμό αισθητήρα. Ειδικότερα, καταλύτες με βάση το Pt και το Pd έχουν παρουσιάσει αξιοσημείωτη καταλυτική δράση προς τη οξείδωση της ντοπαμίνης.

Στην παρούσα διπλωματική παρουσιάζεται η επίδραση των καταλυτών Pt(20%wt)/graphitized carbon, εμπορικού Pd(20wt%)/Vulcan-XC72 καθώς και homemade Pd(20wt%)/Vulcan-XC72 για την οξείδωση ντοπαμίνης σε φυσιολογικό ορό με 7.3 pH. Βρέθηκε ότι και οι τρεις καταλύτες επιδεικνύουν ηλεκτροχημική δραστηριότητα ως προς τη ντοπαμίνη με τις μέγιστες τιμές των ανωδικών ρευμάτων να είναι 1403.7μA cm^{-2}, 1091μA cm^{-2} και 780μA cm^{-2} για μετρήσεις σε διάλυμα 0.5mM ντοπαμίνης σε 0.15M PBS και θερμοκρασία 26ºC για τους τρεις καταλύτες αντίστοιχα. Η αντοχή των καταλυτών ήταν σχετικά ασθενής στο διάλυμα ντοπαμίνης και η σταθερότητα και των καταλυτών ήταν ικανοποιητική. Η άνοδος της θερμοκρασίας προκάλεσε αξιοσημείωτη αύξηση στην ανώτατη τιμή ρεύματος οξείδωσης σε όλα τα κυκλικά βολταμογραφήματα. Συμπερασματικά, ο καταλύτης με βάση τον λευκόχρυσο (Pt(20%wt)/graphitized carbon) παρουσιάζει καλύτερα αποτελέσματα, αναφορικά με την αποτελεματική ανίχνευση της ντοπαμίνης και την αντοχή, σε σχέση με τους καταλύτες παλαδίου (Pd(20wt%)/Vulcan-XC72 εμπορικός και homemade).

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Introduction

Advances in electrochemistry have provided efficient and analytical systems characterized by instrumental simplicity, moderate cost, portability and short analysis time. Electrochemical techniques are proved to be valuable tools for specific applications. One exceptional use of them is for real time detection of biomolecules, such as glucose, dopamine, uric acid, etc. The main challenge of such kind sensors is the improvement of sensitivity, stability and selectivity; factors that can be improved and lead to an accurate detection of the respective electroactive molecules. The main objective of the current thesis is the investigation of Pd(20wt%)/VulcanXC-72 and Pt(20%wt)/graphitized carbon as catalysts for dopamine enzymeless electrochemical sensors.

The thesis is divided into two main sections: i) the theoretical part, which describes the data in the literature regarding the research topics addressed, and ii) the experimental part, which presents the original contributions. The occurrence, role, biological importance of dopamine for its assessment are firstly discussed. Therefore the principals, the theoretical background and the development through the years of biosensors will be extensively analyzed in the following chapters.

The first Chapter describes the motivation of the thesis which is the importance of dopamine detections. The second Chapter discusses general aspects concerning the principals of electrochemistry and the applications of electrochemical cells and their applications. The third Chapter is dedicated to the chemical sensors and specifically to the amperometric electrochemical sensors. Then in the fourth Chapter the electroanalytical methods used for this thesis along with electrochemical thermodynamics and kinetics are explained. In the fifth Chapter the experimental results are presented, while the sixth Chapter discussed the experimental procedures being followed in the current thesis. Finally, in the seventh Chapter draws the conclusions, and suggests some for future research.

Chapter 1

Electrochemistry fundamentals and electrochemical devices

The performance of an electrolytic cell depends on the electrodes and the electrolyte, thus it's a necessity to introduce some of the basic meanings and principals on this field. The first Chapter is dedicated to some of the most common application of electrochemistry. Firstly, the basic principles of electrochemistry and an electrochemical cell are analyzed and. Thereinafter, systems for electrochemical energy storage and conversion such as batteries, fuel cells, and electrochemical capacitors (ECs) are discussed.

1.1   Electrochemical Cells and Reactions

Electroanalytical chemistry along with the use of oxidation–reduction reactions and other charge-transfer phenomena had its origin a century ago. In the last decades, voltammetric methods have been broadly used for multiple application such as electrochemical reactions [1], model studies of enzymatic catalysis [2], solar energy conversion [3], in the determination of trace concentrations of biological and clinically important compounds [4] and others. An electrochemical cell is a device capable of either generating electrical energy from chemical reactions or using electrical energy to cause chemical reactions. In electrochemical systems the subject is the processes and factors that affect the transport of charge across the interface between chemical phases. An electrochemical cell has two main compounds which are an electronic conductor (an electrode) and an ionic conductor (an electrolyte) and we are mostly concerned about the interface electrode/electrolyte and the events that occur there when an electric potential is applied and current passes. Charge is transported through the electrode by the movement of electrons (and holes) [5]. Both the electrode reactions and/or the charge transport can be modulated chemically and serve as the basis of the sensing process [6].
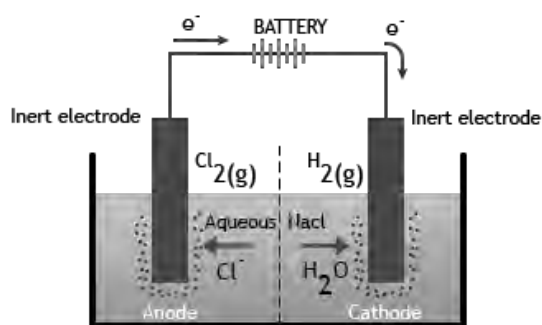
Figure 1.1. Electrolytic cell with aqueous solution of sodium chloride [7].

Many well-known systems follow the same electrochemical principals- all consist of two electrodes in contact with an electrolyte solution. Common features are that the energy-providing processes take place at the phase boundary of the electrode/electrolyte interface and that electron and ion transport is separated [7].

1.2 Electrodes

An electrode in an electrochemical cell is referred to as either an anode or a cathode. The anode is now defined as the electrode at which electrons leave the cell and oxidation occurs and the cathode as the electrode at which electrons enter the cell and reduction occurs [8]. Each electrode may become either the anode or the cathode depending on the direction of current through the cell. For the needs of the experiments a three-electrode cell was used, which is composed from a working, a reference and an auxiliary electrode. Typical electrode materials include solid metals (e.g., Pt, Au), liquid metals (Hg, amalgams), carbon (graphite), and semiconductors (indium-tin oxide, Si). [5]

Working electrode

The working electrode is the electrode in an electrochemical system on which the reaction of interest is occurring [9]. Working electrode materials are selected to provide good electron transfer properties towards the substrate while showing high activation energy for electron transfer in the principal competing reaction [10]. Depending on whether the reaction on the electrode is a reduction or an oxidation, the working electrode is called cathodic or anodic, respectively. Ideally it should have a good signal-to-noise ratio, reproducible response, no interfering reactions over the potential of interest, high electrical stability, low cost, availability, low

toxicity and long-term stability. Due to these factors, the working electrode has been made from noble metals such as Au, Ag, Pt, or Hg and C in the form of carbon paste or graphite [9, [11]. Chemically modified electrodes are employed for the analysis of both organic and inorganic samples [12].

Glassy Carbon electrode (GCE)

Since the experiments are operated for this Thesis are led using a glassy carbon electrode as the working electrode, the following review focuses on it. Glassy carbon is among the most popular carbon electrode materials used as substrates for modification as it provides wide potential window with low background currents, negligible porosity and it is chemically and mechanically stable [10]. Bare glassy carbon electrode has been employed for multy-analyte detection. In general Carbon electrodes are widely used in electroanalysis due to their low background current, wide potential window, chemical inertness, low cost, and suitability for various sensing and detection applications [13].The use of chemically modified electrodes can help in selective, sensitive, and reproducible detection of DA in the presence of other interferences by reduction of ohmic resistance associated with the wide potential window. Factors affecting the response of a modified electrochemical sensor include the nature of coating material, method of synthesis of coating material, mechanism of electrode coating, use of mediators, and nature of sample matrix. As mentioned above simultaneous determination of DA, AA and UA compounds is a special interest in sensor systems [14]. For this purpose, various modified glassy carbon electrodes (GCE) have been constructed. For example, modified GCE with carbon nanotube [15] graphene/Pt-modified GCE [16], polymer film modified GCE [17] and gold nanoparticles modified GCE [18] were used for the electrocatalytic oxidation of DA, AA and UA. Modified electrode resolved the overlapping of the anodic peaks of DA, AA and UA into three well-defined voltammetric peaks. In addition, the modified electrodes showed high electrocatalytic activities toward the oxidation of DA, AA and UA by changing their oxidation potentials and enhancing the peak currents. The chemical and electrochemical pretreatment shows significant changes in physical and electrochemical properties of GCE [19].

Screen printed electrodes (SPE)

Screen printed electrodes are devices based on the screen-printing technology. They are produced by printing different inks on various types of plastic or ceramic substrates. The composition of the various inks used for printing on the electrodes determines the selectivity and sensitivity required for each analysis. Screen printing is an attractive technology for the fabrication of electrochemical sensors since it enables large scale fabrication of low cost, reproducible and disposable electrodes with extended scope for modification [20]. SPEs, known for their adaptability since they can be easily modified to fit multiple purposes, have attracted the scientific interest. They are easy to modify using different inks commercially available for working electrode, giving significant results in sensitive and selective detection of multiple analytes [21]. Disposable working electrodes have attracted attention worldwide. These disposable working electrodes are easy to handle and have economic efficiency [22].

Carbon paste electrodes (CPE)

Carbon paste electrodes (CPE) have been used for many decades. Its advantages include the ease and speed of preparation, the creation of a new producible surface and the low cost of carbon paste [23]. Carbon paste electrodes are convenient conductive matrixes for preparing chemically modified electrodes (CMEs) [24]. CPEs can be modified by a variety of materials in order to achieve better electro-catalytic activity, sensitivity, selectivity and low detection limit than the traditional CPEs. Carbon paste has become one of the most popular electrode materials used for the laboratory preparations of various electrodes, sensors and detectors and generally they are widely used for voltammetric measurements. They are also non-toxic and environment friendly electrodes. Dopamine (DA) has been determined in presence of ascorbic acid (AA) and uric acid (UA) using poly (spands reagent) modified CPE. DA was also determined in real samples using fabricated sensor [25].

Other types of electrodes

Electrodes such as Boron doped diamond electrode, gold electrode, Carbon-Ceramic electrodes, etc. have been reported in literature for the detection of DA in presence of AA and UA [26, [27].

Reference electrodes

Reference electrodes provide a standard well-known electrode potential for the electrochemical measurements. The composition of a good reference electrode (RE) remains effectively constant, unvarying for the duration of an experiment. It is also required to have reversibility (non-polarizability) and reproducibility. For electrochemical sensors, an accurate and stable reference electrode that acts as a half-cell in the measurement circuit is critical to providing a stable reference potential and for measuring the change in potential difference across the redox reaction. A commonly used reference electrode is the Ag/AgCl electrode due to its simplicity, stability, and capability of miniaturization. A conventional Ag/AgCl reference electrode is a silver wire that is coated with a thin layer of silver chloride either by electroplating or by dipping the wire in molten silver chloride. The potential developed is determined by the chloride concentration of the inner solution, as defined by the Nernst equation.

Auxiliary electrode

The auxiliary electrode, often also called the counter electrode, is an electrode which is used only to make a connection to the electrolyte so that a current can be applied to the working electrode. In a two-electrode system, either a known current or potential is applied between the working and auxiliary electrodes and the other variable may be measured. The auxiliary electrode functions as a cathode whenever the working electrode is operating as an anode and vice versa. It often has a surface area much larger than that of the working electrode to ensure that the half-reaction occurring at the auxiliary electrode can occur fast enough so as not to limit the process at the working electrode. When a three electrode cell is used to perform electroanalytical chemistry, the auxiliary electrode, along with the working electrode, provides a circuit over which current is either applied or measured.

Here, the potential of the auxiliary electrode is usually not measured and is adjusted so as to balance the reaction occurring at the working electrode. This configuration allows the potential of the working electrode to be measured against a known reference electrode without compromising the stability of that reference electrode by passing current over it. Auxiliary electrodes are usually made of an inert material, such as a noble metal or graphite, to keep it from dissolving.

1.3 Electrolytes

Electrolytes are omnipresent and (indispensable) necessitous in all electrochemical devices, and their basic function is independent of the much diversified chemistries and applications of these devices. In this sense, electrolytes in electrolytic cells, capacitors, fuel cells, or batteries play always the same role they function as the medium for the transfer of charges, which are in the form of ions, between a pair of electrodes. The vast majority of the electrolytes are electrolytic solution-types that consist of salts dissolved in solvents, either water (aqueous) or organic molecules (nonaqueous) and are in a liquid state in the service-temperature range. The electrolyte, in most situations, defines how fast the energy could be released by controlling the rate of mass flow within the electrolytic cell. Conceptually, the electrolyte should undergo no net chemical changes during the operation of the cell, and all Faradaic processes are expected to occur within the electrodes. While the potencies of electrode materials are usually quantified by the redox potential in volts against some certain reference potential, the stability of an electrolyte can also be quantified by the range in volts between its oxidative and reductive decomposition limits, which is known as the "electrochemical window". It's important for an electrolyte to have a wide electrochemical window, so that electrolyte degradation would not occur within the range of the working potentials of both the cathode and the anode.

Certainly, electrochemical stability is only one of the requirements that an electrolyte should meet Electrolytes is also required to present good ionic conductivity and function as an electronic insulator, so that ion transport can be facile and self-discharge can be kept to a minimum. Finally, it should show resistance against various abuses, such as electrical, mechanical, or thermal ones and its components should preferably be environmentally friendly [28].

Liquid electrolytes

Electrolytes can take several different forms and phases (liquid, polymer, solid, or ionic liquid) depending on the application they are used in. In general, liquid electrolytes have low viscosity, high ionic conductivity, and are extremely flammable. Liquid electrolytes are solutions generally consist of solvated ions and solvent molecules which can be aqueous or not. The departures from ideality in ionic solutions are dominantly caused by the Coulombic interaction between ions. Ions that are oppositely charged attract one another. As a result, anions are more likely to be found near cations in solution, and vice versa. Near any given ions, there is an excess of counter ions (ions of opposite charge), even though the overall solution is electrically neutral [29].

Solid electrolytes

The most popular category of solid electrolytes is the one of Polymer electrolytes. They are known for their significantly higher viscosity, lower ionic conductivity and their favorable mechanical properties, but on the downside, they contain the similar components that make liquid electrolytes extremely flammable. Such materials may be readily fabricated as films and are used as replacements for typical low-molecular-weight liquid-based electrolytes for practical electrochemical devices. The recent years there has been an increasing interest in polymer based electrolytes due to their utilization in abroad variety of their technological applications in electrochemical devices such as thin film batteries and fuel cells [30]. Solid electrolytes can also be ceramic. Certain ceramic structures are capable of li-ion conduction and can serve as an electrolyte; however, the temperature at which the lithium diffusion processes become functional is much higher than the practical use temperatures of a lithium-ion battery.

Ionic liquids

Ionic liquids (molten salts) have the highest conductivity, but must be maintained at a high temperature to remain in the liquid state to be an ionically-conducting electrolyte [31, [32].

1.4 Applications of electrochemistry

Energy consumption/production that relies on the combustion of fossil fuels is forecast to have a severe future impact on world economics and ecology. In research of new environmentally friendly and sustainable energy sources, the electrochemical energy production is a good perspective alternative. Systems for electrochemical energy storage and conversion include batteries, fuel cells, and electrochemical capacitors (ECs).

1.4.1 Batteries

An electric battery is a device consisting of one or more electrochemical cells with external connections provided to power electrical devices [33]. When a battery is supplying electric power, electrons will flow through an external electric circuit from anode to cathode. When a battery is connected to an external electric load, a redox reaction converts high-energy reactants to lower-energy products, and the free-energy difference is delivered to the external circuit as electrical energy [34]. The two main categories of batteries are the primary and the secondary. Primary batteries are disposable which means that they are used once and discarded since the electrode materials are irreversibly changed during discharge (e.g. the alkaline battery. Secondary batteries are rechargeable meaning that they can be discharged and recharged multiple times using an applied electric current since the original composition of the electrodes can be restored by reverse current with the most common example being the lithium-ion batteries.

1.4.2 Fuel cells

A fuel cell is an electrochemical cell that converts the potential energy from a fuel into electricity through an electrochemical reaction of hydrogen fuel with oxygen or another oxidizing agent. It is composed from two electrodes, the anode and the cathode, and one electrolyte that allow positively charged hydrogen ions (protons) to move between the two sides of the fuel cell. Two chemical reactions occur at the interfaces of the three different segments. At the anode a catalyst causes the fuel to oxidase generating protons (positively charged hydrogen ions) and electrons. The protons flow from the anode to the cathode through the electrolyte after the reaction while electrons are drawn from the anode to the cathode through an

external circuit, producing direct current electricity. At the cathode, another cata-lyst causes hydrogen ions, electrons, and oxygen to react, forming water. The re-sult of the process is water, electrical energy and heat. A typical fuel cell produces a voltage from 0.6 V to 0.7 V at full rated load. However, phenomena such as activation loss, ohmic loss and mass transport loss can cause voltage decreases as current increases.

The main characteristic of categorizing the fuel cells is the type of electrolyte they use but despite the differences between the types of the fuel cells, they all work under the same principal. They are made up of three adjacent segments: the anode, the electrolyte, and the cathode. The types of the different fuel cells are given: Proton exchange membrane fuel cells (PEMFCs) [35], Phosphoric acid fuel cell (PAFC), Solid acid fuel cell (SAFC), alkaline fuel cell (AFC) and Solid oxide fuel cell (SOFC), high temperature.

1.4.3 Supercapacitors

A supercapacitor (SC) (also called a supercap, ultracapacitor or Goldcap) is a high-capacity capacitor able to store 10 to 100 times more energy per unit volume or mass than electrolytic capacitors with capacitance but with lower voltage limits they can accept and deliver charge rapidly and tolerate many charge and discharge cycles. Unlike conventional capacitors, ES (electrochemical supercapacitors) elec-trodes are normally composed of high surface porous materials such as carbon particle materials and the separator is either solid or liquid, thus electrode/electro-lyte interfaces are generated. These interfaces, called electric double layer, have higher surface area than dielectric capacitors and thus can store more charges. It is recognized that the improved structure of an ES allows better energy storage than conventional capacitors [36]. The type of the ES is defined by the electrodes used. Electrostatic double-layer capacitors (EDLCs) use carbon electrodes, elec-trochemical pseudocapacitors use metal oxide or conducting polymer electrodes and hybrid capacitors use electrodes with differing characteristics: one exhibiting mostly electrostatic capacitance and the other mostly electrochemical capacitance.

1.4.4 Sensors

A very usual sensor is chemical sensors. A chemical sensor is defined as analytical device that converts chemical information into a useful measurable physical signal, providing a certain type of response directly related to the quantity of specific chemical species [37, [38]. They are the evolved products of many interdisciplinary scientific fields and technologies such as polymer, chemistry, physics, electronics and molecular biology, using principals of many measurement systems [6].

Another category of chemical sensors are the conductometric sensors. They are the simplest of the electroanalytical applications, but fundamentally non-specific and non-selective. Conductometric sensors can observe the changes of electric conductivity of a film or a bulk material, whose conductivity is affected by the present analyte and they have the advantages of simplicity and low cost. Their performance can be improved through electrode modification [39, [40].

It is interesting to say that the main elements of every sensor is presented in Fig.1.2. It consists of a transducer, which transforms the response received, into a detectable signal to an appropriate device and a chemically selective layer, which isolates the response of the analyte from the reaction environment. There is a diversity of recognition elements and signal transducers and they can be categorized as mass, thermal, optical and electrochemical sensors and are designed to detect an analyte in the gaseous, liquid or solid state.
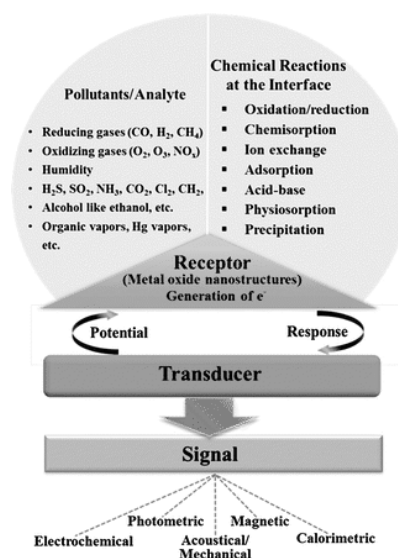
Figure 1.2. Basic Components of a sensor [38].

By definition, thermal sensors are constructional elements for measuring temperature and employ the functional dependence of a certain physical property of the sensor material on temperature, which is customarily recognized and well defined [6, [41].

Essentially an analog temperature sensor converts the ambient temperature into a voltage reading, which can then be sensed by an analog pin of a microcontroller. The temperature sensor is great for applications that record and display temperature information such as a thermometer when you are sick, or the thermostat for your air conditioner [42]. Another important category of chemical sensors is the mass sensors which, as it can be indicated from their name, are used for mass change measurements during chemical reactions. The major advantages of mass sensors are their simplicity of construction and operation, their light weight, and the low power required.

Optical sensors are another significant application of optics technology with applications in industry, medicine and engineering [43]. They can be defined as detectors that convert light, or a change in light, into an electronic signal [44].

In Chapter 2 a more analytical and extensive review of the electrochemical sensors, is given.

Chapter 2

Electrochemical sensors

Electrochemical sensors are the largest and the oldest group of chemical sensors. It is the technology that connects electricity and chemistry, by measuring electrical quantities, such as potential, current and electrical charge and. Electrochemical sensors are devices that give information about the composition of a system in real time by coupling a chemically selective layer (the recognition element) to an electrochemical transducer. In this way, the chemical energy of the selective interaction between the chemical species and the sensor is transduced into an analytically useful signal [45].

The development of electrochemical sensors is one of the most active areas of analytical research in many fields. The first electrochemical sensors were manufactured within the 1950s, mainly for oxygen monitoring. In the 1960s the glass electrode for pH measurements was the most widely used sensors. Clark Jr. introduced the amperometric glucose enzyme electrode in 1962. The interest in electrochemical sensors continues unabated today, stimulated by the wide range of potential applications. In the following sub-section the current challenges of the majority of the electrochemical sensors are reported and discussed.

2.1 Current challenges of electrochemical sensors

Several analytical and physical properties must be considered when developing (and commercializing) chemical and biological sensors. In order for them to be considered as practical and applicable, characteristics such as sensitivity, selectivity, stability, reproducibility, the ability for multi-analyte detection and miniaturization and also cost must be optimized. Sensitivity of a sensor is the slope of its response in the dynamic range whereas the detection limit in the minimum detectable response, usually above three times the standard deviation (i.e.,$>3\sigma$).

Because it's evaluated experimentally, the sensitivity of the sensor has two sources of error: the standard deviation of the response (σ_R) and the standard deviation of

the concentration (σ_C). The sensitivity (m) of the sensor is the first derivative of its response, with respect to the concentration of the analyte, at any concentration ($C_{X,i}$) on the response curve. Therefore the sensitivity goes from zero in the interference region, through a maximum in the dynamic range, to zero in the saturation region [6].

Selectivity is the single most important general issue in chemical sensing. It determines the usefulness of the sensor for any given application. It can be defined as the ability of a sensor to respond primarily to only one species (analyte) in the presence of other species. Its role can be best explained by examining the response curve in a differential pulse voltammetry diagram. In this graph, several responses of the sensor to increasing concentration of the analyte are plotted tor increasing concentration of interfering species. As their concentration increases, the detection limits shift to the higher concentration of the primary species, and the dynamic range becomes entirely nonresponding.

Obviously, one of the most important objectives is to design the selective layer in such a way that it has a wide dynamic range and as low a detection limit as possible. It means that, in the ideal case, the layer should completely reject any interfering species, and respond exclusively to the analyte.

Reproducibility and stability are also two of the most important aspects. It is a measure of the drift in a series of results taken over a period of time. A sensor with great reproducibility should give as results observations that don't greatly differ from one another. The stability of a sensor depends on various factors such as the method of preparation of the sensor, its geometry, along with the applied receptor and transducer and the limiting current response rate and it declares in what degree the sensor characteristics remain constant over time.

Many electrochemical sensors have reached commercial maturity while many are still in various stages of development. Their applications include as diverse as enzyme electrodes, high-temperature oxide sensors, fuel cells, surface conductivity sensors, etc. They are divided by their mode of measurement into potentiometric (measurement of voltage), amperometric (measurement of current), and conductimetric (measurement of conductivity) sensors.

In amperometric sensors, an optimal potential is applied to the electrochemical cell and a corresponding current is obtained due to a reduction or oxidation reaction. The current signal produced is related to the concentration of the analyte by Faraday's law and the laws of mass transport. Amperometric sensors translates the activity of the analytes on the surface into a signal in the form of current. Amperometric sensors will be widely reviewed in the next chapter.

As opposed to the amperometric sensor, potentiometric sensors measure the potential difference between two electrodes under the conditions of no current flow. This potential converts into measurable information about the composition of the sample. These voltages arise because an electrochemical reaction can occur at wires, or at membranes in solid, liquid, or condensed phases. The generated signal is an electromotive force that is dependent on the activity of the analyte, and is described by Nernst's equation. Potentiometry provides information about the activity of ions in an electrochemical reaction. Potentiometric sensors can be used for measuring relatively low concentrations, since they do not react and chemically influencing a sample.
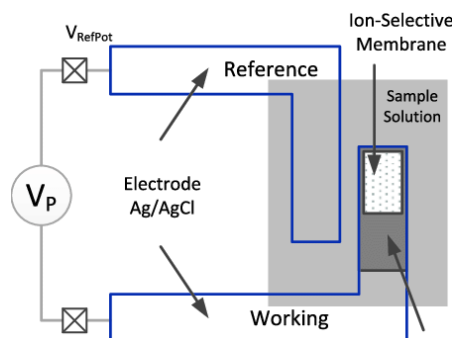
Figure 2.1. A typical potentiometric sensor scheme [41].
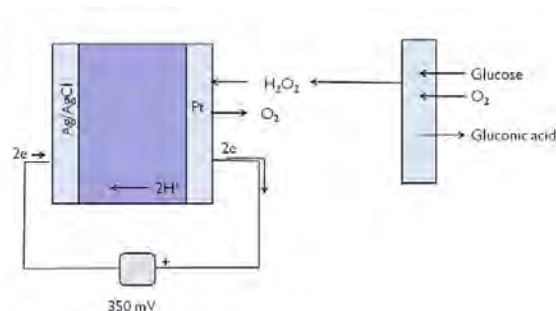
Figure 2.2. Basic setup of an amperometric glucose sensor [36].

The research developed in this area is certainly increasing. The last decades it has been an exponential trend in the number of publications in the field of biosensors emphasizing on the development of novel materials for being applied as electrodes in sensors. In the next sub-section some of the materials that are studied, are discussed. [36]. Materials for sensors electrode

In recent years, chemically modified electrodes have attracted the scientific interest because of wide range of their potential applications, low cost and ease of fabrication and regeneration. Materials used as sensing layers vary from metals and metal oxides and carbon based materials to polymers [46]. Each one of the materials that have been examined is proved to enhance the catalytic properties of the supporting electrode. For over a decade, the carbon materials such as CNTs, graphene, and its derivatives, metal oxides and nanoparticles and polymers have been used as modification materials in the electrochemical detections because of their unique properties such as high electrical conductivity, large surface area, and good mechanical and chemical stabilities.

The simultaneous determination of dopamine, ascorbic acid and uric is a special interest in sensor systems. Thus, an extensive review of the materials used especially for biosensing DA, AA and UA is given in the following chapters.

2.1.1   Carbon nanotubes

A nanotube is a nanometer-scale tube-like structure. Carbon nanotubes (CNTs) have been tested widely as an electrode surface and they display intrinsic properties their high electrical conductivity, large surface area, and mechanical and chemical stability [47]. Generally, Carbon-based electrochemical sensors have been used extensively for neurotransmitter analysis because the surface oxides facilitate electron transfer and readily adsorb neurotransmitters due to electrostatic interactions [48]. Carbon nanomaterial (CN) based sensors exhibit enhanced electrochemical properties and high biocompatibility [49]. The nanostructured surface provides a larger specific surface area, increased interfacial adsorption, and enhanced electrocatalytic activity. Thus, CN-based electrodes have rapid electron transfer, reduced electrode fouling, reduced overpotential, and increased sensitivity and selectivity for neurotransmitter detection [50].

CNTs are available in different forms and shapes (SWCNT, MWCNT) and they are widely used as a sensing surface over bare electrodes. The metallic behavior of MWCNTs can be attributed to the presence of a single metallic layer, while SWCNTs are classified as metallic and semiconducting; separation of the metallic ones will be more beneficial for modifying electrodes [51]. It has been noted from numerous examples that researchers have combined SWCNTs or MWCNTs with other emerging materials to take advantage of the synergistic effects [52]. Samo B. Hočevar et al. modified a single carbon fiber microelectrode with multi-wall carbon nanotubes (MWCNT) and Nafion reporting an attractive electroanalytical performance for enhanced voltammetric detection of dopamine. The preparation was fast and simple and a LOD of 70nM of Dopamine was achieved [53]. Simultaneous and sensitive determination of a quaternary mixture of AA, DA, UA using a modified GCE by iron ion-doped natrolite zeolite-multiwall carbon nanotube has also been achieved [54]. Functionalized carbon nanotubes and nanofibers are prove to be notably efficient biosensing applications [55]. Nanostructured Multi-Walled Carbon Nanotubes (MWNT) Yarns have been reported to be used for the Selective and Sensitive Detection of Dopamine. It's a material highly flexible and conductive with high [56].

2.1.2 Graphene and its derivatives

Graphene is a 2D nanomaterial, thinnest than any other material in the universe, flexible yet harder than diamond. The large electrical conductivity, surface area, high tensile strength, and low cost make it an ideal material. Gr is consisting of a single layer of sp2 network of carbon atoms in a honeycomb crystal lattice and is also the basic structure of all graphitic materials [57]. Among car-bon nanomaterials, graphene has been considered as one of the most promising materials for electrochemical sensing due to its good biocompatibility and conductivity, as well as abundance of inexpensive graphite source materials [58]. Different preparation methods have been adopted to introduce various moieties of graphene on electrodes to obtain altered effects. Substantial progress in carbon nanotube/metal nanoparticle hybrid sensors [59] has been achieved, however, Gr/metal nanoparticle as sensing composites are still to be extensively investigated [60]. The CNT/Gr sensing composites have also been researched [61].

Graphene was fabricated on the bare graphite electrode to achieve the electrochemical oxidation of DA and UA in the presence of high concentrations of AA. The biosensor resulted has the advantages of large surface area, high electrical conductivity, a very stable electrode which is to prepare [62, [63]. A highly sensitive and selective detection of dopamine in the presence of ascorbic acid was reached by using a graphene oxide modified electrode [64]. A sensor modified with porphyrin-functionalized graphene was used for the electrochemical detection of dopamine in the presence of large excess of ascorbic acid and uric acid [65] with good sensitivity and selectivity [66].

The use of graphene oxide [67] and graphene-zinc oxide [58] modified electrode for Simultaneous determination of dopamine, ascorbic acid and uric acid have also been reported in literature as very efficient.

Another Carbon based nanomaterial that has been reported is Fullerene (C60). Fullerene science is one of the fastest growing areas of research and its application can be useful for electrocatalysis. Fullerene has been used for modifying electrode surfaces because it is chemically stable, metallic impurity free and relatively simple to implement and gives rise to reproducible electrocatalytic responses [68].

2.1.3 Metal oxides

Generally NPs are known for having better physical, chemical, and electronic properties than the bulk which makes them useable at numerous potential Applications. Metal nanoparticles (MNPs) in particular have attracted enormous attention in electrocatalysis and they have been widely used as modifiers [69, [70, [71, [72]. They appear to be highly sensitive to the changes occurring on their surface. Major advantages of using NPs in modified electrodes include enhancement of electron kinetics by providing a high surface area, increase in mass transport rates and controllable functionalization of the desired groups [73, [74].

Metal nanoparticles, especially noble metal nanoparticles modified electrodes, usually show high electrocatalytic activity toward the compounds with slow redox process at bare electrodes [75, [76].

In many cases, the use of MNP-modified electrodes helped in resolving over-lapped peaks of analytes with close oxidation potentials. [77] Moreover, such modified electrodes provide good peak-to-peak separations. Here we describe few examples of MNP-modified electrodes for the simultaneous detection of DA, UA, and AA levels. A number of MNP-based materials for electrode modifications can be found in the literature.

3D Porous Graphene Oxide/Gold Nanoparticle Composites were used for the electrochemical detection of dopamine in the presence of several interfering molecules (e.g., ascorbic acid, glucose) [78]. Copper nanoparticle- Graphene/SnO2 nano-composite-modified electrode for electrochemical detection of dopamine, in presence of AA [79, 80] have also been reported as sensors with good electrocatalytic activity, increased sensitivity and selectivity and a limit of detection (LoD) of 1μM. A sensitive electrochemical sensor for dopamine (DA) based on a glassy carbon electrode that was modified with a nanocomposite containing electrochemically reduced graphene oxide (RGO) and palladium nanoparticles (Pd-NPs) resulting a detection limit is 0.233μM has also been reported [81].

Gold nanoparticle-based have been widely used in electrochemical biosensors [82]. The ability of gold nanoparticles to provide a stable immobilization of bio-molecules retaining their bioactivity is a major advantage for the preparation of biosensors. Characteristics of gold nanoparticles such as high surface-to-volume ratio, high surface energy, ability to decrease proteins–metal particles distance have been claimed as reasons to utilize them as electrode modification composites. A reference should be also made to the bimetallic nanoparticles (NPs) with novel physical and chemical properties in electrochemical sensors. Compared to mono-metallic nanoparticles, the bimetallic nanoparticles can retain the functional properties of each component and provide synergistic effects via cooperative interactions, resulting in important features such as increased surface area, enhanced electrocatalytic capability, promoted electron transfer and improved biocompatibility [83].

2.1.4 Polymer nanocomposites

The term polymer nanocomposites (PNCs) commonly referred to a material that consist of conductive polymers with some other nanomaterials possessing electrochemical importance, has resulted in a highly active electroactive compound that exhibit remarkable properties due to the synergic effect from the individual components and is different from their individual counterparts. Electrodes modified with polymer containing different modifiers have been widely used for the quantification of biological substrates such as DA. Electrodes can be modified with different PNCs including electroactive, polyelectrolyte, coordinating, and biological polymers [50].

Electroactive/ conductive polymers such as polypyrrole, polyaniline contain groups that can be oxidized or reduced and have been used as modifiers for the construction of the chemically modified electrodes [26]. Polyelectrolyte polymers contain some ionic groups that can attract charges toward the surface from the solution through an ion-exchange process. Coordinating polymers contain donor atoms that can form coordinate covalent bonds with positive charges. Biological polymers such as enzymes, DNA, antibodies, and aptamers are very specific for certain target analytes and used in typical biosensors. Various polymeric materials such as polypyrrole [84] and overoxidized polymer films [85, [86]have been used for the simultaneous determination of AA, DA, and UA. Polymers are mostly biocompatible which makes them ideal for the use in biosensors.

2.1.5 Ionic liquids

Ionic liquids (ILs) may be conveniently described compounds composed entirely of ions with melting point equal to or below room temperature and possess an organic cation and an organic or inorganic anion [87]. Due to its specific characteristics such as wide electrochemical windows, tunable structures, high ionic conductivity, thermal stability ,good solubility, and biocompatibility make ILs a good electrochemical sensing material [88].

Specific physical and chemical properties can be obtained by combining novel cations and anions. ILs display a potential window of 4.5 V that is much wider

than those of common electrolyte solutions. Water-immiscible ILs can form hydrophobic films over bare electrodes in aqueous solutions. Physical properties of ILs such as water miscibility, viscosity, surface tension, polarity, and melting points are highly dependable on the nature of ions forming these ILs and carbon chain length [89].

Chapter 3

Electrochemical detection of dopamine and its importance

The current thesis focuses on electrochemical methods for dopamine (DA) assessment. Since DA most of the time coexists with other biological compounds such as Uric Acid and Ascorbic Acid, the catalysts have also been tested on detection of DA in the presence of them. The occurrence, role and biological importance of Dopamine for its assessment are firstly discussed.

Dopamine is an important catecholamine neurotransmitter that plays a vital role in maintaining the functional activities of central nervous, cardiovascular, and hormonal systems (Fig. 3.1). It has a significant role in the regulation of cognitive functions such as stress, behavior, and attention. DA exists as organic cations in body fluids and brain tissues.

Neurotransmitters are defined as endogenous chemical messengers that transmit, enhance, and convert specific signals between neurons and other cells and they affect and regulate everyday functions such as sleeping, mood, appetite and many others [90].

The detection of the change of the concentration levels of neurotransmitters is worth of research because it has been proved that numerous mental and physical disorders are associated with them [7]. To give an example the main symptoms of Parkinson's disease are caused by the loss of dopamine-secreting cells in the substantia nigra [91]. Thus, the quantitative detection of the neurotransmitter in different human fluids appears to be important for diagnosis, monitoring disease state and therapeutic interventions.

However, the diagnosis of these diseases necessitates very accurate measurements of the neurotransmitters in biological samples. In case of DA measurements can be operated using electrochemical techniques since DA is electrochemically active. Detection of DA levels is generally interfered by several issues. Firstly, interference of other compounds in biological samples such as uric acid and ascorbic acid it's a common phenomenon. UA is the primary end-product of purine metabolism, and its presence in the biological samples indicates symptoms of various

ailments in human body. AA is a reducing agent and plays a vital role in protection against biological oxidation processes in the body. Furthermore, the oxidation potential of DA, UA, and AA are very close, which makes their electrochemical detection rather a challenging task. Moreover, the concentrations of UA and AA are several hundred to thousand times higher than DA and lastly the oxidation products of these compounds absorb or electropolymerize on the bare electrodes and reduce their reusability and reproducibility [92].
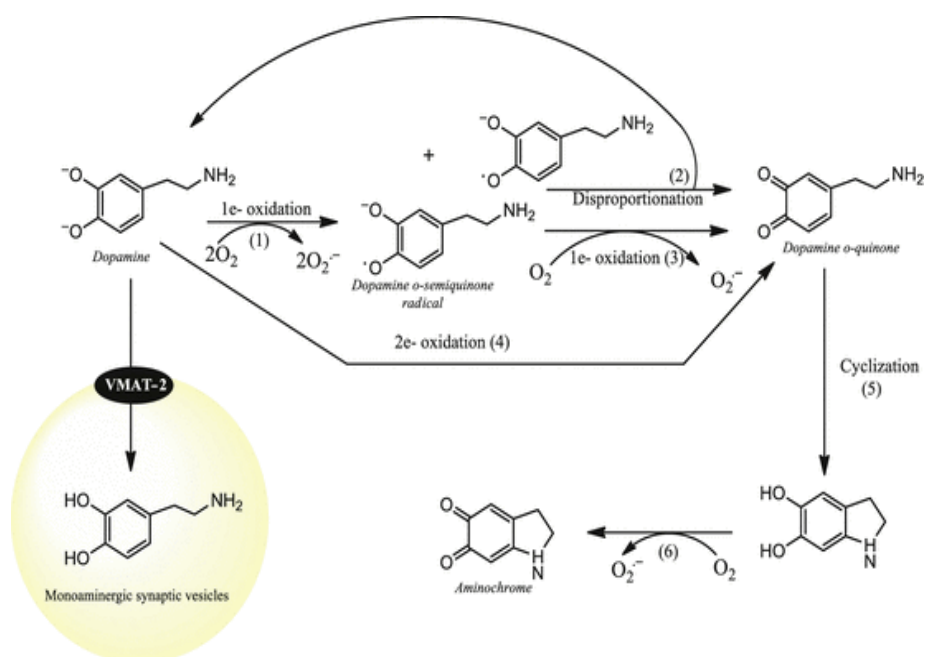
Figure 3.1. Dopamine oxidation to aminochrome, Source: Mechanisms of Dopamine Oxidation and Parkinson's Disease [93].

The fact that DA most of the cases coexists at the human samples, such as blood and Urine, with AA and UA, arises the need for developing a sensitive biosensor capable of detecting simultaneously these three substances. Both AA and UA are also compounds that can cause health problems if they are in abnormal levels so detecting them is also imperative.
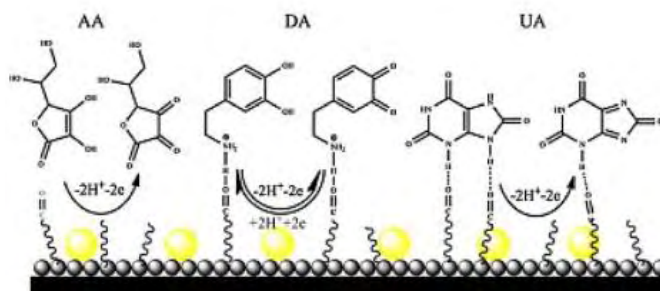
Figure 3.2. Schematic illustrates the electro-oxidation of AA, DA and UA at the surface of the electrocatalyst [7].

Ascorbic Acid, also called Vitamin C (Fig. 3.3), coexists in the human brain with several neurotransmitteramines and is vital component in human diet. Body requires vitamin C for numerous physiological functions, such as lowering blood cholesterol levels, contributing to the synthesis of amino acids and catecholamines, which regulate the nervous system and assisting in the metabolism of important components like tyrosine, folic acid and tryptophan. Its presence is mandatory for the formation and maintenance of collagen, which promotes tissue growth, wound healing, bone strength and aids in the formation of neurotransmitters and increases the absorption of iron in the gut. As an antioxidant, it protects the body from free radicals induced diseases, pollutants and it can break down histamine, which is the inflammatory component that can triggers many allergic reactions.
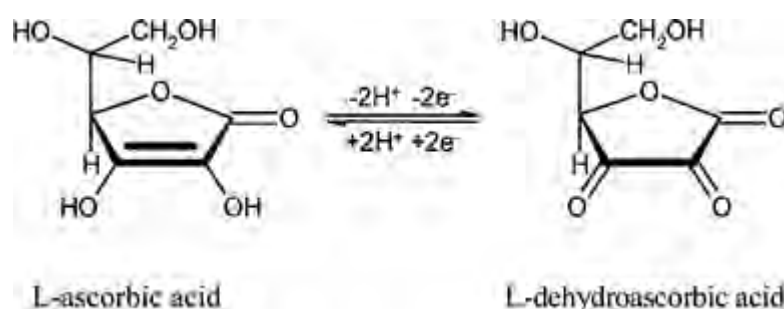
Figure 3.3. The oxidation reaction of L-ascorbic acid source: Ascorbic acid oxidation in SDS micellar systems [94].

Uric acid (C5H4N4O3) is an organic heterocyclic compound (Fig. 3.4) produced in the liver, muscles and intestines. It is the final product of the purine catabolism, deriving from its precursor xanthine, which is degraded by the enzyme xanthine oxidoreductase. Dissimilar to most mammals, humans lack the enzyme that is able to degrade uric acid into allantoin and tend to have much higher uric acid levels,

which is linked to a constellation of clinical conditions and diseases, such as the commonly known gout. However, these higher concentrations in humans also enhance their survival advantage, as hyperuricemia maintains better blood pressure in the face of low dietary salt [83]. Uric acid is considered to be one of the most important antioxidants in the blood and central nervous system. These antioxidant properties results into neuroprotective effects, as uric acid scavenges free radicals in the blood.

Since the last few years, the electrochemical techniques have gained significant attention of researchers for the determination of compounds of biological interest primarily due to their high sensitivity, simplicity, and relatively low cost [7]. Some unwanted species and impurities from aqueous solutions tend to accumulate on the surface of the bare electrode due to their hydrophobic nature or other affinities toward electrode. The severity of the problem increases while dealing with complex biological matrices; the electrodes are easily poisoned or fouled due to the accumulation of interferents on the surface. Most of the studies dealing with the detection of dopamine are carried out in the presence of other neurological interferents such as ascorbic acid and uric acid in order to represent real physiological conditions.
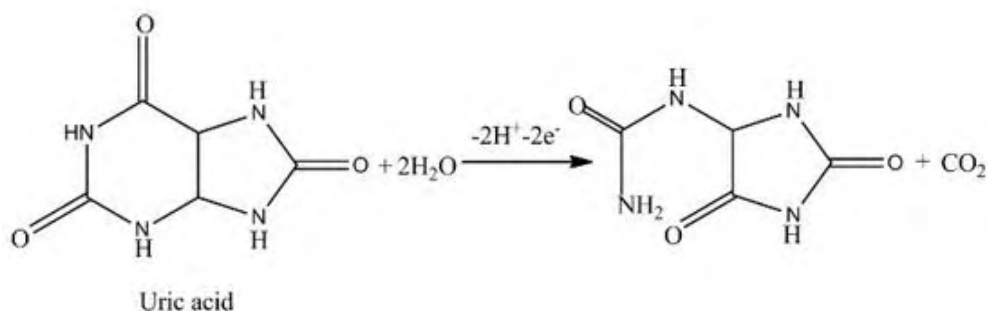
Uric acid

Figure 3.4. Oxidation mechanism for DA and UA. The UA is oxidized to allantoin. Source Simultaneous Detection of Dopamine and Uric Acid Using a Poly(l-lysine)/Graphene Oxide Modified Electrode [95].

As described earlier, simultaneous electrochemical determination of DA, AA, and UA concentrations is challenging because of the closeness of oxidation potentials and the accumulation of the oxidation products at the electrode surface which leads to electrode poisoning [7]. Consequently, it results in loss of reusability, selectivity, and reproducibility of these electrode surfaces. In order to overcome such problems and improve the determination of DA, AA, and UA concentrations,

many materials have been developed and used to fabricate modified electrode sensors. Chemically modified electrodes possess a conductive substrate modified with electroactive thin films, monolayers, or thick coatings. The modified electrodes are fabricated for a particular application which is not possible with a bare conductive electrode. Surface modifications play a catalytic role and very small changes in surface characteristics determine the sensitivity of measurement in electroanalytical applications. The materials can be carbon-based nanomaterials, nanoparticles, polymeric materials and ionic liquids were broadly analyzed in the previous chapter.

Chapter 4

Electroanalytical sensitive techniques

Electroanalytical techniques can be easily adopted to solve many problems of fundamental importance with a high degree of accuracy, precision, sensitivity and selectivity, often in spectacularly reproducible way and low operating cost compared to other analytical methods. In the technique, the electrode surface itself is a powerful tool for quantification of an analyte. By controlling the potential, the electrode can be used as a variable free energy source (or sink) of electrons.

Modern electroanalytical techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) etc. have been proved to be particularly suitable for real time, very sensitive detection of organicmolecules including drugs and related molecules in pollutants pharmaceutical dosage forms and biological fluids. The electrochemical methods decrease the required cost and simplify the measuring process because the electrochemical instrumentations are easy to be operated [5, [96, [97].

Some of the most useful electroanalytical techniques are based on the concept of continuously changing the applied potentials to the electrode-solution interface and the resulting measured current. The most commonly used voltammetric methods are cyclic , linear sweep (LSV), normal pulse (NPV), differential pulse, square wave, and stripping voltammetry [90, [98].

4.1   Cyclic Voltammetry

Cyclic voltammetry is the most effective and commonly used electrochemical technique for studying redox reactions of electroactive compounds. It's a simple, inexpensive and perhaps one of the most versatile electroanalytical techniques employed for studying electroactive species. It is often the first experiment performed in an electrochemical study of a compound, a biological material, or an electrode surface. Accordingly, the technique has been used widely in studying the redox mechanism of many biologically significant molecules [99, [100].

The CV method has become a very popular technique for initial electrochemical studies of new systems since the CV curves give information about electron transfer kinetics and thermodynamics as well as the consequences of electron transfer. It is a direct current (DC) electrochemical technique, which records the response in a current while a potential scan is applied to the working electrode at a constant scan rate in the forward and reverse directions, once or several times (the rate of change of potential with time is referred to as the scan rate) [13]. The potential initially increases and at the end of the first scan is decreased returning to an initial value. Thus, the waveform is usually of the form of an isosceles triangle. In practice, the three parameters that need to be characterized are the starting potential of the scan, the finishing potential and the scan rate in cyclic voltammetric measurements. The important parameters of CV scan are the magnitude of the peak current and the peak potentials. A redox couple in which both species are stable and rapidly exchange electrons with the working electrode is termed an electrochemically reversible couple. During the redox reaction, if the working electrode is driven to a positive potential, relative to the reference electrode, an oxidation reaction can occur at the working electrode of the electrochemical cell and if a negative potential is used to drive the reaction, this results in a reduction reaction. When applied to biosensors, the working electrode monitors the oxidation or reduction of a species near the surface of the electrode. Additionally, adsorption or other surface reactions may need to be investigated.

The peak value of the measured current I_p is directly proportional to the bulk concentration of the analyte. I_p obtained during the CV is affected by the scan rate as described by Randles-Sevcik equation (4.1):

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2} \qquad (4.1)$$

$$\text{for } T = 298° \text{ K} \qquad (4.1)$$

where: I_p is the peak current (A), n is the number of electrons (equivalent/mol) v is the potential scan rate (V/sec), A the electrode area (cm^2), D the diffusion coefficient (cm^2/sec) and C is the analyte concentration (mol/L). According to this equation, the peak current is directly proportional to concentration and increases

with the square root of the scan rate, and the analyte concentration. The diffusion coefficient increases while the temperature increases as it can be proved form Fick's law.

4.2 Deferential Pulse Voltammetry (DPV)

In pulse methods, the procedures are based on the application of pulse changes of potential, and the current response is measured at a suitable time relative to the time of the pulse. Pulse techniques have been increasingly used for electrochemical analysis of electrochemically active substances, such as compounds in pharmaceuticals, tissues, and biological fluids, considering that they provide improved detection limits as they benefit from the different variation of diffusion and capacitive current intensities with time; when carrying out measurements at the pulse end, the capacitive current is practically negligible, the value of the faradaic currents still being significant [9, [101].

DPV is an extremely useful technique for measuring trace levels of analyte compounds. The excitation waveform is a series of regular voltage pulses superimposed on the potential linear sweep applied to the working electrode at a time just before the end of the drop and it can be easily described as a staircase. The current is sampled twice in each pulse period, the first sampling is right before the pulse and the second is done at the end of the pulse. The difference between these two current values as a function of the potential is recorded and displayed. The application of these pulses allows for discrimination of the unwanted capacity current from the required faradaic current [102]. Note that non faradaic current is due to capacitance element and it will not produce any peak (example: flat parallel in the curve) that exists throughout the voltagram. Whereas, faradaic process such as oxidation, reduction involves electron transfer and results in a peak. As Non faradaic exist in background, it has to be subtracted from the faradaic to get the exact contribution of faradaic current [103, [104].

4.3 Chronoamperometry

Chronoamperometry is a time-dependent technique where a fixed potential is applied to the working electrode. Chronoamperometry experiments are carried out

to examine the electrochemical activity and stability of the electrocatalysts [105]. The current of the electrode is measured as a function of time for a period τ [8].

The potential of the working electrode should increase from a value, at which no Faradaic reaction occurs, up to a potential of zero surface concentration. At the initial potential no current flow and no oxidation or reduction of the electrochemically active species takes place. When the potential reaches its final value, the current flows and the electrochemical reaction occurs, which is diffusion limited. The resulted current can be described Cottrell equation:

$$I(t) = \frac{nFAcD^{1/2}}{\pi^{1/2}t^{1/2}} = kt^{-1/2} \tag{4.2}$$

Where n is the stoichiometric number of electrons involved in the reaction, F is the Faraday constant, A is the surface area, c is the concentration of the electroactive species, D is the diffusion coefficient and t represents time.
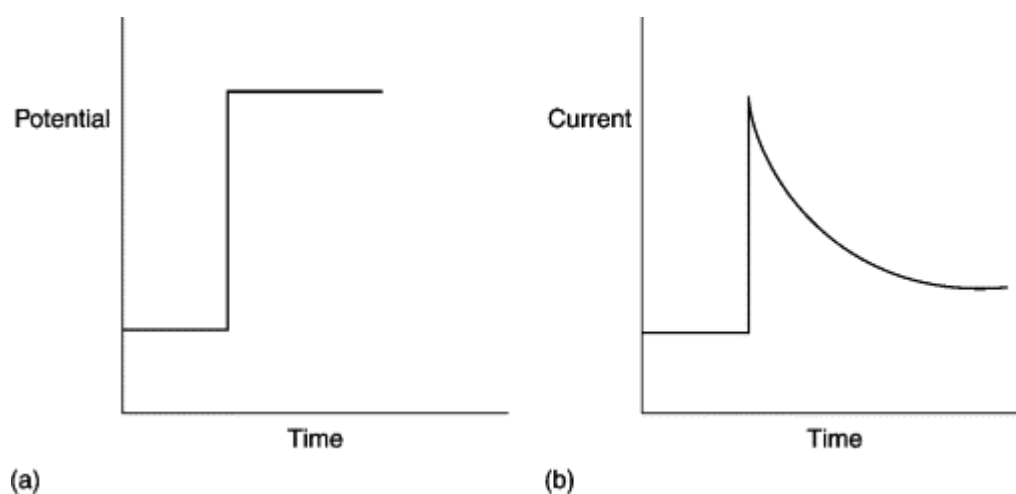
Figure 4.1. Typical input waveforms (a) and output (b) for chronoamperometry. Source [12].

4.4 Amperometry – Amperometric calibration

Amperometry is a powerful electrochemical tool for the analysis of electro active compounds like dopamine, uric acid and ascorbic acid at the range of nanomolar to picomolar level. It is awidely reported electrochemical method often applied in commercial biosensors [8]. Amperometry is the measurement of the resulting current after applying a constant potential to the working electrode in a two- or three-

electrode arrangement. Typically, the current is measured between the working and counter electrodes, at a constant potential, applied between the working and reference electrodes, and this is referred to as amperometry. Amperometric sensing is often used in enzyme-based sensors, where the enzyme catalyses a redox reaction. Selectivity and good detection limits of electroactive substances can be achieved through a careful choice of the detection potential, with the optimal detection potential normally being determined by hydrodynamic voltammetry [106].

4.5   Rotating disk electrodes (RDE)

The rotating disc electrode (RDE) is a hydrodynamic electroanalytical method used to limit the diffusion layer thickness [9]. It is a typical way to estimate the numbers of electrons transferred in faradaic reactions at rotated disk electrodes (RDEs) from the slope of plots of reciprocal current (1/I) vs. reciprocal square root of rotational velocity ($1/w^{1/2}$) [107]. The RDE consists of a disc which can be from a variety of materials such as Pt, Ni, Au, Cu, glassy carbon and graphite, surrounded by an insulating material e.g. PTFE.

The electrode is rotated about its vertical axis, as shown in the figure below, typically between 400 and 10,000 rpm (maintaining laminar flow around the electrode, regardless the speed). At the same figure it is clear that the velocity of each unique point on the surface is linear, increased proportionally by its distance from the center axis of rotation. This has as a result that the rate of mass transport at the electrode surface is even and homogeneous. According to literature, the RDE assumes that the electrode is evenly accessible and provides a precise and reproducible control of the convection and diffusion of reactant to the electrode. Thus, the RDE can be a valuable tool in studying the kinetics of interfacial processes [107].

The collected data from the RDE technique can be described sufficiently from the Koutecky-Levich equation:

$$\frac{1}{I} = \frac{1}{I_K} + \frac{1}{0.201 F A D_0^{2/3} v^{-1/6} C_0^* \omega^{1/2}} \quad \text{with } \omega \text{ in rpm} \qquad (4.3)$$

where I_K is the kinetically controlled current that would be obtained for $\omega^{-1/2} = 0$, i.e. for an infinite rotation speed where the mass transfer would be so efficient that

the surface concentration of C_O would be equal to CO^* [107]. Hence, a plot of $1/I$ vs. $\omega^{-1/2}$ should be linear and extrapolation to $\omega^{-1/2} = 0$ should yield I_K.

It's clear that the shape of the curve depends on ω and this due to the fact that the redox process is kinetically limited. The current can be measured at fixed potentials along the voltammogram and for each potential the inverse of the current vs. $\omega^{1/2}$ can be plotted. When the redox process is reversible, at any potential, the current should, like the limiting current, be proportional to $\omega^{1/2}$ [9].
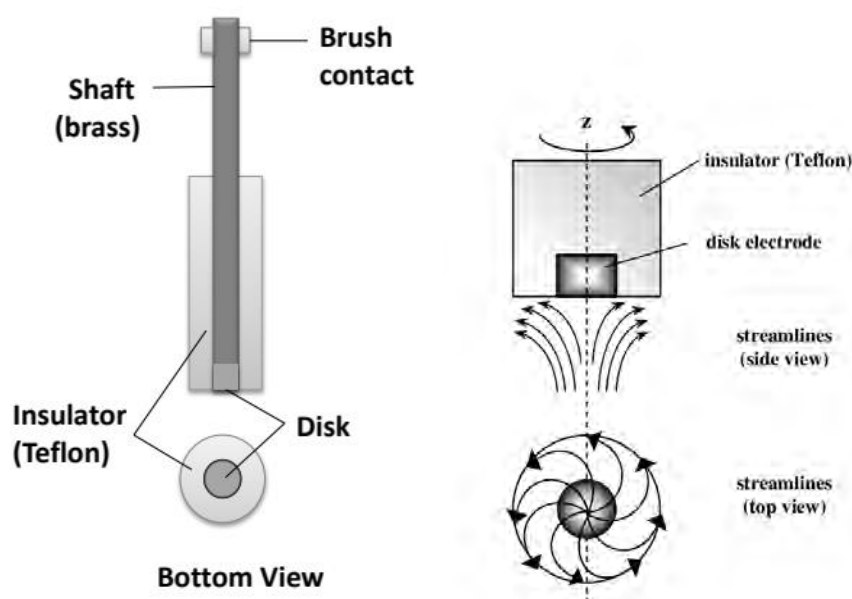
Figure 4.2. streamlines for flow and vector representation of fluid velocities near a rotating disc electrode [7].

4.6 Electrochemical thermodynamics and kinetics

4.6.1 Faraday's Law

Faraday's laws of electrolysis are quantitative relationships based on the electrochemical researches published by Michael Faraday in 1834. Faraday cased two laws of electrolysis. The first states that the mass of a substance altered at an electrode during electrolysis is directly proportional to the quantity of electricity transferred at that electrode. It should be noted that the quantity of electricity refers to electrical charge, typically measured in coulombs, and not to electrical current. Faraday's second law indicates that: For a given quantity of electricity (electric

charge), the mass of an elemental material altered at an electrode is directly proportional to the element's equivalent weight. The equivalent weight of a substance is its molar mass divided by an integer that depends on the reaction undergone by the material [108].The mathematical form of Faraday's laws (4.4) can be represented by

$$m = (\frac{Q}{F})(\frac{M}{z}) \tag{4.4}$$

where m is the mass of the substance altered at an electrode, Q is the total electric charge passed through the substance, $F = 96.485$ C mol−1 is the Faraday constant, M is the molar mass of the substance, and z is the valance number of ions of the substance (electrons transferred per ion).

Note that M/z is the same as the equivalent weight of the substance altered. For Faraday's first law, M, F, and z are constants, so the larger the value of Q, the larger will m be. For Faraday's second law, Q, F, and z are constants, so the larger the value of M/z, the larger will M be [109].

Another useful form of Faraday's law, which relates the total charge Q that passes through an electrochemical cell to the amount of product N is:

$$Q = n \cdot F \cdot N \tag{4.5}$$

where F is Faraday's constant and n is the number of electrons transferred per mole of product [9].

4.6.2 Nernst Equation

The Nernst Equation is the fundamental principle, which relates electromotive force, or potential, to thermodynamic properties and it is the most important equation in the field of electrochemistry. It enables the determination of the cell potential at any moment during a reaction or at conditions other than standard-state. Redox reactions can generally be expressed in the following format where an oxidant, Ox, results to a reductant product, Red, by the charge transfer of n number of electrodes:

$$Ox + ne^- \Leftrightarrow Red \tag{4.6}$$

The determination of the potential of an electrochemical cell, set as E, containing a reversible system with fast kinetics, is given by the following equation. Note that it is valid only at equilibrium and at the surface of the electrode:

$$E = E^{0}_{\text{cell}} + \frac{RT}{nF} \ln \frac{C_{ox}}{C_{red}} \qquad (4.7)$$

where E^0 [V] is the standard potential, R is the gas constant, T is the temperature in Kelvin, n is the number of electrons transferred at the redox reaction, F is Faraday's constant, and C_{ox}, C_{Red} represent the concentration of the oxidation and reduction species at the electrode interface. For this common expression of the Nernst equation, the activity coefficients of Ox and Red are assumed to be at unity.

The basic thermodynamics for a reversible electrochemical reaction is given by:

$$\Delta G = \Delta H - T\Delta S \qquad (4.8)$$

where ΔG is the Gibbs free energy or the available energy in a reaction, ΔH is the enthalpy, or the energy released by the reaction, ΔS is the entropy and T is the absolute temperature, with $T\Delta S$ being the heat associated with the reaction. The terms ΔG, ΔH and ΔS are state functions and depend only by the characteristics of the electrode materials and the initial and final states of the reactions [110]. The Nernst Equation relates the potential of an electrochemical reaction to some fundamental thermodynamic properties, as described in the following equation:

$$\Delta G^{o} = -nFE^{o}_{\text{cell}} \qquad (4.9)$$

where $\Delta G°$ is Gibbs free energy, n represents number of electrons transferred at the redox reaction, F is Faraday's constant, and $E°_{\text{cell}}$ is the abovementioned potential at standard conditions. The Nernst equation applies to a redox reaction at steady state conditions [111].

4.6.3 Butler-Volmer Equation

The Butler-Volmer Equation is a kinetic relationship between current and applied potential of an electrochemical device. It can be expressed as:

$$i = nFAk_0 \left([Ox]exp\left[-\frac{anF}{RT}(E - E_0)\right] - [Red]exp\left[\frac{(1-a)nF}{RT}(E - E_0)\right] \right) \qquad (4.10)$$

where R is the gas constant, T is the temperature, E_0(in V) is the standard potential of the redox reaction, E(in V) is the applied potential, F is the Faraday constant, n is the number of electrons transferred, k_0 is the standard heterogeneous rate constant (in m/s), and α is the transfer coefficient (α=0.5 in this case) [112].

The term $nFAk_0$ equals to i_0 which is the exchange current density and describes the current that the electrochemical cell is theoretically capable of producing without activation influences or other losses. The exchange current density is described at open circuit potential, where there is no net current and the forward and reverse reactions are occurring at the same rate which is equal and opposite in magnitude. The forward and reverse components can be considered idle and upon polarization, will produce a net current. High exchange current density means that the redox reaction occurs rapidly in both directions [113].

4.6.4 Tafel Equation

The Tafel equation was empirically developed by Julius Tafel in 1905 by experimentally measuring the hydrogen evolution reaction on several metal electrodes such as mercury, lead, and cadmium. He researched the relationship of overpotential, rather than the absolute potential, and the logarithm of current density and was aiming to control the rate of electrode reactions by controlling the potential. The equation he reported was:

$$\eta = a + b \log(J) \tag{4.11}$$

where η is the overpotential, a is the intercept of the Tafel plot, b is the slope of the fitted line on a Tafel plot and J is the scalar current density. Since Tafel's reports in 1905, it has been shown that his empirical relationship can be related to fundamental principles. This fact was confirmed after the Butler-Volmer equation establishment, as the Tafel Equation can be derived from the Butler-Volmer equation, which is a relationship of fundamental terms.

In the Tafel region, the electrode polarization is sufficient to suppress the reverse reaction and the forward reaction dominates. Thus, the reverse reaction branch in the Butler-Volmer equation is negligible and the equation is reduced to:

$$i = i_0 exp \frac{\alpha n F}{RT} \eta \tag{4.12}$$

Rearrangement of the terms, with the addition of the quantity 2.303 accounts for the differences in the natural logarithm used in the Butler-Volmer equation and the base 10 logarithm used in the Tafel equation:

$$\log(I) - \log(I_0) = \frac{\alpha nF}{2.303rT}\eta \qquad (4.13)$$

Solving for η yields to:

$$\eta = \frac{2.303rT}{anF}\log(I) - \frac{2.303rT}{anF}\log(I_0) \qquad (4.14)$$

which resembles the form of a line where b is the slope and a is the intercept, where the slope of the line on a Tafel plot is represented by:

$$b = \frac{2.303rT}{anF}\frac{2.303rT}{anF} \qquad (4.15)$$

and the intercept of the line is an expression of these constants

$$a = -\frac{2.303rT}{anF}\frac{2.303rT}{anF}\log(I_0) \qquad (4.16)$$

This derivation of the Tafel equation shows its relationship to kinetic properties. Kinetics Thermodynamics describe reactions at equilibrium and the maximum energy available for a given reaction [114].

Separating the parameter α to α_α and α_c, which are called anodic and cathodic transfer coefficients respectively, we can relate how an applied potential favors one direction of reaction over the other [115]. The following equations can be extracted:

$$\text{Oxidation slope} = -\frac{a_a nF}{2.303rT}n \qquad (4.17)$$

$$\text{Reduction slope} = -\frac{a_C nF}{2.303rT}n \qquad (4.18)$$
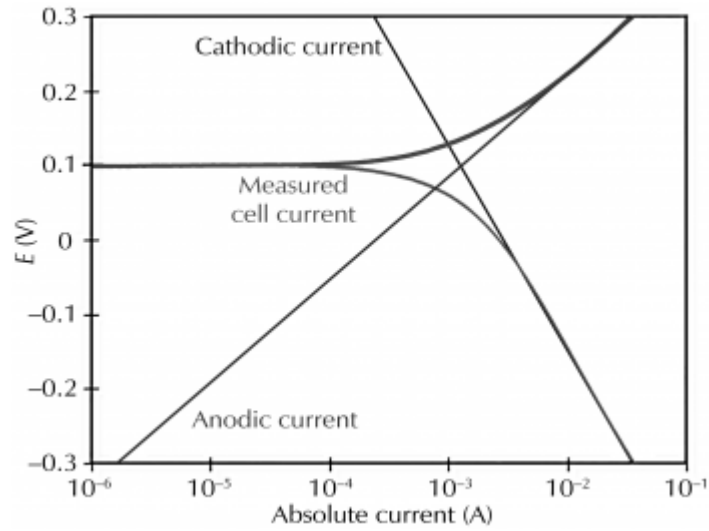
Figure 4.3. Schematic polarization curve showing Tafel extrapolation [7].

4.6.5 Koutecky-Levich Equation

The Koutecký-Levich equation gives a model of the measured electrical current in an electrode by an electrochemical reaction in relation to kinetic activity and mass transfer of the reactants. It is a versatile tool that is commonly used for the correction of mass transport effects in a rotating disc electrode testing to obtain kinetic current densities [7]. The Koutecky-Levich equation can be derived from two basic assumptions. The first is a linear diffusion model of reactants, where the surface reactant concentration C_a can be related to the reactant concentration in the bulk C_b by the measured total current density I_m and the mass transport limiting current density I_{lim}.

$$\frac{C_a}{C_b} = \frac{I\,lim - I\,tot}{I\,lim} \quad \frac{I_{lim} - I_m}{I_{lim}} \tag{4.19}$$

The second assumption is that the electrochemical reaction follows first-order reaction kinetics. Assuming a first order reaction, the local current density I_{loc} is proportional to the multiplication of surface reactant concentration and the kinetic current density I_k, whereby I_k is a function of overpotential η and is a product of reaction rate constant and reactant concentration C_a under standard conditions:

$$I_{loc} = I_k \frac{C_a}{C_b} \tag{4.20}$$

As $I_{tot} = L\ I_{loc}$ and given that the surface roughness factor L of the electrode is 1, the previous equations can be combined to form the Koutecky-Levich equation [116]:

$$\frac{1}{I_m} = \frac{1}{I_{lim}} + \frac{1}{I_k}$$

(4.21)

In the case of the rotating disk electrode, the equation is deduced to:

$$\frac{1}{I_m} = \frac{1}{BL\omega^{0.5}} + \frac{1}{I_k}$$

(4.22)

where BL is the Levich constant and ω is the angular rotation rate of the electrode.

Chapter 5

Experimental procedure

The electrochemical behavior of dopamine is illustrated, followed by a description of the voltammetric and amperometric methods for Dopamine in physiological buffer solution. The electrocatalysts tested include Pt(20%wt)/graphitized carbon, Pd(20wt%)/Vulcan-XC72 commercial and Pd(20wt%)/Vulcan-XC72 homemade. Firstly, the equipment used for the experiments is presented and then the preparation of the catalyst is explained. Therefore, the analytical performances of the modified electrode are highlightened and the results for the two catalysts are discussed.

5.1 Materials and methods

5.1.1 Apparatus and measurements

The electrochemical experiments were performed with an AMEL 7050 (Fig.5.1.A), electrochemical workstation in an electrochemical cell of three electrodes (Fig.5.1.B).

Figure 5.1. A) AMEL 7050 Potentiostat B) Electrochemical cell.

As a working electrode (a working area of 0.07 cm^2) a 3-mm-diameter GC was used and a silver/silver chloride (Ag/AgCl (3mol L^{-1} KCl) electrode and a platinum electrode were used as the reference and the counter electrodes, respectively (Fig.5.2.A, B). All potentials in this study are reported with respect to the Ag/AgCl (3M KCl +0.210V vs (RHE).
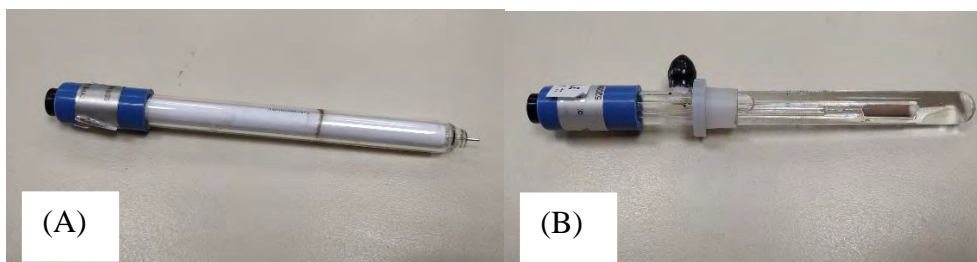
Figure 5.2. A) Counter electrode B) Reference Electrode.

5.1.2 Electrode preparation and characterization

5.1.2.1 Catalyst preparation

Glassy Carbon electrode (GCE) was modified with two different catalysts. The two tested catalysts are Platinum (20%wt) on graphitized carbon and Palladium(20%wt) on Vulcan-XC72. The catalytic ink was composed of 5mg of the selected catalyst powder, 1.8ml Ethanol and 0.2ml Nafion. Nafion is consisted by a tetrafluoroethylene main chain with perfluoroether side chains and terminated with a sulfonic acid group, whose ionization yields an anionic Nafion membrane. The ethanol and the Nafion were added gradually to the mixture. Firstly, the catalyst was mixed with Ethanol and they were homogenized in the magnetic stirrer for 2 minutes and in the ultrasonicator for another 2 minutes (Fig.5.3A, B). This procedure was repeated for 3 times. Thereinafter, Nafion was added and the mixture was placed at the magnetic stirrer for 45 minutes.

The mixed paste was then inserted into a glass tube (d: 1.5 mm). Before use, the surfaces of the electrodes were smoothed using a mixture of alumina and deionized water. Then it was located in the ultrasonicator for 10 minutes.

Figure 5.3. A) Magnetic Stirrer B) Ultrasonicator.

After the electrode was cleaned and dried, 10μl of the mixed paste was placed on it. The modified electrode obtained was inserted in the solution and connected to the electrochemical station ready to use. Before every experiment, the solution was saturated with high-purity N_2 gas for 30 minutes in order to remove the dissolved oxygen in the electrolyte. The electrochemical techniques used for the evaluation and comparison of the performance of the catalysts were cyclic voltammetry, differential pulse voltammetry and chronoamperometry. In detail, the electrocatalytic activity towards dopamine electrooxidation was examined by cyclic voltammetry (CV), while their stability and poisoning rate by chronoamperometric measurements. The range in which the CV measurements were made was 0-1V for both catalysts as it is proposed from the available literature. Moreover, the effects of temperature, concentration of dopamine and electrolyte were also investigated.

5.1.3 Physico-chemical characterization

XRD measurements were carried out with the aid of a D/Max-IIIA (Rigaku Co., Japan) employing Cu K_α ($\lambda = 0.15406$ nm) as the radiation source at 40 kV and 40 mA. Catalysts were investigated by TEM using a Philips CM12 microscope (resolution 0.2 nm), provided with high resolution camera, at an accelerating voltage of 120 kV. Suitable specimens for TEM analyses were prepared by ultrasonic dispersion in *i*-propylic alcohol adding a drop of the resultant suspension onto a holey carbon supported grid.

5.1.4 Electrochemical characterization

All the electrochemical measurements were conducted with AMEL 5000 electrochemical station in a three-electrode model cell 497 (AMEL) with a silver/ silver chloride (Ag/AgCl 3M) (0.204 V *vs.* RHE) and platinum wire as the reference electrode and counter electrode, respectively. The thin catalyst film was prepared onto a glassy carbon disk surface with a diameter of 0.3 cm. The catalyst ink was then quantitatively (10μL) transferred onto the surface of the glassy carbon electrode and dried under infrared lamp to obtain a catalyst thin film. The electrochemical tests were performed initially in 0.15M saline containing 0.5M dopamine (Sigma Aldrich), for the electrochemical activity evaluations. Then the effect of

temperature (26–56°C) on dopamine electrooxidation was also investigated. Moreover, before each measurement, each catalyst was applied under continuous potential cycling until stable electrochemical signals were received. It should be noted that all the potentials are referred to Ag/AgCl reference electrode without specification.

Differential-pulse voltammetry measurements were carried out with a CHI 660B electrochemistry workstation (CHI, USA) in 0.15M saline with a conventional three-electrode system comprised of a platinum wire as the auxiliary electrode, an Ag/AgCl as the reference electrode and the modified GCE used as working elec1trode.

Chapter 6

Results and Discussion

In this chapter the physiochemical and electrochemical characterization of Pt (20wt%)/graphitized carbon and Pd (20%wt)/Vulcan-XC72 electrocatalysts are presented. The methods used are transmission electron microscopy for the depiction of their structure and Cyclic voltammetry, Chronoamperometry and differential pulse voltammetry are the methods which assisted the electrochemical detection of dopamine. At this point should be mentioned that all the electrochemical experiments were also carried out for the characterization of ascorbic acid and uric acid.

6.1 Pt(20%wt)/graphitized carbon electrocatalyst

6.1.1 Physicochemical characterization

Firstly, the structure of Pt(20wt%)/graphitized carbon was characterized by transmission electron microscopy (TEM). The TEM images depicted in Fig.6.1 show the general morphology of the nanomaterial that was used in this study.
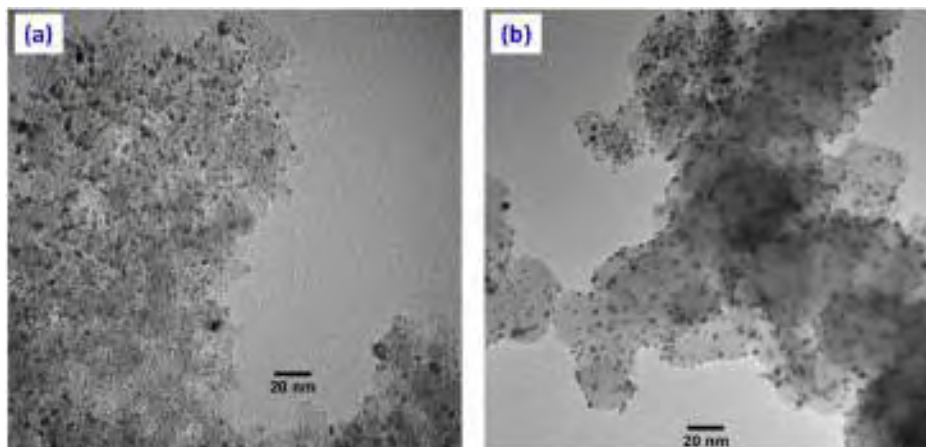

Figure 6.1. TEM images of Pt(20%wt)/graphitized carbon.

In the figure the Pt/C nanoparticles can be observed as dark dots. It can be observed that the Pt/C nanoparticles are well distributed on the carbon surfaces so as to form the nanocomposite, which is believed to be capable of enhancing the electrochemical detection of AA, DA, and UA.
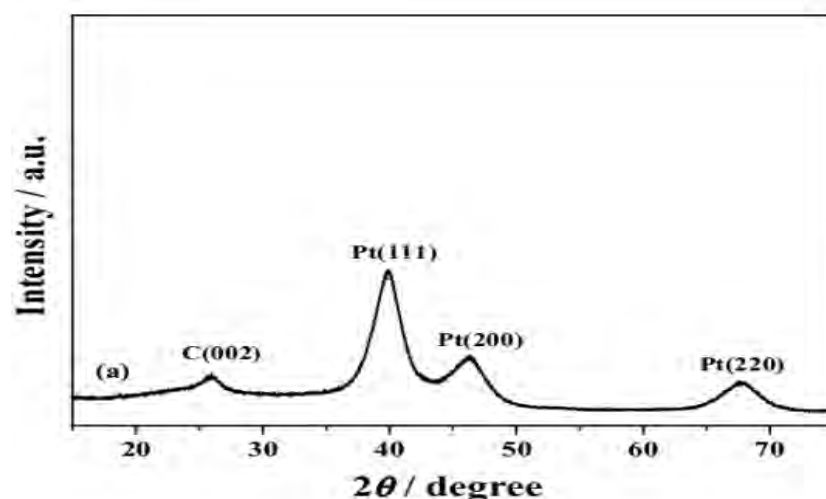
Figure 6.2. X-ray pattern of Pt(20%wt)/graphitized carbon.

In Figure 6.2 the X-ray pattern of the Pt(20%wt)/graphitized carbon is given. Peak positions occur where the X-ray beam has been diffracted by the crystal lattice. The unique set of d-spacings derived from this patter can be used to identify the the compounds of the material. The typical face centered cubic (fcc) crystalline peaks of Pt are identified, namely the planes (111), (200) and (220) at *ca.* 39°, 47°, ans 68°. Apart from the Pt crystallographic structure signals, the graphitized carbon reflection plane (002) at $2\theta = 25.5°$ is also identified, the latter for the Pt1Sn1/C and especially the Pt1Sn3/C samples.

6.1.2 Electrocatalytic activity

The electrocatalytic activity towards dopamine oxidation was examined by cyclic voltammetry. The stability and poisoning rate during time was investigated by chronoamperometry and the selectivity of the catalyst was tested by using DPV. The glassy carbon electrode (GCE) is modified using Pt(20%)/graphitized carbon for the electrochemical determination of low concentration, 0.5 mM of dopamine (DA) in pH 7.3 physiological buffer solution.

During the recent years many researches have reported materials for electrode modification for dopamine detection based on carbon materials, metal oxides, polymers or even a combination of these. In the next table some examples are given:

Table 6.1. Comparison of modified electrodes in the literature in this work.

Working Electrode	Reference Electrode	Peak Potential (V)	Refs
Pd/RGO/GCE	Ag/AgCl	0.2	[117]
Pd₃Pt₁/PDDA-RGO	Ag/AgCl	0.15	[118]
Au@Pd-RGO	Ag/AgCl	0.2	[119]
Graphene -SnO2	Ag/AgCl	0.13	[120]
MWCNT/CCE	Ag/AgCl	0,57	[121]
MgO/Gr/Ta	Ag/AgCl	0,3	[122]
SWCNTs-GCE	Ag/AgCl	0.2	[123]
Pd/CNF-CPE	Ag/AgCl	0,33	[124]
Gr/Pt/GCE	Ag/AgCl	0.24	[16]
Pd³Pt¹/PDDA-RGO	SCE	0.16	[83]
f-SWCNTs/GCE	SCE	0.18	[125]
Pt/RGO/GCE	SCE	0.32	[126]
Pt/C/GCE	Ag/AgCl	0,63	This work
Pd(20wt%)/Vulcan-XC72	Ag/AgCl	0,63	This work

6.1.2.1 *Effect of temperature*

Cyclic voltammetry has been carried out initially, in 0.15 M physiological buffer solution in absence and in presence of an analyte in order to determine were the oxidation of dopamine takes place. It is clearly shown in Fig.6.3 that the oxidation dopamine is observed at a potential 0.63-0.7 V. The following figure also presents the cyclic voltammetric curves for dopamine in saline, 7.3pH in order to investigate the effect of temperature on the oxidation of dopamine. The four temperature values tested were 26ºC, 36ºC, 46ºC and 56ºC.
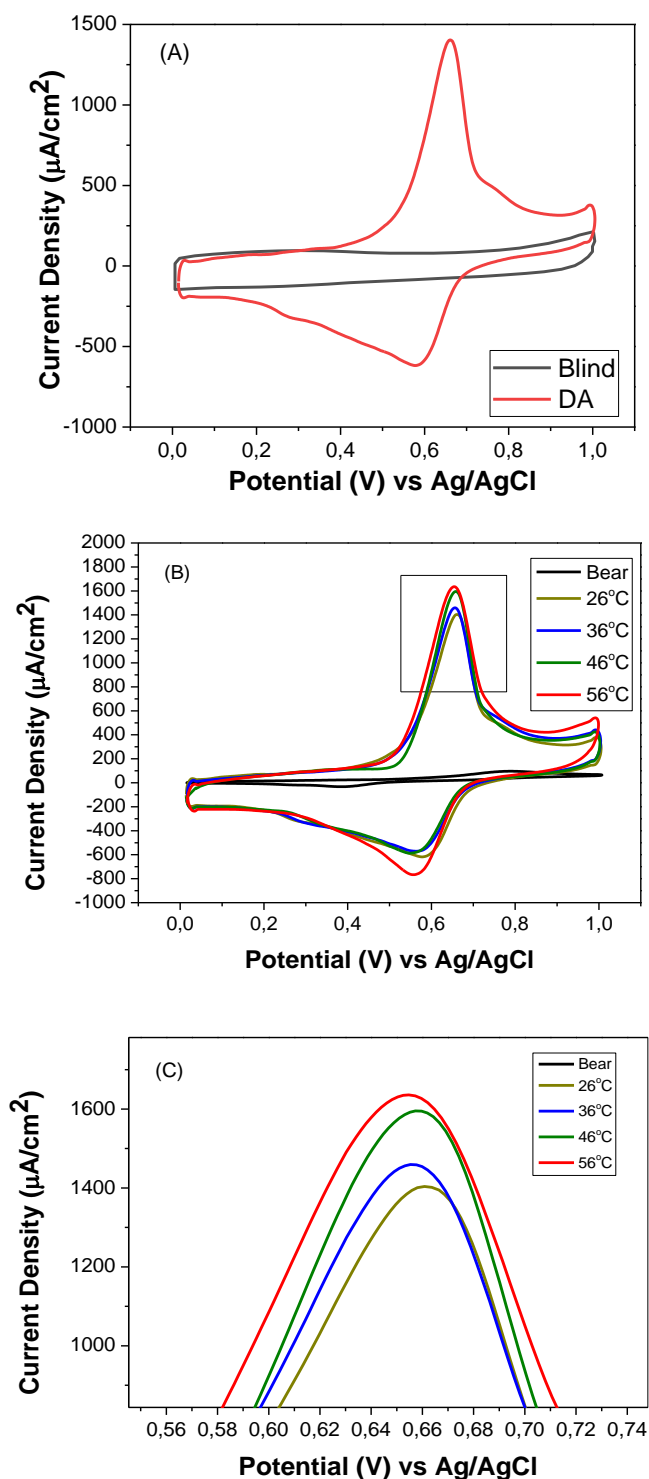
Figure 6.3. Cyclic voltammograms of Pt(20%wt)/graphitized carbon in:(A) 0.5mM dopamine in a 0.15M saline (physiological buffer solution), (20mV s-1, 26°C compared with the cyclic voltammogram of the Pt(20%wt)/graphitized carbon in 0.15 M saline. (B) Comparison of cyclic voltammograms of Pt(20%wt)/graphitized carbon electrocatalyst in 0.5mM dopamine in a 0.15M saline at 26°C, 36°C, 46°C and 56°C, (scan rate 20mV s-1). (C): Focus on oxidation peak current.

A well-defined oxidation peak of DA in the CVs is observed at ∼0. 65 V and the current density values are found to be 1403.7μA cm⁻², 1459.4μA cm⁻², 1595.3μA

cm^{-2} and 1636µA cm^{-2} at 26ºC, 36ºC, 46ºC and 56ºC, respectively. As the temperature of the solution is increased, the peak current is raised as well, demonstrating the increase of the kinetic energy of the dopamine molecules, confirming the dependence of the peak current from temperature. These data illustrate that Pt(20%wt)/graphitized carbon is suitable for dopamine sensing.

Activation energy E_a is calculated from the following equation [7],

$$E_a = R \frac{d(lnI)}{d(^1/_T)} \qquad (5.1)$$

where R is the gas constant, I the current and T the absolute temperature (K). Taking into consideration Fig.6.4. (Arrhenius plot), activation energy E_a was calculated to be 16.3 kJ mol^{-1} which is a satisfactory value according to the literature [7].

Equation	y = a + b*x
Plot	B
Weight	No Weighting
Intercept	9,00037
Slope	-0,53127
Residual Sum of Squares	6,51309E-4
Pearson's r	-0,97909
R-Square(COD)	0,95861
Adj. R-Square	0,93792
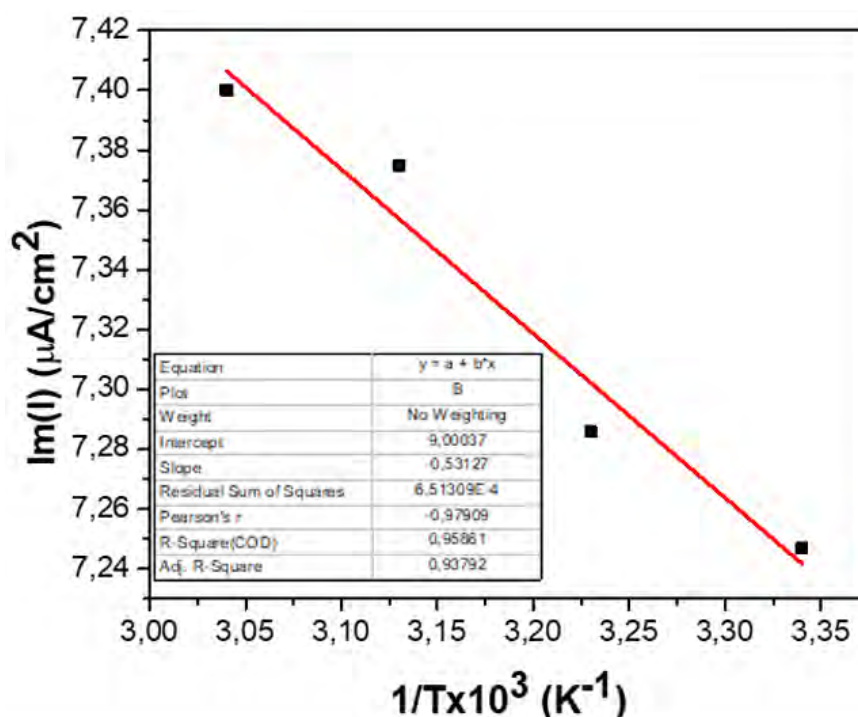
Figure 6.4. Arrhenius plot for the Pt(20%wt)/graphitized carbon electrocatalyst in 0.5mM dopamine in a 0.15 M saline derived from cyclic voltammograms at 20mV s-1.

6.1.2.2   Effect of concentration

The response of the catalyst in different was also tested. The lowest concentration tested was 33 µM but comparing its current with the one of the measurement without the analyte, lead to the conclusion that the catalyst could possibly have a lower limit of detection.
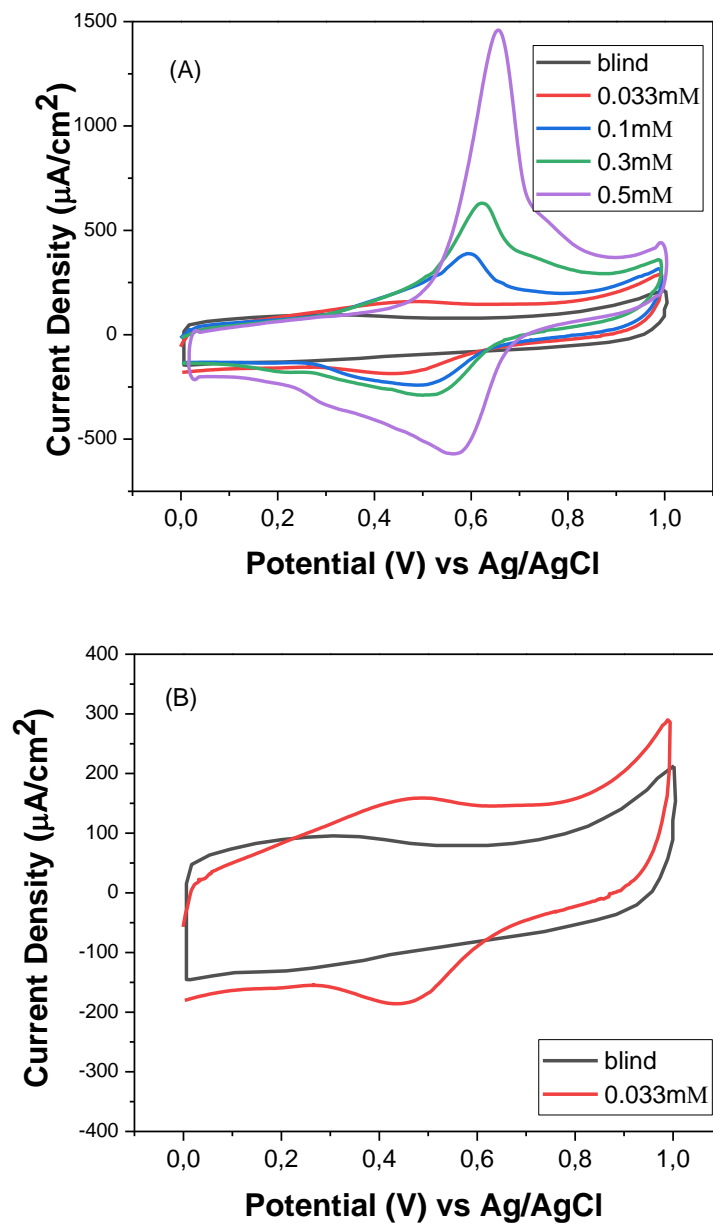
Figure 6.5. (A) Cyclic voltammograms of Pt(20%wt)/graphitized carbon electrocatalyst in different concentration of dopamine in a 0.15 M saline (scan rate 20mV s^{-1}). (B)Comparison between measurements in 0.15 M PBS and in 0.033mM dopamine in a 0.15 M PBS.

6.1.2.3 Effect of Time

Furthermore, chronoamperometry tests were executed in order to observe the stability and possible poisoning of the Pt(20%wt)/graphitized carbon under short time continuous operation. The operation took place for 4000 s at a constant applied potential of 0.6 V (vs Ag/AgCl), as portrayed at Fig.6.6.
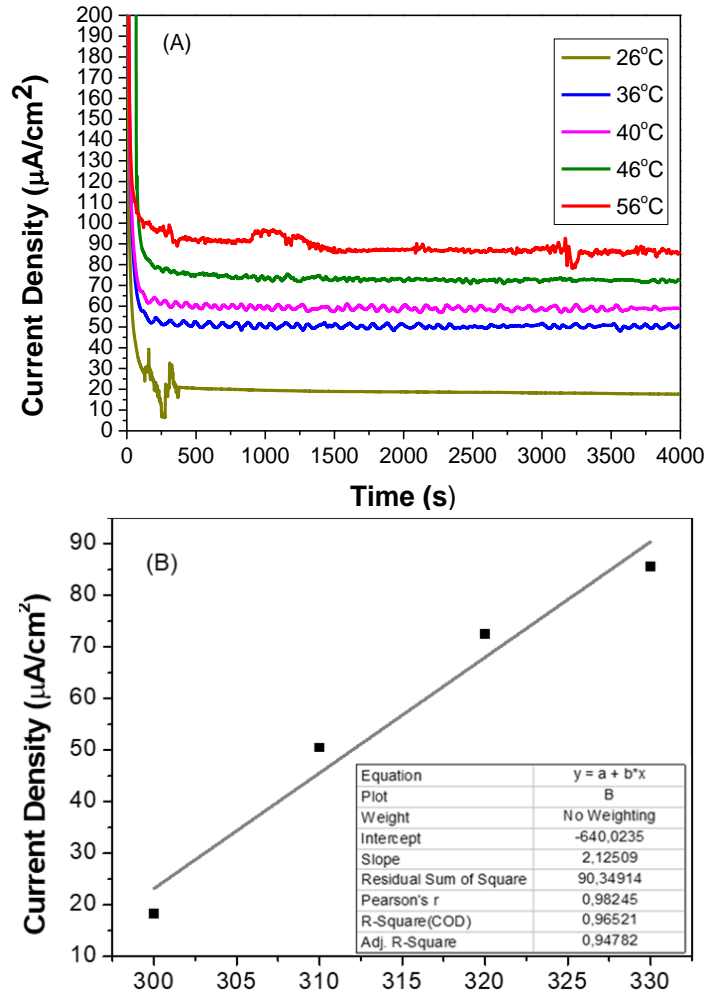
Figure 6.6. (A)C Comparison of chronoamperometric curves of Pt(20%wt)/graphitized carbon electrocatalyst at 0.6V (vs Ag/AgCl) for 4000sec, in 0.5mM dopamine in a 0.15M saline at different temperature values of 26ºC, 36ºC, 46ºC, 56ºC, (20mV s^{-1}), (B): Relationship between current density and temperature.

It is observed that the current density increases with the raise of temperature, while the current density decreases abruptly with time, following a parabolic path and then reaches steady in about 5 minutes.

The poisoning rate (d) obeys the following equation[7]:

$$\delta = \frac{100}{I_o} \left(\frac{dI}{dt}\right) \quad t>500s \tag{5.2}$$

where I$_0$ is the current at the start of polarization back extrapolated from the linear current decay and $\left(\frac{dI}{dt}\right)$ (for t>500s) is the slope of the linear portion of current decay. From the previous equation (5.2), the poisoning rate cam be calculated to be 0.00042 s^{-1}, 0.000064 s^{-1}, 0.0001 s^{-1}, 0.0002 s^{-1} at 26ºC, 36ºC, 46ºC and 56ºC, respectively.

6.1.2.4 Durability Test

As seen from Fig. 6.7, the effect of Pt catalyst slowly weakens, as the cycles of the cyclic voltammetry grows, which means the durability of the catalyst is relatively acceptable.
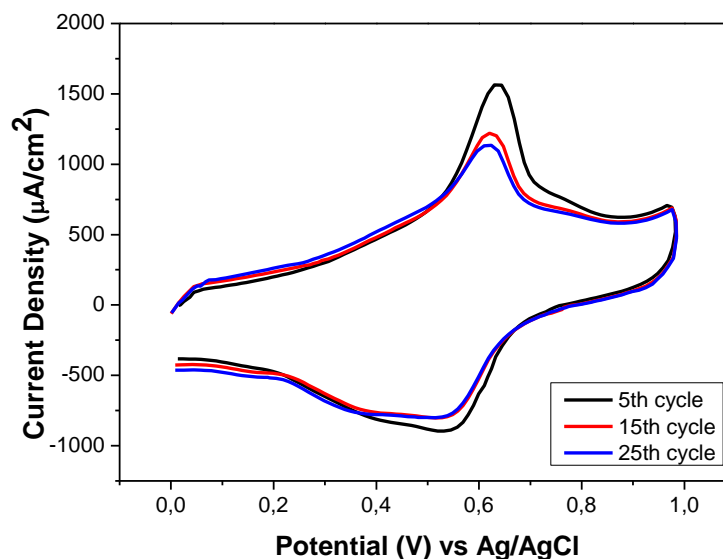
Figure 6.7. Electrochemical durability of Pt(20%wt)/graphitized carbon electrocatalyst in 0.5mM dopamine in a 0.15 M saline: (A) 5th cycle, (B) 15th cycle, (C) 25th cycle (scan rate: 20mV s-1, 36oC). (D): Comparison of all the previous cycles.

6.1.2.5 Selectivity test

Dopamine most of the time coexists in blood and urine samples with ascorbic acid and uric acid. So it is important for the catalyst to be able to detect dopamine regardless the interferences and also give separate redox peaks for each of the substances. Differential pulse (DPV) is a more sensitive technique and was mostly utilized for detection of redox peak of the substances tested [73].

In order to observe the oxidation points of each of the substances, cyclic voltammetry measurements have been carried out initially, in 0.15 M physiological buffer solution in absence and in presence of an analyte. It is clearly shown in Fig.6.8. that the oxidation of ascorbic acid occurs at 0.43 V and the value of the peak current is 173.7μA cm^{-2}, while the reduction occurs at 0.23 V. As it refers to UA, there are two curves that are noteworthy, one around 0.38-0.42 V and the other at 0.77-0.81 V. The first one is explained by hydrogen absorption and the second due to the uric acid oxidation where the current reaches the 193.2μAcm^{-2}.

The measurements were made at a range 0V to 0.9 V and at the figure below, it's shown that three distinct oxidation peaks appear at 0.42V 0.67 V and 0.78 for ascorbic acid, dopamine and uric acid respectively. The peak separation is distinct which makes the catalyst selective enough for the simultaneous detection of the three molecules.
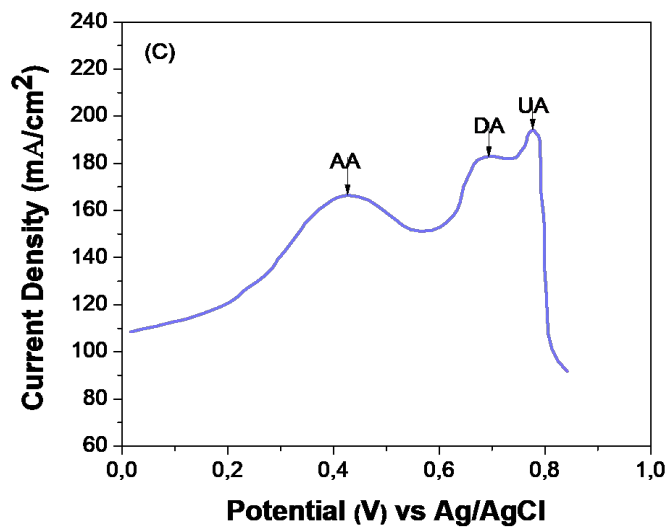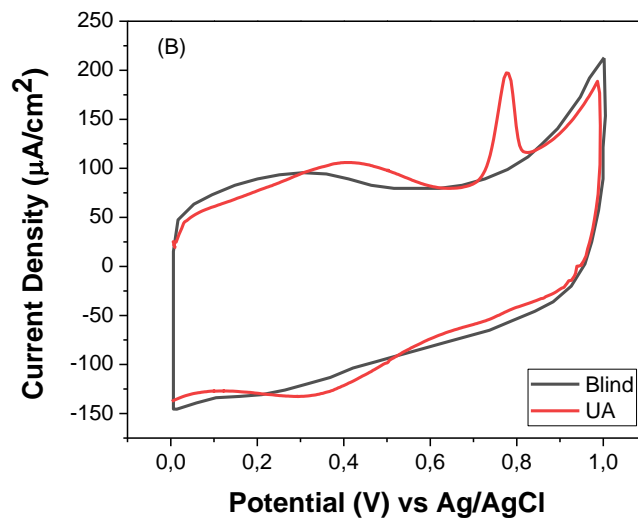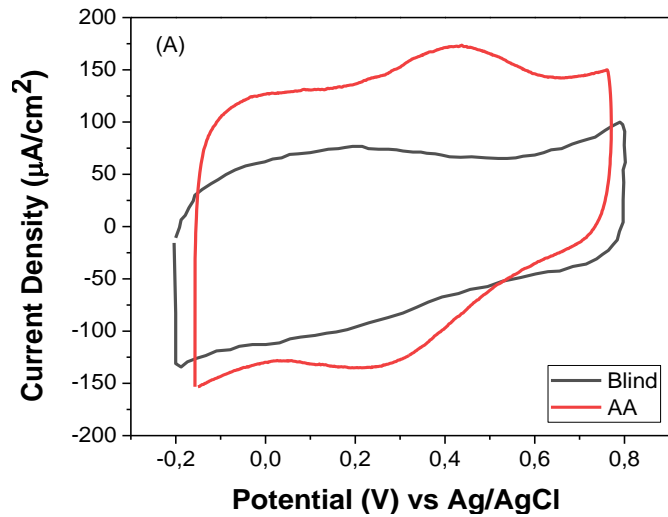
Figure 6.8. (A)Cyclic voltammograms of the Pt(20%wt)/graphitized carbon in 0.5mM ascorbic acid in a 0.15 M saline , (20mV s-1, 26oC), (B) Cyclic voltammograms of the Pt(20%wt)/graphitized carbon in 0.5mM dopamine in a 0.15M saline, (20mV s-1, 26oC , (C) DPV recordings of 30μM DA at a Pt(20%wt)/graphitized carbon in a pH 7.4 saline and in the presence of 5mM AA and 0.5mM UA.

6.2 Pd (20%wt)/Vulcan-XC72 commercial electrocatalyst

In the chapter, the results of the physicochemical and electrochemical characterization of the commercial Pd(20wt%)/Vulcan-XC72 for dopamine electro-oxidation are presented. More precisely, the catalyst structure was characterized by transmission electron microscopy (TEM). The electrochemical characterization conducted via cyclic voltammetry (CV) and chronoamperometry (CA) and rotating disk electrode (RDE) [127].

6.2.1 Physicochemical characterization

The morphology of the surface of the Pd(20wt%)/Vulcan-XC72 prepared catalyst was also examined by TEM and XRD. TEM images in Fig. 6.9.a and Fig. 6.9.b demonstrate a successful loading dispersion of Pd/C nanoparticles onto the surface of carbon substrate [128]. A remarkably uniform and high dispersion of metal particles on the carbon surface is observed in the examined samples.
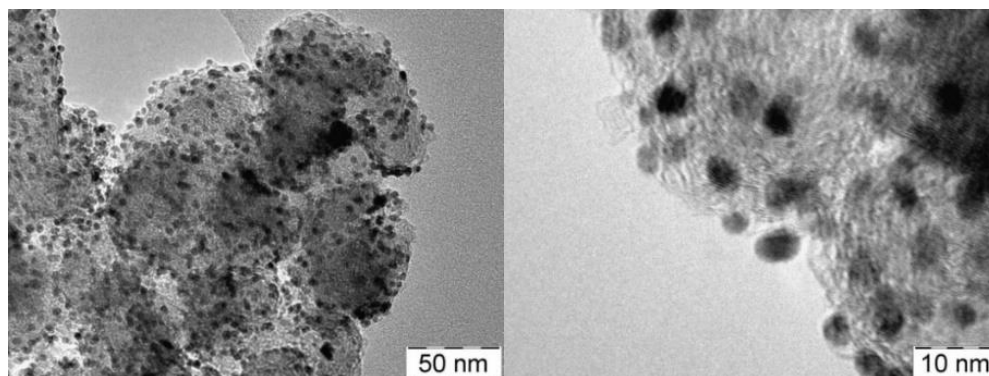
Figure 6.9. TEM images of Pd(20%wt)/Vulcan-XC72.
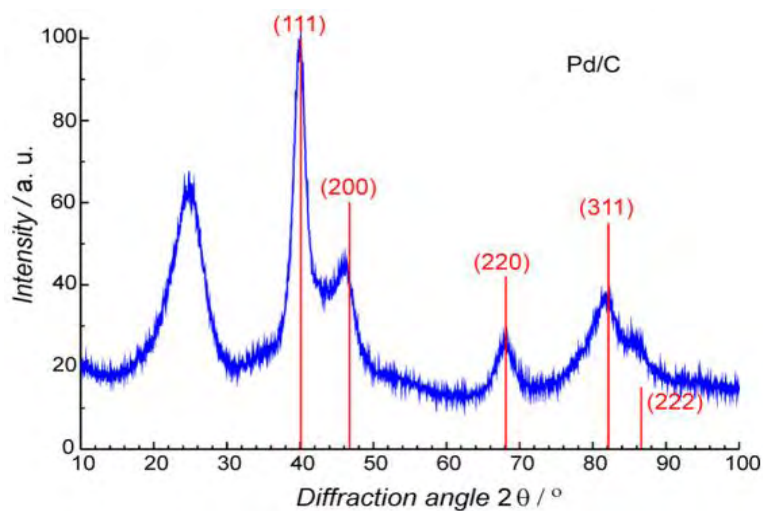
Figure 6.10. X-ray pattern of Pd (20%wt)/Vulcan-XC72 commercial.

XRD patterns of the as-prepared electrocatalysts are shown in Fig. 6.10. There are five observed characteristic diffraction peaks at *ca.* 40°, 47°, 68° 82° and 86° belonging to the face-centered cubic (fcc) phase of Pd (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2), respectively. The Pd (1 1 1) plane has the largest intensity among those planes.

6.2.2 Electrocatalytic activity

The electrochemical detection of dopamine was investigated by cyclic voltammetry and the stability and poisoning rate during time by chronoamperometry. All cyclic voltammetries conducted at 20mV s^{-1} scan rate, with open circuit voltage at 0.23 V.

6.2.2.1 Effect of temperature

Cyclic voltammetry has been carried out initially, in 0.15 M physiological buffer solution (saline) in absence and in presence of an analyte in order to determine were the oxidation of dopamine and the results are displayed in the following figure (Fig. 6.11.)
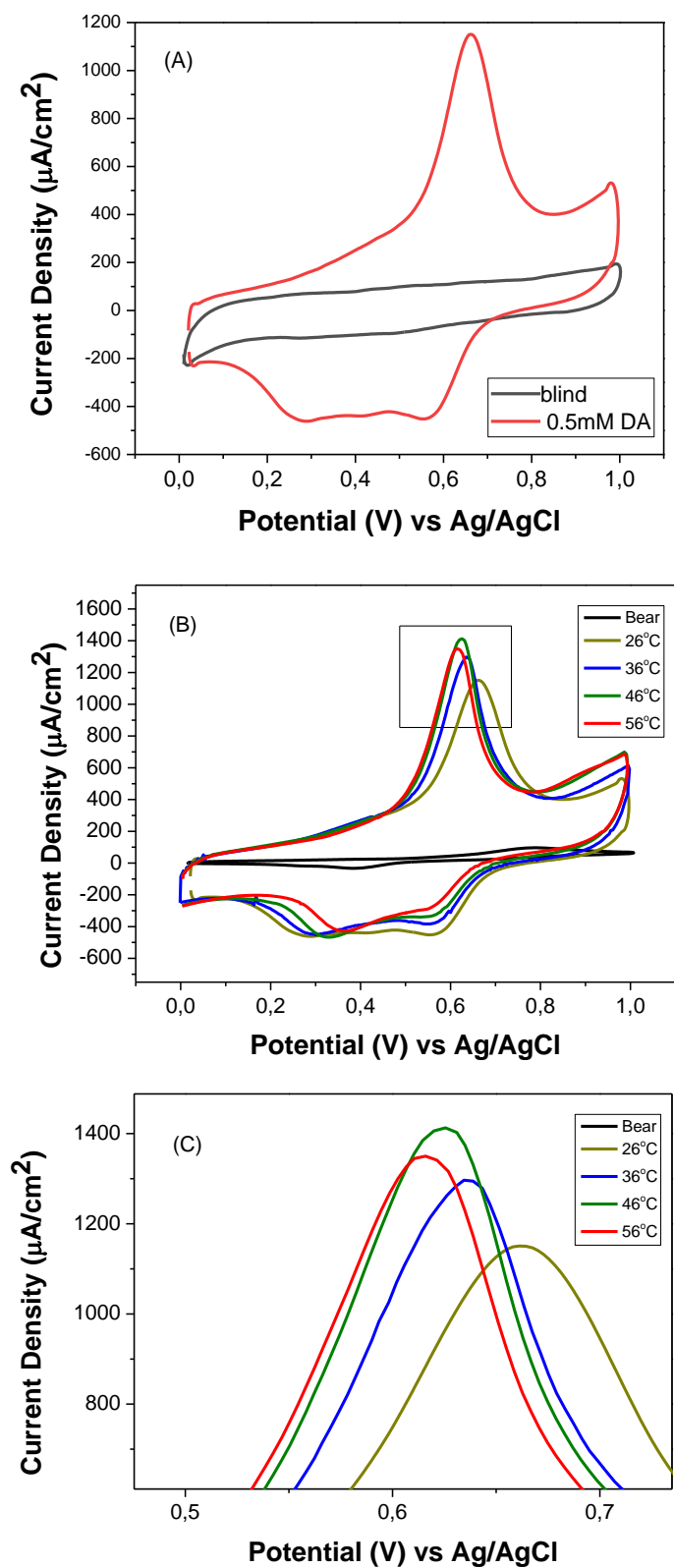
Figure 6.11. (A) CVs of the Pd(20%wt)/ Vulcan-XC72 in 0.5mM dopamine in a 0.15 M saline, scan rate 20mV s^{-1}, temperature 26°C, compared with the cyclic voltammogram of the Pd(20%wt)/ Vulcan-XC72 in 0.15M saline. (B) Comparison of cyclic voltammograms of Pd(20%wt)/ Vulcan-XC72 electrocatalyst in 0.5mM dopamine in a 0.15M saline at 26oC, 36oC, 46oC, 56oC, scan rate 20mV s-1. (C): Focus on the oxidation peak current.

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The oxidation of dopamine clearly appears at 650 mV. The figure also presents the cyclic voltammetric curves for dopamine in saline, 7.3pH in order to investigate the effect of temperature on the oxidation of the analyte. The open circuit voltage of this cyclic voltammetry is 0.23V, the scan rate is 20 mV/s and the voltage is measured by using a reference electrode of Ag/AgCl (+0.210V). The anodic peak current at 26°C, 36°C, 46°C and 56°C appears at 1091μA cm^{-2}, 1295μA cm^{-2}, 1350μA cm^{-2} and 1413μA cm^{-2} respectively.

The peak current is raised as it was expected with the rise of the temperature, demonstrating the increase of the kinetic energy of the dopamine molecules, confirming the dependence of the peak current from temperature. These data illustrate that Pd(20%wt)/ Vulcan-XC72 carbon is also suitable for dopamine sensing.

Using the equation (5.1) and the Fig.6.12. (below) the activation energy is calculated to be at 30,62 kJ mol^{-1} [129].

Equation	$y = a + b*x$
Plot	B
Weight	No Weighting
Intercept	9,50037
Slope	-0,81252
Residual Sum of Squares	0,00388
Pearson's r	-0,94788
R-Square(COD)	0,89847
Adj. R-Square	0,84771
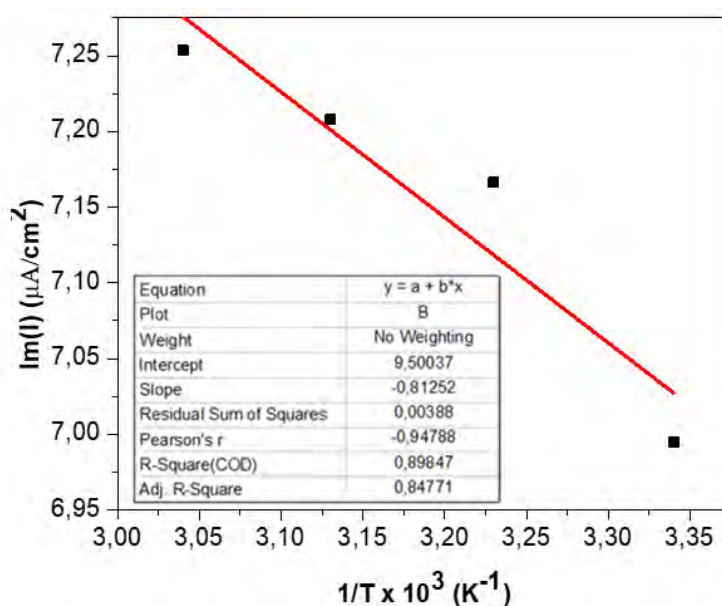
Figure 6.12. Arrhenius plot for the Pd(20%wt)/ Vulcan-XC72 electrocatalyst in 0.5 mM dopamine in a 0.15 M saline derived from cyclic voltammograms at 20mV s-1.

6.2.2.2 Effect of Time

Chronoamperometry technique was also used in order to evaluate the performance, the stability and the poisoning rate of the Pd (20%wt) on glassy carbon electrode of 0.5 mM dopamine in physiological buffer solution at 0.6 V (vs Ag/AgCl).
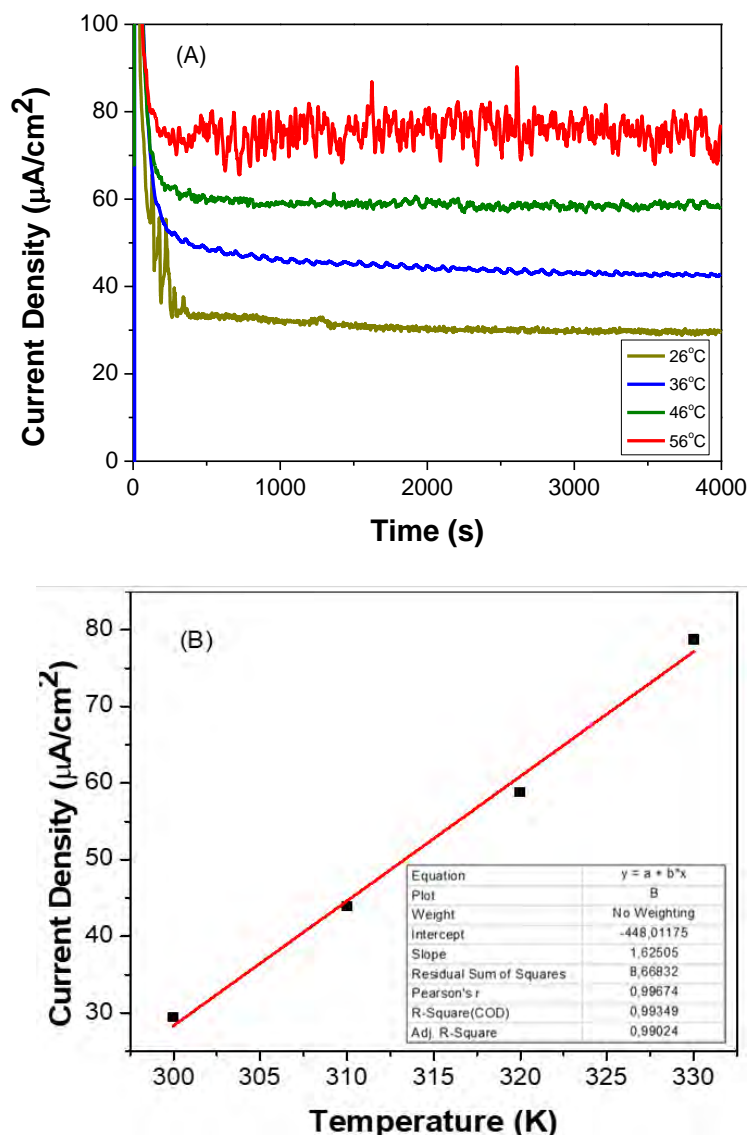
Figure 6.13. Comparison of the chronoamperometric curves Pd (20%wt)/Vulcan-XC72 electrocatalyst at 0.6 V (vs Ag/AgCl) for 4000 sec, in 0.5 mM dopamine in a 0.15 M saline at different temperature values 26oC, 36oC, 46oC, 56oC, scan rate 20 mV s-1, (B): Relationship between current density and temperature.

This method lasted 4000s and according to Fig. 5.15 it was found that the current density decreases abruptly with time, following a parabolic path and then reaches steady in about 5 minutes. Lastly, the poisoning rate was measured from the equation (5.2) and found to be 0.0003, 0.00019, 0.0003, 0.0004 s^{-1} at 26°C, 36°C, 46°C and 56°C, respectively.

6.2.2.3 Durability Test

Fig. 6.14. presents the durability test of the electrocatalyst. The stable duration of it is very low, due to the oxidation and the reduction peak current decreasing with time.
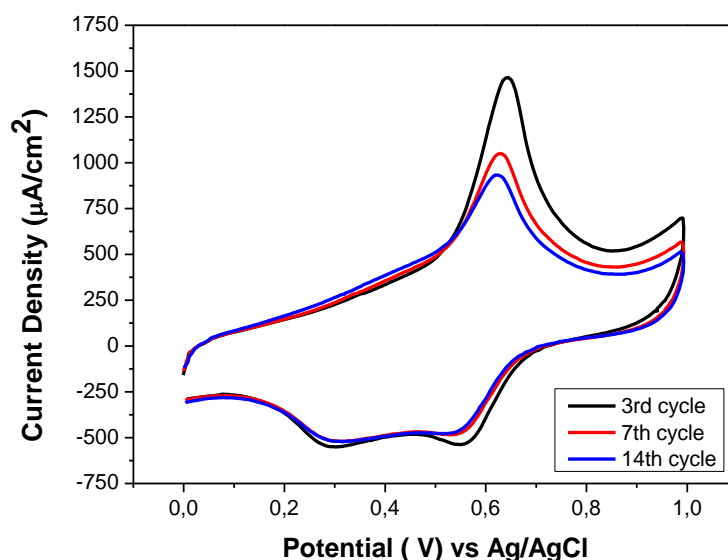
Figure 6.14. Electrochemical durability of Pd(20%wt)/Vulcan-XC72 electrocatalyst in 0.5 mM dopamine in a 0.15 M SALINE: (A) 3rd cycle, (B) 7th cycle, (C) 14th cycle (scan rate 20 mV s-1, 26 oC). (D): Comparison of all the previous cycles. (36.6°C. Scan rate 20 mV/s).

6.2.2.4   Selectivity test

Cyclic voltammetry has been carried out initially, in 0.15 M physiological buffer solution (saline) in absence and in presence of an analyte in order to determine were the oxidation of each of the three substances takes place.   While operating cyclic voltammetry at 0.5mM AA /saline solution an oxidation peak appeared at 0.43V and the peak current value is 125.9μA cm^{-2} (Fig. 6.15 A) At the cyclic volt-ammogram of uric acid, the oxidation peak is not clear, thus it is not presented here, but there is an assumption that occurs from 0.7V to 0.85V.
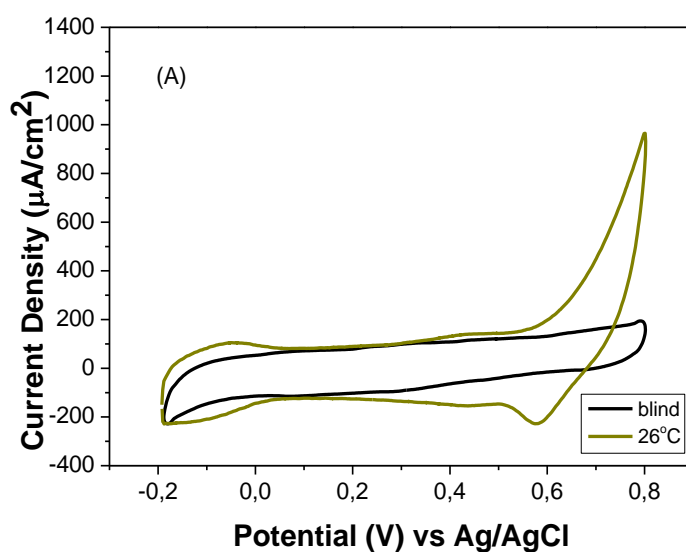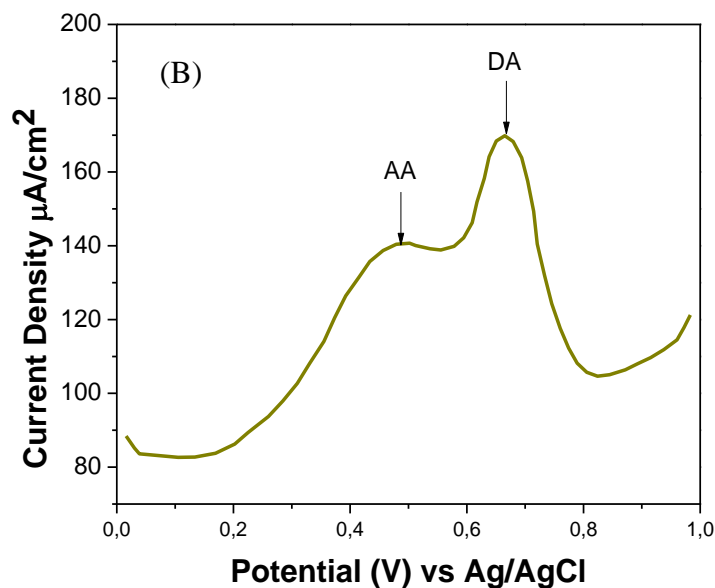
Figure 6.15. (A)Cyclic voltammograms of the Pd(20%wt)/ Vulcan-XC72 in 0.5mM ascorbic acid in a 0.15 M saline, scan rate 20mV s^{-1}, temperature 26°C, compared with the cyclic voltammogram of the Pd(20%wt)/ Vulcan-XC72 in 0.15M saline. (B) DPV recordings of 60µM DA at a Pd(20wt%)/Vulcan-XC72 in a pH 7.4 saline and in the presence of 0.5 mM AA and 0.5 mM UA.

A differential Pulse Voltammetry measurement (Fig. 6.15 B) was carried out to test the selectivity of Pd(20wt%)/Vulcan-XC72 catalyst. As it is obvious from the following figure, the selectivity of the catalyst is not sufficient to observe the oxidation peaks of the analytes. The peaks that are clear are the one of ascorbic acid which appears at 0.5V and the peak value 140.7mA and at 0.66 at 170mA. The measurements were made at a range 0V to 1V and at the figure 6.15 only two distinct oxidation peaks appear at 0.48V and 0.67V for ascorbic acid and dopamine respectively.

6.3   Pd (20%wt)/Vulcan-XC72 homemade electrocatalyst

The performance of Pd(20wt%)/Vulcan-XC72 commercial catalyst in electrochemical detection of dopamine has been compared to Pd(20wt%)/Vulcan-XC72 catalyst manufactured completely at the lab. The electrochemical characterization conducted via cyclic voltammetry (CV) and chronoamperometry (CA) and rotating disk electrode (RDE) [127].

6.3.1 Catalysts preparation

The 20 wt.% Pd/C electrocatalysts were fast and easily prepared by the pulse-microwave assisted polyol synthesis method. The primary steps of this synthesis process are given as follows: The starting precursor ($PdCl_2 \cdot 2H_2O$) was well mixed in a beaker with ethylene glycol (EG) by the aid of an ultrasonic bath, and then XC-72R carbon black was added into the mixture. After the pH value of the system was adjusted to be ~13 by the drop-wise addition of 1.0 mol L^{-1} NaOH/EG, a well-dispersed slurry was obtained by ultrasonic stirring for 30 min. Thereafter, the slurry was microwave-heated for several times in a 10 s-on/10 s-off pulse form. In order to promote the adsorption of the suspended metal nanoparticles onto the support, hydrochloric acid was adopted as the sedimentation promoter and the solution was re-acidified to a pH value of about 3–4. The resulting black solid sample was filtered, washed with hot de-ionized water and dried at 80 °C for 10 h in a vacuum oven. For the sake of comparison, 20 wt.% Pd/C was also prepared in the same way and examined under the same experimental conditions for the dopamine electrooxidation.

6.3.2 Physicochemical characterization of Pd/C

The surface morphology of the prepared Pd/Vulcan XC72 electrocatalyst was examined by TEM. TEM images in Fig. 6.16. show a successful loading dispersion of Pd/C nanoparticles onto the surface of carbon substrate. A remarkably uniform and high dispersion of metal particles on the carbon surface is observed in the examined sample. XRD patterns of the as-prepared electrocatalysts are shown in Fig. 6.17.
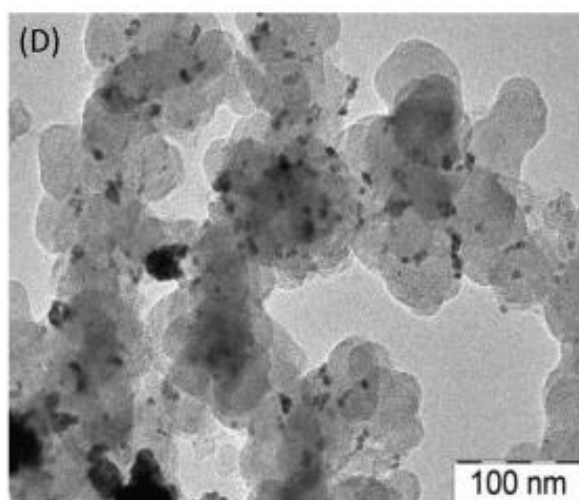
Figure 6.16. TEM images of Pd/C.
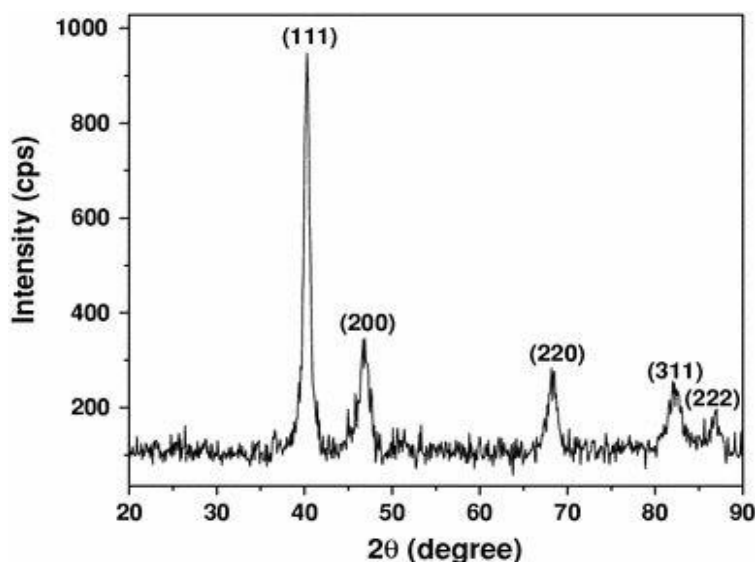
Figure 6.17. XRD results of the as-prepared carbon supported Pd/C electrocatalysts.

XRD patterns of the as-prepared electrocatalysts are shown in Fig. 6.17. There are five observed characteristic diffraction peaks at *ca.* 38°, 47°, 68° 83° and 87° belonging to the face-centred cubic (fcc) phase of Pd (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2), respectively. The Pd (1 1 1) plane has the largest intensity among those planes.

6.3.3 Electrocatalytic activity

The following figure (Fig 6.18) presents the cyclic voltammetric curves for dopamine in saline, 7.3pH in order to investigate the effect of temperature on the oxidation of the analyte. The open circuit voltage of this cyclic voltammetry is 0.23V, the scan rate is 20 mV/s and the voltage is measured by using a reference electrode of Ag/AgCl (+0.210V). The anodic peak current at 26ºC, 36ºC, 46ºC and 56º appears at values 780µA cm^{-2}, 950µA cm^{-2}, 1147µA cm^{-2}, 1305µA cm^{-2} respectively. The effect of the temperature rise of the solution is also clear at this case.
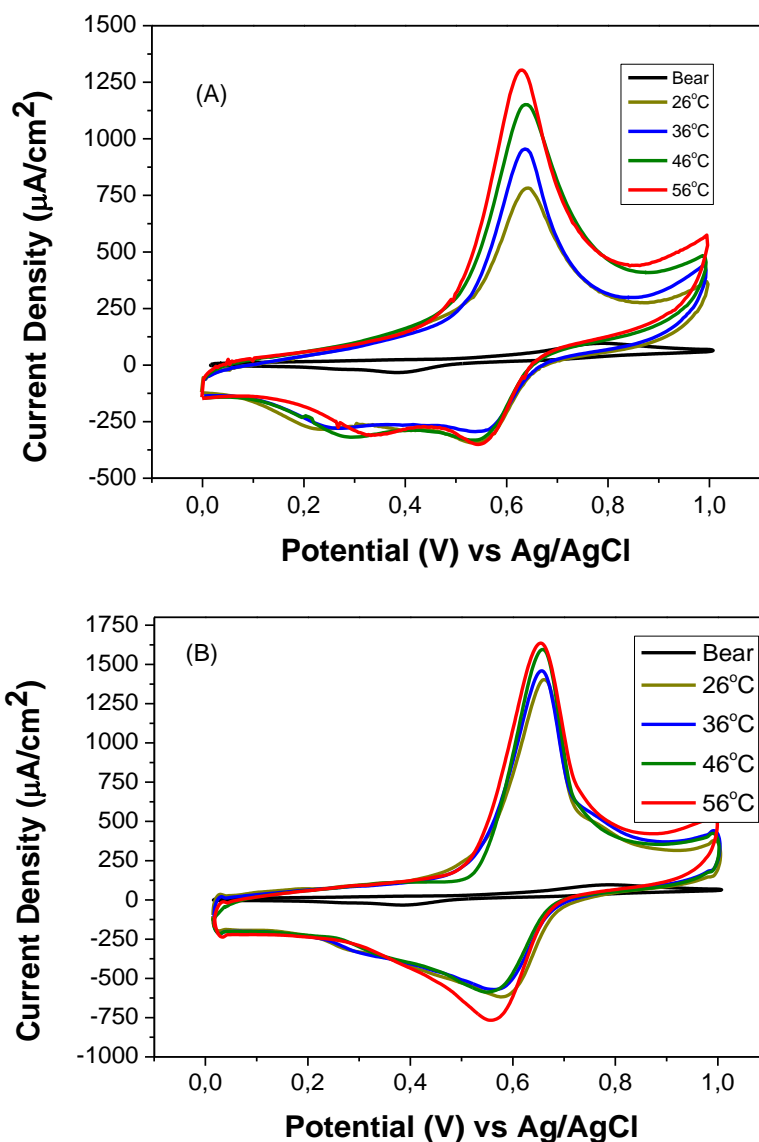
Figure 6.18. Comparison of cyclic voltammograms of Pd(20%wt)/ Vulcan-XC72 commercial and homemade electrocatalyst in 0.5mM dopamine in a 0.15M saline at 26°C, 36°C, 46°C, 56°C, scan rate 20mV s^{-1}.

Comparing the commercial (Fig. 6.18 A,B) with the 'homemade catalyst', it's clear that the performance of the homemade catalyst is sufficient to make it suitable for dopamine sensing. It presents a similar response to the commercial catalyst is observed since the anodic peak appears at the same voltage. The percentage decrease between the peak currents in the four different temperatures, is -28.5%, -26.6% -15% -7.6% respectively.

Using the equation (5.1) and the Fig.6.18.the activation energy is calculated to be at 32.56 kJ mol^{-1} and it can be observed that the value of activation energy is positively good [129].1
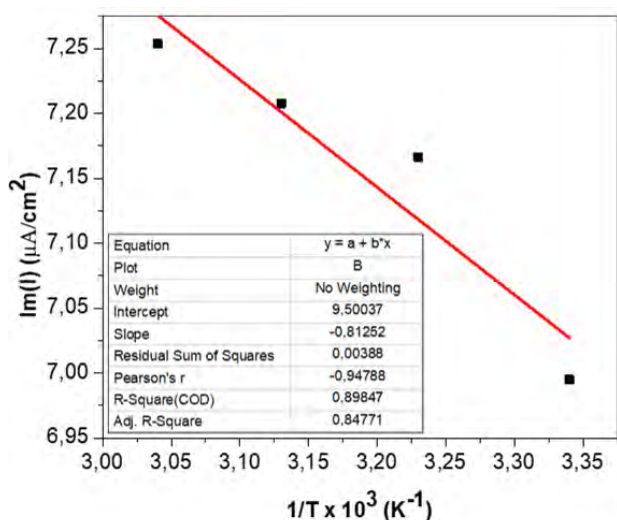
Figure 6.19. Arrhenius plot for the Pd(20%wt)/ Vulcan-XC72 homemade electrocatalyst in 0.5 mM dopamine in a 0.15 M saline derived from cyclic voltammograms at 20mV s-1.
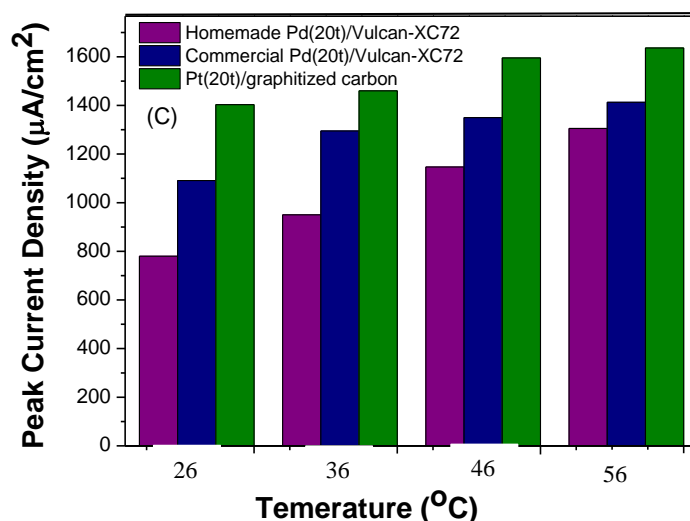
**Figure 6.20.** Cyclic voltammograms of Pd(20%wt)/ Vulcan-XC72 homemade electrocatalyst in 0.5mM dopamine in a 0.15M saline (A) Comparison of cyclic voltammograms at 26°C, 36°C, 46°C, 56°C of the homemade catalyst (B) Comparison of cyclic voltammograms at 26°C, 36°C, 46°C, 56°C of the commercial catalyst. (C) Comparison of the peak current values for palladium homemade, palladium commercial and platinum at 26°C, 36°C, 46°C, 56°C .

The stability and the poisoning rate of the Pd(20%wt) on glassy carbon electrode was also evaluated using chronoamperometry. The solution used was 0.5 mM dopamine in physiological buffer solution at 0.6 V (vs Ag/AgCl) and the method lasted 4000s. The response is also similar to the one of the commercial catalyst and the poisoning rate was measured from the equation (5.2) and found to be 0.0001 s^{-1}, 0.00018 s^{-1}, and 0.00032 s^{-1}, 0.0005 s^{-1} at 26°C, 36°C, 46°C and 56°C, respectively. The comparison of the responses between the two catalysts is given in the next graphs.
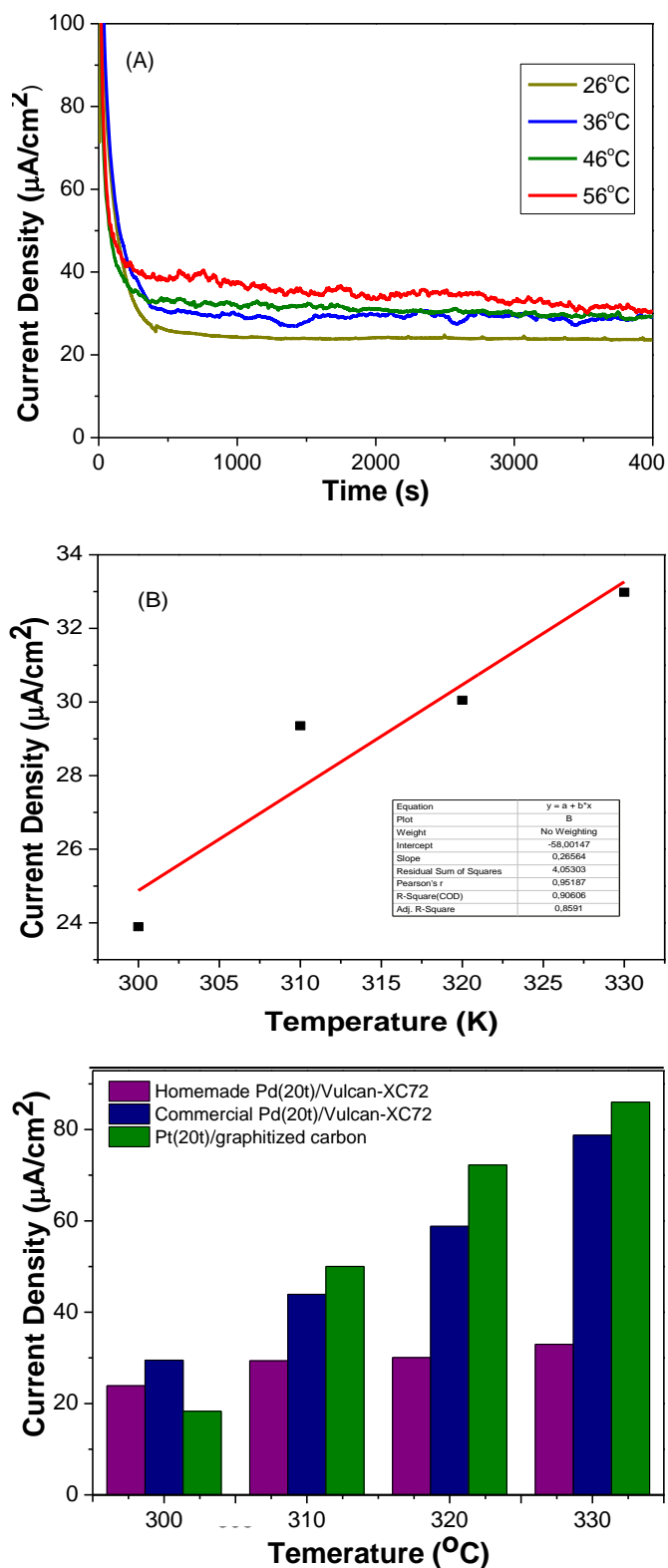
Figure 6.21. (A) Chronoamperometric curves of Pd (20%wt)/Vulcan-XC72 'homemade' electrocatalyst at 0.6 V (vs Ag/AgCl) for 4000sec, in 0.5mM dopamine in a 0.15 M saline at 26ºC, 36ºC, 46ºC, 56ºC, (20 mV s⁻¹), (B) Relationship between current density and temperature. (C)Comparison of the current density values between the palladium commercial, the palladium homemade and platinum catalyst.

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A Comparison of the current density values between the palladium commercial, the palladium homemade and platinum catalyst is also given in Fig.6.21.C.The current values of platinum-based catalyst is higher in every temperature measurement except from the temperature of 26° C, setting the platinum catalyst the most suitable for dopamine detection of the three tested.

Chapter 7

Conclusions

The activity of Pt(20%wt)/graphitized carbon, Pd(20%wt)/Vulcan-XC72 commercial and Pd(20%wt)/Vulcan-XC72 homemade towards dopamine electrooxidation reaction in 0.15M saline solution of 7.3 pH, the effect of temperature, concentration and interference of other substances to the catalysts activity were examined. Moreover, the durability, stability as well as sensitivity of each electrocatalyst were investigated.

From the cyclic voltammograms of the Pt(20%wt)/graphitized carbon in 0.15 M saline with 0.5mM dopamine the oxidation of it was established successfully. At 26°C the anodic current peak for dopamine was 1403.7µA cm^{-2} at ~ 0.65V. By applying higher temperatures, the anodic current peaks were constantly increased. From these results, activation energy was calculated at 16.3kJ mol^{-1}. Also, according to the accelerated cyclic voltammetry measurements, the catalyst exhibited poor durability. On the other hand, its stability over time was found satisfactory, with the poisoning rate of the catalyst to be calculated relatively low. Performing differential pulse voltammetry, for examining catalysts selectivity, three well distinct current peaks appeared rendering the catalyst useful for simultaneous analyte detection and not only for one substance (dopamine).

As for Pd(20%wt)/Vulcan-XC72 commercial catalyst, the cyclic voltammetry in the 0.5mM dopamine in 0.15 M saline solution also demonstrated a well-shaped peak of anodic current. Anodic current peaks were increased as the temperature escalated. The oxidation of dopamine at 26°C resulted to an anodic current peak current of 1091µA cm^{-2} at 0.65V and the activation energy was calculated to be 30,62kJ mol^{-1}. The accelerated cyclic voltammetry's measurements also show that the catalyst exhibited poor durability towards dopamine electrooxidation. Meanwhile, according to the chronoamperometric measurement the poisoning rate of the catalyst was relatively low and its stability over time, very good. Performing differential pulse voltammetry, the current peak of ascorbic acid and dopamine appeared clearly but not the one of uric acid, setting the catalyst inappropriate for simultaneous detection of the three analytes.

While operating cyclic voltammetry for Pd(20%wt)/Vulcan-XC72 homemade catalyst in 0.15 M saline with 0.5mM dopamine the oxidation of it was established successfully. At 26°C the anodic current peak for dopamine was 780μA cm^{-2} at ~ 0.64V. By applying higher temperatures, the anodic current peaks were constantly increased. From these results, activation energy was calculated at 32.56 kJ mol^{-1}. The stability over time was found satisfactory, with the poisoning rate of the catalyst to be calculated relatively low.

To sum up, Pt(20%wt)/graphitized carbon presented better electrochemical activity stability and durability than Pd(20%wt)/Vulcan-XC72 commercial and homemade. However, it can be said that the commercial Pd(20%wt)/Vulcan-XC72 show a little higher selectivity over dopamine, since uric acid could not be detected. Moreover, every electrocatalyst demonstrated high sensitivity towards dopamine. In addition, in each case, the poisoning rate during time was relatively low, which established satisfying stability and the anodic current peaks were constantly increased with the temperature increment.

As for future perspectives the effect of various concentrations, pH, pressure levels and scan rate could be examined. With so many different sensors described in the literature arises the question which one the best is. Of course, there is no simple answer, because there are so many different sensing situations and criteria that must be considered. Instead, a general precept must serve: the best sensor is the one that will do the job, at cost which justifies its use. The cost must be viewed in terms of money, time, and ease of use. These parameters are determined by the characteristics of the sensor [130].

There are definitely many challenges while dealing with real biological samples [92]. It's interesting that although there are numerous biosensors described in the literature, only a relatively small number of them have been tested under realistic conditions. The development of upgraded sensor materials to enhance the selectivity, sensitivity, and stability is also a broad field of research [6, 130, 131], can lead to better biosensors but is not enough. To apply a biosensor in an analytical system involves integration of biosensor technology with sampling sample handling, and data processing. The lack of this integration may to a large extent the so far limited success that biosensors have had on the market. Fouling of sampling as well as sample handling equipment and the biosensor itself may cause severe

disturbances in the analyses. Besides such general compilations, the presence of in interfering substances may further distort the signal. A biosensor analysis consists of the steps of sampling, sampling handling, Biosensor analysis and the last step is the data handling. Thus, the biosensing step is just a small part of the total scheme for an analytical use of a biosensor. In most cases the biosensor is a part of an analytical system and the whole system must be properly treated when designing an analysis [131].

Additionally, in order to operate measurements inside biological systems, organisms and cells minimizing sensors is a necessity. Miniaturized electrode systems have potential for in vivo applications compared to spectroscopic and chromatographic methods.

Although modified electrodes are considered to be a sufficiently matured technology, the suitability of integration of them into miniaturized systems which can be employed in more relevant real applications (e.g. real-time in vivo measurements of DA in POC applications) is still to be investigated. The possibilities of such integrated systems are still far from being fully explored because of the limited studies describing toxicity and biocompatibility of these modification materials. Another major problem is also that most of the original papers lack studies describing the morphological changes at the surface of sensor after detection of analytes [63, [132]. The majority of the reports lack long-term stability data, and many of these modified electrodes have only been fabricated at a laboratory scale.

All these fields are still to be clarified and the modified electrons can be commercialized and solve real problems which is highly desired. For successful commercialization of these sensors, technology that ensures production of a large number of sensors with rigid and reproducible sensing properties to maintain high-quality specifications and cost at an affordable value for the consumers has to be developed. After successfully facing these issues, the construction of commercial electrochemical sensors for real-life applications can finally be achieved and considered a great success.

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