**NEAR EAST UNIVERSITY**

**INSTITUTE OF GRADUATE STUDIES**

**DEPARTMENT OF ANALYTICAL CHEMISTRY**

**GOLD-MODIFIED MOLECULARLY IMPRINTED
N-METHACRYLOYL-(L)-PHENYLALANINE CONTAINING ELECTRODES
FOR ELECTROCHEMICAL DETECTION OF DOPAMINE**

**Ph.D. THESIS**

**Nemah ABUSHAMA**

**Nicosia**

**Fall, 2021**

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**Approval**

We certify that we have read the thesis submitted by Nemah ABUSHAMA titled **"Gold-modified molecularly imprinted n-methacryloyl-(l)-phenylalanine containing electrodes for electrochemical detection of dopamine"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctoral of Educational Sciences.

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## Acknowledgments

**Abstract**

**Gold-modified molecularly imprinted n-methacryloyl-(l)-phenylalanine containing electrodes for electrochemical detection of dopamine**

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Electrochemical measurements have the trend in the recent research with a high competition in order to detect both organic and inorganic analytes in a very low limit of detection by improving the working electrode sensor used in this technique. A molecularly imprinted polymer-based pencil graphite electrode (MIP PGE) sensor, modified with gold nanoparticles, was utilized for the detection of dopamine in the existence of other biochemical combinations by means of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) depending on its strong electroactivity function. The pulse voltammetry methods recorded the highest response. In addition to the high oxidation rate of DA and the other biomolecule interferences available in the sample matrix used, which cause overlapping voltammograms, we aimed to differentiate them in a highly sensitive limit of detection range. The calibration curves used for DA were obtained using the CV and DPV over the concentration range of 0.395-3.96 nM in 0.1 M phosphate buffer solution (PBS) at pH 7.4 with a correlation coefficient of 0.996 and a detection limit of 0.193 nM. The calibration curves for DA were obtained with the CV and DPV over a concentration range of 0.395-3.96 nM in 0.1 M buffer of phosphate solution (PBS) at pH 7.4 with a correlation coefficient of 0.996 and a detection limit of 0.193 nM in 0.1 M phosphate buffer solution (PBS) at pH 7.4 with a correlation coefficient of 0.996 and a limit of detection of 0.193 nM in 0.1 M phosphate Without using any pre-treatment techniques, the electrochemical methodology was used to identify DA molecules quantitatively in human blood plasma selected as actual samples. Even in the presence of epinephrine and norepinephrine competing molecules as well as interferences such as ascorbic acid, MIP electrodes demonstrated their capacity to detect DA with good selectivity (AA). Many biological and pharmacological research rely on the high level of recognition provided by molecularly imprinted polymers (MIPs).

Keywords, cyclic voltammetry, gold nanoparticles, pencil graphite electrode, molecularly imprinted polymer, differential pulse voltammetry

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# List of Abbreviations

| | |
|---|---|
| **DC:** | Direct Current |
| **DPV:** | Differential Pulse Voltammetry |
| **CV:** | Cyclic Voltammetry |
| **SQWV:** | Square Wave Voltammetry |
| **RE:** | Reference Electrode |
| **WE:** | Working Electrode |
| **CE:** | Counter Electrode |
| **PGE:** | Pencil Graphite Electrode |
| **CPE:** | Carbon Paste Electrode |
| **EDTA:** | Ethylenediamine Tetaracetic Acid |
| **DI:** | Deionized Water |
| **$KNO_3$:** | Potassium Nitrate |
| **MIP:** | Molecularly Imprinted Polymer |
| **NIP:** | Molecularly Non-Imprinted Polymer |
| **NPs:** | Nanoparticles |
| **Ia:** | Anodic Current |
| **Ic:** | Cathodic Current |
| **$E_{pa}$:** | Anodic peak potential |
| **$E_{pc}$:** | Cathodic Peak Potential |
| **mM:** | Millimolar |
| **mV/s:** | Millivolt Second |
| **V/s:** | Volt Per Second |
| **$M\Omega/cm$:** | Mega-ohm per Centimetre |

| | |
|---|---|
| **µA:** | Microampere |
| **V:** | Volt |
| **Hz:** | Hertz |
| **DA:** | Dopamine |
| **AA:** | Ascorbic Acid |
| **UA:** | Uric Acid |
| **EP:** | Epinephrine |
| **NE:** | Norepinephrine |
| **EDMA:** | Ethylene glycol dimethacrylate |
| **HEMA:** | 2-Hydroxyethyl methacrylate |
| **MAPA:** | N-methacryloyl-(L)-phenylalanine methyl ester |
| **MAC:** | N-methacryloyl-(L)-cysteine |
| **SD:** | Standard Deviation |
| **RSD:** | Relative Standard Deviation |
| **LOD:** | Limit of Detection |
| **LOQ:** | Limit of Quantification |
| **nM:** | Nano molar |
| **m:** | Slope |
| $R^2$**:** | Correlation Coefficient |

# CHAPTER I

## Introduction

The area of analytical chemistry has risen to prominence in most recent research applications in all aspects of the industrial and scientific worlds. The majority of the research focuses on one aspect of that field, specifically electrochemical approaches. Electrochemical measurements have become increasingly popular in recent studies, owing to the widespread adoption of cost-effective, time-saving, and environmentally friendly methodologies, as well as the high sensitivity and selectivity results obtained (Veloso, et al., 2013). One of the electrochemical subjects on which scientists have worked in a variety of studies and successes is pulse voltammetric methods. Voltammetry states to the electrochemical techniques where a definite voltage pulses are applied to the electrode sensor called working electrode as a function of time needed and current obtained from the analyte in the system (Veloso, et al., 2013). This method usually carried out with a device called a potentiostat or galavanostat, which is very important tool for applying variable values of potential to the working electrode used, whereas the current single is measured which resulting from a chemical reaction occurs on the working electrode surface (Holze, 2002). According to the specific technique, it is feasible to spread over an oxidizing and/or reducing potentials. Once the oxidation reaction occurs, the current obtained called the anodic current (Ia), however, when the reduction reaction occurs, the current obtained here called the cathodic current (Ic). In addition, various voltammetric procedures involve many different voltage profiles (Noël & Laudadio, 2019). Voltammetric methods are amongst the most extensively used electrochemical techniques today. Voltammetric methods come in a range of shapes and sizes. Only three of these approaches will be covered in this unit: linear sweep voltammetry, defferntial pulse voltammetry (DPV), and cyclic voltammetry (CV). Nevertheless, voltammetry approaches include a variety of techniques of differential pulse voltammetry DPV and cyclic voltammetry CV, among others. Each has its own unique set of qualities and applications, and the ones with the highest selectivity are usually the most popular (Li, 2019). Furthermore, the ability to discriminate in contrast to charging current is the fundamental goal of adopting pulse voltammetric techniques, such as differential or standard pulse voltammetry (Trojanowicz, 2009). As a result, redox currents (oxidation-reduction currents) or so-called faradic currents are more sensitive to these pulse approaches than typical Direct Current (DC) voltammetry. For faradic currents, DPV yields voltammogram peaks that differ from the sigmoidal waveform generated using DC or normal pulse approaches (Lee, et al., 1993). One more thing about the voltammetric methods

is that the process by which the moieties in the solution are move up to the working electrode surface, where they are oxidized or reduced according to their electro activity. Here, there are three main commonly progression for the ions movement: electrostatic, diffusion, and convection migration (Ricci, et al., 2012). The electrostatic migration of ions resulting from the attractiveness of the positive ions towards the negative electrode charged or otherwise the negative ions are attract to the positive electrode charged. The second ions migration, which is diffusion that involves motion of the charged species randomly in the solution. The final one is convection migration, which indicates the physical movement produced by somewhat like the magnetic bar stirrer. Usually, the diffusion migration is always occurs in most solutions, others it may not be required to have electrostatic migration or convective movement (Merli, et al., 2012). Voltammetry has unique characteristics that demonstrate its widespread application in analytic chemistry. These characteristics include not only great simplicity, robustness, sensitivity, but also the ability to use the potential in a variety of ways across various waveforms to collect data. As a result, accurate, dependable, and precise quantification of data is required. Extensive changes in contemporary electrochemical techniques have boosted sensitivity (Hoyos-Arbeláez, et al., 2016). In addition, there has been a surge in interest in modern "soft" measuring techniques. They have a number of advantages over traditional methods, such as the capacity to evaluate the status or process superiority. One of these techniques, the electronic tongue, can be used to examine features that are difficult to analyze using conventional methods (Helfrick & Bottomley, 2009). The voltammetric method depends on the electrochemical cell which contains of electrode sensors to detect the single obtained, the point is these electrode sensors can be changed or improved for more sensitive detection results. This electrode sensor can come out in different shape, size and type to detect the current obtained from the chemical of concern in the solution used for detection. For example, in an electrochemical system, the produced current can be measured utilizing voltammetry and an electronic tongue to apply a potential to the sample. Electronic noses, which are similar to electronic tongues and are designed to detect gases, are widely used and commercially accessible. The microelectronic nose is made up of a series with gas sensor with varied sensitivities, an indicator assembly unit, and a operating system for pattern recognition that runs on a processer (Rizk, et al., 2016). Electronic tongues, instead, are made up of three system working electrodes, primarily formed of metallic, as well as counter and reference electrode. The tongue electronics work upon the same principle as electronic tongues, except they're designed for liquid analysis (Enustun & Turkevich, 1963).

Tongue electronics are similar to the sensation of taste, which is explain why they're also called as sensors of taste. It has been consumed to separate liquids (Winquist, et al., 1997), check the nature of milk by detecting viruses (Podrażka, et al., 2018), combine classic methods like conductivity meter and selective ion electrodes (Legin, et al., 1997), and combine another "soft" technology, the electronic nose. As a result, the electronic tongues may be combined with nanomaterials, which are of considerable interest due to their wide range of uses in a variety of sectors, and their compositions and sensitivities will be altered. Systems are regularly encountered in research in a complex form that may be broken into simpler ones, or small pieces can be gathered and united to generate a gigantic one (Winquist, 2008). Electrochemical techniques have been utilized to determine a wide range of analytes in a wide range of matrices, with the most extensively used being diabetics blood glucose detection systems. When compared to spectroscopic approaches, they are analytical assay methods that are significantly faster. Meaning to say, electrochemical approaches mitigate the disadvantages of other methods, such as great sensitivity due to a low limit of detection, low period consumption, besides inexpensive cost of equipment. Because of some compounds such dopamine DA's electro activity, electrochemical techniques for detecting it have been developed in old and recent years (Keithley, 2011; Njagi, 2010; Ouellette, 2019). Dopamine DA which is a neurotransmitter that is linked to the brain's pleasure and our reward system and helps neurons communicate with one another has the trend in many resent research and because DA is a highly oxidative chemicals so electrochemical detection is carried out, but also dealing with them during the voltammetry method can be difficult because of its oxidation rate, so caution is advised. There are two types of electrochemical methods for detecting dopamine: voltammetric and amperometric. When an analyte is reduced otherwise oxidized at a surface of electrodes, voltammetry is used to detect the current created. With reference to a separate electrochemical cell having a known concentration of an appropriate redox mediator, amperometry measures the current created using the oxidation otherwise reduction of an analyte at a surface electrode (Bucher & Wightman, 2015). For the biochemical compound which is DA, many investigations observed biological substances for instance ascorbic acid (AA) and uric acid (UA), which are found in extremely high concentrations in a human fluid along with DA (Strawbridge, et al., 2000), because they are easily oxidized at very similar DA potentials, resulting in voltammogram overlap. Many electrodes, such as modified electrodes with nano-composites (Safavi, et al., 2009), nanoparticles (Shahrokhian & Fotouhi, 2006), and pyrolytic graphite electrodes (Banks & Compton, 2005), have been modified to increase their selectivity and sensitivity response to DA. Despite the fact that these modified electrodes have

distinguished the overlay peaks to a specified level and achieved fast DA detection with other biological chemicals, the detection limit of DA in a real sample remains high. Furthermore, because of DA's high reactive oxidation characteristics, it can quickly react with other chemicals in the real sample, such as ascorbic acid, lowering recognition accuracy (Strawbridge, et al., 2000). To assess DA in the presence of other biological chemicals, a highly selective and sensitive approach is necessary.

Dopamine and many other various compounds and chemicals can be detected by the pulse voltammetric methods as it was mentioned before, especially those which are electro active due to the redox reactions due to the electron transfer which can be detected on the surface of the sensor used. Sensors that play a critical role in the predicted observation, primarily DPV and SQWV, are used to quantify species utilizing electrochemical cells (Sathisha, et al., 2012). Some electronic systems for monitoring various chemical substances have been proposed using this method. The reference electrode (RE), the working electrode (WE), and the counter electrode (CE) are the major components of the sensor for the three electrodes in the electrochemical cell. The electrochemical cell is powered by a potentiostat, and samples are examined based on the current created by the redox reaction (Dungchai, et al., 2009). It is possible to quantify the amount of the chemical of concern in the real sample by identifying an analyte in the recorded voltammogram (current vs. potential), in the volumetric procedure, the potential cycle over each period of the staircase ramp is identified as either forward or backward potential pules (Chougala et al., 2017). For the working electrode sensor, molecularly imprinted polymer (MIP) electrodes are one of the most common sensors for detecting a wide range of biological chemicals in a very sensitive and selective manner, and they have a wide range of applications in voltammetric detection research (Gómez-Chavarín, et al., 2019). Despite existing biological chemical interferences, MIP electrodes indicated that they may be studied for their potential to detect DA with excellent accuracy (Lakshmi, et al., 2009). A BDD which stands for boron-doped diamond film electrode was used to avoid overlapping in the differential pulse voltammetry approach, dopamine was detected as an interference in the presence of AA, albeit with a high detection limit (Guo-hua, et al, 2007). For the improvement of the electrode sensor, nanoparticles were also applied, using the square wave voltammetry (SQWV) approach in a physiological pH media, a gold electrode with a Fullerene-C60 modification was also used to detect the presence of DA in the occurrence of excess AA (Ciszewski & Milczarek, 1999). Despite this, the detection limit was exceeded.

The target of this work was to detect the DA using MIP electrode cell system in a very sensitive and selective manner. The recognition of DA at a very low detection limit is a critical

step in this system. Despite the fact that there have been numerous reports on modified MIP sensors, this study describes the use of an N-methacryloyl-(L)-phenylalanine (MAPA)-containing polymer to produce a pencil graphite electrode surface and accelerate the electron trans-fer mechanism to achieve higher sensitivity for DA molecules. In a 0.193 nM design, other neurotransmitter rivals, including as epinephrine (EP) and norepinephrine (NE), were detected alongside DA. Furthermore, recognizing DA molecules in a complex media like human blood plasma is a challenge.

**Statement of the Problem**

Electrode sensitivity is the degree of accuracy which can be achieved with a particular type of detection. Electrodes can be divided into two general types: those with direct and those with indirect detection (Shaikh, et al., 2006). In the direct case, the chemical being measured is bound directly to the electrode surface. Typical examples include ion exchange resins for pH determination, or mercuric and silver electrodes for conducting electrolysis. The indirect case generally involves a chemical that interacts with a second material such as porogen on an electrode surface (Kinga Kaczmarska, et al., 2017). The sensitivity of an electrochemical detector, for example, is often measured in units called millivolts per square meter ($mV/m^2$) or volts per square meter ($V/m^2$). Measured in this way, the sensitivity reflects how accurately the device detects changes in its environment. Hence, the problem of the study indicates on hoe to decrease the limit of detection of the chemical of interest of the working electrode used for the voltammetric measurements, to enhance the sensitivity of the whole electrochemical technique.

**Purpose of the Study**

The aims of this study can be summarized as follows:

- To achieve a highly sensitive and selective determination of inorganic and organic chemicals in the electrochemical cell system.

- Differentiate between the modified and unmodified pencil graphite electrodes output.

- The recognition of the biochemical compounds at a very low detection limit.

- Comparison between the imprinted and non-imprinted polymer electrodes measurements.

- Detecting the biochemical molecule with its other interferences.

- Detect the biochemical compound in a real sample such as human's plasma.

**Research Questions/Hypotheses**

Generally, the main research hypothesis can be determined as follows:

- The sensitivity and selectivity of the study can be improved for the detection of the analyte used by pulse voltammetric techniques.

- The pencil graphite working electrodes PGE can be modified its surface to enhance its sensitivity and detect the analyte at a very low limit of detection.

- The addition of nanoparticles NPs to the working electrode will enhance the sensitivity of the measurement.

- The analyte can be detected by using the modified PGE in the existence of other interferences compounds.

- The analyte can be detected in a very complex matrix sample as human's plasma.

**Significance of the Study**

The main significance of this study illustrated in the preparation of a film molecularly imprinted surrounded the surface of the PGE in order to enhance the sensitivity of the working electrode used for the detection of both chemical and biochemical compounds. The imprinted polymer can be synthesized by using functional monomers with and without the addition of the chemical of concern to the template in order to prepare non-imprinted polymers and imprinted polymers for the working electrode. Once the molecularly imprinted besides molecularly non imprinted polymers base on PGE, measurement can be carried out to detect the analyte in a very low concentration and in a very complicated matrix sample in order to increase the selectivity as well.

**Limitations**

Each study has some restrictions needed to be recovered. The main limitation in this work indicates in the detection of the chemical of concern in a very complicated matrix where many interferences are available and competitors to the analyte used that has same peak potential, hence, the analyte peak is affected by the other available interferences and so, the

peak obtained will be noisy and shifted, as a result, the selectivity should be improved more in this type of samples.

**Definition of Terms**

The main terms are related to this work can be defined as follows:

- Electrochemical detection: An electrochemical detection method in which an electric potential is utilised across electrodes and the consequent current created by an electro active target analyte surrounding the electrodes is measured.

- Electrochemical cell: system contains from three main electrodes dipped in the analyte solution to obtain a current signal for the chemical of concern.

- Voltammetric detection: is a part of the electrochemical detection that used in analytical chemistry and a variety of industrial operations. Voltammetry is a technique for obtaining data about an analyte by evaluating the current when a potential is changed.

- Cyclic voltammetry CV: is an electrochemical method for determining the current response of a redox active solution between two or more defined values to a linearly cyclic potential sweep. It's a quick technique to determine out redox reaction thermodynamics, analyte energy levels, and electronic-transfer reaction kinetics.

- Differential pulse voltammetry DPV: is an electrochemical voltammetry method that uses a set of periodic voltage pulses overlaid on a potential linear sweep or stair step, and is a derivative of linear sweep voltammetry or staircase voltammetry.

- Square wave voltammetry SQWV: is a type of the voltammetric technique that indicates a wave shape formed of a symmetrical square wave overlaid on a base staircase potential is applied to the working electrode in this large-amplitude differential approach.

- Redox reaction: indicates a chemical reaction of both oxidation and reduction where the transfer of electrons occur.

- Faradic current: the current which resulting from an electron transfer occurs due to the presences of an analyte in the solution.

- Non-faradic current: the current where no electron transfer is happens because of the absence of the analyte in an electrolyte solution.

- Pencil graphite electrodes PGE: indicate the current name for working electrodes (PGEs). PGEs are not only less expensive, but they are easier to use and more suitable, with no need for time-wasting surface electrode wiping and enhancing.

- Carbon Paste Electrode CPE: indicates a type of electrode can be used as a working electrode in the electrochemical cell, it contains from mixing carbon power with a mineral oil.

- Nanoparticles NPs: A nanoparticle is a very small particle with only a dimension of 1 to 100 nanometers. Nanoparticles, which are imperceptible to the naked eye, can have vastly different chemical and physical properties than their larger counterparts. Only a few hundred atoms make up the majority of nanoparticles.

- Gold Nanoparticles AuNPs: a tiny gold particles that have a diameter less than 100 nm, used to improve the electrode sensitivity because of the high surface area they have.

- Conductivity: the ability for a substance to conduct electricity.

- Molecularly imprinted polymer MIP: indicates a type of synthetic materials that have a, reusability, higher thermal stability and selectivity than biological receptors, hence, they are in charged as distinguishing elements in the sensors design.

- Functional monomers: are those with reactive side chains that can be used to create more complex vinyl compounds, such as macro-monomers, or to improve the functionality of prefabricated polymer chains.

- Molecularly non-imprinted polymer NIP: synthetic polymers except in the non-existence of a template, the synthesis technique is similar as for MIPs. As a result, NIPs have the same composition as MIPs but do not contain any cavities.

# CHAPTER II

## Literature Review

Due to an overuse of thermodynamic concepts in understanding the procedures that occur at locations in the technique where the various numerous fragments form interfaces, electrochemistry starts in the 18th century, peaked in the first 20th period, and then declined. Electrochemistry has changed significantly since 1950. The analysis of methods in solutions has received less attention, while the research of electron transfers between metals and liquids has exploded in popularity. Electrochemistry becomes a fundamental science as a result of this new focus. The analytical chemistry field has a vital function in the improvement of the compounds formation from their synthesis step as a part of chemical analysis. The known four main techniques for the analyte detection drop into four main classifications: spectrophotometry, chromatography, radiometry, and electrochemical methods (Trullols, et al., 2004). The electrochemical chemistry laterally with the redox reactions and the charge transfer mechanism which had been started eight years ago, which indicates one of the primary analytical chemistry sub-disciplines. Traditionally, the electro analytical analysis using the pulse voltammetric methods with typically an electrochemical cell contains of three main electrodes system which is immersed in an electrolyte solution with a high conductivity under optimized conditions. The main item of the pulse voltammetry electrodes is prepared admission to a circular diffusion where the effect of the voltage applied which is connected to the current response. In addition, and under adjusted parameters, the electrochemical technique resulting a current of insignificant charging. Therefore, providing and preserving the electrodes with a very high property and electrical interactions, that a response can be achieved with a simple electrochemical cell electrodes. The requirement for the complex potentiostat apparatus for the instrumentation combination, cause a reduction in alternative current used (Turner, et al., 2009). In the updated analytical chemistry, the electrochemical techniques achieved a high performance in increasing the sensitivity of the results obtained via changing the working electrode used for the analayte detection. Moreover, the electrochemical field which also plays an important rules in analytical chemistry and drugs analysis stage till its marketing final stage. The electrochemistry methods as a part of the analytical chemistry studies have been used recently due to the economically and environmentally friendly benefits in many studies (Shrivastava & Gupta, 2011). On the other hand, the application of voltammetric techniques permits important reduction of the destructive chemicals, which works in parallel to the green

chemistry principles. In addition, to use a safe martials and reagents in the preparation of working electrodes used for the detection, the enhancement of the number of researches concentrate on the development of nontoxic, new electrode materials such as carbon, ceramics, and polymer compounds, that propose a similar or superior performance in the electrochemical applications (Zhang, et al., 2021). Many different types of materials were used for the working electrodes application preparations, for instance, pencil graphite electrodes, glass carbon electrodes, and carbon paste electrodes have been lately used in the electrochemical detection. Long time ago, a Jaroslav Heyrovsky, a Czech scientist, who discovered the voltammetry method, in which the materials are tested by determining the current by way of function of the potential applied, in 1920s. Since a quarter-century, Jaroslav Heyrovsky has been awarded the Nobel Prize in Chemistry for his improvements to polarographic techniques for analysis, in 1959 (Zuman & Heyrovsky, 1959). Voltammetric measurements have become a famous device for the electrochemical reactions study (Ianniello, et al., 1982), the survey of electrochemically produced the free radicals (Floyd, et al., 1984), solar energy conversion (Crabtree & Lewis, 2007), model studies of enzymatic catalysis (Kaminskaia & Lippard, 2000), environmental observing (Tilak, et al., 2007), industrial quality control (Melin & Castillo, 2007), and the detection of ultratrace concentrations of the chemically and biological essential compounds (El-Shahawi & Al-Saidi, 2013). Electrochemistry provides a number of benefits that make it a viable option for pharmaceutical analysis (Gil & Melo, 2010).

Analytical techniques based on electrochemistry have long been characterized by instrument simplicity, low cost, and portability. The most promising strategies for specific applications have been introduced using these techniques (Zhang, et al., 2013). Since biological reactions and electrochemical reactions are so similar, it's reasonable to hypothesize that the reduction/oxidation the electrode and body mechanisms both work on the very same principles. Voltammetry can be used to analyze biologically relevant substances electro analytically to detect the molecules in various techniques. The electrochemistry also has other applications, such as determining mechanisms of the electrode. The reduction/oxidation characteristics of pharmaceuticals can provide information on their metabolic fate or electrochemical activity in in vivo redox processes. Additional, the electrochemical methods have been presented to be brilliant for the detection of chemical and biochemical compounds in diverse matrices. Many chemicals and biochemical are electrically active, and be able to be oxidized easily. Selectivity of this technique is usually tremendous because the chemical of concern can be freely recognised by its peak potential. The developments in the electrochemical experimental techniques in the analysis area are because of their low cost, simplicity, and less time needed

for analysis. The use of different types of electrodes especially the modified ones for the electrochemical methods has been enhanced in current years due to their ability in the detection of chemically dynamic compounds which endure redox reactions. Ozkan (2020) seriously revised the application of the advanced electrochemical methods in the analysis of biochemical compounds and biological liquids. The novelists revised about approximately 200 articles begins from 90s till 2000s. The usage and benefits of the voltammetric techniques to the chemicals inside the biological media. Though, the author did not obviously delivers the matrix in which medicines were analysed. In another studies, included the voltammetric detection of different biochemical and pharmaceutical materials of numerous antiallergics (Sakthivel, 2018), hypoglycemic (Gonzalez, et al., 1992), antibiotics (El-Maali, et al., 2005), antiamoebic (Maurya, et al., 2006), antidepressant (Farghaly, 2000), vitamins (Siddiqui & Pitre, 2001), sedatives (Jain & Yadav, 2012), and others. Cyclic voltammetry CV method is usually the initial experiment accomplished for a compound in the electrochemical techniques. It is efficiently employed in the areas of inorganic chemistry, biochemistry, organic chemistry and environmental chemistry. The efficiency of CV outcome obtained from its ability for quick monitoring the oxidation-reduction reactions over a very broad range of potential. The outcome which results for CV method called voltammograms which indicates potential applied vs. current obtained. Since the last 40 years, CV has become a famous device for the studying of the electrochemical reactions (Qiu, et al., 2000). And it's very popular for the detection of biological (Kohen, et al., 1999) and pharmaceutical compounds (Norouzi, et al., 2007). The stability of the anion radical of metronidazole was studied by Bollo et al. (2004) by the usage of CV for the detection.

The use of CV technique for the studying of the hydrolytic decomposition kinetics for the creation of pharmacological action in the human by Acuna et al. (2002). Another study for the determination of acetaminophen in paracetamol doses was detected using CV in a PBS phosphate buffer solution, and limit of detection was obtained around 3-240 mg/mL. Wang et al. used CV for the detection of nicotinic acid and nicotinamide by studying the redox reactions occurred in electrolytes solutions (Sarker & Ehsan, 2017). The study of iso-hramnetin behaviour was carried out by using CV method at a glassy carbon electrode (Liu, et al., 2008), the oxidation peak current presented a linear curve on an ISO concentration at 1.0 V in a real tablet samples. By another study, voltammetric technoques were used for the detection of (Ch-HCl) chlorphenoxamine hydrochloride in caffeine tablets by using CV and DPV using both glassy carbon and platinum working electrodes, standard addition method was used for detection of Ch-HCl concentration in a raw dray sample, Overall average recoveries for glassy

carbon electrodes and platinum were 101.44 percent and 100.49 percent, respectively, with SD 0.45 and 0.38 (n = 4). The Ip and Ep were also studied in relation to scan rate, sample concentration, and supporting electrolyte (Abdel-Ghani, et al., 2010). The DPV, CV, and SQWV were also carried out for the detection of the oxidation of Loracarbef, the effect of pH, concentration, scan rate, and buffer nature on peak currents and potentials was examined. Loracarbef quantitative determination methods based on square-wave voltammetry and differential pulse voltammetry were developed. Different characteristics were investigated in order to find the best circumstances for determining Loracarbef, Loracarbef's quantitative determination was proposed in 0.1 M $H_2SO_4$, which allowed quantification in the range of $6 \times 10^6$ to $2 \times 10^4$ M. Accuracy, Precision, repeatability, selectivity and sensitivity were all examined. The techniques for determining Loracarbef inside pharmacological dose formulations were proposed (Topal, et al., 2009). The pulse voltammetric method was studied by Gardner and Barker (1992) to enhance the sensitivity of the method used for the electro active chemicals. DPV was very beneficial for the detection of electroactive compounds traces in real samples. There are various studies focused in the electrochemical determination of sparfloxacin in medicine (Girish Kumar, et al., 2006). Many cephalosporins have been detected by the voltammetric techniques and high accuracy was obtained (Reddy, et al., 2003). The DPV method approach was successfully used to validate the consistent amount of bromhexine in individual tablet assays (Turchan, et al., 2007). DPV was also used to establish and study the electro activity of tamsulosin on an electrode made of glassy carbon (Özkan, et al., 2003). Actually, the methods mentioned were less time consumed, taking less than 5 minutes to complete. It shown that this drug molecule could be monitored, creating the process is helpful for pharmacodynamics and pharmacokinetic studies. Another easy, precise, and economical pulse differential voltammetric method for determining hydroxychloroquine was achieved, and to detect the active principle, no extensive pre-treatment is required. CV technique was improved by Jain et al. (2008) for the detection of pyrantel pamoate in medicine, the number of electrons exchanged in the reduction method was computed, and a mechanism for reduction was proposed. Over the concentration range of $4 \times 10^4$ to $2 \times 10^2$ M, the current of the peak was discovered to be linear, with a limit of detection $2.45 \times 10^5$ M. DPV's main advantages are its ease of use and the availability of low-cost instruments. A waveform formed of symmetrical square waves is used in a large amplitude differential technique and square-wave voltammetry SQWV. The reality that total combined current is considerable when compared to forward or backward current, as well as efficient charging current discriminating, results in

excellent sensitivity in SQWV. The obtained peak currents obtained by the differential pulse response is 4 times greater than the differential pulse responses.

Many recent studies illustrated the use of SQWV in the determination of my chemical and biological compounds. The measurement of the concentration of Lumivudine in the human serum was carried out by using SQWV method depends on the redox reaction across the HMDE, matching to the cytosine moiety. Because no pre-treatment or time using abstraction processes were used, it may be used for both pharmacokinetics and quality control laboratory experiments (Jadreško D, et al., 2010). The SQWV method for methimazole determination was improved with a limit of detection around 0.5 mM and a low RSD (2.89 %). With strong consistency, precision, and accuracy, the SQWV method was used to determine resveratrol (Mirceski V, et al., 2013) in Chinese patent medication and diluted wine. Both DPV and SQWV were improved via Uslu et al. (2016) for the detection of antiviral medicine in the man fluid's, the medicine is highly electro active chemical that was oxidized of the glassy carbon electrode surface, and the limit of detection obtained were For DPV and SQWV, the values were $1.04 \times 10^7$ and $4.6 \times 10^8$ M, respectively. Golcu et al. (2009) established an anodic voltammetric behaviour for determining Cefixime in biological fluids and in the pharmaceutical dosage forms. In all media, the approaches', reproducibility, repeatability, accuracy, and precision, were tested. Inside the pharmacological dose formulations and biological real samples, there were no interferences from excipients or endogenous chemicals. For the detection of trepibutone via SQWV method by using pencil graphite electrode PGE. The PGE used gives superior sensitivity and reproducibility without any supplementary steps for the electrode surface renewal. In an additional research, a quick and simple SQWV technique was carried out for the determination of captopril in a very low concentration in a reconstituted serum. For both a supportive electrolyte and an oxygen-removing agent, sodium sulphite was used. Dopamine, Abacavir, etodolac, tramadol, and nefazodone hydrochloride (Manjunatha, et al., 2014), and have all been determined using SQWV voltammetric methods. Table 1 illustrated many uses chemicals and biochemical material were detected by the pulse voltammetric methods. Vinod K. Gupta (2010) in India, talked about the usage of the voltammetric methods for instance square wave voltammetry SQWV , cyclic voltammetry CV, and deferential pulse voltammetry DPV in different drug types by using various electrode for detection followed by the results of each technique used in analysis. Data were gathered and compared related to different parameters as the type of electrode used and the type of voltammetric techniques, and the results were varied among all these and published to show the importance of analytical part in pharmaceutical analysis (Vinod K. Gupta et al., 2010).

In another studies, which summarized the main concepts of the usage and important goals that voltammetric techniques were achieved in analysis. Many recent articles described the quantitative and qualitative analysis in details of each voltammetric method used for analysis in many filed, in addition, to the advantages and disadvantages of each technique in details (Fritz Scholz, 2015). Pharmaceutical field indicates one part of the voltammetric applications, food chemistry also takes a second part of the voltammetric method application, and where the determination of food contaminates indicates an important target in food industries such as residuals of hazardous metals, veterinary medications, trace necessary minerals, food additive dyes, and other biologically vital chemical compounds. Using various electrode such as Gold film electrode, Glassy carbon electrode, adjusted glassy carbon electrode, and Modified silver electrode for the determination of different food samples and the result s were documented and compared (Alghamdi, 2010). K. Srivastava, 2019 also discusses studies on nano-materials-based CMEs for drug analysis from 2000 to the present, using a variety of voltammetric methodologies for different classes of pharmaceuticals (K. Srivastava et al,. 2019). The current study looks at how analgesics, anthelmintics, cardiovascular, anti-TB, antipsychotics, gastrointestinal, anti-allergic, and antibiotic medications are determined. Enantio-analysis of certain chiral medicines employing voltammetry is also covered in a separate section. For each class of drug, thorough information on the voltammetric determination is provided (K. Srivastava et al., 2019).

Speciation also take a part in electrochemical methods applications in many recent articles due to the sensitivity of the voltammetric techniques used in determination an comparison in various metal speciation's (JOHN R. DONAT et al., 1990). Differential pulse anodic stripping voltammetry (DPASV) and differential pulse cathodic stripping voltammetry (DPCSV) were used for the determination of zinc speciation by using compatible ligand complexing for each zinc type in ocean water samples. Additional studies, were done on the determination and the separation of Selenium Se for also speciation analysis by voltammetric method (Analytica Chimica Acta, 2002). Depends on the suspended mercury drop electrode (HMDE) and the differential pulse cathodic stripping voltammetry (DPCSV) in addition to the extraction process for the isolation of the Selenium species. In this study, several voltammetric detection parameters, such as accumulation duration and voltage, type of electrolyte and sample acidity, along with the type of extraction for efficient isolation of the Selenium species, were studied in the organic and aqueous phases for the technique optimization. Furthermore, the electrochemically inert Se was quantitatively transformed and various reducing agents were used to convert immobile Se (VI) to Se (IV) (Analytica Chimica Acta, 2002). Complexation

reaction analysis via voltammetric methods was interviewed widely and specifically. By using the voltammetric techniques by measuring the current obtained, indicates a basic speciation parameters (H. van Leeuwen, et al., 2009). Furthermore, Three distinct research groups used voltammetric methods to assess dissolved copper chemical speciation in a coastal saline sample, through different participating ligands; 8 hydroxyquinoline , benzoylacetone, and salicylaldoxime, the three study groups used different Methodologies for selective ligand equilibration/adsorptive cathodic voltammetric (CLE-ACSV) methodologies, two of which utilised a variety of additional ligand concentrations. As a result, Copper was largely powerful Copper-binding ligands complexed less than 99.9% according to the data obtained with each electro analytic technique (whether CLE-ACSV otherwise ASV) (Bruland, et al., 2000).

Table 1.

*Various Compounds Detected by Pulse Voltammetric Techniques by Using Different WEs.*

| Compound | WE | Method | LOD (M) | Ref. |
|---|---|---|---|---|
| Ascorbic acid | PGE | DPV | $0.88 \times 10^5$ | (Erdurak-Kilic, et al., 2006) |
| Acetaminophen | GCE | CV | $3.0 \times 10^4$ | (Jain, et al., 2006) |
| Catechol hydroxyquinone | GCE | DPV | $3.0 \times 10^6$ | (Peng, et al., 2006) |
| Cefotaxime | CPE | CV | $2.0 \times 10^5$ | (Aleksic, et al., 2006) |
| Dopamine | GCE | SQWV | $0.08 \times 10^6$ | (Shahrokhian, et al., 2006) |
| Dopamine | CPE | DPV | $5.0 \times 10^6$ | (Alarcon, et al., 2008) |
| Ethinylestradiol | CPE | CV | $3.0 \times 10^8$ | (Chunya, et al., 2007) |
| Flupenthixol | GCE | DPV | $1.17 \times 10^7$ | (Uslu, et al., 2005) |
| Natamycin | CPE | DPV | $1.5 \times 10^6$ | (Uslu, et al., 2001) |
| Niocotinic acid | PGE | CV | $1.17 \times 10^7$ | (El-Desoky, et al., 2005) |
| Nicotinamide | PGE | SQWV | $0.27 \times 10^6$ | (Wang, et al., 2006) |
| Pantoprazole | CPE | DPV | $4.0 \times 10^7$ | (Nevin, et al., 2003) |
| Repaglinide | CPE | DPV | $1.348 \times 10^7$ | (El-Ries, et al., 2008) |
| Vitamins 6 | GCE | CV | $1.0 \times 10^7$ | (Song, 2008) |
| Tryptophan | CPE | DPV | $1.7 \times 10^6$ | (Fiorucci, et al., 2002) |

**Related Research**

Molecularly imprinted polymers MIPs, indicate the greatest that can be explained as synthetically equivalents to the living, antigen-antibody system, and natural. As known, they works by a form of key and lock manner to a selective attach to the molecules in which they were attached to template during synthesis. MIPs usually, deliver the selectivity and specificity of the receptors with an obvious benefits of robustness regard to the surroundings circumstances and costless. For instance, temperature in the human's body range and storage are required for a natural receptor, whereas, the MIPs that depend on a hosting polymer can be stored for ever, hence no special conditions and surroundings are required to increase their shelf life (Uzun & Turner, 2016). They can be available even in a broad temperature range. There are commonly synthesis procedures for the MIPs production. All procedures have the same core of in MIPs producing; first, the polymer is synthesized containing the template for the molecule of concern, bind covalently or non-covalently, to the host's functional group, secondly; the molecules template is totally extracted from the hosting polymer, departing the specific aim cavity free to rebind again, and finally; the MIPs are subjected to the chemical of concern sample, and the selectivity of the cavity acceptances the molecule of concern from the complicated sample. Generally, sensor can be defined according to the Oxford dictionary: "a tool which measures a record and physical thing that respond to it". The physical and the chemical sensor thing indicates the existence of the analyte (Yan & Row, 2006). Meaning to say, that the most important parameter in the MIPs application in sensors contains the imprinting feature, time response, and capacity of binding. The imprinting factor indicates the binding ratio of the template in the in the MIPs to its binding to the NIPs which indicates the non-imprinting polymer. Whereas, the capacity of binding can be calculated as a ration of the analyte concentration absorbed divided by its initial concentration in the solution times 100% (Whitcombe, et al., 2011). According to Chemical Abstracts, B. Sellergren & K. Mosbach (1984) published the first paper describing a imprinted polymer, Despite the fact that G. Wulff had been publishing studies Enzyme Analogue Constructed Polymer is a series of enzyme analog built polymers which is from 1973, the phrase "imprinted polymer" was first used in a study by G. Wulff (1985). These pioneering scientists pioneered many fields of science. Mosbach (1995) concentrate on the non-covalent interaction between both the hosts and the targeted, whereas Wulff (1985) chosen covalent binding to imprint. The most obvious variance relating to the two procedures is the chemistry required to eliminate the template molecules from the MIP. Significantly, the synthesis of the covalent should create a more consistent

Greater target-specific MIPs occur from the accumulation of rebinding holes. Wulff (1985) widely use the imprinted polymers were in catalysts, as demonstrated by the title of his early series of publications, and K. Shea (2010) stated on quite rare biological uses, for example the plastic antibody injected into an alive mice, in a similar vein, beginning. Although publications by this author explaining the creation of MIPs in over-all appear in Russian recent studies, Piletsky et al. (2002) appears to have coined the phrase of the sensor of the molecularly imprinted polymer. The study of these groups goes back to the 1990s and has resulted in studies on a number of uses of this technology. Synthesis is the most frequent manufacturing method, which includes forming a network of covalently or non-covalently binding complexes by combining the target molecule with the functional monomer in solution.

The functional monomer can, for instance, provide the functionality of hydrogen bonding or a sensitive substituent that forms a covalent connection with the template. The functional monomers include methyl methacrylate (MMA), acrylamide, methacrylic acid (MAA), pyrrole, and aniline. Then, thorough mixing, a cross-linking material as ethylene glycol dimethacrylate (EGDMA) and an initiator for the polymerization as azobis (isobutyronitrile) (AIBN) are commanded, UV radiation-driven polymerization or heating is started, and the reaction continues to accomplishment, yielding processed material appropriate for isolations. Rather than creating powders, the reaction mixture can be covered onto a variety of substrates and photo-initiation employed to initiate polymerization in situ (BelBruno, 2018). The process is for the manufacture of 1-(2-hydroxyethyl)-2-methyl5-nitroimidazole, a MIP that is used to treat protozoan infections and targets metronidazole. The template, the functional monomer the EGDMA cross-linker, MAA and the AIBN initiation are all dissolved, deoxygenated, and heated for 24 hours in the DMF porogen solvent. Soxhlet extraction is used to eliminate the template after crushing and sifting, keeping binding holes left.

Other studies preview the several common modifying agents and their use in voltammetric and amperometric sensors for clinical and physiologically significant targets presides the usage of some modifier to the electrode used in the voltammtric methods such as MIPs (molecularly imprinted polymers), polymers, metal complexes, nanomaterial's, and composite films are all examples of molecularly imprinted polymers (Chillawar et al, 2015). Especially, in the last few decades molecularly imprinted polymers MIP becomes more familiar in the electrochemistry analysis, due to the selectivity to such chemicals and biochemical compounds which are important in many fields as pharmaceutical and drug analysis. By modifying the electrode sensors used with MIP also preferred due to the simplicity to be stored and operated, and they have a long shelf life. In many studies, carbon paste

electrode CPE was used in detection of pharmaceutical compounds in the form of MIP-CPE; Promethazine, metronidazole, sulfasalazine, chloramphenicol, and lamotrigine were all deactivated using it. Several parameters were gives and discussed among those chemicals by using the modified electrodes with MIP, for instance, the measurement technique used, linear detection range (LDR), and limit of detection (LOD). In general, molecularly imprinted polymers preparation can undergoes via various polymerization steps. For instance, the 2D which is surface imprinting and the 3D which is the bulk imprinting. In the surface imprinting procedure, the templates are situated on the material surface, whereas, the bulk imprinting, monolithic solids are produced with the template surrounded into the structure cross-linked. Particularly, in the MIPs manufacturing for the biomolecules compounds, Surface imprinting is still advantageous because of the analyte diffusion and binding are more favorable and faster near the polymer surface than inside its porous structure. Soft lithography, self-assembled monolayers, core-shell particle preparation, and emulsion polymerization are the most often used surface imprinting techniques (Mujahid, et al., 2013). For effectively scheming the recognized positions in MIPs, various methodology through the interactions between the template molecules used and the functional monomers in the process. The achievement of the non-covalent imprinting by Mosbach et al. (1995) based on the interaction of the template and the functional monomers throughout the polymerization stage within weak forces for example π-π interactions or Van der Waals forces that consequences in the removal of the template used. Moreover, selective distinguishing through the chemical of concern binding stage depends on the non-covalent interactions. The non-covalent imprinting indicates an efficient and simple achievement, and despite the availability of some matters related to moderately simple disruptions through the formation of the template monomer complex of these interactions, it stills the most familiar used as an up to date approach (Wulff & Knorr, 2001). The functional monomers can be neutral, acids and bases such as 2-hydroxyethyl methacrylate, acrylamide, and acrylic acid), that can be used in the preparation of non-covalent imprinting, just the functional monomers which have the ability to produce reversible condensation reaction with the used template for instance; boronic esters (Mayes & Whitcombe, 2005). In addition, the binding in a slow motion and a more boring removing of the template used, are connected to the covalent imprinting procedure, but, it Has been established that the strong noncovalent interactions can give homogeneous distributed the cavities of the molecularly imprinted, the covalent achievement stills the most commonly used recently to obtain a constant orientation of the distinguishing sites. To overcome the both achievements restrictions, a semi-covalent achievement was established by involves a covalent binding between the template and the

functional monomer through the MIPs preparation and during the analyte binding. For the synthesis of MIP with nanoparticles NP, two main methods are used in the polymerization process; emulsion and precipitation polymerization (Priego-Capote, et al., 2008), whereas the bulk polymerization is used in MIPs synthesis as monoliths porous. The initiated step in both polymerization process is done by UV energy and thermal in the existence of a free radical initiator such as acrylate polymers. In contrast, in the preparation of the thin polymeric layers, electro-polymerization is more commonly used for this purpose (Cardoso, et al., 2018).

The need for more safe life and good health, causes countless research about antioxidants compounds due to the importance of these materials to human's life, hence, the seek to produce synthetic antioxidants. Some of the synthetic antioxidants are very sensitive to the voltammetric waves for instance; butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Nevertheless, the waves of these antioxidants are heavily overlapping, making it difficult to separate them all at once. In a quantitative simultaneous assessment of antioxidants in alcoholic mixes and genuine sample foods, the impact of several factors such as the working electrode and the voltammetric technique used was investigated. The output of the findings reveal that the parameters tested were adequate for quantitative determination using square wave voltammetry (SWV) for some food samples (Valentin Mirceski, et al., 2019).

Environmental chemistry specify one of the voltammetric application. Several soil and water samples were introduced to be detected by some voltammetric techniques to measure the herbicides and pesticides quantity. In a process to find the contamination of these compounds in soil, ground water, and lake samples. Square wave voltammetry SQWV and Cyclic voltammetry CV were used to detect the availability of these compound in the biological samples. In addition, the most commonly utilized materials in their electrodes are briefly discussed, including polymers, carbon, clay materials, metal oxides, biomolecules, nanostructured, and micro materials. There are some recent studies, researches, and new approaches for all of those techniques and materials, because of their great sensitivity and specificity in detecting pesticides and herbicides. Some researchers point to the contamination of aquatic system by metal speciation's, in addition to review the voltammetric methods in the detection of the metals in samples provided. In more detailed, this study enumerates the precise conceptual, analytical, and technological criteria that must be addressed and/or met in order to design tough, field deployable, non-perturbing sensors and probes (Tercier & Taillefert, 2008). The voltammetric techniques show a powerful weapon in detection both the inorganic and the organic materials with high sensitivity and selectivity; Anastasia D. Pournara pointed to the

efficiency of the working electrodes in the voltemmetric methods used for the determination of the inorganic and organic analytes, especially when those electrodes were modified by polymers called Metal organic frameworks MOFs, which principles can be seen in appealing as sensors because they may combine highly porous structures with a range of functional groups, resulting in rapid analyte diffusion into their pores and improved framework interactions among the analytes used, in addition, not this study only talked about the modified electrodes with MOFs, similarly, There have been a number of articles that detail the modification of electrodes with MOFs, however there is often little information on the MOF's stability in the working environment (Pournara et al., 2019).

**Theoretical Framework**

**Electrochemical Methods**

The word electrochemical delivered from the words "electron" and "chemistry" which are Greek words. The electrons are negatively charged particles that revolve around an atom's nucleus. Chemicals are substances that are created or utilized in the course of a chemical reaction. When both words connected to each other, it becomes a branch of analytical chemistry which indicates a methods that employ electricity to produce or change a chemical reaction to be called as electrochemical methods (Kubáň & Hauser, 2009). Electrochemical technologies can be used to make novel products like plastics and fertilizers, as well as for analytical applications like spectroscopy and chromatography. Tilak et al. shows that the interplay of electrical energy and matter underpins all electrochemical processes. The measurements are carried out in the electrochemical cell, which contains the sample as well as a minimum two electrodes. The electrochemical cell has a wide range of concentration-dependent physical properties that can be used in chemical analysis. The techniques are mostly used to analyse aqueous samples, although they can also be used to analyse gases and non-aqueous solutions. Most techniques monitor one concentration-dependent electrical parameter, such as current, resistance, and voltage, while the others are held constant or modified to obtain the preferred response that associates to the composition of the sample (Hirst & Koonce, 1996). In the solution of the chemical of concern, the conductivity approach is not specific because all anions, cations, and ions, provide to the conductance. The measurement of the cell's electrical capacitance is also a measurement of the impedance, and the response, which is not precise but rather a bulkiness characteristic attribute, is linked to the sample's dielectric stable. The main advantages of electrochemical approaches include the fact that the indication is

electrical and that no conversion is necessary for information to be transferred to controller units, for both quantitative and qualitative information about the chemical of concern. Because some of the procedures are quite specific, they can only be used to determine the chemicals of interest. Other approaches are less specific, but they can be used to identify only specific sorts of substances in conduct metric measurements, such as ionic species (Shul'ga, et al., 1994).

**Electrochemical Reactions**

Hudson et al. illustrated that in order to obtain a current single from an electrochemical method, a chemical reaction should be occurred in the analyte solution. Hence, any process defines the transfer of an electron between two materials, one solid as well as the other liquid, and which is either generated or facilitated by the passage of electric current, is known as an electrochemical reaction. Under normal circumstances, a chemical reaction results that there are several reactions that occur, when allowed to progress in contact with two electronic transmission lines separated by conducting wires, release electrical energy rather than some other form of energy; however, there are several chemical reactions that, when allowed to progress in contact with two electronic conductors separated by electrical conductors, release electrical energy and generate an electric current (Hudson & Tsotsis, 1994). An electric current's energy, on the other hand, can be utilized to initiate a variety of chemical reactions that do not occur naturally. An electrical cell is a process that involves the direct conversion of chemical energy when properly arranged. Electrolysis, or electrolysis, is a process in which electrical energy is turned directly into chemical energy which can be obtained. The products of the process of an electrolytic that have a tendency to spontaneously react with one another due to their combined chemical energy, recreating the materials that were reactants and so consumed through the electrolysis (Heidrich, et al., 2013).

A great portion of the electrical energy used in electrolysis may be renewed if this reverse reaction is allowed to occur under proper conditions. This capability is utilized in collectors or storage cells, that are grouped together to form storage batteries. The charging of an accumulator is an electrolysis process in which the electric current passing through it causes a chemical reaction. The accumulator acts as a cell that creates an electric current during the discharge of the cell, resulting in the reverse chemical reaction. Regardless to the type of chemical cell used, the main favourable reaction is redox reaction where the electron transfer cause a current which is then recorded as a signal. In an electrochemical cell, electrochemical processes require an electron transfer between a solid catalyst capable of electron conduction,

usually a metal-based substance, and the electrolyte, which can be a liquid solution, a polymer, or a ceramic capable of ionic conduction. The catalyst, the electrolyte, and the micro scale interface between them all have an impact on the reaction's kinetics. The nature of the nanoparticle surface determines not only the majority of reaction properties such as activity, rate, and product selectivity, but also how those Nano objects will stand on the support and how electrons will flow from electrode to active sites and from active sites to adsorbed molecules (and vice versa). When choosing a synthetic process to construct a nano-electrocatalyst, the balance between the electronic conductivity and the electronic conductivity of the nano-electrocatalyst should be examined (Ebbesen, et al., 1996).

**Oxidation-Reduction of the Biomolecule Compounds**

As it is known, the oxidation reaction generally means the losing of electron for a species, whereas, if it gains electrons means it is undergoes a reduction reaction. Meanwhile, that the electrons are preserved in the chemical reactions; meaning to say that they are not created or destroyed. Heidrich et al. mentioned that when one species is losing electrons means that the other is gained. Hence, an oxidation can't happens with a consequent reduction, and vice versa. The species that enrolled in redox reactions can be inorganic or organic, and they may be available in all states of matter solid, liquid, or gas. In addition, the reduction of the species taking place when the reductant which is the electron donor convert the electrons to the oxidant which is the electron acceptor. Naturally, the organic compounds preform as an oxidant, whereas the abiotic reductants such as sulphur minerals and organic compounds. Additionally, the extracellular biomolecules act as reducing agents as porphyrins. Mostly, the reducing agents are available in an anaerobic conditions anywhere the anaerobic bacteria are reducing chemicals by themselves. Hence, it is not that easy to differentiate abiotic and biotic reductive process in the environment. Many essential abiotic reductive transformation to surroundings such as dehalogenation and the reduction of azoaromatic and quinones. In this work, the biomolecule which is DA will be oxidized at pH 7.4, where the hydroxyl groups in its chemical structure will be transformed into quinones, resulting an electron transfer producing current which is later measured and studied.

**The Electrochemical Cell**

The voltammetric method is a technique for determining an analyte's concentration in a solution. According to Hwang et al. study that the voltammetric method uses three electrodes

immersed in an electrolyte solution to detect the potential difference between them. The current flowing between the electrodes is proportional to the potential difference, and this current is determined by the concentration of ions, which can be determined by their ability to carry electric charge. For many years, this technique has been used to quantify hydrogen ions (H+) and hydroxide ions (OH-) in natural waters, as well as other chemicals such heavy metals, organic compounds, pesticides, and medications (Hwang, 2007). The advanced voltammetry based on three electrode system as illustrated if figure 1 below. The three electrodes should be immersed in the solution of the chemical of concern, in addition to the connection between the potentiostat and the three electrodes. The system of the three electrode are counter electrode CE, working electrode WE, and the reference electrode RE. The working electrode is the most critical part of an electrochemical cell since it is where the electrically induced chemical reaction and electron transfer takes place.

Figure 1

*Electrochemical Cell Used In Pulse Electrochemical Techniques.*
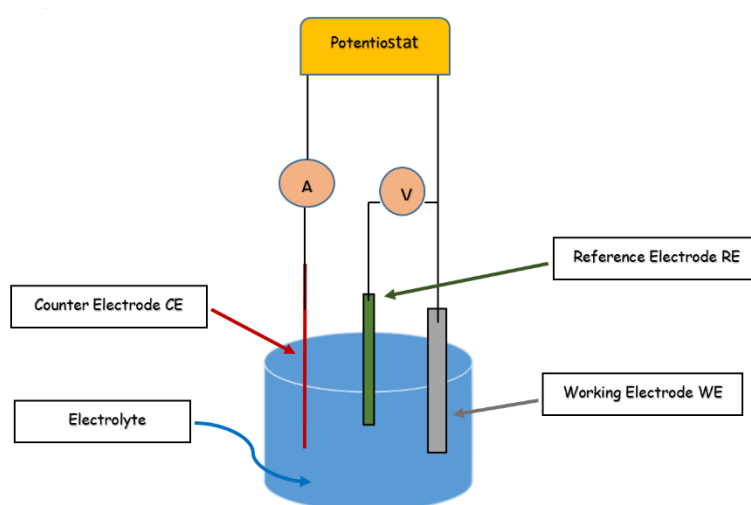


Typically, the chemical reaction occurs on the surface of the working electrode, where a large surface area is preferred for increasing the rate of the chemical reaction which usually indicates transfer of an electron due to the redox reaction (oxidation-reduction reaction). Whereas the current measured by using the counter electrode, besides the measurement of the voltage in a comparison of the working electrode with the reference electrode (Kim, et al., 2004). Hence, through the experiment is running, the charge which indicates the current usually flows between the counter or auxiliary electrode and the working electrode. And the potential

is carried out by the reference and the working electrode. More explanation about the types of the pulse voltammetric techniques in more details as follows.

**Cyclic Voltammetry CV**

Generally, CV which stands for cyclic voltammetry, indicates one of the electrochemical techniques which measures the current developed from a redox reaction due to the electron transfer. CV has been a non-standard instrument for investigating electrochemical reactions over the recent fifteen years. Organic chemists have used the technology to investigate electrochemically generated free radicals and high-temperature reaction routes. Cyclic voltammetry CV is being used by a rising number of inorganic chemists to assess the effect of ligands on the oxidation/reduction capacity of the central metal ion in complex and multinuclear clusters as shown in figure 2.

Figure 2

*CV measurement excitation signal by potential vs. time and CV measurement for redox reaction (Wang, 2006)*
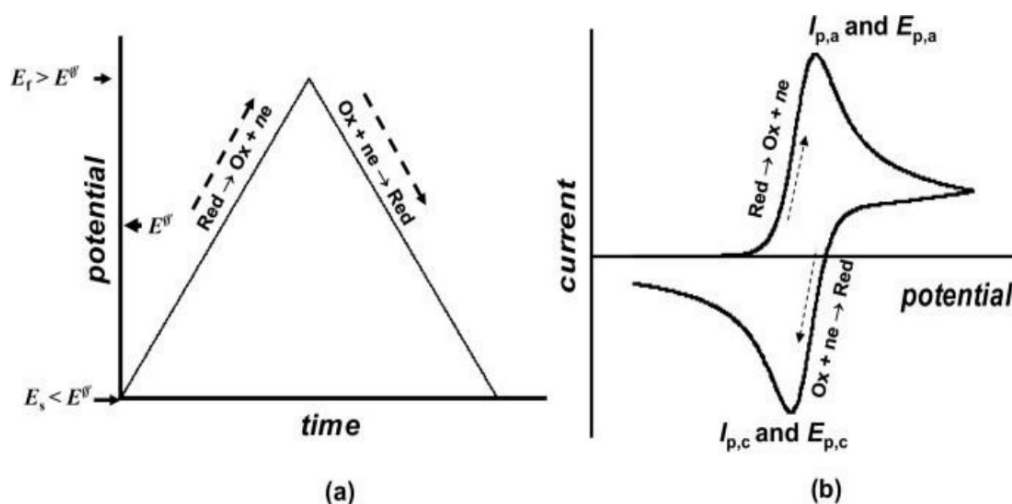


This type of information is used in several approaches in the electrochemical analysis for the chemical of concern. CV which indicates a method for determining the current response of a redox active solution to a linearly cyclic potential sweep between two or more defined values. It's a quick technique to determine out redox reaction thermodynamics, electronic-transfer reaction kinetics and analyte energy levels. It indicates a very useful method to determine the

quantitative information about a chemical reaction occurs on the electrode surface. The resulting voltammogram comes out in a shape called the duck shape; due to the reversible reaction that occurs as can be shown in figure 3 for the Potassium ferricyanide solution. The oxidation reaction takes place first followed by the reduction reaction latter to form a cyclic shape, and due to the electron transfer between the specious in the electrolyte solutions, the duck shape obtained with a signal for the oxidation peak current which should be equal to the reduction peak current when there is one electron is transfer (n=1) (Shama, et al., 2019). Hence, when a chemical reaction has more than one electron transfer, the currents obtained wouldn't be in an equal form.

Figure 3

*Detection of 2mM Potassium Ferricyanide by Cyclic Voltammetry CV Method*
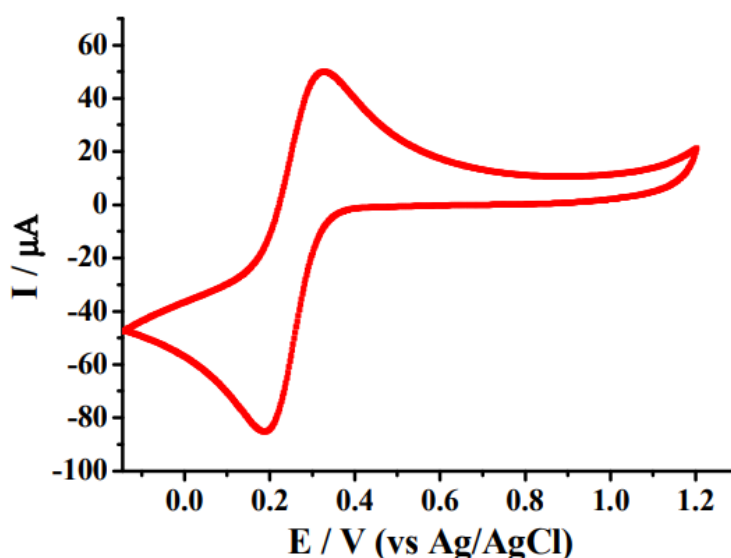


The CV is determined by many parameters; the cathodic peak potential ($E_{pc}$), the anodic peak potential ($E_{pa}$), the cathodic peak current ($I_{pc}$), the anodic peak current ($I_{pa}$), the cathodic half-peak potential ($E_{p/2}$), and the half wave potential ($E_{1/2}$) which should be adjusted (Sayyar Muhammad et al., 2021). The potential initial scan is started where in electrolysis first, then the scan is continued to the acceptable linear scan rate to the switching potential, after that its reverse the direction to go back again to the first potential. The reaction is regulated by the diffusion rate of the ions on the surface area of the electrode, instead the kinetic controller may be regulated according to the rate of the reaction and the potential scan rate (Shama, et al., 2020).

**Differential Pulse Voltammetry DPV**

By applying the differential pulse voltammetry DPV, which indicates another category in the pulse voltammetric techniques but with a high sensitive performance, due to the elimination ability the DPV has to eliminate any back current which indicates an interference for the result obtained for the detection of analyte. This indicates the reason of the popularity behind using DPV for most of the analysis measurements. Baranowska et al. has shown for DPV, the current here is evaluated at two points in each pulse, the former is measured before the pulse application, and the second is measured at the termination of the pulse. The total measurement is calculated by calculating the differences between the two measures of current in each pulse stage, then the net current is plotted vs. the potential applied as described in figure 4.

Figure 4

*Voltammogram for 2 mM of Potassium Ferrocyanide by DPV. AUTO LAB Potentiostat PGSTAT101 with NOVA 2.1.2 Software.*
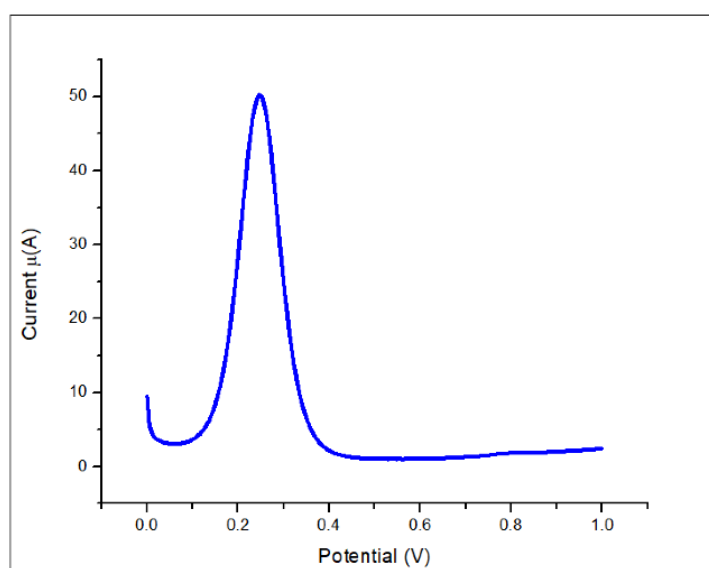


Differential pulse voltammetry DPV is considered to be a recipient technique. It enables to detect up to Nano-scale to be done. It is because of the usage of differentiation when there is a difference even at a low current that also could be observed. Nevertheless, as known it cannot be done so using the traditional method of pulse voltammetry. It was because of the small scale of differences that could be noticed (Baranowska, et al., 2008). Plus, the ability to detect the electron transfer via the redox reaction that occurs on the working surface electrodes, which increases the application of this technique to be used widely.

Figure 5

*Potential Wave for DPV Measurement (Kissinger & Heinemann, 1996)*
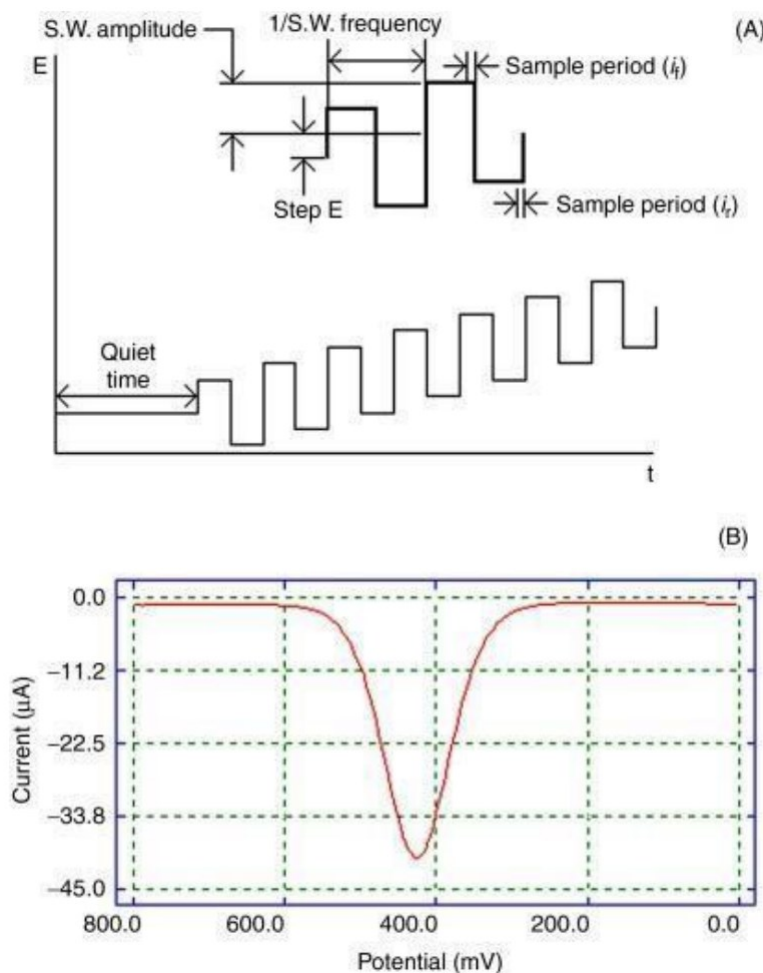


In particular, differential pulse voltammetry minimize the capacitive currents, that why sensitivity is also high and improved. Hence, a quantitative determination is also increased, because of its easy operation, the use of simple instrumentation and to the low limit of determination (Blasco, et al., 2004).

**Working Electrode**

The working electrode in the electrochemical cell, indicates the most essential part which is in charge for the sensitivity and selectivity of the method carried out. In addition, the research compete in how to select and synthesize a working electrode which has high affinity to the chemical of concern (analyte) and costless at the same time. In this work the followings working electrodes were used to carry out the detection of dopamine measurements.
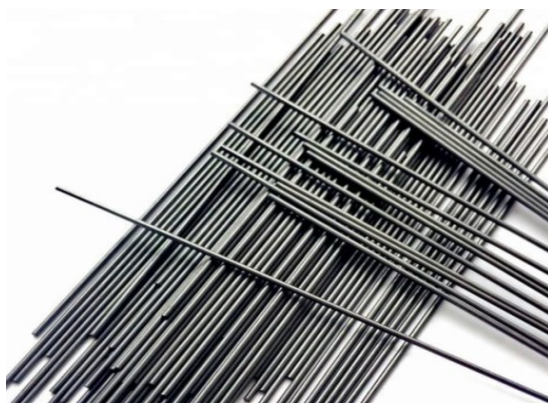
**Pencil Graphite Electrode PGE**

Pencil graphite electrode indicates one of the commonly used as electrode sensor the recent electrochemical research detection of both inorganic and organic compounds with an low limit of detection. Because of their high sensitivity, low backgrounds current, and high reproducibility, in addition to their electro active surface area. Additionally, pencils can be an ultranative for other expensive electrode sensors such as gold electrodes and glassy carbon ones. That's the reason behind that the Pencil graphite electrodes PGEs have piqued interest due to its electrochemical and cost-effective features. These electrodes are simple to use and discard, removing the time-consuming surface cleaning required by solid electrodes (David, et al., 2017). As a result, one of the most inventive techniques to reduce ePAD manufacturing costs was to use pencil graphite as a carbon-based material for electrode creation on paper devices. The method is straightforward, and a pencil or pencil lead is readily available. The percentages of graphite, clay, and resin in pencil graphite fluctuate according to a grading scale that takes hardness (H) and darkness (D) into account (B). The quantity of clay used to the pencil leads throughout the manufacturing process impacts its hardness; the more clay added, the harder the pencil. The harder the pencil lead is, the higher the amount next to the 'H,' the softer the pencil lead is, the higher the number next to the 'B,' enabling more graphite to be left on the board. An ePAD can be made by scratching the paper surface with pencil graphite using a specified template pattern. Pencil-drawn electrodes are made without the use of waste, organic solvents, or inks (Kariuki, 2012). PGEs easily undergoes in the development of the electrochemical sensor electrodes for the detection of both inorganic and organic compounds, many electrodes were used and modified with the suitable materials to get the needed improvement of selectivity and sensitivity in various electrochemical techniques such as DPV and CV. For an electrochemical cell, several electrodes would be used for electrochemical detections, in this study all the measurement where done by using the PGEs and due to their characteristics; for instance the highly surface area which enhancing the selectivity and sensitivity of the measurement (Koyun, et al., 2018). The highly electrochemically active and the economically properties PGEs wide used in chemical detections recently. Aoki et al. [8] reported on the usage of graphite reinforcement carbon (GRC) as voltammetric electrodes for the first time. GRC is commonly used as mechanical pencil leads. The invention of disposable sensors was prompted by the widespread trade availability of GRC by way of graphite leads for mechanical pencils with low weighty metal impurities in addition to uniform quality, as well as all of the other electrochemical types of carbon based electrodes. According to Barbeira

and Tavares, most pencil leads, autonomous of manufacturer or hardness, have a resistance to the electrical properties of less than 5Ω, making them perfect as electrode material. Consequently of their cyclic voltammetry (CV) research, these writers discovered that tougher pencil leads create larger voltammetric indications and that the isolation of peak potentials for reversible systems is closer to the predicted value. Due to variable interactions between common components of a graphite pencil lead and an analyte, electroactive features may display voltammetric activity on graphite pencil leads of the same hardness but created by various manufacturers. PGEs are becoming increasingly valuable in the electroanalysis of a wide range of analytes from diverse matrices and beyond, as previously indicated. Several reviews on various types of carbon-based electrodes and their diverse applications were located in a thorough search of the literature, with some of them referencing PGEs (Özcan & Şahin, 2019). A recent paper offered a history of the usage of graphite pencils as electrodes and emphasized the use of PGEs in environmental sample analysis, but no work to our knowledge provides a summary of general PGE applications and developments (Iulia, et al., 2017).

Figure 6

*Pencil Graphite Electrodes PGEs.*



The material of the electrode for this sort of working electrode is graphite pencil leads, which are well-known and readily available commercially. PGEs are so inexpensive and environmentally friendly, and they can be used as non-refundable electrodes, eliminating the time-using procedure of wiping the surface of solid electrodes between readings. The PGEs ingredients are composite materials made up of clay (30%), graphite (65%), and a binder such as wax (David et al., 2017). Due to the suitability of the PGEs they can be available in several forms and modified forms to increase the sensitivity of the measurement. Furthermore, PHEs

are capable to be modified by other substances such as nanoparticles NPs and polymers in order to improve their sensitivity.

## Modified Pencil Graphite Electrode

The durability of the PGE surface allows alteration to be done easily to improve the characteristics of the electrode used for the detection. The improvement would make the electrodes more flexible, conductive, and sensitive. Various methods can be used in addition to the variety of chemicals can be added to these electrodes as nanoparticles and polymers. Hence, the surface modification using a caly/sol-gel (triethoxysilane) matrix (Ozsoz, et al., 2003), molecularly imprinted polypyrrole (Tsai, et al., 2012), polypyrrole nanofiber, polypyrrole nanofibers are mixed with metal complexes (Akanda, et al., 2016), poly vinyl ferrocenium ions, pyronin Y polymeric films (Kawde, et al, 2016), chlorpyrifos-imprinted polypyrrole nanofiber (PPyNF). Critically, the surface of the PGE may be polymerized with low-cost materials and easy procedures that require little technology. The sensitivity of the bare pencil graphite electrodes stills low especially when PGE is used for the detection of the biomolecules compounds as DA, hence, recent approach is going towards the increasing of the sensitivity of the PGE surface. The modification of the PGE surface helps in enhancing the detectability for the chemical of concern by adding for example some magnetic nanoparticles of synthesis some molecularly imprinted polymer to obtain a very low limit of detection. Therefore, after the addition these types of modifications, the PGE called modified PGE (Akanda, et al., 2016). In this work, the PGE were modified by surrounding the surface with a film contains cavities are very sensitive to the analyte (DA), template was used for the imprinted polymer synthesis by using main functional monomers for the preparation of both molecularly imprinted polymer MIP and molecularly non-imprinted polymer NIP.

## Functional Monomers

Functional monomers are widely used due to their ability to increase the functionality to the final performance obtained. MAPA which stands for N-methacryloyl-(L)-phenylalanine methyl ester indicates one of the functional monomers commonly used, and it was chosen in this work for the preparation of the MIPs electrodes by the template form. The MAPA properties allow it to be a part of many industrial applications and a part of the synthesis procedures as well. In cooperation with MAC which stands for N-methacryloyl-(L)-cysteine which is also a functional monomer and together are applied into a polymerization process.

Both monomers can be easily under go through a complexation reactions with a heavy metal and helps in many applications in speciation and metal complex studies. EDMA which stands for ethylene glycol dimethacrylate indicates one of the functional monomers commonly used, and it was chosen in this work for the preparation of the MIPs electrodes by the template form. The EDMA properties allow it to be a part of many industrial applications and a part of the synthesis procedures as well. In cooperation with HEMA which stands for 2-hydroxyethyl methacrylate which is also a functional monomer and together are applied into a polymerization process. Both monomers can be easily under go through a complexation reactions with a heavy metal and helps in many applications in speciation and metal complex studies.

**Nanoparticles NPs**

Nanotechnology has been the subject of substantial research and a lot of attention among scientists in recent years. The term "nanoparticles" refers to a type of intermediary atom between bulk materials that allows them to have distinct photonic, electrical, catalytic, and medicinal properties. NPs are very tiny materials that range in size from 1 to 100 nm. They can be classified into several categories based on their features, forms, and sizes. Fullerenes, metal NPs, ceramic NPs, and polymeric NPs are among the various groups. Because of their large surface area and nano-scale size, NPs have unique chemical and physical characteristics. According to reports, their optical qualities are affected by their size, which results in variable hues due to absorption in the visible area. Their unique shape, size, and composition have an impact on their reactivity, durability, and other characteristics. Because of these characteristics, they are suitable for a wide range of military and commercial applications, including catalysis, imaging, pharmaceutical uses, energy-based research, and environmental services. The NPs were utilized in this study to boost the sensitivity of the working electrode PGE in order to lower the detection limit. The NPs used in this study were golden nanoparticles AuNPs (Beveridge, et al., 2011).

**Gold Nanoparticles AuNPs**

Long time ago, gold collide particles has long been studied for its potential medical applications. Scientists, on the other hand, have only recently become interested in the fabrication and testing of various gold nanoparticles. The Gold nanoparticles (AuNPs) are very appealing and versatile nanoparticles because they have extraordinary facility to scatter and

absorb light. Additionally, AuNPs have chemistries in their surfaces that can be exploited to make them act as drug carriers, and they can also use non-radiative electron relaxing dynamics to transform optical energy into heat. Because of their biocompatibility, gold nanoparticles (Au NPs) are widely used (Daniel & Astruc, 2004). Photothermal heating of Au NPs, nanocages, nanoshells, and nanorods was employed to regulate infection caused by bacteria as well as cancer cells using near infrared light (Rai & Bai, 2015). AuNPs' antibacterial effect has been linked to their high electrostatic attraction to negatively charged cell membrane bilayers (Johnston, et al., 2010). The capability to modify the surface of gold nano-particles with diverse targeting and functional compounds greatly widens the potential analysis applications. For the analysis and detection part, AuNPs can be used for modification to modify the analytical method used for detection. The AuNPs' unique physical and chemical species make them ideal for developing new and enhanced sensing instrument, mainly electrochemical biosensors and sensors. Many various types of Au nanoparticles have been utilized to build electrochemical biosensors and sensors, including oxide and metal, and these AuNPs' play diverse functions in various sensing structures, catalysis of a electrochemical reaction, biomolecules' Immobilization, increase of electron transfer between electrode surfaces and proteins, labelling of biomolecules, and even serving as a reactant are all significant activities supplied by Au nanoparticles. Due to the rapid growing of the nanotechnology, the synthesis and use of Au nanoparticles has increased tremendously. Au Nanoparticles have been created via a number of physiochemical techniques, many of which have resulted in major environmentally demands. Because of their lengthy history of therapeutic applications, gold nanoparticles are the most important of the metal nanoparticles described above. Gold nanoparticles are the most extensively documented type in the literature. A variety of physical, chemical, and biological synthesis approaches have been established for the producing of AuNPs, nevertheless, the most commonly way used for the preparation was established since In 1951, a method was devised for producing AuNPs by combining hydrogen tetrachloroaurate ($HAuCl_4$) with citric acid in boiled water, with the citrate serving as a stabilizing agent and reducing as well (Espinosa, et al., 2010).

## Molecularly Imprinted Polymer MIP

The molecularly imprinted polymer present a type of is a polymer that has undergone molecular imprinting processing, resulting in holes in the polymer matrix with a specific similarity for a template molecule. In most cases, the technique entails polymerizing monomers

in the existence of a template molecule, which is then removed, leaving holes in its place. Because of their affinity for the original molecule, these polymers have been used in chemical isolations, molecular sensors and catalysis. In the 1930s, the first books on the subject were released. In addition, the MIPs can be employed in sensors because they mimic biological molecular recognition units. That's the reason of using MIPs in this work, and because the analyte is a biomolecule which is dopamine so MIPs help a lot to get the target of high sensitivity. MIPs are theatrically synthesised substances to imitate the recognition of an anlyate molecule process of biochemical macromolecules and macromolecules as antigen and its antibody. The hyphenation of these substances with the electrochemical methods has permitted the improvement of sensors electrodes, which meaningfully develop the functioning of a bare electrodes such as PGEs, to be able for a new application. Nevertheless, in spite of the MIPs great selectivity, these still display some annoyed response near some other compounds. Thus, the combination of voltammetric devices with the MIPs sensors gives the room for the improvement of a new type of electrochemical sensor electrodes. In this direction, the electrochemical electrode sensors gives an accurate and quick data in a very costless manner. In addition, to their wide linear range, high sensitivity, and simplicity. For these reasons, the area of bio sensing and sensing is having acceptance among other techniques in the scientific field in current publications. To develop the selectivity and sensitivity of the electrode sensors, various materials and biomolecules have been used through the improving of the electrochemical electrode sensor. On the other hand, the usage of the biocatalysts which especially catalyse the chemical of concern conversion into another product that is easier to be detected, by lowering the potentials of the reduction/oxidation reactions or enhancing the sensitivity. In this logic, the process in the nanotechnology has directed to a variety of modern nanostructured substances which have developed an alternatives to the big substances given its highest surface ratio and developed the properties of the electrochemical techniques. On the other hand, the use of the biochemical recognition substances that has no effect to the response of the electrochemical methods directly, nevertheless, they enhance the sensitivity of the modified electrode sensor by the binding to the required analyte. Many synthetic bicomounds have been assessed from the philosophies of the chemistry of host guest, including the use of MIPs, metal organic frameworks (MOFs) and cyclodextrins. Although, the week chemical stability in solutions and have very low conductivity of the hinder their usage in the system of electrochemistry, hence, MIPs show a high performance and potential among the others mentioned. Additionally to their costless and higher stability. The synthesis of these polymers depends on formation of template induced of the free recognition cavities that are hence created

particular in size, shape, and working against the analyte. Definitely, the distinguishing of the template by the MIPs has been associated to the Fischer's lock which used for the description of the substrate and its enzyme interaction. The synthesis of MIPs begins by the mixing of the template with the functional monomers chosen in a suitable solvent, which permit the solubilisation and formation of the ore-polymerization composite. Later on, an initiator should be added or a cross linker, usually under free oxygen condition. The following step is the template molecule removing, to obtain a substance that is able to a selective binding to the chemical of concern with superior selectivity even in the existence of interferences compounds. For the Non-imprinted polymers NIPs, which are manufactured in the same manner of MIPs, however without the step of template availability for the comparison aims.

**Molecularly Imprinting Technique**

The imprinting technique can be defined generally as a process of making an impression in a solid or a gel that is identical to a particularly during the polymerization process, the template molecule in terms of the shape, size, and charge distribution. The result is a synthetic receptor with excellent affinity and specificity at the binding site for attaching to a target molecule. The interactions between the template and the polymer, which are akin to these between antigens and antibodies, are characterized by Van der Waals forces, electrostatic interactions, hydrogen bonds and hydrophobic interactions. For the preparation of this method, the followings should be provided in order to obtain a success steps; a functional monomer, template, polymerization initiator as radical, cross-linker, porogenic solvent, and extraction solvent and according to the polymerization method aimed, one or more of the reagents can be avoided. The functional monomers are firstly arranged spontaneously round the template in order to gain tailor made cavity fixed to it.

Figure 7

*Sketch Representation of the Synthesis of MIPs Electrodes.*
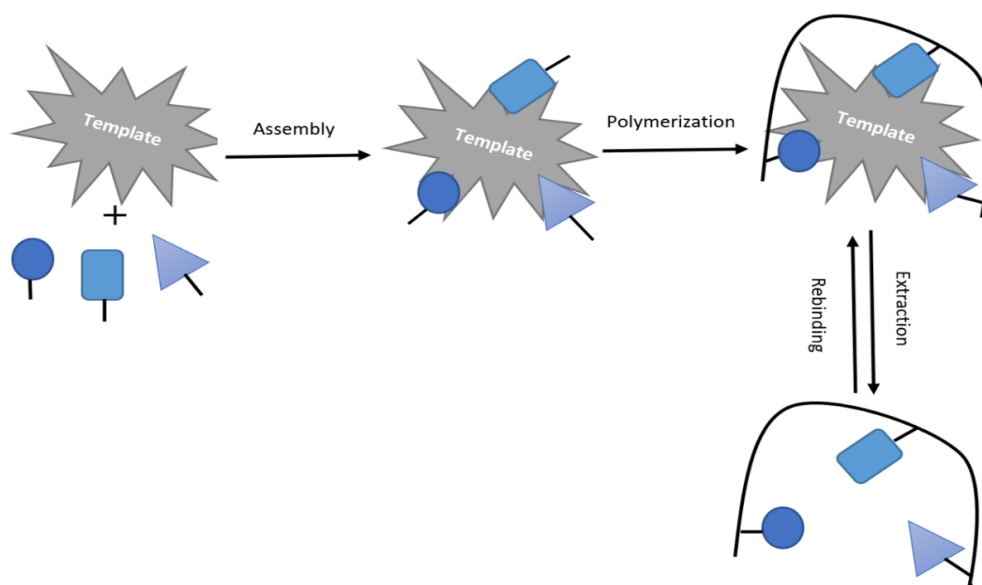
According to the NIPs consideration, there is no arrangement step because there is no template, meaning to say, synthesizing a polymer without an imprinting, consequently less functional cavity available. To the end with, in order to be effective for the electrochemical electrode sensors, by the way of the preparation of these polymers which alter elastic insulator substances, the appropriate combination into the electrode chemical sensors is needed. This is the cause of behind the popularity of the optical applications when MIPs are used.  In spite of their weak electrical goods, the selectivity and sensitivity are developed when MIPs are involve into the chemical electrode sensors, hence allowing an enrichment and subsequent divesting methodology.

Figure 8

*Sketch Representation of the Synthesis of NIPs Electrodes.*
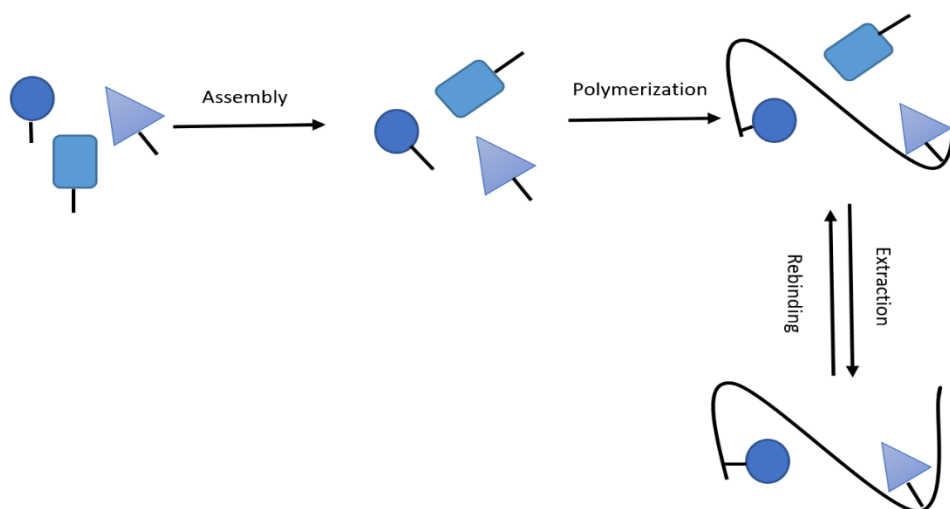
By means of these substances reward attention, updated synthetic ways, immobilization and polymerization are being discovered to develop the total performance of the electrochemical electrode sensors. In this way, this work aims to show and specifically the achievements of more familiar MIPs synthetic process and their involvement in the electrochemical electrode sensors for the detection of DA analyte in a high performance quantitative and qualitative analysis achievements. For the binding relative aims, NIPs must be prepared equivalent. The purpose is to establish that there is originally an imprinting influence on the polymer synthesis process. In this way, by the usage of NIPs as a reference for the MIPs in our work, it will be easy to calculate the imprinting factor IF as follows:

$$IF = K_{MIP}/K_{NIP} \tag{1}$$

Where K indicates the sensor response or the affinity constant. Which indicates the measurement of the imprinted polymer interaction strength regarding the template molecule. Thus, the highest IF presents the best MIP, and if the IF equals 1; means that the interaction of MIPs to NIPs is insignificant, and negligible between the MIPs and the template as well due to the surface interactions.
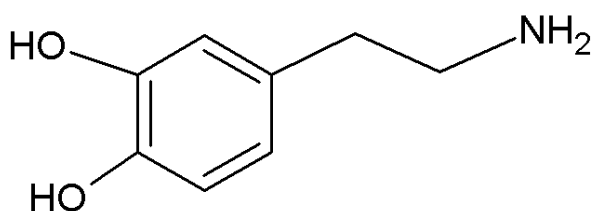
**Dopamine DA**

Dopamine (DA) is a monoamine neurotransmitter (chemical messenger between neurons) that occurs naturally in humans and is currently being investigated in depth. It is most commonly found in the brain tissue of the human body's central nervous system, which serves

a variety of vital tasks, including executive functions, blood flow, reinforcement, motivation, behavior, and reward (Sathisha, et al., 2012). Dopamine, also known as hydroxytyramine, is a nitrogen-containing chemical molecule generated even during breakdown of the amino acid tyrosine as an intermediary step from dihydroxyphenylalanine (DOPA). Dopamine is a neurotransmitter that aids in our happiness. It's critical to our ability to reason and organize as humans. It motivates us to put in long hours, focus, and discover experience. As a result, abnormal DA levels can lead to a variety of complex-functioning illnesses, including depression, Parkinson's disease, Alzheimer's disease and others (Manjunatha, et al., 2014). As shown in figure 9, DA is a polar molecule with a melting point around 128 $^0$C which belongs to the phenethylamine and catecholamine functional groups of organic chemicals. Dopamine accounts for approximately 80% of the catecholamine content i the brain. It's an amine made by removing a carboxyl group from a precursor chemical molecule. These hydroxyl group alter the DA for the high oxidative property, and converted into Quinone's at pH 7.4 which is the physical pH for the human's blood. In addition, Organic solvents including such ethanol, DMSO, and dimethyl formamide (DMF) are soluble in this compound, which should be purged with an inert gas. Dopamine (hydrochloride) has a solubility of around 1 mg/ml in ethanol and about 30 mg/ml in DMSO and DMF.

Figure 9

*Dopamine Chemical Structure*



Because of its capacity to oxidize to amino-chrome compound, dopamine DA is both a necessary neurotransmitter for the movements regulator and a neurotoxic substance that causes apoptosis and toxicity in cell lines in human's body (Ouellette, et al., 2019).The dopamine hydroxyl protons groups that are disconnected when dopamine is in the cytoplasm where the pH media is 7.4 cause dopamine to spontaneously oxidize to aminochrome in the existence of oxygen. Monoamine oxidase (MAO) and catechol ortho-methyltransferase (COMT) soluble isoforms may both breakdown dopamine in the cytosol. Since the neuromelanin colorant is

located in the substantia nigra in the human, dopamine can oxidize to amino-chrome form, the precursor of neuro-melanin, under certain unknown circumstances.

Figure 10

*Mechanism of DA Oxidation*
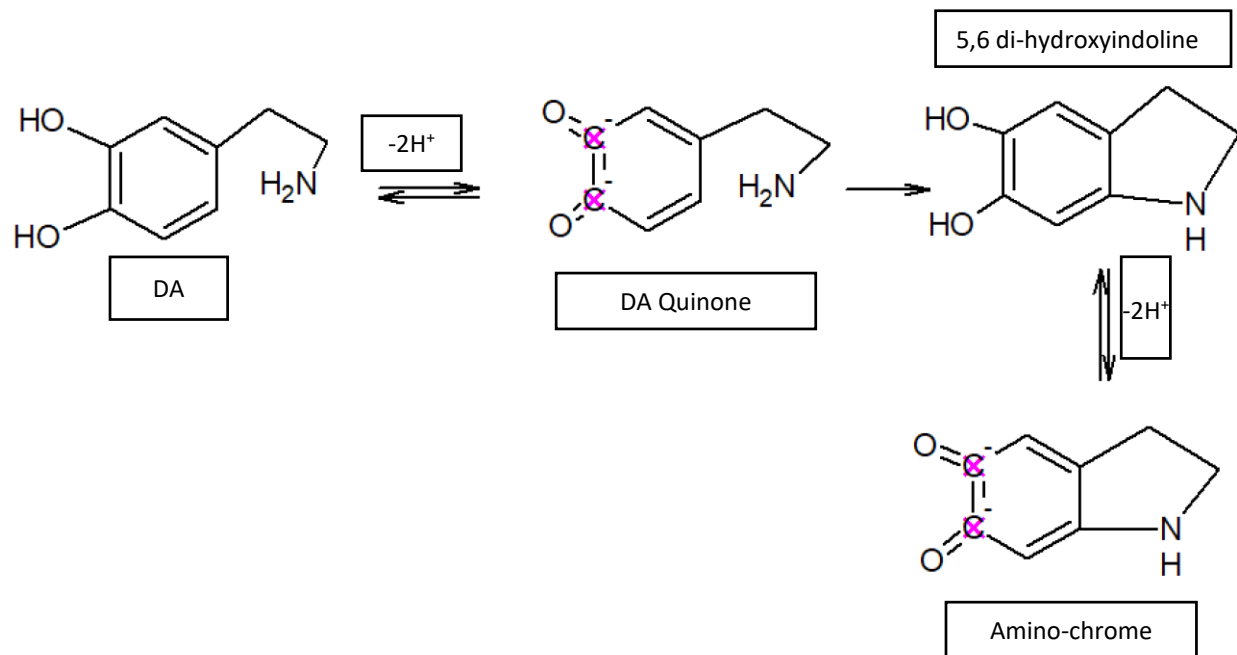


In the absence of enzymes for example catechol-O-methyltransferases (COMT), MAO or di-amine oxidases, the dopamine DA autoxidation occurs in an aqueous solution (DAO). Dopamine interacts with oxygen first, forming the Quinone form and the superoxide anion, which then decomposes into different reactive oxygen species ROS. The Quinone form can undergo intermolecular Michael addition (cyclization), resulting in aminochrome, which is the rate-determining step under physiological conditions. The aminochrome can polymerize further to form the dark neuromelanin polymer (Keithley, et al., 2011).

**Epinephrine EP & Norepinephrine NE**

Despite their molecular similarity, norepinephrine and epinephrine have different actions. Noradrenaline acts more specifically on the alpha receptors to raise and preserve blood pressure, while epinephrine has a broader range of consequences. Norepinephrine is discharged into rotation at low amounts all of the time, whereas epinephrine is only released during

stressful situations. Noradrenaline is another name for norepinephrine. It's a hormone as well as the sympathetic nervous system's most frequent neurotransmitter. Adrenaline is another name for epinephrine. It is mostly manufactured in the adrenal medulla and functions as a hormone, while minor quantities are also produced in nerve fibers and function as a neurotransmitter (Bucher & Wightman, 2015).

Figure 11

*EP and NE Chemical Structures Respectively.*



Norepinephrine is produced frequently inside nerve axons (the nerve's channel), accumulated in vesicles (little fluid-filled sacs), and then released when an action potential (an electrical impulse) travels down the nerve. The Noradrenaline crosses the distance between two nerves, binding to a receptor on the second nerve and stimulating it to respond. This is the neurotransmitter norepinephrine in action. Because norepinephrine promotes vasoconstriction (narrowing of blood vessels), it is effective for both providing and boosting blood pressure in times of stress. Inside the adrenal medulla, norepinephrine is converted to epinephrine (the inner portion of the adrenal gland, a tiny gland that is connected to the kidneys). The adrenal medulla aids in the management of physical and mental stress. During times of stress, the production of epinephrine increases. Epinephrine affects nearly all tissues of the human, but its effects vary depending on the tissue. For example, the hormone epinephrine relaxes the breathing tubes, making it easier to breathe, but it also constricts the blood vessels, making breath harder (preserving blood pressure up and ensuring brain and heart are perfused with blood). The Epinephrine also boosts heart rate and contraction force, blood supply to the muscles and brain, and the liver's translation of glycogen (a accumulated type of strength) to glucose (Cui & Zhang, 2012).
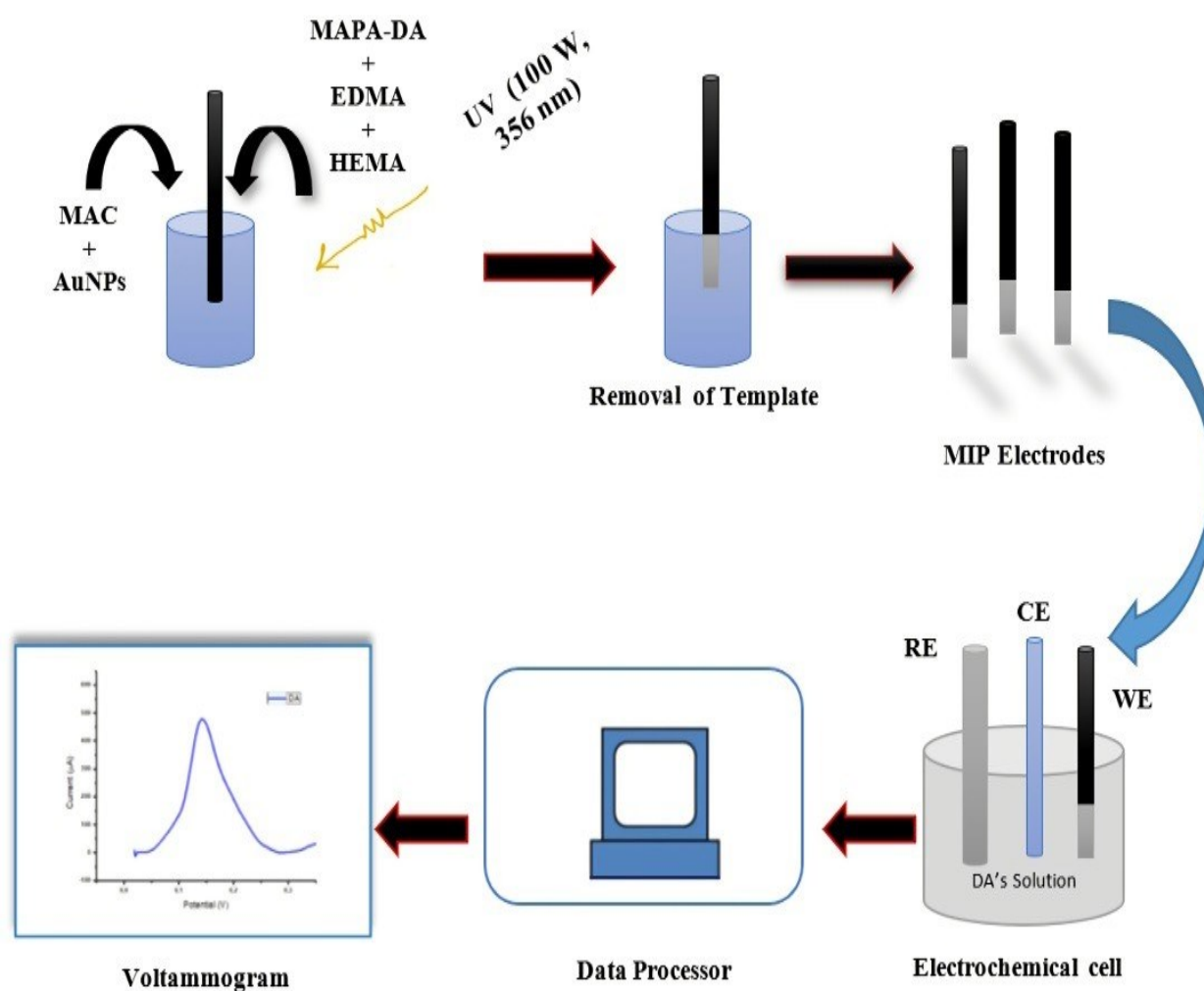
# CHAPTER III

## Methodology

**Research Design**

**Dopamine Analyte Detection**

The proposed electro-chemo-metric flowchart system for the detection of biochemical Dopamine molecules, which starts from the preparation of the MIP based on PGEs, ends to the voltammetric techniques used for the detection of DA solution.

## Materials & Chemicals

Dopamine hydrochloride (DA, 98%), Epinephrine hydrochloride (EP, 98%), Norepinephrine hydrochloride (NE, 98%), (L)-phenylalanine methyl ester, (L)-cysteine methyl ester, Ethylene glycol dimethacrylate (EDMA), 2-Hydroxyethyl methacrylate (HEMA), methanol, and acetic acid were supplied from Merck (Darmstadt, Germany). The chemicals used to prepare buffers in this research are sodium di-hydrogen phosphate adjusted with phosphoric acid to get a pH of 1.5. Acetic acid and sodium acetate for the preparation of acetate buffer to obtain a pH of 5.5, potassium phosphate monobasic and potassium phosphate dibasic for the preparation of phosphate buffer to achieve pH of 7.4. Ammonium chloride was adjusted by ammonia to obtain pH of 8.5, and finally, carbonic acid and sodium hydroxide adjusted the pH to 10.5, which were obtained from Merck (Darmstadt, Germany). Deionized (DI) water (18.2 $M\Omega.cm$) was utilized to make all of the aqueous solutions and treated with pure lab Ultra Analytic (ELGA Lab Water, UK). The pH meter was used to measure the pH, and the sonic water bath was also used in solution preparations. Pencil graphite electrodes (PGEs) were used in pencil tips (Tom-bow 0.5, HB), obtained from local stationery stores.

## Apparatus

The AUTOLAB-PGSTAT204 potentiostat/galvanostat with NOVA 2.1.2 software (Metrohm, Utrecht, The Netherlands) performed DPV and CV, measurements. Electrochemical pulse measurements for both techniques were carried out using an electrochemical cell consisting of three main electrodes; the first electrode, called a counter electrode (CE), or auxiliary electrode, was a platinum wire. The second electrode shows the reference electrode (RE), made of Ag/AgCl (3M KCl). The latter is called the working electrode (WE). The solutions were prepared freshly before each measurement for each DA concentration to avoid any oxidation reaction of the analyte of concern. Furthermore, all detection methods were verified at a room temperature of 25°C. The parameters used for both pulse voltammetric techniques, DPV and CV, were adjusted for measurements. The upper and lower vertex potentials for the CV method were +0.6 V and 0.4 V, respectively, whereas the number of scans and the scan rate were 1 and 0.05 V/s, respectively. The DPV was carried out under a start potential and stop the potential of 0 V and 0.4 V, respectively, in addition to the step height of 0.005 mV and the amplitude of 0.025 V in a time interval of 0.5 s. All received data and peaks were evaluated with After MathTM software, OriginPro8, and Microsoft Excel

2013. Centrifuge and sonicator devices were used for solutions and samples preparation. A Century India Ltd. digital pH meter (Model CP-901) was employed for pH optimization, moreover, all the detection procedures were detected under normal room temperature 25˚C.

Figure 12

*AUTOLAB-PGSTAT204 Potentiostat/Galvanostat with NOVA 2.1.2 Software for the Electrochemical Detection*
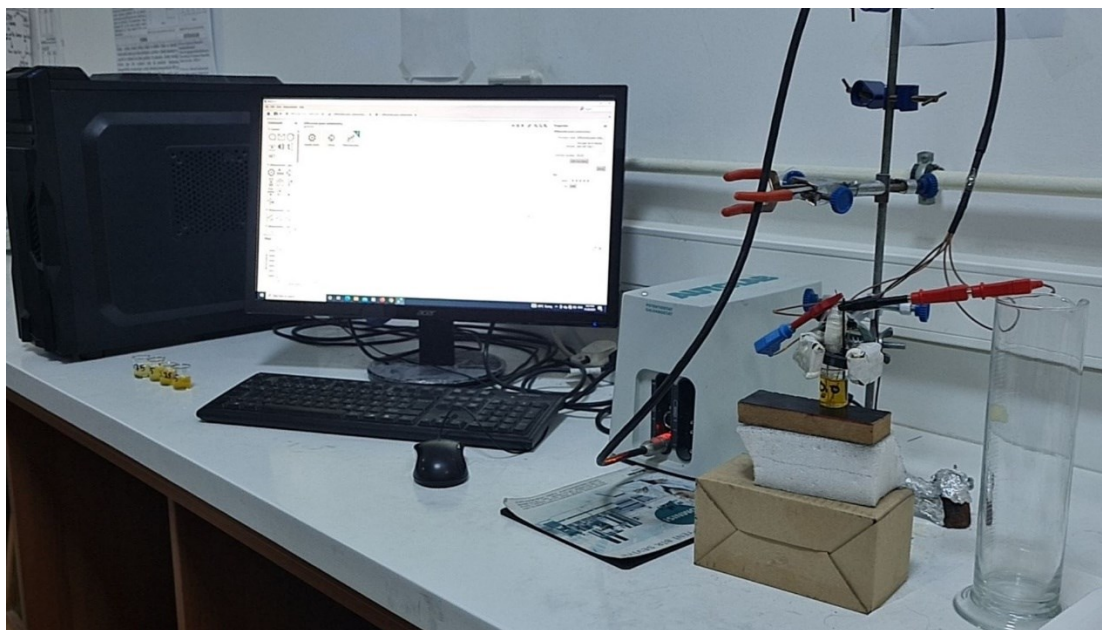


**Preparation of Stock Solutions and Real Sample**

For the preparation of the stock solution, the pH should be adjusted first. To modify the pH to the physiological pH of 7.4, potassium phosphate and potassium dihydrogen phosphate were used to make the buffer solution. The DA standard stock solution was then made by dissolving the appropriate amount of DA.HCl powder in an adequate amount of $D.H_2O$ and phosphate buffer solution (PBS) to the adjusted volume utilized for the subsequent dilution portion. For both neurotransmitters, the EP and NE solutions are prepared in the same way, by dissolving a sufficient amount of each in PBS. Human blood samples were taken from volunteers at NEU hospital after gaining authorization from the Institute Ethical Clearance Committee. The blood samples were centrifuged for 5 minutes at 1000 rpm. The supernatant was collected using the DPV method at the modified MIP PGE sensor. The DA levels were determined using the supernatant blood plasma. Before analysis, blood plasma samples were spiked with various amounts of dopamine at the same pH which is 7.4.

**Preparation of Desorbing Agent**

In order to increase the affinity of the working electrode, the surface of the electrode should be empty in order to accept the dopamine molecules. After each measurement the electrode surface cavities full with the dopamine molecules after the measurement, hence, to increase the repeatability of the electrode to be used again, a desorbing solution mixture was prepare. The desorbing solution contains from both methanol and acetic acid in a ratio of (9:1 v/v). between each measurement, the MIPs based PGEs were immersed in the desorbing mixture for 30 seconds in order to remove all the DA attached to the moieties on the electrode surface.

**Preparation of Working Electrodes**

The working electrode in the electrochemical cell is made of pencil graphite electrode PGE as mentioned before. The length of each PGE is 6cm.  Each single pencil graphite was cut into two pieces to be 3 cm long. The next step was to clean each pencil graphite, so deionized water was used, followed by immersed each pencil graphite in phosphate buffer and run into chronoamperometry measurement with a potential of +1.4 V for 30 seconds to prepare the surface of each electrode for the detection of the oxidation of DA's solutions as its demonstrated in the figure below.

Figure 13

*Chronoampermetry for Each Pencil Graphite Electrode PGE*
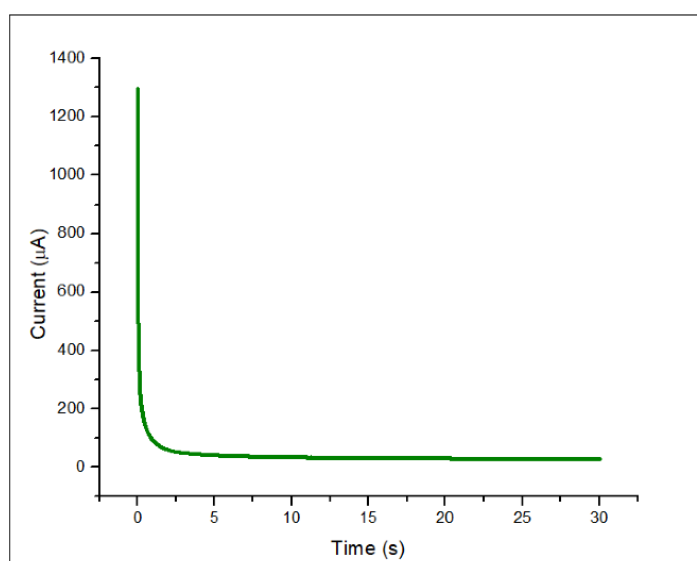
**Production of Gold Nanoparticles**

The gold nanoparticles (AuNPs) were synthesized using the Turkevich method with gold (III) chloride trihydrate (HAuCl$_4$.3H$_2$O) salt, which was reduced to Au with sodium citrate (Enustun & Turkevich, 1963; Sener, et al., 2014). In a nutshell, preheated sodium citrate solution (12 mL, 1% w/w) and 8.5 mg of HAuCl$_4$ salt were added to 100 mL boiling water. This solution was heated vigorously until it turned a deep red color. The prepared AuNPs solution was cooled in the final step, and the volume of the final solution was adjusted to 100 mL. The Nano Zetasizer equipment was used to measure the size of AuNPs and perform zeta-size analysis (Nano S, Malvern Instruments, London, England). The concentration of AuNPs was calculated using the dimension of AuNPs determined by zeta average size measurement (Liu, et al., 2007). The concentration of AuNPs was estimated after the average size of AuNPs was determined. Equation 2 is used to compute the number of Au atoms per nanoparticle (N), whereas equation 3 is used to get the concentration of AuNPs (C). NT is for the total number of Au atoms, V for the solution volume, and NA for Avogadro's number in the equations, where A represents for Au density (fcc, 19.3 g/cm3), M for Au atomic weight, and D for the diameter of AuNPs.

$$N = \frac{\pi}{6} \frac{\rho D^3}{M} \qquad (2)$$

$$C = \frac{N_T}{NVN_A} \qquad (3)$$

**Synthesis of MAPA and MAC Functional Monomers**

N-methacryloyl-(L)-phenylalanine (MAPA), a functional monomer, was chosen to interact with the DA molecules. In brief, (5.0 g) of L-phenylalanine methyl ester and (0.2 g) of NaNO$_2$ were liquefied in 30 mL of K$_2$CO$_3$ aqueous solution (5 percent, w/v) using the practical procedure described elsewhere in (Öncel, et al., 2005). Following that, the solution was refrigerated to 0°C. 4.0 mL of methacryloyl chloride was added to this solution under nitrogen gas, then magnetically swirled for roughly 2 hours at room temperature. At the end of the chemical reaction, the pH of the solution was corrected to 7.0. The solution was then isolated using an organic solvent, ethyl acetate. The aqueous phase was then evaporated in a rotary evaporator. Finally, for crystallization of MAPA, cyclo-hexane and ether solvents were utilized. The functional monomer N-methacryloyl-(L)-cysteine (MAC) was synthesized

according to a previously published technique (Candan, et al., 2009). In a nutshell, 30 mL of a prepared 5 percent v/v $K_2CO_3$ aqueous solution was diluted in 0.2 g $NaNO_2$ and 5.0 g (L)-cysteine methyl ester and refrigerated to 0°C. Then, under nitrogen, 4.0 mL of methacryloyl chloride was gently added to the $NaNO_2$ aqueous solution. After that, the solution is magnetically stirred at 22°C for roughly 2 hours. The unreacted methacryloyl chloride was separated using ethyl acetate after the reaction mixture was neutralized at pH 7.0. The aqueous phase was evaporated in a rotating evaporator unit. The residue (MAC) was crys-tallized and collected in a 1:1 (v/v) of ethanol: ethyl acetate ratio.

## Preparation of Molecularly Imprinted Polymer-Based Pencil Graphite Electrode (MIP PGE) Sensor

PGE 0.5/HB pencil tips were used to make the MIP PGE Sensor. To make the MAC-AuNPs pre-complex, the functional MAC monomer was combined with AuNPs (0.05 mmol: 0.05 nmol). A UV-VIS spectrophotometer was used to measure the complex formation of MAC monomers with AuNPs. MAPA and DA (0.2 mmol/0.02 mmol) were additionally reacted for 1 hour to generate the N methacryloyl-(L)-phenylalanine-Dopamine (MAPADA) pre-complex, then EDMA (2 mmol) and HEMA (0.4 mmol) monomers were mixed with the pre-complexed monomers. Finally, two prepared mixtures were combined with 5 mg of AIBN and mixed for 1 hour. Tips were dipped in the produced monomer mixture to manufacture the MIP PGE sensor. The polymerization process was started with UV light and allowed to run for 1 hour on the tips for converting monomer mixtures to polymeric films. After the polymerization was completed, the DA was removed from the matrices using a desorption agent of methanol/acetic acid (80:20, v/v percent). Desorbed DA in the desorption solution was measured using a UV-VIS spectrophotometer until no DA absorption was recorded at 285 nm (Guo, et al., 2009). A similar technique was used to make a non-imprinted polymer-based pencil graphite electrode (NIP PGE) sensor, with the exception of the insertion of DA, a template molecule. For the control studies, an NIP PGE sensor was employed.

## Characterization of MIP PGE and NIP PGE Sensors

A Fourier transform infrared spectrophotometer with attenuated total reflection (FTIR-ATR) spectrophotometer was used to characterize the MIP PGE and NIP PGE sensors (wavenumber range of 400-4000 cm-1) (Thermo Fisher Scientific, Nicolet iS10, USA). The surface characterization of MIP PGE and NIP PGE sensors was investigated using the Kruss DSA100 contact angle (CA) device (Hamburg, Germany). The CA measurements of MIP PGE

and NIP PGE sensors were estimated using the sessile drop method from various sections of the sensor surfaces. The typical drop angles were evaluated using DSA2 software. A scanning electron microscope was used to evaluate the surface morphology of MIP PGE and NIP PGE sensors (JSM-6400, JEOL). A thin gold-palladium (Au-Pd) alloy coating was applied to the samples.

# CHAPTER IV

## Findings and Discussion

The following sections indicate the detection of dopamine molecules used in this work by the pulse voltammetric techniques by using the MIPs based on PGE as mentioned in the previous sections.

## Dopamine Measurements

## Characterization Studies

To make the MAC-Au pre-complex, the MAC monomer was combined with AuNPs. MIP-PGE and NIP-PGE sensors successfully integrated the designed MAC-Au pre-complex into the polymeric composition. To examine the size distribution, Zeta sizer measurements were used to approximate the molar ratio and concentration of AuNPs. Monodisperse particles with a uniform average nanoparticle size distribution were formed since there were no distinct sizes. Aggregation was not observed in the system, as evidenced by the low polydispersity index value (PDI= 0.287) in Figure 14. The average size of the nanoparticles was determined to be 56.48 nm after 3 times (n= 3) repeated testing. Using the average AuNPs dimension by zeta sizer, the concentration of the AuNPs solution was estimated to be $1.57 \times 10^{-7}$ M. Similarly, the functional monomer MAPA had a 1:1 ratio with the template DA molecule. (1 mmol = 0.1 mmol) MIP PGE and NIP PGE sensors were created by reacting functional monomers, MAC and MAPA monomers, with Au and DA to generate pre-complexes, which were then reacted with EDMA (2 mmol) cross linker and HEMA (0.4 mmol) monomer. The polymerization process was used to manufacture NIP PGE sensors, with the exception of DA molecules.

Figure 14

*DLS Analysis of AuNPs*

**Results**

|  | Z-Average (d.nm): 34.42 |  | Size (d.nm): | % Number: | St Dev (d.n... |
|---|---|---|---|---|---|
|  |  | Peak 1: | 31.22 | 100.0 | 9.343 |
|  | PdI: 0.279 | Peak 2: | 0.000 | 0.0 | 0.000 |
|  | Intercept: 0.897 | Peak 3: | 0.000 | 0.0 | 0.000 |
|  | Result quality : Good |  |  |  |  |
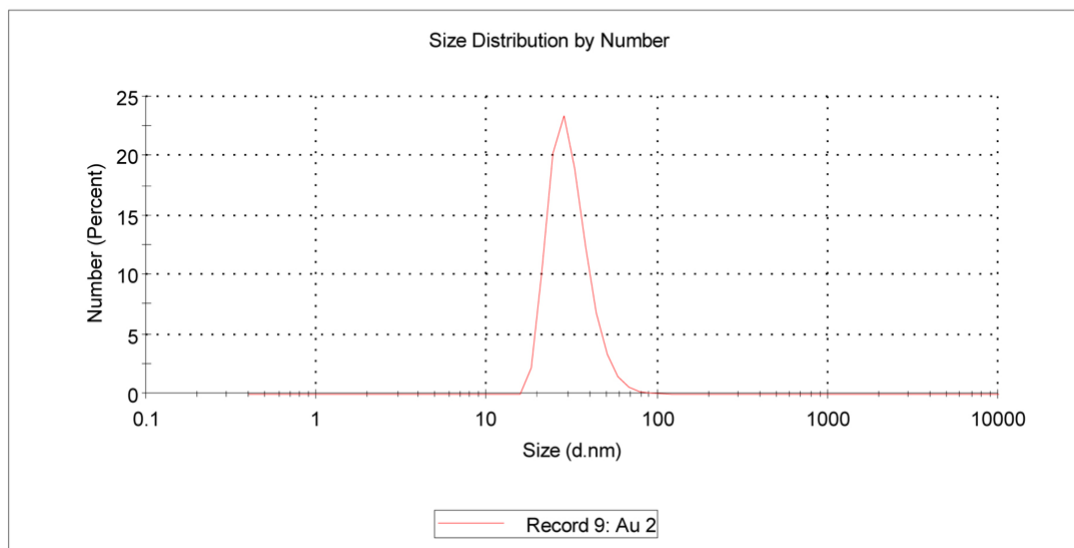


Because functional $-C_6H_5$, $-NH_2$, and $-OH$ groups are present in both MIP and NIP polymeric structures, identical signals were obtained when the FTIR-ATR spectra of the non-imprinted polymer (NIP) and molecularly imprinted polymer (MIP) were compared. The relocation of some functional groups was observed after the complexation of DA. For example, in the NIP, the $-C=O$ band shifting from 1729 cm-1 to 1726 cm-1 to the amide I band shifting from 1452 cm-1 to 1449 cm-1 indicates that the DA molecule has been integrated into the MIP structure. The surface characterization images of MIP PGE and NIP PGE sensors are shown in figure 14 and figures 15 exhibit CA pictures of MIP PGE and NIP PGE sensors, respectively. The CA value for the MIP PGE sensor was estimated to be 69.3°2.52, whereas the CA value for the NIP PGE sensor was estimated to be 57.5°1.98. The CA rose when the hydrophobicity of the MIP PGE sensor surface improved as a result of the DA coupling to MAPA via molecular imprinting. The surface morphology of the MIP PGE and NIP PGE sensors was examined using a scanning electron microscope (SEM) at 5kx magnification for each PGE. The SEM images of MIP PGE and NIP PGE sensors are shown in Figure 14 and 2D, respectively.
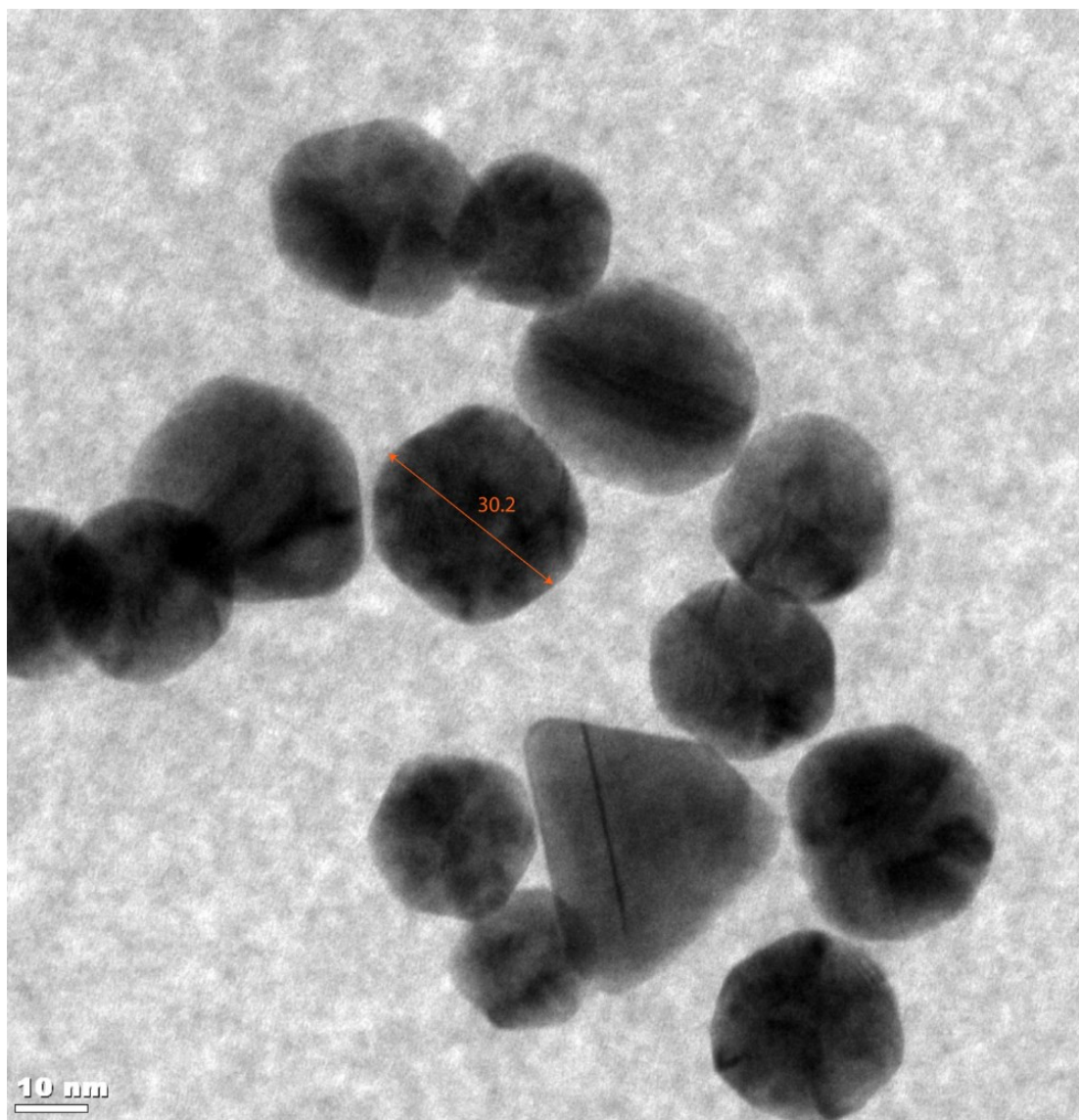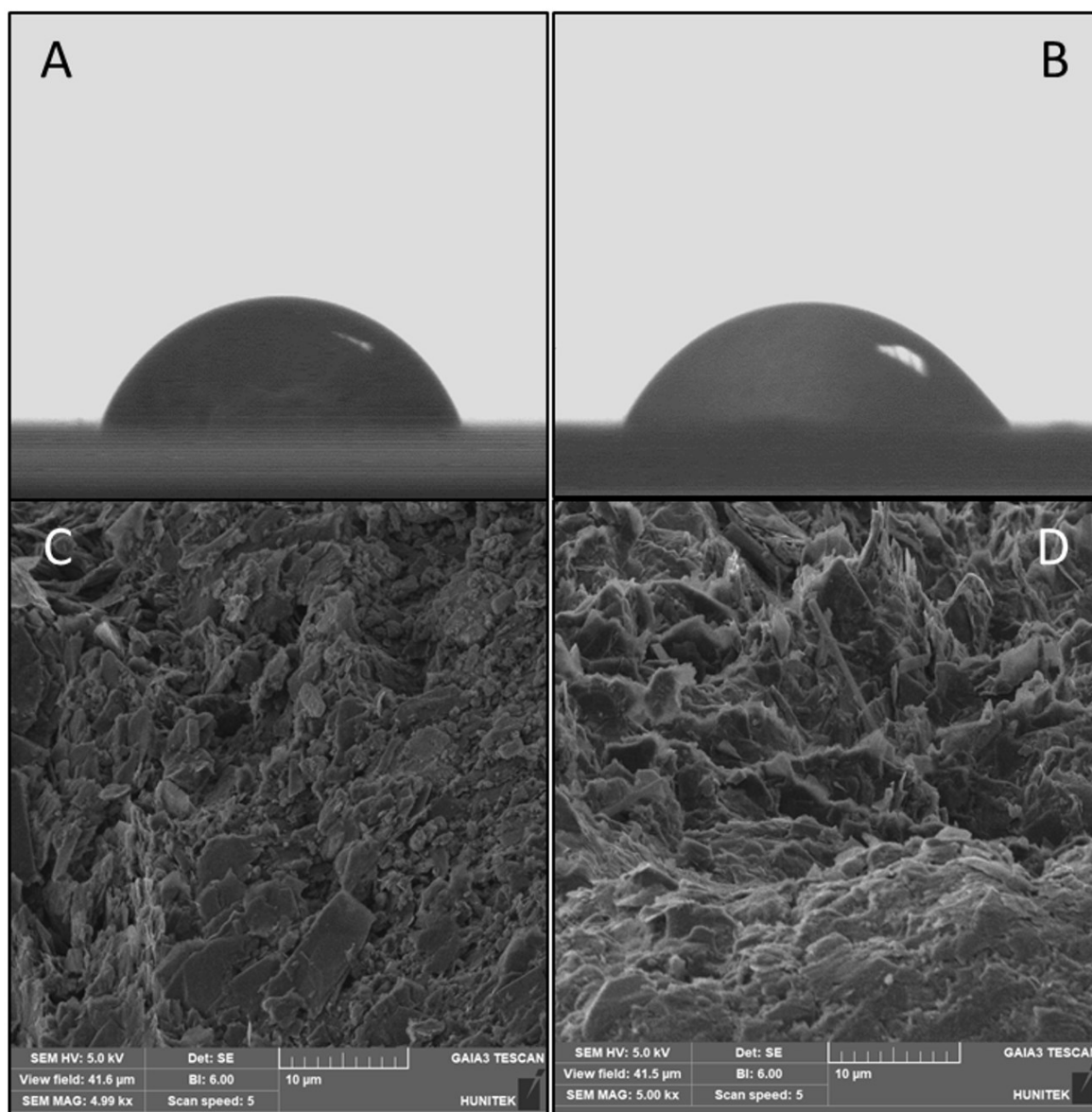
Figure 15

*TEM Image*

Figure 16

*The surface characterization images of MIP PGE and NIP PGE sensors A) CA image of MIP PGE sensor B) CA image of NIP PGE sensor C) SEM micrograph of MIP PGE sensor D) SEM micrograph of NIP PGE sensor.*

## Optimization and Kinetic Analysis by MIP PGE Sensor

Because DA is available in the human body's brain in a blood fluids media with a pH of 7.2 to 7.4, electrochemical techniques must be optimized to identify the highest electroactivity of DA. As a result, buffers with various pH values (1.5, 5.5, 7.4, 8.5, and 10.5) were made using phosphate, acetate, and ammonium solutions. The standard stock of DA at a concentration of 189.6 mg/ml was made using double distilled water and pH-neutral PBS and kept at 4°C in the refrigerator. For the calibration curve (figure 23), several concentrations of DA were generated from the stock solution, ranging from (0.395-3.96 nM) using PBS buffer solution. Figure 17 shows the detection of DA at various pH values, as well as the responses

to each voltammetric technique utilized in the electro-chemical cell with PGE 0.5/HB as a WE. The maximum voltammogram peak height was higher at pH 7.4 than at other pHs, indicating that the protonated form of DA compound alters the electroactivity of its moieties to the potential applied. Furthermore, the peak potential position was found to shift to lower positive potentials with pH variation.

Figure 17

*Optimization of working pH for DA by voltammetry methods in different buffers at room temperature. The detection of DA by CV in the different pH ranges of buffer solutions*
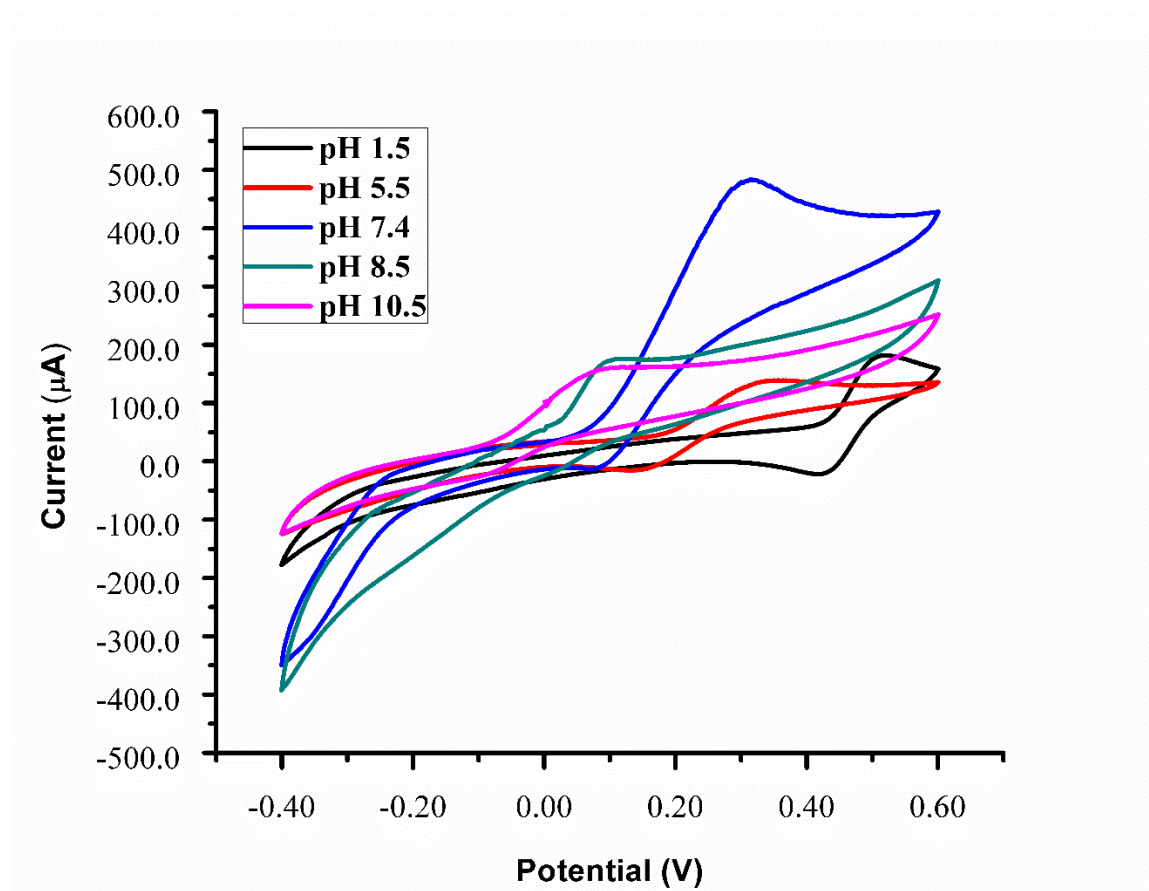


The following figure indicates the relation between the responses obtained from the CV voltammograms vs. the pH media prepared for the DA analyte. The response was different for each pH value. As it is illustrated in figure 18 that the highest response for DA molecules was at the physical pH body which is 7.4.

Figure 18

*Optimization of working pH for DA by voltammetry methods in different buffers at room temperature.*
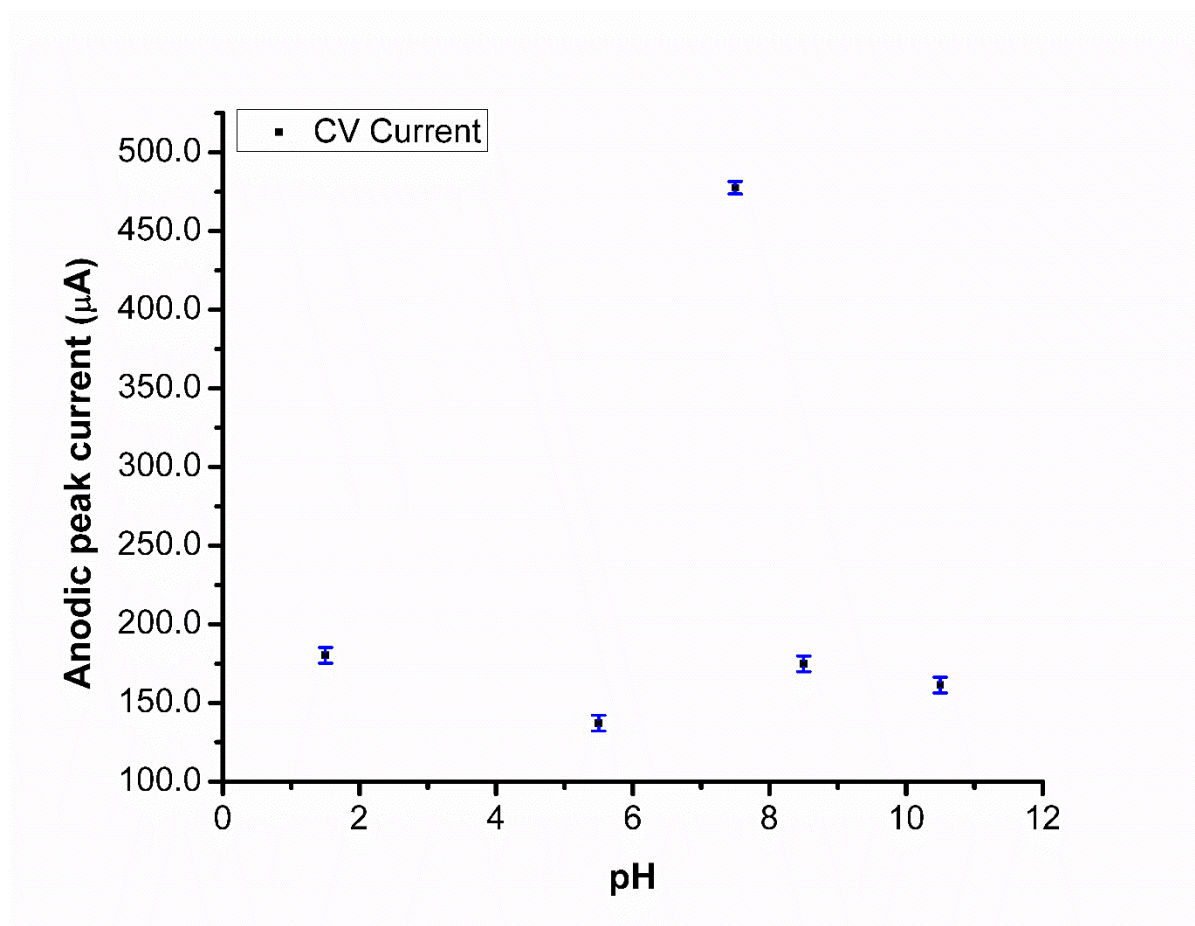


       Five different concentrations of DA (0.395, 0.791, 1.32, 2.64, 3.96 nM) were produced in 0.1 M PBS in 7.4 pH conditions for the kinetic study using the MIP PGE sensor. The concentrations were measured using the same DPV approach, with a modulation duration of 0.05s and start and stop potentials of 0 and 0.4, respectively. Furthermore, the desorption solution, which comprises methanol: acetic acid in the right proportion of 9:1 (v/v) solution, was utilized to de-sorb the DA molecule from the MIP PGE sensor cavities between each measurement, therefore enhancing the electrode affinity. To compute the relative standard deviation RSD percent and repeatability accuracy, three times the measurements were performed (n= 3).

Figure 19

*Optimization of working pH for DA by voltammetry methods in different buffers at room temperature. DA by DPV in the different pH ranges of buffer solutions.*
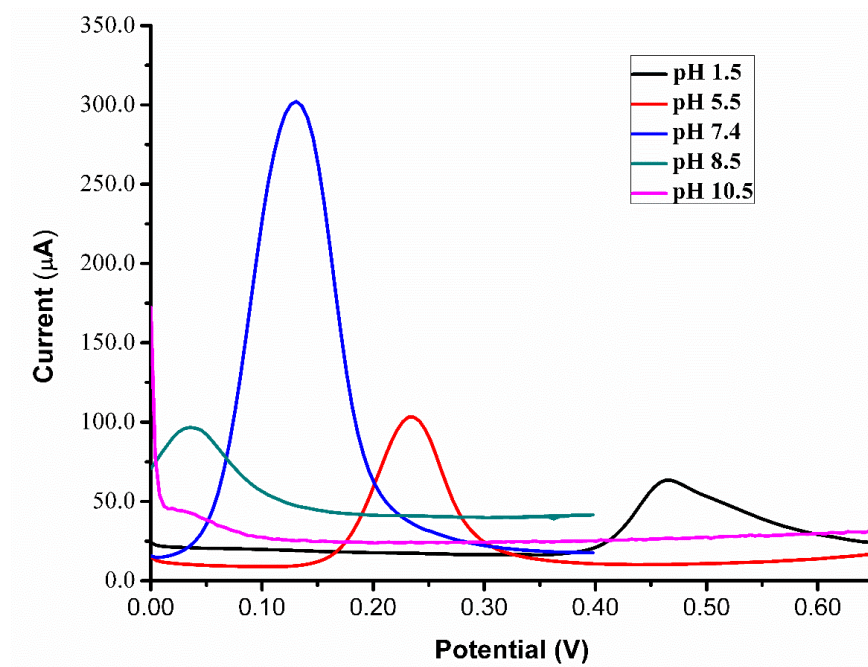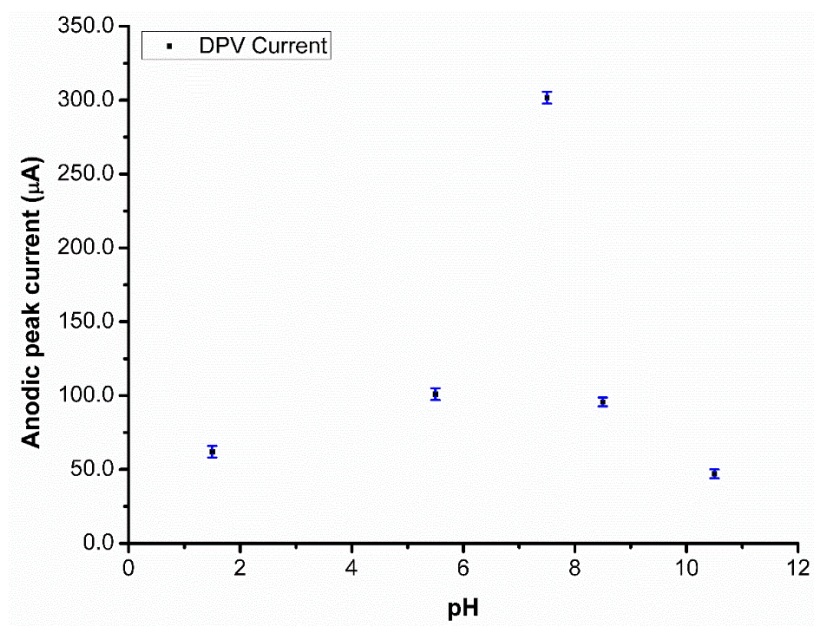


Figure 20

*Optimization of working pH for DA by voltammetry methods in different buffers at room temperature.*

**Potential Response for Different pH Media**

The pH value can affect the peak potential position for the analyte of concern, the figure 21 below illustrated the potential response vs. the changing in the pH media, Meaning to say the value of the pH causes shifting in the peak position, and it may be right or left shifting according to the pH value if it is acidic or basic or even neutral. The pH range were as follow: 1.5, 2.5, 5.5, 7.4, 8.5, and 10.5 were prepared to see the effect to pH on the peak position of dopamine.

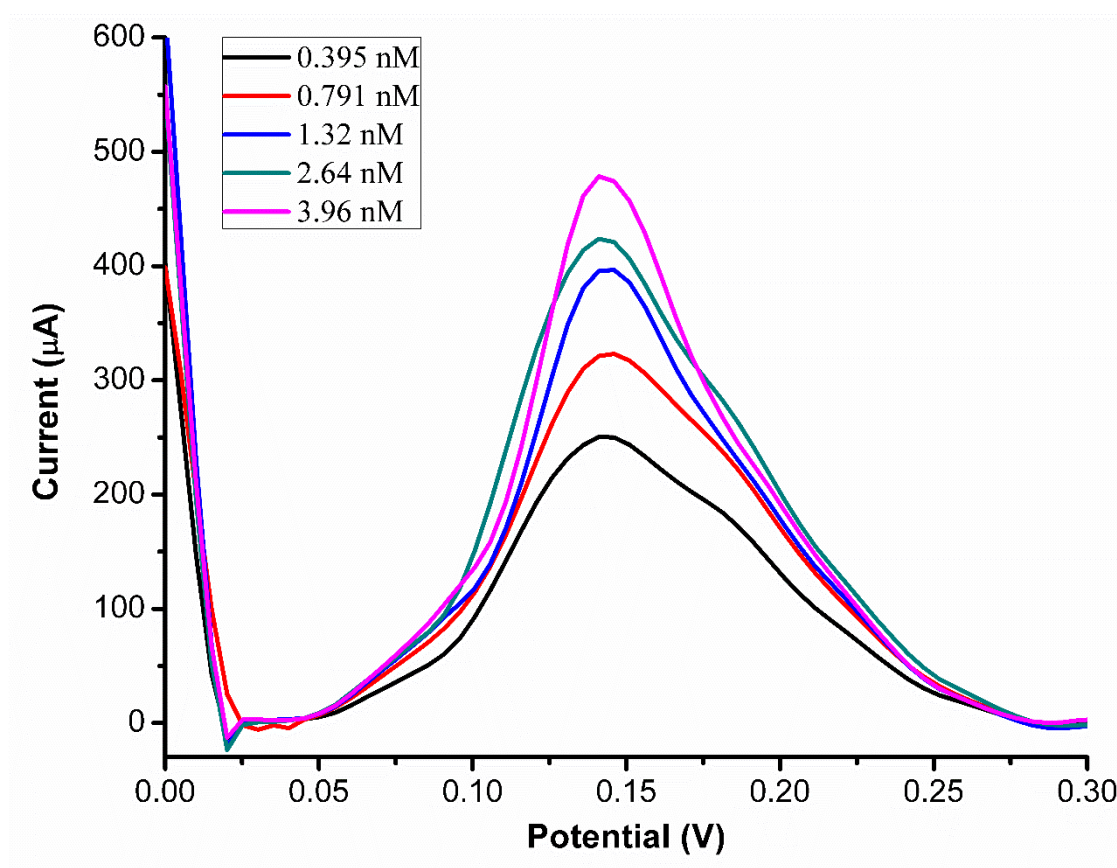Figure 21

*The Potential Peak Position vs. pH Values*
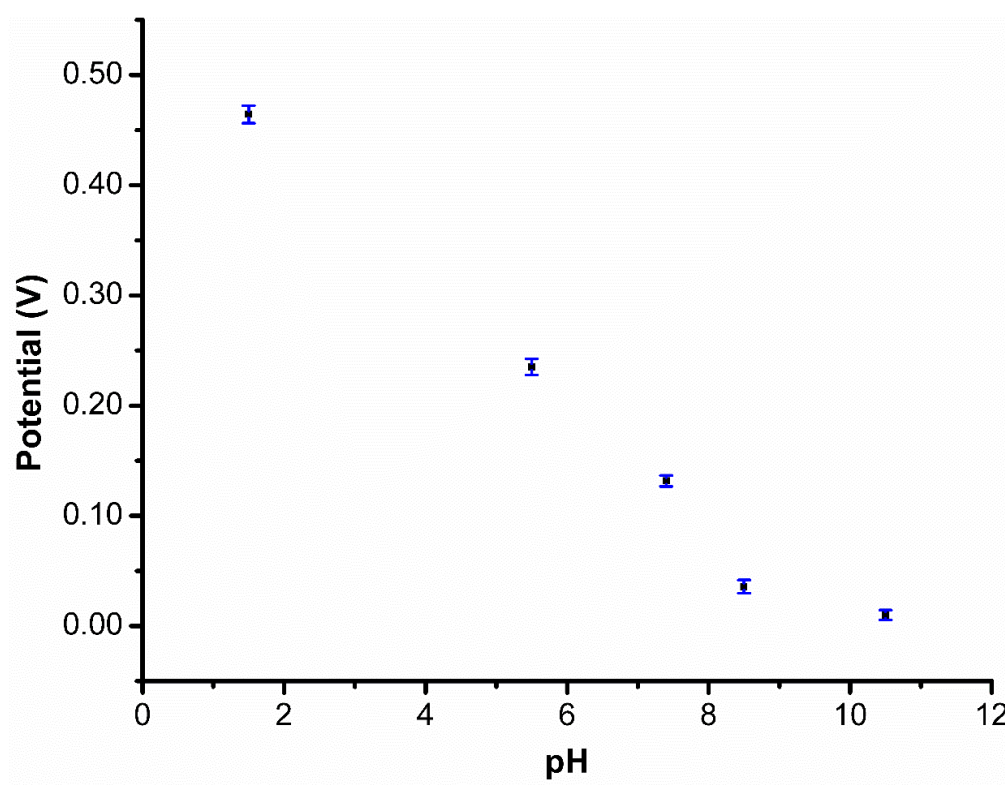


**DPV Response of MIP PGE Sensor for Different DA Concentration**

The negatively charged modified polymer electrode surface assisted in adsorbing the positive-charged DA molecules, improving electrode selectivity to the chemical of interest. Phenylala-nine is the amino acid component of MAPA monomer, consisting of a basic amino group (NH2) and an acidic carboxyl group (COOH). The amino (–NH2) and carboxylic acid (–COOH) functional groups of the phenylalanine component of the MAPA monomer are responsible for the negative charge on the modified polymer electrode surface derivatized with

MAPA. The pI of phenylalanine is 5.48. (pKa1: 2.58 and pKa2: 9.24). The rate of deprotonation of acid functions increases as the mobile phase's pH rises. At 0.1 M PBS (pH: 7.4), phenylalanine is negatively charged (pI: 5.48). Figure 22 displays voltammograms derived by DPV, a highly sensitive technique among various analytical techniques, for five different DA concentrations (0.395 nM-3.96 nM). Around 0.15 V, a potential peak was observed, indicating that increasing DA concentration increased DA oxidation.

Figure 22

*Voltammograms of MIP PGE sensor response for DA concentrations by DPV*



For the characterization of the voltammetric method carried out, figures of merit were performed. Hence, for this analytical evaluation, accuracy, sensitivity, precision, linear dynamic range, limit of detection, and selectivity were evaluated and calculated in term of predictive ability and detection capabilities as illustrated in table 2 below.

Table 2.

*Figures of Merit of DA Detection by the DPV Method.*

| Method | [a]$R^2$ | [b]m ($\mu A/\mu M$) | [c]SD ($\mu A$) | [d]LR (nM) | [e]LOD(nM) | [f]LOQ (nM) |
|--------|----------|---------------------|-----------------|------------|------------|-------------|
| DPV | 0.9955 | $1.0 \times 10^{-04}$ | $1.102 \times 10^{-06}$ | 0.395-3.93 | 0.193 | 0.581 |

[a]Correlation coefficient, [b]Slope, [c] Standard deviation, [d]linear range, [e]limit of detection, [f]limit of quantitation
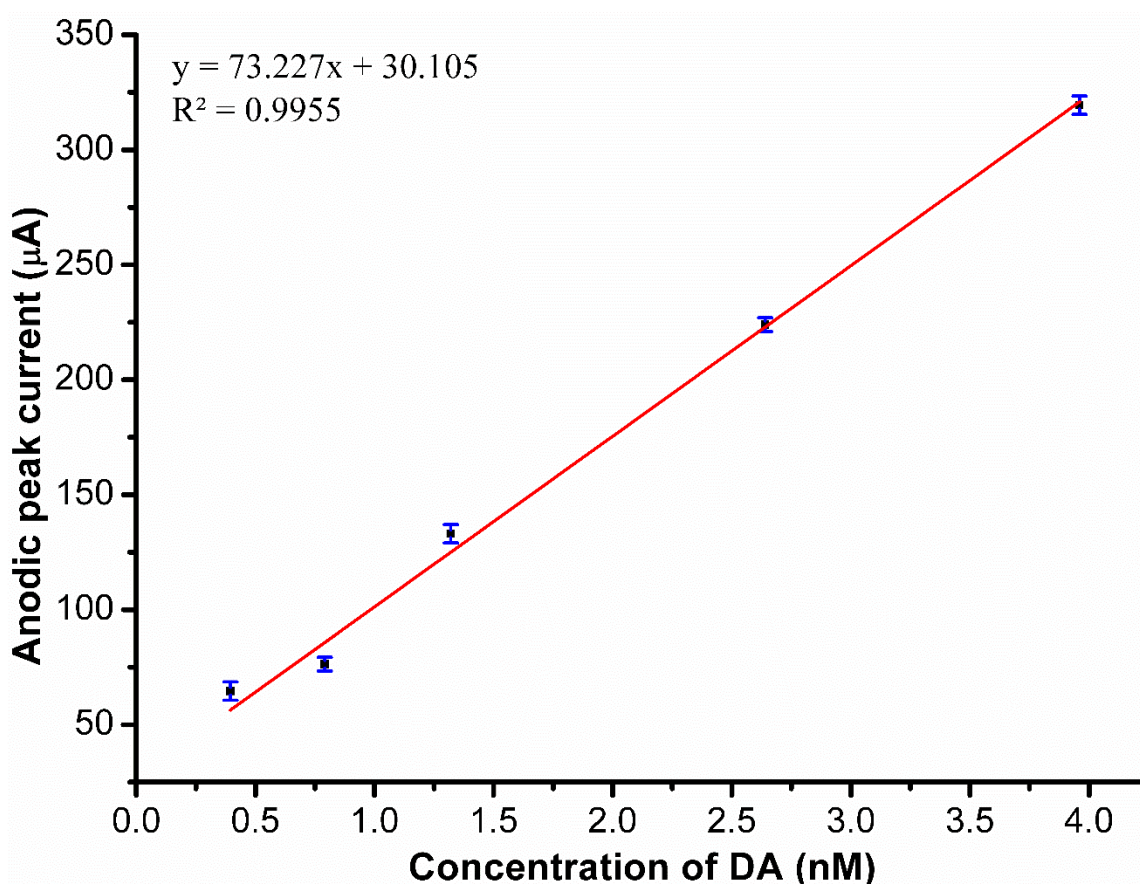
Because of its sensitivity and low background current availability, the DPV result are highly performed. The calculations below can be used to calculate the detection limit (LOD) and limit of quantitation (LOQ) using the linear regression equation:

$$LOD = 3.33 \times \frac{SD}{m} \qquad (4)$$

$$LOQ = 10 \times \frac{SD}{m} \qquad (5)$$

Figure 23

*Calibration of DA Conc. In a range of (0.395-3.96 nM).*



The standard deviation of the intercept of the five observations is SD, and the slope of the calibration curves is m. Table 2 shows the LOD values obtained from the analysis for DA

detection using both approaches, based on calibration curve in figure 23. Because DA solutions were synthesized in the mM unit, both LOD and LOQ are provided in mM. The results revealed a sensitivity difference between the two pulse electrochemical techniques. In the analytical behavior study, there was a linear association between the DPV response and the DA concentration employed, which ranged from 0.395 nM to 3.96 nM, with a limit of detection of less than 0.193 nM (Table 2), which is better than prior studies (Ratnam, et al., 2020). These discoveries recommend that the N-methacryloyl-(L)-phenylalanine monomer can improve DA molecule electrode selectivity. Time was also used to test the stability and sensitivity of the MIP PGE sensor to DA. Even after ten usage, the peak height (current) remained at 97%.

**Estimation of Selectivity and Imprinting Efficiency**

The MIP PGE sensor's selectivity was tested by detecting additional neurotransmitters with comparable chemical and structural properties to DA, such as NE and EP molecules. 1.32 nM of DA, NE, and EP were produced in 0.1 M PBS at a pH of 7.4 to exhibit the MIP PGE sensor. The competitive behaviour of both compounds was analysed using the same process as the kinetic research, as well as the same technique. The selectivity of neurotransmitters at a concentration of 1.32 nM were administered to both MIP PGE and NIP PGE sensors to observe the imprinting effect. The availability of DA concentrations in human plasma is extremely low. The matrix contains a large number of interference chemicals, indicating that there is a compelling need for a selectivity measurement. As a result, a simple, low-cost, effective, and selective DA recognition method should be developed. As a result, even at micro-molar concentration levels of DA, the MAPA monomer, which includes phenylalanine, this MIP PGE sensor can detect DA among other neurotransmitters molecules.

Figure 24

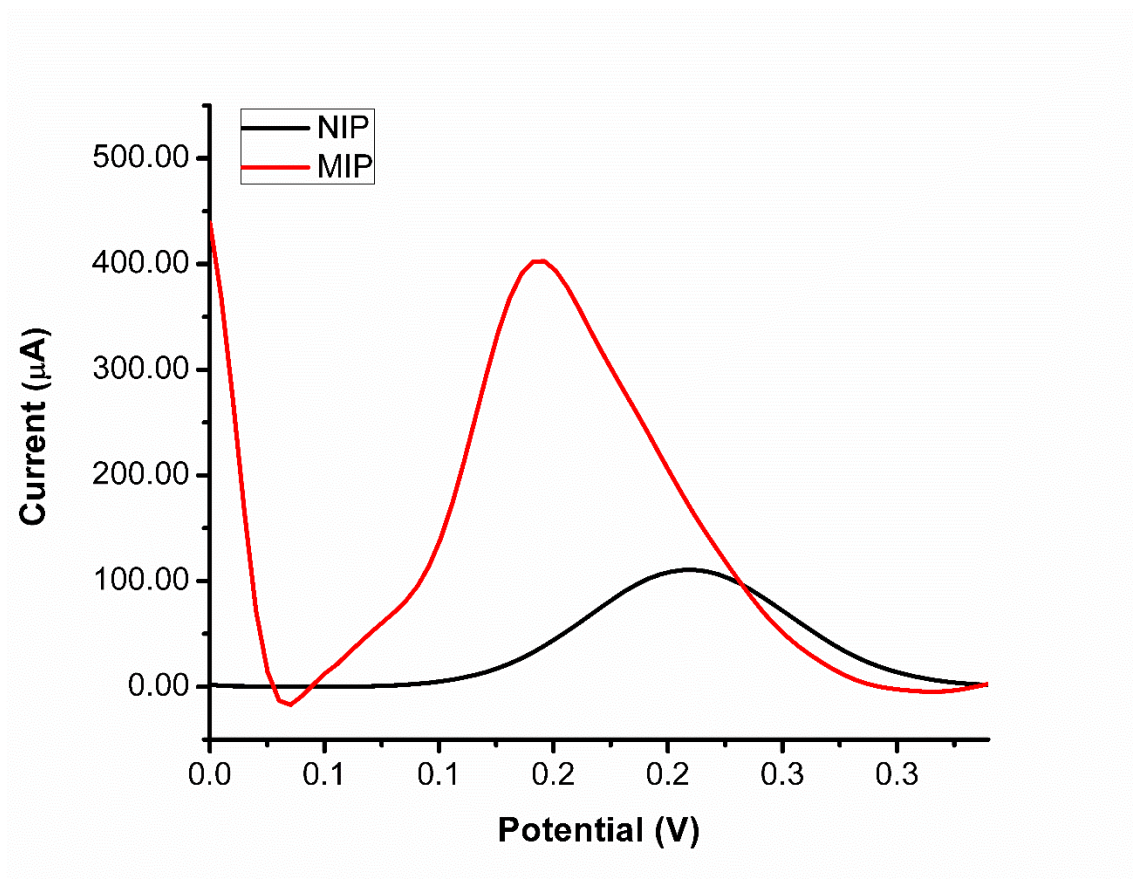*Modified Electrodes Response; MIP PGE and NIP PGE Sensor Response for DA Molecule*

Figure 25

*Modified Electrodes Response; Error Bar for MIP PGE and NIP PGE Sensor Response (n= 3).*
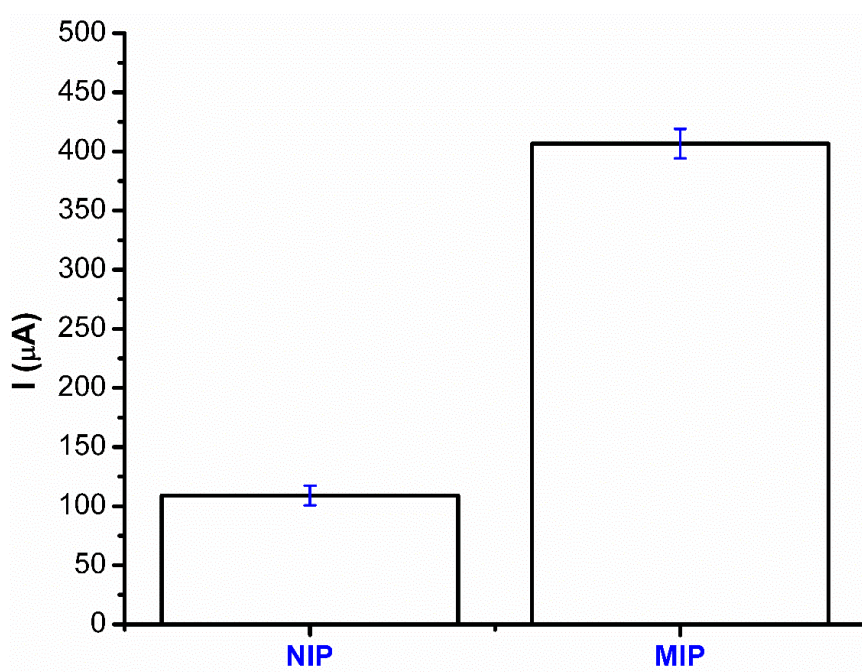
Figure 26

*Selectivity study; MIP PGE sensor response for DA (1.32 nM) NE (1.32 nM) and EP (1.32 nM) competitors*
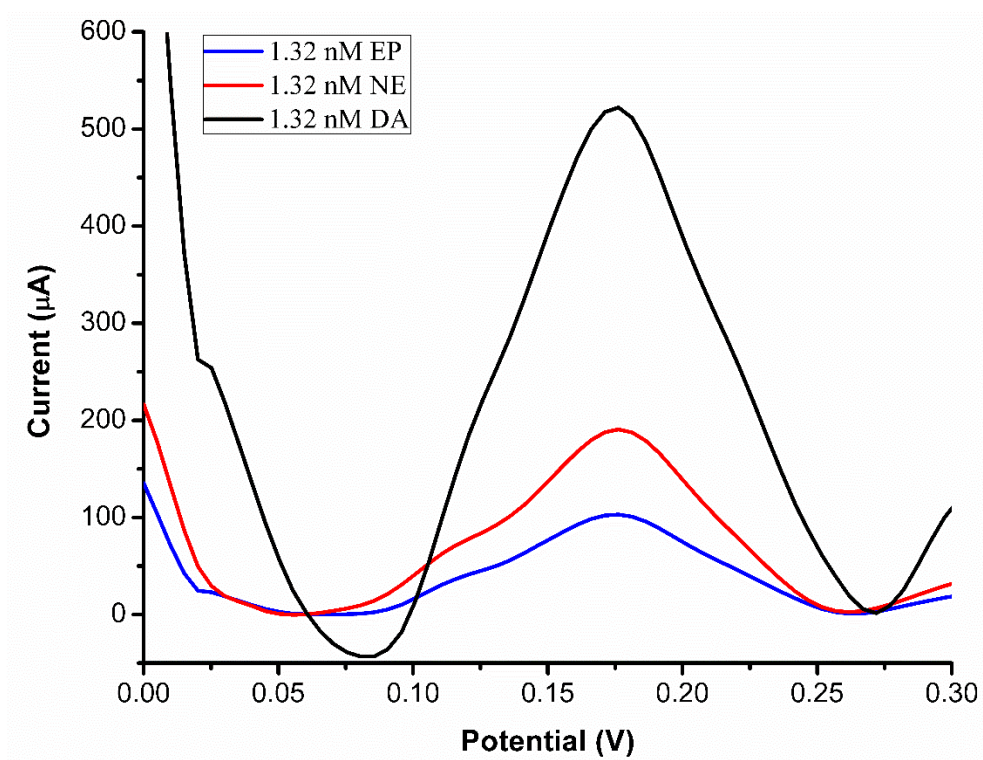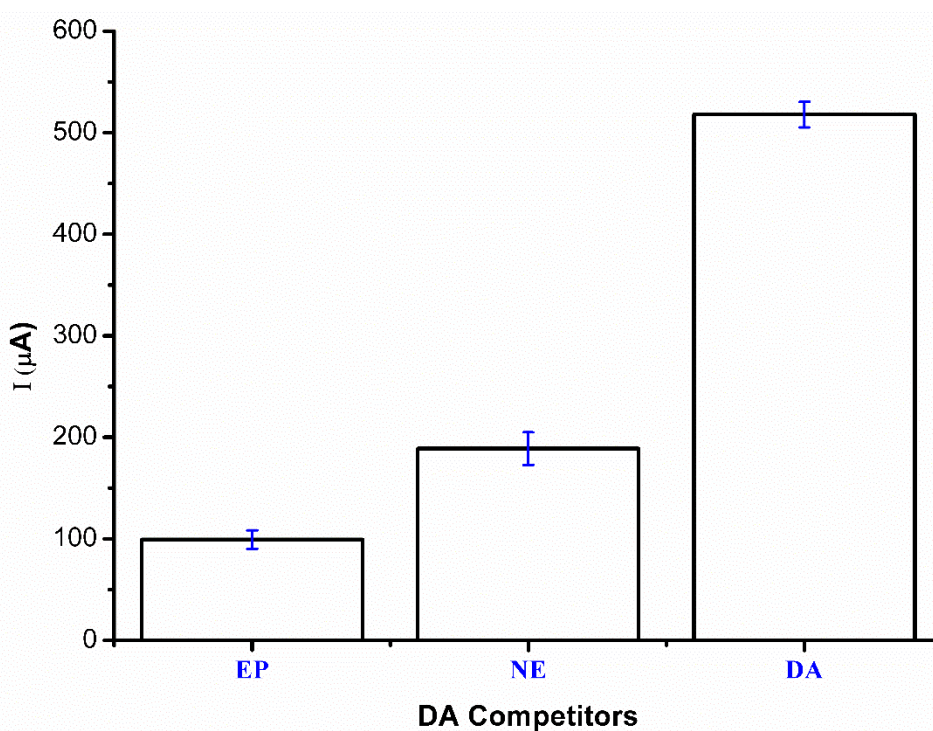


Figure 27

*Selectivity study; Error bar for MIP PGE sensor response (n= 3).*

The MIPs electrode is utilized as a recognition element to create imprinted cavities that are identical in form and size to the template molecule. These imprinted sites perform the selectivity function and improve the analyte rebinding process' efficacy. Figure 26 shows the MIP PGE sensor's selectivity evaluation of the DA detection. Some competitor compounds, such as NE and EP, were studied for competitive adsorption. The MIP PGE sensor was used to test 1.32 nM NE and 1.32 nM EP solutions for this purpose. As illustrated in Figure 27, the imprinting effectiveness was assessed by comparing the MIP PGE sensor to the NIP PGE sensor for detecting DA (1.32 nM DA). EP and NE were chosen as competitive compounds in the DA selectivity research of the MIP PGE sensor. The prepared sensor was 1.45 times more selective than EP and 1.90 times more selective than NE molecules, according to the results. The calculated imprinting factor, IF: 5.06, indicated that the imprinting process was completed effectively. In addition, the MIP PGE sensor detected DA more selectively than the NIP PGE sensor. As shown in Table 3, the selectivity parameters were tabulated.

Table 3.

*Selectivity parameters of MIP PGE and NIP PGE sensors for 1.32 nM DA.*

|  | MIP PGE | | NIP PGE | | |
|---|---|---|---|---|---|
|  | Current, $\mu$A | K | Current, $\mu$A | k | k' |
| **DA** | 521.25 | - | 103 | - | - |
| **EP** | 189.07 | 2.76 | 54 | 1.91 | 1.45 |
| **NE** | 101.24 | 5.15 | 38 | 2.71 | 1.90 |

**Repeatability of MIP PGE Sensor**

The use of a polymer to modify the electrode surface for component recognition is limited due to electrode fouling during the measurement and regeneration stages. Passivation of the three-dimensional cavities produced for the biological analyte of interest on the electrode surfaces during these processes is also feasible. By protecting the graphite surface from the harsh environment and operating conditions, the electrode structure employed in this study can make the pencil graphite electrode reusable. The MIP PGE sensor's repeatability was tested using a 5.27 nM solution, and the response of the MIP PGE sensor was plotted as I (current) versus (V) potential applied, as shown in Figure 28.

Figure 28

*Repeatability study of MIP PGE sensor, the response of the sensor electrode is to 5.27 nM DA.*
*The measurements were repeated 10 times (n= 10).*
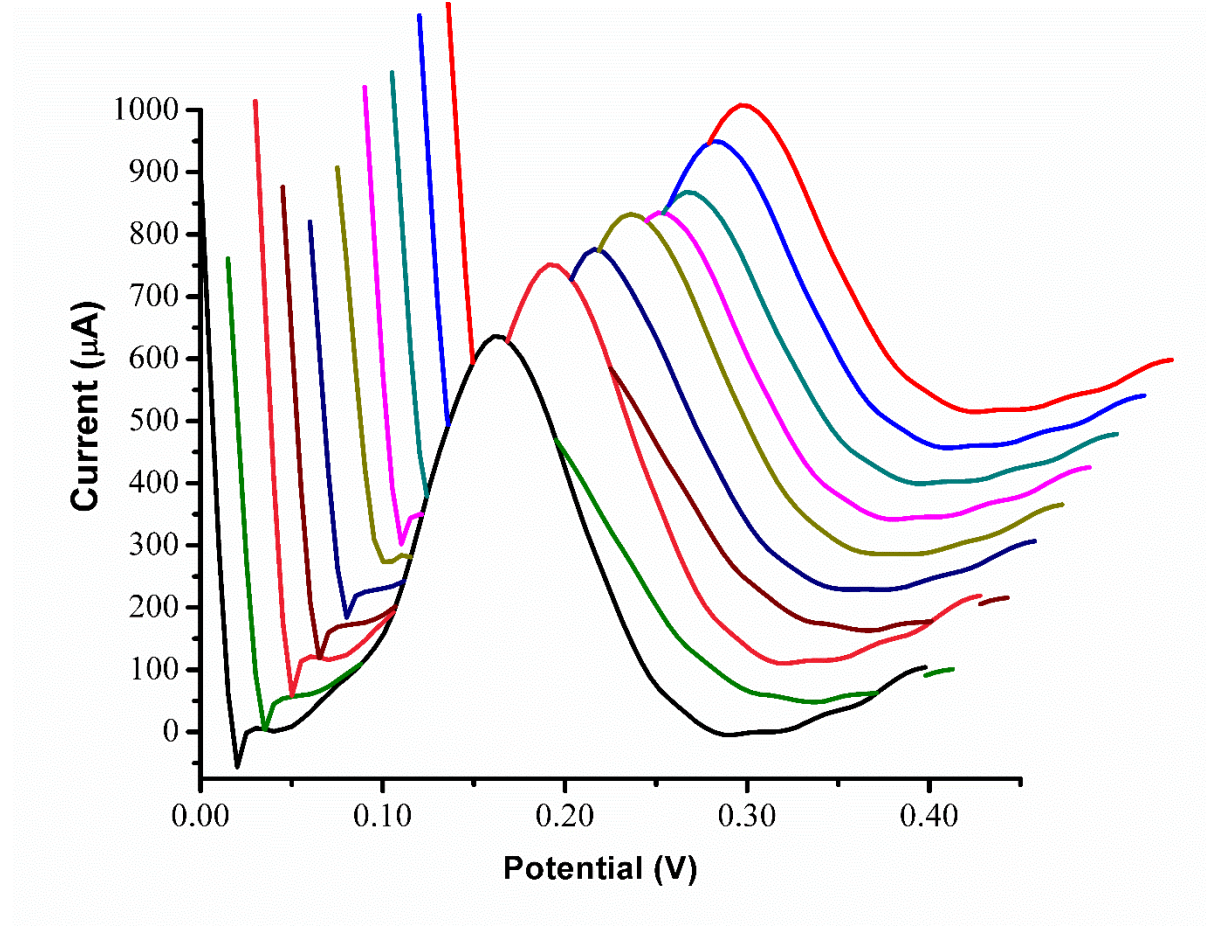


The MIP PGE sensor was immersed in newly generated DA solution (5.27 nM) and de-
tested by DPV technique 10 times (n=10) to demonstrate the stability of the MIP PGE sensor
used for DA detection. Between each measurement, desorption step was performed by
immersing the electrode in a desorption solution of methanol and acetic acid at a ratio of (1:9,
v/v). The voltammograms collected for the 10 times were analyzed using the following
equation ratio:

$$Peak._{Ratio} = Peak._{h\,(n-1)} / Peak._{h\,(n)} \tag{6}$$

In Equation 3, h is the measured signal's peak height after detection, and n denotes the
number of measurements taken. The results of the experiments revealed that the current (I)
signal collected had a relative standard deviation of 3.44 % for DA, indicating strong electrode
stability. The modified electrodes were used ten times to detect the same concentration of DA,

and the MIP PGE sensor's efficiency was determined to be 97 percent. This demonstrates that the MIP PGE sensor can be reused up to ten times without sacrificing efficiency or affinity. The great stability of the electrodes for the biological substance of interest is demonstrated by calculating the relative standard deviation RSD percent value, which is around 4.131 %.

**DA Determination in a Real Biological Sample**

In human plasma samples, the MIP PGE sensor was designed to distinguish DA molecules from other compounds with similar functional groups (catecholamine). Furthermore, MIP has a high selectivity and sensitivity for detecting DA molecules. The voltammetric approach was used to detect DA molecules in the plasma of human blood. The DA level in healthy human blood plasma was found to be 23.1 nM in a recent study (Phung, et al., 2018). The MIP PGE sensor was preserved with human blood plasma solutions spiked with different amounts of DA solutions (0.395, 0.791, 1.32, 2.64, and 3.96 nM) produced at PBS pH 7.4 to determine the DA concentration level. Due to the natural existence of NE and EP molecules in the serum plasma utilized, a minor shift (0.13 V) occurred in the non-spiked sample, as shown in Figure 29. The rise in DA concentrations utilized for spiking the plasma samples used caused the change in peak height (current response). The plasma sample without DA solution is indicated by the lowest peak height.
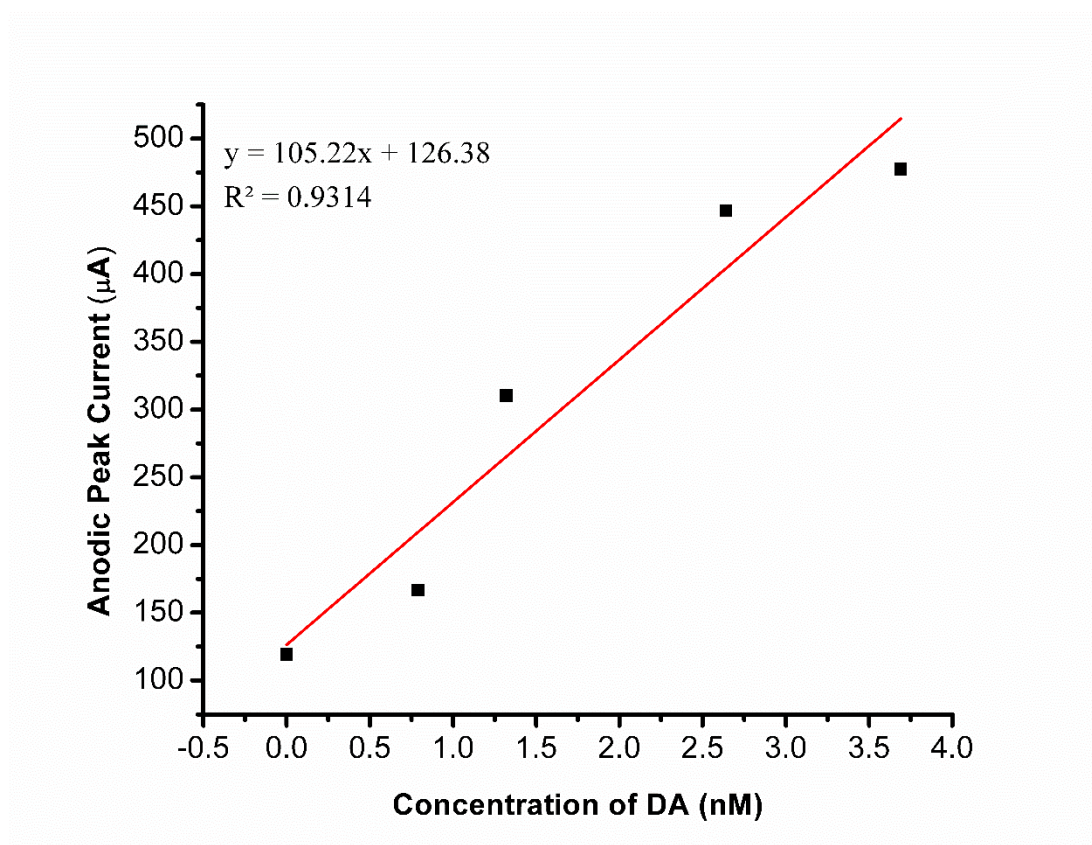
Figure 29

*Voltammograms of Spiked Plasma Samples Range from 0.0 nM to 3.96 nM DA.*
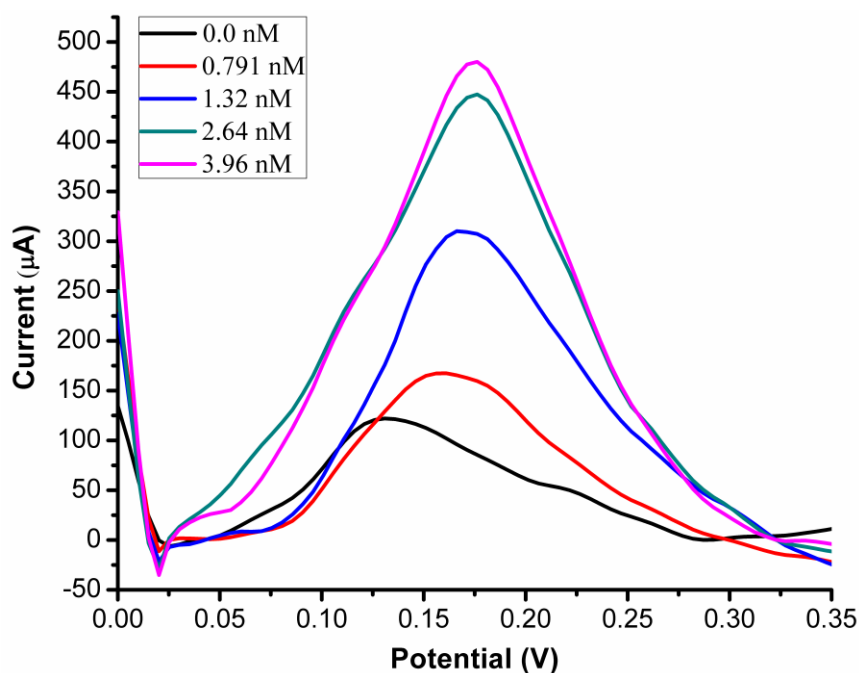
The MIP PGE sensor was preserved with human blood plasma solutions spiked with different amounts of DA solutions (0.395, 0.791, 1.32, 2.64, and 3.96 nM) produced at PBS pH 7.4 to determine the DA concentration level. Due to the natural existence of NE and EP molecules in the serum plasma utilized, a minor shift (0.13 V) occurred in the non-spiked sample, as shown in Figure 30.

Figure 30

*Calibration Curve of the Spiked Plasma Samples Range from 0.0 nM to 3.96 nM DA.*



The rise in DA concentrations utilized for spiking the plasma samples used caused the change in peak height (current response). The plasma sample without DA solution is indicated by the lowest peak height, and figure 29 illustrates the calibration study by adding to DA to the plasma samples. When compared to other imprinted electrode sensors mentioned in the literature (Peeters, et al., 2016; Jain, et al., 2020) , the MAPA monomer, which has a similar molecular structure to DA through its various binding sites presented by phenylalanine, offers greater sensitivity with a lower limit of detection for the MIP PGE sensor for DA detection.

**CHAPTER V**

**Discussion**

The development of the voltammetric detection output indicates the main purpose in this work in order to obtain high selectivity and sensitivity. Many recent researches done on improving the measurements, but this work still has the competition among them. Several methods were employed for comparison and precision verification. For the biomolecule dopamine outcome, nonetheless, a number of comparable imprinted polymer electrode sensors with paral-lel or superior detection limits have been discovered (Table 4). The film was made by incorporating an AA through the electropolymerization of pyrrole onto a PE in aqueous solution using a CV technique to display great selectivity and sensitivity to the analyte AA, and the electrode performance was monitored using DPV (Kumara Swamy, 2015).

Table 4.

*Different Modified Electrode Sensors for DA Detection.*

| Method | Material | LOD | Ref. |
|--------|----------|-----|------|
| DPV | $CO_2$ laser-induced graphene (LIG) electrodes modified with g old nanoparticles (AuNPs) and (MIP) | 3.16 nM | (Zheng, et al., 2021) |
| DPV | α-cyclodextrin (α-CD) incorporated carbon nanotube (CNT)-coated electrode | 1000 nM | (Ge-Yun,e t al., 2005) |
| DPV | (MIP) electropolymer of over-oxidized polypyrrole (OPPy) | 4.5 nM | (Tsai, et al., 2012) |
| CV | Molecularly Imprinted Polymer (MIP) screen-printed electrodes (SPEs) | 470 nM | (Peeters, et al., 2016) |
| CV | Poly(procaterol hydrochloride) (p-ProH) membrane modified electrode. | 300 nM | (Kong, et al., 2018) |
| CV | poly (sudan III) modified carbon paste electrode (PS/MCPE) | 9300 nM | (Sunil, et al., 2018) |
| DPV | MIP PGE sensor | 0.193 nM | This study |

In another study, a carbon nanotube (CNT)-coated electrode with -cyclodextrin (-CD) was modified and used to detect EP and DA simultaneously in concentration ranges of (2.010-6 - 1.010-3) mol/L and (1.010-6 - 1.010-3) mol/L, respectively, with a LOD of roughly 10-9 M (Sriwichai, et al., 2016). When compared to the detection of DA by MIP PGE sensor, the majority of the reported studies in the literature had an almost higher limit of detection. Despite the fact that another investigation on highly sensitive DA detection was conducted (Tsai, et al., 2012). The presence of EP and NE neurotransmitters was successfully identified and detected by the MIP PGE sensor in this work, which has a very good selectivity and sensitivity with a detection limit of 0.193 nM.

# CHAPTER VI

# Conclusion and Recommendations

## Conclusion

The molecularly imprinted polymers have the trend in recent electrode modification research due of their innate specific for the inherent template molecules, the development of a new sensor electrode that has a very high sensitivity and selectivity to the chemical of concern was achieved. Although, high-performance liquid chromatography (HPLC), UV-visible spectrophotometry and electrochemical techniques have all been used to determine neurotransmitters. Electrochemical methods proved out as a costless, easy-to-miniaturize, and simple technique with a quick response time. In addition, to the importance of the biochemical molecules in the human's body, especially, the neurotransmitters due to their prominence stems from their neurological significance as well as their roles as hormones in the circulatory system. Dopamine (DA), epinephrine (EP) and norepinephrine (NE), have all been related to memory, sleep, attention, learning, heart rate, emotion regulation and mood. These essential functions alter research to improve their process in the detection of them in a very high sensitivity.  Different process have been used for the modification of electrode sensor for the synthesis of MIPs, but the mostly common ones are related to the usage of functional monomer within a template of the chemical of concern. On the other hand, the selectivity of the many electrodes that have been created over the years has been a major cause of concern. The addition of the gold nanoparticles that helps to increase the electrodes sensitivity because of their high surface are which also helps in the electrode improving. The synthesis of the molecular imprinted polymer indicates a very wide method due to the variety of functional monomers that can be used. The types of functional monomers can be decided according to the type of the analyte of concern, due the cavities built up during the synthesis process because of the template with the analyte form. Hundreds of articles on the issue show that dopamine detection in real-world applications is a favourable and exciting field of research. Electrochemical biosensors are a promising tool for detecting dopamine and other neurotransmitters. In this study, we outline recent experiments on dopamine detection utilizing nanomaterial-modified electrodes. The majority of research in recent years has concentrated on developing updated sensing substances to develop the biosensor performance. As a result, a variety of nanomaterial's such as polymers and metals have been reported to improve the sensitivity and selectivity of modified electrodes. The AuNPs exhibited

their potential to increase the identification of discrete oxidation peaks or significant currents in electrochemical detection by facilitating electron transfer processes on the electrode. Modified electrodes have now been shown to be capable of detecting dopamine in standard circumstances, with a LOD in the low range and selectivity for dopamine even when interferences are available.

To conclude, we developed an electrochemical sensor electrode coated with MAPA contains MIP for the specific binding of DA molecules, which increased the sensitivity and selectivity for DA molecule detection even in the occurrence of NE and EP molecules, thanks to specific cavities on the electrode surfaces. It has been used to identify DA in human blood plasma samples with great success. The polymer layer, which is formed on the electrode surface, has a number of advantages over traditional electrode sensors for detecting DA. The promising electrostatic attraction between the positively charged dopamine and the negatively charged modified polymer provides for better analyte contact with the electrode surface and accelerates the electron transfer mechanism.

**Recommendations**

According to the results obtained from this research, the work can be improved more by improving the selectivity of the working electrode used, especially the MIP can be enhanced to detect the biomolecule of interest which dopamine in a very complicated matrix such as human's blood and urine, where various interferences and competitors available that affect the peak potential position to be shifted for example. With the purpose of increasing the selectivity of the MIP based PGE by improving the functional monomer used or combine more than monomer to synthesis more selective working electrode to detect in a very dense real sample for the analyte detection, hence, high selective results may be obtained. For the inorganic chemicals detection with a ML as a validation method, and despite these remarkable results, machine learning models that apply for pulse electrochemical methods DPV and SQWV based classification, able to identify large samples in chemical and biological systems, and trying to improve the access of algorithms model used to be available for further fields as a target for our future studies, are still needed to be improved in performance. Thus, wide range of matrixes can be detected regardless to the high complexity they may contain, which help diagnosis and may branches sciences in the near future for the fast, easy , and high precise of detection of the analyte.

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# APPENDICES

**APPENDIX 1**

**TURNITIN SIMILARITY REPORT**

**APPENDIX 2**

**ETHICAL APPROVAL DOCUMENT**

**BİLİMSEL ARAŞTIRMALAR ETİK KURULU**

04.08.2020

Dear Mehmet Özsöz,

Your application titled **"Gold-modified Molecularly Imprinted Methacryloyl-(L)-phenylalanine containing Electrodes for Electrochemical Detection of Dopamine"** with the application number YDÜ/FB/2020/495 has been evaluated by the Scientific Research Ethics Committee and granted approval. You can start your research on the condition that you will abide by the information provided in your application form.

Assoc. Prof. Dr. Direnç Kanol

Rapporteur of the Scientific Research Ethics Committee

**Note:** If you need to provide an official letter to an institution with the signature of the Head of NEU Scientific Research Ethics Committee, please apply to the secretariat of the ethics committee by showing this document.
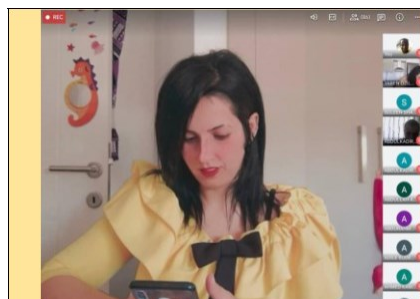
# Nimet/Nemah Shama

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**Nationality- Palestinian**

## Profile

I was graduated from The Department of Chemistry, Faculty of Science at Bethlehem University in Palestine. I finished my master's degree in Industrial Chemistry from Al-Quds University under the supervision of Assist. Prof. Dr. Amir Marei and the co-supervision of Prof. Dr. Fuad Al-rimawi. my thesis was entitled "Assessment of Commonly Used Pesticides in the Ground Water of the Shallow Aquifer Systems in Jericho and Jeftlik areas/ Lower Jordan Valley, Occupied Palestinian Territories".

I was working as teaching assistant in the pharmacy faculty at Near East University in Cyprus during my PhD studies. I have obtained my Ph.D. within 3 years and 3 months in Analytical chemistry at Near East University in Cyprus at the same Department where I am currently working as a Doctor at Near East University at the pharmacy faculty. My research is focused on electro-chemistry, green analytical chemistry, and Artificial intelligence in analytical chemistry with a cooperation with Assist. Prof. Dr Mehmet Ozsoz and the co-supervision of Prof. Dr. Sulieman Ajir.

## Research Interests

✓ Analytical chemistry and detections methods.
✓ Industrial chemistry
✓ Environmental chemistry and analysis
✓ Samples analysis and extractions.
✓ Artificial intelligence and logarithms

## Papers Published

- Assessment of Commonly Used Pesticides in the Ground Water of the Shallow Aquifer Systems in Jericho and Jeftlik areas/ Lower Jordan Valley, Occupied Palestinian Territories,2017.
- Electrochemical Determination of Potassium Ferricyanide using Artificial Intelligence, 2019.

- Potassium Ferrocyanide electrochemically detected by Differential Pulse and Square Wave Voltammetry in a Competition using Gradient Boosting as Machine Learning Algorithm, 2020.
- Gold-Modified Molecularly Imprinted N-methacryloyl-(l)-phenylalanine-Containing Electrodes for Electrochemical Detection of Dopamine, 2021.

# Conferences

- IEEE Institute of Electrical and Electronics Engineers. October 2019 in Ankara in Turkey, a presentation about the Electro-chemical Determination of Potassium Ferricyanide using Artificial Intelligence.
- IEEE Institute of Electrical and Electronics Engineers. October 2020 in Ankara in Turkey, a presentation about the Potassium Ferrocyanide electrochemically detected by Differential Pulse and Square Wave Voltammetry in a Competition using Gradient Boosting as Machine Learning Algorithm.

# Employment History

### Laboratory Assistant, Bethlehem/Palestine

During my four years of my B.A study, I was working in the chemistry department in Bethlehem University as a technician assistant. I worked well independently and on a team to solve problems.

### Teaching Assistant, Jerusalem/Palestine.

During my master's studies, I was working as a teaching assistant in the chemistry department. I Served as a friendly, hardworking, and punctual employee and I Organized and prioritized work to complete assignments in a timely, efficient manner.

### Teaching Assistant, Nicosia/Cyprus.

Nowadays, and during my PhD's studies, I was working as a teaching assistant in the Analytical chemistry department in the faculty of pharmacy at Near East University. I am teaching undergraduate students how to use different types of detection apparatus such as HPLC, Atomic absorption, and UV-Vis.

### Instructor, Nicosia/Cyprus.

Nowadays, I am working as an Instructor in the Analytical chemistry department in the faculty of pharmacy at Near East University. I am teaching undergraduate students how to use different types of detection apparatus such as HPLC, Atomic absorption, and UV-Vis and courses in addition to electrochemistry courses.

## Teaching Experience

<u>Undergraduate Courses Taught</u>

- General chemistry.      Spring 2015
- Analytical chemistry.  Fall 2015
- Organic chemistry.      Spring 2016
- Industrial chemistry.   Fall 2016
- Analytical chemistry    Fall 2018- Fall 2021

# Education

Diploma in English Skills and communications.

Bachelor, Bethlehem University, Palestine: B.A in CHEMSTRY

Master, Al-Quds University/Jerusalem, Palestine: Masters in Industrial and pharmaceutical chemistry

Ph.D., Near East University/Nicosia, Cyprus: Phd in Analytical chemistry

# Activities

Master of graduation ceremonies.

Reviewing papers for some journals.

Supervise undergraduate projects.

# Languages

Arabic: Native language

English: excellent

Hebrew: very good

Turkish: good

French: good

## Skills

Computer, internet, social media and emailing skills

Communication skills

Public Speech

Cosmatics and detergent manufacturing

## Hopes

Sports as swimming, spinning, and tennis

Dancing

Reading

Social media and Tik tok videos.