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TOPIC

Novel Multifunctional Molecular Recognition Elements Based on Molecularly Imprinted Poly (aniline-co-3,4-ethylenedioxy-thiophene) Composite Thin Film for Salicylamide Electrochemical Detection

Thesis Submitted and Publicly Defended in Fulfilment of the Requirements for the Award of a Master's Degree in Chemical Engineering

Presented by

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"In the Name of Allah, the Most Beneficent, the Most Merciful. All the praises and thanks be to Allah, the Lord of the 'Alamin (mankind, jinns and all that exists)

First, I am thankful to **ALLAH ALMIGHTY**, for the love, blessings, mercies and chance to finish this work. Salam and Shalawat are addressed to the highly prophet, Muhammad SAW, the last messenger of God who has guided us from the darkness to the brightness, and from stupidity to the cleverness.

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ABSTRACT

The combination of co-electropolymerization and molecularly imprinting technology provides functional materials with improved properties and can enhance the number of binding sites than the individual monomer. In this work, molecularly imprinted poly (aniline-co-3,4-ethylenedioxy-thiophene) MI-P(AnEDOT) composite thin film was synthesized on gold electrode by using an in-situ co-electropolymerization method using salicylamide (SMD) as a template. The formation of the thin film was monitored and characterized by electrochemical methods; SWV and CV. Solvent extraction of the template generated the binding cavities in the polymer matrix which fit the target in size, shape and functionality. After the rebinding of SMD, the novel sensor shows a linear range between 2×10^{-6} to $1 \times$ 68.5 ppm, limit of detection (LOD) of 7.88×10^{-6} ppm. The composite film showed high affinity towards SMD through multiple noncovalent interactions, good stability and reproducibility with RSD 2.1 %. The developed sensor has an interesting potential for real sample testing applications with good RSD of 6.01 % and a LOD of 9.29×10^{-6} ppm. The new sensing composite material has great sensitivity and selectivity due to the synergistic effects of multi-functionality from the two polymers, porous thin film and imprinting effect. To this end, the MI-P(AnEDOT) can be regarded as a potential functional material for the chemical sensor development in future.

Key words: Molecular recognition, Poly (aniline-co-EDOT) composite, Salicylamide detection, Molecular imprinted copolymer, Co-electropolymerization

RÉSUMÉ

La combinaison de la co-électropolymérisation et de la technologie d'impression moléculaire fournit des matériaux fonctionnels aux propriétés améliorées et peut augmenter le nombre de sites de liaison par rapport au monomère individuel. Dans ce travail, un film mince composite poly (aniline-co-3,4-éthylènedioxy-thiophène) MI-P(AnEDOT) à empreinte moléculaire a été synthétisé sur une électrode en or en utilisant une méthode de co-électropolymérisation in situ utilisant du salicylamide (SMD) comme un modèle. La formation du film mince a été suivie et caractérisée par des méthodes électrochimiques; SWV et CV. L'extraction par solvant du modèle a généré les cavités de liaison dans la matrice polymère qui correspondent à la cible en termes de taille, de forme et de fonctionnalité. Après la reliaison du SMD, le nouveau capteur montre une plage linéaire entre 2×10^{-6} à $1 \times 68,5$ ppm, limite de détection (LOD) de $7,88 \times 10^{-6}$ ppm. Le film composite a montré une affinité élevée envers SMD grâce à de multiples interactions non covalentes, une bonne stabilité et reproductibilité avec RSD 2,1%. Le capteur développé a un potentiel intéressant pour les applications de test d'échantillons réels avec un bon RSD de 6,01 % et un LOD de $9,29 \times 10^{-6}$ ppm. Le nouveau matériau composite de détection a une grande sensibilité et sélectivité en raison des effets synergiques de la multifonctionnalité des deux polymères, du film mince poreux et de l'effet d'impression. À cette fin, le MI-P(AnEDOT) peut être considéré comme un matériau fonctionnel potentiel pour le développement futur de capteurs chimiques.

ملخص

يوفر الجمع بين تقنية البلمرة الكهربية المشتركة والطبع الجزيئي مواد وظيفية بخصائص محسنة ويمكن أن يعزز عدد مواقع الربط أكثر من المونومر الفردي. في هذا العمل، تم تصنيع غشاء رقيق مركب مطبوع جزيئيًا بولي(أنيلين-إيتيلين ديوكسي ثيوفين) على قطب من الذهب باستخدام طريقة البلمرة الكهربائية المشتركة في الموقع باستخدام ساليسيلاميد مثل قالب. تمت مراقبة تكوين الغشاء الرقيق وتمييزه بالطرق الكهروكيميائية والسيرة الذاتية. أدى استخراج المذيبات للقالب إلى توليد تجاويف الربط في مصفوفة البوليمر والتي تناسب الهدف من حيث الحجم والشكل والوظيفة. بعد إعادة ربط ساليسيلاميد، يُظهر المستشعر الجديد نطاقًا خطيًا بين 2 × 10^{-6} إلى 68.5 جزء في المليون، ويبلغ حد الكشف 7.88 × 10^{-6} جزء في المليون. أظهر الفيلم المركب تقاربًا كبيرًا تجاه ساليسيلاميد من خلال تفاعلات غير تساهمية متعددة واستقرار جيد وقابلية للتكاثر مع إنحراف معياري نسبي 2.1٪. يتمتع المستشعر المطور بإمكانيات مثيرة للاهتمام لتطبيقات اختبار العينة الحقيقية مع إنحراف معياري نسبي جيد بنسبة 6.01٪ وحد كشف 9.29 × 10^{-6} جزء في المليون. تتميز المادة المركبة المستشعرة الجديدة بحساسية وانتقائية كبيرة بسبب التأثيرات التآزرية للوظائف المتعددة من البوليمرين، والغشاء الرقيق المسامي وتأثير الطباعة. تحقيقا لهذه الغاية، يمكن اعتبار بولي(أنيلين-إيتيلين ديوكسي ثيوفين) كمواد وظيفية محتملة لتطوير المستشعر الكيميائي في المستقبل.

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ABBREVIATIONS AND ACRONYMS

MIPs : Molecularly Imprinted Polymers.

MIT : Molecular Imprinnting Technology.

MIECS : Molecularly imprinted electrochemical Sensor.

MICP : Molecularly Imprinted Conducting Polymers.

NIPs : Non-Imprinted Polymers.

NICP : Non-Imprinted Conducting Polymers.

FMs : Functional Monomers.

EIS : Electrochemical Impedance Spectroscopy.

SPR : Surface Plasmon Resonance.

ILs : Incorporation of Ionic Liquids.

HPLC : High Performance Liquid Chromatography.

TLC : Thin Layer Chromatography.

IR : Infrared.

UV : Ultraviolet.

An : Aniline.

Pan : Polyaniline.

EDOT : 3,4-ethylenedioxy-thiophene.

PEDOT : Poly (3,4-ethylenedioxy-thiophene).

ECs : Emerging Contaminants.

PCs : Pharmaceutical Compounds.

WWTP : Waste Water Treatment Plant.

SMD : Salicylamide.

IBP: Ibuprofen.

TMP : Trimethoprim.

DPA : Diphenylamine.

AZM : Azithromycin.

PHAs : Polycyclic Aromatic Hydrocarbons.

4VP : 4-vinyl pyridine.

BaP : Benzo[a]pyren.

MP : Methyl-parathion.

Gly : Glyphosate.

SMX : Sulfamethoxazole.

AZY : Azithromycin.

ERY : Erythromycin.

DFT : Density Functional Theory.

SPAuE : Screen Printed Gold Electrode.

m-PD : m-phenylenediamine.

PY : Pyrole.

PPY: Polypyrole.

GCE : Glassy Carbon Electrode.

CPE : Carbon Paste Electrode.

GNPs : Graphene.

AA : Ascorbic Acid.

SN : Sulfanilamide.

NA : Naproxen.

ACN : Acetonitrile.

CPs : Conductive Polymers.

ECPs : Electrochemichal Conductive Polymers.

NCPs : Non-Conductive Polymers.

LOD : Limit Of Detection.

I : Current.

ΔI : Differential Current.

E : Potential.

E^0 : Standard Potential.

ΔE : Pulse Amplitude.

R : Universal Gas Constant.

T : Temperature.

n : number of electrons.

F : Faraday Constant.

RED : Activity of the Reduced Species.

OXI : Activity of the Oxidized Species.

t : time.

CV : Cyclic Voltammetry.

SWV : Square Wave Voltammetry.

DPV : Differential Pulse Voltammetry.

BDDE : Boron Doped Diamond Electrode.

PATP : N-(4-Pyridylcarbonylamino)-1,2,3,6-tetrahydropyridine.

IF : Imprinting Factor.

S_b : Standard Deviation.

I_{MIP} : Current of molecularly imprinted polymer.

I_{NIP} : Current of non-imprinted polymer.

m : Slope of the Curve.

RSD : Relative Standard Deviation.

ppm : Parts Per Million.

pH : Power of Hydrogen.

M : Molarity.

V : Volt.

mV : Millivolt.

µA : Microampere.

Introduction

The widespread occurrence of pharmaceutical contaminants in environmental fresh water samples is a direct consequence of improper disposal of unused pharmaceuticals and excretion of these compounds by humans and livestock. Pharmaceuticals in the fresh water environment, although present in trace amounts, are recognized as emerging contaminants since their persistent exposure can adversely affect human health and the environment. For example, pregnant women and children are at particular risk due to the possible effects that pharmaceutical contaminants can pose to a young child's physical development.2 Plant, animal, and microbial life are also adversely affected as a result of exposure to water contaminated with these drugs.

Current analytical tools used to study the occurrence of pharmaceutical contaminants in environmental water samples involve liquid or gas chromatographic separation followed by quantification using a mass spectrometer. Depending on the starting material, additional steps such as sample extraction, purification, concentration, and derivitization may be necessary prior to analysis. This process lacks efficiency due to the inability of being carried out in the field. The sample needs to be transported to a well-equipped laboratory and analyzed by highly skilled personnel. The entire process can be time consuming and expensive. Constraints associated with the rapid evaluation of large numbers of water samples limits the ability to routinely check for pharmaceutical contaminants in the environment. A field deployable monitoring device for the real-time analysis of water quality can have a significant impact on the proficiency with which public water is examined. Sensors based on electrochemical detection methods are particularly promising due to their relative simplicity compared to chromatography/mass spectrometry methods and can prove to be a direct, quick, reliable as well as cost-effective analytical tool.

In this regard, the development of original analytical technologies is of prime importance in modern scientific research, specially, in chemical and biological sensors, where a considerable effort has to be paid to improve the performances of the sensors at the levels of both sensing layers and transduction technologies.

In this context, development of sensing layers based on molecularly imprinted polymers (MIP) knows a considerable interest in the detection of molecules of different types. These sensing layers mimic the natural bio-receptors, with interesting properties. In addition to their stability against a wide range of environments, the advantage of molecularly imprinted polymers-based sensors is that the binding affinity of MIP is comparable to

biological recognition elements. Besides, these materials are easily synthesized in a tailor–made manner for a given template (analyte) and they can be developed against various targets such as pharmaceuticals, proteins and vitamins. The needed features in the MIP based sensing layers, particularly, in terms of minimization of the sensor, easiness of use and low cost of realization, are compatible with the use of electrochemical, piezoelectrical and optical transduction methods.

In this work, an effort has been made to improve the sensing performance of a sensor based on molecularly imprinted conducting polymers (MICP), dedicated for the detection of small organic molecules, through the combination of different approaches. The preparation of MICP sensing layers as well as the detection of salicylamide pharmaceutical molecules, by differently functionalized polymers and co-polymers, as MICP based sensing films, was studied as a physico-chemical model for the involved recognition phenomena. Then, electrochemical transductions of salicylamide sensing process was developed in order to improve the performance of our sensor.

This effort has been discussed throughout this thesis. First chapter overviews the state of art of molecularly imprinted sensors and sensing layers dedicated to the detection of small organic molecules and proposes an introduction to the role of MICP in the development of chemical sensors.

Second chapter demonstrates the different experimental procedures used for the preparation of the sensing layers, with a brief introduction to the theoretical method used in this work. In addition, the electrochemical characterization techniques used in this study are described. Also, the different electronic assemblies developed and used for the electrochemical transductions are presented.

The content of the third chapter is presented in two parts: the first part is reserved for the quantitative and qualitative interpretation of the results obtained. the second part focuses on the development and characterization of the molecularly imprinted conducting polymers and co-polymers films.

Finally, the properties of the developed MICP as sensing layers in electrochemical and gravimetrical sensors are summarized and the perspectives based on this work are outlined and oriented towards overriding the actual limitations of MICP based sensors.

Chapter 1

Bibliographic Synthesis

1. Introduction

The focus of environmental research in the past two decades has extended beyond the classic environmental pollutants, such as polychlorinated biphenyls, dioxins, organochlorine and organophosphorus pesticides, and has shifted to pharmaceuticals and personal-care products that enter the environment via domestic use, some of these "contaminants of emerging concern" had not previously been detected (or were previously found in far lesser concentrations), they are important because the risk they pose to human health and the environment is not fully understood. Therefore, development of sensing technologies is needed for precise monitoring and control of these chemicals. In this chapter, a study on the state of art of MIP Sensors with a special attention to the electrochemical sensors will be paid. A brief demonstration of different types of sensors classified by their transduction methods will be followed by the role of imprinting technology in the chemical sensors field. Then a description of the role of electrochemistry in the sensing applications will be presented. The development of a novel MIP sensor for the detection of "Salicylamide" as an example of the application of molecularly imprinted polymers in the detection of pharmaceutical residues in the aquatic environment will be studied in order to introduce our contribution in the sensing of this kind of molecules by combination of co-electropolymerization of aniline (An) and 3,4-ethylenedioxy-thiophene (EDOT) and molecularly imprinting technology, which provides functional materials with improved sensitivity and selectivity and enhanced number of binding sites due to the synergistic effects of multi-functionality from the two polymers, porous thin film and imprinting effect. Molecularly imprinted poly (AnEDOT) composite thin film was synthesized on gold electrode using an in-situ co- electropolymerization method using salicylamide as a template.

1.1. Emerging contaminants

Emerging contaminants (ECs) or "compounds of emerging concern" are defined as chemicals that are not currently (or have been only recently) regulated and about which there exist concerns regarding their impact on human or ecological health. Examples of emerging contaminants include disinfection by-products, pharmaceutical and personal care products, persistent organic chemicals, and mercury, as well as their degradation products. These chemicals make it into our nation's lakes and rivers, and have a detrimental affect on fish and other aquatic species. They have also been shown to bioaccumulate up the food web - putting even non-aquatic species at risk when they eat contaminated fish [1]. Thus, there is

a great need for low-cost and highly efficient tools for quick, reliable, and accurate detection of these contaminating bioactive agents.

The investigation of new sensing principles and technologies for the molecular binding events detection has created great expectations on numerous major industrial sectors, such as healthcare, food, water and agriculture. Combining many of these advances with the potential of the immunochemical systems has allowed developing novel sensors that provide interesting advantages against the traditional strategies for analysis, such as the possibility of multianalysis, development of field analytical methods and fabrication of easy end-user devices [2]. Specifically, many efforts have been lately invested to control residues of pharmaceuticals in environmental samples [3].

1.1.1. Pharmaceuticals as emerging contaminants in the aquatic environment

Pharmaceutical compounds (PCs) are environmentally ubiquitous around the world, in the past three decades, trace concentrations of PCs have been discovered in almost all environmental matrices on every continent. This includes surface water (lakes, rivers, streams, estuaries, and seawater) [4], groundwater [5], wastewater treatment plant (WWTP) effluents and influents [32], however there is still little knowledge of the magnitude, occurrence and their consequences in the aquatic environment.

Pharmaceuticals are still unregulated [7]. Their residues in the environment are considered to be "compounds of emerging concern" because they have the potential to cause considerable impact on human health and ecosystems [8]. The hazardous potential of pharmaceutical compounds on ecosystems was recently established [9]. Advanced analytical techniques have permitted the determination that some environmental effects of pharmaceuticals can be established in the μg/L and ng/L concentration ranges [10]. These techniques enabled the determination and quantification of almost 3000 biologically active compounds in the environment [11]. These pharmaceutically active compounds are pseudo-persistent because of their continuous influx into environmental matrices despite their continuous degradation and removal by various processes. This causes the development of "a complex pharmaceutical pool" in many natural matrices [12]. Once pharmaceutical residues enter water, they become incorporated into plants grown in these waters, therefore, an urgent requirement exists to develop sensitive and selective devices for the detection of ECs in aqueous environment.

1.1.2. Salicylamide as emerging pharmaceutical contaminant

Salicylamide (SMD) (o-hydroxybenzamide or amide of salicyl) is a non-prescription anti-inflammatory drug usually classified inside the salicylates group. This group of drugs has similar pharmacologic effects due to the fact that the principal metabolite of these drugs is the salicylic acid. This group of drugs possesses anti-inflammatory, antipyretic and analgesic effects due to the inhibition of the prostaglandin synthesis by the ciclooxygenase enzymes [13]. SMD is also used in combination with both aspirin and caffeine in the over-the-counter pain remedies, it appears as odorless white or slightly pink crystals. Bitter taste, leaves a sensation of warmth on the tongue. Salicylamide is soluble in hot water, ether alcohol and chloroform [14].

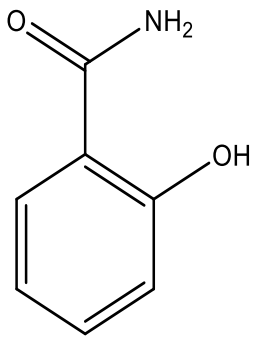

Figure 1. Salicylamide structure.

The existence of salicylates derivatives in wastewater effluents and potable water origin is quiet frequent, and it's now a global challenge [15], these compounds have been identified as a contaminants of emerging concern. This proves the necessity of water monitoring techniques for SMD determination. In previous works different techniques were used for the determination of salicylates compounds, most of them are based on chromatographic methods; like gas liquid chromatography [16] used for the determination of acetylsalicylic acid, salicylic acid and salicylamide in plasma. HPLC for the determination of SMD, acetylsalicylic acid and the impurities of salicylic acid in pharmaceutical preparations [16]. Spectrophotomy and Fluorescence methods has been also used for the determination of these compounds, so Kenneth et al. published the determination of acetylsalicylic acid, salicylamide and salicylic acid as impurities on pharmaceutical preparations using prior separation techniques [17]. Also Murillo et al. developed a FIA-fluorescence method for the determination of salicylamide and salicylic acid in biological fluids [18]. But no environmental monitoring systems for salicylamide determination were found in the literature, which encouraged us to develop of a novel robust anomaly detection

model of this emerging contaminant in water using molecularly imprinting smart technology.

1.2. Sensing devices for environmental monitoring

The field of environmental monitoring and control requires an exact and fast acquisition of different variables covering various areas and multiple applications. Sensors which are becoming more and more sophisticated and varied represent excellent candidates to answer this demand.

A Sensor is a dispositive or a device that provides a signal related to a measurand and converts the sensed chemical, biological or physical manifestation into measurable signals, generally converted into an electric signal. The electric signals are intended to deliver the necessary information of the measured variable recognition [19]. With a large panel of physical, chemical and biological phenomena, a classification of sensors can be proposed as follows:

Table 1. Classification of the different types of sensors with examples of applications.

Sensor category	Applications	Mesurand
Physical sensors	Thermal sensors Radiation sensors Mechanical sensors Magnetic sensors Electrical sensors	Temperature, heat, thermal flux γ ray, X ray, UV visible, IR displacement, velocity, acoustic waves magnetic field, permeability potential, current, charge, inductance.
Biological sensors	Recognition	Cells, proteins, antigens, hormones
Chemical sensors	Recognition ph Humidity	Pollutants, gas, vapors, VOC

1.2.1. Development of chemical and biological sensors

Over the last decades, many types of sensors were developed. starting with the pH glass electrode, which appeared early in the 1930s till the onward development of lab-on-chip and sensor arrays, becoming an indispensable part of our technology driven society, they can be found in chemical process, pharmaceutical, food, biomedical, environmental, security, industrial safety, clinical and indoor monitoring [20, 21]. In addition to environmental applications such as the fuel mixtures analysis, identification of toxic wastes, the detection

of oil leaks [22], analysis of natural waters [23] and monitoring of heavy metals [24]. Like many fields in science, chemical sensors have benefited from the growing power of computers, integrated electronics, new materials, novel designs and processing tools. Manifestation of such technological changes can be seen in the development of miniaturized, inexpensive, portable and mass manufacturable devices that are able to deliver qualitative as well as quantitative informations regarding the chemical, biological or physical state of a material [25]. Moreover, research on nanostructured materials [26] and the use of sensor arrays in electronic nose (e-nose) systems [27] is addressing the need for better analyte selectivity. Breakthroughs over the last decade have pushed sensors into new markets, as well as new applications [28].

Chemical sensors and Biosensors involve a recognition layer and a transducer in an integrated system. A range of selective and specific recognition layers have been interfaced onto the surface of transduction system. The target species interaction, being measured by the recognition layers induces a change in the latter, which is transduced (converted) into a measurable electronic signal. In biosensors, the recognition material is usually a natural biologically derived system such as microorganisms, enzymes, anti-bodies, oligonucleotides, or other natural molecular binding systems [29]. In chemical sensors, the recognition layer is a chemical (synthetic) recognition layer, such as a functionalized polymer film, a supramolecular host system, or a self assembled arrangement that is designed for selective interaction with the substance to be sensed.

A chemical sensor can be defined [30] as a device, which responds to a particular analyte in a selective way by means of a reversible chemical interaction and that can be used for the quantitative and qualitative determination of the analyte. Sensors also involve the transduction of physicochemical properties at an interface into usable information [30]. Thus, two main features define the detection process (Figure 2):

i) Chemio-, bio- or physico-sensitive layer which provides selectivity and sensitivity of the recognition process;

ii) Transducer, which converts the chemical, biological or physical information into an electrical signal.

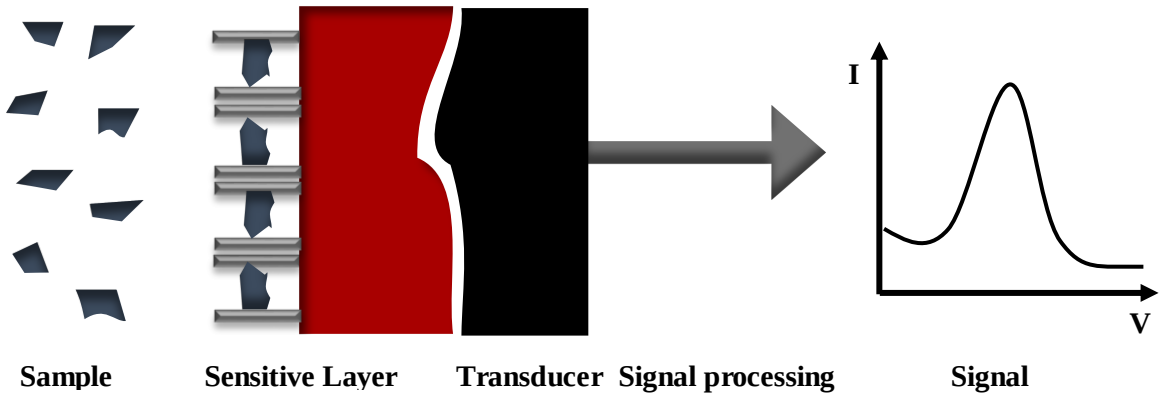

Figure 2. Chemical (biological) multistep sensing process: sensing of the target molecules by the recognition element (the sensitive layer), transduction of the recognition into a signal and processing of the signal, then exploiting the resulting signal [85].

The large molecules systems detection such as biologically active entities like oligonucleotides involve a large set of probe-target interactions (hydrogen bonds), which enabled the development of various sensors showing high selectivity and sensitivity. On the other hand, the detection of small organic molecules, such as: gas molecules, volatile compounds, organic solvents, pharmaceuticals, toxic substances, pesticides, herbicides, explosives or organophosphorus compounds, remains one of the most challenging questions in the sensor field, regarding the relative small molecular mass and the relative limited capacity of these molecules to interact specifically with the recognition sites on the sensing layer. The synthetic materials design as recognition elements in the design of sensors, has become an important and active area of research making molecular imprinting one of the strategies followed to create materials with recognition ability comparable to the natural systems in recent years [31].

1.2.2. Molecular Imprinting

Molecular Imprinting Technology (MIT) is today a viable synthetic approach to design robust molecular recognition materials [32], based on the formation of a complex between an analyte (template) and a functional monomer. In the presence of a large excess of a cross-linking agent, a three-dimensional polymer network is formed [33]. After polymerization process, removal of the print molecule by extraction leaves sites specific for print molecules and free for binding [34, 35] complementary in shape, size and chemical functionality to the template molecule [32, 36]. The resulting MIP is therefore capable of selective recognition of the target analyte in the template-derived sites.The recognition properties of the imprinted polymers are not affected by acid, base, heat or organic phase treatment [37], some application areas of this technology include: separation sciences and purification [38,39–44],

chemical sensors [45], catalysis [46], drug delivery [47], biological antibodies and receptors system [55, 48, 49].

1.2.2.1. MIPs in sensors

With the ability of MIPs to resist pH, organic environment, and ionic strength, their usage in sensor technology is very beneficial [50]. Since biocompounds are expensive, hard to immobilize onto transducers, and challenging to study on their optimum conditions, the idea to use MIPs on transducer surfaces and avoid these disabilities came up to the scientists [51], the resulting receptor materials have frequently been referred to as "artificial antibodies" [52, 53, 54]. Their advantages over natural receptor molecules are listed in Table 2, in which they mainly include superior stability, low cost, and ease of preparation. Therefore, versatility and ability to recognize not only biological but chemical molecules as well is also one of their main advantages, including amino acids and proteins [55-57], nucleotide derivatives [57], pollutants [58, 59], drugs and food [60, 61].

Table 2. Comparison of Natural Biomolecules Used in Sensors and MIPs.

Natural biomolecules	**MIPs**
Low stability	Stable at low/high pHs, pressure, and temperature (<140°C)
High price of enzymes and receptors	Inexpensive and easy to prepare
Poor performance in nanoaqueous media	Can work in organic solvents
Different natural biomolecules have different operational requirements (pH, ionic strength, temperature, substrate)	Polymers for different targets could operate in the same specific environment
For some important analytes natural receptors and enzymes do not exist and antibodies cannot be prepared	Polymers could be prepared for practically any compound
Poor compatibility with micromachining technology and miniaturization	Polymers are fully compatible with micromachining technology

The first attempts to use bulk-imprinted polymers in sensors were made in 1992 [62]. It was shown that templates such as amino acids, nucleic acids, and cholesterol increase the current passing through membranes made of imprinted polymers [63]. Since then, more than 1200 papers focusing on MIP applications on sensors have been published worldwide, which clearly indicates growing interest in this area. The standard polymerization reaction for MIP

synthesis takes place within a complex mixture which contains a proper functional monomer along with a cross-linker, an initiator, and the target molecule, all blended in an appropriately chosen solvent or mixtures of different solvents. Firstly, a pre-polymerization complex is formed, where the template is bounded to the monomer by different types of interactions, through covalent, semi-covalent, non-covalent or metal coordinated associations [29].

Depending on the type of bonding, the energy required for removing the template is different, being the highest in case of covalent bonds and the lowest in case of non-covalent ones. Therefore, non-covalent bonding is more versatile, which allowed it to become the preferred strategy for MIPs preparations [25]. Upon removal of the template molecule from the polymeric matrix, complementary cavities result, with specific shape, structure and functional groups, which will act as specific binding sites for the molecules previously removed [64]. The schematic representation of MIP synthesis is presented in Figure 3.

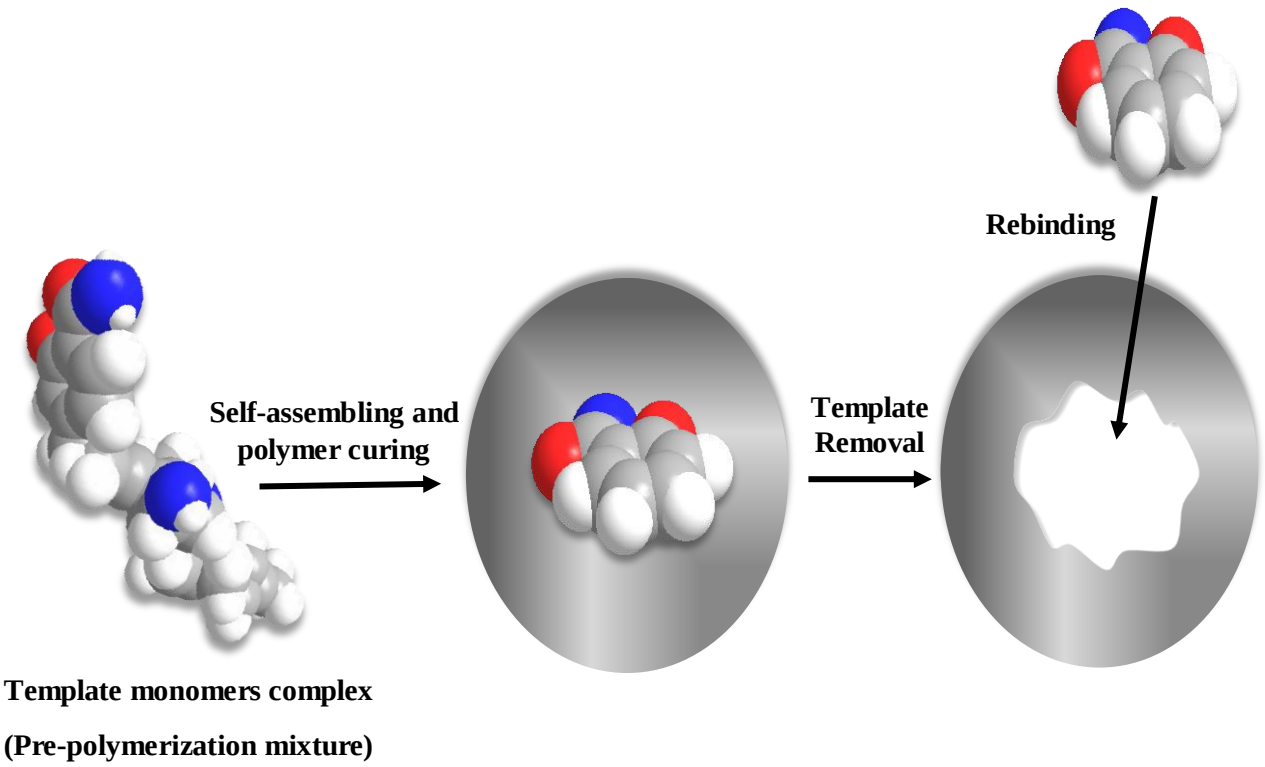

Figure 3. A schematic representation of the molecular imprinting method [100].

1.2.2.2.Synthesis methods for molecularly imprinted polymers

1.2.2.2.1. Molecularly imprinted polymers constituents

Depending on the nature of the template molecule, the elements for the polymerization mixture are selected: the monomer, the cross-linker, the initiator and the appropriate solvent which facilitates the binding of the components. Each ingredient added within the

polymerization blend has its particular influence over the properties and performances of the final MIP [47].

1.2.2.2.1.1. Template

The template is central importance and it directs organization of the functional groups pendent to the functional monomers in all molecular imprinting processes. In terms of compatibility with free radical polymerization, templates should ideally be chemically inert under the polymerization conditions [65], thus alternative imprinting strategies may have to be sought if the template can participate in radical reactions or is for any other reason unstable under the polymerization conditions. The following are legitimate questions to ask of a template: (1) Does the template bear any polymerisable groups? (2) Does the template bear functionality that could potentially inhibit or retard a free radical polymerization? (3) Will the template be stable at moderately elevated temperatures or upon exposure to UV irradiation? The imprinting of small, organic molecules (e.g., pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids, and sugars) is now well established and considered almost routine [66]. Optically active templates have been used in most cases during optimization. In these cases the accuracy of the structure of the imprint (the cavity with binding sites) could be measured by its ability for racemic resolution, which was tested either in a batch procedure or by using the polymeric materials as chromatographic supports.

1.2.2.2.1.2. Monomers

The careful choice of functional monomer is one of the utmost importance to provide complementary interactions with the template and substrates (Figure 4). The monomer interacts with the template molecule due to their functional groups, leading to the development of the pre-polymerization complex, this being a crucial step in the MIP synthesis. Within its structure, two types of elements can be identified: the ones capable of recognizing and interacting with the template and the polymerizable unit [57].

From the general mechanism of formation of MIP binding sites, functional monomers are responsible for the binding interactions in the imprinted binding sites, and for non-covalent molecular imprinting protocols, are normally used in excess relative to the number of moles of template to favor the formation of template-functional monomer assemblies [67]. It is very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion (e.g. H-bond donor with H-bond acceptor) in order to maximise complex formation and thus the imprinting effect. Higher retention and resolution was finding by the two co-monomer imprinting polymer than the single monomer imprinting polymer, which indicated an increase in the affinity of the MIP with the sample

as a result of the cooperation effect of the binding sites. However, it's important to bear reactivity ratios of the monomers to ensure those co-polymerisations are feasible [68, 69].

1.2.2.2.1.3. Cross -linkers

A cross-linker is an organic (rarely inorganic) compound which is added within the polymerization blend, with the main purpose of fixing the monomer molecules around the template ones. Cross-linker (Figure 5) have a crucial role in the polymer stability, which is why is added in almost all mixtures for MIP fabrication [70, 71].

1.2.2.2.1.4. Initiators

Polymerization is a chain reaction that starts with the activation of a single monomer's molecule that becomes the active center of the entire reaction. The triggering of the reactive species is generally due to the presence of an initiator in the polymerization mixture. The initiators (Figure 6) can be classified in three major classes: thermal initiators (the most commonly used being benzoyl peroxide and azo-bis-isobutyronitrile [57]), redox initiators and photo-initiators.

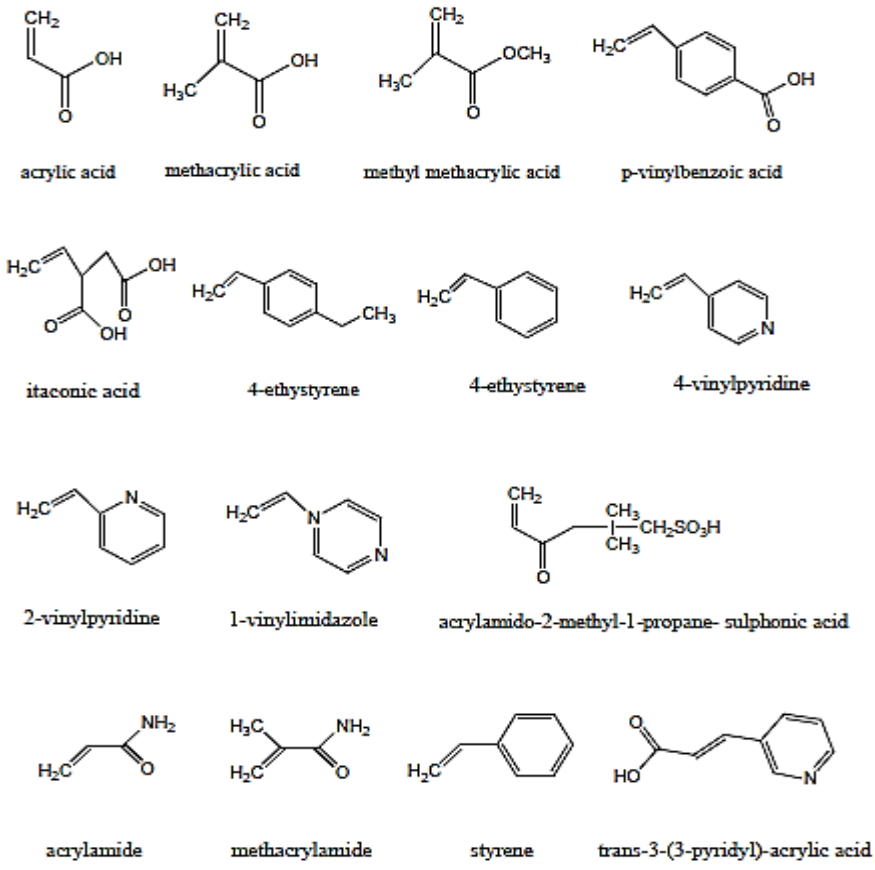

Figure 4. Common functional monomers used in molecular imprinting procedures [67].

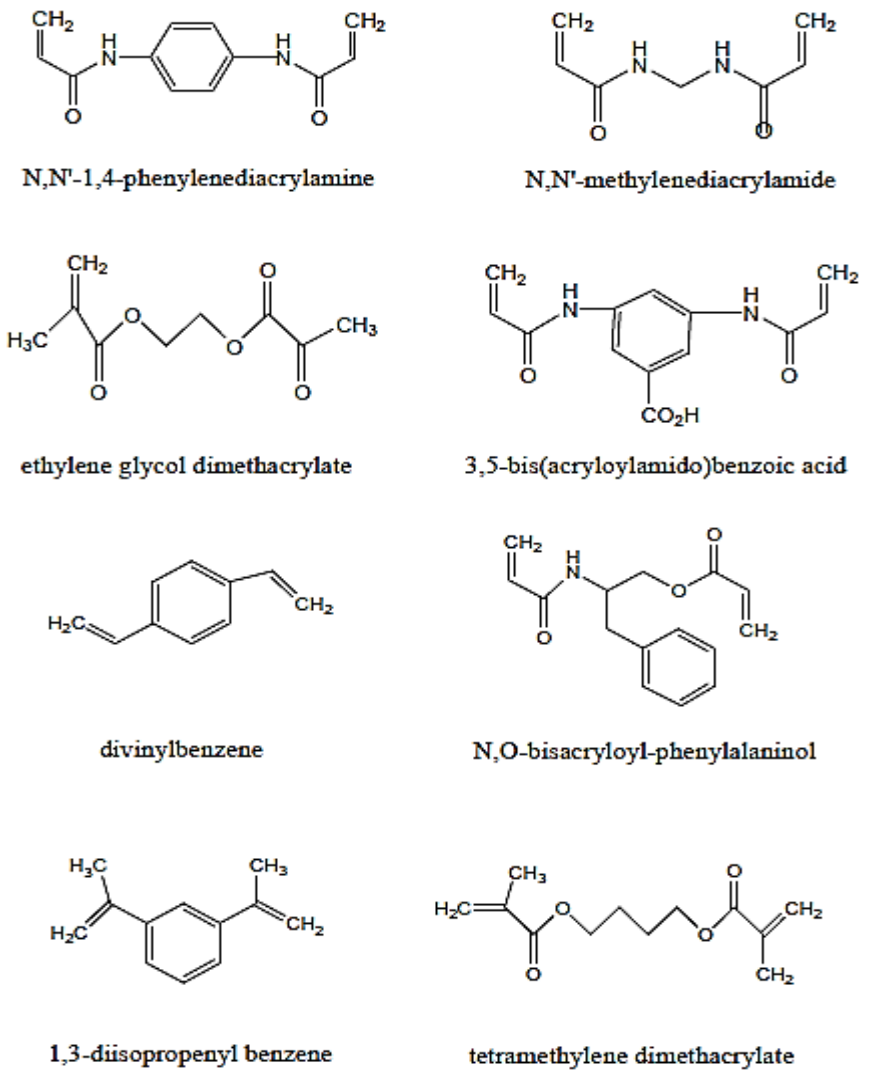

Figure 5. Chemical structure of common cross-linkers used in molecular imprinting [67].

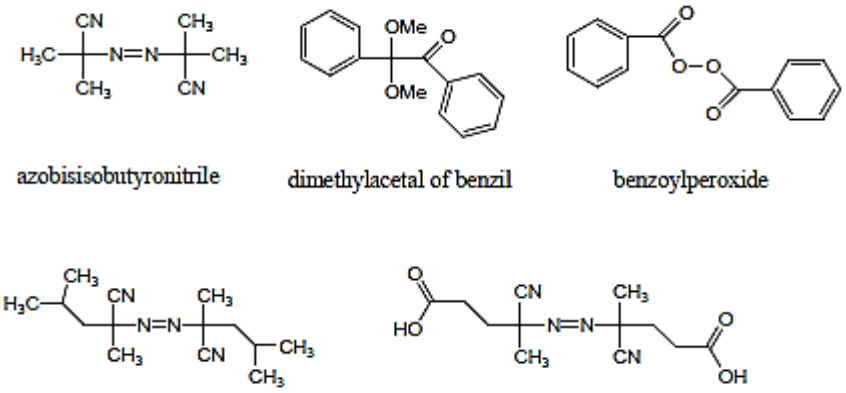

Figure 6. Chemical structure of common initiators used in molecular imprinting [67].

1.2.2.3.Preparing strategies for MIPs

1.2.2.3.1. Bulk Polymerization

Molecularly imprinted polymers can be prepared in a variety of physical forms to suit the final application desired. The conventional method for preparing MIP is via solution polymerization followed by mechanical grinding of the resulting bulk polymer generated to give small particles and sieve the particles into the desired size ranges, which diameters usually in the micrometer range [3, 72]. This method, by far the most popular, presents many attractive properties, especially to newcomers. In fact, it is fast and simple in its practical execution and it does not require particular operator skills or sophisticated instrumentation. Particle sizes < 25 μm are usually used in chromatographic studies [73]. Such ground and sieved particles have been packed into conventional HPLC columns, immobilized on TLC plates, and entrapped in capillary columns using acrylamide gels or silicate matrices [74].

Although bulk polymerization is simple, and optimization of imprinting conditions is relatively straightforward, however, bulk polymerization method presents many drawbacks anyway. First of all, the particles obtained after the last sieving step have a highly irregular in size and shape, some interaction sites are destroyed during grinding, and thus lead to a negative impact on chromatographic performance and lower MIP loading capacity with respect to theoretical values. Moreover, the procedure of grinding and sieving is cumbersome, and it causes a substantial loss of useful polymer, that can be estimated between 50 and 75% of the initial amount of bulk material. Since a portion of polymer can only be used as packing material, this method suffered high consumption of the template molecules. Last, but not least, due to its exothermical nature, bulk polymerization cannot be scaled-up without danger of sample overheating [75, 76].

1.2.2.3.2. Suspension Polymerization

A rather simple method for the preparation of imprinted supports not requiring mechanical grinding is suspension polymerization, which yields aggregates of spherical particles, if the system is sufficiently dilute, uniformly sized microspheres. To avoid above interference in multi-step swelling method, suspension polymerization in perfluorocarbon solvents has been studied [65, 66]. In two-phase systems, the use of liquid perfluorocarbons instead of water as the continuous phase might be preferred since water may have a detrimental effect on the non-covalent complex between monomers and imprint molecule. Although regular molecularly imprinted microspheres have been prepared and excellent chromatographic performance was obtained from polymer beads produced by use of these methods and selectivity was good even at high flow rates, unfortunately, the specialized

perfluorocarbon solvent and fluorinated surfactant impose limits on the applicability and practicality of this method.

1.2.2.3.3. Precipitation Polymerization

MIP microspherical shapes with more uniform size can be obtained by the method of precipitation polymerization, which offers a higher active surface area by manipulating its compositions. As regards precipitation polymerization, this technique involves coagulation of nano-gel beads followed by ordered particle growth due to capture of oligomers from surrounding solution [77, 78]. In this manner, near-monodispersed spherical beads can be prepared, and size and porosity can be fine-tuned thereby changing the polymerization conditions. This technique has been reported in MIP-based competition assays [79, 80] and capillary electrochromatography [81, 82], but only recently works have been published, in which it is clearly shown that precipitation polymerization can be a potentially fruitful technique for preparing chromatography-grade molecularly imprinted beads [82].

1.2.2.3.4. Electropolymerization

Electropolymerization is typically conducted by applying a suitable potential or range of potentials to a solution containing the template with the monomer, originating a film formation on the surface of the electrode [83]. This simple approach is useful since by adjusting the electrochemical conditions (e.g., potential range, number of cycles and scan rate) and by using different conductive materials of various shape/size one can achieve a close control of the polymer thickness [40]. The electropolymerization around the template normally requires the use of a functional monomer, a porogenic solvent, and sometimes a cross-linking monomer in contact with the transducer surface. Also, no initiator is required, nor UV light or heat. Thus, electropolymerization is another method with plenty attractive features to overcome the difficulties of producing MIPs for macromolecules such as proteins, as reviewed elsewhere [12].

The electrosynthesis of MIPs involves conductive polymers (ECP) and insulators/non-conductive polymers (NCP). The charge transfer between the electrode substrate and the analyte that occupies the molecular cavities of the MIP is assured by incorporating ECP matrices in the film. Several research works in the literature report the use of electroactive monomers like 3,4-ethylenedioxythiophene (EDOT) [84, 86], pyrrole [67], aniline [87, 53], thiophene [83, 67], and dopamine [25] for MIP electrosynthesis of organic compounds (Figure 7).

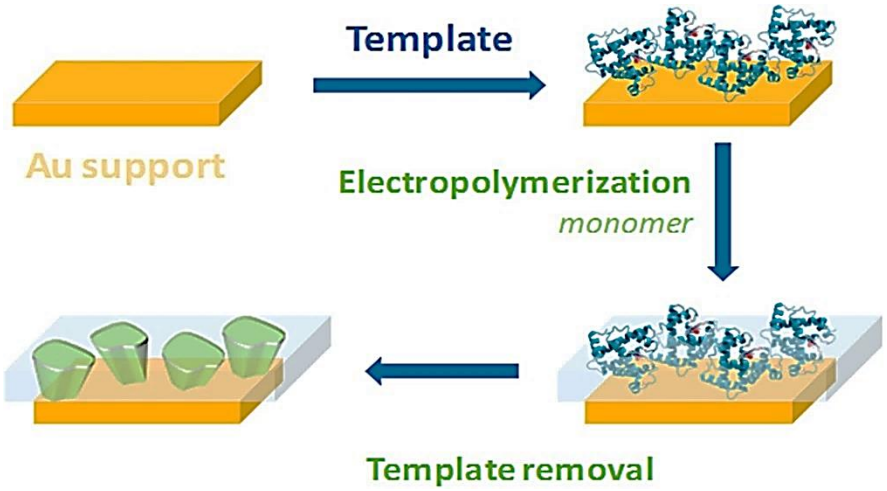

Figure 7. Electrosynthesis of MIPs [52].

Several non-conducting MIP films from the electro-synthesis of non-conductive monomers as phenol [53], aminophenol [83], phenylenediamine [25] among others have also been reported. Electropolymerization resulting in non-conducting MIP films are widely used for capacity chemosensors specially polyphenylenediamine and polyphenol. These materials form non-conductive, compact MIP films after electropolymerization. The prepared MIP biorecognition element can detect the analyte at nanoscale level with notable selectivity and low LOD (limit of detection). The selectivity of the electrosynthesized MIP can be improved by modifying the monomers with additional functional groups.

The resulting conducting or non-conducting MIPs show advantages and disadvantages. The deposition by electropolymerization of non-conducting MIP films has to be tightly controlled related to the polymer thickness. This self-limiting characteristic is due to the need of stopping the deposition upon reaching a thickness at which the polymer insulates the fundamental conducting electrode surface. On the other hand, deposition of ECPs by electropolymerization may occur indeterminately as the deposition conditions control the polymer thickness. The method of choice for signal transduction is related with the conductivity of the polymer [74].

1.2.2. Advantages of Electrochemical Conducting Polymer Based Layer Formation

Conducting polymers (Figure 9) are poorly soluble in usual solvents, for this reason it is not very easy to apply CPs in the formation of sensing layers (Figure 8). These technological problems can be solved by electrodeposition, which is more reliable for the formation of CP-based structures on conducting substrates. The electrodeposition methods selection [61] and the adjustment of parameters that are used during the deposition of CP-based films enables to form sensing layers with very different analytical characteristics. The

most easily adjustable synthesis parameters are: (i) the voltage of applied potential, (ii) the duration of potential pulses or potential sweep rate used when potential cycling is applied, (iii) the limitation of electrical current passing through the electrochemical system, [62,63], (iv) some other additionally applied external factors (e.g., treatment by ultrasound) [64]. The variation of all these parameters enables changing many physicochemical properties of polymeric layers. Hence, some CP-based layers electrochemical characteristics can be adjusted by the concentrations adaptation of all materials, which are used in polymerization bulk solution [75, 76]. The most important characteristics including sensitivity and linear range of CP-based sensors are predetermined by the thickness, density, permeability and other properties of CP-based layers. Therefore, by variation of above mentioned and some other polymerization conditions (such as thickness and morphology), the deposited conducting polymer layer porosity can be easily changed [78, 79]. The formed layer morphology control enables to change the CP-based films permeability [12,69]. The diffusion of target/analyte and some other compounds through CP-based matrix is very important for the affinity sensors action based on these structures. Conducting polymers from this point of view are very attractive, because by the selection of proper synthesis conditions porous structures based on CPs can be formed [80]. In addition, such porous structures mostly are amorphous and do not display long-range order of polymer-film forming molecules. Some researchers are reporting the possibility to adjust the porosity of CPs by using some organic compounds as spacers, which are interlinking different polymer chains [81]. Conducting polymers of high porosity were exploited in the sensor design dedicated for the determination antibiotic-aminoglycoside, which was evaluated in aqueous samples [82]. Hence, CP-based structures electro-deposition offers many possibilities for the sensors design with tunable analytical characteristics. In addition to above mentioned advantages, there are many other serious reasons to choose electropolymerization for the formation of CP-based layers, because: this technique is much faster than the classical oxidative-chemical polymerization in the bulk but also it can be carried out in situ on the working electrode's surface [83], and if potentiostat/galvanostat is controlled by properly developed software, then the whole process can be clearly observed on computer screen and evaluated/controlled using elaborated mathematical algorithms [12]. The most recently used electrochemically deposited polymers are: polyaniline [36,56], polypyrrole [39], polythiophene and poly(3,4-ethylenedioxythiophene) (PEDOT) derivatives [36,74], Poly-9,10-phenanthrenequinone [42]. Some derivatives of these polymers can be electropolymerized and/or electro-copolymerized with some other monomers, which themselves are also forming conducting polymers.

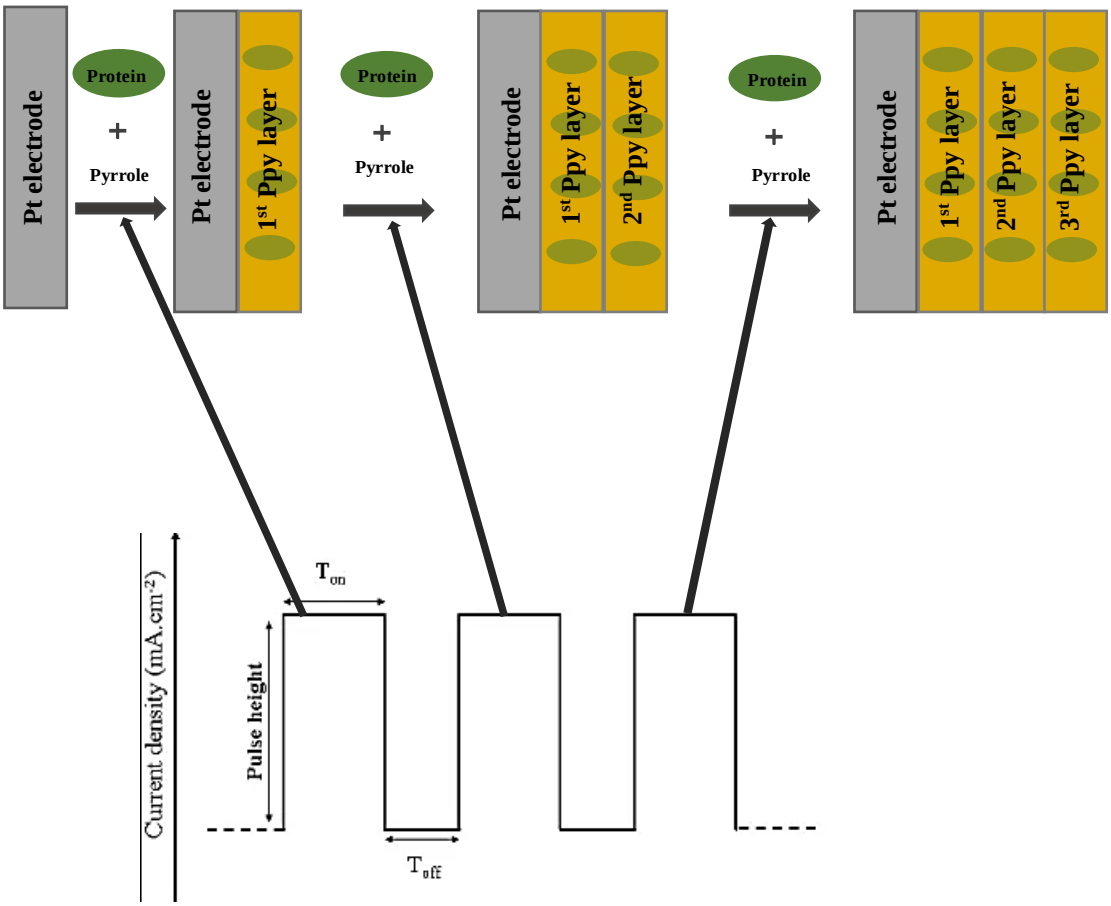

Figure 8. The scheme of Ppy electrochemical deposition by potential pulses and entrapment of proteins within the formed Ppy layer [45].

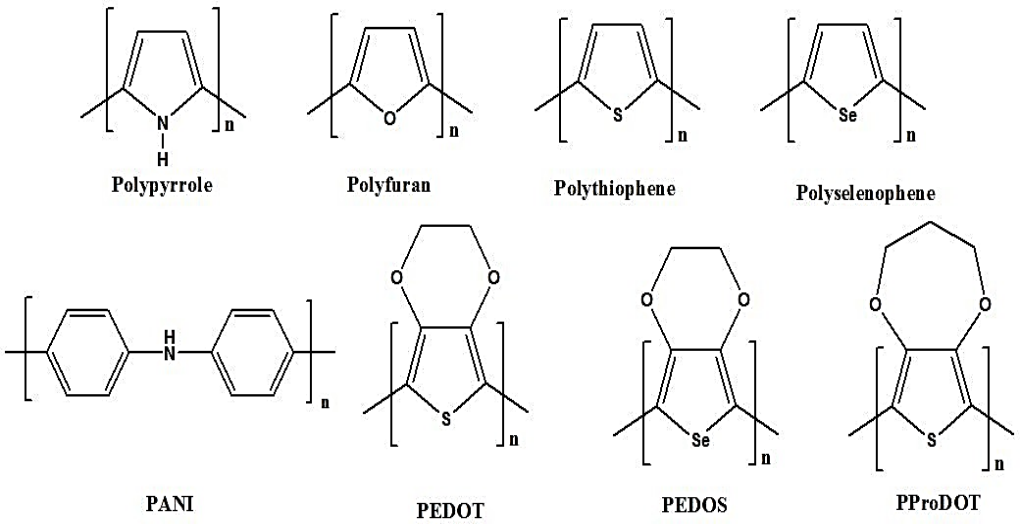

Figure 9. Chemical structures of representative conductive polymers [46].

1.3. Electrochemistry

The electrochemistry fundamentals are to study the interaction between matter and electricity. An electrochemical sensor is a device that transforms the interaction of an analyte with a receptor on the electrode surface into a useful analytical signal (from electrochem nd mips) [84]. Table 3 lists some of the main electrochemical methods and their respective monitored electrical signals [85], indicating that in general, electrochemical responses monitored by different methods are based primarily on potential, resistance and electrical current. Coulometry and capacitance methods, for example, have their responses derived from potential, resistance, and electrical current.

Table 3. Main Electrochemical Methods, Monitored Electrical Properties, and Respective Units.

Electrochemical methods	**Monitored electrical properties**	**units**
Potentiometry	Potential difference (volts)	V
Conductometry	Resistance (ohms)	Ω
Amperometry and voltammetry	Current (amps) as a function of applied potential	I
Coulometry (Q)	Current as a function of time (coulombs)	C = I . S
Capacitance (C)	Potential load (farads)	$F = C. V^{-1}$

When we treat electrochemical techniques as analysis methods, we can divide them into two main groups: interfacial methods and noninterfacial methods (measuring the solution as a whole) [86]. From the examples mentioned in Table 2, conductometry is a noninterfacial method, as it is based essentially on a cell of known size, with two equidistant electrodes that measure the electrical conductance (essentially, the resistance) of a solution as a whole. When applying an alternating current signal, there is no electrode polarization and the response is given in terms of the solution electric resistance, as a cell constant function, which is based on the electrodes surface area, their spacing and the solution volume in the electrochemical cell [87]. In turn, the interfacial methods respond directly or indirectly to the presence of the analyte on the electrode surface (sensory unit), resulting in a disturbance of an electric signal that can be measured [88]. We can divide the interfacial methods into two main groups: the static methods and the dynamic methods. The static methods are defined as those in which there is no disturbance and the electric current is zero (i = 0). The dynamic methods are those that exploit a redox reaction; that is, electron transfer

occurs between the electrode and the analyte [89]. Figure 10 shows the main electrochemical methods and their subdivisions.

1.3.1. Voltammetric Methods

Historically, voltammetric methods were developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, who received the chemistry Nobel Prize in 1959 [13]. Polarography studies electrolysis solutions and substances that are reduced or oxidized in a dropping mercury electrode and a reference electrode. The potential between these electrodes is varied, and the resulting changes in the current flow are measured. By plotting the changes in current flow against the potential variation, a current versus potential polarographic graph is obtained (I × E) [8]. During the 1960s and 1970s, theories, methods, and instrumentation were developed for the voltammetry field, thus increasing the sensitivity and repertoire of electroanalytical methods [14]. The common characteristic of all voltammetric techniques is that they involve the potential application (E) on an electrode and the monitoring of the resulting current (I) flowing through the electrochemical cell. In many cases, the applied potential is varied or the current is controlled for a period of time (t). Thus, all voltammetric techniques can be described as a function of potential, current, and time (E, I, and t). The analytical advantages of the many voltammetric techniques include the following: excellent sensitivity with the detectable concentration range of organic and inorganic species; a large number of useful solvents and electrolytes; a wide temperatures range; fast analysis times (seconds); simultaneous determination of several analytes; the ability to determine kinetic parameters and estimate unknown parameters; the ease with which different potential wavelength shapes can be generated; and the small currents measurement [8]. The potential sweep methods, also known as voltammetric methods, consist of the potential varying continuously application with time on a working electrode, which leads to the oxidation occurrence or reduction reactions of electroactive species in the solution (faradaic reactions), in accordance with the species adsorption with the potential and a capacitive current due to the electrical double layer. The observed current is therefore different from the current in the steady state. These methods are commonly used for the processes occurring study in the working electrode and can be used with linear, pulse, and cyclic sweep, in addition to cyclic voltammetry (CV). Their main use has been for the diagnosis of electrochemical reaction mechanisms, for the identification of species present in solution and for semiquantitative reaction speeds analysis [15]. In addition to these applications, these methods are also widely used for the kinetic and constant rates measurement, the determination of adsorption processes in surfaces, the study of

electron transfers and reaction mechanisms, the determination of thermodynamic solvated species properties, essential studies of oxidation and reduction processes in several ways and the complexing values and coordination determination.

1.3.1.1. Cyclic Voltammetry

In linear sweep voltammetry, the potential sweep is performed in only one direction, stopping at a chosen value E_f, for example, for $t = t_1$. The sweep direction can be positive or negative, and the sweep speed, at first, can take any arbitrary value [90]. In CV, when reaching $t = t_1$, the sweep direction is reversed and changed until Emin is reached, then reversed and changed to $E_{máx}$, and so on, generating cycles with several sweeps. The basic diagram involving the application of a potential sweep is shown in Figure 11.

In a cyclic voltammogram, the most analyzed parameters are the following:

• initial potential, E_i;

• initial sweep direction;

• sweeping speed, ν;

• maximum potential, $E_{máx}$;

• minimum potential, E_{min}; and

• final potential, E_f.

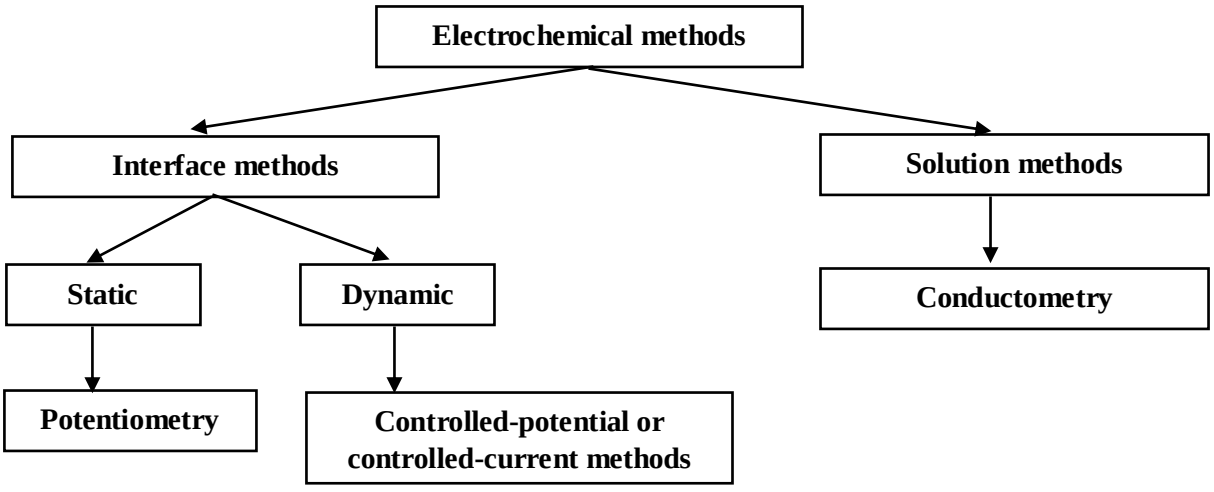

Figure 10. Main electrochemical methods and their subdivisions [8].

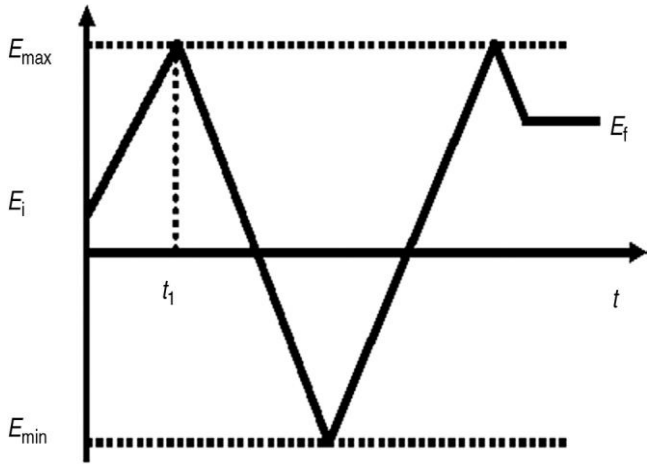

Figure 11. Potential applied as a function of time in cyclic voltammetry (CV) [92].

An example of a cyclic voltammogram, that is, the measured current response as a function of the potential for a reversible system, is shown in Figure 12. A faradaic current (I_f), resulting from the electrode reaction is recorded in the potential window during the reaction of the analyte on the electrode surface. There is also a capacitive contribution (I_c) because when sweeping the potential, the charge of the electrical double layer (C_d) changes. This contribution increases with increasing sweeping speed. The total current is a sum of the capacitive and faradaic currents [91]. Both currents tend to increase with increasing sweeping speed. This relationship limits the technique sensitivity because the high capacitive current can interfere in the sensitivity of the faradaic current, which, in the linearity region, is proportional to the analyte concentration. The potential obeys the Nernst equation and is therefore characteristic of a given redox process or an analyte. Thus, CV can be used for quantitative determinations; because of its limitations, however, it is more generally used for exploratory purposes, that is, to determine the redox process of different analytes. To minimize the contribution of the capacitive current and therefore increase the sensitivity of voltammetric methods, potential impulse (or pulse) methods were developed, including pulse voltammetry and square wave voltammetry (SWV) [92].

$$\mathbf{E=E° + RT/nF \ln [RED] / [OXI]}$$

where E, cell potential; E^0, standard potential of a half-reaction; R, universal gas constant; T, temperature; n, number of electrons (eq. mol^{-1}) involved in the half-reaction; F, Faraday constant; [RED] = activity of the reduced species; and [OXI] = activity of the oxidized species.

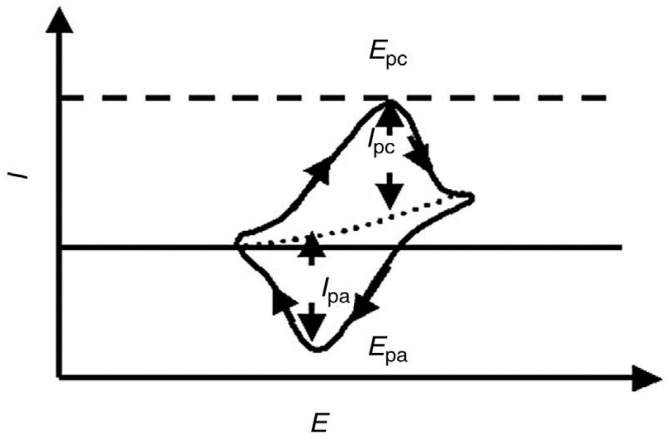

Figure 12. Cyclic voltammogram for a reversible system, where E is the potential and I is current [92].

1.3.1.2. Square Wave Voltammetry

SWV is one of the fastest and most sensitive pulse voltammetry techniques. The detection limits can be compared with those of chromatographic and spectroscopic techniques. In addition, the analysis of the characteristic parameters of this technique also enables the evaluation of the kinetics and mechanism of the electrode process under study [9,17]. In SWV, the shape of the potential current curve is derived from the application of potentials of height ΔE (pulse amplitude), which vary according to a potential step Estep (in mV) and τ duration (period). On the potential–time curve, the pulse width (τ/2) is denoted by t, and the frequency of pulse application is denoted by f and is given by (1/t). The electric currents are measured at the end of the direct (I_1) and reverse (I_2) pulses, and the signal is obtained as an intensity of the resulting differential current (ΔI); this technique offers excellent sensitivity and high rejection to capacitive currents. This measurement precedes an initial time (t_i) at which the working electrode is polarized at a potential for which the redox reaction does not occur [93].

Figure 13 shows details of the potential application of SWV, with the definition of the used parameters, whereas Figure 14 shows the theoretical voltammograms associated with (A) a reversible system and (B) an irreversible system, with observed separation of direct, reverse, and resulting currents, and both voltammetric profiles have similarities to those obtained in square wave polarography.

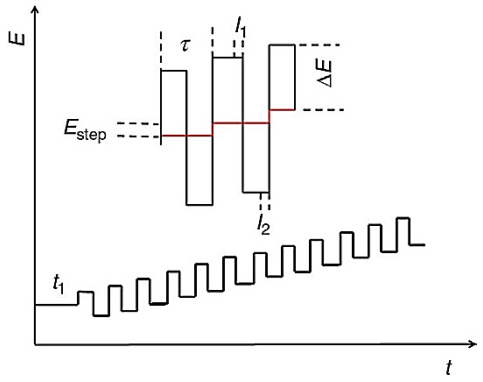

Figure 13. Application of potentials in square wave voltammetry (SWV) [9].

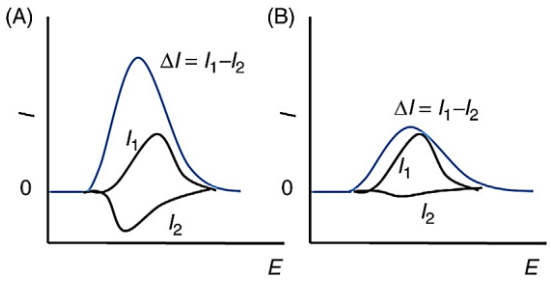

Figure 14. Schematic square wave voltammogram, where (A) represents a redox process of a reversible system and (B) represents that of an irreversible system [92].

Current-potential curves display well-defined profiles and are usually symmetrical. This is due to all currents being measured only at the end of each semiperiod and the variations in the height and width of the potential pulse being always constant for a determined potential range [94]. Thus, electrochemical techniques can be used in the synthesis and characterization of materials through voltammetric methods that relate the current and the electric potential in the electrochemical cell. In amperometric sensors, for instance, a constant electric potential is applied to the electrochemical cell and a corresponding current appears because of the redox reactions that occur on the surface of the working electrode. This current can be used to quantify the reactions involved. Amperometric sensors can be operated through CV, another powerful technique for the synthesis and characterization of different electroactive species, and the relationship between the current-potential characteristic of each oxidation or reduction reaction involved. In general, voltammetric sensors are used in the detection of species in redox reactions that occur in the electrochemical cell. The SWV technique has also been used in the develop ment of sensors and biosensors because of its high sensitivity and selectivity [18]. It is currently of great interest to the pharmaceutical industry for the use of sensors in the

detection of disease, environmental pollutants, such as heavy metals, and other chemical contaminants that are part of the environmental liability in contemporary societies.

1.2.3. The role of electrochemistry in the integration of chemically synthesised MIPs in sensors

An interesting approach in which electrochemistry meets imprinting technology has led to the design of composite sensing materials based on the entrapment of chemically prepared MIP particles into electropolymerised films [98]. Mosbach and Haupt [95] in 1999 first combined the electrochemical sensor with MIPs to develop a MIPs-coated electrode, which is called a molecularly imprinted electrochemical sensor (MIECS). The MIP on electrochemical sensor has both recognition and transduction properties, that is, the MIPs as a recognition element can specifically bind target analytes and generate a chemical or physical signal. A transducer then translates this signal into a quantifiable output signal such as potential, current, conductivity, or impedance change for detection [96].

1.2.4. MIPs as recognition elements in electrochemical sensors

The MIECS has the advantages of high selectivity, high sensitivity, low detect limit, ease of miniaturization and automation, and lower cost in use due to the combination of the molecularly imprinted technique and the electrochemical detection [98, 99]. Molecularly imprinted polymer is the sensitive membrane of MIECS. An electrochemical response is obtained when the MIP membrane is recognized and combined with the target molecules (i.e., template molecule or imprinted molecule); then the electro-signal can be recorded, and the concentration of target molecules can be determined. MIECS is commonly classified as capacitive, potentiometric, and amperometric sensors in accordance with the determination process of target molecules. The detection of target molecules by MIECS is based on electrochemical signal changes, including current, capacitance, and potential of molecular imprinting sensitive film before and after the specific recognition of target molecules [100]. The advantage of the capacitance and potentiometric sensors is that no other additional reagents or markers should be added besides the analyte; the sensor is also simple in operation with a low-price cost. The amperometric MIP sensor is the most widely used sensor among MIECS. It can be used to detect the electroactive target molecules by the electrode reaction of target molecules, or detect the no electroactive target molecules indirectly [38, 39]. In this section we gave examples of imprinted electrochemical sensors.

1.2.5. MIP Electrochemical Sensors

Electrochemical sensors generally possess higher sensitivity than piezoelectric sensors. The first potentiometric MIP sensors based on electropolymerized materials were described by Boyle and co-authors and Vinokurov [101, 93]. Here the monomers reacted with themselves and other molecules as templates forming specific polymers. Sensors for pyrrole, aromatic amines, and substituted phenols, based on polypyrrole, polyaniline, and aniline-p-aminophenol copolymers, respectively, were prepared. A similar MIP-based amperometric device was developed by Piletsky and co-authors for the detection of aniline and phenol [94]. The sensors relied on preferential adsorbance of the electroactive template by conductive MIP. Differential pulse voltammetry has been used for the detection of clenbuterol in bovine liver samples [102]. The sensor approach relied on displacement of clenbuterol specifically adsorbed by a MIP with an electroinactive analogue-isoxsuprine.

Silva et al designed a novel electrochemical sensor for the determination of trimethoprim (TMP) by electro polymerization of pyrrole (PY) and molecularly imprinted polymer (MIP) which was synthesized onto a glassy carbon electrode (GCE) in aqueous solution using cyclic voltammetry. In their study, they used graphene (GNPs) in order to enhance the sensitivity of the sensor by an increase in the electrochemical conductivity. The performance of the imprinted and non-imprinted (NIP) films was investigated by electrochemical impedance spectroscopy (EIS) and the cyclic voltammetry (CV) of a ferric solution. The sensor they developed presented a linear range between peak current intensity and logarithm of TMP concentration with a range from 10^{-6} to 10^{-4} M. The results were accurate (with recoveries higher than 94%), precise (with standard deviations less than 5%), and the detection limit was 1.3×10^{-7} M [40].

A case was observed with a MIP nanofilm sensor for transferrin prepared by electro polymerization of scopolamine on gold electrodes [41]. Transferrin was detected using cyclic voltammetry (CV) and square-wave voltammetry as well as by surface plasmon resonance (SPR), with the sensor response depending on the permeability changes of the MIP film and the redox couple ferri/ferrocyanide. In another experiment the electrochemical probe was immobilized within the polymer matrix itself.

One of the objectives in molecular recognition is to achieve stimulus-responsive recognition behavior; as a result, Recent advances in stimulus-responsive materials have made this aim possible [42, 43]. Pilla et al reported a modulated molecular recognition achieved in a temperature-sensitive molecularly-imprinted polymer [44]. Using PNIPA

(poly(N-isopropylacrylamide) as the temperature-sensitive element, the adenine-imprinted polymer (i.e., MIP-S) was prepared and characterized. The MIP-S exhibited a temperature-responsive molecular recognition behavior because of the thermal phase-transition within the MIP-S network. Specifically, below the transition temperature (e.g., 20 °C), the MIP-S showed a highly specific recognition for the imprint species (adenine). However, the MIP-S did not show any significant resolution for the imprint species (adenine) and its analogue (1-methyladenine) above the transition temperature (e.g., 40 °C). Wei and colleagues also proposed a temperature-sensitive MIP electrochemical sensor for the detection of bovine serum albumin (BSA) [45]. The sensor was based on a thermoresponsive memory hydrogel prepared on a glassy carbon electrode (GCE) with a free radical polymerization method. The sensing process for BSA relies on a reversible structural change of the MIP when an external temperature stimulus is applied, which can be monitored by CV and electrochemical impedance spectroscopy (EIS) in aqueous media. The proposed BSA sensor exhibited satisfactory performance in milk products with high recovery ratios.

Incorporation of ionic liquids (ILs) in the MIP framework has also been the focus of many studies. By exploring their good electrocatalytic activity, adsorption capacity and multiple interactions with targets, there are some works adopting polymerisable ILs as functional monomers and crosslinkers to prepare MIPs [73]. Liu et al introduced a new IL composite using IL 1-vinyl-3-butylimidazolium hexafluorophosphate as functional monomer, IL 1,4-butanediyl-3,3'-bis-l-vinylimidazolium dihexafluorophosphate as crosslinker and Fe_3O_4 as support, to detect diphenylamine (DPA) in water samples [74]. Although, ILs have been considered alternative green solvents, it is important to note that ILs are quite expensive and their recycling is difficult.

Encouragingly, there has been an increase in the application of MIP sensors to biological samples. Recently, a cocaine potentiometric sensor based on MIPs was reported to accurately measure cocaine in blood serum in a linear range of 1 nM to 1 mM [46]. The low polymer yield did, however, require multiple syntheses of MIPs for preparation of a PVC membrane. Analyte was detected with high sensitivity by combining MIP nanomaterials with transducers. Likewise, Peeters et al have developed a biomimetic sensor for the detection of serotonin in human blood plasma, based on impedance spectroscopy in combination with MIP-based synthetic receptors, has the potential to become a fast and low cost alternative to chromatographic techniques. At the receptor side, they have shown that a blend of two functional monomers, methacrylic acid and acrylamide, is essential to achieve selective molecular recognition [47]. Another example reported a disposable MIP sensor to

monitor heparin levels in blood based on a graphite paste electrode [48]. The voltammetric sensor was tested in physiological saline and bovine whole blood containing 5 mM ferrocyanide, displaying high selectivity in a range of 0 - 8 unit mL^{-1} of heparin and no crossreactivity.

In addition to biological samples Zhou et al reported the fabrication of a new selective and sensitive sensor based on molecularly imprinted polymer/acetylene black (MIP/AB) for the determination of azithromycin (AZM) in pharmaceuticals samples as well. The MIP of AZM was synthesized by precipitation polymerization. MIP and AB were then respectively introduced as selective and sensitive elements for the preparation of MIP/AB-modified carbon paste (MIP/ABP) electrode. under the optimized conditions, the prepared sensor showed two dynamic linear ranges of 1.0×10^{-7} mol L^{-1} to 2.0×10^{-6} mol L^{-1} and 2.0×10^{-6} mol L^{-1} to 2.0×10^{-5} mol L^{-1}, with a limit of detection of 1.1×10^{-8} mol L^{-1} [49]. For the fast determination of bisoprolol fumarate in analogous samples, Frag et al prepared a novel molecularly imprinted potentiometric sensor by bulk polymerization with methacrylic acid as the functional monomer [50], the obtained results indicated that the sensor modified by the MIP have much higher recognition power for the BF molecules than the NIP based sensor where the MIP based CPE exhibited a Nernstian response 29.50 ± 0.55 mV decade within a concentration range of 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1} and pH independence in the range 3.50 - 7.15, the detection limit was 5.0×10^{-8} mol L^{-1}.

Due to the alarming abundance in the environment and adverse health effects, sensitive and selective analytical methodologies for the determination of numerous pollutants in air, water and sediments and analyzing the ever-increasing presence of contaminants in environmental waters have been pursued [51, 52]. The lack of functionality in the chemical structure of Polycyclic Aromatic Hydrocarbons (PHAs) is one of the difficulties in imprinting PAHs is which prevents the formation of well-defined, functionalized and size-specific binding cavities in the polymer, therefore, only a limited number of studies have demonstrated molecular imprinting of PAHs [53-56]. While Munawar et al have reported a highly sensitive sensor based on molecularly imprinted polymer film to detect ultra-trace concentration of PAHs in environmental samples by electropolymerisation of 4-vinyl pyridine (4VP) and target, pyrene, using cyclic voltammeter in electrolyte medium, forming the pyrene imprinted polymer [57] Branger et al developed an e-MIP by distillation–precipitation polymerization using ferrocene functionalized with a vinyl group as a monomer capable of forming aromatic stacking interactions with a benzo[a]pyrene (BaP) template. These interactions caused detectable modifications of the ferrocene redox properties

following BaP recognition, as the reversible oxidation to a ferricenium ion is sensitive to the environment. Using this method, MIPs were able to detect BaP at concentrations down to 90 nM [58]. Tiu et al prepared a pyrene-imprinted polythiophene by electrochemical deposition and used for highly sensitive detection of pyrene and its analogues. Using a molecular imprinting technique, pyrene-specific recognition sites were formed throughout the oligo/polythiophene film deposited onto a gold surface. The developed sensory materials showed high sensitivity to pyrene in the presence of its structural analogues in low concentration range (0.1-100 μM) have great potential in in-situ environmental monitoring of these carcinogenic pollutants in aqueous solutions [51].

Over the past 50 years, global sales of pesticides and Insecticides have increased because they are an important component of agricultural production. However, repeated applications result in their accumulation in soils and can be transported to the aquatic environment by surface runoff [59]. Amongst the many publications that have been reported about their use for the trace analysis and in other examples of the use of nanomaterials, electrochemical MIP sensors for the analysis of methyl-parathion (MP) were prepared using both electro and bulk polymerisation. Wu et al constructed a sensor based on AuNPs decorated with CNTs. After electrodeposition of functionalized AuNPs on a MWCNTs/GCE surface, the electrode was immersed in a solution containing PATP. Since MP is electroactive, its recognition by the MIP could be directly monitored by LSV [60]. The proposed sensor allowed a low LOD (0.30 nM) and was successfully applied to the determination of MP in spiked distilled and tap water and apple and cucumber samples with recoveries ranging from 95 to 106 %. Motaharian et al synthesized nanoparticles of diazinon imprinted polymer by suspension polymerization and then used for modification of carbon paste electrode (CPE) composition in order to prepare the sensor [61]. Cyclic voltammetry (CV) and square wave voltammetry (SWV) methods were applied for electrochemical measurements. The obtained results showed that the carbon paste electrode modified by MIP nanoparticles (nano-MIP-CP) has much higher adsorption ability for diazinon than the CPE based non-imprinted polymer nanoparticles (nano-NIP-CP). Under optimized extraction and analysis conditions, the proposed sensor exhibited excellent sensitivity (95.08 μA L μmol^{-1}) for diazinon and a detection limit of 7.9×10^{-10} $mol.L^{-1}$. The sensor was successfully applied for determination of diaznon in well water with recovery values in the range of 92.53 - 100.86 %.

Glyphosate (Gly) is among the mostly widely used herbicides by farmers during the past 40 years. Although not proven, Gly has been associated with cancer in humans [62].

Due to its persistence in seawater, the need to identify trace levels of Gly in drinking water is also urgent [63]. To the best of our acknowledge, the most recent electrochemical MIP-sensor used for Gly detection in water samples was reported by Zhang et al [64]. In this work, a simple electropolymerisation procedure, using Py as functional monomer, on an Au electrode was performed. The sensor presented good binding kinetics to Gly and showed good stability, selectivity, and sensitivity, and an LOD of 1.60 nM. The analytical signal was based on the use of the $[Fe(CN)_6]^{3-/4-}$ redox probe and DPV.

Pharmaceuticals are widespread micropollutants and are ubiquitous in waters and soils [65, 66]. They have been released into the environment for decades through various ways. Antibiotics are the most frequently studied group of pharmaceuticals for electrochemical detection using MIP-based sensors, showing the concern about their presence in waters and the importance of their detection. Sulfamethoxazole (SMX) is one of the most frequently sulfonamide bacteriostatic antibiotic detected in the aquatic environment. The first electrochemical MIP-sensor, based on a PPy modified Boron Doped Diamond Electrode (BDDE), was developed for the determination of SMX in spiked lake water [67]. This sensor was prepared by electropolymerisation of Py and the determination of SMX was achieved through its direct oxidation using SWV. A good sensitivity, a limit of detection (LOD) of 24 nM and minimal interferences of structurally similar sulfonamides (sulfadimethoxine, sulfadiazine and sulfafurazole) were observed. Sulfanilamide (SN) is another member of the sulfonamide family and has mainly been detected in surface waters, with high detection rates in Chinese rivers in comparison with other countries. Tadi et al also used electropolymerisation of Py for the fabrication of a sensor for the analysis of SN. In this case, a pencil graphite electrode (PGE) was used. DPV was used for its direct analysis (oxidation), achieving an LOD of 20 nM. This sensor showed good selectivity towards SN; species with analogue structures such as SMX, sulfathiazole and sulfadiazine did not significantly interfere in the analysis. The applicability of this sensor was tested in spiked human serum and ground water samples, obtaining recoveries between 98 and 115% [68].

Azithromycin (AZY) and erythromycin (ERY) are frequently prescribed macrolide antibiotics to treat many different bacterial infections. Due to their wide use, the difficulty of removing them by common wastewater treatments and their frequent detection in water bodies, AZY and ERY were included in the EU Watch List (Decision 2018/840/ EU) of substances that could pose a significant risk to aquatic environments [69, 70]. Recently, Rebelo et al reported a low-cost and user-friendly electrochemical MIP sensor to detect AZY in water. By a computational study based on density functional theory (DFT), 4-

aminobenzoic acid (4-ABA) was chosen as the most suitable monomer, which was electropolymerised on the surface of a SPCE. The analysis of AZY was performed by its oxidation using DPV (LOD = 80 nM) and the sensor displayed great recognition behaviour in the presence of other interfering compounds. It was successfully applied to the analysis of spiked tap water and water samples collected upstream of a WWTP output in the Ave river (Portugal) [70]. A similar system was used by Ayankojo et al to obtain the first ERY-selective MIP film integrated with a screen printed gold electrode (SPAuE). In their work, the MIP was generated directly on the SPAuE via electropolymerisation of m-phenylenediamine (m-PD). DPV measurements performed using a $[Fe(CN)_6]^{3-/4-}$ redox probe, allowed the determination ERY with an LOD of 0.1 nM [69].

Other substances present in environmental waters at concentrations of toxicological and carcinogenic concern are hormones, especially 17-β-estradiol (E2). E2 is commonly used in contraceptive pills and its release in water has gained notable attention [71]. In a recent study, MIP magnetic composites (Fe_3O_4-MIPs) have been employed for a range of molecules, with an application for E2 in river water [72]. The parameters affecting these composites have been explored, with pH and incubation time having the most notable influence. The Fe_3O_4-MIP sensor operating mode relies on amplification of the 17-b-estradiol oxidation current using square-wave voltammetry, reaching a LOD of 20 nM.

In this direction, we designed novel biomimetic co-polymer sensing layers for the selective molecular recognition of salicylamide; an emerging pharmaceutical contaminant in aqueous medium. Combining co-electropolymerization and molecular imprinting technology provides improved properties of functional materials and enhanced number of binding sites. Electrosynthesis of molecularly imprinted poly (aniline-co-3,4-ethylenedioxy-thiophene) will be described and studied in the next chapter.

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Chapter 2

Instruments and Experimental Techniques

1. Introduction

A presentation of the different techniques and experimental protocols used in the present work will be given in this chapter. Electrochemical measurements and characterization techniques are demonstrated with the preparation procedures of electrodes and solutions. Then, electrochemical transduction will be described with a brief presentation of the different electronic configurations and used procedures. Preparation of electrosynthesized molecularly imprinted conducting polymers and copolymer; polyaniline (PAn), poly(3,4ethylenedioxythiophene) (PEDOT) and poly(AnEDOT) as well as Non imprinted conducting copolymer film will be demonstrated, in order to investigate our intelligent and novel MICP sensor performance, characteristics and practicality in the detection of salicylamide in water.

1.1. Chemicals

The 3,4-Ethylenedioxythiophene, EDOT and aniline used as functional monomers (FM) were purchased from Sigma-Aldrich. Perchloric acid used as supporting electrolyte during both co-electropolymerization of EDOT and aniline as well as electropolymerization of EDOT was purchased from Fluka, sulfuric acid was provided from Fluka and utilized as supporting electrolyte during electropolymerization of aniline. Pharmaceuticals used as target molecules (Figure 15): Salicylamide, SMD, Ibuprofen, IBP, (isobutylphenylpropionic acid), Sulfanilamide, SN, (p-Aminobenzenesulfonamide), Naproxen, NA, (Naprox Sodium), Ascorbic acid, AA, (ascorbate), were obtained from Aldrich. Ethanol, was obtained from Honeywell Riedel-de-Haën and was used as solvent. Acetonitrile, ACN, used as extraction solvent was purchased from Merck.

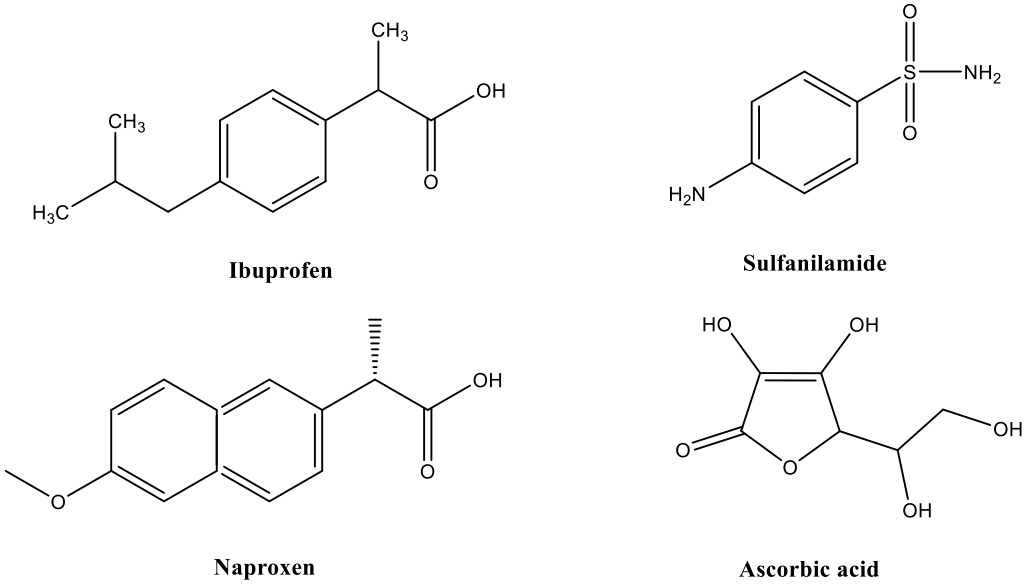

Figure 15. Schematic representation of the cleaning process of a gold electrode.

1.2. Electrodes and electrochemical apparatus

Different types of electrodes were used: platinum counter electrodes and gold working electrodes with an active surface area of 0,52 cm^2 and 0.19 cm^2 respectively, and Thickness of 300 nm were provided from Laboratory for Analysis and Architecture of Systems: Toulouse, France.

1.2.1. Preparation of working electrodes

Before electrochemical deposition or starting any surface modifications, the gold electrodes must be pre-cleaned in order to activate the surface and obtain a good electrochemical response. The cleaning is done in two stages; a first step which consists in removing the resin layer on the gold surface, the electrode is then entirely dipped in acetone for 10 min. In a second step, the electrode is cleaned with a "piranha" mixture. This solution is composed of (2/3) volumes of concentrated sulfuric acid (96%), H_2SO_4, with 3 volumes of hydrogen peroxide, H_2O_2. The electrode is then left for 1 minute in the solution. After this treatment, the electrode is rinsed extensively with distilled water and then ethanol and is left to dry in the open air [103] (Figure 16).

With piranha solution Being both strongly acidic and a strong oxidizer, it is extremely energetic and explosive if not handled with extreme caution (exothermic reaction). This mixture is prepared beforehand, putting sulfuric acid first, followed by hydrogen peroxide [104].

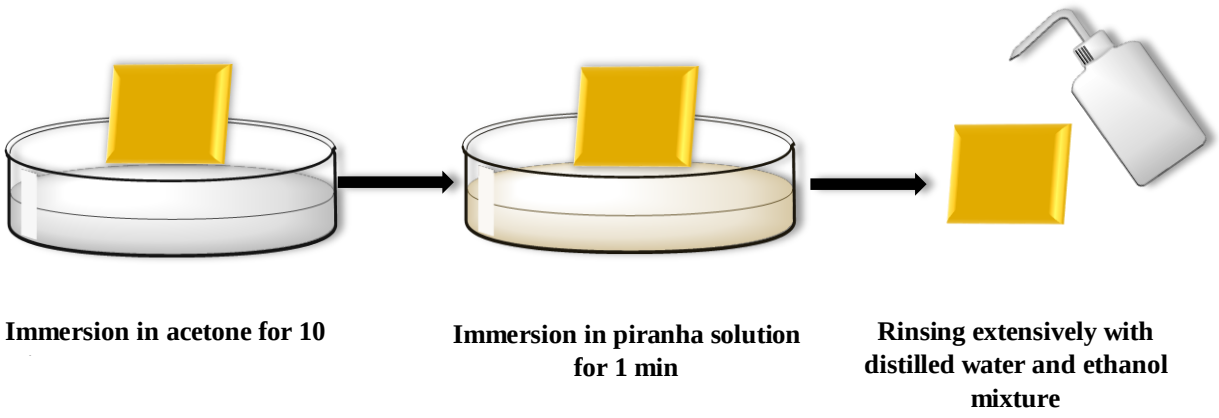

Figure 16. Schematic representation of the cleaning process of a gold electrode.

1.2.2. Instrumentation, Hardware and Software

The working principle of an electrochemical sensor is to measure and analyse certain variables of an electrochemical cell. This is purely done by analysing the difference in potential, current, or concentration by using three-electrode sensing system [105]. Reference

electrode, counter/auxiliary electrode, and the working electrode are the key components that made up the three-electrode system. Each electrode has its own unique purpose to the system. Reference electrode points to the electrode that has an established electrode potential. Meanwhile, counter electrode is responsible in ensuring that the current will not be able to get through the reference electrode. Along with the working electrode, which is the medium of transportation for the electrons, the three-electrode system will eventually produce the redox reaction inside the electrochemical cell [106], [107]. The redox reaction occurs when there is an electron transfer from one space to another. The three-electrode system work together as the potential of a working electrode is sustained at the same level compared to the Reference electrode by adjusting and balancing the current at counter electrode [108]. When the potential is applied to the system, the electrons will be transferred from one electrode to another inside the solution, which in turn will produce the current through the system.

The potentiostat cell cable can be configured for three-electrode experiments by appropriate connection of the drive and sense lines. To drive current between the working and counter electrodes, the working electrode drive is connected to the working electrode, and the counter electrode drive is connected to the counter electrode. To measure potential between the working and reference electrodes, the working electrode sense is also connected to the working electrode, and the reference electrode sense is connected to the reference electrode [109].

Noting that the three-electrode cell configuration requires both the working electrode drive and sense to be connected at a point very near the working electrode. An easy way to make this connection is to stack the plugs for the working electrode drive and sense together before connecting to the working electrode. Both of these leads must be connected to the working electrode for the potentiostat to properly control the electrochemical cell [110].

Just as mentioned above, our electrochemical set involved a three-electrode cell, where gold electrodes acted as working electrodes, and the platinum ones as counter electrodes. All potentials were controlled vs. Ag/AgCl reference electrode. All measurements were performed at room temperature.

Cyclic voltammetry, square wave voltammetry measurements were performed on a PalmSens (Emstat3) instrument controlled by Ps Trace software.

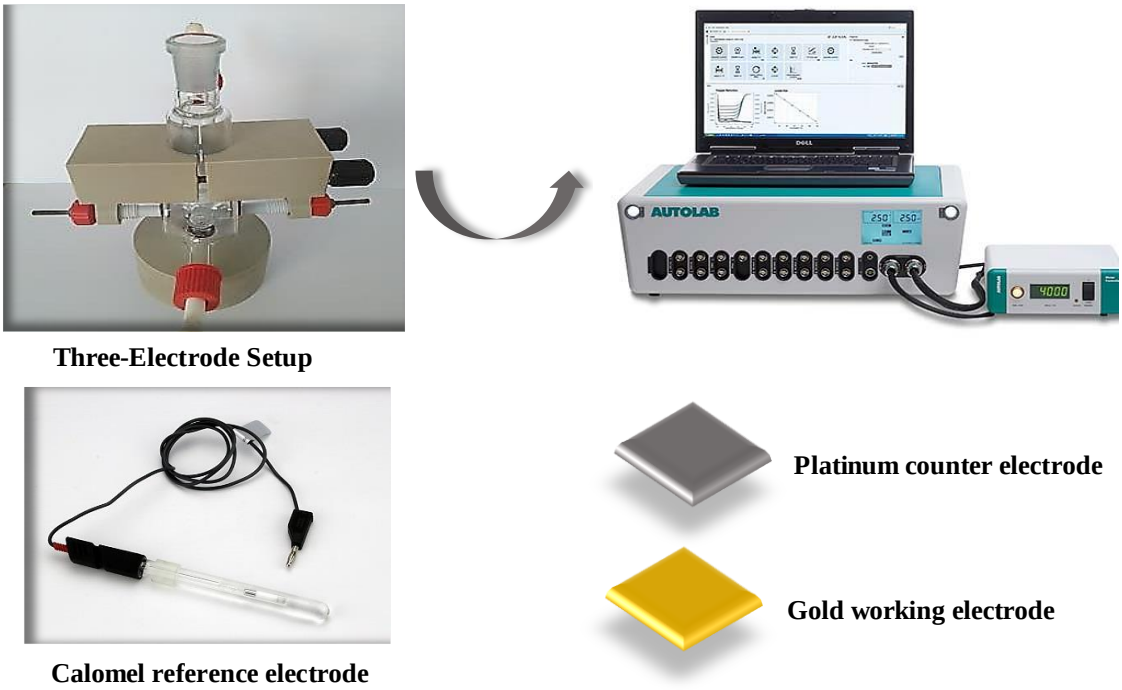

Figure 17. Schematic representation of the construction of electrochemical MIP sensor.

1.3. Poly(3,4ethylenedioxythiophene) and Polyaniline electrosynthesis

Poly(3,4ethylenedioxythiophene) (PEDOT) is one of the widely used conducting polymers for detection devices [111]. It has a low oxidation potential and moderate band gap with good stability and transparency in the oxidized state, high electrical conductivity, excellent thermal stability, intrinsically low thermal conductivity and low price [112]. In parallel, electropolymerization is one of the methods used for the preparation of polymer film with good quality. It allows the reproducible formation of organic polymer films with precise spatial resolution. Moreover, film thicknesses are easily controlled by the deposition charge and the polymer is directly obtained in his conducting state. Thus, electrodeposition protocol of Poly(EDOT) is easier compared to others strategies of electrode modifications. Finally, ethylenedioxythiophene (EDOT) is a commercially available monomer that eliminates synthesis steps [113].

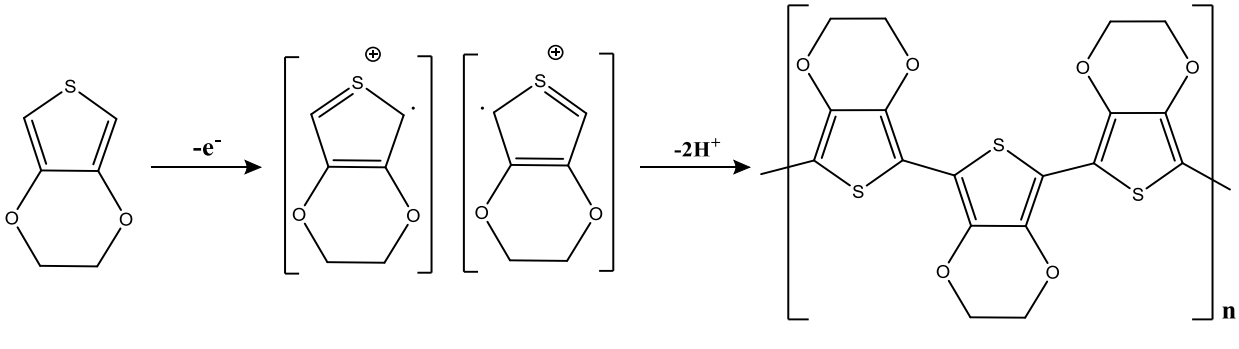

Figure 18. Proposed mechanism of EDOT electropolymerization.

Polyaniline is probably the eldest known electro-conducting polymer, since it was used for textile coloring one century ago. The great interest in research of polyaniline is connected to discovery of its conductivity in the form of emeraldine salt and existence of different oxidation forms Polyaniline obtained by electrochemical polymerization is usually deposited on the electrode [114], however electro-hydrodinamic route was also developed resulting in polyaniline colloids of specific functionalities. Electrochemical polymerization of aniline is routinely carried out in strongly acidic aqueous electrolytes, through generally accepted mechanism which involves formation of anilinium radical cation by aniline oxidation on the electrode. Electrochemical polymerization of aniline is proved to be auto-catalyzed. The experimental conditions, such as: electrode material, electrolyte composition, dopant anions, pH of the electrolyte etc., all have strong influence on the nature of the polymerization process [115]. The low pH is almost always needed for preparation of the conductive polyaniline in the form of emeraldine salt, since it is evidenced that at higher pH, the deposited film is consisted of low chain oligomeric material. The doping anion incorporated into polymer usually determines the morphology, conductivity, rate of the polyaniline growth during electrochemical polymerization, and has influence on degradation process [115].

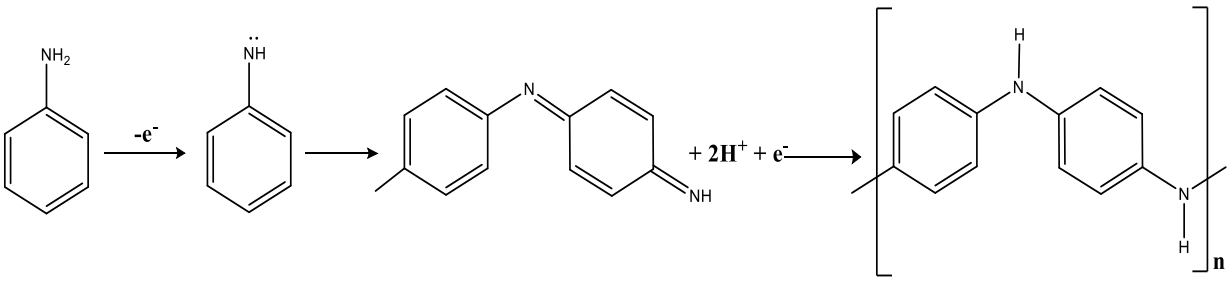

Figure 19. Proposed mechanism of aniline electropolymerization.

However, Poly(EDOT) and Poly(An) present some limitations associated with their low functionality, compability and stability are usually synthesized in the presence of other molecules such as polymer derivatives, dopants and stabilizers. In order to overcome this deficiency in a facial one step approach, we synthesized aniline–EDOT copolymer through in situ co-electropolymerization method. Electrochemical characterizations of the product proved that copolymerization of aniline with EDOT led to a considerable improvement in conductivity, binding properties and stability of the deposited film on gold electrodes compared with poly(An) and poly(EDOT) films [116].

The main motivation for preparing copolymer composites lies in the possibility that these materials will display better properties and also to overcome the limitation of the rareness of new conjugated -bond-containing monomers. The preparation of copolymers from a pair of monomers will lead to an increase of the number of conductive polymers obtained from the same set of monomers. Copolymerization allows chemical modification as well as the introduction of specific functional groups to side substituents to control the physicochemical properties of materials [117, 118].

The co-electropolymerization of EDOT and aniline to copolymer poly(AnEDOT) proceeds through the formation of radical cations by redox reaction, which causes the reorganization of electronic structures, to give two semiquinone radical cations [119]. These radical cations lead to the formation of dimer, which was further oxidized to form stable electrically conducting polymer (Figure).

1.4. Electrosynthesis of Molecularly imprinted conducting polymers and copolymers

In this work, molecularly imprinted conducting polymers or copolymers, MICP-based sensitive layers, were electrochemically obtained by electropolymerization of EDOT monomer in the presence of SMD target molecules (functional monomer, FM = EDOT), by electropolymerization of aniline monomer in the presence of SMD target molecules (functional monomer, FM = An) or by co-electropolymerization of EDOT with An associated by non covalent interactions with SMD target molecules. For clarity, for all functional monomers (FM) used, the obtained Poly(EDOT/SMD) polymers, Poly(An/SMD) polymers and Poly(AnEDOT/SMD) copolymers are noted FM-MICP (or MICP).

For Poly(EDOT/SMD) preparation, FM functional monomer (EDOT) at a concentration of 0.1 M were dissolved in solvent (ethanol), in the presence of $HClO_4$ (0.1 M as supporting electrolyte), together with SMD molecules at a concentration of 0.05 M. A sufficient lap of time of 30 minutes with continuous stirring at a constant speed was used in order to favor the association between FM and SMD, through non covalent interactions. After that, EDOT with template molecules was deposited on the gold electrode surface using cyclic voltammetry in potential range from -0.4 to 1.3 V during 20 cycles (Figure 21).

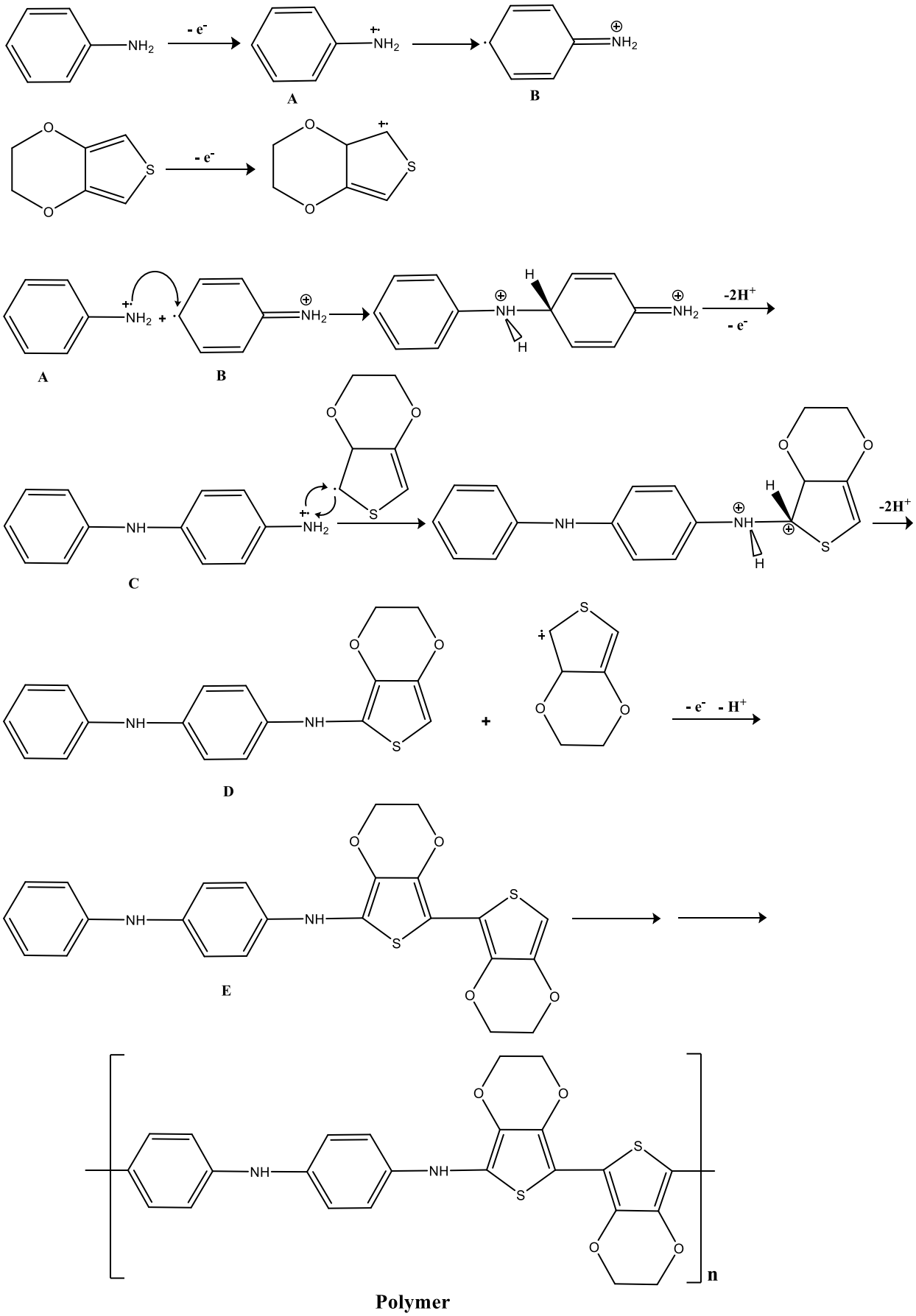

Figure 20. Proposed mechanism of copolymerization of aniline and EDOT.

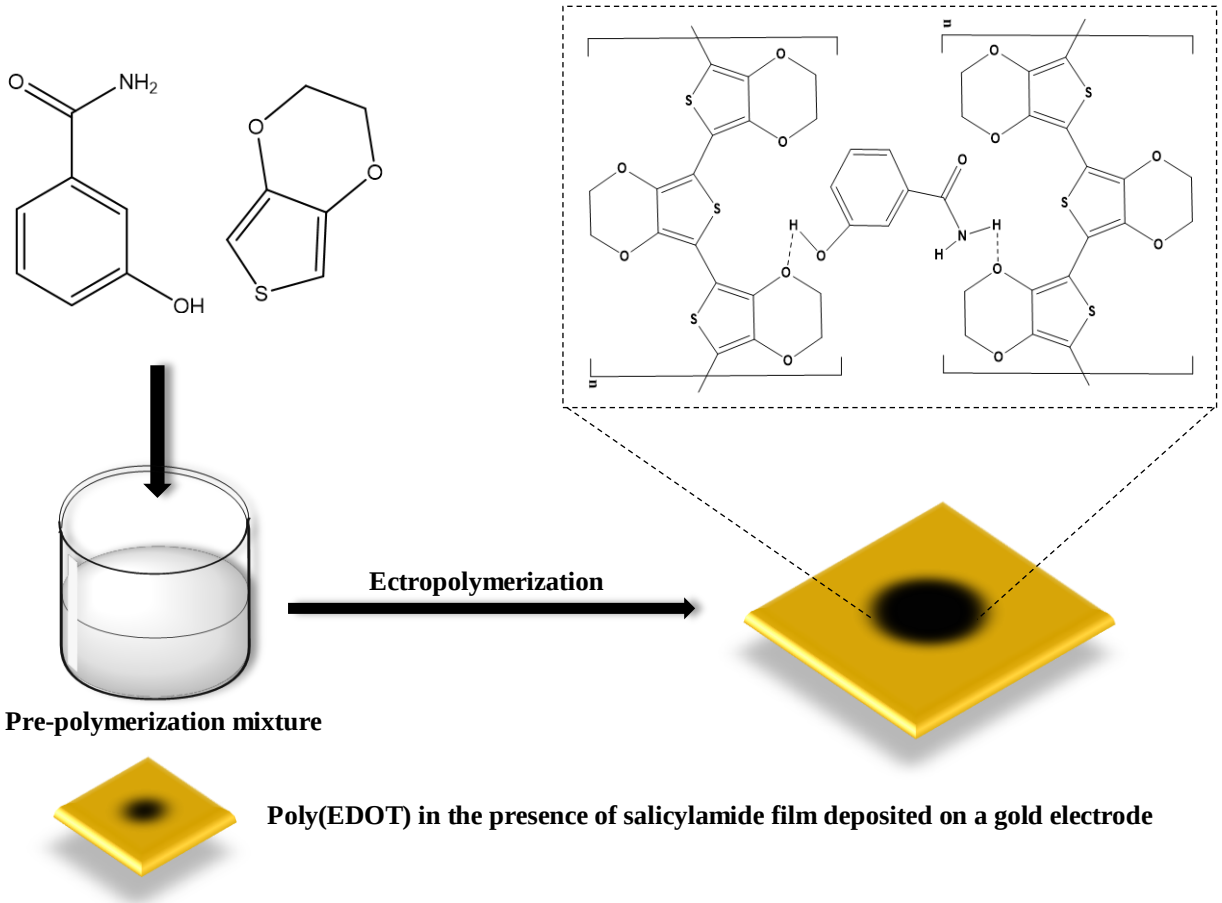

Figure 21. Schematic of the electrosynthesis process of the MI-poly(EDOT) sensor for salicylamide detection.

Aniline and SMD molecules deposition on gold electrode surface was also executed using cyclic voltammetry in potential range from -0.2 to 0.7 V during 20 cycles, and the pre polymerization mixture was formed under similar conditions of electrolyte compositions in the presence of H_2SO_4 (0.1 M as supporting electrolyte), with FM functional monomer (An) at a concentration of 0.1 M. non covalent interactions between FM and SMD as shown in Figure 23.

Poly(AnEDOT/SMD) film was obtained by co-electropolymerization of functional monomers (EDOT) and (An) at a concentration of 0.1 M each, dissolved in solvent (ethanol), in the presence of 0.5 M $HClO_4$ as supporting electrolyte, together with SMD molecules at a concentration of 0.1 M. This time a sufficient lap of time of 45 minutes with continuous stirring at a constant speed was used in order to favor the association between co-polymer and SMD, through non covalent interactions, in FM/SMD pre-polymerization complexe. Electrosynthesis of poly(AnEDOT/SMD) was achieved by electrodeposition on the gold electrode surface using cyclic voltammetry in potential range from -0.6 to 1.4 V during 20 cycles. An illustration of hydrogen bonding between SMD template and co-polymer is presented in Figure 22.

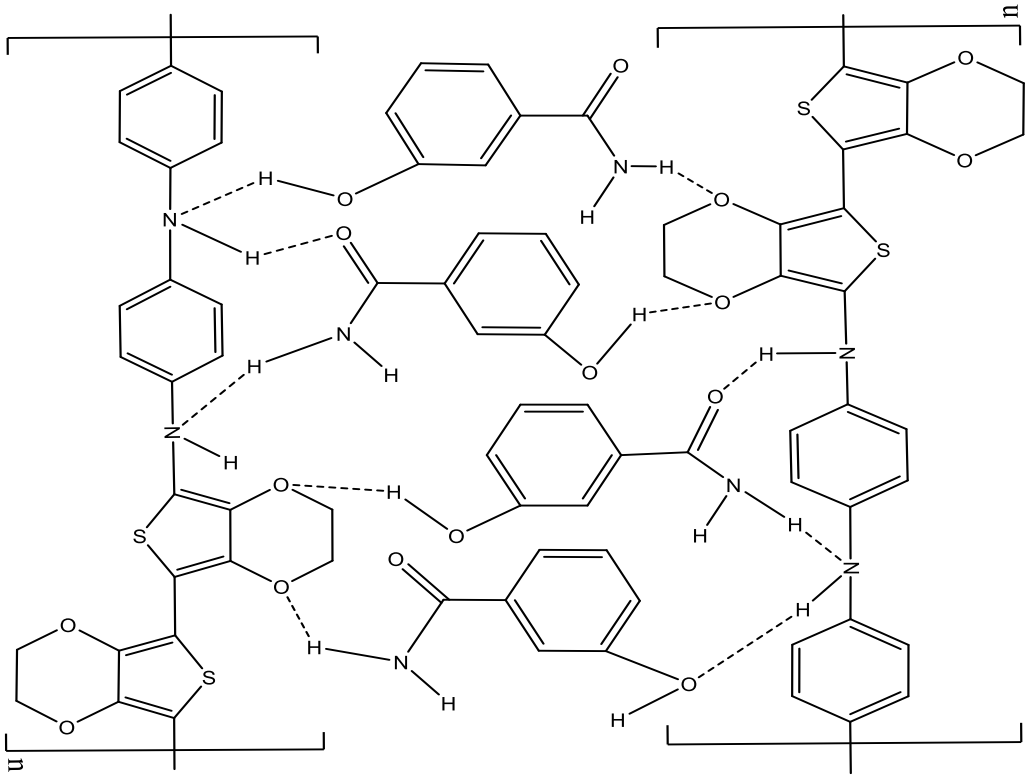

Figure 22. Proposed interaction between salicylamide and poly(AnEDOT) complex.

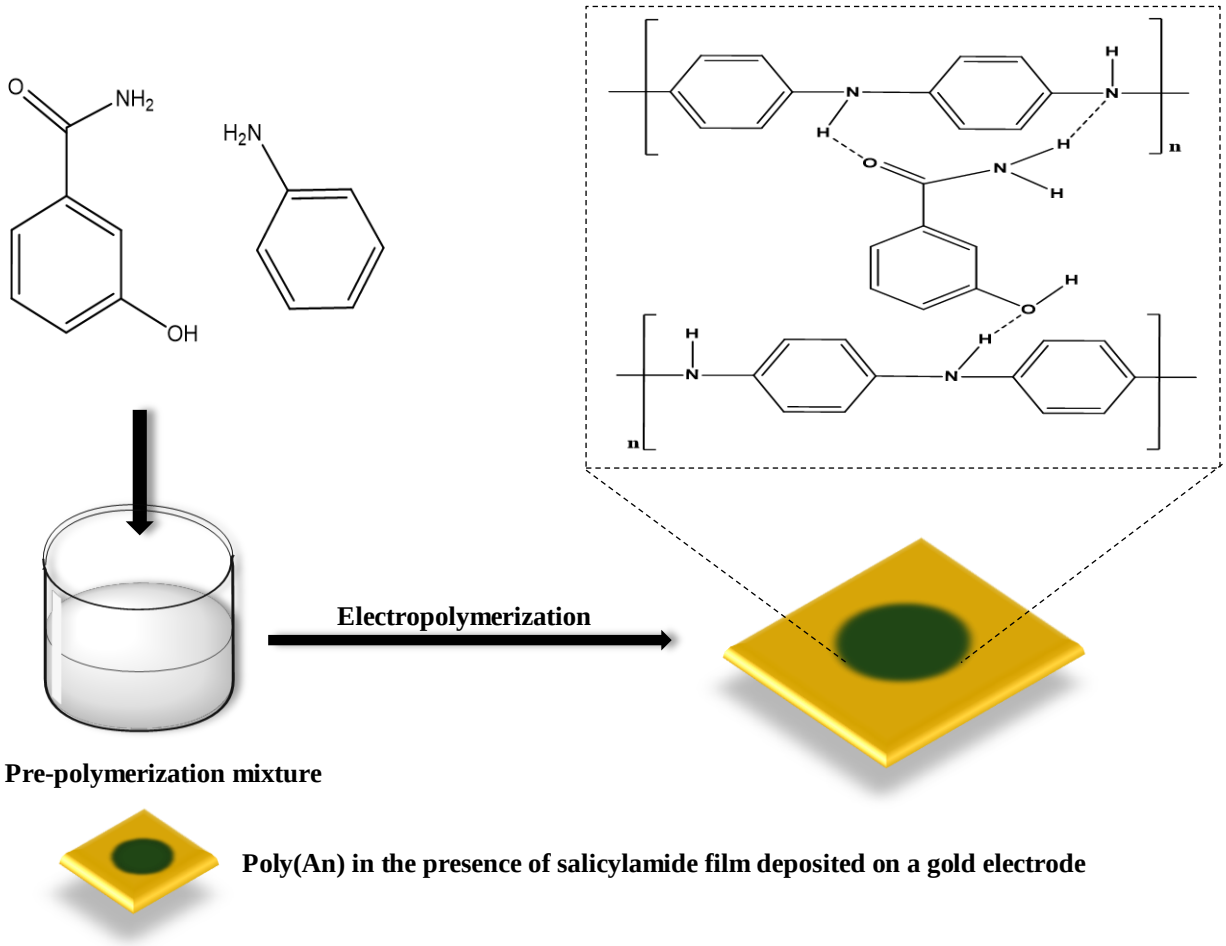

Figure 23. Schematic of the electrosynthesis process of the MI-poly(An) sensor for salicylamide detection.

1.5. Template removal

Template removal is a critical step in the preparation of most molecularly imprinted polymers. The polymer network itself and the affinity of the imprinted cavities for the template make its removal hard. If there are remaining template molecules in the MIPs, less cavities will be available for rebinding, which decreases efficiency. Furthermore, if template bleeding occurs during analytical applications, errors will arise. Despite the relevance to the MIPs performance, template removal has received scarce attention and is currently the least cost-effective step of the MIP development. Attempts to reach complete template removal may involve the use of too drastic conditions in conventional extraction techniques, resulting in the damage or the collapse of the imprinted cavities [120, 121].

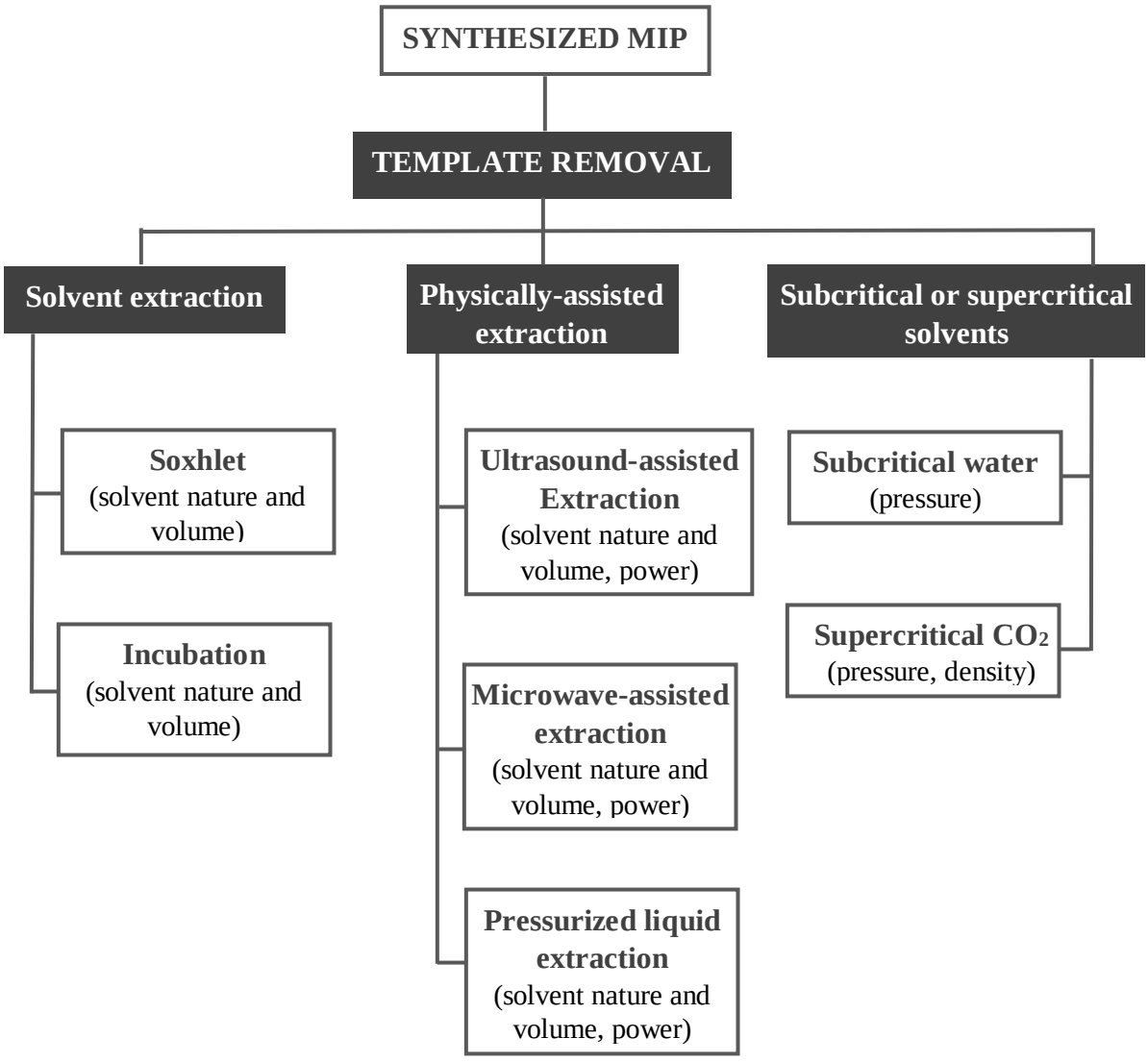

Figure 24. The three main approaches available for template removal: extraction with common solvents, physically-assisted solvent extraction, and extraction with subcritical or supercritical fluids. The variables that determine the yield of each process, in add in [124].

The current state-of-the-art on extraction comprises a wide variety techniques [122], some of them already well implanted in analytical chemistry but still scarcely applied for template removal. Each extraction technique has its own merits and to establish a decision tree for their selection is not easy since the nature and the stability of both the template and the MIP should be considered. Nevertheless, the following general rules should be taken into account: Simplicity of use, short operation time, environmentally friendly solvents, minimum amount of solvent, low economic cost, and possibility of becoming used at the industrial scale [123].

In order to remove the target molecules from FM-MICP matrixes, by destroying the non covalent intermolecular interactions which involve SMD and polymerized functional monomers, acetonitrile was used to wash the polymer-coated electrodes for 30 min at room temperature. This washing step leads to the desired creation of molecular imprinted cavities within the copolymer matrixes of FM-MICP (Figure 25). These cavities keep the memory of the interactions between FM functional monomers and SMD molecules. Indeed, the spatial distribution of the polymerized functional monomers into the polymer matrixes allows the precise matching of additional SMD molecules. Thus the so-prepared washed FM-MICP modified electrodes can act as sensors to quantify the presence of new additional SMD molecules thanks to the establishment of new intermolecular FM/SMD interactions between the probe-functionalized monomer units and the target molecules (Figure 26, Electrosynthesis of MI poly(AnEDOT), SMD detection).

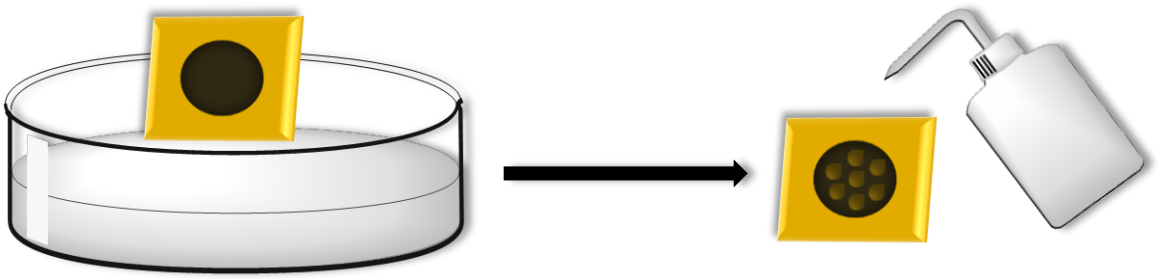

Figure 25. Schematic representation of salicylamide solvent extraction process.

In order to check the ability of MI-poly(AnEDOT) to interact with newly added SMD targets, the modified electrode was immersed for 10 min in 13.7 ppm SMD solution at room temperature. The presence of added template targets and consequently the specific recognition process was then analyzed using an electrochemical method (chapters 3).

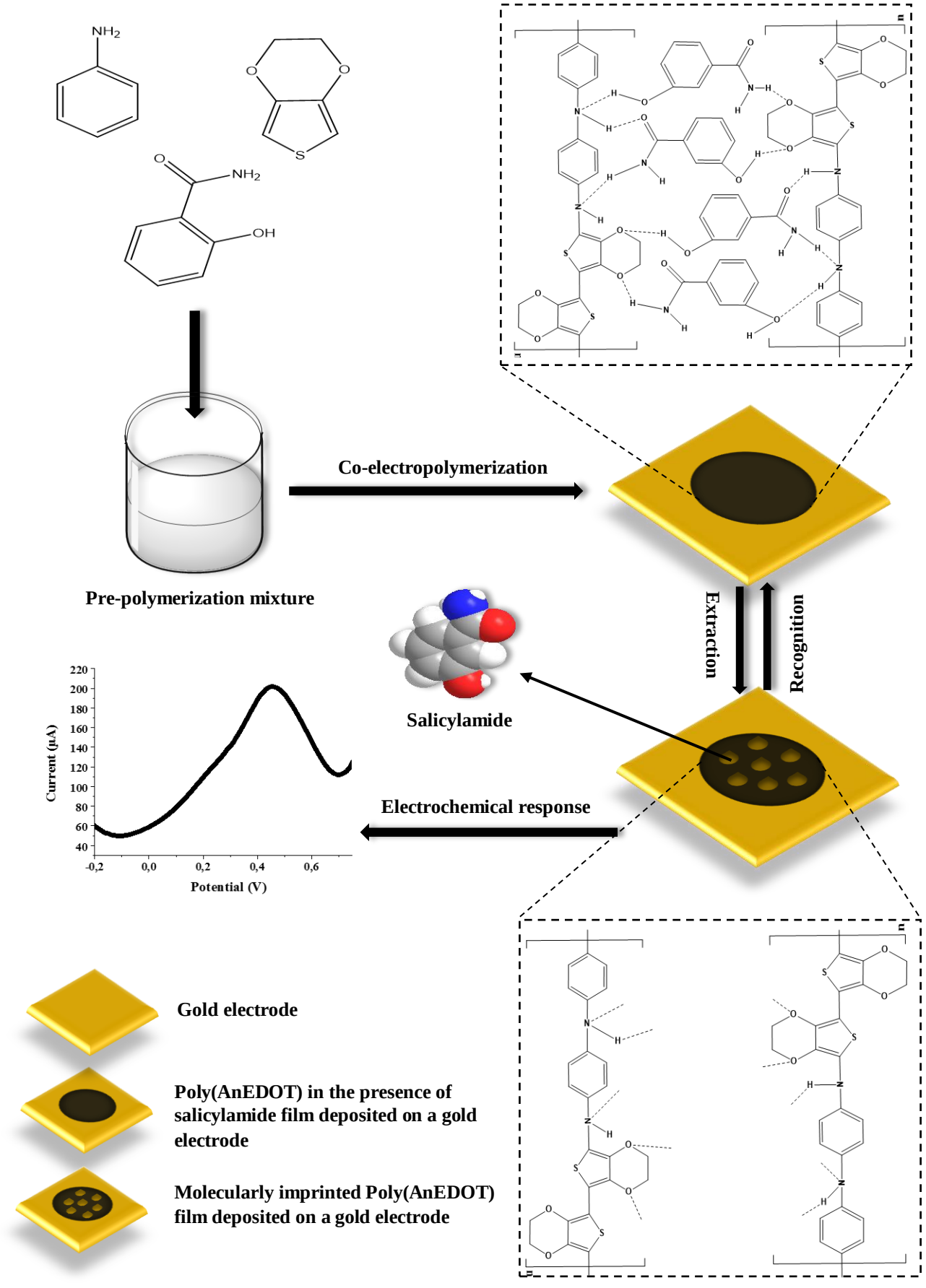

Figure 26. Schematic of the electrosynthesis process of the MI-poly(AnEDOT) sensor for salicylamide detection.

Contrarily to MI-poly(AnEDOT), Non-imprinted -poly(AnEDOT) layers are synthesized in the absence of SMD molecules. As a consequence, no specific cavities are presented in the structure of FM-NICP layers. In order to check the ability of FM-NICP to interact with newly added SMD target molecules, the modified electrodes were immersed for 10 min in 10^{-4} M SMD solution at room temperature. The presence of added pesticides and consequently the non-specific recognition process was analyzed using an electrochemical method (chapter3).

1.6. Electrosynthesis of Non-imprinted conducting polymers

Another type of film, non-imprinted conducting copolymer, NICP, was also electrochemically prepared, but in the absence of target molecules in the pre-polymerization media (Figure 21, electrosynthesis). NICP polymers were synthesized by co-electropolymerization of EDOT and An in the absence of SMD molecules.

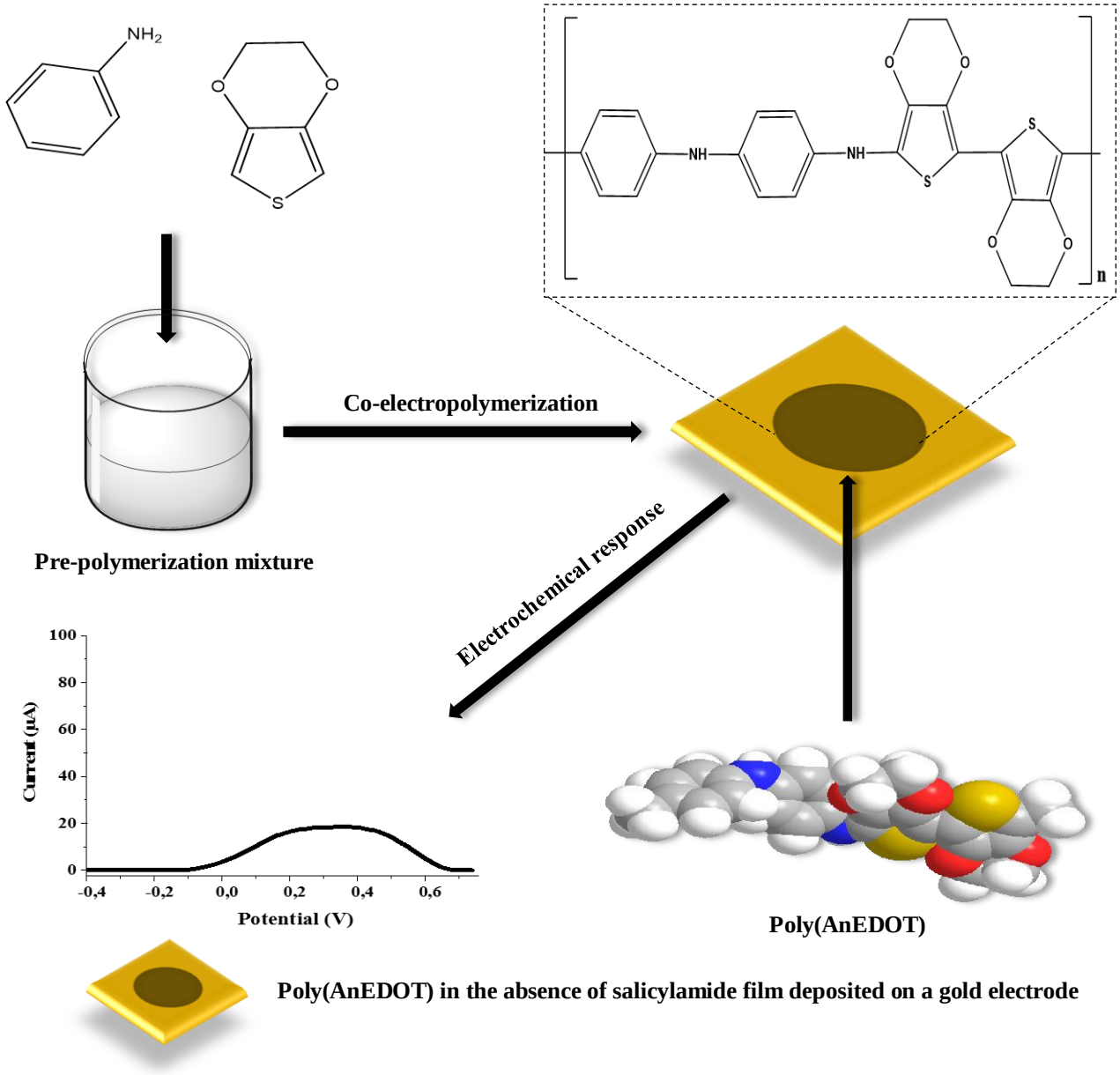

Figure 27. Schematic of the electrosynthesis process of the NI-poly(AnEDOT) sensor for SMD detection.

For NICP preparation, FM functional monomers (EDOT, An) at a concentration of 0.1 M each were dissolved in solvent (ethanol), in the presence of $HClO_4$ (0.5 M) but in the absence of SMD molecules. The electrodeposition on the gold electrode surface was executed using cyclic voltammetry in potential range from -0.6 to 1.4 V during 20 cycles.

1.7. Measurement of salicylamide using poly(AnEDOT) film coated electrode

The fabricated sensor was tested at different concentration of salicylamide ranging from 2×10^{-6} to 1×68.5 ppm, using electrochemical cell with SWV mode. the sensor response was monitored by PS Trace software. The potential range employed was -0.6 to +1 V vs. Ag/AgCl with the modulation amplitude at 0.05 V. The measurements were carried out using pH 5 PBS (0.5 M) solution at the electrochemical medium. The electrode coated with poly(AnEDOT) possessing salicylamide imprinted sites was on the working Au electrode and the platinum and Ag/AgCl was the counter and reference electrodes. The potential change in the electrochemical cell by different concentrations of salicylamide was determined and the resulted data was normalised to construct standard calibration curve. Besides, the limit of detection was calculated using the equation described in the next chapter.

1.8. Selectivity of the MICP sensor

The MICP sensor was tested for other pharmaceuticals such as ibuprofen, naproxen, sulfanilamide and ascorbic acid with the concentration ranged from 2×10^{-6} to 1×68.5 ppm. The MICP sensor response was compared to non-NICP sensor to determine the degree of effect of salicylamide imprinting [125].

1.9. Analysis of real samples

The prepared MICP sensor was used to test Tap water samples for salicylamide with the concentration ranged from 2×10^{-6} to 1×68.5 ppm and the resulted data was normalised to construct standard calibration curve. It is worth nothing that the pH of the water samples needs to be adjusted to 5. The salicylamide in the real water sample was measured by SWV, and the was calculated to confirm the accuracy of the prepared MICP sensor for practical use [126].

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Chapter 3

Results and Discussion

1. Introduction

In this chapter experimental results will be presented and discussed in order to optimize the electrosynthesis as well as the properties of the sensing layers based on molecularly imprinted conducting polymers. A detailed discussion will be presented on the characteristics of the sensing layers which lead to the obtained properties of the sensor. Also, the electrochemical transduction of the sensor will be demonstrated with a study of the recognition properties, selectivity, sensitivity, repeatability and reproducibility.

1.1. Results and discussions

1.1.1. Electrochemical polymerization of composite thin films

1.1.1.1. Molecularly imprinted poly(EDOT) thin film electrosynthesis

Figure 28 shows the cyclic voltammogram registered during the electropolymerization of poly(EDOT) on the surface of gold electrode within the potential ranging from -0.4 V to 1.3 V at a fixed scan rate of 0.08 V/s in 0.1 M $HClO_4$ electrolyte consisting of 0.1 M EDOT and 0.05 M SMD. The amount of current at given potential differs, and the charge increased as a function of the number of cycles, which is typical during the electrochemical formation of conducting polymers [112]. Two broad peaks of current density are observed due to doping-undoping process. After 20 cycles in ethanol, a dark coloured polymer film is observed on the gold electrode surface.

1.1.1.2. Molecularly imprinted poly(An) thin film electrosynthesis

Voltammogram of aniline electropolymerization for 20 cycles within the potential ranging from -0.2 V to 0.7 V at a fixed scan rate of 0.05 V/s in 0.1 M H_2SO_4 electrolyte consisting of 0.1 M An and 0.05 M SMD are shown at Figure 29. The current is increased gradually for further cycles. It means the polyaniline layers have formed on the surface of gold electrode successfully. There are two peaks observed in anodic and only one peak in cathodic sweep. Anodic peak observed at +0.37 V with current responses from 212 to 367 μA, and at +0.42 mV with current responses from 215 to 400 μA. The cathodic peak was found at 0.2 V and with current responses from -220 to -370 μA. Peak at +0.42 V is the oxidation peak of salicylamide while peak at +0.37 V is the oxidation peak of leucoemeraldine (Polyaniline in fully reduced state) which is oxidized into emeraldine (Polyaniline in semioxidized state) [115].

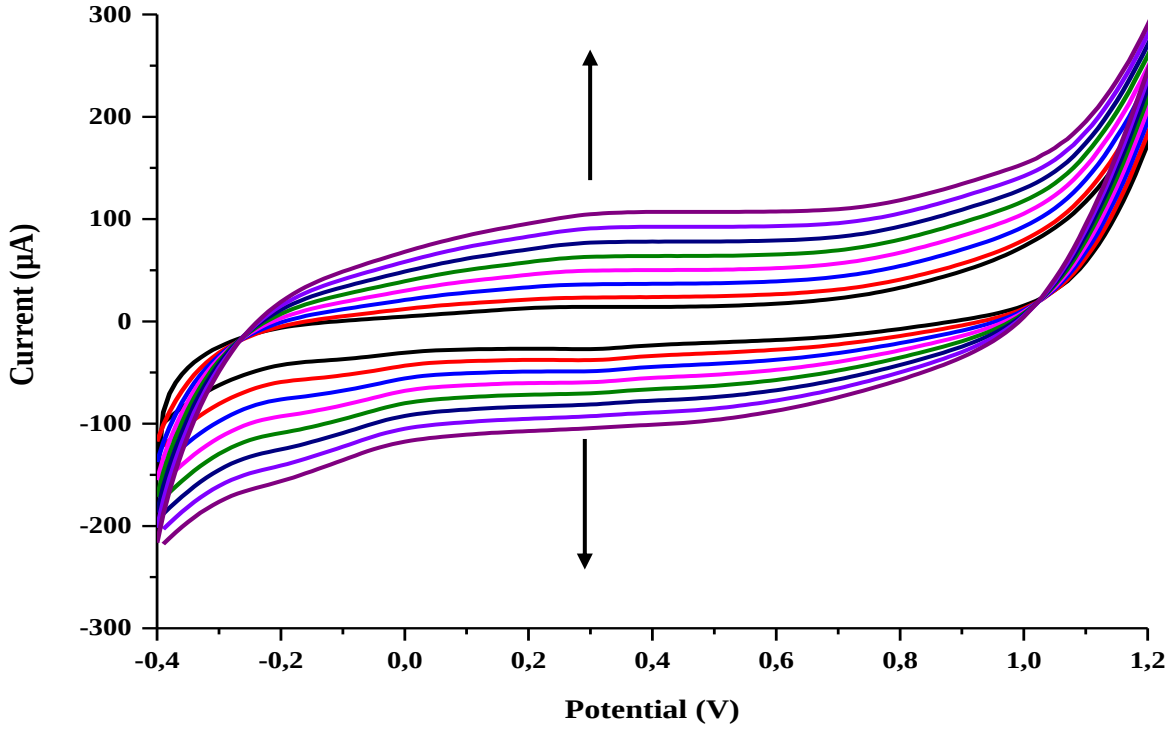

Figure 28. Voltammogram of EDOT electropolymerization on the surface of gold electrode in the presence of salicylamide template.

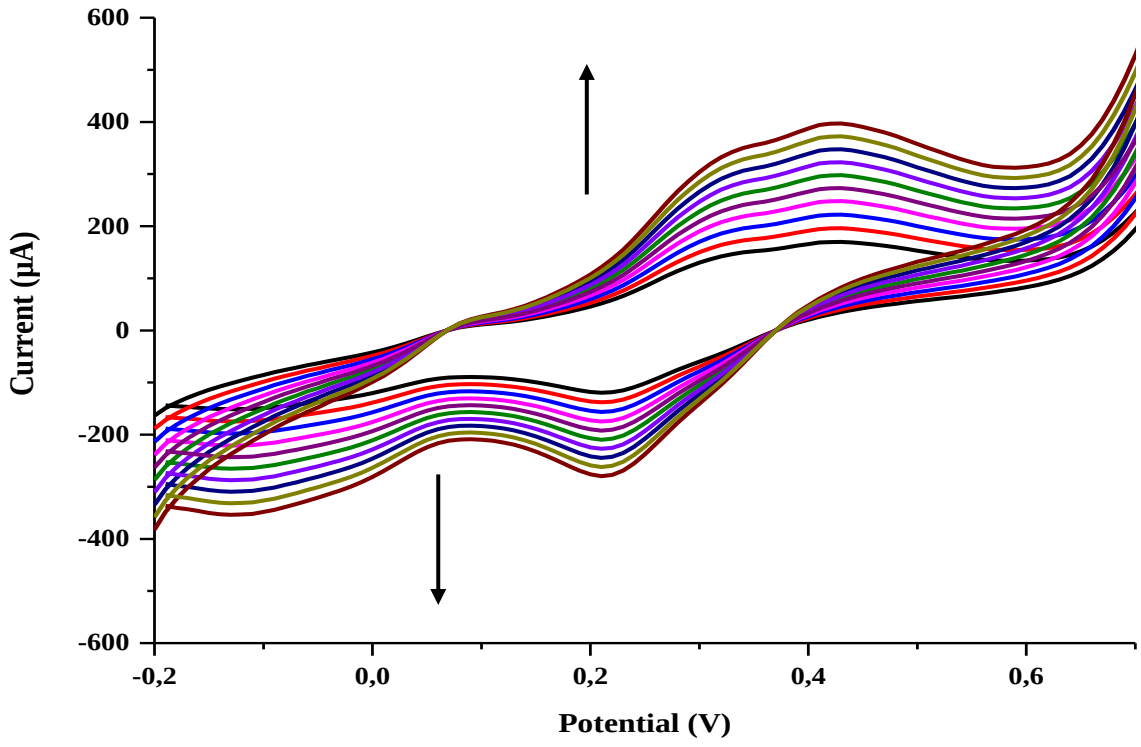

Figure 29. Voltammogram of aniline electropolymerization on the surface of gold electrode in the presenc salicylamide template.

1.1.1.3. Molecularly imprinted poly(AnEDOT) thin film electrosynthesis

The co-electropolymerization of aniline and EDOT in the presence of salicymalide leads to the inclusion of the template molecule in the copolymer matrix during its growth. The intention of co-electrosynthesizing poly(AnEDOT) thin film (following the protocol mentioned in chapter 2) was to bring together different units such as -C=O-, -N-, -NH- and -OH to interact with SMD in multiple ways (chapter 2, Figure 26), the electrochemical co-polymerization on the gold electrode surface was performed by cyclic voltammetry with and without the presence of the template SMD in the co-monomer solution, see Figures 30 and 31. In both cases, we can observe the anodic current that increase after 0.38 V vs Ag/AgCl due to the oxidation of the aniline on gold electrode with the current peak at 180 µA (both MIP and NIP), and the increase in the anodic current after 0.9 V vs Ag/AgCl is due to the oxidation of EDOT with the current peak at 138 and 200 µA respectively in the solutions without and with 0.1 M of the SMD template. The well-known reversible oxidation-reduction behavior of polyaniline [115] is also clearly observed at around 0.25 V vs Ag/AgCl for the reduction process in our MIP-SMD electrode. The potential shift is probably due to the presence of salicylamide in the solution which itself is oxidized at a potential around 0.4 V, see below, Figure 30. In both voltammograms of MIP and NIP, co-electropolymerization shows that the aniline and EDOT oxidation peaks increase continuously due to the change in the nature of the electrode surface after the deposition of the copolymer.

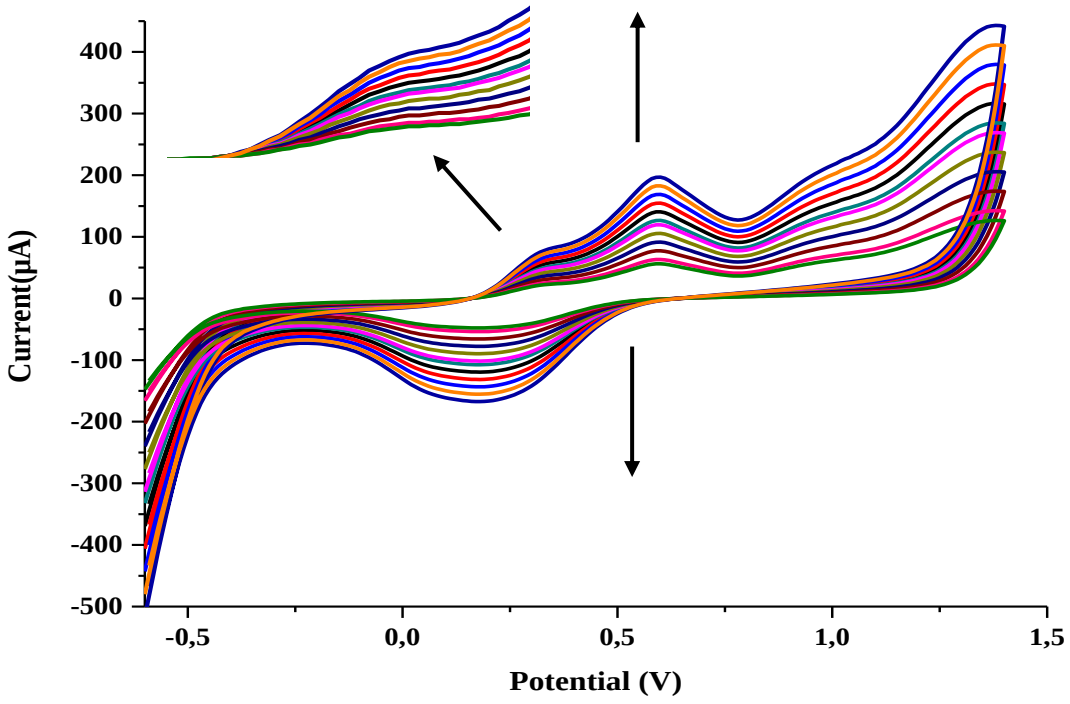

Figure 30. Voltammogram of aniline and EDOT co-electropolymerization on the surface of gold electrode in the presence of salicylamide template.

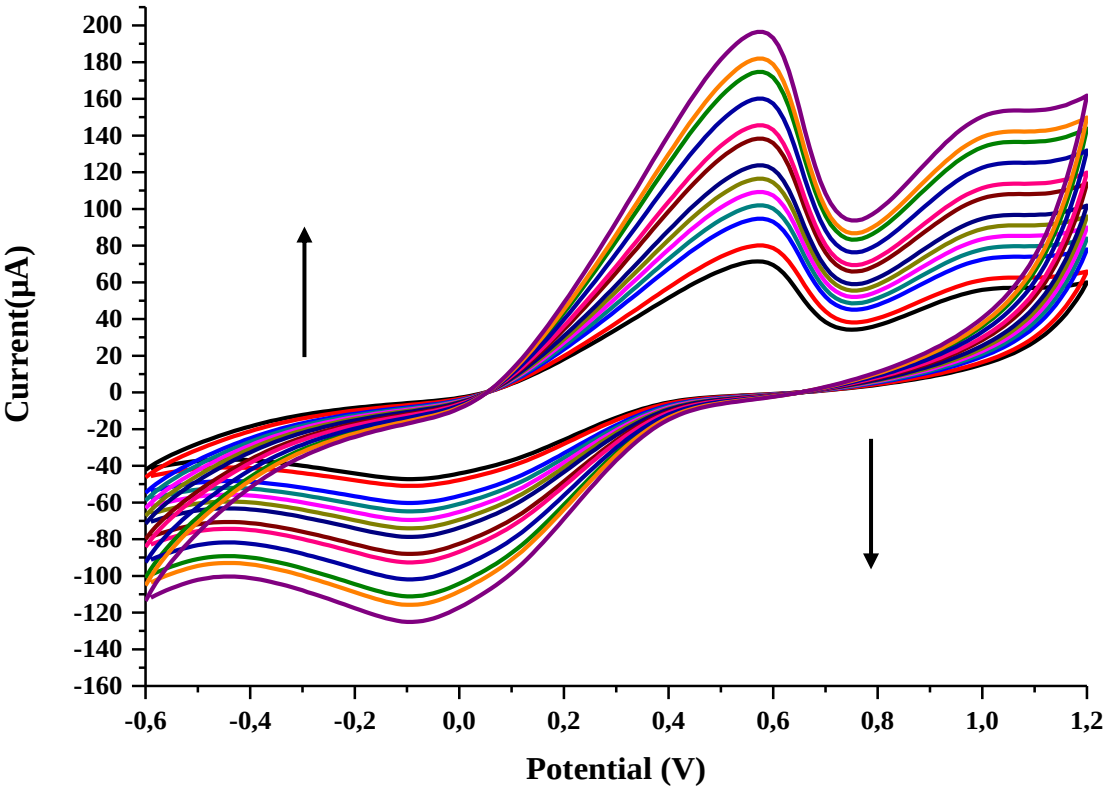

Figure 31. Voltammogram of aniline and EDOT co-electropolymerization on the surface of gold electrode in the absence of salicylamide template.

1.1.2. Extraction of the template molecule

The template extraction was achieved by the procedure described in chapter 2 by solvent extraction. From SWV measurements We observed an oxidation peak, at around 0.45 V vs Ag/AgCl, corresponding to the salicylamide trapped within the copolymeric matrix (Figure 32). The follow up of variations of peak currents determined, versus extraction duration in acetonitrile solvent, indicate that extracting SMD during30 min is the most appropriate compromise between template extraction and keeping intact the poly(AnEDOT) matrix. Actually, a duration of 30 min permits the reduction in the current peak attributed to SMD oxidation by about 93% (Figure 33) without altering the morphological structure of the MICP. This method of template extraction is easy to achieve and avoids the use of a variety of chemical reagents [127]. Moreover, the voltammograms obtained validated the removal of the template.

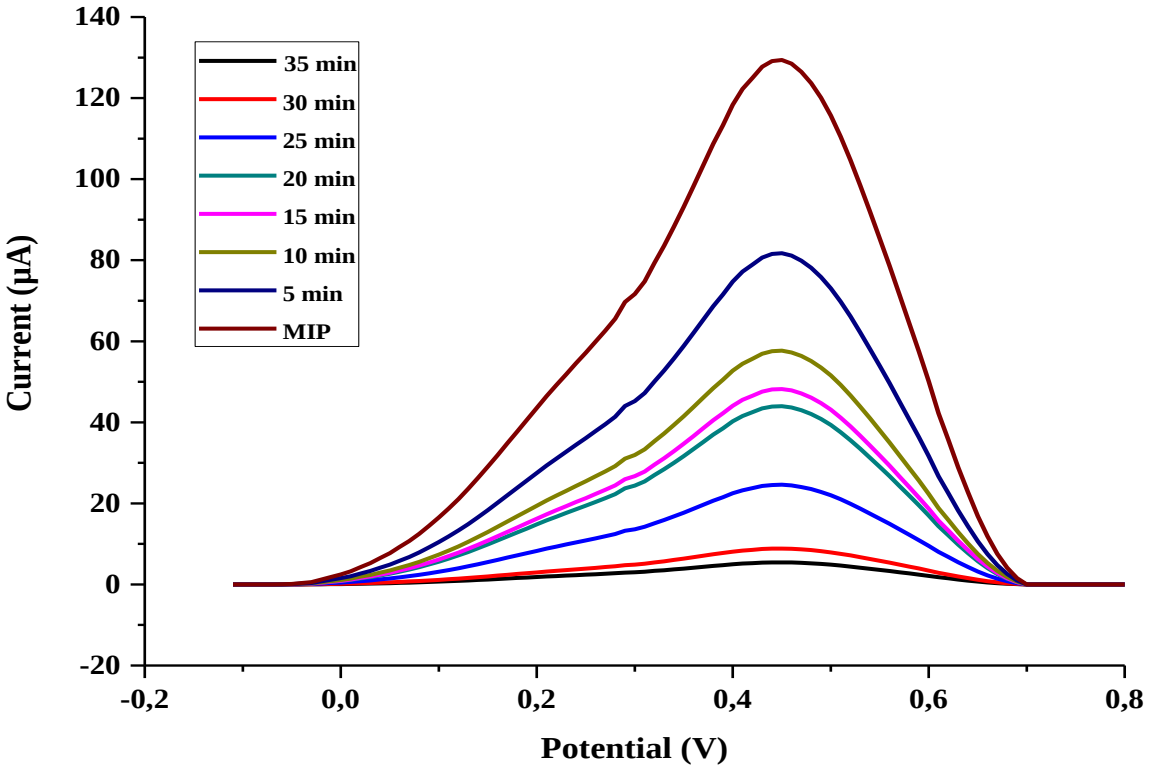

Figure 32. Follow-up of square wave voltammetric peak current variation during the extraction in acetonitrile.

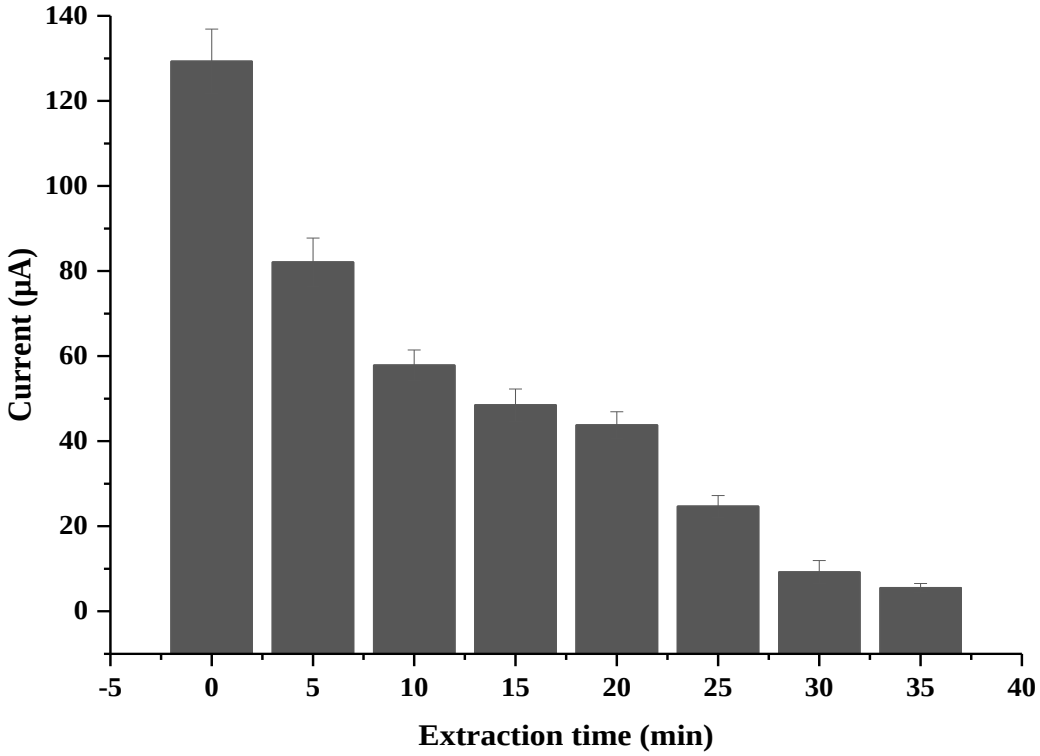

Figure 33. Follow-up of the peak current variation versus the extraction duration in acetonitrile. Each value of the histogram, represented with its error bar, corresponds to the mean value obtained from three experiments.

1.1.3. Electrochemical Characterization of Modified Gold Electrodes

Electrochemical technique namely CV was used for the characterization of modified gold electrodes using both MICP and NICP technologies in the presence of $[Fe(CN)_6]^{3}$ /$[Fe(CN)_6]^{4-}$ as a probe (Figure 34). Electrocatalytic property of bare gold electrode surface without any modification, MCIP modified gold electrode toward SMD and NICP was checked using CV by cycling within the potential range from -0.6 V to 1.4 V at a fixed scan rate of 0.2 V/s. The gold bare electrode shows a pair of well-shaped redox peaks (curve in black color), The formation of MIP film on gold electrode resulted in an incitement of the ferro/ferricyanide redox peaks, indicating the deposition of a highly conducting polymer film on the gold surface, which impeded the electron transfer of the redox probe (curve in blue color). When the MICP modified electrode soaked in acetonitrile for 30 min to remove template molecules, the oxidation and reduction peak current increase obviously with the disappearance of the oxidation peak of salicylamide due to the emptied cavities, which formed after template removal (curve in fuchsia color), and the absent of SMD oxidation peak in case of NICP film (curve in maroon color) confirms the formation of SMD imprinted poly(AnEDOT) membrane on the gold electrode surface.

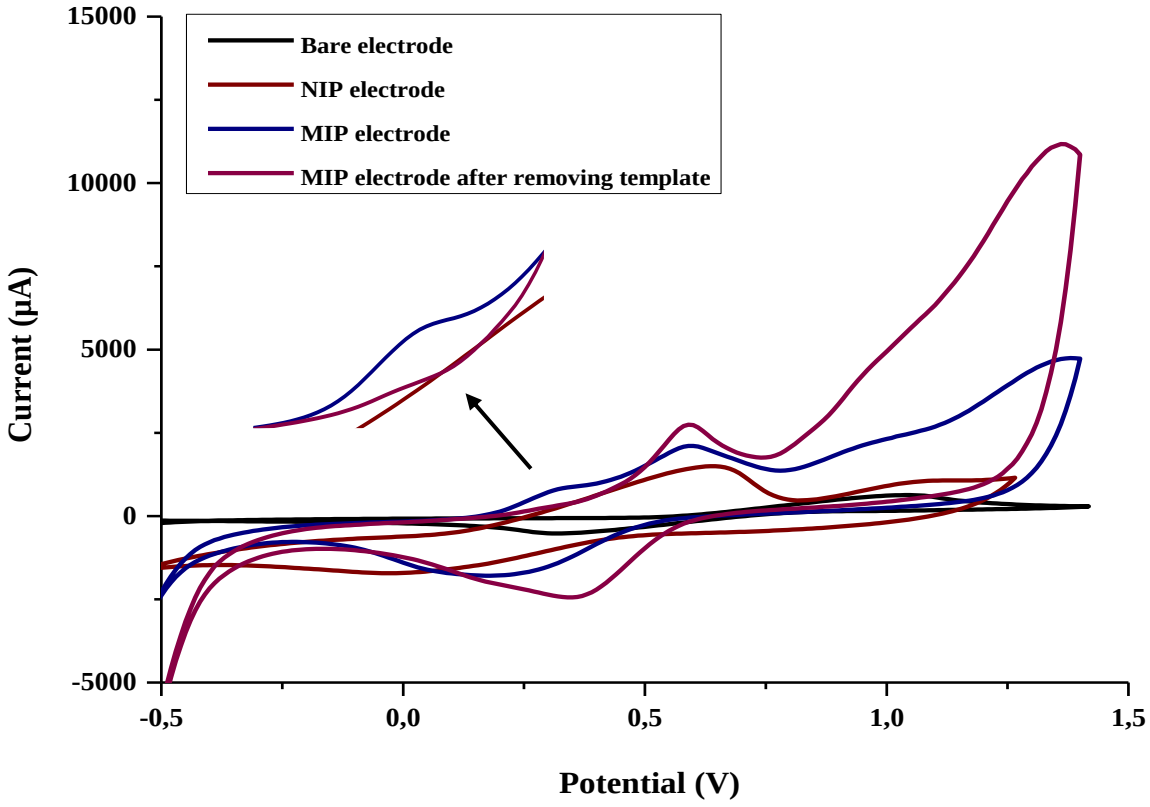

Figure 34. Cyclic voltammograms in at a fixed scan rate of 0.2 V/s for bare gold electrode without any modification, NICP electrode, MICP electrode before and after template extraction.

1.2. Salicylamide Detection

Prior to SMD detection, we have tried to assess the possible effects of non-specific adsorption between SMD analytes and the poly(AnEDOT) matrix. For this purpose, we have prepared two electrodes. The first one was functionalized with a poly(AnEDOT) MIP before being extracted with the acetonitrile solvent and then incubated during 30 min in a 13.7 ppm solution of SMD. This electrode was characterized by SWV at each of the above-mentioned steps. The second one, coated with NIP, was also characterized by the same technique before and after incubation.

The examination of the voltammograms presented in Figure 35 clearly shows the oxidation peak of SMD at 0.45 V, appearing for the MIP containing the template. This peak is quasi-absent in the response of the extracted MIP (red line), indicating an almost total extraction of the template. The oxidation peak reappears after the incubation of the extracted polymer in a SMD solution, with the same current peak magnitude than the MIP (before extraction). These results indicate that it is possible to perfectly remove and uptake SMD molecules from the vicinity of the designed MIP, thus offering accessible sensing cavities.

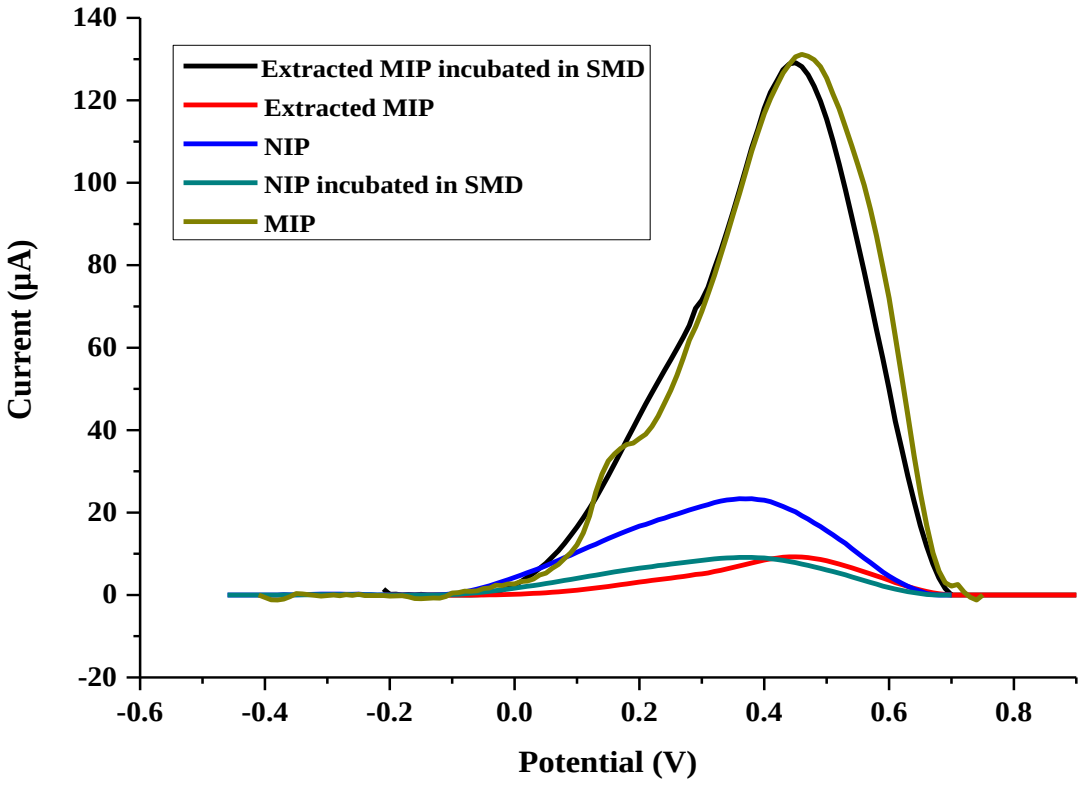

Figure 35. Square wave voltammograms of a MIP-SMD film coated electrode, before and after SMD extraction, a NIP, and the further extracted MIP incubated in a 13.7 ppm solution of SMD.

As expected, the voltammogram of the non-imprinted polymer (NIP) incubated in a SMD solution shows a slight oxidation peak, which is comparable to that of the extracted MIP. This can be attributed to the nonspecific adsorption, and can be explained by the fact that at pH 5 (the pH at which the measurements were done), the functional carboxyl groups of SMDs are negatively charged and can interact with the positively-charged N-H groups of the polyaniline units present at the surface. This value remains, however, largely lower than that of the MIP after incubation in SMD solution, indicating the weak character of the above-mentioned interaction.

The sensing performance of MICP and NICP films was determined using salicylamide solution of different strengths i.e., 2×10^{-6} to 1×68.5 ppm in the electrochemical cell in the presence of pH 5 PBS (0.5 M) buffer solution (pH adjusted using 0.1 M H_2SO_4).

SWV measurements were used to determine the corresponding oxidation current for several additions of SMD concentrations (Figure 36). For the sensor based on molecularly imprinted poly(AnEDOT) film, the voltammograms exhibit a single well-defined anodic peak at about 4.5 V, which is gradually increasing with increase in concentrations of SMD, translating the selective binding of salicylamide molecules, the MIP's functionalized cavities have, thus, recognized the further SMD analytes. Calibration curve for SMD detection is obtained by plotting the oxidation current determined from SWV against logarithms of SMD concentrations using the poly(AnEDOT) film modified gold electrode. As shown in Figure 38, a linear range was observed with the regression equation I (μA) = 134.19666+ 22.22482 Log [SMD] ppm (R^2=0.99926). The linearity might be due to the presence of homogeneous binding sites for SMD formed during the co- electropolymerization.

For the sensor based on non-imprinted poly(AnEDOT) film, SWV voltammograms showed a slight oxidation peak that can be explained by the non-selective adsorption of SMD anlytes. Which is incomparable to the intense response of the sensor based on extracted molecularly imprinted poly(AnEDOT) membrane. a linear range was observed with the regression equation I (μA) = 18.03835 + 2.51058 Log [SMD] ppm (R^2=0.9997) from the calibration curve showed in Figure 37, which can be explained in this case by the linearity that might be due to the presence of homogeneous non-selective binding sites during co-electropolymerization of aniline and EDOT.

Figure 39 shows the relationship between the peak currents measured by SWV and the logarithms of the corresponding SMD concentrations. It can be noticed that the current response has a good linear relationship with logarithms of SMD concentrations.

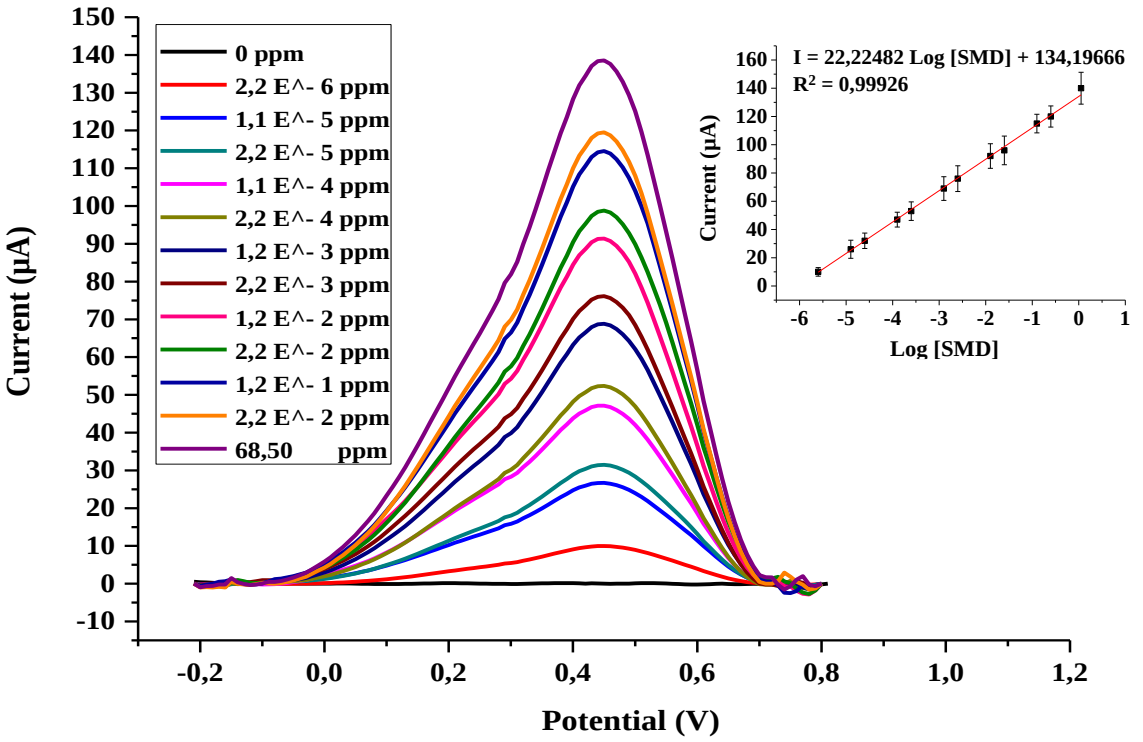

Figure 36. Square wave voltammetry response to the SMD analyte of different concentrations at the MIP-SMD film coated electrode in pH 5 phosphate-buffered saline solution.

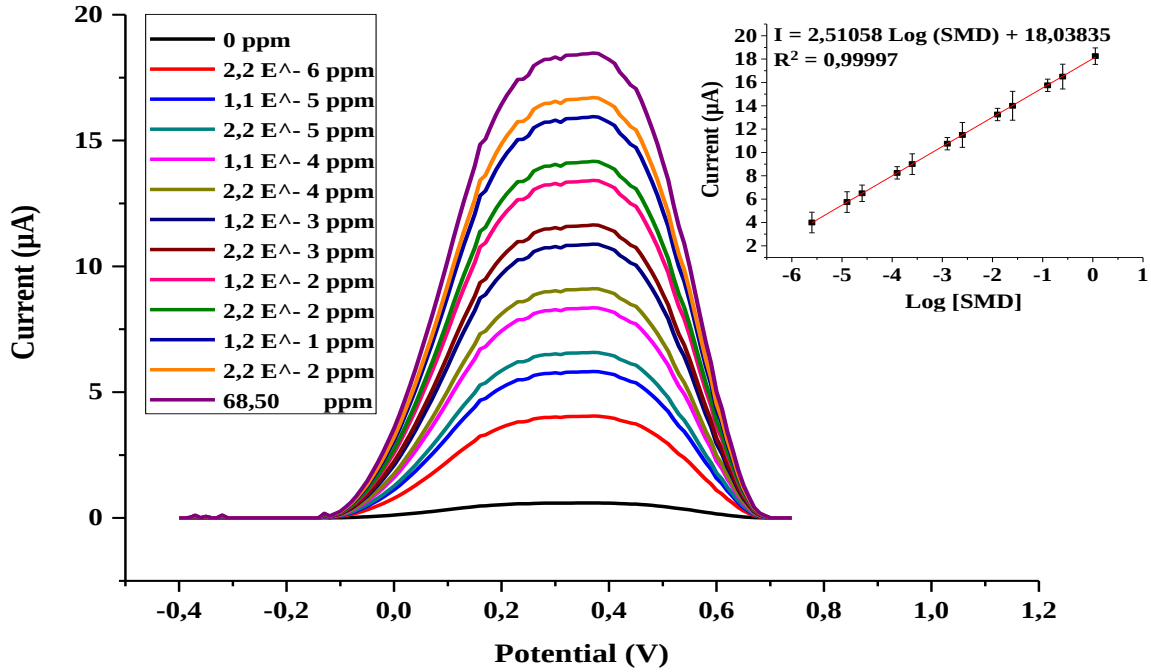

Figure 37. Square wave voltammetry response to the SMD analyte of different concentrations at the NIP film coated electrode in pH 5 phosphate-buffered saline solution.

The great and obvious difference between NICP and their relative MICP shown in Figures 38 and 39 was definitely attributed to imprinting easily proving that the MICP exhibited a higher adsorption capacity for SMD.

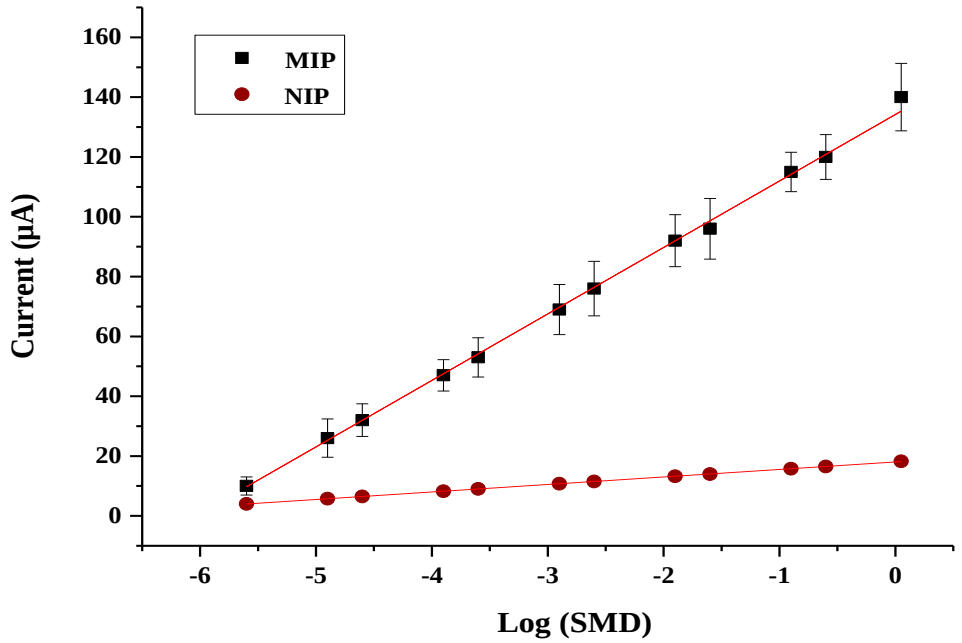

Figure 38. Calibration curves for SMD at the MIP-SMD and NIP film coated electrodes in pH 5 phosphate-buffered saline solution. Each value of the histogram, represented with its error bar, corresponds to the mean value obtained from three experiments.

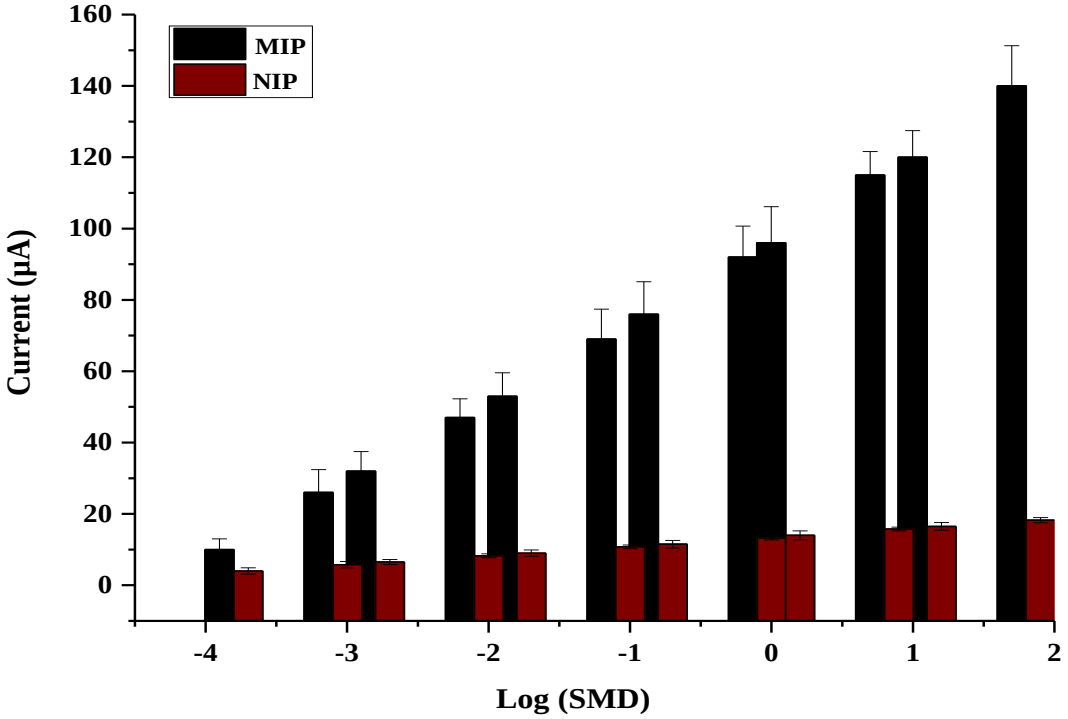

Figure 39. Relative charges corresponding to the detection by molecularly imprinted poly(AnEDOT/SMD) and non imprinted poly(AnEDOT) of Salicylamide (SMD), at pH 5 PBS buffer. Relative charges were deduced from SWV. Each value of the histogram, represented with its error bar, corresponds to the mean value obtained from three experiments.

1.3. Salicylamide detection mechanism by molecularly imprinted poly(AnEDOT) film

There are hydrogen bond donor/acceptor interaction between molecularly imprinted poly(AnEDOT) film and SMD as well as π-donor/π-acceptor interaction between polyaniline moieties and SMD molecules as has been shown in the previous chapter. The conductivity of the poly(AnEDOT) film is partly based on polyaniline, a conjugated polymer electron donor. This conductivity is increased when the concentration of SMD molecules in the polymer increases which is reasoned out to be due to the electroactivity of the SMD molecule. Electroactive dopants have a significant effect on the conductivity, morphology and stability of polyaniline-based polymer films [128]. These properties depend on the molecular size and polar characteristics of the dopants. Furthermore, the delocalization of charges forms acceptor type sites and causes the events of electron transfer rates of to be increased when the amount of SMD increases. This event has been supported by the increase of electron transfer rate for poly(AnEDOT) film when concentration of nitrogen containing aromatic compounds increases [129].

1.4. Selectivity of molecularly imprinted poly(AnEDOT) film towards SMD

The important performance of MIP sensors is the selective recognition of template molecules [125]. The MICP-based sensitive layer enables the quantitative detection of SMD, due to the presence of pre-shaped functionalized cavities in the MICP backbone. The washing step of MICP allows the extraction of SMD molecules from the copolymer, which leaves molecular prints of SMD in the matrix of the copolymer. These prints are specific, and possess a precise 3-Dimensional distribution of the functionalities able to engage hydrogen bonding with their counterparts, in SMD target molecules.

In order to evaluate the selectivity of our proposed sensor to SMD template, four different sensors based on molecularly imprinted poly(AnEDOT) using SMD as the template were prepared with the same characteristics following the same conditions, interference experiments were carried out in the presence of two anti-inflammatory drugs; ibuprofen (IBP) and naproxen (NA), an antibacterial medication; sulfanilamide (SN) and ascorbic acid (AA), which usually exist in environmental water. these molecules differ by their molecular size and structure, although presenting the same chemical functionalities able to establish hydrogen-bonds by with the preformed functionalized cavities by the functional carbonyl and amino groups linked to the aromatic structure, but their size, their structure and the spatial distribution of the functionalities are very different from those of SMD molecule.

In order to check and to compare the ability of "washed MICP" to detect the presence of these molecules, the same range of concentration of each interference was injected in the electrochemical cell containing pH 5 PBS buffer. Then, SWV was carried out, as previously indicated, in order to quantify the detection of these target molecules (Figures 40-43). Hence, the relative charges corresponding to each target molecule were displayed on histogram in Figure 45.

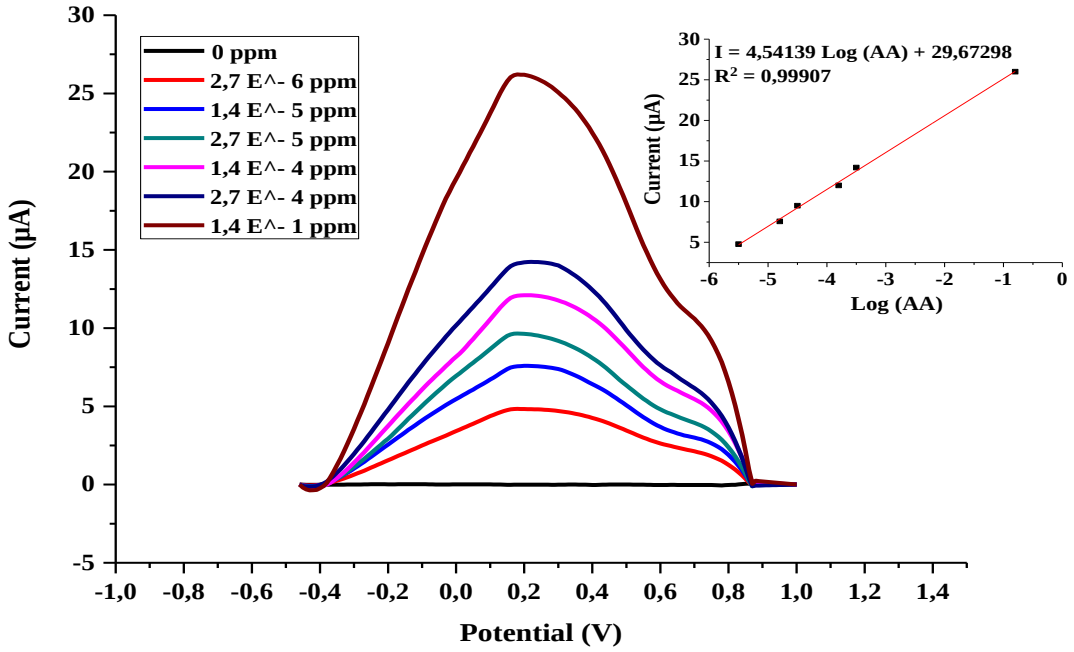

Figure 40. Square wave voltammetry response to the ascorbic acid analyte of different concentrations at the MIP-SMD film coated electrode in pH 5 phosphate-buffered saline solution.

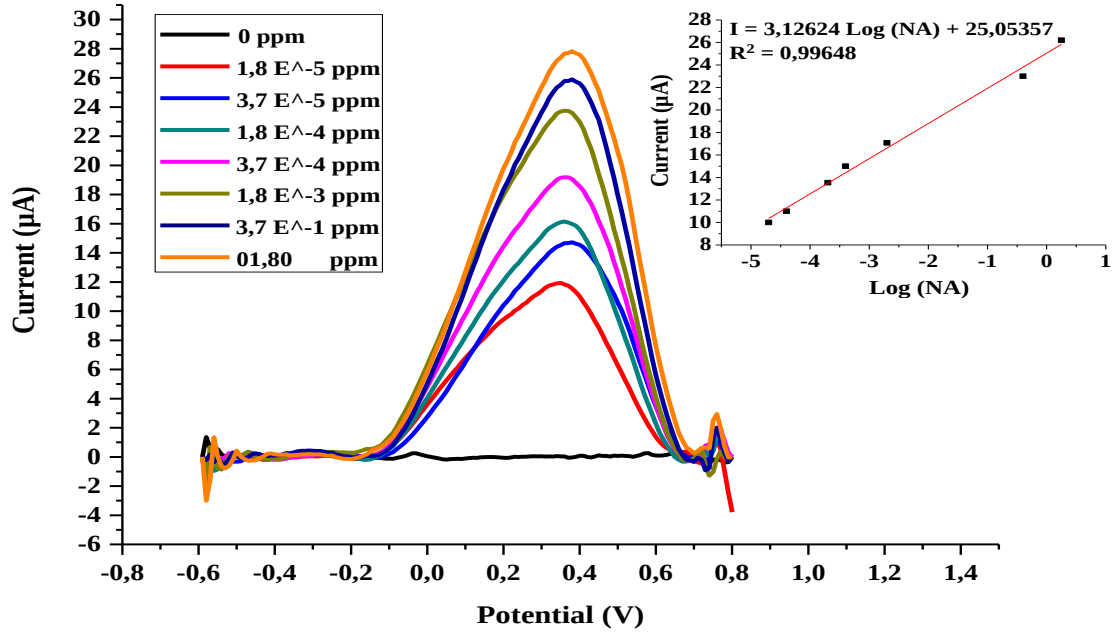

Figure 41. Square wave voltammetry response to the naproxen analyte of different concentrations at the MIP-SMD film coated electrode in pH 5 phosphate-buffered saline solution.

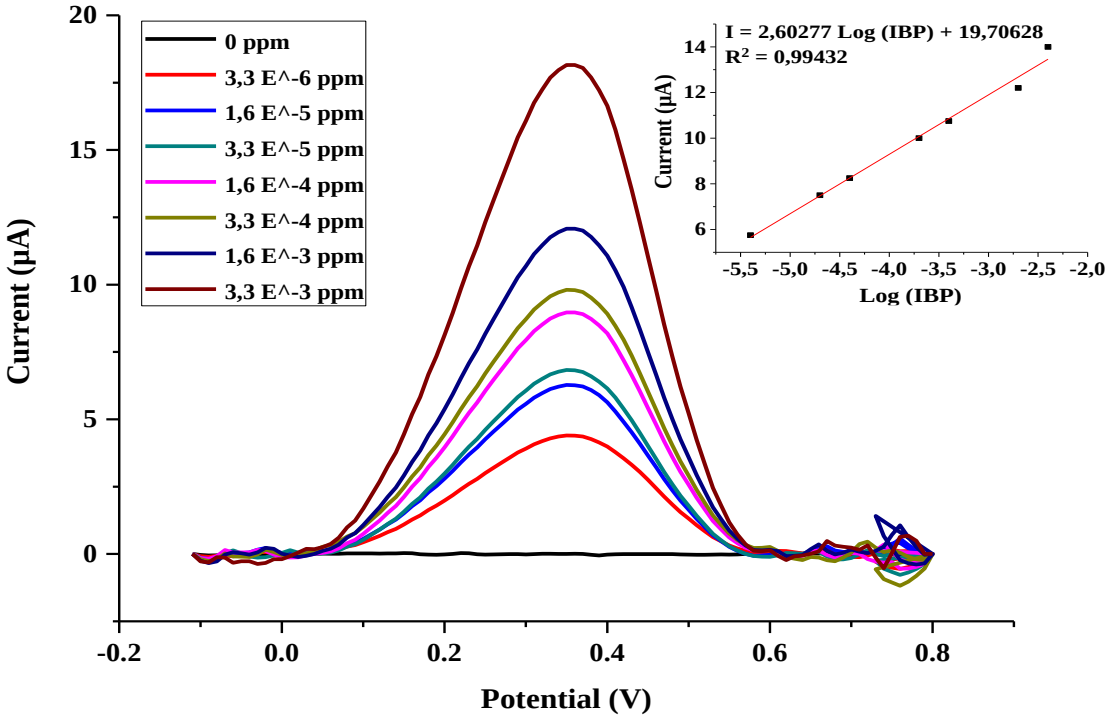

Figure 42. Square wave voltammetry response to the ibuprofen analyte of different concentrations at the MIP-SMD film coated electrode in pH 5 phosphate-buffered saline solution

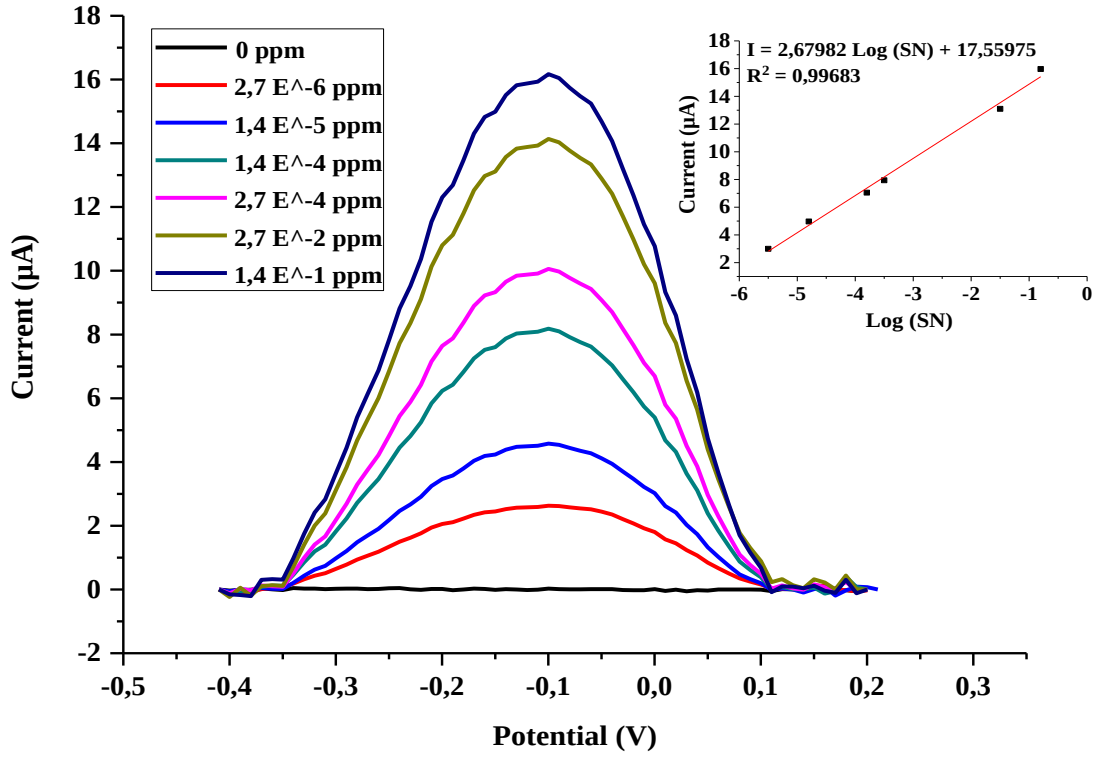

Figure 43. Square wave voltammetry response to the sulfanilamide analyte of different concentrations at the MIP-SMD film coated electrode in pH 5 phosphate-buffered saline solution.

As shown in the four Figures above, the affinity of the molecularly imprinted poly(AnEDOT/SMD) based sensor depends on the nature of pharmaceutical target. The low relative charge of detection of each interference molecules indicates that their recognition is far less important when compared to SMD molecules. Results suggest that the steric bulkyness of the targets plays an important role on the recognition process. The weaker recognition of the four components when compared to SMD can be understood in terms of molecular size of the lateral chain. Although the presence of some of the same functionalities on IBP, AA, SN, NA and SMD molecules, the size difference prevents the specific recognition of these molecules by molecularly imprinted poly(AnEDOT/SMD) films.

As a conclusion, interference molecules adsorb only at the polymer surface and are not specifically detected by imprinted cavities, thus, we can consider a good selectivity of molecularly imprinted poly(AnEDOT) towards SMD molecules, and the high sensitivity of the sensor towards MA was achieved despite the high similarity between these five molecules. This proves the effect of imprinting and template extraction processes to obtain cavities with high affinity for the template.

Figure 44 shows the calibration curves for current intensity response, ΔI_{anodic}, to increased concentrations of IBP, AA, SN, NA and SMD with the molecularly imprinted poly(AnEDOT/SMD) sensors. Each of the linear equations obtained were shown in Figures (40-43).

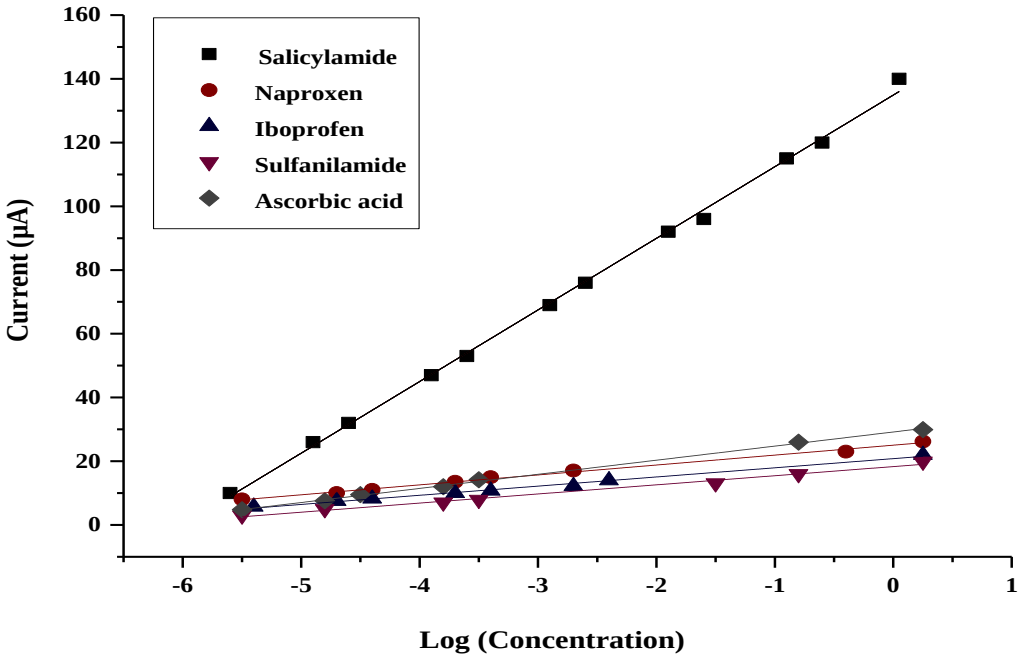

Figure 44. Calibration curves at five different MIP-SMD film coated electrodes for Salicylamide, ascorbic acid, naproxen, sulfanilamide, ibuprofen in pH 5 phosphate-buffered saline solution.

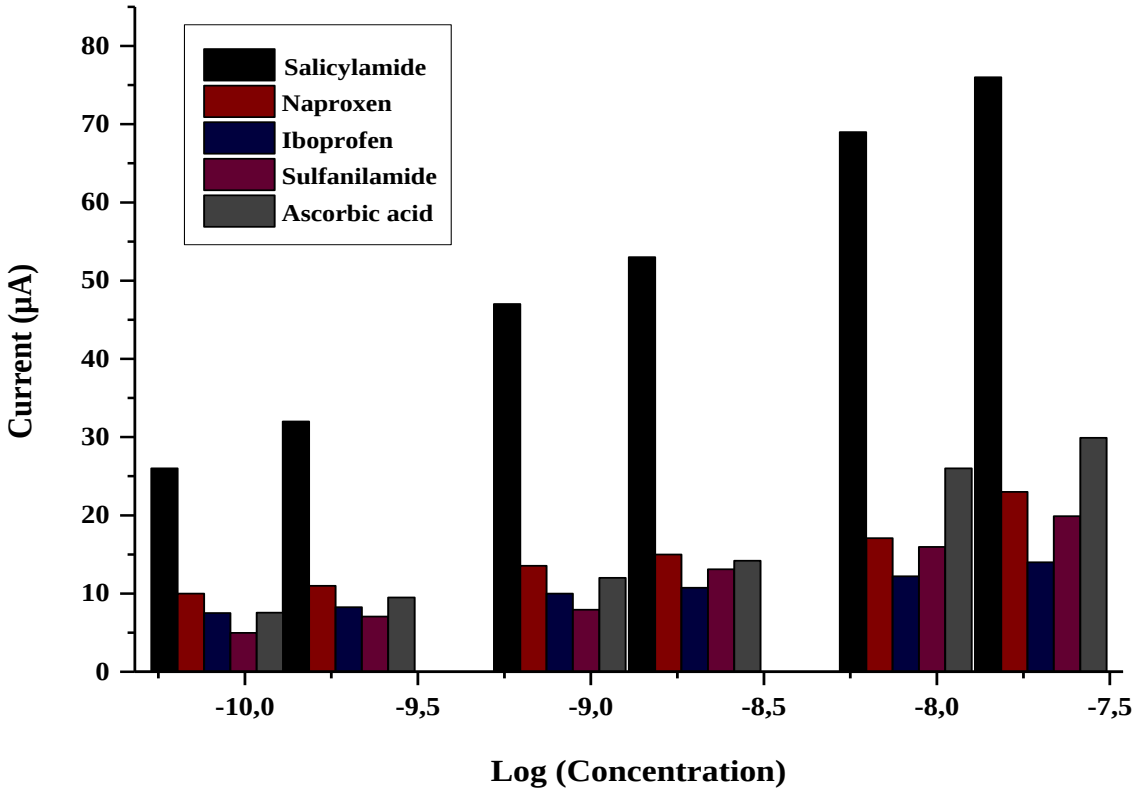

Figure 45. Relative charges corresponding to the detection by molecularly imprinted poly(AnEDOT/SMD) of Ibuprofen (IBP), Sulfnilamide (SN), Naproxen (NA), Ascorbic acid (AA), Salicylamide (SMD), pharmaceuticalsat pH 5 PBS buffer. Relative charges were deduced from SWV.

1.5. Reproducibility and stability of sensor

Reproducibility of imprinted polymers has a crucial role in developing applications that are reliable, economic and sustainable [130]. The fabrication reproducibility of poly(AnEDOT/SMD) was evaluated by preparing two electrodes under the same conditions and used for the detection of SMD within the same concentration range in PBS with pH 5 by SWV (Figures 46 and 47) and the relative standard deviation (RSD) of the current responses were found to be 2.1 % for SMD, confirming that the developed fabrication method was highly repeatable with good precision. Measurement reproducibility was also tested for the proposed sensor by measuring the same quantity of SMD five times using the same electrode. The RSD of the measurements was 3.2% which is < 5 showing that there is good agreement among the individual measurements [131]. The obtained results demonstrate the excellent performance of the elaborated sensor to recognize the detection molecule with several uses, indicating good durability and robustness.

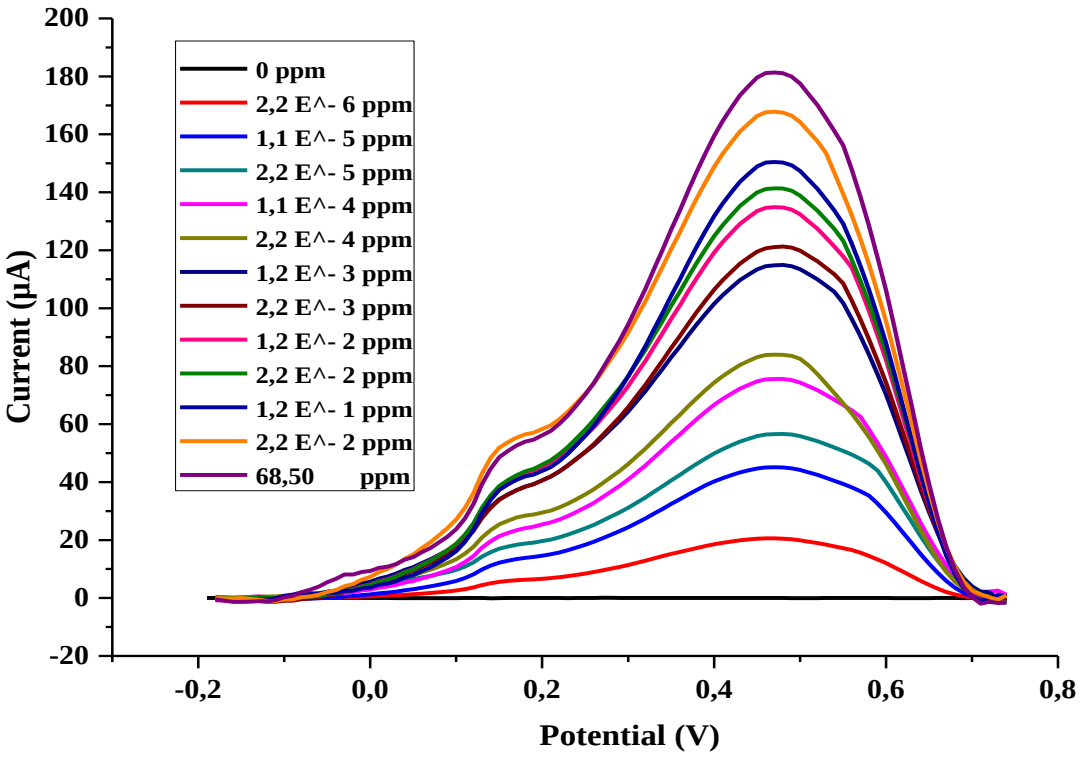

Figure 46. Square wave voltammetry response to the SMD analyte of different concentrations at the MIP-SMD film coated electrode in pH 5 phosphate-buffered saline solution (Reproducibility test 1).

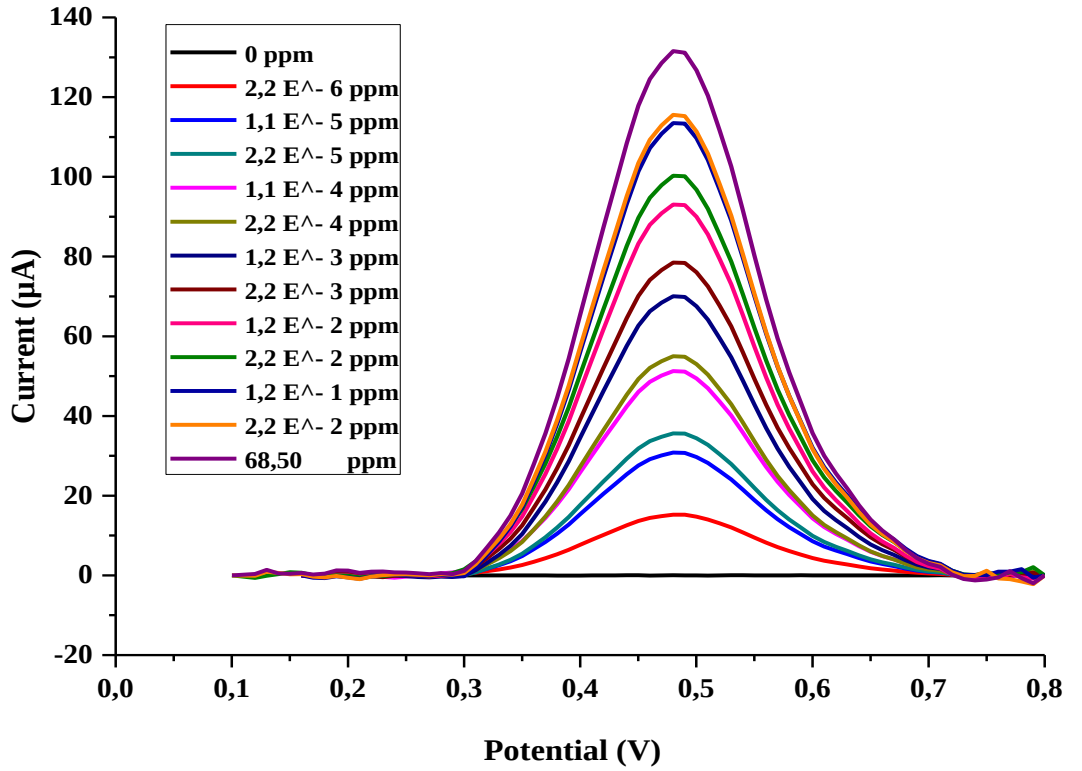

Figure 47. Square wave voltammetry response to the SMD analyte of different concentrations at the MIP-SMD film coated electrode in pH 5 phosphate-buffered saline solution (Reproducibility test 2).

1.6. Limit of detection and imprinting factor

The limit of detection (LOD) of **7.88×10^{-6} ppm** was achieved based on the LOD = $3S_b/m$, where S_b is the standard deviation of 10 blank measurements and m is the slope of the curve. To further verify the recognition selectivity, the efficiency of the imprinting effect was quantified by evaluating the imprint factor (IF) of the imprinted film. The average IF value for SMD detected by the molecularly imprinted poly(AnEDOT/SMD) sensor was determined using the eq. IF = $\Delta I_{MIP}/\Delta I_{NIP}$ [132]. Where, I_{MIP} represents the current of molecularly imprinted poly(AnEDOT/SMD) film and I_{NIP} represents the current of non imprinted poly(AnEDOT) film which are directly proportional to the concentration of the bound template SMD.

The average IF for the present sensor is 2.74 for the concentrations of 1.1×10^{-5} and 2.2×10^{-5} ppm. A high increase of the current value with the increase in the concentration of SMD is observed due to the presence of selective binding cavities on the surface of molecularly imprinted poly(AnEDOT/SMD).

1.7. Real sample analysis

The applicability of this novel system for the detection of pharmaceutical emerging contaminant SMD in aqueous samples was determined by MIP analysis in fresh tap water. The proposed electrochemical SMD sensor was evaluated for real sample analysis using tap water samples free from SMD with adjusted pH of 5, used directly without any pretreatment steps. SWV voltammograms exhibited a single well-defined anodic peak at about 4.5 V, which was gradually increasing with increase in concentrations of SMD as shown in Figure 48, confirming the selective binding of salicylamide molecules, Figure 49 shows a linear relationship between current and the logarithms of SMD concentrations in the range from 2×10^{-6} to 1×68.5 ppm with a linear equation of I (μA) = 110.85122 + 18.45127 Log [SMD] ppm (R^2= 0.999838) and the detection limit is found to be **9.29×10^{-6} ppm.**

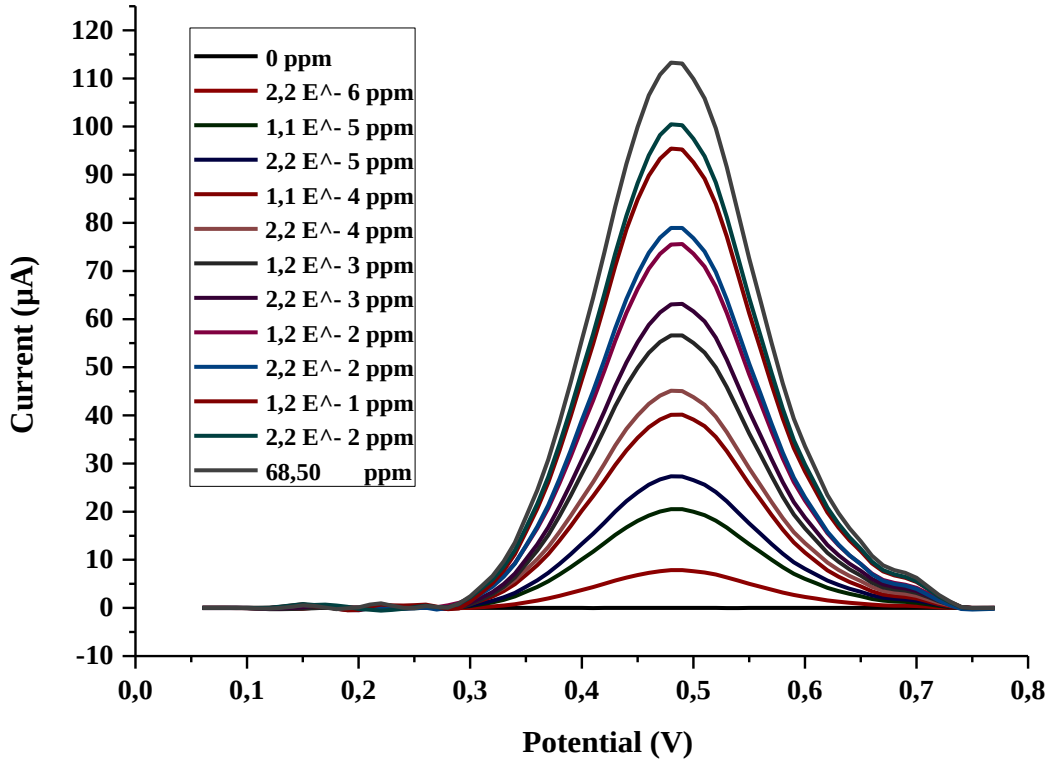

Figure 48. Square wave voltammetry response to the SMD analyte of different concentrations at the MIP film coated electrode in pH 5 tap water sample.

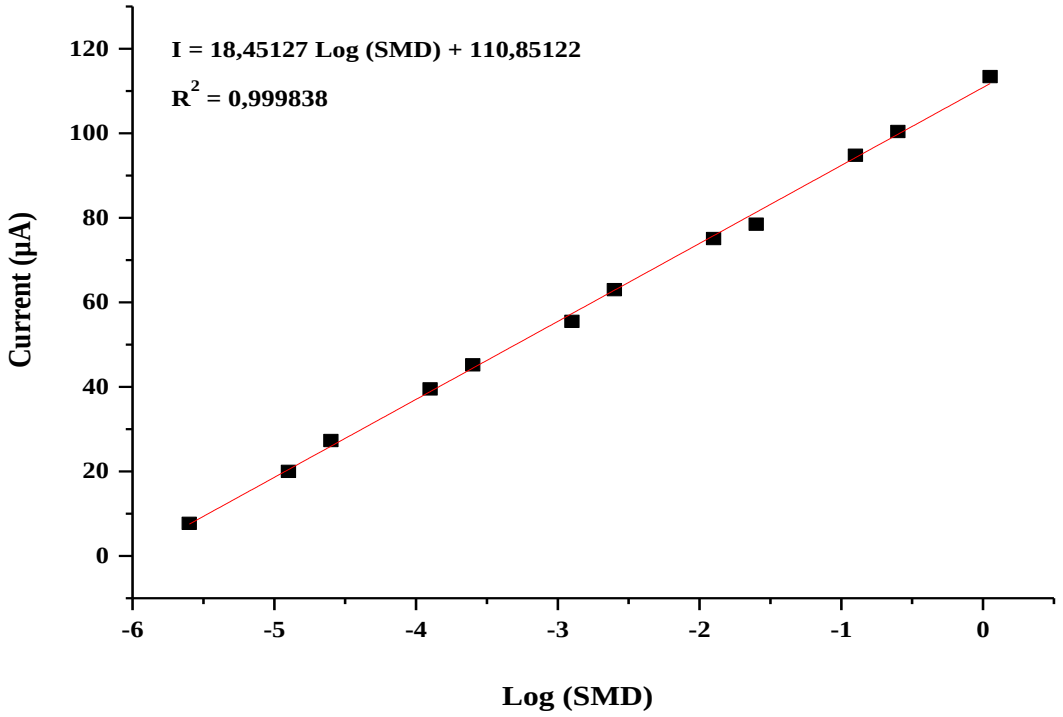

Figure 49. Calibration curve for SMD at the MIP-SMD film coated electrodes in pH 5 tap water sample.

Further the repeatability of SMD imprinted poly(AnEDOT) film was investigated for three fixed concentrations of SMD solution for three SWV consecutive measurements (Figure 50). The peak current response to SMD detection with a single electrode resulted in the relative standard deviation (RSD) of 6.01 % demonstrating a very good reproducibility for this SMD sensor, which confirms that it would definitely be applied to for the accurate and reliable determination of SMD in real water samples (results are presented in the Table 4 below).

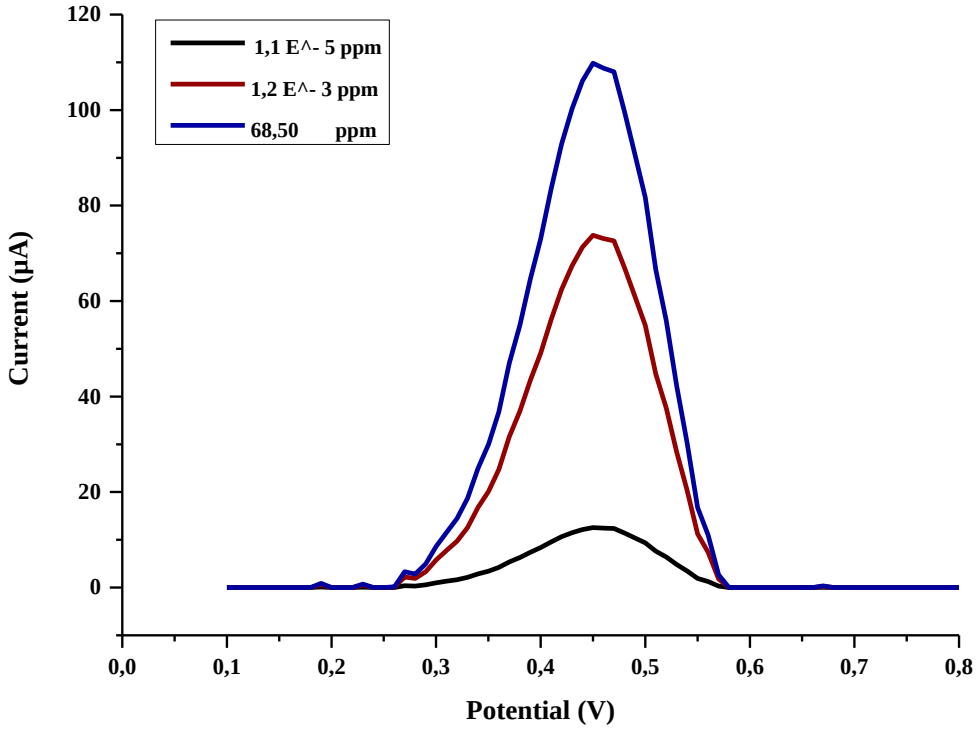

Figure 50. Square wave voltammetry response to the SMD analyte of different three concentrations at the MIP film coated electrode in pH 5 tap water sample.

Table4. Determination of SMD in tap water sample.

Samples	Added (ppm)	Founded (ppm)	RSD (%)
Tap water 1	0	Not detected	-
Tap water 2	1.1×10^{-5}	6.7×10^{-6}	2.27
Tap water 3	1.2×10^{-3}	5.5×10^{-4}	3.01
Tap water 4	68.5	92.1	6.2

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Conclusion

The increased demand to more performing and accurate chemical sensors leads to the development of new materials dedicated for sensing applications. Functionalized Molecularly Imprinted Conducting Polymers (MICP) represent interesting candidates for the fabrication of such new sensing layers since they present a specific recognition ability and a compatibility with various transduction techniques.

In this context, we developed molecularly imprinted poly(AnEDOT) sensing layers dedicated for the sensitive and specific detection of small electroactive organic molecules, such as salicylamide. Indeed, in such small molecules, the limited number of potential interactions with the sensing layers complexifies the recognition process, making it less sensitive and less selective.

In this work, profound investigations were carried out on the synthesis, the characterization and the evaluation of the sensing properties towards salicylamide of four kinds of differently functionalized conducting polymers-based sensitive layers, PEDOT, NICP and MICP:

i) Imprinted conducting polymer, PEDOT, was synthesized by electropolymerization of EDOT in the presence of salicylamide target molecules.

ii) Imprinted conducting polymer, polyaniline, was electrosynthesized by electropolymerization of An in the presence of salicylamide target molecules.

iii) Imprinted conducting copolymers, MICP, were prepared by electrochemical co-polymerization of EDOT and An in the presence of SMD.

iv) Non-imprinted conducting copolymers, MICP, were prepared by electrochemical co-polymerization of EDOT and An in the absence of SMD.

Characterizations and structural investigations of all these sensing polymer layers were carried out using electrochemical surface characterization techniques in order to understand the physico-chemistry of the recognition process and with the aim to improve the performance of the co-polymer layers in both their sensing and transducing (electrochemical) functionalities. To our knowledge, such a physico-chemical approach is very original in the field of sensors.

We first optimized the synthesis of all the considered sensing layers (PEDOT, PAn, MI-poly(AnEDOT) and NICP) in ethanol solvent. All these layers were electrochemically

grown, which enabled a precise control of their thicknesses through the adjustment of the electropolymerization charges. The sensing properties of all layers were quantified through electrochemical measurements (by cyclic voltammetry and square wave voltammetry). The observed dependence of the recognition properties of the conducting layers with their thickness and roughness indicates that the interaction between the target molecules and the sensing groups of the sensor layers takes place, not only at the surface, but also into the bulk of the porous conducting polymer matrixes in a tri-dimensional geometry. However, the involved recognition process appears to be always limited by the diffusion of the target molecules into the polymer matrixes.

In the case of PEDOT and PAn, the polymer layers do not favor the recognition process since only weak interactions can be involved between the EDOT and An units and the salicylamide target molecules. On the contrary, the presence of stronger interactions, due to FM residues, in the MI-poly(AnEDOT) layers enables the significant recognition of salicylamide through hydrogen-bonds. In comparison with NICP, the superior sensing properties of MICP layers result from the presence of pre-shaped imprinted cavities in their backbones which keep the memory of the salicylamide targets.

In addition to their sensitivity, the so-developed MICP electrochemical sensing layers, when used for the detection of salicylamide in the optimal conditions, present a low limit of detection ($7{,}88 \times 10^{-6}$ ppm) and a large dynamic range (2×10^{-6} to 1×68.5 ppm), as shown by square wave voltammetry measurements. Nevertheless, non-specific adsorption onto the surface of the sensing layers takes place systematically, which affects the specificity and the selectivity of the recognition process.

We demonstrated that, in order to fabricate the best sensing layers based on FM-MICP, strong interactions between FM and the template molecules are necessary in the pre-polymerization complex and in the functionalized cavities into the MICP matrixes. However, these strong interactions are not sufficient since non-specific adsorption systematically exists and should be minimized. Among all the conducting layers studied in this work, we showed that the MI-poly(AnEDOT) based sensing layers are the best candidates for the preparation of highly sensitive sensors since they offer the best compromise between high level of specific detection and low level of non-specific adsorption.

In order to validate our sensitive layers, and in particular the An/EDOT-functionalized MICP ones, we applied the developed sensors for the analysis of tap water matrices evidencing the applicability of the sensor for the detection of SMD in real samples. The

fabricated MI-poly(AnEDOT) based sensor is simple to fabricate, easy to operate, sensitive and selective towards the target analyte. Finally, the sensing layer is considered as the potential material for sensor fabrication for other analytes also.