Winter 2017

SELECTIVE DETECTION OF MULTI-ANALYTE NEUROTRANSMITTERS USING MOLECULARLY IMPRINTED POLYMERS

Bo Si
University of New Hampshire, Durham

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SELECTIVE DETECTION OF MULTI-ANALYTE NEUROTRANSMITTERS USING MOLECULARLY IMPRINTED POLYMERS

BY

Bo Si
B.S., Sichuan University, 2013

THESIS

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

Master of Science in

Electrical and Computer Engineering

December, 2017
This thesis has been examined and approved in partial fulfillment of the requirements for the degree of master of science in Electrical and Computer Engineering by:

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On December 7, 2017

Original approval signatures are on file with the University of New Hampshire Graduate School.
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This thesis is dedicated to my beloved parents. The support of my parents helped me accomplish all the research work in these meaningful two years.
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Neurotransmitters are chemical messengers produced in the brain that plays an important role in human’s physical, psychological and emotional conditions. The balance of neurotransmitters can affect the brain function, mood, pain response and exercise performances. In particular, dopamine (DA), norepinephrine (NE), and epinephrine (EP) are three well-known neurotransmitters that control various functions in the nervous system. Therefore, the detection of DA, NE and EP is extremely important for many instances of mental disease treatment and diagnosis. One of the most promising sensing techniques for neurotransmitter monitoring is the electrochemical detection method because of its high sensitivity, relatively low-cost and ease of operation. However, the similarities in redox potentials among different neurotransmitters limit the selectivity of the electrochemical detection. One way to enhance the chemical selectivity in multi-analyte detection is to utilize a molecularly imprinted polymer (MIP) as a selective molecular recognition motif. This thesis explores the capability of MIP-based electrochemical sensor for multiple neurotransmitter sensing. Pyrrole (PPy) and o-phenylenediamine (o-PD) are used as functional monomers for the MIP sensor development, and the characteristics of those sensors are analyzed. The results show that MIP sensors possessed higher sensitivity than non-
imprinted (NIP) sensors due to the unique molecular receptors. The detection limits of the developed MIP sensors are less than $1.3 \times 10^{-5}$ M. These results demonstrate the possibility of implementing a multi-analyte sensing platform for simultaneous detection of multiple neurotransmitters.

December, 2017
CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

Neurotransmitters are important chemicals which can transfer information between neuron cells. Otto Loewi was the first person to discover one type of neurotransmitter called acetylcholine in 1921 [1]. His work greatly impacted human perception about information transmission in animals. There are many types of neurotransmitters, and each one is essential for animals to maintain health. Monoamine is one major class of neurotransmitters which contains one amino group. It includes dopamine (DA), norepinephrine (NE), epinephrine (EP), serotonin (5-HT), melatonin, phenylethylamine, etc [2]. In 1957, Montagu discovered dopamine which belongs in the reward system and controls the motion of human being [3]. Dopamine concentration can increase when people feel pleasure or anxious. Some drugs such as methamphetamine and heroin can also stimulate dopamine neuron activity [4]. The lack of dopamine in brain may cause some horrible disorders like Parkinson’s disease, Alzheimer’s disease, and Huntington’s disease [5]. Norepinephrine is the main factor of fight-or-flight response, which responds to the sense of danger. The decreasing concentration of norepinephrine may cause depression and hypotension [6]. Epinephrine is another crucial neurotransmitter in the stress system, that controls anxiety and sweating [7]. Hence, each type of neurotransmitter is essential for a human, and the quantitative detection of neurotransmitter is necessary for medical treatment and clinical analysis.

Nowadays, monitoring neurotransmitters is still a hot topic, and a large number of sensors have sprung up in the market [8]. Epipen is a widely used epinephrine autoinjector used by millions of people in recent years [9]. The price for a two-pack Epipen has
increased from $100 in 2009 to $600 in 2016, which causes a current national conversation [10]. Some side effects such as dizziness, headache or vomiting might occur after epinephrine injection by Epipen, hence, monitoring epinephrine level after injection can be an effective way for planning further medical treatment. Developing a new type of sensor for neurotransmitter recognition is therefore urgently needed. The sensor should be sensitive enough to monitor the neurotransmitter concentration with a short response time, and also be selective toward the selected analyte without responding to other interferences. One way to enhance the chemical selectivity in multi-analyte detection is introducing a molecularly imprinting polymer (MIP) during sensor modification [11]. MIP is a polymer containing molecular cavities whose shape resembles the molecular structure of the target analyte. Due to molecular shape-based specificity, the MIP can greatly enhance the sensitivity and selectivity for a specific molecule [12,13].

1.2. PROBLEM DEFINITION

The common method for neurotransmitter detection is fast scan cyclic voltammetry (FSCV) [14]. This measurement is able to record neurotransmitter concentration in real time. However, there are still some limitations for FSCV. For example, the background current of the measurement is always high, and it is hard to quantify the concentrations of electroactive analyte. What’s more, it is hard to detect multiple analytes at the same time when using FSCV.

Most work focuses on single analyte detection, and that can limit the application of the developed sensor, because other species with similar properties might interfere with the signal, and it is complicated when monitoring multiple analytes in real applications because of the selectivity problem. In real biological sample, the similarities in redox potentials
among different chemicals can limit the selectivity of the electrochemical sensors. Therefore, simultaneous electrochemical detection of neurotransmitters is a challenging task. In addition, the redox potential for each neurotransmitter can vary from test to test even if the testing is done with the same device, which can be one of the most difficult challenges in neurotransmitter detection. In this work, we are trying to detect multiple neurotransmitters with high sensitivity and selectivity via an electrochemical approach. The target analytes are chosen to be dopamine, epinephrine and norepinephrine. These were chosen as our targets because they are electroactive, structurally similar, possess similar electrochemical properties, and are essential for brain function.

1.3. OBJECTIVE

The goal of this work is to develop sensors with high sensitivity and selectivity for neurotransmitter detection using molecularly imprinted polymers (MIP), and to demonstrate MIP can improve the electrochemical behavior of the modified sensor. The specific objectives of this work are the following:

1. Develop two types of MIP, including one conductive polymer and one non-conductive polymer.
2. Investigate the optimal growth condition, for MIP polymerization.
3. Evaluate the sensitivity, selectivity, and limit of detection (LOD) for developed MIPs.
4. Demonstrate whether MIPs exhibit higher sensitivity than NIPs toward target analyte.
5. Quantify the selectivity of the sensor when interfered with other species.
6. Compare different types of MIPs regarding the sensitivity, selectivity and LOD.
CHAPTER 2: THEORETICAL BACKGROUND

2.1. NEUROTRANSMITTERS

There are about 100 billion neurons in the human brain [15], and we need them all the time. The neurons communicate to each other via electrical and chemical signals when we eat, study, and even sleep. However, those neurons do not physically contact each other, so they use some chemicals for communication. Neurotransmitters are chemicals that act as a messenger in the synaptic transmission process [16]. With the development of neurobiology, a large number of neuroactive substances were found in the nervous system [17]. All types of neurotransmitters can be synthesized in the neuron, and then stored in a nerve cell called presynaptic neuron [18]. After presynaptic neurons release neurotransmitters, postsynaptic neurons receive these neurotransmitters [19].

Neurotransmitters can be divided into three categories: monoamines, amino acids, and peptides [20]. Monoamines are the first to be found among all categories, including dopamine, epinephrine, norepinephrine, serotonin, etc [21]. Dopamine is essential to the reward system, and is released when people feel happy [22]. Epinephrine and norepinephrine controls the stress system in the human body, and the concentration levels can change when people become nervous or anxious [23]. Amino acids include gamma-aminobutyric acid (GABA), aminobutyric acid, acetylcholine, etc [24]. GABA is an important inhibitory neurotransmitter in the mammalian central nervous system. People will feel tired or anxious when GABA level decreases [25]. Peptides, on the other hand, contain cholecystokinin, somatostatin, oxytocin, etc [26]. Somatostatin is also important for humans, because it can inhibit the level of hormones, and control of the digestive system.
Hence, all types of neurotransmitters are essential for the human body, and it is necessary to monitor them for medical diagnosis.

In this work, DA, NE, and EP are chosen as analytes for electrochemical analysis because they are all electroactive. Table 2-1 summarizes the oxidation process of those three neurotransmitters [28–30]. For all cases, analytes lose two electrons and get oxidized. The lost electron can cause a current peak during the electrochemical measurement, and the current density will be affected due to the concentration of the analyte.

Table 2-1. The oxidation DA, NE, and EP

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Structure</th>
<th>Oxidation Process</th>
</tr>
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<tbody>
<tr>
<td>Dopamine</td>
<td><img src="image" alt="Dopamine structure" /></td>
<td>-2H, -2e⁻</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td><img src="image" alt="Norepinephrine structure" /></td>
<td>-2H, -2e⁻</td>
</tr>
<tr>
<td>Epinephrine</td>
<td><img src="image" alt="Epinephrine structure" /></td>
<td>-2H, -2e⁻</td>
</tr>
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</table>
2.2. ELECTROCHEMICAL BASIS

2.2.1. ELECTROCHEMICAL SENSORS

In order to quantify the biochemical processes in the field of biological and medical engineering, electrochemical biosensing became a hot topic in recent years [31–34]. Electrochemical biosensors can convert the biological information to an electric signal, so they can be highly sensitive because only the desired molecule can interact with the sensor substrate. Bioelectroanalysis with electrochemical biosensors can provide a promising measurement with the advantages of rapid response, high sensitivity, high selectivity and low cost [35–38].

The first generation of electrochemical biosensors was developed by Leland C. Clark in 1962 [39]. Clark’s electrode was designed to detect oxygen. The net reaction, which occurred on the platinum surface, was $O_2 + 4 e^- + 2H_2O = 4 OH^-$. The oxygen gains four electrons on the platinum surface to form hydroxyl when applying a voltage. This reaction can cause a current flow, so one can easily know how much oxygen was consumed by measuring the current density between the electrodes. This setup is very simple for a biosensor, and this pioneer work is crucial for electrochemical biosensor development.

Thanks to the work from Clark, the study of electrochemical biosensors has become one of the most important directions in the field of sensing. There are many advantages for an electrochemical biosensor compared with other types of sensors [40–43]. First, most of the electrochemical biosensors are relatively low cost compared to the other sensors. Second, the electrode of the sensor can be easily modified to improve the sensitivity and selectivity towards a certain analyte. Third, most electrochemical biosensors are portable. The size of a potentiostat can be smaller than a pencil case. Also, the biosensors can be
disposable because of the low cost. According to a survey collected from 2013, there is a total of 12455.8 USD million in the field of biosensors [44]. Electrochemical biosensors are surely the most attractive sensor, as they accounted for a larger portion of the market. As a result, it is necessary to develop electrochemical biosensors to meet the market demand.

In our work, the potentiostat is purchased from DropSens company. Figure 2-1 shows the photograph of the potentiostat used in this work. The whole detection system is portable and simple. The potentiostat sends electric signals to the device and the response is sent back to a computer for analysis.

![Figure 2-1. The potentiostat used in this project](image)

2.2.2. ELECTROCHEMICAL CELL

All of the electrochemical measurements need an electrochemical cell. An electrochemical cell is defined as a device which can transduce a chemical reaction to an electrical signal [45]. Also, the electrochemical biosensor always contains an analyzer to measure the current, voltage, or impedance in the cell. One good example of an electrochemical cell involves copper and zinc [46]. The electrons from zinc will transfer to copper through the wire spontaneously, causing a current flow. An electrochemical
A biosensor contains a working electrode (WE), counter electrode (CE), and reference electrode (RE) [47].

The WE is where the chemical reaction of interest will take place [48]. The WE can also be called anodic or cathodic when the reaction is oxidation or reduction, respectively. As a result, the material of a WE needs to be carefully chosen for an electrochemical sensor to minimize the background noise and stabilize the current response. Carbon, gold, and platinum are three materials commonly used for a WE because of their good electroconductivity and surface reproducibility [49, 50]. It is also common to modify WE to improve the sensitivity and selectivity towards a certain target analyte.

The CE is also known as an auxiliary electrode. A current will be applied or measured between the WE and CE [51]. The material of a CE will always be the same as the WE. The RE, on the other hand, is used to form a constant cell potential, so that the WE potential can be accurately controlled [52]. Calomel and silver/silver chloride are commonly used as reference electrode materials because of their high electrochemical stability.

Figure 2-2 shows a screen printed carbon electrode (SPCE) that was used in this work. The WE and CE are made of carbon, and the RE is silver. This kind of electrode is widely used in research because of the ease of modification, stability of electrical properties, and low cost. During the electrochemical measurement, 50 μL of solution will be dropped on the surface of the device, fully covering the WE, CE, and RE.
2.2.3. ELECTROCHEMICAL MEASUREMENTS

In our work, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are utilized to study the electrochemical properties of the modified sensor and detect the neurotransmitter concentrations. These two electroanalytical methods belong to voltammetry, which measures the current when the potential is changing [53]. The desired potential will be applied on the WE of the electrochemical cell, and the RE is used to control the potential of the working electrode without any current flow. The current between the WE and CE was measured to determine the concentration of the analyte. The electrochemical reaction during experiments can be presented by the Nernst equation, which can relates the equilibrium potential to the concentration of the analyte. During electrochemical measurement, analyte A can be oxidized at the working electrode:

\[ \text{A} - \text{ne}^- \rightarrow \text{B} \]

The Nernst equation for this reaction is:

\[ E = E^{0'} + \frac{RT}{nF} \ln \left( \frac{C_A}{C_B} \right) \]
$E^0$ represents the redox potential, $C_A$ is the concentration of analyte A, $C_B$ is the concentration of oxidation product B, $R$ is the universal gas constant (8.314 J K$^{-1}$ mol$^{-1}$), $T$ is the temperature in kelvins, and $n$ is the number of electrons that A lost during the reaction. For both DPV or CV measurement, the current at WE is changing over time. The electrochemical reaction takes place rapidly at the surface of the electrode when the potential reaches a certain level, and then the concentration of analyte A near the electrode surface is reduced. In this case, $C_A$ near WE is less than that in bulk solution, leading to analyte A transfer from the bulk solution to meet the equilibrium state. This phenomenon can be explained by Fick’s first law: the analyte transport from regions of high concentration to regions of low concentration [54]. The diffusion flux $J$ determined by amount of analyte and surface area.: 

$$J = -D \frac{\partial C}{\partial x} \bigg|_{x=0}$$

D represent the diffusion coefficient, C represent the concentration of the analyte, $x$ represent the distance to the surface.

The current, on the other hand, is determined by the mass transfer and electrochemical reaction occurs on the WE surface:

$$I = \frac{dQ}{dt} = -nF \frac{dN}{dt} = -nFAD \frac{\partial C}{\partial x} \bigg|_{x=0}$$

Figure 2-3 shows an example of a CV diagram. The plot is potential versus current. A potential is applied on the working electrode and the current is measured between the counter electrode and working electrode [55]. The current will increase when the potential increases, and reach a maximum value when the analyte is oxidized. After that, the current will decrease because all analytes near the electrode are oxidized, and it will take some time for them to diffuse into the solution. The potential will scan back after $t_1$, and a
reduction peak will occur in the same way as oxidation. CV is a very important electrochemical measurement because it can obtain a lot of information from the analyte solution and the electrode [56]. The electrical properties of the analyte can be obtained by observing the oxidation peak of the CV, and the electron transfer kinetics can be determined using CV. However, the detection limit of CV is larger than other detection methods, such as DPV or square wave voltammetry. As a result, we mainly used CV to examine the electrochemical properties of the modified electrode or do some preliminary measurements.
Figure 2-3. An example of CV diagram (a), and the applied potential during CV scans (b).

Figure 2-4 shows an example of a DPV diagram. The main difference between CV and DPV is the applied potential. For DPV measurements, the potential on the working electrode is increased step by step. $E_1-E_0$ is the height of pulse, which is called the pulse amplitude. The current is measured before and after the pulse, and the difference is
calculated. The background current is minimized for DPV measurements, and that leads to a relatively low detection limit.

![DPV curve](image)

Figure 2-4. An example of DPV diagram (a), and the applied potential during CV scans (b).

2.3. MOLECULARLY IMPRINTED POLYMER

Molecular imprinted polymer (MIP) is a polymer that is coated with a particular type of template molecule, which is then extracted to create artificial recognition sites in the polymer. Only the original molecule can bond to the cavities when the polymer is tested.
with several types of species, so the selectivity and sensitivity of the sensor increases due to MIP [57]. The first work related to MIP was published by Polyakov in 1931. He discovered an imprinting effect when he preparing the porous silica with different solvents [58]. Several years later, Dickey further explored the synthesis process of sodium silicate cooperated with four different dyes including methyl, ethyl, n-propyl and n-butyl orange. After extracting the imprinted dyes, the silica selectively rebinds the template [59]. Their work has spurred the development of molecular imprinted technique, and the MIP is still a hot topic in the field of bio-sensing in recent years.

Li et. al. reported a three-dimensional electrochemical sensor developed by carbon nanotubes (CNT), graphene foam, and o-phenylenediamine (o-PD). O-PD works as a functional monomer for MIP polymerization, and dopamine (DA) is chosen as template analyte. The selectivity of the modified sensor was improved by molecular imprinting and the sensitivity of the sensor was promoted by carbon nanotubes and graphene foam. The detection limit for this work was 6.67 x 10^{-16} M [60].

Wang et. al. developed a ZnO nanotube supported MIP array for dopamine detection. Pyrrole (Py) was electropolymerized on the ZnO nanotubes in the presence of DA. This developed MIP shows excellent selectivity towards DA [61].

Figure 2-5 demonstrates the working principle of a molecular imprinted polymer sensor [62–64]. Before polymerization, the monomer and template are mixed together for self-assembly. After polymerization, the template molecules will be embedded inside the polymer layer. Some special cavities with a specific shape and size will form after the template molecules are removed from the polymer. Only the template molecules can rebind to the recognition sites once the MIP is exposed to multiple species, and the rebinding can
enhance the template signal. A large number of MIP chemical sensors for analyte detection were explored in recent years. There are a large numbers of functional monomers for MIP synthesis, such as methacrylic acid, pyrrole, o-phenylenediamine, etc [65–68]. In this work, we explored the electrochemical properties of pyrrole and o-phenylenediamine as functional monomers, and evaluated the imprinting capacity for several types of neurotransmitters.

Figure 2-5. The working principle of molecular imprinted polymer sensor
CHAPTER 3: CHEMICAL SYNTHESIS OF POLYPYRROLE AND DOPAMINE IMPRINTING

3.1. INTRODUCTION

Polypyrrole (PPy) is one of the most attractive conducting polymers because it has good stability in air and high electrical conductivity. Also, PPy can be used for many applications because of its electrochemical properties. PPy is commonly used in the field of biosensing due to the promising conductivity [69–71]. The high physical and chemical stability also make PPy a material for anti-corrosion [72]. Nanostructures of the PPy can greatly improve the performance in some applications [73]. As a result, the method to synthesize 1-dimensional PPy and how to utilize it have become attractive topics in recent years. In this work, we choose PPy as a sensing material because it is conductive, which can amplify the electrochemical signal when detecting neurotransmitters. Also, PPy is a partially crosslinked MIP, so no crosslinking agent is needed during polymerization [74].

In this chapter, the objective is to explore the proper growth condition for PPy synthesis. 1-dimensional PPy structure is preferred for bio-sensing application because the high surface-area-to-volume ratio can improve the sensitivity of the sensor. Several chemical synthesis approaches will be examined to develop PPy nanowires. The goal of the work presented in this chapter is to optimize the protocol for PPy polymerization and evaluate the dopamine imprinting capability of the PPy nanowires.
3.2. REVIEW OF PIONEERING STUDY FOR POLYPYRROLE SYNTHESIS

The chemical structure of PPy is showing below:

![Chemical structure of PPy]

There are many approaches to polymerizing PPy nanostructures. According to the literature, the three main methods to synthesize PPy nanostructures include using Vanadium oxide ($V_2O_5$), cetyl trimethylammonium bromide (CTAB), and multiwall carbon nanotubes (MWCNT). The goal in this section is to find out the best way to synthesize PPy nanofibers (PPyNF) in a simple, efficient, and cost-effective way. Also, the resulting nanofibers should have diameters in the range of around 20 - 100 nanometers.

Henry et al. discussed a template-free route to synthesize PPy nanofibers [75]. They utilized bipyrrrole as a surfactant and FeCl$_3$ as an oxidant to polymerize pyrrole in their experiments. The product is a nanowire with a diameter around 10-50 nanometers. They also explored how molar ratios of bipyrrrole to pyrrole might influence the morphology. The network of the PPy becomes more interpenetrated and interwoven when the percentage of bipyrrrole is higher. As a result, the ratio of pyrrole and template might affect the morphology of the product. Also, the diameter of the PPy nanofibers might change based on oxidant concentration.

Aimei et al. synthesized PPy nanofibers by using a CTAB/HCl/Pyrrole combination [76]. CTAB was the soft template during the experiment, and ammonium persulfate (APS) was used as an oxidant. Their approach also produced a good nanofiber morphology with approximately 100 nm diameter. They also tried to use FeCl$_3$ as an
oxidant during the experiment, but no precipitate was formed. The morphology of the product is threadlike or rod like micellar aggregates when they used FeCl$_3$ as an oxidant. In this case, a different oxidant might influence the structure of the PPy. Quan et. al. also polymerized the PPy nanowire by using CTAB as a template [77]. Their whole experiment was performed at low temperature. Well-formed PPy nanowires were obtained. The SEM image shows that the diameter of the nanowires is around 30-50 nanometers. They also analyzed the electrochemistry properties of PPy nanowires’ by cyclic voltammetry (CV). The CV curve indicated that the output current changes based on different PPy structures. The PPy nanowire-based electrodes possessed higher capacitance than that of bulk PPy. According to basic chemical knowledge, temperature affects the reaction rate. Also, the reaction rate might influence the product structure.

Zhang et. al. also provided a method to synthesize wire/ribbon-like PPy nanostructures [78]. They used the CTAB/ammonium persulfate(APS)/Pyrrole system, and their result shows that the product’s morphology is affected by the concentration of the pyrrole monomer and APS. Another PPy polymerization method was provided by Cui et. al. [79]. They used vanadium oxide (V$_2$O$_5$) as a soft template, and FeCl$_3$ as an oxidant. They also test the AC conductivity of composite PPy and V$_2$O$_5$. The result shows that the conductivity of PPy can be changed based on the ratio of monomer and oxidant. Their study indicated that the morphology of the product will change by adjusting the concentration of the reagents, and the conductivity can also be changed because of it.

Qian et. al. reported a polypyrrole sensor for dopamine sensing. The chemical polymerization of polypyrrole was completed by using pyrrole, FeCl$_2$, H$_2$O$_2$, and multi-walled carbon nanotubes (MWCNT) [80]. MWCNT works as a template for polypyrrole
growth, and H_{2}O_{2} is the oxidant to start the reaction. Compared to the PPy-FeCl_{3} system, the oxidant was replaced by a weaker chemical H_{2}O_{2}, which may cause a slower growth rate. Also, dopamine was imprinted inside the PPy layer to enhance the sensitivity and selectivity of dopamine sensing. The imprinted sensor obtained a better current signal when compared with the non-imprinted one, and the limit of detection reached 1.0 \times 10^{-11} \text{ M}.

The capability of dopamine imprinting has also been proven by Kan et. al. [81]. Their work reported an electrochemical synthesis method to develop polypyrrole-dopamine sensors. The factors for polymerization and the optimal experimental conditions were discussed extensively, and DPV was used as a detection method for dopamine detection. Their sensor possessed good stability and selectivity with a detection limit of 6 \times 10^{-8} \text{ M}.

Thanks to the previous work, we are able to determine the necessary factors that influence PPy polymerization. In our research, we explore the optimal conditions to polymerize PPy nanofibers by adjusting those factors, and then try dopamine imprinting. Cyclic voltammetry and differential pulse voltammetry are used to verify if dopamine is successfully imprinted in the polypyrrole layer.

3.3. POLYPYRROLE SYNTHESIS CONDITIONS

According to the pioneering studies for polypyrrole synthesis, we summarize all possible materials for PPy polymerization in Table 3-1. FeCl_{3}, H_{2}O_{2}, and APS are candidates for the oxidant. Vanadium oxide (V_{2}O_{5}), MWCNT, and Cetyl trimethylammonium bromide (CTAB) are possible choices for the template along which
the polymer will grow. In addition, other factors such as temperature, oxidant to monomer ratio, and synthesis time are also discussed in this work.

Table 3-1. All possible materials for PPy polymerization

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Template</th>
<th>Supporting materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₃</td>
<td>V₂O₅</td>
<td>HCl</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>MWCNT</td>
<td>FeCl₂</td>
</tr>
<tr>
<td>APS</td>
<td>CTAB</td>
<td>(SDS)</td>
</tr>
</tbody>
</table>

In order to obtain reliable results, only one specific parameter is changed while all other conditions are kept the same for each experiment. Then, an SEM is used to observe the morphology of the product.

3.4. **PYRROLE-CTAB COMBINATION**

3.4.1. OPTIMAL GROWTH CONDITION FOR PYRROLE-CTAB COMBINATION

The first step in the experiment design is to choose a proper template for the PPy growth. In order to obtain the nanowire structure of PPy product, the template should possess a long-chained chemical structure. Three options are available based on the previous literature study, which are V₂O₅-nanotube, MWCNT, and CTAB [82–87]. However, it is hard to obtain V₂O₅-nanotubes commercially, so it is necessary to synthesize V₂O₅-nanotubes before PPy synthesis. That makes the experimental work more complicated. Also, V₂O₅ is a toxic chemical which could cause serious injuries. Therefore, V₂O₅ was not used. Hence, CTAB and MWCNT were tried as templates for PPy synthesis.
The first PPy polymerization method was carried out using CTAB as a template. In brief, 0.1M pyrrole monomer was added with 0.05 M CTAB to a 10 mL 1 M HCl solution. After stirring for about 5 mins, a solution of cooled APS (0.025 M) in aqueous 1M HCl was added, then the mixed solution was stirred for 5 mins and cooled for about 24 h. After the PPy was fully synthesized, acetone was used to wash the nanofibers and remove the acid and other unreacted residues. Figure 3-1 shows the SEM image for Py-CTAB-APS combination. The PPy nanofibers are well polymerized with approximately 100 nm diameters.

Figure 3-1 The SEM image for Py-CTAB-APS combination in optimal growth condition
Cooled environments are needed for PPy nanofiber synthesis. No nanofiber morphology was obtained when using the room temperature (Figure 3-2). The reason is that the reaction rate can be influenced by the temperature. The reaction rate is slower in a cooled environment, and that can help PPy nanofibers grow on the surface of the CTAB and then polymerize into PPy nanowires.

An acidic environment is also necessary for PPy nanofiber growth. PPy particles are formed rather than the nanofibers when synthesized in non-acidic solution (Figure 3-3).
The molar ratio of pyrrole and the template molecules also affected the results. Some plate-like structures were obtained around the PPy nanofibers when the molar ratio of pyrrole to the template was 1:2 (Figure 3-5). These “plates” were determined to be the undissolved CTAB. CTAB are difficult to dissolve in HCl solution, so we minimized the amount of CTAB, and changed the Py to CTAB ratio to 2:1.

Figure 3-3 The SEM image for Py-CTAB-APS combination in non-acidic environment.
Different oxidants were tested in our experiments. Only particle-like structures are observed when FeCl₃ were used as an oxidant (Figure 3-4). The SEM images demonstrates

Figure 3-5 The SEM image for Py-CTAB-APS combination when the ratio of Py and template decreased to 1:2

Figure 3-4 SEM image for Py-CTAB-FeCl₃ combination.
that APS is best choice for oxidant, it produces 1-dimensional PPy nanofibers when using CTAB as template molecules.

The ratio of pyrrole and the oxidant does not affect the diameter of the resulting nanofibers, however it influences the morphology of the PPy nanofibers. PPy tends to aggregate when the ratio of pyrrole and oxidant becomes 1:2, and the nanofiber structure no longer exists (Figure 3-6).

Figure 3-6 The SEM image for Py-CTAB-APS combination when the ratio of Py and oxidant decreased to 1:2
3.4.2. PRELIMINARY STUDY OF MOLECULARLY IMPRINTING FOR PYRROLE-CTAB COMBINATION

Once the growth condition is fixed for the Py-CTAB combination, dopamine is added during the synthesis process. The hypothesis of this section is that dopamine can be imprinted in the PPy nanofibers, and the product can enhance the dopamine signal after extraction of the imprinted dopamine. The dopamine doped PPy nanofibers is named as DA-PPy-CTAB-NT, and the pure PPy nanofibers will be called PPy-CTAB-NT. The SEM image of DA-PPy-CTAB-NT is shown in Figure 3-7, where the PPy is in nanotube form.

In order to compare the capability of dopamine detection for DA-PPy-CTAB-NT and PPy-CTAB-NT, 5 μL of washed products were dropped on the working electrode of the screen printed sensors. For DA-PPy-CTAB-NT, the imbedded dopamine molecules are extracted by scanning cyclic voltammetry from the potential between -0.2V and +0.7V in phosphate buffered saline (PBS 1X) solution for 20 cycles. After the polymer is dried, the electrode was incubated in 50 μL dopamine solution for 2 minutes, and cyclic voltammetry
is carried out for electrochemical measurements. The potential is scanned from -0.3 V to +0.7 V with a scan rate of 50 mV/s. Figure 3-8 shows the CV measurements for blank, DA-PPy-CTAB-NT and PPy-CTAB-NT devices incubated in 10 mM dopamine solution with different pH. In all cases, the blank device exhibits the lowest redox response indicating the PPy modification can amplify the dopamine signal. Also, the DA-PPy-CTAB-NT sensor further improved the dopamine signal, especially when the pH of the solution was 7.
Figure 3-8. Cyclic voltammetry measurements for blank, PPy-CTAB-NT, and DA-PPy-CTAB-NT devices incubated in 10 mM dopamine solution with different pH (a) pH 4, (b) pH 7, and (c) pH 10.
More measurements were carried out when different types of devices were incubated in varying concentrations of dopamine solutions. Figure 3-9 (a) shows the cyclic voltammetry plot of PPy-CTAB-NT incubating in 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mM DA solutions. The obtained oxidation peak at 0.24 V is flat indicating the reaction of dopamine is slow. DA-PPy-CTAB-NT, on the other hand, obtained a sharp peak at 0.13 V. However, the calibration curve shows that the non-imprinted PPy has a better sensitivity than the imprinted one. As a result, the Py-CTAB combination is not suitable for molecular imprinting. Another polymer synthesis method needs to be developed.
Figure 3-9. Cyclic voltammetry measurement for PPy-CTAB-NT (a), and DA-PPy-CTAB-NT (b) incubated in varying concentrations of dopamine solution. Calibration curve of the current readings at 0.13V (c).
3.5. PYRROLE-MWCNT COMBINATION

In order to improve the imprinting capability of polypyrrole, multi-walled carbon nanotubes were selected as a template for polymerization and two oxidants were tested for polymerization. Each method obtained promising nanostructures for dopamine imprinting.

3.5.1. PYRROLE-MWCNT-APS METHOD

The optimal growth condition is developed as follows: 33 mM of pyrrole monomer and 1 mg/mL of CNT is added to 1M HCl at low temperature (4°C). After sonication for about 5 mins, 0.05 mM sodium dodecyl sulfate and 0.025M ammonium persulfate (APS) in 1M HCl is added. The mixture is then sonicated for another 10 mins and then left in low temperature (4°C) for 12 hours to react completely. For dopamine-imprinted polypyrrole, 1 mM of dopamine is added before the reaction. PPy-MWCNT-APS-DA represents the dopamine imprinted nanotube sensor, and PPy-MWCNT-APS-NIP represents the non-imprinted nanotube sensor. As can be seen in Figure 3-10, PPy-MWCNT-APS-DA is well synthesized with the diameter of 100 nm.
Different amounts of imprinted dopamine can affect the diameter of the nanotube. Figure 3-11 displays the SEM images for Py-MWCNT-APS imprinted with 10 mM or 1 mM dopamine. The average diameter of 1mM DA imprinted nanotubes was about 100 nM, while the 10 mM sample reached 200 nM. As a result, increasing the amount of dopamine increases the nanotube diameter. CV was carried out to further evaluate the performance of each sensor.
Figure 3-11. The SEM image of Py-MWCNT-APS imprinted with (a) 10 mM and (b) 1 mM dopamine.

Figure 3-12 (a) and (b) display 20 CV scans from -0.2V to +0.7V for 1 mM and 10 mM DA imprinted sensors incubated in 1X phosphate buffered saline (No sodium dodecyl sulfate was added during polymerization). This step, commonly known as overoxidation process, is an excessive oxidation at large positive potential to remove the imprinted template molecules [88]. The overoxidation of polypyrrole can extract the imbedded molecules, forming molecular cavities for specific target recognition [89]. As can be seen in Figure 3-12 (a), the current decreases as the number of CV cycles increases and the CV becomes stable after 10 cycles, which indicates the extraction of DA and the degradation of polypyrrole. Figure 3-12 (b), on the other hand, shows the overoxidation for the 10 mM imbedded Py-MWCNT-APS sensor. An oxidation peak of DA is observed at 0.28 V due to the massive amount of DA molecules embedded inside the PPy nanotubes. The electrochemical signal then decreased sharply when scanning the working electrode potential continuously, and it was hard to reach a stable value even after 15 cycles. After the extraction of DA, the sensors were incubated in 0 mM, 10 mM, 50 mM, and 100 mM DA solutions to verify the sensitivity of the sensor. In Figure 3-12
(c), the 1mM DA imprinted sensor exhibits an increasing signal as the concentration of DA increases. However, the 10mM DA imprinted sensor cannot be easily stabilized, and the current density become lower when DA concentration increased from 0 mM to 10 mM.
Figure 3-12. Cyclic voltammetry diagram for Py-MWCNT-APS combination (without SDS) imprinted with 1 mM or 10 mM dopamine when doing overoxidation and dopamine sensing (scan rate: 50 mV/s).
Figure 3-13 summarizes the current responses at the potential of 0.26 V for 1 mM and 10 mM DA imprinted Py-MWCNT-APS sensors when exposed to various concentrations of DA solutions. The trend line of those two sensors are parallel, indicating similar sensitivities for both sensors. However, the current response for the 10 mM DA imprinted sensor detecting 0 mM DA is at 27.8 μA, which is even higher than the response for 50 mM solution. As a result, 1 mM DA is preferred for Py-MWCNT-APS imprinting because of the good stability.

![Calibration curve](image)

**Calibration curve**

Figure 3-13. Calibration curves when 1 mM or 10 mM dopamine imprinted Py-MWCNT-APS sensors (no SDS) were exposed to 0 μM, 10 μM, 50 μM, and 100 μM DA solutions.

According to Figure 3-13, the linear regression equation for the 1 mM DA imprinted sensor is $I = 0.06C_{DA} + 51.64\,\mu A$. The slope of the equation is too low for DA recognition, so it is necessary to introduce a catalyst to improve the sensitivity of the sensor. Hence, sodium dodecyl sulfate (SDS) was added into the synthesis protocol to enhance the dispersion of MWCNT.
Figure 3-14 (a-c) displays the CV responses of SDS-DA imprinted sensors after exposing the device to varying concentrations of DA, which produced some clear oxidation peaks around 0.17V. Compared to the CV response produced from 1 mM DA imprinted with Py-MWCNT-APS combination, the sensitivity improved a lot for all cases, which demonstrates that SDS can improve the sensitivity of the PPy sensor.
Figure 3-14. Cyclic voltammetry curves for (a) 0.01mM, (b) 0.05mM, and (c) 0.1 mM SDS with Py-MWCNT-APS combination incubated in 0µM, 10µM, 50µM, and 100µM DA solutions. (scan rate: 50 mV/s)
Figure 3-15 (a) displays the DA sensing performance for DA-imprinted sensors synthesized with and without SDS. The linear regression equation for Py-MWCNT-APS without SDS is \( I = 0.0628 C_{DA} + 51.642 \mu A \), while the equation for Py-MWCNT-APS with 0.05mM SDS is \( I = 0.404 C_{DA} + 52.963 \mu A \). The slope increased 6.4 times after introducing SDS, because the PPy polymer was more dispersed in the solution. Hence, it is necessary to add SDS in the polymerization protocol. Figure 3-15 (b) discusses the sensor performance when different concentrations of SDS were added. The slopes are similar for all cases, so the sensitivity of the sensor was not affected by the SDS concentration, as long as the SDS reached a certain amount. 0.05mM SDS improved the sensitivity slightly more than other concentrations of SDS, so 0.05mM SDS was chosen as the final concentration for PPy polymerization.
In conclusion, the Py-MWCNT-APS combination is an optimal protocol for polypyrrole polymerization, and has a potential for molecular imprinting. The stability of the PPy production is not perfect, because more than 10 CV cycles are needed to stabilize the PPy. Also, the background current for CV is about 40 µA, which is relatively high for analyte detection.
3.5.2 PYRROLE-MWCNT-H₂O₂ COMBINATION

In order to increase the stability of the PPy and lower the background current, another oxidant was tested for PPy polymerization. Hydrogen peroxide (H₂O₂) has been used for PPy polymerization as well as for dopamine imprinting [89]. Hence, this work also used H₂O₂ for PPy polymerization and further explore the possibility of imprinting other neurotransmitter species.

The polymerization protocol was as follows: 33 mM of pyrrole monomer, 0.1 mg/mL of FeCl₂, and 1mg/mL of CNT were added to 5 mM DA (NE, EP) in DI water at room temperature. After that, the mixture was sonicated for about 5 mins, and 0.1mL of hydrogen peroxide (H₂O₂) was added. The mixture was vigorously stirred for 5 mins and left to react for about 6 h at 23 ºC. Afterward, the molecularly imprinted polypyrrole nanofibers were rinsed with deionized water. We overoxidized the PPy-CNT nanofibers by scanning the potential between -0.2 V and +0.7 V in phosphate buffered saline (PBS 1X) solution for 10 cycles to remove the embedded template molecules. The imprinting capacity of this protocol is further discussed in Chapter 4.

SUMMARY

In this chapter, three synthesis protocols are discussed (Table 3-2). All of them result in nanotube structures. Also, Py-MWCNT combinations are able to imprint dopamine inside the PPy layer, which indicates the potential for other neurotransmitters to be imprinted. As a result, the next chapter will further explore the electro-chemical property of PPy made by Py-MWCNT combinations.
Table 3-2. The summary of three PPy synthesis protocol

<table>
<thead>
<tr>
<th>Synthesis protocol</th>
<th>Nano-structure</th>
<th>DA imprinting capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Py-CTAB</td>
<td>Yes</td>
<td>Poor</td>
</tr>
<tr>
<td>Py-MWCNT-APS</td>
<td>Yes</td>
<td>Good</td>
</tr>
<tr>
<td>Py-MWCNT-H$_2$O$_2$</td>
<td>Yes</td>
<td>Good</td>
</tr>
</tbody>
</table>
CHAPTER 4: THE ANALYSIS OF POLYPYRROLE BASED MOLECULARLY IMPRINTED SENSOR

4.1. INTRODUCTION

This chapter mainly focuses on the electrochemical analysis for neurotransmitter detection using polypyrrole. Electrochemical detection methods are preferred for neurotransmitter detection because of good sensitivity, and ease of operation [90], [91]. Electrochemical measurements produce much useful data such as current, voltage or impedance. Those data can be analyzed to study the electrical and chemical properties of the sensing material and analyte [92]. Fast scan cyclic voltammetry (FSCV) has been a common tool for measuring monoamine neurotransmitters in recent years [93]. However, there are several limitations of this technique, such as high background noise and relatively low sensitivity. Furthermore, it is difficult to use FSCV to detect multiple neurotransmitters simultaneously. Hence, a more selective and reproducible electrochemical analysis method is needed for multi-analyte monoamine neurotransmitter sensing. Differential pulse voltammetry (DPV) is carried out because it is very sensitive [94]. Wu reported on ascorbic acid, dopamine and uric acid detection [95] where they modified a glassy carbon electrode (GCE) with reduced graphene oxide and imidazolium groups to improve the selectivity of the sensor, and the modified sensor was able to detect those three species simultaneously. CV was utilized to analyze the electrochemical properties of the modified sensor, and DPV was used for analyte detection. Their results showed the detection limit of 10 µM, 0.03 µM, and 5.0 µM for ascorbic acid, dopamine, and uric acid, respectively. Their work demonstrates the potential for multi-analyte detection using DPV as a sensing mechanism. However, ascorbic acid and uric acid are common interfering species.
whose concentrations are generally much higher in brain tissues compared to the neurotransmitters of interest, therefore, an improved chemical sensor that is highly selective toward target neurotransmitters and is minimally responsive to interferents is needed. As a possible strategy to enhance the chemical selectivity, the molecularly imprinted polypyrrole is introduced in this chapter for selective neurotransmitter sensing. The goal of this chapter is to investigate the simultaneous detection capability of multiple neurotransmitters (dopamine and norepinephrine mixture) using molecularly imprinted polypyrrole sensors.

4.2 BACKGROUND

Much work has been done in neurotransmitter detection using the molecular imprinting technique in recent years. Li et. al. introduced an o-aminophenol-based molecularly imprinted sensor for dopamine detection [96]. The selectivity of the modified sensor was improved by both molecular imprinting and the inclusion of copper oxide nanoparticles. Their results show a detection limit of 8 nM for dopamine. The electrochemical signals from ascorbic acid and uric are minimized because only dopamine recognition sites are created in o-aminophenol layer. Tadi et. al. have successfully developed an epinephrine sensor using a molecularly imprinted polymer [97] using 2,4,6-trisacrylamido-1,3,5-triazine as a functional monomer. The detection limit of the modified sensor reached 1.2 nM. Sacramento et al. also reported an electrochemical sensor for acetylcholine (Ach) recognition. Polyaniline was synthesized with Ach and multiwalled carbon nanotubes to form a molecularly imprinted material. The detection limit for this work was 34.5 µM. These aforementioned work demonstrated the practicality of using the molecular imprinting technology for neurotransmitter recognition. However, most previously reported work
were only focused on single analyte detection, and did not mention the possible interference associated with other types of neurotransmitters with similar chemical structures and properties. Hence, this thesis explores the feasibility of multi-analyte detection of neurotransmitters using various molecularly imprinted polymers.

4.3 EXPERIMENTAL SETUP

As mentioned in chapter 3, Py-MWCNT-APS, and Py-MWCNT-H_2O_2 combinations have good potential for neurotransmitter imprinting. Hence, both methods were used for molecular imprinting and electrochemical measurement. Figure 4-1 shows the overall experimental process for neurotransmitter sensing. The analyte solution is first mixed with PPy and MWCNT. Once the PPy was fully grown on the MWCNT with template, the product was washed with water. Afterward, 1 µl of the product was deposited uniformly on the working electrode of the glassy carbon electrode (GCE). Then, the GCE was connected to a potentiostat for electrochemical treatments.
Figure 4-1. Experimental process of neurotransmitter recognition. (a) reactant preparation; (b) oxidant injection; (c) mix and leave undisturbed for 12 hours; (d) MIP pipetting; (e) PPy MIP illustration; (f) rinse and deposit on the screen printed electrode, and (g) detection of analyte.
4.4 NEUROTRANSMITTER DETECTION USING PY-MWCNT-APS COMBINATION.

4.4.1 DOPAMINE DETECTION

This section focuses on the dopamine sensing performance of the MIP based on Py-MWCNT-APS combination. Firstly, 1 µl droplet of dopamine imprinted polypyrrole (DA-PPy-MIP) was dropped and dried on the working electrode. A 50 µl droplet of 1X PBS aqueous solution was dropped on the device covering the working, counter, and reference electrode. The embedded DA molecules were extracted by scanning CV from -0.2V to +0.6V for 20 cycles. Figure 4-2 shows the CV diagram of DA-PPy-MIP overoxidation. The current drops after each cycle due to the extraction of embedded DA, and is finally stabilized after 20 cycles because no DA is left in the MIP layer. The same CV treatments were also applied to the non-imprinted polypyrrole (PPy-NIP) for the fair comparison.

![Overoxidation of DA-PPy-MIP](image)

Figure 4-2. Overoxidation of DA-PPy-MIP, scan rate: 50 mV/s.
After the extraction of DA, DPV was carried out for dopamine sensing. Figure 4-3 (a) shows the DPV curves for the non-imprinted Py-MWCNT-APS sensor when exposed to 0, 10, 50, and 100 \( \mu \text{M} \) of dopamine solutions. The background current was in the neighborhood of 400\( \mu \text{A} \), with some degree of variation for each DPV run. The oxidation peak of DA occurs at 0.04V. Figure 4-3 (b), on the other hand, shows the DPV curves when DA-PPy-MIP expose to the same concentrations of DA solutions. A sharp current response occurs at 0.04V, and the amplitude of the current peak is clearly larger than that of the NIP’s.

![Non-imprinted Py-MWCNT-APS combination](image1)

![DA-imprinted Py-MWCNT-APS combination](image2)

Figure 4-3. DPV curves when (a) non-imprinted Py-MWCNT-APS and (b) DA-imprinted Py-MWCNT-APS were exposed to 0, 10, 50, 100 \( \mu \text{M} \) DA solutions. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms.
Figure 4-4 compares the sensitivity of DA-PPy-MIP and PPy-NIP when they were each exposed to 0, 10, 50, and 100 µM dopamine solutions. The current ∆I is calculated using the formula ∆I = I (peak) – I (background), where both I(peak) and I (background) were obtained at 0.04V. Error bars indicate standard deviation obtained from three independent experiments (n=3). The linear regression equations for PPy-DA-MIP and PPy-NIP are ∆I = 1.408 C_{DA} (µM)+8.80 and ∆I = 0.627 C_{DA} (µM)+0.389, therefore the sensitivity of the PPy-DA-MIP sensor has more than doubled compared to that of the PPy-NIP sensor. The detection limits for the PPy-DA-MIP and the PPy-NIP sensors are 7.851 µM and 16.487 µM, respectively. In conclusion, the DA-imprinted sensor possessed better sensitivity than the non-imprinted counterpart.

![DA-PPy-MIP vs PPy-NIP](Image)

**Figure 4-4.** The calibration curves for DA imprinted and non-imprinted sensors. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms.

### 4.4.2 NOREPINEPHRINE AND EPINEPHRINE DETECTION

Due to the successful imprinting of DA, other types of neurotransmitters were tested for the imprinting in PPy. Figure 4-5. (a) shows the DPV response of the PPy-
NIP sensor when incubated in different concentrations of norepinephrine (NE) solutions. The oxidation peak occurs at 0.05V, and the change in peak current for 100 µM NE is approximately 56 µA with respect to the background current. For PPy-NE-MIP (Figure 4-5 (b)), the peak current is also obtained at 0.05V, while the difference between 100 µM NE and 0 µM NE increased to 65 µA. Figure 4-5 (c) and (d) shows the DPV curves of NIP and PPy-EP-MIP sensors when incubated in various concentrations of EP. The oxidation peak of EP occurs at -0.01V, and the current enhancement between 100 µM EP and the background for NIP and PPy-EP-MIP is 15 µA and 21 µA, respectively. In all cases, the NE and EP imprinted sensors showed little improvement over the non-imprinted sensors, and the result is not as promising as that of the DA-imprinted PPy-based MIP.
Figure 4-5. DPV curves for (a) NIP, (b) NE-MIP sensors when exposed to 0, 10, 50, 100 µM NE solutions and (c) NIP, (d) EP-MIP sensors when exposed to 0, 10, 50, 100 µM EP solutions. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms.
Figure 4-6 (a) compares the calibration curves between the PPy-NE-MIP and NIP sensors. The slope of the calibration curve for PPy-NE-MIP is slightly higher than the NIP sensors. For EP detection, both PPy-EP-MIP and NIP sensors were neither stable nor sensitive. Although current $\Delta I$ of the imprinted sensors was always higher than the non-imprinted sensors, the error bars overlapped, indicating minimal improvement in sensing performance due to the imprinting.

![Graph](image)

In summary, the PPy-MWCNT-APS combination is shown to be capable of dopamine imprinting. The PPy-DA-MIP greatly improved the sensitivity of DA
detection. However, the improvement for NE detection is not very significant when compared between PPy-NE-MIP and NIP. For EP detection, both PPy-EP-MIP and NIP curves exhibited instability and poor sensitivity, possibly due to the poor electroactivity of EP. Furthermore, the background current in all tested samples was relatively high, making the measurements unstable and non-reproducible.

4.5 NEUROTRANSMITTER DETECTION USING PY-MWCNT-H2O2 COMBINATION

Although the DA-imprinted Py-MWCNT-APS combination exhibited good improvement in sensitivity for DA detection compared to the non-imprinted sensor (NIP), similar improvements were not obtained in NE and EP imprinting. Hence, a new strategy was attempted where Py-MWCNT-H2O2 combination was synthesized to see if using a different oxidant would enhance the sensing performance. 30% H2O2 is a weaker oxidant compared to APS, allowing a slower reaction rate. In addition, the slower reaction rate is expected to capture more template molecules during the polymerization process thereby creating more imprinted receptors. Figure 4-7 compares the sensitivity of molecularly imprinted sensors (MIP) and non-imprinted sensors (NIP). Figure 4-7 (a) shows the DPV curves when the DA-MIP and NIP sensors were both exposed to 100 μM DA solution. MIP shows a better current response than NIP, because the created cavities can provide more recognition sites towards DA molecule resulting a larger surface for electron transfer. The oxidation peak for the MIP occurs at 0.15V, while for the NIP, it occurs at 0.2V. This potential shift indicates a higher reaction rate for MIP, which may be due to the larger surface area for DA oxidation. Figure 4-7 (b) compares the current responses of NE-MIP and NIP for 100 μM NE solution. The peak current for NE-MIP is 9.9 μA, while the peak response for NIP is only 4.9 μA. Hence, the NE-MIP shows improvement in performance after adjusting
the polymerization protocol. For EP detection, peak current for MIP was better than NIP, but the improvement was still not promising because the EP is not as electro-active as other analytes. The peak current only increased 1.4 μA when compare the magnitude of the current responses between MIP and NIP. Therefore, we introduced Fe₃O₄ nanoparticles as a catalyst to further improve the reaction rate [98]. The sensitivity of EP detection increased because of the recognition sites and the catalyst. The detection limits of PPy-DA-MIP, PPy-NE-MIP, and PPy-EP-MIP were 7.97 μM, 3.05 μM, and 7.08 μM, respectively. In all cases, the magnitude of the current responses for MIPs was greater than those of the NIPs, supporting the hypothesis that molecularly imprinting can enhance the sensitivity towards the target neurotransmitter.
Figure 4-7. DPV curves of each neurotransmitter imprinted sensor (MIP) vs. non-imprinted sensor (NIP): (a) DA imprinted and non-imprinted sensors when exposed to 100 μM DA solution. (b) NE imprinted and non-imprinted sensors when exposed to 100 μM NE solution, and (c) EP MIP with Fe₃O₄, EP MIP and non-imprinted sensors when exposed to 100 μM EP solution. PPy was polymerized via Py-MWCNT-H₂O₂ method. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms.
Figure 4-8 compares the calibration curves between imprinted versus non-imprinted sensors. The linear regression equations of DA-MIP for dopamine detection is $\Delta I = I(\text{peak}) - I(\text{background}) = 0.079 \ C_{\text{DA}} (\mu\text{M})+0.178$ where the slope is 0.079. This value is higher than that of the NIP response whose regression has the slope of 0.0504. We calculated the limit of detection (LOD) by taking three times the standard deviation over the slope of the regression line [99]. The LOD of the DA-MIP-based sensor is 7.972 µM. Figure 4-8 (b) shows the detection results for NE-MIP and NIP materials when they were exposed to various concentrations of NE solutions. The imprinted sensor exhibits higher sensitivity than the non-imprinted sensor, with a detection limit of 3.047 µM. The better response for MIP indicates more recognition sites during NE sensing. The calibration curves of NE-MIP and NIP can be described by the linear equations $\Delta I = 0.0534 \ C_{\text{NE}} (\mu\text{M}) + 0.079$ and $\Delta I = 0.0367 \ C_{\text{NE}} (\mu\text{M}) + 0.117$, respectively. However, there is significant overlap in error bars between the two, which may be due to a small number of NE-imprinted cavities in the PPy layer suggesting that NE imprinting is more difficult than other template molecules. For EP detection (Figure 4-8 (c)), Fe$_3$O$_4$ modified EP-MIP material shows the highest sensitivity and the linear regression equation for that sensor is $\Delta I = 0.0693 \ C_{\text{EP}} (\mu\text{M}) - 0.089$ with the LoD of 7.081 µM. The calibration curves for EP-MIP and NIP sensors are $\Delta I = 0.0497 \ C_{\text{EP}} (\mu\text{M}) - 0.102$ and $\Delta I = 0.0346 \ C_{\text{EP}} (\mu\text{M}) + 0.030$, respectively. The sensitivity of the Fe$_3$O$_4$ modified EP-MIP is nearly doubled compared to the NIP. Therefore, the molecular imprinting technique improved the sensitivity for neurotransmitter detection, including DA, NE, and EP.
Figure 4-8. Calibration curves for neurotransmitter imprinted (MIP) vs. non-imprinted (NIP) sensors incubated in 0 µM, 10 µM, 50 µM, and 100 µM concentrations of solutions: (a) DA imprinted and non-imprinted sensor when exposed to DA solutions, (b) NE imprinted and non-imprinted sensor when exposed to NE solutions, and (c) EP imprinted sensor with Fe₃O₄, EP imprinted sensor and non-imprinted sensor when exposed to EP solutions. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms,
4.6 CROSS REACTIVITY OF PPY-IMPRINTED SENSORS.

For \textit{in vivo} measurement in the brain, many different species of neurotransmitters exist in the sample fluid. Therefore, it is necessary to investigate the selectivity of the developed sensors. This section discusses the sensing ability of three molecularly imprinted sensors when other analytes are present in the solutions. Figure 4-9 (a) shows the calibration curves when the DA-MIP sensor is exposed to each of the three analytes (DA, NE, or EP). The current $\Delta I = I_{\text{peak}} - I_{\text{background}}$ is obtained at 0.15V, which is the oxidation potential for DA. The DA-MIP based sensor does exhibit cross-reactivity toward NE and EP since they are also electro-active and can produce some level of redox current even if they are not specifically bound to the molecular cavity. However, the current response for DA is much higher than the other analytes because of the molecularly imprinted motifs. The oxidation process of NE and EP mainly occur on the surface of the PPy nanofibers while DA can rebind to the cavities inside PPy, which may cause the response improvement. Figure 4-9 (b) shows NE-MIP sensor performance on multi-analyte sensing at 0.22 V. The selectivity of NE-MIP is not promising because the current response for three different analytes cannot be distinguished from one another. The reason is that the peak oxidation potential of NE occurs at 0.22V, which is in between those of DA (0.15V) and EP (0.3V). The similarity in the oxidation potentials among the three analytes makes the detection of NE particularly difficult. In Figure 4-8 (c), the EP-MIP-Fe$_3$O$_4$-based sensor shows much improvement in terms of selectivity as well as the sensitivity where the linear regression equation is $\Delta I = 0.0693C_{EP} (\mu M)$. 
Figure 4-9. The calibration curves for (a) DA-MIP; (b) NE-MIP, and (c) Fe₃O₄-modified EP-MIP when exposed to 0 µM, 10 µM, 50 µM, 100 µM of DA, NE, and EP solutions.
In order to quantify the selectivity of the three neurotransmitter-imprinted sensors, we used an analytical method developed by Danzer [100]. A matrix of sensitivity M is introduced to calculate the selectivity of the three sensors. Each column j represents the three individual analytes (DA, NE, and EP), and each row i represents the three sensing materials imprinted with each analyte (PPy-DA-MIP, PPy-NE-MIP, and PPy-EP-MIP-Fe$_3$O$_4$). The coefficients A$_{ij}$ are the slopes of the calibration curves of each sensor detecting a single analyte.

$$
\begin{array}{ccc}
\text{DA} & \text{NE} & \text{EP} \\
M_1 &=& \begin{bmatrix} A_{11} & A_{12} & A_{13} \\ A_{21} & A_{22} & A_{23} \\ A_{31} & A_{32} & A_{33} \end{bmatrix} \\
&=& \begin{bmatrix} 0.0794 & 0.0486 & 0.0405 \\ 0.0601 & 0.0534 & 0.0592 \\ 0.0348 & 0.0446 & 0.0693 \end{bmatrix}
\end{array}
$$

The selectivity S of the each imprinted sensor is calculated as follows:

$$S(j) = \frac{A_{ij}}{\sum_{l=1}^{n} A_{lj}}$$

$$S(\text{DA}) = \frac{A_{11}}{A_{11} + A_{12} + A_{13}} = 0.471$$

$$S(\text{NE}) = \frac{A_{22}}{A_{21} + A_{22} + A_{23}} = 0.309$$

$$S(\text{EP}) = \frac{A_{33}}{A_{31} + A_{32} + A_{33}} = 0.466$$

The selectivity of the three MIP-based sensor array was calculated as follows:

$$S(\text{total}) = \frac{\sum_{i=1}^{n} A_{ii}}{\sum_{l=1,j=1}^{n} A_{lj}} = 0.413$$

The calculated selectivity ranges between 0 and 1, with a larger value indicating a better selectivity. The DA-MIP sensor exhibited the best selectivity, followed by PPy-EP-MIP-Fe$_3$O$_4$. However, NE-MIP showed a poor selectivity towards the three analytes.
hence further improvements in the imprinted sensors were required to enhance the selectivity of the MIP-based sensors.

In order to further investigate the selectivity of the MIPs in a more complicated environment, we developed a DA-MIP and NE-MIP array then test with DA and NE mixture. Figure 4-10 illustrates the experimental setup for detecting DA and NE simultaneously. The DPV signal was generated by a LabVIEW system using four electrodes. The system included two working electrodes, one counter electrode, and one reference electrode which were exposed to an analyte solution for DPV measurements. The resulting current responses were then used with the Gamry instrument for data analysis.

Figure 4-10 The experimental setup for simultaneous detection of DA and NE

DA-MIP was deposited on working electrode 1 (W1), while NE-MIP was deposited on working electrode 2 (W2), and these two sensors were working simultaneously when measuring the current responses. Figure 4-11 shows the calibration curves for the sensor arrays when they were exposed to a mixture of NE and DA solution which contained a fixed concentration of NE with various amounts of DA. In all cases, DA-MIP showed better selectivity than NE-MIP with respect to DA
detection, demonstrating improved selectivity of dopamine detection due to the molecular imprinting.
Figure 4-11. Calibration curves for DA-MIP and NE-MIP arrays incubated in (a) 0 µM, (b) 20 µM, and (c) 50 µM NE solutions mixed with various concentrations of DA.
Figure 4-12 compares the responses when DA-MIP and NE-MIP were each exposed to various concentrations of NE mixed with (a) 0 µM, (b) 20 µM, and (c) 50 µM of DA solutions. Although the NE-MIP sensor did not show significant improvements in the selectivity for the single analyte experiments, it did exhibit better responses than DA-MIP for norepinephrine detection in the mixed samples.

Figure 4-13 summarizes the results of simultaneous detection of dopamine and norepinephrine using a DA-MIP- and NE-MIP-based sensor array from a multi-analyte sample. The slopes of the calibration curves are utilized to evaluate the sensitivity of each sensor. In Figure 4-13 (a), DA-MIP possessed a higher sensitivity than NE-MIP for DA detection. Also, regardless of the amount of NE present in the solution, the sensitivity for NE-MIP with DA detection was constant, which suggested that DA molecules are poorly recognized by the NE-imprinted molecular receptors in NE-MIP. For NE measurements (Figure 4-13(b)), the sensitivity of NE-MIP was better than DA-MIP, although the improvement was not significant when 0 µM or 20 µM DA was in the solution. Therefore, the NE-MIP sensor is also selective toward NE detection, and the signal interference can be attributed to the similar oxidation potentials of DA and NE.
Figure 4-12. Calibration curves for DA-MIP and NE-MIP arrays incubated in (a) 0 µM, (b) 20 µM, and (c) 50µM DA solutions mixed with various concentrations of NE.
4.7 CONCLUSION

This chapter discussed in detail the sensitivity and selectivity of PPy based molecularly imprinted sensors. It is certain that DA can be easily imprinted into PPy polymer layers to improve the sensitivity as well as the selectivity. However, the PPy-MWCNT-APS combination faced problems with NE and EP imprinting, and the sensitivity of NE and EP detection did not improve as expected. Hence, the polymerization protocol was adjusted to PPy-MWCNT-H₂O₂. Motivated by Qian’s work and its preliminary work on DA imprinting, this thesis further explored other neurotransmitters capable of molecular imprinting [89]. Fe₃O₄ was introduced to
improve the sensitivity for the EP sensor. All three MIP-based sensors, namely, DA-MIP, NE-MIP, and EP-MIP-Fe$_3$O$_4$, exhibited improved sensitivity compared to the NIP for each target analyte. The fact that the oxidation peak for NE sits in between those of DA and EP makes the NE-MIP less selective than other MIPs. Other approaches to solve this problem may be to introduce nano-particle-based catalysts or adjust the polymerization conditions for the PPy-based MIP. Overall, PPy is a promising material for implementing the MIP-based neurotransmitter sensor.
CHAPTER 5: ELECTROCHEMICAL POLYMERIZATION OF O-PHENYLENEDIAMINE

5.1 INTRODUCTION

In the previous chapter, polypyrrole has been extensively used to develop molecularly imprinted polymers (MIPs). In this chapter, the use of o-phenylenediamine (o-PD) as a functional monomer in MIP is investigated. O-PD has been used for molecular imprinting previously [13]. Unlike PPy, which possesses high electrical conductivities, poly-o-phenylenediamine (P-o-PD) is an electrical insulator that can be polymerized on surfaces of the electrodes to block charge conduction. Hence, the non-conducting property of P-o-PD can be effective in minimizing the background noise in the current when measuring electrochemical signals. Moreover, the polymerized o-PD can have a high packing density, preventing the penetration of unwanted molecules through the P-o-PD layer. This trait is beneficial to the MIP-based sensing technique because the imprinted molecular cavity would become a molecular receptor accepting charge transfer towards the rebinding molecule [101]. The rebinding process occurs when the MIP is exposed to the template solution: only the imprinted molecule is capable to fit inside the o-PD layer, while other interferents will stay on the o-PD surface due to the densely packed layer. One advantage of this kind of polymer is that it minimizes the interference from other electro-active species in the real sample. Therefore, it is necessary to study the selective molecular recognition capability of P-o-PD for neurotransmitter detection. There has been work published on P-o-PD-based MIPs for selective chemical sensors due to the excellent stability and ease of polymerization of P-o-PD [102–106].

Kong et al. introduced a P-o-PD based MIP for imidacloprid detection [107]. They electro-polymerized the o-PD with imidacloprid on a reduced graphene oxide electrode surface. Imidacloprid is an insecticide widely used in agriculture. High
concentrations of imidacloprid residue in crops are harmful to human health. CV and linear sweep voltammetry were utilized to study the properties of the imprinted polymer. The detection limit of this sensor is 0.4 μM, and the linear detection range of the sensor was between 0.75 μM and 70 μM.

Peng et al. developed a paracetamol imprinted sensor by electro-polymerizing o-PD on MWCNT modified carbon electrodes. Paracetamol is a medication used for pain relief [108]. The polymerization method is to scan several CV cycles in o-PD and paracetamol solutions. After 20 CV cycles, the current is stable near 0, indicating the o-PD is covering the MWCNT surface, preventing charge transfer. Once the target molecule was removed from the P-o-PD layer, the sensor became sensitive towards ferrocyanide solution. When the target molecules are rebinding, the paracetamol would fill the molecular cavities, partially blocking the electron transfer which leads to an increase in the charge transfer resistance. Here, the ferrocyanide solution is used as a redox probe for paracetamol detection. Their sensor is selective towards other interferents, and the detection limit of their sensor is 0.5 nM.

Li et al. also reported a P-o-PD sensor using the molecular imprinting technique [109]. CV was used to polymerize the P-o-PD with atrazine, a persistent organic pollutant, on gold electrode. Their study showed that different CV scan rate and template-monomer ratio affected the performance of the modified sensor. The optimized monomer and atrazine ratio was 1:5, and 15 cycles of CV scans were performed for polymerization. Once the sensor was prepared, differential pulse voltammetry (DPV) was used to detect the binding of the atrazine. A linear calibration curve for atrazine detection was obtained, and the detection limit of the sensor was reported to be 1 nM.
Wu et al. introduced a selective P-o-PD sensor for dopamine detection. Their work demonstrated the feasibility of o-PD for preparing a dopamine-imprinted MIP. A uniformly coated P-o-PD layer was polymerized on a gold electrode using repeated potential sweep, and the electrochemical properties of the product was affected by the template to monomer ratio, pH, and the number of CV scans. 30 cycles with 100 mV/s scan rate for CV were performed during P-o-PD polymerization, and 0.5M H$_2$SO$_4$ was introduced for template removal. Their modified sensor was found to be selective towards dopamine, and the detection limit of the sensor was 0.11 mg/L.

Thanks to the pioneering works for P-o-PD imprinting, the electrochemical properties of P-o-PD are well-characterized. Several factors that affect the polymerization of the P-o-PD-based MIP have also been reported such as the template to monomer molar ratio and the number of potential scans. Motivated by the aforementioned previous work, this chapter presents the development of an o-PD-based MIP to see if it is capable of imprinting other neurotransmitters besides dopamine.

This chapter will mainly discuss the proper polymerization approach for P-o-PD synthesis. For PPy polymerization, we introduced a chemical synthesis approach because Py monomers can be easily assembled on the surfaces of conductive template such as CNTs, forming a nanofiber structure. For P-o-PD polymerization, it is desirable grow a uniformly coated insulating layer with high packing density, therefore an electro-polymerization on the working electrode was used. The goal of the work presented in this chapter is to optimize the protocol for o-PD polymerization.
5.2 GROWTH FACTORS FOR POLY-O-PHENYLENEDIAMINE

According to the literature, the polymerization method for P-o-PD can be accomplished using CV scans [110]. Several factors might influence the electrochemical properties of the product such as numbers of CV cycles, scan range, monomer to template ratio, the electrode material, pH, and the washing solution.

The number of potential scan cycles and the scan range are directly related to the degree of the polymerization and hence the thickness of the polymerized P-o-PD layer. An efficient scan range for CV is also necessary for P-o-PD synthesis, because o-PD needs to be fully polymerized at a potential above 0.8V.

Monomer to target ratio and pH might influence the sensitivity of the produced sensor due to the varying number of created recognition sites. The material of the electrode can affect the affinity between the P-o-PD layer and the electrode. The washing solution also needs to be carefully chosen to remove the residues and embedded target molecules without damaging the MIP layer.

All of these factors need to be considered during P-o-PD polymerization to enhance the electro-chemical properties of the sensor.

5.3 EFFECT OF ELECTRODE MATERIAL

Gold and carbon electrodes were used to electropolymerize pure P-o-PD synthesis in this section, and the initial polymerization protocol was as follows: 50 μL of 5 mM o-phenylenediamine monomer was dropped onto either a gold or carbon electrode. Afterward, the electro-polymerization was performed by cycling the working electrode potential 20 times from 0 V to +1.2 V versus the reference electrode with a scan rate of 50 mV/s. Figure 5-1 shows the CV curves when o-PD is polymerized on gold (a) and carbon (b) electrodes. The rate of polymerization was much faster on the
gold electrode than on the carbon electrode. After 1 cycle, the current of the CV curve dropped significantly, and subsequent cycles between the 10th cycle and 20th cycle resulted in minimal oxidation current, indicating that the P-o-PD has fully coated the working electrode thereby blocking the charge transfer between the solution and working electrode. On the other hand, in Figure 5-1 (b), the polymerization rate on the carbon electrode was slower compared to the gold electrode possibly due to the higher resistivity of the electrode. There is a significant change in the CV curve between the 10th cycle and 20th cycle, indicating that the polymerization of P-o-PD is progressing even after 10 cycles of potential sweep. This result proved that the material of the electrode can affect the growth rate of the P-o-PD layer and the electrode. However, the growth rate on the gold electrode was too fast, making it hard to control the P-o-PD thickness on the electrode. Therefore, the carbon electrode was preferred in this work.
Figure 5-1. CV diagram of o-PD polymerized on (a) gold and (b) carbon electrodes after 1, 2, 10, and 20 cycles. Scan rate: 50 mV/s.
5.4 EFFECT OF SCAN RANGE

The scan range is also an important parameter in determining the growth rate of P-o-PD. Figure 5-2 shows the CV curves when o-PD monomers was polymerized on carbon electrodes with scan ranges of (a) 0 V to +1.2 V and (b) 0 V to +0.9 V. The growth rate of P-o-PD layer is higher with a larger potential scan range, as evidenced by the negligible oxidation current after 10 cycles. In both cases, the current decreased nearly to zero after 20 CV cycles, so the scan range during polymerization was not a main factor that affected the P-o-PD product. Hence, the scan range was chosen to be between 0 V to +1.2 V for the P-o-PD polymerization.
Figure 5-2. CV diagram of o-PD polymerized on carbon electrode after 1, 2, 10, and 20 cycles with a scan range of (a) 0V to 1.2V and (b) 0V to 0.9V. Scan rate 50 mV/s.

5.5 EFFECT OF PH

The pH of the polymerizing solution can also influence the electrochemical properties of the P-o-PD product. Moreover, dopamine can form polydopamine when the pH of the solution is above 6.0 [111], and this will affect the structures of the
molecularly imprinted receptors during polymerization. Figure 5-3 shows the CV diagram of NE-templated P-o-PD polymerization. 1 M HCl was introduced in the P-o-PD synthesis protocol. With 1 M HCl, a sharp oxidation peak is obtained at 0.8V, and this peak indicates the imprinting phenomenon of NE. In Figure 5-3 (b), the current peak around 0.8V is not pronounced, and this may be due to the lower imprinting rate. In the case of EP, an acidic environment is needed to dissolve EP in aqueous solutions. Therefore, 1 M HCl was added to the P-o-PD polymerization protocol, as was done for the NE-imprinted polymer. The presence of 1 M HCl reduced the pH of the solution to 0, promote the imprinting effect.
Figure 5-3. Cyclic voltammetry diagram when NE-P-o-PD MIP was polymerized with and without 1 M HCl solution. Scan rate 50 mV/s.

5.6 THE CHOICE OF ELUENT

After the synthesis of the molecularly imprinted P-o-PD, the embedded template molecules needs to be extracted from the P-o-PD layer. Therefore, a proper extractant needs to be used for device regeneration without damaging the polymer.

Figure 5-4 shows the cyclic voltammetry curves of NE-P-o-PD-MIP treated with different extractants. Prior to washing, the polymer layer is uniformly covered on
the working electrode, therefore the redox current is nearly zero due to the insulating nature of P-o-PD. After washing the electrode with DI water or 1 X PBS, the background current increased only slightly, so these two solutions are not effective eluents. However, when the polymer was incubated in methanol for 10 mins while stirring at 100 rpm, the polymer was damaged as the charges were able to flow between the PBS solution and working electrode. Although DI water and 1X PBS are mild solutions for washing, it might take several hours to extract NE molecules in the polymer layer. In order to remove all of the embedded NE molecules inside the P-o-PD layer in a short period of time, it is necessary to use diluted methanol as an eluent.

![NE-P-o-PD-MIP, test with 1xPBS](image)

Figure 5-4. CV diagrams when NE-P-o-PD-MIP devices were exposed to 1X PBS solution before and after washing with different eluents (DI water, PBS, and methanol). Scan rate: 50 mV/s.

Figure 5-5 shows the DPV responses after polymerized o-PD-MIP was washed with DI water containing 0, 10%, 30%, or 50% methanol, and exposed to 1 mM Fe(CN)₆. The oxidation peak of Fe(CN)₆ occurred when the methanol concentration reached 30 %. Therefore, 10% methanol in DI water solution is the most effective
The eluent of the solutions tested for template extraction without damaging the polymer layer.

![Graph showing DPV curves after NE-P-o-PD-MIP was washed with different eluents, then exposed to 1mM Fe(CN)$_6$ solution.]

5.7 CONCLUSION

This chapter mainly discusses the P-o-PD polymerization processes. The polymerization is performed by scanning the potential repeatedly on a screen printed carbon electrode. Several parameters affected the properties of the final product. The scan range and the number of cycles play a major role in the thickness of the insulating polymer layer. Also, the P-o-PD grew faster on gold electrodes than on carbon electrodes, and an acidic environment was required for neurotransmitter imprinting. What’s more, the eluent solution should be mild in order to prevent the polymer from being destroyed, while still being able to remove the embedded molecule. Based on numerous iterations of optimizing the synthesis parameters, the following polymerization protocol was obtained: 5 mM of the template molecule (either DA, NE
or EP) was added to an aqueous solution containing 5 mM o-phenylenediamine monomer, and 0.1 M HCl at room temperature. Afterwards, 50 μL of the mixture was dropped onto the carbon working electrode. Then, the electro-polymerization was performed by scanning the potential from 0 V to +1.2 V vs. a silver pseudo-reference electrode for 30 cycles. Following the synthesis, the molecularly imprinted P-o-PD layer was rinsed in 10% methanol for 10 minutes while stirring at 100 rpm to remove the embedded template molecule.
CHAPTER 6: DETECTION OF NEUROTTRANSMITTER USING O-PHENYLENEDIAMINE-BASED MOLECULARLY IMPRINTED POLYMER

6.1 INTRODUCTION

In this chapter, the objective is to evaluate the performance of the neurotransmitter detection using P-o-PD-based MIPs in conjunction with electrochemical sensing techniques. We propose two hypotheses: first, the molecularly imprinted P-o-PD (P-o-PD-MIP) possesses higher sensitivity towards the target neurotransmitter than the non-imprinted (P-o-PD-NIP) sensor and second, the P-o-PD-MIP can minimize the electrochemical transduction signal from other interfering species.

P-o-PD is capable of dopamine imprinting. Wu et. al. polymerized the P-o-PD with dopamine on gold electrodes via an electrochemical method [112]. They utilized cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) to examine the properties of the sensor. The detection limit was found to be 0.11 mg/L, and the sensor was selective to dopamine when ascorbic acid was present in the solution.

In this chapter, we explore the use of o-PD as an MIP layer for the detection of dopamine (DA), epinephrine (EP) and norepinephrine (NE). Several studies have been done previously for dopamine detection. However, no work on EP and NE imprinting using o-PD has been reported. Figure 6-1 shows the cyclic voltammetry diagram of a P-o-PD-coated screen-printed carbon electrode and a blank carbon electrode when each is exposed to 1 mM of ferro-/ferricyanide solution. A clear oxidation peak of ferro-/ferricyanide was observed from a blank carbon device whereas no obvious redox current was found for the P-o-PD-modified electrode. This confirms that P-o-PD is an insulator that prevents charge transfer between the solution and the electrode.
Figure 6-1. Cyclic voltammetry (CV) of a blank carbon electrode and the Poly(o-PD) coated electrode when each device was exposed to 1 mM ferro-/ferricyanide solution. Scan rate: 50 mV/s.

6.2 o-PD-MIP-BASED SENSOR DEVELOPMENT

The experimental procedure for testing the P-o-PD-modified sensors is illustrated in Figure 6-2. 50 μL of 5 mM o-PD monomer and 5 mM template mixture was deposited on the surface of the screen-printed carbon electrode, followed by the electropolymerization process with the same parameters given in Section 5.7. Then, the template was removed by incubating the device in a 10% methanol solution. Before analyte detection, the prepared sensor was exposed to 1 mM ferro-/ferricyanide solution for CV measurements to make sure the polymer was still fully covered on the working electrode after washing. The device was ready for analyte detection if no clear oxidation peak appeared on the CV diagram.
6.3 NEUROTRANSMITTER DETECTION

DPV responses of P-o-PD-DA-MIP based-sensors when exposed to various concentrations of DA solutions are given in Figure 6-3 (a). The oxidation peak occurs at 0.02 V and increases proportionally to the DA concentration. The curve for 0 μM DA was considered to be the background current, and the change in peak current for 100 μM DA was approximately 7.8 μA with respect to the background current. Figure 6-3 (b) shows the current response when the P-o-PD-NE-MIP device was exposed to NE solutions. NE exhibited its oxidation peak at 0.08 V, at which the maximum oxidation current of 14.9 μA was observed when the NE concentration was 100 μM.
The DPV responses for P-o-PD-EP-MIP for EP detection are summarized in Figure 6-3 (c). The peak oxidation current was obtained at 0.1 V while the magnitude of the difference in current between 0 µM and 100 µM of EP concentrations was 7.46 µA. For all molecularly imprinted sensors, clear oxidation peaks for each type of neurotransmitter were clearly obtained from the DPV curves. The results indicated that P-o-PD is capable of detecting all three analytes. The non-imprinted (P-o-PD-NIP) sensor was also prepared as a reference. Figure 6-4 shows the DPV responses of the NIP based-sensor incubated in various concentrations of (a) DA, (b) NE, and (c) EP solutions. The magnitudes of the current response from these analytes were significantly reduced compared to their MIP counterparts by the densely coated and non-conducting P-o-PD layer without molecular cavities.
Figure 6-3 DPV diagrams for (a) P-o-PD-DA-MIP; (e) P-o-PD-NE-MIP, and (f) P-o-PD-EP-MIP based-sensors as functions of the working electrode potentials when exposed to 1, 5, 10, 20, 50, 100 µM analyte solutions. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms,
Figure 6-4 DPV diagrams for (a) P-o-PD-DA-MIP; (e) P-o-PD-NE-MIP, and (f) P-o-PD-EP-MIP based-sensors as functions of the working electrode potentials when exposed to 1, 5, 10, 20, 50, 100 µM analyte solutions. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms,
Figure 6-5 compares the DPV responses between P-o-PD-MIP and P-o-PD-NIP for the three types of neurotransmitters. In Figure 6-5 (a), when both PPy-DA-MIP and NIP were exposed to 100 µM of dopamine, the oxidation peak occurred at -0.02 V and 0.02 V for MIP and NIP, respectively. The peak current of the P-o-PD-DA-MIP response was approximately 10.2 µA, which is much larger than that of the P-o-PD-NIP response. Figure 6-5 (b) shows the DPV responses of P-o-PD-NE-MIP and P-o-PD-NIP when exposed to 100 µM of norepinephrine. A sharp oxidation peak was observed at 0.08 V for NE-MIP and the peak current was not very pronounced for P-o-PD-NIP, indicating a sluggish reaction. The peak current for P-o-PD-NE-MIP was 10.2 µA, which is much higher than that for the PPy-NIP. Therefore, the P-o-PD-NE-MIP demonstrated a better sensing performance than the PPy-NIP with respect to NE detection, showing improved sensitivity. Figure 6-5(c) shows the current response for P-o-PD-EP-MIP versus P-o-PD-NIP when detecting EP. The MIP based-sensor exhibited a clear oxidation peak at 0.1 V, with a current of 10.8 µA, while the sensor based on NIP hardly showed any response from EP exposure. In all three cases of analyte detection, the DPV results showed obvious increases in the oxidation peak currents for the imprinted sensors compared to the non-imprinted sensors.
Figure 6-5 DPV responses of each neurotransmitter imprinted sensor (MIP) compared with non-imprinted (NIP) sensors: (a) P-o-PD-DA imprinted vs. non-imprinted sensors when exposed to 100 µM of DA; (b) P-o-PD-NE imprinted vs. non-imprinted sensors when exposed to 100 µM of NE, and (c) P-o-PD-EP-imprinted MIP vs. non-imprinted sensor when exposed to 100 µM of EP. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms.
Figure 6-6 summarizes the calibration curves for P-o-PD MIP versus P-o-PD-NIP when detecting their target analytes; the data was obtained with three independent trials (n = 3). Figure 6-6 (a) shows the current response for P-o-PD-DA-MIP- and P-o-PD-NIP-based sensors for DA detection. The current peaks were obtained at working electrode potentials of 0.02 V, and their linear regression equations were calculated as
\[ \Delta I = I_{\text{peak}} - I_{\text{background}} = 0.0735 \, C_{\text{DA}} \, (\mu M) + 0.371 \]
and
\[ \Delta I = 0.0206 \, C_{\text{DA}} \, (\mu M) + 0.075, \]
respectively. The slope for the MIP is approximately 3 times as great as that of the NIP, indicating better sensitivity for the MIP based-sensor. In Figure 6-6 (b), the linear regression equations for P-o-PD-NE-MIP and NIP are
\[ 0.065 \, C_{\text{NE}} \, (\mu M) + 0.294 \]
and
\[ \Delta I = 0.0208 \, C_{\text{NE}} \, (\mu M) + 0.009. \]
The MIP based-sensor performed better than the NIP based-sensor due to the created recognition cavities for NE molecules. The calibration curves for P-o-PD-EP-MIP and NIP detecting EP is shown in Figure 6-6 (c), and all the current data were obtained at 0.1 V where the oxidation peak occurred for EP. The linear regression equations for the MIP and NIP based-sensors are
\[ 0.077 \, C_{\text{EP}} \, (\mu M) + 0.100, \]
and
\[ \Delta I = 0.0102 \, C_{\text{EP}} \, (\mu M) + 0.196, \]
respectively. In all cases, MIP material showed better sensitivity towards the target analyte than NIP. Also, the linear regression equations for the NIP based-sensors detecting the three analytes are very close indicating that the pure P-o-PD layer is not very selective to those neurotransmitters.
Figure 6-6. Calibration curves for neurotransmitter imprinted (MIP) vs. non-imprinted (NIP) sensors incubated in 0 µM, 1 µM, 5 µM, 10 µM, 20 µM, 50 µM, and 100 µM concentrations of solutions: (a) DA imprinted and non-imprinted sensor when exposed to DA solutions, (b) NE imprinted and non-imprinted sensor when exposed to NE solutions, and (c) EP imprinted and non-imprinted sensor when exposed to NE solutions.
6.4 CROSS REACTION OF P-O-PD IMPRINTED SENSORS.

The cross-reactivity of each MIP-based sensor is further discussed in this section to evaluate the selectivity of each MIP based-sensor. Figure 6-7 (a) shows the DPV response when the P-o-PD-DA-MIP was exposed to 100 µM DA, NE, or EP solutions. The oxidation peak for DA is at 0.02 V, while NE and EP have their oxidation peaks at 0.12 V and 0.14 V, respectively. It is clear that DA produced the highest peak, indicating that the created recognition cavities in the P-o-PD-DA-MIP is most selective toward DA molecules.

Figure 6-7 (b) summarizes the DPV responses of the P-o-PD-NE-MIP based-sensor detecting 100 µM of DA, NE, or EP solutions. The oxidation peaks for DA, NE, and EP occurred at 0.02 V, 0.08 V, and 0.1 V, respectively. However, the peak current of NE was the lowest when compared to the other two analytes, so the P-o-PD-NE-MIP was not selective. The reason for this lack of selectivity may be because of the partially imprinting of NE molecules. In Figure 6-7 (c), the P-o-PD-EP-MIP based-sensor was exposed to 100 µM of DA, NE, or EP solutions and showed three distinguished oxidation peaks. Although EP is not as electro-active as DA in nature, the DPV curves for these two analytes were still distinguishable due to their different oxidation potentials. Hence, it can be concluded that the P-o-PD-EP-MIP is still selective toward EP at 0.1 V.

As a result, both P-o-PD-DA-MIP and P-o-PD-EP-MIP based-sensors were found to be selective toward their respective imprinted analyte. The P-o-PD-NE-MIP based-sensor, on the other hand, was not particularly selective towards NE molecule. The peak oxidation potential of NE occurred at 0.8 V, which is located between that of DA and EP, therefore, DA and EP could easily interfere with NE.
Figure 6-7 DPV responses of each neurotransmitter imprinted sensor (MIP) when exposed to 100 µM DA, NE, and EP solutions. Scan rate: 50 mV/s, step potential: 0.01 V, pulse potential: 0.2 V, pulse time: 100 ms,
Figure 6-8 summarizes the cross-reactivity results for the three types of imprinted sensors. In Figure 6-8 (a), the current response at 0.02 V was obtained when P-o-PD-DA-MIP was exposed to DA, NE, or EP, and the linear regression equations are \( \Delta I = 0.0735 \, C_{DA} \, (\mu M) + 0.3715 \), \( \Delta I = 0.0374 \, C_{NE} \, (\mu M) + 0.1388 \), and \( \Delta I = 0.0371 \, C_{EP} \, (\mu M) + 0.105 \), respectively. DA resulted in the highest sensitivity of the three MIPs as the slope of its regression equation is almost twice that of either NE or EP. Figure 6-8 (b) shows the calibration curves for P-o-PD-NE-MIP at 0.08 V. The current response for each analyte was almost the same, indicating poor selectivity for the P-o-PD-NE-MIP based-sensor. Figure 6-8 (c) shows the P-o-PD-EP-MIP based-sensor performance on multi-analyte sensing at 0.1 V, and the linear regression equations based on its response to DA, NE and EP are \( \Delta I = 0.0486 \, C_{DA} + 0.0804 \), \( \Delta I = 0.0465 \, C_{NE} \, (\mu M) + 0.1126 \), and \( \Delta I = 0.0772 \, C_{EP} \, (\mu M) + 0.0998 \), respectively. The current response of the unimprinted analytes were minimized by the P-o-PD-EP-NIP layer.

In summary, it can be concluded that P-o-PD-DA-MIP and P-o-PD-EP-MIP were selective towards their imprinted target species, and therefore, a multi-analyte sensing device consisting of these two MIPs for simultaneous detection of DA and EP can be developed.
Figure 6-8 Cross-reactivity response for (a) P-o-PD-DA-MIP, and (b) P-o-PD-NE-MIP, and (c) P-o-PD-EP-MIP for different concentrations of DA, NE, and EP.
The selectivity of three neurotransmitter imprinted sensors are quantified by Danzer [100]. As described in Section 4.6, matrix M is the matrix of sensitivity calculated from each sensor. Each column represents a single analyte (DA, NE, and EP), and each row represents a specific sensor (P-o-PD-DA-MIP, P-o-PD -NE-MIP, and P-o-PD -EP-MIP). The coefficients Aij are the slopes of the calibration curves of each sensor detecting a single analyte.

\[
M1 = \begin{bmatrix}
A_{11} & A_{12} & A_{13} \\
A_{21} & A_{22} & A_{23} \\
A_{31} & A_{32} & A_{33}
\end{bmatrix}
\]

\[
= \begin{bmatrix}
0.0735 & 0.0374 & 0.0371 \\
0.0637 & 0.0650 & 0.0679 \\
0.0486 & 0.0456 & 0.0772
\end{bmatrix}
\]

The selectivity of each imprinted sensor was calculated as follows:

\[
S(j) = \frac{\sum_{i=1}^{n} A_{ij}}{\sum_{i=1,j=1}^{n} A_{ij}}
\]

\[
S(DA) = \frac{A_{11}}{A_{11} + A_{12} + A_{13}} = 0.497
\]

\[
S(NE) = \frac{A_{22}}{A_{21} + A_{22} + A_{23}} = 0.331
\]

\[
S(EP) = \frac{A_{22}}{A_{31} + A_{32} + A_{33}} = 0.450
\]

The total selectivity of the three MIP based-sensor array was calculated as follows:

\[
S(\text{total}) = \frac{\sum_{i=1}^{n} A_{ii}}{\sum_{i=1,j=1}^{n} A_{ij}} = 0.426
\]

The calculated selectivity indicates a relatively good selectivity for both DA-MIP and EP-MIP. The selectivity of NE-MIP was not as promising as the other MIPs. However, one possible strategy to improve its selectivity could be to introduce a catalyst to the NE-MIP material.
6.5 CONCLUSION

This chapter discussed the imprinting capability of P-o-PD-based neurotransmitter sensors. P-o-PD was proven to be a promising imprinting material after electrochemical polymerization. The coated polymer layer can prevent the charge transfer between the solution and the electrode, allowing the sensor to minimize the current signal and unwanted noise from other interfering species. P-o-PD-DA-MIP-, P-o-PD-NE-MIP-, and P-o-PD-EP-MIP-based sensors were developed and tested using electrochemical methods. For all three molecularly imprinted sensors, the current responses showed better sensitivity compared to the non-imprinted sensor due to the created recognition cavities and the insulating property of o-PD layer.

P-o-PD-DA-MIP and P-o-PD-EP-MIP also exhibited a good selectivity towards the imprinted analyte, suggesting that it is possible to develop a sensor array for simultaneous detection of DA and EP for future work. However, P-o-PD-NE-MIP showed poor selectivity towards NE molecules.
CHAPTER 7: CONCLUSION

This thesis work demonstrates the capability of using molecular imprinting technology for neurotransmitter detection. Pyrrole and o-phenylenediamine were used as functional monomers for sensor development. Dopamine, norepinephrine, and epinephrine were selected as the model neurotransmitters.

For pyrrole-based sensors, several chemical synthesis approaches were developed. We have optimized the growth conditions to develop a molecularly imprinted nanowire structure for PPy to improve the surface area of the material. Also, the polymerized polymer was tested to verify the imprinting capability. Py-MWCNT-H$_2$O$_2$ combinations showed a promising potential for neurotransmitter imprinting, so the protocol was adopted to develop DA-, NE-, and EP-imprinted sensors. The DPV responses showed that the imprinted sensors improved the sensitivity toward the target molecule when compared to the non-imprinted sensor. The detection limits of PPy-DA-MIP, PPy-NE-MIP, and PPy-EP-MIP were 7.97 µM, 3.05 µM, and 7.08 µM, respectively. Also, the cross reaction test proved that PPy-DA-MIP and PPy-NE-MIP based-sensors were selective towards the imprinted analyte because of the created recognition cavities. Although the PPy-NE-MIP based-sensor did not perform as well as the other two analytes in terms of selectivity during the cross-reaction test, it did show some level of selectivity when PPy-DA-MIP and PPy-NE-MIP sensor arrays were tested for simultaneous detection.

O-phenylenediamine based sensors were polymerized via an electrochemical method due to the uniform coating on the working electrode. Several parameters would influence the electrochemical properties of the modified sensor, such as electrode material, scan range and pH. The choice of eluent is also important in extracting the
imbedded analyte without damaging the polymer. The optimized polymerization approach is proved to be capable of neurotransmitter imprinting because a pure P-o-PD layer grew uniformly on the working electrode, preventing all charge transfer between the sample fluid and the electrode. The molecularly imprinted P-o-PD layer could also extract the embedded molecule, forming unique cavities for analyte recognition. The impact of molecularly imprinting is further discussed by comparing the DPV responses of MIP and NIP based-sensors. All types of MIP based-sensors exhibited far better sensitivity than NIP based-sensors because of the created cavities inside the P-o-PD layer. Also, the cross-reaction test confirmed a good sensitivity for P-o-PD-DA-MIP and P-o-PD-EP-MIP based-sensors towards the imprinted analytes.

For all MIP based-sensors, the sensitivity for target analyte recognition was enhanced with respect to the NIP based-sensors, and that is due to the unique cavities created inside the polymer layer. DA and EP MIPs were selective toward their respective imprinted analyte, hence the development of a sensor array for the simultaneous multi-analyte detection of those two species may be possible.

Table 7-1. The sensitivity and detection limit for all MIPs

<table>
<thead>
<tr>
<th></th>
<th>Linear Regression Equations</th>
<th>Slope</th>
<th>Sensitivity</th>
<th>Detection limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPy-DA-MIP</td>
<td>( \Delta I = 0.080 C_{DA} + 0.1768 )</td>
<td>0.080</td>
<td>1.27 ( \mu A \cdot \text{mM}^{-1} \cdot \text{cm}^2 )</td>
<td>7.972 ( \mu M )</td>
</tr>
<tr>
<td>PPy-NE-MIP</td>
<td>( \Delta I = 0.053 C_{NE} + 0.0792 )</td>
<td>0.053</td>
<td>0.84 ( \mu A \cdot \text{mM}^{-1} \cdot \text{cm}^2 )</td>
<td>3.047 ( \mu M )</td>
</tr>
<tr>
<td>PPy-EP-MIP</td>
<td>( \Delta I = 0.069 C_{EP} - 0.0885 )</td>
<td>0.069</td>
<td>1.10 ( \mu A \cdot \text{mM}^{-1} \cdot \text{cm}^2 )</td>
<td>7.081 ( \mu M )</td>
</tr>
<tr>
<td>o-PD-DA-MIP</td>
<td>( \Delta I = 0.074 C_{DA} + 0.3715 )</td>
<td>0.074</td>
<td>0.59 ( \mu A \cdot \text{mM}^{-1} \cdot \text{cm}^2 )</td>
<td>3.97 ( \mu M )</td>
</tr>
<tr>
<td>o-PD-NE-MIP</td>
<td>( \Delta I = 0.065 C_{NE} + 0.2941 )</td>
<td>0.065</td>
<td>0.52 ( \mu A \cdot \text{mM}^{-1} \cdot \text{cm}^2 )</td>
<td>12.18 ( \mu M )</td>
</tr>
<tr>
<td>o-PD-EP-MIP</td>
<td>( \Delta I = 0.077 C_{EP} + 0.0998 )</td>
<td>0.077</td>
<td>0.61 ( \mu A \cdot \text{mM}^{-1} \cdot \text{cm}^2 )</td>
<td>5.56 ( \mu M )</td>
</tr>
</tbody>
</table>
Table 7-1 summarizes the linear regression equations, sensitivity and detection limit of all MIPs synthesized. The sensitivity is calculated using the slope over the surface area of the MIP (PPy-MIP had an average surface area of 0.063 cm\(^2\) while o-PD-MIP had a surface area of 0.126 cm\(^2\)). The detection limit is calculated by taking three times standard deviation over slope. The sensitivities of PPy-MIPs are higher than o-PD-MIPs due to the highly conductive nature of PPy and also to the nanowire morphology which increases the surface area. The detection limits are less than 1.3×10\(^{-5}\) M for MIPs.

Several limitations remain unsolved in this work, and will be addressed in our future work. First of all, the PPy based-sensor is still not stable enough during analyte recognition; the background current changed slightly between each test. Although we stabilized the newly made PPy with several DPV scans, a new strategy needs to be developed. The LOD of PPy-MIP based-sensors can reach nM level when the background current is fixed below a certain value. We are confident that PPy MIPs have a potential for real sample analysis when the challenge of detection limit is fixed. Also, the P-o-PD layer can still suffer some slight damage during washing, and there was a chance that the P-o-PD sensor was unable to fully cover the working electrode after washing. The probability was close to 20 percent. We still need to find a better solution to extract the embedded molecule without damaging the polymer. What’s more, both PPy-NE-MIP and P-o-PD-NE-MIP did not work well during cross-reaction tests. The reason for this might be due to the fact that the created receptor site is too large and allows DA and EP to interfere, and the oxidation potential for NE is too close to DA and EP. One possible solution for this is to introduce a catalyst to improve the electrochemical response of NE alone, so that the selectivity of the sensor could improve.
The future work stemming from this thesis include enhancing the detection limit of PPy sensors to meet the requirements of commercial use. Also, the selectivity of all of the developed MIP based-sensors need to be further improved. Once the interference of other analytes is minimized, it will be feasible to develop a MIP based-sensor array for multi-analyte detection from a mixture of analytes. Yan et al. has tried detecting ascorbic acid using PPy and P-o-PD copolymer [113], and one possibility is to attempt a two-step polymerization to improve the performance of MIP. The idea of a two-step polymerization is to synthesize PPy-MIP on the electrode first, then cover the PPy-MIP-grown electrode with P-o-PD MIP layer. This strategy would keep the good electrochemical properties of PPy, while further improving the selectivity of the sensor.
Appendix

The LabVIEW block diagram:
REFERENCES


[76] Chemical Synthesis of Highly Conducting Polypyrrole Nanofiber Film - Macromolecules (ACS Publications), (n.d.).


