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Understanding the surface fouling mechanism of ultrananocrystalline diamond microelectrodes using microfluidics for neurochemical detection

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UNDERSTANDING THE SURFACE FOULING MECHANISM OF ULTRANANOCRYSTALLINE DIAMOND MICROELECTRODES USING MICROFLUIDICS FOR NEUROCHEMICAL DETECTION

by

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A Dissertation Presented in Partial Fulfillment of the Requirements of the Degree Doctor of Philosophy

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We hereby recommend that the dissertation prepared under our supervision by An-Yi Chang, M.S. entitled UNDERSTANDING THE SURFACE FOULING MECHANISM OF ULTRANANOCRYSTALLINE DIAMOND MICROELECTRODES USING MICROFLUIDICS FOR NEUROCHEMICAL DETECTION be accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Engineering

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ABSTRACT

Electrochemical methods are widely used for chronic neurochemical sensing, but thus far, the organic solution redox reactions fouled the electrodes' surface. It caused the reduction of sensitivity and the electrodes’ lifetime.

Here, we present the boron-doped nanocrystalline diamond microelectrodes (BDUNCD) as the next generation electrode material for neurochemical sensor development. To aid in long-term chronic monitoring of neurochemicals, they have a wide window of electrochemical potential, extremely low background current, and excellent chemical inertness. The main research goal is to reduce the rate of electrode fouling due to the reaction by-products, and significantly extend their useful lifetime.

We systematically characterize the fouling mechanism at the BDUNCD microelectrode surface by investigating silver particles deposited on BDUNCD surface at different fouling conditions using customized microfluidic device. The fouling rates were carefully studied using two electrochemical techniques Fast Scan Cyclic Voltammetry (FSCV) and Amperometry (AM).

Furthermore, in-situ electrode cleaning methods were developed in PBS buffer solution. Under optimal conditions, the cleaning method extended the electrode sensitivity from ~6.5 hours to ~28 hours.

We developed a droplet based microfluidic platform to characterize three types of microelectrodes BDUNCD, nafion modified BDUNCD, and nafion multi wall carbon
nanotube modified. BDUNCD dopamine signals enhance two to three times by 
Electrophoretic deposition (EPD) of nafion layer (50 nM), and enhance about 10 times in 
complementary nafion multi wall carbon nanotube modified BDUNCD. Specifically, the 
sensitivity, response time, and clearance rate of dopamine were determined in 9 hours of 
monitoring. In the presence of ascorbic acid and serotonin was studied using differential 
pulse voltammetry method, the selectivity comparison performs the multi-wall carbon 
nanotube superiority and achieved long-lasting 9 hours monitoring after nafion layer 
coating, it has the initial sensitivity of oxidation current DA (0.58 nA) and 5-HT 
(1.03 nA) with a sensitivity value of DA 1.18 μA μM⁻¹ cm⁻² and 5-HT 
2.09 μA μM⁻¹ cm⁻². The other advantage of multi-wall carbon nanotube modified 
BDUNCD electrode reduces the limit of detection to 5.4 nM (DA) and with nitric acid 
treatment 1.78 nM (DA).
APPROVAL FOR SCHOLARLY DISSEMINATION

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Author

Date 7/26/2017
DEDICATION

This dissertation is dedicated to my parents, Chen-Land Chang and Lien-Ti Liu, my brother Kai Chang, whose support and encouragement provided me strength to overcome the difficulties to complete my research successfully.
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CHAPTER 1
INTRODUCTION

1.1 Chronic Neurochemical Detection

1.1.1 Neurochemical Influence on Human Brain Disease

In the 19th Century, the nervous system has been studied by chemical techniques; it is the constituents of neuronal tissue and as "chemical statics" by J.W.L. Thudichum. However, the brain is processing information dynamically rather than statically [1]. Many years of studying neurons focused on the domain of electrophysiology [2, 3, 4]. Nevertheless, in the 20th century, scientists have clearly understood the information of neurons involved in the chemical signals transferred in the brain; it developed from whole brain analyzation to microdissection [5]. By continuing to improve the technique, it gave prominence to the importance of neurochemistry.

Presently, we know that the nervous system transfers information by neurochemicals such as dopamine, Glutamate, Serotonin, etc. [6]. Those neurochemicals are important because they affect our mood, focus, and memory. Furthermore, scientists discovered that many brain diseases are related to the neurochemical such as Dopamine concerning Parkinson [7, 8]. In the human brain, neurons have diverse sizes and shapes. After learning more about neurons, it has two types of projections which are dendrites and axon; the dendrites are the input for information and axons have an output function.
In the neurons, the information is transferred by electrical potentials on the cell membrane [9]. Ordinarily, neurons have a negative potential and the outside ions can cause the membrane to depolarize; it causes a potential action, which transfers to another neuron and has a propagation rate of 50 m/s [10].

Currently, there are many neurotransmitters to be analyzed to develop the distinct methods. Here, we will focus on the specific neurotransmitter dopamine because it is the main neurochemical, and it is key to understanding the human brain [11]; discovered as a neurotransmitter in the late 1950s, in the 1960s, it is confirmed that it is related to Parkinson's disease [12]. Parkinson's disease was discovered in 1817 by James Parkinson and usually occurs at age 60+; it is caused by aging and damaged neurons [13]. In the tegmentum, the Substantia Nigra can generate dopamine and transfer information in the human brain. It also inhibits Acetylcholine [10]. When Substantia Nigra reduces, it will affect the generated of dopamine. The lack of dopamine causes the increase of Acetylcholine; it causes tremor and muscle rigidity. Parkinson's patients have Freezing of Gait problem; it causes difficulty in walking. Moreover, there are other symptoms of Parkinson’s patients such as arm and leg tremors, and muscle stiffness. It also affects cognitive skills, judgment, and memory. However, levodopa has been used to aid dopamine generated [14, 15]. The treatment successfully reduces the symptoms even with some exercise. Likewise, there are other treatments for Parkinson's patients such as dopamine receptor agonists [16], COMT inhibitors [17], MAO-B inhibitors [18], Amantadine [19], and Anticholinergics [20].
1.1.2 Dopamine Redox Reaction and Fouling Mechanism

Dopamine is important in several brain processes, and it is a neurotransmitter. It also plays a critical role in the central nervous, cardiovascular, renal, and hormonal systems. In the 1970s, the seminal work of Ralph Adams has stated the DA is electroactive and it can be monitored \textit{in vitro} and \textit{in vivo} by electrochemical detection [21]. Dopamine is easily oxidized, and it can be analyzed by using electrochemistry. For example, fast scan cyclic voltammetry was able to monitor dopamine in the brain of an anesthetized rat selectively and simultaneously (Bledsoe \textit{et al.} [22]). FSCV offers the possibility of carrying out precise chemical identification. Amperometry is another method for biological assays. It offers the capability to measure the quantitative in sub millisecond for time resolution. Cyclic voltammetry is used to measure the current by cycling the potential of a working electrode.

In the human brain, Dopamine (DA) is one of the neurochemicals, which transfer information in order to control the body [23]. Therefore, it is easy to be oxidized and it can be analyzed electrochemically. In the DA redox reaction on carbon nanotube fiber (CNTF) electrodes, it fouls the electrode surface and results in a reduction of the dopamine current, which is the detection signal. The fouling (rate) is dependent on the dopamine concentration, the applied potential at the working electrode, and the time of the potential is applied (i.e. detection/monitoring time). For real-time sensing either \textit{in vitro} or \textit{in vivo}, one of the challenges is to understand this fouling mechanism and the fouling rates under various experimental conditions. In the dopamine oxidation processes, there are two electron transfers forming the o-dopaminoquinone (o-DQ) (1). Then the amine is protonated, resulting in a cyclization leading to the formation of
leucodopaminochrome (LDC) (2). Finally, the LDC transfers two electrons to form dopaminochrome (DC). The DC has free radical polymerization to melanin, which forms a polymeric film on the surface and limits the electrochemical detection of DA (Fig. 1-1) [24].

Melanin has particles whose diameters range from 100 nm to 200 nm [25] and they are insoluble and non-conductive. Therefore, during bio-sensing, the generated of melanin adheres on the electrodes' surface and reduces the electrodes' sensitivity and conductivity.

![Melanin formation (electrode fouling)](image)

Fig. 1-1. This is the dopamine oxidation process with final melanin formation. Reaction (1) there are two electron transfers forming the o-dopaminoquinone (o-DQ), which has a positive charge; reaction (2) the amine is protonated, resulting in a cyclization leading to the formation of leucodopaminochrome (LDC); reaction (3) the LDC transfers two electrons to form dopaminochrome (DC), and finally The DC has free radical polymerization to melanin, which forms a polymeric film on the surface and limits the electrochemical detection of DA [24].

1.2 Chronic Neurochemical Sensing

The treatment of neurologic disorders and restoration of the normal neuronal condition have been pursued by several research groups over three decades [26-30]. Carbon nanomaterials – nanotubes [31-33], nano fibers [34-36], nano diamonds [37-41] are frequently used as electrode materials in neuromonitoring devices, which
demonstrated high sensitivity during DA sensing and during electrochemical sensing along with better resistance towards chemical etching and surface fouling due to DA oxidative product such as melanin [42, 43]. Simulation driven neurochemical release in neuronal environment preferably measured by Fast scan cyclic voltammetry (FSCV) [44, 45], but the existing FSCV relies on carbon fiber micro electrodes (CFM) which are not suitable for chronic neurochemical recording due to severe susceptibility towards fouling and degradation [43, 44] in a simple buffer solution due to Kolbe like electrolysis [44, 45] at the electrodes’ surface. Previous study has shown boron doped diamond has greater fouling resistance than non-doped graphene and boron doped graphene [46].

1.3 Microfluidic Device Engineering

A microfluidic system was introduced into the simulation research in order to provide a controllable flow rate in the laminar flow. The main idea of microfluidics involves reducing the sample sizes and use of solutions; some of the solutions are expensive. Moreover, some of the samples are difficult to obtain, such as cancer cells from patients or sample cells from babies. It has been used in many research such as chemical reaction [47], cell separating [48], cell transfection [49, 50], screening the effective concentration [51], controlled release of drugs from microcapsules [52], and cell reactions [53], etc. Microfluidics can be used to inject more than one solution in a channel. On the other hand, the solution can be mixed in the channel by the different channel designs [51]. In the microfluidics, channel designs are very significant. Erroneous designs may cause the malfunction of the devices.

Microfluidic of biosensors have been successfully developed for clinical applications such as electrochemical blood glucose sensors (Kissinger 2005) [124], and
spinning blood device set to safeguard astronaut health [125]. It uses widespread as clinical diagnostic tool because of real-time measurement, quick diagnosis, multi-target analyses, automation, and the lower costs (Luong et al. 2008) [126], and it is a high-performance technology for early diagnosis (Teles and Fonseca 2008 [127]; Schasfoort 2004; Wang 2006) [128, 129]. However, the acknowledged drawbacks of investigating neural function and structure are the cells’ chemical microenvironment in a real-time sensing. Therefore, the microfluidic provide the capability to simulate the chemical environment with controllable distinct concentration and flow conditions [130]
CHAPTER 2

BACKGROUND

2.1 Fast Scan Cyclic Voltammetry (FSCV) and Amperometry (AM) Methods

Current electrochemical micro sensors have been employed to investigate neurochemicals which are important in the human brain to understand the effects involved in the disease [54]. In the electrochemical, Cyclic Voltammetry (CV) and Amperometry is powerful and common methods in the study of electrochemistry, inorganic chemistry, organic chemistry, and biochemistry. CV is employed to analyze the biological material, or an electrode’s surface. The analysis has the capability of investigating the redox behavior over a setup potential window; it has the result of a current versus potential which changes with time. From the results of the CV, it provided the current peak of anionic/cationic and peak potential. The current peak depends on the solution’s concentration or the electrode’s material. However, peak potential does not vary with different concentrations. Nevertheless, CV can measure data in different scan rate from 0.0175 μV/s (GAMRY instruments, PHE 200™) to $10^6$ V/s [55]. Fast Scan Cyclic Voltammetry (FSCV) is the progress data at a high scan rate. In order to analyze the rapid change of signals, we deal with complicated analysis such as the human brain. However, AM is a fundamental technique of electrochemical. It provides real time study by giving a constant voltage. This technique has the measured current, which is the
direct ratio with concentration and it can measure rapid changes in concentration. It also
gives a good signal to noise ratio.

2.2 Effect of BDUNCD on Long-term Detection

There are many other research groups that have attempted to improve the fouling problem in Carbon Nanotube Fiber Microelectrodes [56], and Hydrogenated Conical-tip Carbon Electrodes [57]. They showed two times improvements in the electrode's lifetime due to the lack of long-lasting electrodes to analyze neurochemicals. Here, we introduce the BDUNCD microelectrodes, which has ultra-small diamond grains (2–5 nm) and very smooth surface characteristics [52]. The smooth surface can exhibit low friction in most applications. The grains have a sp³ character and their boundaries have a mixture of sp², sp³ and amorphous carbon with sp² being predominant [58]. The decrease in diamond grain size increases the grain boundary (GB) volume fraction that contains the non-diamond carbon to improve electrical properties of these films [59]. UNCD has different properties which cannot be found in other carbon based materials. The properties can be adjusted and optimized for different applications such as Nitrogen UNCD (N-UNCD) [60]. BDUNCD film electrodes can be formed on different substrates (Ta, Si, Nb, W, Ti). However, the substrate stability is different by the order Ta > Si > Nb > W >> Ti [61]. To fabricate BDUNCD on the Si surface, apply boron gas while the BDUNCD form on the surface. In the procedure, it results in a high re-nucleation rate [62]. The re-nucleation occurs in two stages (1) Nanoparticles are created in the boron gas phase that causes re-nucleation. (2) The different species at the surface produce defect or re-nucleation.

BDUNCD film electrodes have been widely used in electrochemistry because of the advantages of a wide potential window, low background currents, and chemical
inertness. However, BDUNCD electrode conductivity is enhanced by increasing with the boron concentration [63]. In this study, I systematically studied the fouling rates on boron-doped ultra nanocrystalline diamond (BD UNCD) microelectrodes.

2.3 Introduction to Droplets’ Diffusion Studies

In the stream status, it provides a constant flow rate pass through the electrodes, and it supplies fresh DA at a controllable flow rate. It maintains the DA concentration during the redox reaction. On the other hand, the redox reaction is an effect factor in this research because we concentrate on determining the concentration, the sensitivity, and the response time. It detects electrons from DA oxidation. Nevertheless, the DA redox reaction is an advantage, which can increase signals by double-walls’ sensing.

In the droplets’ diffusion sensing, it releases a DA droplet at a constant time in a background solution in order to investigate the different effect factors such as the flow rate, the droplet volume, the scan rate, Signal-to-noise ratio, and concentration. Furthermore, a finalized procedure by studying the effect factors used to evaluate the best electrodes that have an excellent sensitivity, response time, and clearance rate.

2.4 Dopamine Fouling Mechanism on BDUNCD Surface

Neurochemicals are released in the human brain to deliver the message, and to have a reaction on human body or emotion. Therefore, neurochemicals have chemical properties that easily oxide to transfer the message. As well, electrochemical sensing takes advantage of these chemical properties to investigate oxidation current to estimate the signals. However, this causes the critical issue which is the electrodes’ fouling. Proteins, phenols, amino acids, neurotransmitters, and other biological molecules are regarded as potential fouling agents [32] of electrodes. Electrode fouling can severely
affect the sensitivity, detection limit, reproducibility, and reliability of electrode material by passivating the active electrode sites on the surface hindering contact of sensing analyte for electron transfer [32]. Neurochemicals can foul the electrodes’ surface without applying the potential or the currents. Consequently, present electron materials have the same arduous task in the fouling issue.

Recently developed chemical inertness surface is one of the directions such as nafion deposition on the electrodes’ surface [64]. On the other hand, by inventing a new electrode material, it is an option. Thence, BDUNCD takes advantage of chemical inertness [65]. However, neurochemicals fouling any type of material include plastic, metal, alloy, or polymer electrodes. In the fouling mechanism of the dopamine, it produces polymer films on the electrodes’ surface [66]; this procedure occurs in several hours which depend on the concentration. Nevertheless, it generates faster at sensing the electrodes’ surface. The reason is the chemical intermediates the charge. In the beginning, poly-dopamine produces a thin film adsorption on the surface, then slowly increases the thickness and fresh dopamine is one of the major chemicals to produce the final melanin particles on the surface [91]; it causes the critical fouling. By studying the material of BDUNCD, it can assist in understanding the fouling effect to the signals and the lifetime on distinct technologies, which can be used to compare with other materials and to develop the new electrode's surface treatment or materials.

2.5 FSCV Cleaning Methods

We considered boron doped ultra nanocrystalline diamond (BDUNCD) films as our electrode material with 2-5 nm grains & ultra-smooth surface (3-5 nm rams), where boron doping has been achieved by keeping fixed C/H ratio of ~3000 ppm to achieve the
minimum film resistivity of \( \sim 0.08 \Omega \cdot \text{cm} \) \([40, 67, 68]\) to investigate DA related surface fouling and in-situ surface reactivation techniques. Previously, an inviolate BDUNCD surface without changing surface orientation and property having signs of surface reactivation during in-situ cleaning by FSCV cycling \([69]\) for several hours have been reported by our group. BDUNCD considered as a very potent platform for neurosensing research with superior dimensional stability at higher current densities (> 300 mA cm\(^{-2}\)) for hundreds of hours \([67]\) which is conducive for chronic FSCV cycling. In this study, the effect of FSCV (from \(-0.4\) to \(+1.0\) V at \(400\) V/s, \(60\) Hz) cycling in a controlled manner for 30 minutes in 100 \(\mu\)M dopamine solution within integrated microfluidic channel (MFC) with a steady flow rate (0.2 ml/ minutes) of analyte is reported to qualitatively understand the signal behavior and DA related surface fouling of BDUNCD surface. This is followed by visualizing and understanding the effect of different in-situ reactivation techniques comprised of different ranges of \(-0.4\) to \(+1.0\) V \([45, 70]\), \(-0.5\) to \(+0.8\) V, and \(-0.5\) to \(+1.2\) V.

It also studies the positive potential effect, which fixes negative’s potential at \(-0.5\) V and studies the different positive potentials. Negative potential effect fixes positive’s potential at \(+1.0\) and studies the different negative potentials. The rationale behind keeping the resting potential at \(-0.4\) V and \(-0.5\) V is to observe the modification of BDUNCD surface resulting in a change in electrostatic effect which further increases the dopamine adsorption, resulting in an increase in the DA peak current \([43, 71, 72]\). The outcome of such an investigation would lead us towards a better cognition about the texture of BDUNCD fouling followed by proper in-situ cleaning/reactivation to estimate the alteration in the chronic DA monitoring time in the micro environment.
The reactivation scheme is comprised of the abovementioned waveforms FSCV cycling at 60 Hz, 400 V/s scan rate as it is effective in attenuating the oxidative peak current of the dopamine more rapidly in carbon nano material with an increase in frequency [89]. Detection of DA before and after fouling followed by reactivation was performed by applying a triangular waveform at 10 Hz, from −0.4 V to +1.0 V at 400 V/s scan rate as it is traditionally used and provides certain advantages in the detection of neuromodulators reported previously [70, 71, 73-78]. This approach is novel in the sense of strategizing and qualitatively estimating better reactivation protocol for BDUNCD during chronic monitoring of DA in accordance to fouling associated with it. The effect of high or less anodically dominated potential window during in-situ FSCV cleaning on BDUNCD surface and on monitoring life of BDUNCD in vitro has also been observed. Since boron doping on the diamond’s surface is not homogeneous and doping infuses defects, impurities upon the diamond’s surface reside near grain boundaries which give rise to surface oxygen functionalities affect charge carrier conduction mechanism significantly on the diamond’s surface is not yet well understood [63, 79-83]. This phenomenon ensuing inhomogeneous surface electro activity is known as deposited BDUNCD. Investigation on alteration of inhomogeneity regarding electro activeness of BDUNCD surface and its effect on electrochemical response, DA sensing were realized by exposing it in acidic medium and simultaneously electrochemically biasing the working electrode (BDUNCD) in cathodic and anodic environment to investigate the effect of electrochemical surface termination or functionalization (H terminated & O terminated) of BDUNCD surface [84,85].
2.6 Identify the Microelectrode Properties by Droplets Microfluidic Sensing

The neurochemicals are released in the human brain as chemical messengers. Therefore, messengers are transferred between the nerve cells, which transfer electrical signals called impulses by axon and received by another nerve cell’s dendrite [10]. The chemical messengers are carried by nerve cells with a positive charge as impulses and transfer in the negative charge of the nerve cells. Then, we introduce the droplets which can be used to sense the real time neurochemicals signals in 0.2 seconds in order to develop a suitable electrode for neurochemicals sensing.

The neurochemicals have been studied in a previous study of the steady state cell, and in the continuous constant flow rate in 0.2 ml/min. However, we want the condition to be similar to the human brain and to understand the neurochemicals and our electrodes’ properties. The electrodes’ properties are defined as sensitivity, response time, clearance rate, selectivity, and limited of detection (LOD). In this study, there are two treatments, nafion deposition, and nitric acid treatment, which have been employed to the electrodes’ surface to enhance the sensitivity and selectivity [64]. The treatment of the thin nafion layer has the advantage of enhancing the chemical resistance and increase sensitivity. Because the nafion layer has a negative charge, it attracts the positive charge of neurochemicals and refuses the negative chemical intermediates, which is the reason for the surface fouling.

However, the thick nafion layer can cause the reduction of sensitivity and increase oxidation’s potential. The neurochemicals have to diffuse through the nafion layer into the electrodes’ surface, which will be discussed in the results. Nevertheless, the nitric acid treatment has been employed to treat the carbon’s surface which can remove the
impurity and oxidize the carbon to increase the carboxyl group (COOH) [86]. Therefore, the carboxyl group which is an active component in the MWCNT enhances the selectivity and sensitivity. The carboxyl group in the solution has a negative charge (COO⁻), which attracts a positive charge of neurochemicals to enhance sensitivity and refuse ascorbic acid to increase selectivity.

The electrodes have been developed into three types of electrodes, BDUNCD, nafion-BDUNCD, and nafion-MWCNT-BDUNCD (Fig. 2-1) in order to study the sensitivity, response time, clearance rate, selectivity, and limited of detection (LOD). The sensitivity, response time, and clearance rate are studied using amperometry, CV, and FSCV. The selectivity and limited of detection (LOD) are studied using differential pulse voltammetry (DPV).

Fig. 2-1. Three types of microelectrodes BDUNCD, nafion modified BDUNCD, and nafion multi wall carbon nanotube modified.

2.7 Outline

The main goal of this research is to design and micro fabricates a microfluidic chip that can be used to develop a fundamental understanding of the fouling of boron-doped nanocrystalline diamond microelectrodes during chronic neurochemical sensing. To aid long-term monitoring of dopamine and other neurochemicals, it is critical to reduce the rate of electrode fouling due to reaction by-products and significantly extend their useful lifetime. The fouling rates were carefully studied using Fast Scan Cyclic Voltammetry (FSCV) and amperometry techniques. Furthermore, in-situ electrode
cleaning methods were developed in 1x PBS buffer. Under optimal conditions, the cleaning method extended the electrode sensitivity from 6.5 hours to 28 hours.

Microfluidic devices that can detect diseases from diabetes to heart disease are widely applied in health care. Recently, they have been successfully applied to determine the amount of dopamine (DA) which is important for an understanding of brain disorders such as Parkinson's disease. However, there are some defects that can be improved in dopamine detection. This paper introduces Boron Doped Ultra nanocrystalline Diamond (BDUNCD) which has a wide electrochemical potential window, extremely low background current, and excellent chemical inertness, making it an ideal electrode material. Boron doped diamond (BDD) films with dominant sp³ structure, sufficient boron doping with low surface polar functional groups results in less passivation by fouling agent's adsorption hampering the surface conductivity [32,87,88].

Some research shows high density boron doped diamonds with superior sensitivity are more prone to surface fouling due to dopamine oxidation forming more electrocatalytically active high density defect sites inactivated by fouling agents comparatively fast, diminishing the electrochemical detection of analyte [32]. Conversely, low level boron doped diamond will limit adsorption of fouling agent on active electrode sites [88-90]. For the first time, we integrated a BDUNCD (diameter 250 μm) microarray chip with a microfluidic device and analyzed sensitivity on neurochemical dynamics in a fluid condition. Accordingly, we investigated the response times that relate to monitoring time.

Eventually, the fouling mechanism on BDUNCD has been carefully studied in the different techniques of AM, CV, FSCV, and AG particles mapping; it determined the
fouling effect to the signals and the electrode’s surface active area changed.

Consequently, the 5 minutes reactivation protocol has been developed in different ranges and potentials’ effect to achieve the best clean effectiveness, which extended the electrode lifetime from 6.5 hours to 28 hours.

The next study, microfluidic of droplets sensing protocol, has been developed to investigate the different electrodes’ properties. The signal’s effect factors were studied individually, the background flow rate, the signal to noise ratio, and the droplet’s volumes by AM in the three different electrodes. Three types of microelectrodes are BDUNCD, nafion modified BDUNCD, and nafion multi wall carbon nanotube modified. The 50 nm of nafion modified BDUNCD enhance DA sensitivity two to three times increases chemical resistant and blocks the AA signals. The nafion multi wall carbon nanotube modified BDUNCD has a total increasing of DA sensitivity 10 times. Three electrodes were investigated in 9 hours of droplets’ monitoring with a total of 270 droplets; it includes sensitivity, response time, clearance rate, and selectivity.

After all, the selectivity and limit of detection (LOD) were studied in differential pulse voltammetry (DPV). The nafion multi wall carbon nanotube modified BDUNCD of the multi wall carbon nanotube layer has a functional group of (COOH), which increase the selectivity of DA and 5-HT by distinct potentials −83.47 mV and 62.56 mV. Also, the nafion layer blocks the AA signals. However, with the addition of the selectivity study, the nafion multi wall carbon nanotube modified BDUNCD achieved long-lasting 9 hours monitoring which has an excellent DA sensitivity Value of 0.63 μA μM⁻¹ cm⁻² and 5-HT Sensitivity Value of 1.55 μA μM⁻¹ cm⁻² after 9 hours. The other advantage of multi-wall carbon nanotube modified BDUNCD electrode, it has the lowest concentration
detection of 1 nM (DA) and LOD of 5.4 nM (DA). By the nitric acid treatment, the LOD can be reduced to 1.78 nM (DA). The 10 M nitric acid treatment can increase the functional group of (COOH) on the multi wall carbon nanotube layer to enhance the selectivity and LOD.

Finally, the developing protocol of microfluidic droplets sensor successfully evaluates the three different electrodes' properties of sensitivity, response time, clearance rate, selectivity, and LOD. The best electrode is nafion multi wall carbon nanotube modified BDUNCD electrode which has the highest sensitivity of FSCV 15.01 μA (100 μM DA) and AM 43.7 nA (100 μM DA), quick response time (2 ± 8% s), and excellent selectivity of DA Sensitivity Value of 1.18 μA μM⁻¹ cm⁻² and 5-HT Sensitivity Value of 2.09 μA μM⁻¹ cm⁻².
CHAPTER 3

MICROFLUIDIC DEVICE AND BDUNCD: DESIGN AND FABRICATION

3.1 BDUNCD Chip Design

On the UNCD chip design, there are nine electrodes separated on one chip, and each of them has electrodes with a length of 500 µm and a width of 500 µm, and the UNCD, which has a diameter of 250 µm, is in the center of the electrodes. To link the electrodes conveniently, the nine electrodes are connected to the wires by a row of electrodes; this is shown in Fig. 3-1.

Fig. 3-1. Left is the real design of BDUNCD chip with nine electrodes and the right is the design in the L-Edit. The BDUNCD has a diameter of 250 µm [121].
3.2 Microfluidic Channel Design

The microfluidic device channel designs are based on Equation (1). In the microfluidic channels, there are two inlets and one outlet. By using Equation (1), it can calculate the channels’ resistance with the channels’ dimensions and employ the solution’s density combined. The ideal of channel design is to have the same resistance for all inlets. The reason is that the different resistance can cause the wrong flow in direction. It may flow from one inlet to another inlet and have an unstable flow. On the other hand, it causes difficulty in the droplets generated.

\[
R = \frac{12\mu L}{h^2w} \left[ 1 - \sum_{n, odd}^{\infty} \frac{1}{n^5} \times \frac{192}{\pi^5} \times \frac{h}{w} \tanh \frac{n\pi w}{2h} \right]^{-1}
\]  

(1)[92]

3.2.1 Generation 1 Channel Design

In the generation 1 Channel design, it has two inlets (height 65 \( \mu \text{m} \) and width 1800 \( \mu \text{m} \)), one outlet, and storage design (Fig. 3-2). The storage is designed to catch the droplet and storage in the channel; the catch droplet will stock in the chamber and increase the channel’s resistance to change the flow of the direction to another channel. After the analysis, the droplet can easily remove and catch the new one. This design is used for single droplet analysis, and it can be applied to the cells analyzed and drug release.
3.2.2 Generation 2 Channel Design

In generation 2, the design revises the storage section to a straight channel (height 65 μm and width 1800 μm) (Fig. 3-3). The design provides the constant flow rate in the laminar flow. In the research of electrodes' fouling mechanism, it employed only one type of solution, and it requires a constant flow. In order to study the electrode surface, it is significant in the velocity and a stable condition. Therefore, the velocity in the channel can be calculated by the channel's dimensions and flow rate as well as the straight channel to support a stable flow condition.

3.2.3 Generation 3 Channel Design

In generation 3, this design has three inlets with the same resistance design (height 65 μm and width 800 μm) (Fig. 3-4). To study more than one solution, this
design can generate two types of droplets at the same time. In the second project regarding droplets study, the narrow channel's width aided the droplet generated and carried in the background solution.

Fig. 3-4. The third-generation channel design, was used for droplets sensing; it is reduced to 800 μm and has three inlets, which can apply to three different solutions at the same time.

3.3 Microfluidic Device - Double Layer Design

Due to the limited BDUNCD chip magnitude, it is arduous to design the inlet, outlet and reference electrode on top of the chip. Consequently, this limitation is a major issue to extend the chip. Moreover, the PDMS has weak binding strength on the BDUNCD chip; it can cause leakage when increasing the solution's pressure in the channel. To suit both conditions, a thin PDMS layer (100 μm) is prepared for the first layer. It suits in extending the chip and has a more forceful binding strength from PDMS to PDMS.

3.4 Reference Electrode Design

The potential was established by the results of DA signals in 100 mV/S (Fig. 3-5). The oxidation peaks' potentials are usually around 0.25 V to 0.3 V. Therefore, to reach the highest signal in AM, it selected 0.3 V as the highest potential to reach the oxidation peak and verify the signal current as the highest current. Higher potential can cause the electrode oxidation faster. Therefore, the potential was set up at 0.3 V.
In the reference electrodes, it selected Pt as the reference electrode and the counter electrode because the microfluidic dimension is not suitable to add three electrode systems on top of the BDUNCD electrode. However, the potential of the counter electrode may change by the flow. Therefore, the flow rate was fixed at 0.2 ml/min to reduce the effect. In the material, it selected Pt wire because it is low corrosively, high biocompatibility, and good mechanical resistance. It is also used in medical applications.

The microfluidic devices contain a BDUNCD chip with Polydimethylsiloxane (PDMS) channels: the PDMS with a channel (height 65 µm and width 1800 µm) and a platinum wire (diameter 1.5 mm) on top of the BDUNCD chip with extended PDMS (100 µm thickness). The distance between the Pt wire and the UNCD electrode can be measured by the thickness of the extended PDMS and PDMS with the channel (Fig. 3-5). It also shows the solutions’ flow direction the solutions’ flow in the channel pass through the Pt wire and electrode at a certain speed (0.2 ml/min).
Fig. 3-5. The microfluidic device has two inlets and one outlet; the channel is passing through the BDUNCD electrode with a Pt wire at the top as reference and counter electrode. A) overlook of channels and wire. B) side view of the device. C) 3D of the device [121].

3.5 Microfabrication of Molds for Microfluidic Device Development

3.5.1 Spin Coater

SU 8 2025 was first added on the silicon wafer to remove the bubbles. After that it was spin coating on the silicon wafer with the protocol of (1) 500 rpm, 100 r/s, and 10 s, (2) 2000 rpm, 300 r/s, and 30 s. Finally, SU 8 2025 formed a layer on the wafer with a thickness around 60 μm [93]. It had to be placed for 4 hours to confirm that it had a uniform thickness. After spin coating, it usually had higher thickness on the edge.

3.5.2 Lithography

After the spin coating process, the silicon wafer with SU 8 2025 layer was baked on the hot plate with the protocol of (1) 65°C, 5 minutes, (2) 75°C, 1 min, (3) 85°C, 1 minutes, and (4) 90°C, 15 minutes [93]. After baking, the silicon wafer with SU 8 2025 layer was developed by lithography with a designed mask. It was in contact mode, and 10 seconds’ exposure time, six times, which had a rest time of 10 seconds.
3.5.3 SU 8 Development

After lithography, the wafer was baked for 5 min at 90°C, and it used SU 8 development solution to remove the unexposed parts. Finally, it was washed by acetone, IPA, and DI water and dried by a nitrogen gun (Fig. 3.6 (C)).

![Image of SU 8 Development](image)

Fig. 3-6. Real device A) UNCD chip cover with 100 µm thickness PDMS and the electrodes are exposed, and B) PDMS channel with high 65 µm and width 1800 µm bond on a UNCD chip. C) the procedure of fabricated microfluidic devices.

3.6 Microfluidic Device Fabrication

3.6.1 First Layer Fabrication

There are two layers of PDMS in the bonding processes. First, the first layer extends the UNCD chip size and increases bonding strength because the PDMS bonding strength is weak on the UNCD surface. However, early on, PDMS formed on the UNCD chip, which was cleaned by acetone, IPA, and DI water and further dried in the oven at 50°C. While PDMS formed on the UNCD surface, the PDMS will adhere on the UNCD surface. It mixes PDMS with a curing agent at a 10:1 ratio followed by 2 hours of curing at a temperature of 60°C. Moreover, the PDMS on the electrode areas and connected parts were removed (Fig. 3-6 (C)). This is shown in Fig. 3-6 (A). The UNCD chip which
is the first layer is covered by 100 μm PDMS. It has electrodes with a diameter of 250 μm.

3.6.2 Second Layer Fabrication

The second layer is formed PDMS on the mold. It mixes PDMS with a curing agent at a 10:1 ratio followed by 4 hours of curing at a temperature of 60°C. After that, the PDMS channel layer is peeled off from the SU-8 2025 mold. Before bonding, the inlet, outlet, and wire holes are made on the PDMS channel layer.

3.6.3 Plasma Treatment

After fabricating first and second layers, the PDMS channel layer is bonded on the prepared first layer of BDUNCD chip, using oxygen plasma treatment for 10 seconds at 40 W [93]; this is shown in Fig. 3-6 (B).

3.7 Advantage of Microfluidic in Biosensing

The microfluidic has the capability to reduce the sample volumes (down to nanoliter), control the flow conditions (enhancing the transport), mixing different reagents (automating sample preparation), increase sensitivity of detection (increasing the aspect ratio) [131], high-throughput (experimentation is parallelization) [132], reducing processing time, and lower cost. The merger of microfluidics and biosensor technologies offer the advantages of combining electrochemical and biological technologies into a single platform to offer the portability, disposability, real-time detection, advantageous accuracies, and incorporating different analytes in a single device to measure one or more analytes at the same time [133]. However, the combination provides the capability of laminar flow reduce the effect of response errors due to the non-specific adsorption, offer the continuous or intermittent fresh solution, contain high sensitivity and selectivity with
controllable flow rates and droplet’s volumes, and supply engineered physiological microenvironment for *in vivo* and *in vitro* researches [134].

The study of neurochemical, microfluidic provide the similar of flowing and diffusion to the human brain, it improves to develop new materials of electrodes and new treatment strategies for *in vivo* and *in vitro*. By using the microfluidic to identify the electrode’s properties such as response time, sensitivity, and selectivity; it verifies the improvement of electrodes such as nafion modified BDUNCD and nafion multi-wall carbon nanotube modified BDUNCD electrode in real-time sensing. However, the flowing condition directly affects the sensitivity, diffusion, signal to noise ratio, and mass transfer effect. Therefore, the microfluidic illustrates the advantages of controllable flowing condition to investigate the best condition and parameters. Consequently, the PDMS has the ability to fit any shape of electrode and material from micro to nanoscale and without any leakage up to one week for long-lasting sensing. In this study, we use the combination of BDUNCD microelectrodes and microfluidic technology and microfluidic and neurochemical (DA and 5-HT) simulations to simulate Brain condition by employing distinct concentration, flow rate, and mix solutions. As well, by releasing droplets to simulate neurochemical generated and diffusion as Brain in the microfluidic devices [135].
4.1 Microfluidic Device: Setup, Preparation, and Protocol

4.1.1 Microfluidic Connection

The BDUNCD chips were designed by connecting to the cell. The cell has the advantage to connect all nine electrodes, and it fixed the microfluidic device on the cell. It is significant to fix the device because moving the device can cause an unstable flow in the channel and immediately affect the signals (Fig. 4-1). Therefore, the nine wires were labeled to determine which signal came from which electrode.

![Microfluidic device](image)

Fig. 4-1. Microfluidic device connects to nine electrodes wires and set on the cell.
4.1.1.1 **Solutions and Computer Connection**

The setup of the device is in Fig. 4-2, which connects nine wires, two inlet tubes, one outlet tube, and pumps. Also, the Autolab wires were connected to the UNCD chip on the stage (Fig. 4-3), and Pt wire is placed on the top of the device (Fig. 4-4). It is the close group of tubes and wires connected to the device, and the nine electron wires are fixed on the stage.

![Fig. 4-2. Two inlets and one outlet tube connected to the device.](image1)

![Fig. 4-3. Microfluidic device connected to the pumps and Autolab electrochemical workstation.](image2)
4.1.2 Dopamine and Background Solution Preparation

The background solution is prepared by 10x PBS and diluted with DI water to 1x PBS. After dilution, the 1x PBS is pumped as the background solution by a 60 ml syringe with .030" ID tube. Consequently, dopamine is prepared with 100 μM in 1x PBS solution, and 100 μM DA is also inserted by a 60 ml syringe with .030" ID tube.

4.1.3 Dopamine Detection Studies

The electrochemical technicalities of CV, FSCV, and amperometry have been employed to understand the fouling behavior on BDUNC electrode with a constant concentration of 100 μM DA. Currently, there is no research discussing BDUNC electrode fouling in long-term sensing. Therefore, the BDUNC surface fouling behavior in the dopamine is one of the studies to evaluate the material’s properties. The fouling behavior includes electrode lifetime, fouling rate, signal effect (current peak), and impedance.
4.1.3.1  Protocol of AM

First, the amperometry has been employed to study the lifetime. In the procedure, the total operational time is 4 hours and 20 minutes; 1x PBS has been applied for 10 minutes as the background current at the beginning and last operational which is shown in Fig. 4-5. We applied 100 μM DA as the fouling solution for 4 hours. As well, the potential has been applied at 0.3 V for all operations, and both fluid rates are 0.2 ml/min. The solution’s fluid rate is controlled by syringe pumps, and it only introduces one solution at the same time.

4.1.3.2  Protocol of CV and FSCV

Second are the FSCV and CV technical focus on fouling rate and signal effect. In this procedure, both signals of 100 μM (DA) and 1x PBS is taken every 30 minutes at two scan rates at 100 mV/S and 400 V/s (flow rate 0.2 ml/min), and the potential ranges from −0.4 V to +1.0 V; for FSCV data analysis, the 1x PBS solution is the background signals which are used to subtract from the dopamine signal. In this study, the signals were taken until the signal reduced more than 50% of the initial signal. After electrode reduced 50% of sensitivity, it needs to be replaced or cleaned and it is not suitable for doing sensing.

4.1.4  Surface Oxidation of Microelectrode in PBS buffer solution

In the PBS cycles, the 100 μM DA signal was taken initially and every 30 minutes by FSCV (−0.4 to 1.0 V), and each 30 minutes we employed 5 minutes of reactivation (FSCV, −0.4 to 1.0V). The 6.5 hours (13 cycles) scanning used FSCV (−0.4 to 1.0V, 60 Hz) with a flow rate of 0.2 ml/min (1x PBS solution).
4.1.5 **Ag Particles Deposition Method**

To map the electrode surface, the Ag particles has been introduced by using electrophoretic deposition (EPD) on BDUNCN. The procedure was developed by 3 µM AgNO₃ with 0.01 M per chloric acid solution, and using EPD for 200 seconds at potential −0.5 V; the AgNO₃ concentration varies by the electrode’s area.

4.1.6 **Signals Analysis Method**

4.1.6.1 **AM Signal Analysis**

AM is a different technique. It has a result of a current with time. In this technique, it applies a constant potential to only investigate the oxidation current ($I_{pa}$).

Furthermore, in the AM, the oxidation current is the result of the current peak (100 µM DA) subtracted by the background current (1x PBS). It has the first 10 minutes’ background solution currents (Fig. 4-5).

![Graph showing current (A) over time (S)](image)

Fig. 4-5. The signals of 100 µM (DA) in amperometry with a potential 0.3 V and a flow rate of 0.2 ml/min. The first 10 minutes applied 1x PBS solution with a flow rate of 0.2 ml/min. After that it switched to 100 µM (DA) with a flow rate of 0.2 ml/min; it is fouling the electrode for 4 hours. After 4 hours of fouling, we switched back to 1x PBS solution with a flow rate of 0.2 ml/min.
4.1.6.2 \textit{CV Signal Analysis}

In CV measurement, the scan speed is using 100 mV/s (Fig. 4-6). In the CV signal, the results have a positive current ($I_{pa}$) with energy potential ($E_{pa}$) and a negative current ($I_{pc}$) with energy potential ($E_{pc}$). The current $I_{pa}$ and $I_{pc}$ are the results of the current peak subtracted by the background current, which is shown in the figure as a dotted line. As well, the $E_{pa}$ is the oxidation potential and $E_{pc}$ is reduction potential. Nevertheless, the current is proportional to the scan rate, but the potentials do not vary by the scan rate.

![Graph showing CV signal analysis](image)

Fig. 4-6. The signal of cyclic voltammetry had scan rate of 100 mV/S in 100 μM dopamine solution with a flow rate of 0.2 ml/min. In the beginning, there is a background current; after potential increasing, the currents start increase which is caused by dopamine redox until reaching the oxidation peak, and the signal went down. Therefore, the oxidation peak had the oxidation potential ($E_{pa}$) and signal current ($I_{pa}$). Otherwise, the potential reduced from the highest potential; it is the reduction background current. After potential reducing, the currents reduced until reaching the reduction peak, and the signal went back; the reduction peak had the reduction potential ($E_{pc}$) and the signal current ($I_{pc}$).
4.1.6.3  **FSCV Signal Analysis**

In the FSCV experiment, the scan rate is enhanced to 400 V/s. However, the results cannot be observed directly like CV signals. It requires two signals which are the background signal (1x PBS) and the investigating signal (100 μM DA), and we subtract two signals to have the result (Fig. 4-7). It has the current peak ($I_{pa}$, $I_{pc}$) and energy potential ($E_{pa}$, $E_{pc}$).

![Graph](image)

Fig. 4-7. The signal of 100 μM (DA) with scan rate 400 V/s, and a flow rate of 0.2 ml/min, there are oxidation current $I_p$ and oxidation potential $E_p$; it requires two signals which is the background signal (1x PBS) and investigating signal (100 μM DA), and subtracted by two signals to have the result.

4.2  **In-Situ Electrode Cleaning Methods**

4.2.1  **Distinct Cleaning Periods in AM**

To investigate the suitable clean technique, the procedure has been set up in the regularize fouling condition and reactivation with different fouling periods, 50 minutes and 30 minutes, respectively. In the microfluidic device, BDUNCND electrode fouling condition is in the AM at potential +0.3 V with 100 μM dopamine and the flow rate of 0.2 ml/min. Therefore, the reactivation is employed in the FSCV for 5 minutes at the potential range of −0.4 V to +1.0 V (60 Hz) with 1x PBS solution and the flow rate of
0.2 ml/min. The 5 minutes' reactivation is employed in the different fouling periods 50 minutes and 30 minutes during the electrode fouling in the AM to investigate the signal's effect and electrode lifetime effect. The signals of each period are analyzed by CV, and FSCV. In the CV sensing, the scan rate is 100 mV/S, which has a potential range of −0.4 V to +0.8 V, and takes the signals from both background solution 1x PBS and 100 μM dopamine solution. However, in the FSCV sensing, the scan rate is increased to 400 V/s, which has a potential range of −0.4 V to +1.0 V and it takes both background solution 1x PBS and 100 μM dopamine solution.

4.2.2 FSCV with Distinct Potential Ranges Influence on BDUNCD

After employing the reactivation method, the best reactivation can be found by applying the different reactivation potential range. Three different potential range have been discussed in recent publications, which are −0.5 V to +0.8 V, −0.4 V to +1.0 V and −0.4 V to +1.2 V. The three different reactivations will be discussed and compared in the same condition of FSCV reactivation (400V/s, 60 Hz). It employs 1x PBS at a flow rate of 0.2 ml/min for 5 minutes. The signals take both background solution 1x PBS and 100 μM dopamine solution.

Three different reactivations will be employed in fouling BDUNCD electrode every 30 minutes. The BDUNCD electrode is fouling in the microfluidic device by FSCV (400 V/s, 60 Hz), and it is flowing 100 μM dopamine solution at a flow rate of 0.2 ml/min. Each reactivation potential range is repeated three times with 6.5 hours’ fouling study; in the 6.5 hours study, each 30 minutes takes signals, and the total has 14 signals, which are used to compare the current change, potential change, and the behavior.
4.2.3 Effect of Electrochemical Potential Window

4.2.3.1 Effect of Positive Potential Window

To investigate the positive window influence in the reactivation effects, it employs one different potential range, which is $-0.5 \text{ V}$ to $+1.0 \text{ V}$, to compare the results. In the fixed of negative potential $-0.5 \text{ V}$, the three potential ranges, $-0.5 \text{ V}$ to $+0.8 \text{ V}$, $-0.5 \text{ V}$ to $+1.0 \text{ V}$, and $-0.5 \text{ V}$ to $+1.2 \text{ V}$, are employed in the same condition of 1x PBS at a flow rate of 0.2 ml/min for 5 minutes reactivation, which is introduced in 6.5 hours electrode's fouling at every 30 minutes; in the fouling condition of FSCV (400 V/s, 60 Hz), it is employing 100 μM dopamine solution at a flow rate of 0.2 ml/min.

4.2.3.2 Effect of Negative Potential Window

To investigate the negative window influence in reactivation effects, it employs another different potential range, which is $-0.6 \text{ V}$ to $+1.0 \text{ V}$, to compare the results. In the fixed of positive potential $+1.0 \text{ V}$, the three potential ranges, $-0.4 \text{ V}$ to $+1.0 \text{ V}$, $-0.5 \text{ V}$ to $+1.0 \text{ V}$, and $-0.6 \text{ V}$ to $+1.0 \text{ V}$, are employed in the same condition of 1x PBS at a flow rate of 0.2 ml/min for 5 minutes reactivation, which is introduced in 6.5 hours electrode's fouling at every 30 minutes; in the fouling condition of FSCV (400 V/s, 60 Hz), it is employing 100 μM dopamine solution at a flow rate 0.2 ml/min.

4.2.4 Study BDUNCDB by the Best Window In Long-Term (28 h)

The final best potential range will be employed to study the electrodes' signal for long-term. In the long-term study, the BDUNCDB electrode is fouling in the FSCV (400 V/s, 60 Hz) with a flowing 100 μM dopamine at a flow rate of 0.2 ml/min. The experiment is set up in a 28 hours study to investigate the signals' behavior, potential
change, and electrode’s lifetime. During the 28 hours study, the signals are taken every 35 minutes after reactivation.

4.3 Microfluidic Device of Droplets Sensing

4.3.1 Electrodes’ Electrophoretic Deposition (EPD)

4.3.1.1 Nafion Layer Electrophoretic Deposition (EPD)

The nafion layer was deposited by the two electrodes method; it used Ag/AgCl electrode. The nafion solution (5wt.% in lower aliphatic alcohols and water) was added on the electrode’s surface for 2 minutes EPD at potential +0.5 V. After deposition, the BDUNCND was carefully rinsed by DI water and baked for 10 minutes at 70°C.

4.3.1.2 MWCNT Layer Electrophoretic Deposition (EPD)

The electrode CNT layer was deposited using Electrophoretic deposition (EPD) with potential −6 V and a deposition time of 10 minutes by the two electrodes method; it used Pt wire as account electrode and reference electrode. After EPD, the CNT-UNCD electrode was baked for 10 minutes at 70°C.

4.3.1.3 AM Real Time Droplets’ Sensing

In the beginning of the experiment, we set up a parameter of the flow rate (0.2 ml/min) and droplets’ volume (0.005 ml, 100 μM DA) with potential +0.3V in amperometry in order to study the droplets’ signals. Amperometry is a suitable technique to sensing droplets pass through the electrode, it detects the signals every 0.2 seconds and it investigates the signals’ change in real time. However, the droplets of dopamine (DA) are diffusion in the 1x PBS solution during the droplets carried through the electrode. Therefore, the droplets’ volume and flow rate will affect the signals accurately.
Consequently, the first step is to investigate the suitable potential, droplets’ volume and flow rate.

4.3.1.4 **Evaluate the Best Potential by S/N Ratio**

The suitable potential can be found by highest S/N Ratio. In this experiment, the parameter has been set up with a flow rate (0.2 ml/min) and droplets’ volume (0.005 ml, 100 μM DA). The different potentials are 0.2 V, 0.25 V, 0.3 V, 0.35 V, 0.4 V, 0.45 V, 0.5 V, 0.55 V, and 0.6 V, which have been applied to three different electrodes (BDUNCD, nafion-BDUNCD, nafion-MWCNT-BDUNCD), and each potential have six signals. Therefore, the S/N Ratio can be defined as Equation (2).

\[
\frac{S}{N} \text{ Ratio} = \frac{\text{signal- A + Noise}}{2 \ \text{Noise}} \quad (2)[120]
\]

4.3.1.5 **Background Flow Rate and Droplet’s Volume**

After the best potential has been found, we applied it to investigate the background flow rate and droplet’s volume. First step is to set up the droplets’ volume (0.005 ml, 100 μM DA) and investigate the different flow rates: 0.025 ml/min, 0.05 ml/min, 0.1 ml/min, 0.15 ml/min, 0.2 ml/min, and 0.25 ml/min. Therefore, the suitable background flow rate can find the base on the higher signal in three different electrodes. After that, the second step is to find the best potential and background flow rate to investigate the droplet’s volume: 0.005 ml, 0.01 ml, 0.02 ml, and 0.04 ml. Finally, the best droplet’s volume can be found by the higher signal with lower droplets’ volume in three different electrodes.
4.3.1.6 *BDUNCD Sensitivity, Response Time, and Clearance Rate*

After creating the suitable potential, background flow rate, and droplets' volume, this parameter setup is used in a future experiment to develop three different electrodes (BDUNCD, nafion-BDUNCD, nafion-MWCNT-BDUNCD) and each electrode is studied using AM, CV, and FSCV. All three properties, sensitivity, response time, and clearance rate, are studied at the same time in 9 hours of AM. The frequency is every 2 minutes with one droplet pass through the electrode. The frequency is set up every 2 minutes because the signals have to be verified as the background current with no chemical remaining on the electrode’s surface. Therefore, the sensitivity is defined as the ratio of the signal divided by the initial signal which is Equation (3):

\[
sensitivity = \frac{signal}{initial\ signal}
\]

(3)[95]

The response time is defined as the time of the signals from beginning to the real signal; the beginning is the first points change from the background which usually has lower current than the background, because of the diffusion limited. However, the highest current is not the real signal. If there are two peaks or a soothe current reduced, it is caused by the mass transfers. The mass transfers include diffusion, migration, and convection which can be explained by Cottrell Equation which is Equation (4).

\[
i = \frac{nFAC_0j}{\sqrt{Dt}} \sqrt{D_j}
\]

(4)[94]

\[i = \text{current}\]

\[n = \text{number of electrons}\]
F = Faraday constant, 96,485 C/mol

A = area of the electrode in cm

$c^0_j = \text{initial concentration in } \text{mol/cm}^3$

$D_j = \text{diffusion coefficient in } \text{cm}^2/\text{s}$

$t = \text{time in s.}$

The clearance rate is the time the chemical is removed from the electrode’s surface; it is defined as the time between $T_{20}$ to $T_{60}$ [96]; $T_{20}$ is the signals reduced to 20% of the real signal and $T_{60}$ is the signals reduced to 60%.

### 4.3.2 Time Study of Electrode Properties

### 4.3.2.1 Three Types of Modified BDUNCD Microelectrodes

The electrodes are studied separately. Each electrode is studied by Am for 9 hours; the background solution 1x PBS flow rate is 0.1 ml/min, and the droplets’ volume is 0.02 ml at potential +0.35 V. The droplets are released every 2 minutes and the signals are determined by Autolab every 0.2 seconds. The droplets’ concentration is 100 μM dopamine. Therefore, the 1x PBS solutions (30 ml syringe) and 100 μM dopamine solution (3 ml syringe) are refilled every 3 hours, and at the same time the FSCV and CV signals are taken.

### 4.3.3 Differential Pulse Voltammetry

All the electrochemical measurements were determined using Autolab; the measurements were taken using two electrode setups. The platinum was used as account and the reference electrode as the BDUNCD, nafion-BDUNCD, or nafion-MWCNT-BDUNCD working electrode. All electrodes were prepared 3 x 3 arrays with a diameter
of 250 \mu m and fabricated in the microfluidic devices. The selectivity of DA, 5-HT, and AA detection were investigated by using differential pulse voltammetry (DPV) with a 20 mV modulation amplitude and 5 mV step potential [86].

4.3.3.1 *Selectivity*

The electrochemical measurement used two electrode configurations, a platinum counter electrode, and a working electrode, which are BDUNCD, nafion-BDUNCD, or nafion-MWCNT-BDUNCD. Differential pulse voltammetry (DPV) with a 20 mV modulation amplitude and 5 mV step potential was used to characterize DA, 5-HT and AA detection [86]. Therefore, the signals were studied separately: DA was 1 \mu M and 10 \mu M, 5-HT was 1 \mu M and 3 \mu M, and AA was 100 \mu M. As well, it mixed the study in two conditions, (1) 1 \mu M DA, 1 \mu M 5-HT, and 100 \mu M AA, and (2) 10 \mu M DA, 3 \mu M 5-HT, and 100 \mu M AA; the DPV was employed in the three different electrodes surfaces, BDUNCD, nafion BDUNCD, and nafion-MWCNT-BDUNCD to compare the selectivity in the microfluidic at the same flow rate of 0.1 ml/min. The mix solution of DA, 5-HT, and AA was prepared before the experiment using a 30 ml syringe. The 1x PBS solution was used to clean the channel and electrode’s surface before the change to a different solution or concentration.

4.3.3.2 *Limit of Detection (LOD)*

To investigate the limit of detection on three different electrodes, BDUNCD, nafion BDUNCD, and nafion-MWCNT-BDUNCD; the LOD of BDUNCD, and nafion BDUNCD were studied by the FSCV in the microfluidic at the same flow of 0.1 ml/min at the different DA concentrations of 10 \mu M, 50 \mu M, 100 \mu M, 500 \mu M, and 1 mM. However, DPV was used to study nafion-MWCNT-BDUNCD at a concentration starting
at 1 nM DA; the DPV with was used to study the LOD at different DA concentrations of 1 nM, 5 nM, and 10 nM in the microfluidic at a flow rate of 0.1 ml/min.

The 10 M nitric acid for 5 minutes to oxidize the surface functional groups in nafion-MWCNT-BDUNCD was used to compare the different LOD with original nafion-MWCNT-BDUNCD; the oxidation process was employed after MWCNT deposition, and finally, nafion is deposited on the electrode. The MWCNT-BDUNCD electrode carefully added one drop of nitric acid on the surface for 5 minutes. After 5 minutes the electrode was gently wash two times with DI water.
CHAPTER 5
RESULTS AND DISCUSSION

5.1 Dopamine Fouling Mechanism on BDUNCD

5.1.1 Fouling Behavior

To understand the material of BDUNCD electrodes, the first experiment is to investigate the signals and lifetime of DA sensing in different techniques of AM, CV, and FSCV. The oxidation current is decreased by surface fouling and electrode oxidation. However, in the FSCV, the surface fouling and electrode oxidation can be observed by the current decreased and potential shifted. Boron doped diamond electrode has functional groups aliphatic bonded hydrogen, ketones, alcohols and carboxylic acids (-COOH) resides in grains (sp$^3$) and grain boundaries. It is also composed of non-diamond impurity/sp$^2$ hybridized carbon species having some aromatic bonded hydrogen, phenols, and Quinone and aromatic acids on the BDUNCD surface. It greatly affects the electron transfer process during any electrochemical process [97, 98]. During the electrochemical reaction, the sp$^2$ have a much faster reaction rate constant compared to the diamond (sp$^3$) [98, 99]. Boron doped diamond has been reported to have a higher chemical resistance than other peer carbon electrode materials [69, 99]; it also has better dimensional stability during rapid FSCV cycling [43, 69] and has a CH$_4$/H$_2$ ratio $\sim$ 5% [68, 100].
During the long-term diffusion controlled process, DA adsorbed on the sp² hybridized carbon species, which resulted in slow kinetics, paves way for electrode fouling [100].

5.1.1.1 AM Fouling Behavior

The first time studying the fouling mechanism in BDUNC, it was critical to understand how the electrode surface is fouled and the effect it has on the signals and lifetime of a new electrode. By developing the fouling mechanism, it can assist people in understanding the properties of an electrode to develop a new treatment or material. In the study, it first chose AM technology to analyze the electrodes’ lifetime. Therefore, AM applies the high current density on the electrodes’ surface and result in a faster electrode fouling; the expected lifetime was no more than 4 hours.

In the experiment, it employed a higher concentration of 100 μM dopamine because it is also a method to increase the fouling rate (dopamine usually exists at nM in rat brain [118, 119]). In the result, the BDUNC electrodes were more than 90% electrode fouling in 4 hours, and it slowly decreased in 4 hours. After 4 hours, the signals reduced as low as the background current, which was applied in the first 10 minutes of the experiment. The results were reproducible, repeated more than five times, and they had the same results of fouling in 4 hours. After switching to the DA solution, the signals immediately increased to the highest current, and it starts reducing slowly in 4 hours (Fig. 5-1). The lifetime of BDUNC was about 1 hour to 1.5 hours in AM, and it had a continuing flow of 100 μM (DA); after 1.5 hours, the signals were reduced more than 50% of the initial signal, which was inappropriate in sensing.
However, the result also shows a phenomenon in AM sensing which happens when the solution is added, switch the solutions in the microfluidic is switched, or change the fluid rate; the current immediately increases to a higher value and decreases to stable currents. This phenomenon can be explained by the Cottrell Equation shown in Equation (4). The immediate increased current is caused by mass transport which includes the diffusion, migration, and convection. Based on the equation, the correct oxidation current is at the stable current.

![Graph](image)

**Fig. 5-1.** Study the BDUNCD fouling in AM. Initial 10 minutes were employed 1x PBS solution with potential +0.3 V and a flow rate of 0.2 ml/min. After that, it switched to dopamine solution (100 μM) at a flow rate of 0.2 ml/min, and finally switched back to 1x PBS solution for 10 minutes. The experiment was repeated more than three times. In AM the sensing time was about 1.5 hours (50% fouling) [121].

5.1.1.2 **CV Fouling Behavior**

After studying the fouling rate in the AM, it employed CV and FSCV at the same time. It was significant to consider more than one technology. In the CV sensing, it was fouling in the FSCV in 100 μM (DA) at potential from −0.4 V to +1.0 V (60Hz), and a
flow rate of 0.2 ml/min. The CV signals were taken every 30 minutes until the signal decreased more than 50% of the initial signal. The signals increased in the first 2 hours from 25.00 ± 25 % nA to 29.00 ± 20% nA (vary by the different BDUNCD chips), but the oxidation peak potentials also increased from 0.21 ± 10% V to 0.26 ± 10% V, and currents’ charge were also increasing. The currents’ charge increasing was caused by electrode’s oxidation, and it also contributed in increasing the current signals (Fig. 5-2). The potential was increased by the electrode’s oxidation and rate constants. After the electrode oxidized, it required high potential to oxidize the chemical solution. It resulted in the electrode’s active area reduction. After 2 hours, the signals initiated decreased with no evident oxidation current peak at 4 hours’ signal. It is caused by the electrode oxidation and dopamine fouling the electrode’s surface. However, after 6.5 hours, there was no oxidation peak with a linear increase signal; the electrode was fouling more than 50%, and it needed to be replace or clean.

![Current charge vs. Potential](image)

Fig. 5-2. Signals of the BDUNCD fouling in CV; it was fouling in the FSCV (potential range -0.4 V to +1.0 V, 400 V/s, 60 Hz) condition with flow dopamine (100 μM) solution and a flow rate of 0.2 ml/min. The signals were the dopamine (100 μM) signals; it is in the microfluidic with flow rate 0.2 ml/min, and potential ranges from −0.2 V to +0.8 V. The signal was taken every 30 minutes in FSCV fouling condition.
5.1.1.3 **FSCV Fouling Behavior**

The advantages of the FSCV were that the increased of scan rate increased the signals’ currents and reduced the scan time. The FACV were taken at the same time as the CV with the same condition. The FSCV signals (1.00 ± 5.4% μA) were increased about 40 times compared to the CV signals (25.00 ± 25% nA) in 100 μM DA, and the oxidation peak potential was shifted to about +0.65 ± 2% V (vary by different electrodes) at the initial signal (Table 5-1) [117]. The first two hours’ signals were increasing, and the 2 hours’ signal was about 2 times increased than the initial signal; there was the same behavior as the CV signals. After 4 hours of fouling, the signals were reduced as the initial signal, but the oxidation peak potential was shifted to about +0.81 ± 2%, which was caused by the electrode oxidation and fouling. However, after 6.5 hours, the signal was reduced more than 50%, but the difference with the CV was that there was an oxidation peak at 6.5 hours with a lower current peak.

BDUNCD has the $I_{pc}$ to $I_{pa}$ ratio approximately at 1 in. on the fresh surface; oxidation potential is +0.65 ± 2% V and reduction potential is −0.29 ± 2% V. After 2 h, the oxidation current increased to 1.9 ± 10.8% μA and oxidation potential shifted to +0.84 ± 2% V (Table 5-1). The oxidation current increased by consuming the functional groups which were oxidized during the monitoring of FSCV (from −0.4 V to +1.0V) on the BDUNCD electrode surface, and polydopamine which has a negative charge formed on the electrode’s surface. However, by increasing the positive potential of FSCV, the oxidation current increased, which will be discussed in the positive potential effect of reactivation. Because the dopamine has a positive charge, the negative charge film of polydopamine attracted the dopamine to increase the oxidation current. Therefore, after
the dopamine’s film thickness increased as time went on, and chemical reaction produced no conductivity melanin, it finally reduced the electrical conductivity (Fig. 5-3). After 4 hours, the signal returned to 1.00 ± 3.5% μA, but the oxidation potential increased to 0.81 ± 2% V, which was caused by fouling and surface oxidation. Finally, the signal reduced more than 50% of the initial signal after 6.5 hours. The 50% was the ideal sensor electrode property, but below 50%, it needed to be replaced or cleaned.

Fig. 5-3. Signals of the BDUNCD fouling in FSCV; it was fouling in the FSCV (potential range −0.4 V to +1.0 V, 400 V/s, 60 Hz) condition with flow dopamine (100 μM) solution and a flow rate of 0.2 ml/min. The signals were the dopamine (100 μM) signals subtracted by the 1x PBS signals; it’s in the microfluidic with flow rate 0.2 ml/min, and potential ranges from −0.4 V to +1.0 V. The signal was taken every 30 minutes in FSCV fouling condition [121].

Table 5-1. The FSCV signals analyzed of the BDUNCD fouling at 0, 2, 4, and 6.5 hours.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$I_{pa}$ (μA)</th>
<th>$I_{pc}$ (μA)</th>
<th>$I_{pc}/I_{pa}$</th>
<th>$E_{pa}$ (V)</th>
<th>$E_{pc}$ (V)</th>
<th>$\Delta E_p$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00 ± 5.4%</td>
<td>−1.10 ± 2.0%</td>
<td>1.1</td>
<td>0.65 ± 2%</td>
<td>−0.29 ± 2%</td>
<td>0.94</td>
</tr>
<tr>
<td>2</td>
<td>1.90 ± 10.8%</td>
<td>−1.66 ± 10.9%</td>
<td>0.87</td>
<td>0.84 ± 2%</td>
<td>−0.30 ± 2%</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>1.00 ± 3.5%</td>
<td>−1.17 ± 2.9%</td>
<td>1.17</td>
<td>0.81 ± 2%</td>
<td>−0.29 ± 2%</td>
<td>1.10</td>
</tr>
<tr>
<td>6.5</td>
<td>0.44 ± 9.4%</td>
<td>−1.00 ± 9.7%</td>
<td>2.27</td>
<td>0.76 ± 2%</td>
<td>−0.30 ± 2%</td>
<td>1.06</td>
</tr>
</tbody>
</table>
5.1.1.4 *Surface Oxidation of Microelectrode*

To compare electrode’s oxidation and electrode’s fouling by dopamine, the PBS cycles provided the idea of the electrode’s oxidation effect (Fig. 5-4). After 20 minutes, the oxidation current increased 0.02 μA with 0.01 V oxidation potential changed (Table 5-2). Therefore, there was 98% of oxidation current increased by the polydopamine film, and it resulted in the potential shift since the dopamine must diffuse through the negative charge polydopamine film. After 65 minutes, the oxidation current was reduced by 11% caused by electrode’ oxidation, and 44% current’s reduction was caused by dopamine fouling of the total 55% fouling in 65 minutes.

The difference between fouling and oxidation is the reduction current. There is maintaining the reduction current in 65 minutes in dopamine fouling condition. However, electrode’s oxidation reduced the reduction current as time went on (Fig. 5-4); it reduced 39% of the reduction current compared to the reduction current of the fouling’s surface by 10%. The effect caused by the polydopamine film having a reverse chemical reaction will be discussed in the negative potential effect.
Fig. 5-4. The FSCV Signals of the BDUNCD oxidation in PBS solution at initial, 4 cycles (20 minutes), 8 cycles (40 minutes), and 13 cycles (65 minutes). The signals were the dopamine (100 μM) signals subtracted by the 1x PBS signals; it is in the microfluidic with a flow rate of 0.2 ml/min for both DA and PBS solutions, and potential ranges are from -0.4 V to +1.0 V.

Table 5-2. The FSCV Signals analyzed of the BDUNCD oxidation in PBS solution initial, 4 cycles (20 minutes), 8 cycles (40 minutes), and 13 cycles (65 minutes)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>$I_{pa}$ (μA)</th>
<th>$I_{pc}$ (μA)</th>
<th>$I_{pc}/I_{pa}$</th>
<th>$E_{pa}$ (V)</th>
<th>$E_{pc}$ (V)</th>
<th>$\Delta E_p$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.98 ± 11.1%</td>
<td>-1.21 ± 4.8%</td>
<td>1.23</td>
<td>0.71 ± 2%</td>
<td>-0.24 ± 2%</td>
<td>0.95</td>
</tr>
<tr>
<td>20</td>
<td>1.00 ± 8.3%</td>
<td>-1.02 ± 3.5%</td>
<td>1.02</td>
<td>0.70 ± 2%</td>
<td>-0.23 ± 2%</td>
<td>0.93</td>
</tr>
<tr>
<td>40</td>
<td>0.95 ± 6.2%</td>
<td>-0.90 ± 5.7%</td>
<td>0.95</td>
<td>0.69 ± 2%</td>
<td>-0.23 ± 2%</td>
<td>0.92</td>
</tr>
<tr>
<td>65</td>
<td>0.87 ± 6.3%</td>
<td>-0.74 ± 5.1%</td>
<td>0.85</td>
<td>0.69 ± 2%</td>
<td>-0.23 ± 2%</td>
<td>0.92</td>
</tr>
</tbody>
</table>

5.1.2 BDUNCD Fouling Mechanism

In the previous study, dopamine has a chemical reaction to form α-DQ, LDC, DC, and final melanin. The melanin has particles with diameters from 100 nm to 200 nm [25], and it is insoluble and non-conductivity. The quasi reversible DOQ is pH dependent and not thermodynamically stable [101, 102]. Therefore, higher pH environment allows for faster DOQ desorption rate producing a sufficient amount of non-protonated DOQ, thereby facilitating the required flux for adsorbed DA reactant to make DA/DOQ
cyclization favorable within analyte and BDUNCD interface [103]; it can also through the self-catalytic effect cause a redox mediated adsorbed by-product such as DOQ or some other interaction between adsorbed and solution species [103]. Therefore, during bio-sensing, the generated of poly-dopamine adheres on the electrodes’ surface, and finally produces melanin, which reduces the electrodes’ sensitivity and conductivity. However, the 4 hour fouling study in AM had identified the melanin on the electrode’s surface by the EDX (Fig. 5-5). After 4 hours fouling, the electrode’s surface was washed with DI water and baked for 10 minutes at 60°C. The melanin was identified by the nitrogen and oxygen, which is in the chemical structure of melanin. The fouling effect has been carefully studied by AM, CV, FSCV, and Ag particles deposition. Therefore, it developed a 4-hour study of BDUNCD fouling mechanism. In the investigated 4 hours of AM, the signals were taken initially at 1, 3, and 4 hours. Therefore, it was using Ag particles deposition to pattern the active BDUNCD electrode surface. In the 4 hours fouling, it determined the electrode’s fouling effect to the signals and electrode’s surface. Electrode fouling reduces the signals and active electrode surface. Finally, it compared the fresh BDUNCD, FSCV fouling in 6.5 hours, and AM fouling in 4 hours. In AM of 4 hours fouling (90% fouling), a weak signal remained, and only a few positions had small particles of Ag deposition. However, FSCV fouling had signal reduction of more than 50%, and as seen in the Fig. 5-6, the Ag deposition was concentrated in certain positions because there were high currents that resulted in the deposition of large particles.
Fig. 5-5. EDX of the BDUNCD surface and after 4 hours fouling in AM condition with 100 µM of dopamine solution at a flow rate of 0.2 ml/min and potential +0.3 V. (a)(b) were initial BDUNCD electrode surface. (c)(d) were after 4 hours fouling in AM with (100 µM DA).

Fig. 5-6. The Ag particles deposition on BDUNCD electrodes. In the FSCV condition (potential range −0.4 V to +1.0 V, 400V/S, 60Hz), it is fouling with flow dopamine (100 µM) solution and a flow rate of 0.2 ml/min. In the AM, it is fouling at +0.3 V for 4 hours with flow dopamine (100 µM) solution and a flow rate of 0.2 ml/min. The compared to initial signal, after 6.5 hours fouling (FSCV), after 4 hours fouling (AM), and Ag particles deposition [121].
5.1.2.1  **BDUNCD Fouling Mechanism in AM**

To understand the nature of fouling on electrode surface [24, 43, 46, 89] AM has been successfully used by various research groups. Here, we report AM-based surface fouling process of 250 μm BDUNCD microelectrode in 100 μM DA solution at a potential of +0.3 V for 4 hours, and FSCV current measurements were taken at 0th, 1st, 3rd and 4th h during the abovementioned AM fouling (Fig. 5-7) where FSCV detection data was obtained by applying a potential range of −0.4 V to +1.0V at a scan rate of 400V/s, 60 Hz. After the 1st hours of AM, the subsequent FSCV anodic signal is 1.15 μA ± 3.9% compared to 0th hours signal of 0.95 μA ± 8.1% with both having a sufficient reduction signal which indicates a favorable DA/DOQ cyclization (Table 5-3). Peak anodic potential shift > 100 mV from 0.75 ± 2% V (0th hours) to 0.85 ± 2% V (1st hours) is in agreement in depicting the presence of DA/DA Quinone couple on the carbon’s surface [96, 99]; the increased sensitivity was caused by DOQ desorption, whose similarity was found in the previous researches having ~ 0.8 V anodic potential Vs Ag/AgCl for DA/DOQ redox stability. At the 3rd and 4th hours of AM, BDUNCD surface would generate enough reactive oxygen and hydroxyl radicals, which helps in oxidizing adsorbed organic and increased forming melanin like film results in decreased FSCV currents of 0.43 μA ± 2.2% (3rd hours) and 0.1 μA ± 1.8% (4th hours), respectively. However, during amperometry it is also difficult to maintain an oxide free carbon surface in an aqueous environment since carbon can oxidize in the presence of water at fairly mild anodic potential [97]. After 4 hours fouling, BDUNCD samples are also subjected to EDX (Energy dispersive X ray spectroscopy) at different spots on the samples to track the prominent presence of dopamine based melanin material polydopamine film with a
reaction between intermediate indole species 5,6 dihydroxyindole or 5,6 dihydroxyquinone [104-108]; Various tautomers of these molecules can coexist within melanin [109], and it was difficult to determine the structure of polydopamine due to the heterogeneity and insolubility of the material. The polydopamine may be composed of oligomers by covalently bonded dimers and higher oligomers of 5,6-hydroxyindole and 5,6-indolequinone held together by charge transfer, π-stacking, and hydrogen bonding [109-112].

Electrodes are frequently fouling faster in AM because it employs higher positive current on the electrodes' surface which significantly attract polydopamine film and form faster and thick film on the electrodes' surface, thus resulting in reducing signals by about 90% after 4 hours (Fig. 5-7). The polydopamine film can be identified in two fouling effects which are affected by a thin and a thick film. In the thin film, the fresh dopamine solution can diffuse into the polydopamine layer and have chemical redox reaction which generates the signals. As well, the thin polydopamine film had a reverse chemical reaction, which resulted in maintaining the reduction current by 90% and the reduction potential shifted −0.01 V after 6.5 hours (Table 5-1), while thin film was easily removed compared to the thick film; it occurred in periods of 30 minutes’ monitoring with 5 minutes’ reactivation, fouling in a 100 µM DA solution by FSCV (−0.4 V to +1.0 V, 60 Hz). The fix of 5 minutes’ reactivation could not properly clean the electrode’s surface, which had more of a thick film from 50 minutes of fouling. However, 4 hours of AM generated the more thick film, both oxidation and reduction current reduced. It compared to oxidation reduced by 90% (4th hours). The reduction current reduced 53% (4th hours) because the thick film also had a reverse chemical reaction; this
is due to the thick film being more stable on the electrode’s surface, and the thin film was easily removed to generate new polydopamine film or has redox reaction on the film in order to maintain the reduction’s signals. The thick film resulted in oxidation potential shifting 0.13 V and reduction potential shifting -0.05 V, which blocked the fresh dopamine diffusing into the BDUNCD surface and reduced signals (Table 5-3).

Therefore, reducing thick film generated is the key in extending the lifetime.

![Figure 5-7](image)

**Fig. 5-7.** The BDUNCD fouling mechanism; it is fouling in 4 hours of AM condition with 100 μM of dopamine solution at a flow rate of 0.2 ml/min and potential +0.3 V. The FSCV signals were taken at initial, 1, 3, and 4 hours, and compare with initial signal; there were Ag particles deposition on the four different periods.

**Table 5-3.** The FSCV signals analyzed of the BDUNCD fouling in AM at 0, 1, 3, and 4 hours.

<table>
<thead>
<tr>
<th>Time(h)</th>
<th>I_{pa} (μA)</th>
<th>I_{pc} (μA)</th>
<th>I_{pc}/I_{pa}</th>
<th>E_{pa} (V)</th>
<th>E_{pc} (V)</th>
<th>ΔE_p (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.95 ± 8.1%</td>
<td>-0.97 ± 6.1%</td>
<td>1.02</td>
<td>0.75 ± 0.2%</td>
<td>-0.23 ± 0.2%</td>
<td>0.98</td>
</tr>
<tr>
<td>1</td>
<td>1.15 ± 3.9%</td>
<td>-0.78 ± 2.0%</td>
<td>0.68</td>
<td>0.85 ± 0.2%</td>
<td>-0.20 ± 0.2%</td>
<td>1.05</td>
</tr>
<tr>
<td>3</td>
<td>0.43 ± 2.2%</td>
<td>-0.38 ± 11.0%</td>
<td>0.88</td>
<td>0.95 ± 0.2%</td>
<td>-0.27 ± 0.2%</td>
<td>1.22</td>
</tr>
<tr>
<td>4</td>
<td>0.10 ± 1.8%</td>
<td>-0.46 ± 1.5%</td>
<td>4.6</td>
<td>0.88 ± 0.2%</td>
<td>-0.28 ± 0.2%</td>
<td>1.16</td>
</tr>
</tbody>
</table>

5.1.2.2 **BDUNCD Fouling Mechanism in EDX**

The EDX spectrums were collected from SEM micrograph at a scale of 200 nm, in which minor components are compared to fresh BDUNCD surface components like
Nitrogen (N) and oxygen (O) which show an increase in atomic weight% from 1.22 ± 12.81% and 0.67 ± 49.73% to 4.55 ± 7.18% and 6.03 ± 2.82% more than four times, respectively, in both cases (Fig. 5-5) [25]. The increase in N and O after 4 hours amperometry in DA solution compared to fresh surface due to surface oxides and melanin dopamine moieties from the surface give rise to functionalities containing N and O [25, 91]; it is expected to form polydopamine or melanin film and other surface oxide moieties which might give rise to N and O. However, it was expected to give rise in Na and Cl by 1x PBS solution (pH 7.4, NaCl of 0.5M / L, KCl of 0.0027 M/L, and phosphate of 0.01 M/Lin), which can be removed by carefully washing. The fouling of BDUNCD gave rise to N/C atomic weight ratio from ~ 0.015 to 0.074 along, and gave rise to O/C ratio from ~ 0.017 to 0.123. The increase in functionalities of C and O is abundant in melanin or DA oxidative by products [25].

To identify the melanin on the electrode’s surface, we investigated different magnifications of EDX from 2 μm (15 KV, 20 Kx) to 100 nm (15 KV, 50 Kx) (Fig. 5-8). The black film was identified melanin film and white was identified salt. After increasing magnification from 2 μm to 100 nm, the atomic weight of salt (Na, Cl) reduced from 9.64 Wt% and 13.12 Wt% to 7.99 Wt% and 4.60 Wt%, and melanin (N, O) was increased from 3.68 Wt% and 4.31 Wt% to 10.23 Wt% and 14.12 Wt% (Table 5-4); from low magnifications to higher magnifications, the N increased 178%, O increased 228%, Na reduced 17%, and Cl reduced 65%. The magnifications from low to high gave rise to N/C atomic weight ratio from ~ 0.06 to 0.19 and gave rise to O/C atomic weight ratio from ~ 0.07 to 0.26. The increase of N and O was used to identify the melanin fouling on the electrode’s surface which was authentic. To prevent authentic melanin from forming
during the fouling, no dopamine solution remained on the electrode’s surface. After fouling, the BDUNCD chip was carefully sinking in DI water five times to remove salt from the surface. After cleaning, the BDUNCD was dried in an oven at 50°C for 20 minutes. To remove the salt, it also removed some of the melanin, but melanin still adhered to the surface. However, the result of EDX data has only remained C, N and O peaks in locations 1 and 2 (Fig. 5-9); they have N and O of 2.4 Wt% and 4.1 Wt%, and 2.2 Wt% and 4.8 Wt%. The fresh BDUNCD (N, O) was 1.22 ± 12.81 Wt% and 0.67 ± 49.73 Wt% (Table 5-5). The thin film of polydopamine can also be determined by investigating fouling BDUNCD. The fouling BEUNCD surface was chosen without seeing any melanin on the surface (Fig. 5-9). However, in the EDX analysis, the fouling BEUNCD has the N and O of 2.14 Wt% and 3.61 Wt% which was a polydopamine film on the surface (Table 5-5).

![Fig. 5-8. Comparison of EDX peaks at different SEM magnification scales.](image)

![Table 5-4. Comparison of EDX chemical composition at different SEM magnification.](table)

<table>
<thead>
<tr>
<th>Element</th>
<th>2 µm (Wt%)</th>
<th>0.5 µm (Wt%)</th>
<th>0.5 µm (Wt%)</th>
<th>100 nm (Wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>07.31</td>
<td>07.25</td>
<td>06.51</td>
<td>08.54</td>
</tr>
<tr>
<td>C</td>
<td>61.68</td>
<td>53.87</td>
<td>52.02</td>
<td>53.62</td>
</tr>
<tr>
<td>N</td>
<td>03.68</td>
<td>04.89</td>
<td>07.05</td>
<td>10.23</td>
</tr>
<tr>
<td>O</td>
<td>04.31</td>
<td>09.22</td>
<td>13.78</td>
<td>14.12</td>
</tr>
<tr>
<td>Na</td>
<td>09.64</td>
<td>11.01</td>
<td>11.21</td>
<td>07.99</td>
</tr>
<tr>
<td>Si</td>
<td>00.25</td>
<td>00.85</td>
<td>00.58</td>
<td>00.90</td>
</tr>
<tr>
<td>Cl</td>
<td>13.12</td>
<td>12.93</td>
<td>08.85</td>
<td>04.60</td>
</tr>
</tbody>
</table>
Fig. 5-9. After 4 hours fouling in AM at 100 μM DA, the EDX peaks and SEM images on melanin and BDUNCD surface. BDUNCD electrode surface cleaned by sinking in DI water 5 times to remove salt.

Table 5-5. Comparison of EDX chemical composition at fresh BDUNCD and after 2 hours fouling BDUNCD surface.

<table>
<thead>
<tr>
<th>Element</th>
<th>Location 1 (Wt%)</th>
<th>Location 2 (Wt%)</th>
<th>Fouling BDUNCD (Wt%)</th>
<th>Fresh BDUNCD (Wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>13.31</td>
<td>14.32</td>
<td>14.06</td>
<td>30.65</td>
</tr>
<tr>
<td>C</td>
<td>79.86</td>
<td>78.00</td>
<td>80.03</td>
<td>66.94</td>
</tr>
<tr>
<td>N</td>
<td>02.41</td>
<td>02.20</td>
<td>02.14</td>
<td>01.22</td>
</tr>
<tr>
<td>O</td>
<td>04.10</td>
<td>04.80</td>
<td>03.61</td>
<td>00.67</td>
</tr>
<tr>
<td>Na</td>
<td>00.17</td>
<td>00.45</td>
<td>00.05</td>
<td>00.22</td>
</tr>
<tr>
<td>Si</td>
<td>00.12</td>
<td>00.13</td>
<td>00.11</td>
<td>00.07</td>
</tr>
<tr>
<td>Cl</td>
<td>00.03</td>
<td>00.10</td>
<td>00.00</td>
<td>00.22</td>
</tr>
</tbody>
</table>

5.1.2.3  **BDUNCD Fouling Mechanism in Ag Particles' Mapping**

Electrochemical deposition of Ag particle also gave us a qualitative idea of change in the electroactive area on the BDUNCD surface during dopamine fouling and surface oxidation. It is captured by SEM at 0th, 1st, 3rd and 4th hours during the abovementioned AM; it also has as deposited BDUNCD surface. As deposited BDUNCD has heterogeneous electro activity associated with boron dopant which is not symmetric
and it incorporated by substitution in carbon lattice, interstitials or bonded with NDC impurities in grain boundaries [81], which might give rise to surface oxide groups providing different intensity in the fresh surface [114, 115]; frequently, the higher current happen on the edge of the electrode, which can also be investigated in our results. The different deposition potential affects the surface’s coverage and particles’ size; the higher current charge caused irregular shapes and is not spherical [116]. After electrode’s fouling, 1st reduce the electrode active surfaces about 50%, and the currents were focused on the active area, which increased particle’s dimension from 500 ±18% nm to about 1± 20% μm and resulted in a flower-like structure. However, after the 3rd and 4th hours, the Ag particles become difficult to deposit on the BDUNCD surface, which is due to the no conductivity fouling film cover on the electrode surface by increasing the fouling film’s thickness, which reduced the Ag particles’ size.

5.2 In-Situ Electrode Cleaning Methods

5.2.1 the Effect of Cleaning Periods in AM

The 5 minutes’ reactivation −0.4 V to 1.0V (400 V/s, 60 Hz) with 1x PBS solution has been employed to investigate the suitable fouling time (monitoring time) in AM; it is fouling in the 100 μM dopamine solution at potential +0.3V and a flow rate of 0.2 ml/min. The reactivation was employed in both 30 minutes and 50 minutes; it was first employed in the 50 minutes monitoring time. However, the 5 minutes reactivation cannot properly clean the electrode’s surface in 50 minutes monitoring time, and it resulted in reducing sensitivity by more than 50% in 4 hours. It requested longer reactivation time to clean the thick poly-dopamine on the electrode’s surface. After that, the monitoring time was reduced to 30 minutes, and it successfully cleaned the
electrode's surface and extended the lifetime to 8 hours (Fig. 5-10). During the 8 hours monitoring time, the reactivation cleaned the electrode's surface and maintained the signals initially in 7 hours. It had the similar behavior as the previous study, in which the signals increased after 3 hours and slowly decreased and finally decreased to 50% at 8 hours (Fig. 5-11). The signal dropped at 4 hours. It was the bubble effect. After removing the bubble, the signals were returned to the regular signals. In the future, it can use the same protocol to study the different scan rate effect, which is fix at 400 V/s in this study.

Fig. 5-10. The reactivation was employed every 30 minutes during the electrode fouling in AM to extend the lifetime and maintain the signals. (1) the signals in 3 hours with a total of 30 minutes reactivation time, (2) the signals from 3 hours to 6 hours with a total of 30 minutes' reactivation time, and (3) the signals from 6 hours to 9 hours with a total 30 minutes' reactivation time [121].

Fig. 5-11. The current signals were measured in the beginning of every 30 minutes and 50 minutes electrode fouling in AM. The circle was the 30 minutes of electrode fouling, and the triangle was the 50 minutes of electrode fouling [121].
5.2.2 BDUNCD Reactivation in FSCV Mode

The 5 minutes' reactivation was employed to remove the polydopamine film and extended the lifetime in FSCV fouling study. After 2 hours, the oxidation current (2.49 ± 6.7% μA) increased about two times of the initial signal (1.25 ± 2.6% μA) with the potential shifting 0.11 V (Table 5-6). Because the surface is not 100% clean, the remaining polydopamine film enhance the signals and increased the oxidation potential, the electrode also slowly oxidized during the monitoring and clean process which resulted in shifting oxidation potential and it contributed to enhance the oxidation current signals. However, after 6.5 hours, the oxidation current (1.37 ± 1.3% μA) increased by 10% with the reduction current increased (−1.66 ± 1.1% μA) by 14% compared to the initial reduction current (−1.45 ± 2.8% μA). The reactivation cleaned surface also can be investigated by the potential shifting 0.07 V after 6.5 hours. It was compared without reactivation shifting 0.11 V in FSCV (6.5 hours) and 0.13 V in AM (4 hours), the reactivation cleaned the electrode's surface by increasing both the oxidation and reduction current and reducing oxidation potential shifting which resulted in ΔE_p ≈ 1. The reactivation successfully cleans the electrode's surface (remove the polydopamine film) and maintaining the sensitivity as initial signals after 6.5 hours (Fig. 5-12).
Fig. 5-12. The comparison of with/without 5 min reactivation in 6.5 hours of FSCV: current signals were measured in the beginning of every 30 minutes at potential range −0.4 V to +1.0 V. The 5 min reactivation is using 1x PBS between cycles to clean and reactive the electrode surface at a flow rate of 0.2 ml/min at potential range −0.4 V to +1.0 V. Each cycle represents a continuous application of the FSCV waveform (−0.4 V to +1.0 V) at 60 Hz for 30 min in 100 μM DA. The voltammogram is shown after the background subtraction in 1x PBS.

Table 5-6. The FSCV signals analyzed of the BDUNCD fouling with 5 minutes reactivation at 0, 2, 4, and 6.5 hours.

<table>
<thead>
<tr>
<th>Time(h)</th>
<th>(I_{pc} , \mu A)</th>
<th>(I_{pc} / I_{pa})</th>
<th>(E_{pc} , V)</th>
<th>(E_{pa} , V)</th>
<th>(\Delta E_p , V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.25 ± 2.6%</td>
<td>-1.45 ± 2.8%</td>
<td>1.16</td>
<td>0.68 ± 2%</td>
<td>-0.23 ± 2%</td>
</tr>
<tr>
<td>2</td>
<td>2.49 ± 6.7%</td>
<td>-2.21 ± 10.0%</td>
<td>0.89</td>
<td>0.77 ± 2%</td>
<td>-0.25 ± 2%</td>
</tr>
<tr>
<td>4</td>
<td>2.32 ± 5.5%</td>
<td>-1.86 ± 12.9%</td>
<td>0.80</td>
<td>0.83 ± 2%</td>
<td>-0.25 ± 2%</td>
</tr>
<tr>
<td>6.5</td>
<td>1.37 ± 1.3%</td>
<td>-1.66 ± 1.1%</td>
<td>1.21</td>
<td>0.75 ± 2%</td>
<td>-0.24 ± 2%</td>
</tr>
</tbody>
</table>

5.2.3 FSCV Clean Effectiveness in Three Different Potential Ranges

Previous study suggests that during FSCV detection −0.4 V cathodic start potential plays a crucial role in attaining a maximum saturation rate of dopamine sensitivity irrespective of anodic limit with increased sensitivity and better response time
[70-72], and +1.0V anodic limit provides stability in dopamine signal gain and faradic response coupled with better temporal resolution & good signal to noise ratio during both *in vitro* and *in vivo* [70].

The reactivations were employed in three different ranges to compare the effects; three waveforms -0.5 V to +0.8V, -0.4 V to +1.0V and -0.5 V to +1.2V are used for 5 min in 1x PBS between cycles to clean and re-activate the electrode’s surface. They included a small range of potential -0.5 V to +0.8V and a large range of potential -0.5 V to +1.2V. However, the lower positive potential reduced the reactivation’s effectiveness, which can be observed by the immediate current signals increasing, the negatively charged poly-dopamine fouling the electrode surface, and adhered on the electrode surface, which briefly enhanced the signals (Figs. 5-13, 5-14). After that, the fouling rate increased and the signals quickly went down with oxidation potential increasing (Fig. 5-15); the oxidation potential was one of the methods to identify the electrodes’ oxidation in both dopamine fouling and electrode self-oxidation. In the large range of potential -0.5 V to +1.2V, the higher positive potential was not the ideal potential to be employed to the real brain. It can damage the cells in the brain. However, in the 6.5 hours studied, the sensitivity immediately increased with increasing oxidation potential and decreasing (Fig. 5-16). After 2 hours, the reactivation (-0.5 V to +1.2 V) had higher current signals compared to other reactivations, but it resulted in a higher oxidation potential, and it oxidized the electrode faster by applying higher potential, which caused the final signal’s sensitivity to reduce by more than the potential range of -0.4 V to +1.0V.
Therefore, the results evidently confirmed the positive potential had the effect to the reactivation effectiveness, but higher positive potential could cause faster electrode's oxidation. Compared to the two potential ranges, the potential range of $\text{-0.4 V to +1.0V}$ has smooth signals and oxidation potential increasing, and it had a smooth decreasing of signals after 4 hours. Based on the sensitivity of the current signals and oxidation potential, the potential range of $\text{-0.4 V to +1.0V}$ was the best reactivation by maintaining the sensitivity (Fig. 5-16).

![Fig. 5-13. Effect of electrochemical cycling on surface fouling and DA signal sensitivity. (a-c) Three waveforms $\text{-0.5 V to +0.8V; -0.4 V to +1.0V and -0.5 V to +1.2V}$ are used for 5 min in 1x PBS between cycles to clean and re-active the electrode surface. The PBS buffer was flown in the microfluidic channel at a flow rate of 0.2 ml/min. Each cycle represents a continuous application of the FSCV waveform ($\text{-0.4 V to +1.0 V}$) at 60 Hz for 30 min in 100 $\mu$M DA. Legends: green curve – fresh surface, initial surface or $0^{th}$ cycle; brown curve – after $4^{th}$ cycle; red curve – after $8^{th}$ cycle and black curve – after $13^{th}$ cycle. Voltammograms are recorded using a 250 $\mu$m BDUNCD microelectrode and 400 V/s scan rate. The voltammogram is shown after the background subtraction in 1x PBS [121].]
Fig. 5-14. The change in DA peak current from $0^{th}$ to $13^{th}$ cycle. The three different reactivation range, green is reactivation from $-0.5$ V to $+1.2$ V; the blue is reactivation from $-0.4$ V to $+1.0$ V; the red is reactivation from $-0.5$ V to $+0.8$ V, and orange is without reactivation. Voltammograms are recorded using a 250 µm BDUNC microelectrode, 100 µM DA in 1x PBS buffer solution and 400 V/s scan rate.

Fig. 5-15. The change in DA peak potential from $0^{th}$ to $13^{th}$ cycle. The three different reactivation range, green is reactivation from $-0.5$ V to $+1.2$ V; the blue is reactivation from $-0.4$ V to $+1.0$ V; the red is reactivation from $-0.5$ V to $+0.8$ V, and orange is without reactivation. Voltammograms are recorded using a 250 µm BDUNC microelectrode, 100 µM DA in 1x PBS buffer solution and 400 V/s scan rate.
Fig. 5-16. The change in DA peak sensitivity from 0th to 13th cycle of the three different reactivation ranges, green is the reactivation from -0.5 V to +1.2 V; blue is the reactivation from -0.4 V to +1.0 V; red is the reactivation from -0.5 V to +0.8 V, and orange is without reactivation. Voltammograms are recorded using a 250 μm BDUNC microelectrode, 100 μM DA in 1x PBS buffer solution and 400 V/s scan rate.

5.2.3.1 **Three Different Potential Ranges in Ag Particles’ Mapping**

The Ag particles’ mapping was used to identify the electrode’s fouling surface (Fig. 5-17). It was difficult to investigate the poly-dopamine film and its thickness on the electrodes’ surface. In the comparison of the different potential ranges effect, the Ag particles’ mapping was employed to map the electrodes’ active area. In this study, it added a clean surface without reactivation surface to compare with the three different ranges of reactivation. It demonstrated the clean surface had 100% coverage and uniform Ag particles’ size and shape, and without the reactivation the electrodes’ active area reduced to more than 60% which caused the higher current to charge on a few locations and resulted in a flower-shaped of Ag particle’s (Fig. 5-18). However, with reactivation they had different Ag particles sizes, but had similar shapes; the reactivation cleaned the surface to avoid the fouling film block. The surface resulted in a higher current charge on the exposed surface. To compare the reactivation’s effectiveness, the best reactivation
had a higher coverage of Ag particles about 80% to 90% in the 6.5 hours. However, the 
−0.5 V to +1.2V reactivation had oxidized the electrodes' surface reducing the functional 
group on the BDUNCD to result in the active area reducing, and the −0.5 V to +0.8V 
reactivation did not fully clean the electrodes' surface, which resulted in some blocked 
areas by the fouling products.

![Fig. 5-17. SEM images of Ag particles' mapping on the new BDUNCD electrode's surface, without reactivation surface, and three different reactivations at scale bar 100 µm. The Ag particles were deposited by EPD at potential −0.5 V for 200 seconds.](image-url)
Fig. 5-18. SEM images of Ag particles’ mapping on the new BDUNCD electrode’s surface, without reactivation surface, and three different reactivations at scale bar 5 μm. The Ag particles were deposited by EPD at potential −0.5 V for 200 seconds.

5.2.4 Positive Window Influence

5.2.4.1 Positive Window Influence and Negative Window Influence

In the comparison of positive window influence, the negative potential was set up at the same condition of −0.5 V, and changes the positive potential from +0.8 V to +1.2 V. However, by increasing the positive potential, it was also increasing the reactivation effectiveness and electrodes’ lifetime (Fig. 5-19); the sensitivity was increased by increasing positive potential. However, the higher potential +1.2 V caused the electrode self-oxidation and finally resulted in a lower signal than +1.0 V. In the negative window influence, the positive potential was set up at the same condition of +1.0 V, and changed the negative potential from −0.4 V to −0.6 V (Fig. 5-20). They had similar signals increasing at the beginning, but the signal’s sensitivity decreased for both −0.5 V and −0.6 V, which explained the negative charge was not affected by reactivation,
but it affected the poly-dopamine formed on the electrode surface. It resulted in reducing the sensitivity in both −0.5 V and −0.6 V. The negative potential increased the positive charge of dopamine, and increased the chemical reaction to form the melanin, which finally fouled the electrodes’ surface reducing the electrodes’ sensitivity.

Fig. 5-19. The change in the DA peak’s current from 0th to 13th cycles of the three different reactivation ranges, green is the reactivation from −0.5 V to +1.2 V; dark is the reactivation from −0.5 V to +1.0 V; red is the reactivation from −0.5 V to +0.8 V, and orange is without reactivation. Voltammograms are recorded using a 250 μm BDUNCD microelectrode, 100 μM DA in 1x PBS buffer solution and 400 V/s scan rate.

Fig. 5-20. The change in the DA peak’s current from 0th to 13th cycles of the three different reactivation ranges, purple is the reactivation from −0.6 V to +1.0 V; dark is the reactivation from −0.5 V to +1.0 V; blue is the reactivation from −0.4 V to +1.0 V, and orange is without reactivation. Voltammograms are recorded using a 250 μm BDUNCD microelectrode, 100 μM DA in 1x PBS buffer solution and 400 V/s scan rate.
5.2.5 The Best Window Clean Effectiveness in Long-Term BDUNCD (28 h)

After finding the best reactivation $-0.4 \, \text{V} \rightarrow +1.0 \, \text{V}$, it was employed in a long-term study to investigate the electrode's behavior and lifetime in 28 hours (Fig. 5-21). The reactivation was applied every 30 min by FSCV (400 V/s, 60 Hz) with 1x PBS solution at a flow rate of 0.2 ml/min. The electrode was fouling (monitoring) in FSCV (400 V/s, 60 Hz) with 100 $\mu$M dopamine solution at a flow rate of 0.2 ml/min. The signals behaved as previous. It increased in the first 3 hours, then slowly decreased. However, the difference was that, every day the signals went back to higher sensitivity than the day before. It explained that there was new fouling produced before the removal of fouling product. By stopping the fresh dopamine, the fouling products were slowly removed by an unstable structure adhered to the electrodes' surface. However, this only happened when it was applied with reactivation. Without the reactivation, the dopamine will form a thick structure, and it had stronger adhering on the electrodes' surface. However, the best reactivation was also slowly oxidizing the electrode by investigating the oxidation potential increasing (Fig. 5-22).

After 14th cycles, it cleaned and increased the signals of 44% from $1.23 \pm 17\% \, \mu\text{A}$ to $1.77 \pm 10\% \, \mu\text{A}$ (Table 5-7). It maintained the signals until 28 cycles ($1.21 \pm 18\% \, \mu\text{A}$) and then slowly decreased. Finally, the electrode successfully expanded the lifetime from 6.5 hours to 28 hours with 37% of signal decreased from $1.23 \pm 17\% \, \mu\text{A}$ to $0.78 \pm 2\% \, \mu\text{A}$; for every day of 1-hour monitoring, it is about 1 month in lifetime.
Fig. 5-21. The change in DA peak current from 0th to 57th cycles. The green was the best reactivation range -0.4 V to +1.0V, and orange is without reactivation. Voltammograms are recorded using a 250 μm BDUNCD microelectrode, 100 μM DA in 1x PBS buffer solution and 400 V/s scan rate [121].

Fig. 5-22. The change in DA peak potential from 0th to 57th cycles. Voltammograms are recorded using a 250 μm BDUNCD microelectrode, 100 μM DA in 1x PBS buffer solution and 400 V/s scan rate.
Table 5-7. The FSCV signals analyzed of the BDUNCD fouling with 5 minutes the best reactivation (−0.4 V to +1.0 V) for long-term sensing in 57 cycles (28 hours).

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>No of cycle</th>
<th>Peak anodic Current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peak cathodic Current</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anodic peak potential</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$I_{pa}(\mu A)$</td>
</tr>
<tr>
<td>Reactivation from</td>
<td>Initial data</td>
<td>1.23 ± 17%</td>
</tr>
<tr>
<td>−0.4 to +1.0 V for 57 cycles</td>
<td>After 14th cycles</td>
<td>1.77 ± 10%</td>
</tr>
<tr>
<td></td>
<td>After 18th cycles</td>
<td>1.39 ± 15%</td>
</tr>
<tr>
<td></td>
<td>After 23rd cycles</td>
<td>1.67 ± 16%</td>
</tr>
<tr>
<td></td>
<td>After 28th cycles</td>
<td>1.21 ± 18%</td>
</tr>
<tr>
<td></td>
<td>After 32nd cycles</td>
<td>1.00 ± 21%</td>
</tr>
<tr>
<td></td>
<td>After 43rd cycles</td>
<td>0.76 ± 4%</td>
</tr>
<tr>
<td></td>
<td>After 49th cycles</td>
<td>0.98 ± 1%</td>
</tr>
<tr>
<td></td>
<td>After 57th cycles</td>
<td>0.78 ± 2%</td>
</tr>
</tbody>
</table>

5.3 Microfluidic Device of Droplets Sensing

The advantages of the droplets sensing were sensing the single signal per droplet every 2 minutes. The separate time was set up for 2 minutes to wait for the signals to go return to the background signals. However, if it adds a droplet before the sensing signal goes back to the background signals, it will superimpose both signals, and produce a higher signal than the real signal. As well, the background signals were a significant data, which were required to determine the electrodes’ surface fouling by DA. However, droplets’ volume and background also affect the signals’ stable. Therefore, it is critical to maintain the concentration in the center of the droplets. It can be simply affected by the droplets’ volume and background flow rate. Hence, in this study, the essential conditions have to be studied properly in order to increase the signals stable and reduce the flow’s effects.
5.3.1 Electrodes' Electrophoretic Deposition (EPD)

5.3.1.1 Nafion Layer Electrophoretic Deposition (EPD)

In the beginning of the experiment, I developed the protocol of the nafion layer electrophoretic deposition (EPD) for 250 µm BDUNCD electrodes. The first time the nafion layer deposition was carefully studied by three different deposition times 2 minutes, 4 minutes, and 6 minutes in EPD with potential +1.5 V, and compared to the fresh BDUNCD (Fig. 5-23). In the EPD, the BDUNCD electrodes' surface was cleaned by H₂SO₄ clean process, and nafion (5wt.% in lower aliphatic alcohols and water) has been added on the electrode's surface, final connect with Pt wire and Ag/AgCl reference electrode. In Fig. 5-23 both 2 minutes' and 4 minutes' deposition time have increased the electrodes' sensitivity. However, 6 minutes of EPD of nafion layer resulted in no increase sensitivity. The disadvantage of the thick nafion layer was that it increased the arduous task of dopamine diffusion through the nafion layer. Therefore, by comparing the remaining 2 minutes and 4 minutes, both increased the oxidation current about three to four times. Nevertheless, in the reduction current, 2 minutes EPD of nafion layer indicated about 2 times the increase than 4 minutes EPD layer because the negative charge of the nafion layer refused the oxidized dopamine which has a negative charge, and resulted in the reduction of the current and shift reduction potential to more negative. Finally, I developed the best nafion layer by 2 minutes EPD which increased both the oxidation current and the reduction current about three to four times.
Fig. 5-23. The nafion layer using electrophoretic deposition on 250 μm BDUNCD electrodes’ surface. After deposition, the chip was carefully rinsed with DI water and baked 10 minutes at 70°C. The green line was the fresh BDUNCD (100 μM DA); the orange line was the 2 minutes nafion deposition; the blue line was the 4 minutes nafion deposition, and the red line was 6 minutes nafion deposition [121].

5.3.1.2 **MWCNT Layer Electrophoretic Deposition (EPD)**

The CNT-BDUNCD had an injury due to CNT deposited loss by the time. However, nafion- CNT-BDUNCD has addressed the problem and enhanced CNT-BDUNCD signals about three to four times with a total of 10 times of comparing with BDUNCD. **Fig. 5-24 (a)** shows the optical microscopy’s image 20X that of the CNT-UNCD’s surface; the CNT covered 100% of the UNCD’s surface. However, after nafion layer deposition, some areas became light in the optical microscopy’s image which is shown in **Figs. 5-24 (b) (c)**. Some CNTs have been removed during the nafion layer deposition because it used opposite potential which removed the relatively unfixed CNT. Nevertheless, it is significant to investigate the light area in the nafion-CNT-UNCD surface; even after nafion deposition, CNT layer must be 100% covered on the UNCD’s surface. SEM image of **Fig. 5-25 (a)** (3.0 KV, 7.9 mm X 800) was the UNCD surface.
which shows a dark surface and Fig. 5-25 (b) (3.0 KV, 7.9 mm X 900) was the CNT-UNCD surface which shows a 100% coverage.

However, after nafion layer deposition, Fig. 5-25 (c) (3.0 KV, 7.9 mm X 900) had some dark areas, which CNTs have been removed by EPD process. To investigate the dark area in Fig. 5-25 (d) (3.0 KV, 5.6 mm X 7.0 K), there were two spots have been taken to investigate in high magnification which shown in Figs. 5-25 (e) (f) (3.0 KV, 5.6 mm X 60.0 K); in the high magnification of SEM image, as seen there were thin CNT layer cover on the UNCD electrode, and there was no UNCD surface exposure on the nafion-CNT-UNCD electrode. Therefore, we successfully developed a protocol of nafion-CNT-UNCD sandwich electrode without UNCD surface exposure.

Fig. 5-24. The optical microscopes' images have been applied to investigate the MWCNT deposition on BDUNCD electrode’s surface. (a) the MWCNT-BDUNCD electrode in 20X of the optical microscopes' images with 100% coverage on BDUNCD surface. (b) the nafion- MWCNT-BDUNCD electrode in 20X of the optical microscopes' images. (c) 50X of the nafion- MWCNT-BDUNCD electrode. Light area lost MWCNT materials.
Fig. 5-25. The SEM image of nafion and MWCNT deposition to investigate the MWCNT coverage percentage after nafion deposition. (a) the image of BDUNCD (3.0 KV, 7.9 mm X 800) had dark color and clean surface. (b) after MWCNT deposition 10 minutes with potential $-6$ V (3.0 KV, 7.8 mm X 900). (c) after nafion deposition 2 minutes with potential $+1.5$ V. (3.0 KV, 5.2 mm X 900) (d) after nafion deposition, the high magnification image in dark area of blue box of the (c) (3.0 KV, 5.6 mm X 7.0 K). (e) and (f) were two spots in the (d) of orange boxes (3.0 KV, 5.6 mm X 60.0 K).

5.3.1.3 Advantages of Nafion and MWCNT layers

The nafion layer was used to increase chemical resistance while applying positive potential, and the negative charge nafion layer can refuse polydopamine which has a negative charge adherence on the electrode’s surface; it reduces the chemical intermediates of o-DQ, LDC, and DC remaining on the surface that can reject the chemical intermediates to form the melanin on the surface. However, the negative charge nafion layer also can increase dopamine signals about two to three times by attracting positive dopamine (Fig. 5-26); the signal was taken by FSCV ($-0.4$ V to $+1.0$ V, 400 V/s) with a flow rate of 0.1 ml/min, and 100 μM DA. Therefore, the nafion-MWCNT-BDUNCD has increased about 10 times compared to BDUNCD.
Fig. 5-26. Comparison of sensitivity of after nafion and nafion-MWCNT layers deposition on BDUNCD with fresh BDUNCD. The signals were taken by FSCV (−0.4 to +1.0 V for BDUNCD and nafion-BDUNCD, −0.4 V to +2.0 V for nafion-MWCNT-BDUNCD) at 100 μM DA in a flow rate of 0.2 ml/min [121].

5.3.1.4 Advantages of Nafion on MWCNT-BDUNCD Microelectrode

The MWCNT layer was using electrophoretic deposition (EPD) at −6 V for 10 minutes, so the different deposition time affects the thickness of MWCNT layer in order to influence the signal's sensitivity. In our procedure, the MWCNT layer increased about three times by FSCV (−0.4 V to +1.0 V, 400 V/s) with a flow rate of 0.1 ml/min, and 100 μM DA (Fig. 5-27). However, the disadvantage is with the MWCNT layer removed during the sensing, it is hard to achieve long-term in vivo sensing. Therefore, the nafion layer was introduced to solve the issue. The 50 nm nafion layer covered the MWCNT surface to maintain the MWCNT layer on the electrodes; the thin nafion layer increased dopamine signals ~10 times by FSCV (−0.4 V to +1.8 V, 400 V/s) with a flow rate of 0.1 ml/min, and 100 μM DA.
Fig. 5-27. Comparison of sensitivity of after MWCNT and nafion-MWCNT layers deposition on BDUNCD with fresh BDUNCD. The signals was taken by FSCV (−0.4 V to +1.0 V for BDUNCD, −0.4 V to +2.0 V for MWCNT-BDUNCD and nafion-MWCNT-BDUNCD) at 100 μM DA in a flow rate of 0.2 ml/min.

5.3.2 Droplets’ Sensing

5.3.2.1 Analyzed Droplet’s Signals

The droplet’s sensing was developed to verify the different electrode’s properties which are sensitivity, response time, and clearance rate (Fig. 5-28). The peak’s currents were determined at the second peak which does not vary by the mass transfer effect. The first peaks varied by the mass transfer effect which resulted in an unstable signal’s current peak. The response time was defined as the time of first response’s signal to the second peak; it can be easily affected by unstable background flow which highlights the importance of the microfluidic design. Then, the clearance rate was defined as the time between $T_{20}$ to $T_{60}$; $T_{20}$ was the 20% reduction of the peak’s current and $T_{60}$ was the 60% reduction of the peak’s current. The clearance rate represented the time of dopamine
solution removed from the electrode's surface, the disadvantage of low clearance rate was a longer waiting time for the signal to return to the background signals.

![Droplet signal diagram]

Fig. 5-28. The droplet's signal analyzed; it had a background flow rate of 0.1 ml/min (1x PBS), droplets' volumes were 0.02 ml (100 μM, DA), and the potential was +0.35 V.

5.3.2.2 **Analyzed S/N Ratio**

The signals were defined from half of noise to current peak which is 18 ± 59% nA subtract the higher noise signals 0.075 ± 0.2% nA (A) and plus half of noise (A - B)/2, it is the total signal current (Fig. 5-29) [120]. The noises were defined the higher noise signals 0.075 ± 0.2% nA (A) subtract the lowest noise signals 0.063 ± 0.2% nA (B) which is 0.012 ± 0.2% nA. The metrohm-autolab has number of current ranges remark from 10 nA to 1 A with current resolution of 0.0003 % (of current range) and the accuracy is 0.2 % of current range. It can measure the current to pA range with 0.2 % accuracy of the current range.
5.3.2.3 Evaluate the Best Potential by S/N Ratio

In the droplets' study, the first step was to determine an optimal potential for amperometry analysis of the DA, which can be found by the S/N ratio. There was only select one potential, which had to be suitable at three different electrodes, and it had a higher S/N ratio. The measurement was performed using 100 μM DA in 1x PBS solution. In Fig. 5-30, it is plotted in the potential range of 0.2 V to 0.6 V, and each point corresponds to six droplets' signals. As seen from Fig. 5-30 the +0.35 V had the highest S/N ratio in BDUNCD and nafion-CNT-UNCD. However, the nafion-UNCD had the higher S/N ratios which were from potential 0.25 V to 0.35 V. Based on the theorem, only one selected potential was employed in the three electrodes. Therefore, the 0.35 V was chosen as the best potential which was suitable in three different electrodes in the droplet system.
Fig. 5-30. The S/N ratio was used to identify the best potential in three different electrodes. Each potential took six signals at 0.2 V, 0.25 V, 0.3 V, 0.35 V, 0.4 V, 0.45 V, 0.5 V, 0.55 V, and 0.6 V; the background flow rate was 0.2 ml/min (1x PBS) with droplets’ volume 0.005 ml (100 μM, DA). The S/N ratio was calculated by Equation (2). The blue line was nafion-WMCNT-BDUNCD electrode; the red line was BDUNCD, and the green line was nafion-BDUNCD [121].

5.3.2.4 Background Flow Rate and Droplet’s Volume

To determine the background flow rate for the DA droplets sensing in AM, each electrode has been studied in the different flow rates of 0.025 ml/min, 0.05 ml/min, 0.1 ml/min, 0.15 ml/min, 0.2 ml/min, and 0.25 ml/min; it had the same condition of droplets (0.005 ml, 100 μM DA) with potential +0.35 V, and each point corresponded to six droplets’ signals. In Fig. 5-31 (a) (UNCD), the current changed a direct ratio to the flow rate. However, the accuracy was reduced after the background flow rate became higher than 0.1 ml/min. As seen in both Fig. 5-31 (B) (nafion-UNCD) and (C) (nafion-CNT-UNCD), the electrodes had a higher current at a flow rate of 0.1 ml/min. Therefore, the suitable background flow rate was 0.1 ml/min for the higher accuracy of UNCD and higher current for both nafion-UNCD and nafion-CNT-UNCD.
The last parameter condition was to determine the droplets’ volume. Each electrode employed the different volume of droplets 0.005 ml, 0.01 ml, 0.02 ml, and 0.04 ml. The system condition has been developed with a background flow rate of 0.01 ml/min (1x PBS) at potential +0.35 V, and each droplet’s volume employed 6 droplets (100 μM DA) for the signals' sensing. As seen in Fig. 5-32, the 0.005 ml droplet has diluted current signals which result in a lower current compared to the other droplets’ volume. The other droplets’ volume 0.01 ml, 0.02 ml and 0.04 ml had the mass transfer limited, which had a rush current increase initially and it can be explained by Cottrell’s Equation. Therefore, the real signal can be identified by the second peak which all three electrodes show similar current peaks. However, the increase of droplets’ volume also increased the pressure in the channel. It might have caused leakage during the experiment. Therefore, the 0.01 ml was the best droplets’ volume in the microfluidic of droplets’ sensing.

Fig. 5-31. The different background flow rates were applied to identify the one suitable background flow rate in three electrodes; the background flow rates (1x PBS) were employed 0.025 ml/min, 0.05 ml/min, 0.1 ml/min, 0.15 ml/min, 0.2 ml/min, 0.25 ml/min, and droplets’ volume 0.005 (100 μM, DA) with potential +0.35 V, each flow rate took six signals. (a) has different flow rates in BDUNCD electrode; (b) has different flow rates in nafion-BDUNCD electrode, and (c) has different flow rates in nafion-MWCNT-BDUNCD electrode.
Fig. 5-32. The four different droplets’ volumes which were 0.005 ml, 0.01 ml, 0.02 ml, and 0.04 ml (100 μM, DA) have been employed to identify the best volumes; the background flow rate was 0.1 ml/min (1x PBS) with potential +0.35 V, and each volume had six signals. The dark blue was 0.005 ml; orange was 0.01 ml; green was 0.02 ml, and light blue was 0.04 ml [121].

5.3.3 Determined and Compared the Electrodes’ Properties (AM)

5.3.3.1 Sensitivity

In the Figs. 5-33, 5-34, and 5-35, the 9 hours’ signals have been analyzed by sensitivity, response time, and clearance rate. In the Figs. 5-36, 5-37 reach point was taken every 30 minutes with five signals, and overlay three different electrodes’ signals, and the sensitivity was calculated by Equation (3). The overlay of the sensitivity, the nafion-UNCD had the best signals stable and nafion-CNT-UNCD had the highest sensitivity compared to other electrodes.

After three different types of electrodes have been developed, each electrode was employed in the microfluidic of droplets sensing for 9 hours’ study. In the microfluidic setup, the channel width was 1800 μm, and droplets’ volume was 0.02 ml (100 μM DA) with a background flow rate of 0.1 ml/min (1x PBS). Each droplet was employed every 2 minutes in the channel and passed through the electrode in seconds. As seen in
Fig. 5-33, the UNCD electrode sensed droplets’ signals in real time in 9 hours with a total of 270 droplets’ signals. In the UNCD electrode sensing, the signals were stable during the first 3 hours. However, after 3 hours, the signals were increased about 3.26 times from 7.75 ± 2% nA to 25.3 ± 2% nA which corresponded to the results from the previous work of UNCD fouling mechanism; the poly-dopamine stuck on the UNCD surface, which caused the background current to shift to negative charge with unstable droplets’ signals. Therefore, UNCD electrode was fouling after 3 hours, and the poly-dopamine layer thickness increased during the experiment with the background current shifting to more negative. Poly-dopamine finally blocked the electrode’s signals by chemical reaction during the final stage of the product melanin which has been mentioned in UNCD fouling mechanism. However, in Fig. 5-34, the nafion-UNCD has indicated the advantage of chemical resistance with a constant background current in 9 hours; it did not only increase the sensitivity about two times (18.1 ± 2% nA) compared to BDUNCD (7.75 ± 2% nA) but also increase the signals stable. Therefore, nafion layer refused the poly-dopamine fouling and increased the electrode’s lifetime. In Figs. 5-35, 5-36 the third nafion-CNT-UNCD electrode had the highest current signals about 5 times (43.7 ± 2% nA) compared to UNCD signals (7.75 ± 2% nA), and the nafion layer indicated the advantage again with no changes to the background’s current. Nevertheless, the signals stable was not the best compared to the three electrodes, it varies with monitoring time which shown in Fig. 5-37; it may be due to the non-uniform CNT layer and surface roughness, which affected the dopamine flow on the electrode’s surface and resulted in a different flow rate or direction on the electrodes’ surface. It can be improved in future work.
Fig. 5-33. The microfluidic of droplets' sensing in BDUNCD electrode with a frequency of 1 drop/2 minutes; the total was 270 droplets. The parameter of the experiment had a background flow rate of 0.1 ml/min (1x PBS), droplets' volumes were 0.02 ml (100 μM, DA), and potential was +0.35 V; every 3 hours solutions in the syringes were refilled. The green lines were the first three hours; the red lines were three to six hours, and the blue lines were the final three hours. The total was nine hours' experiment [121].

Fig. 5-34. The microfluidic of droplets' sensing in nafion-BDUNCD electrode with a frequency of 1 drop/2 minutes; the total was 270 droplets. The parameter of the experiment had a background flow rate of 0.1 ml/min (1x PBS), droplets' volumes was 0.02 ml (100 μM, DA), and potential was +0.35 V; every 3 hours solutions in the syringes were refilled. The green lines were the first three hours; the red lines were three to six hours, and the blue lines were the final three hours. The total was nine hours' experiment [121].
Fig. 5-35. The microfluidic of droplets’ sensing in nafion-MWCNT-BDUNCD electrode with a frequency of 1 drop/2 minutes; the total was 270 droplets. The parameter of the experiment had a background flow rate of 0.1 ml/min (1x PBS), droplets' volumes were 0.02 ml (100 µM, DA), and potential was +0.35 V; every 3 hours solutions in the syringes were refilled. The green lines were the first three hours; the red lines were three to six hours, and the blue lines were the final three hours. The total was nine hours’ experiment [121].

Signal Currents

Fig. 5-36. The comparison of three different electrodes signals currents in microfluidic of droplets’ sensing with a frequency of 1 drop/2 minutes. The parameter of the experiment had a background flow rate of 0.1 ml/min (1x PBS), droplets' volumes were 0.02 ml (100 µM, DA), and potential was +0.35 V; every 30 minutes we took five signals and calculated the error bar in the nine hours’ experiment. The red line was BDUNCD electrode’s sensitivity in 9 hours; the green line was nafion-BDUNCD electrode’s sensitivity in 9 hours, and the blue line was nafion-MWCNT-BDUNCD electrode’s sensitivity in 9 hours [121].
Fig. 5-37. The comparison of three different electrodes sensitivity in microfluidic of droplets' sensing with a frequency of 1 drop/2 minutes; the red line was BDUNCD electrode's sensitivity in 9 hours, the green line was nafion-BDUNCD electrode's sensitivity in 9 hours, and the blue line was nafion-MWCNT-BDUNCD electrode's sensitivity in 9 hours [121].

5.3.3.2 Response Time

The sensors cannot immediately change the output state when an input signals change, it takes a period of time to reach the real signals, called the response time; it can be defined as the time required to increase from background current to a final oxidation current [122].

The response time was defined from first changing the current's point to a real signal. The real signal can be identified in the droplet signal of second oxidation peak, the first peak is varied by the mass transfer effect. Fig. 5-38 shows the nafion-CNT-UNCD had a fast response time (2 ± 8% s) and stability compared to the other electrodes BDUNCD (3.5 ± 6% s) and nafion-BDUNCD (2.5 ± 6% s).
**Response Time**

![Graph showing response time for different electrodes](image)

Fig. 5-38. The comparison of three different electrodes response time in microfluidic of droplets’ sensing with a frequency of 1 drop/2 minutes. The parameter of the experiment had a background flow rate of 0.1 ml/min (1x PBS), droplets' volumes were 0.02 ml (100 μM, DA), and potential was +0.35 V; every 30 minutes we took five signals and calculated the error bar in the nine hours’ experiment. The red line was BDUNCD electrode’s response time in 9 hours; the green line was nafion-BDUNCD electrode’s response time in 9 hours, and the blue line was nafion-MWCNT-BDUNCD electrode’s response time in 9 hours [121].

5.3.3.3 **Clearance Rate**

The clearance rate was defined by the time from the T<sub>20</sub> to T<sub>60</sub>; the T<sub>20</sub> was the signal reduced to 20% of the real signal, and T<sub>60</sub> was reduced to 60%. As seen in Fig. 5-39, the nafion-UNCN had the faster clearance rate. It confirmed the truth that nafion can increase the DA resistant and also can be used to increase the clearance rate by refusing chemical intermediate remain on the surface to occur chemical reaction on surface. However, nafion-CNT-UNCN had a slower clearance rate (3.35 ± 54% s) compare to BDUNCD (1.7 ± 20% s) and nafion-BDUNCD (0.65 ± 15% s); it is due to the high sensitivity and surface roughness which results in DA remain on the electrode surface. Therefore, the nafion-CNT-UNCN had the highest sensitivity but the stability and clearance rate can be improved in further work by improving the surface roughness.
Fig. 5-39. The comparison of three different electrodes clearance rate in microfluidic of droplets' sensing with a frequency of 1 drop/2 minutes. The parameter of the experiment had a background flow rate of 0.1 ml/min (1x PBS), droplets' volumes were 0.02 ml (100 μM, DA), and potential was +0.35 V; every 3 hours we took signals. The red line was BDUNCD electrode's clearance rate in 9 hours; the green line was nafion-BDUNCD electrode's clearance rate in 9 hours, and the blue line was nafion-MWCNT-BDUNCD electrode's clearance rate in 9 hours.

5.3.3.4 Comparison of Electrodes' Properties in 9 hours Monitoring

The microfluidic of droplets' sensing successfully developed the three electrodes' properties in sensitivity, response time, and clearance rate by the real-time study. By using the same protocol, the electrodes' properties can be identified and improved in the future.

The sandwich of the electrode nafion-MWCNT-BDUNCD has the highest sensitivity (47.8 ± 16% nA) compared with BDUNCD (15 ± 59% nA) and nafion-BDUNCD (19.4 ± 15% nA) (Table 5-8). The BDUNCD electrodes have higher error bars due to the electrode's surface fouling with polydopamine film (negative charge) on the electrode's surface increasing the signals after 3 hours about three times from 7.75 ± 2% nA to 25.3 ± 2% nA. As a result of increasing the thickness of the polydopamine and the generating of melanin will reduce the electrode's conductivity.
However, the difference between the droplets sensing and continuing flow conditions, it is the continuing flow that offers fresh DA solutions for the polydopamine film on the electrode’s surface, but the droplets sensing only employed fresh DA to pass through electrode in 2 seconds, and the final chemical reaction requires fresh DA to have the chemical reaction with Dopaminochrome (DC) to generate melanin on the electrode’s surface.

Table 5-8. Comparison of sensitivity, response time, and clearance rate on BDUNCD, nafion-BDUNCD, and nafion-MWCNT-BDUNCD electrodes.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Sensitivity (nA)</th>
<th>Response Time (s)</th>
<th>Clearance Rate (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDUNCD</td>
<td>15 ± 59%</td>
<td>3.5 ± 6%</td>
<td>1.7 ± 20%</td>
</tr>
<tr>
<td>Nafion-BDUNCD</td>
<td>19.4 ± 15%</td>
<td>2.5 ± 6%</td>
<td>0.65 ± 15%</td>
</tr>
<tr>
<td>Nafion-BDUNCD-MWCNT</td>
<td>47.8 ± 16%</td>
<td>2 ± 8%</td>
<td>3.35 ± 54%</td>
</tr>
</tbody>
</table>

Therefore, in the droplets sensing condition, it applied 0.02 ml every 2 minutes with about 2 second to pass through the electrode’s surface. It limited the polydopamine to form the melanin and limited the thickness of the polydopamine film; during the 2 minutes, the remaining dopamine and polydopamine film have been removed slowly from the electrode’s surface by reducing the separated time from 2 minutes to 30 seconds. It can increase the thickness of the polydopamine film with background current shifting to a more negative current (Fig. 5-40). The frequency of every 2 minutes employed one droplet, the background current was reduced from 0.012 nA to −0.6 nA after 6 hours. of sensing with a total of 180 droplets. The fresh solutions were refilled after 6 hours and took CV and FSCV signals. Afterwards, the starting background increased to −0.12 nA by removing polydopamine from the electrode’s surface during refilling and took signals of CV and FSCV. However, after 9 hours., the background
current signals shifting to $-2.51 \text{ nA}$ with a total of 270 droplets. Compared to the frequency of employing one droplet every 2 minutes (the background current $-2.51 \text{ nA}$ with total 270 droplets), the frequency of employing one droplet every 30 seconds has a thick polydopamine film by shifting the background current to $-6.20 \text{ nA}$ with a total of 720 droplets after 6 hrs.

![Graph](image)

Fig. 5-40. The droplet sensing has the droplets volume 0.02 ml, a background flow rate of 0.1 ml/min, and potential 0.35 V with the frequency of every 30 seconds employed one droplet. The total is 720 droplets in 6 hours.

In the neurochemical sensing, it requires quickly response time to measure the rapid signals change in human brain, and the nafion-CNT-UNCD had a fast response time ($2 \pm 8\% \text{ s}$) and stability compared to the other electrodes BDUNCD ($3.5 \pm 6\% \text{ s}$) and nafion-BDUNCD ($2.5 \pm 6\% \text{ s}$) (Table 5-8); both sensitivity and response time have been improved by adding MWCNT layer. However, during nafion layer deposition of EPD, it is removing some of the MWCNT on the electrode and increase the surface roughness in order to increase the clearance time ($3.35 \pm 54\% \text{ s}$) compare to BDUNCD ($1.7 \pm 20\% \text{ s}$)
and nafion-BDUNCD (0.65 ± 15% s). The surface roughness caused the DA more easily remain on the electrode’s surface which results in need more time to remove it.

5.3.4 Differential Pulse Voltammetry (DPV)

5.3.4.1 Selectivity

In the selectivity study, the signals of DA, 5-HT, and AA were detected individually, and all three electrodes have detection signals using DPV. In Table 5-9, the BDUNCD electrode detected oxidation potential of 1 µM DA at 52.49 mV, 1 µM 5-HT at 82.7 mV, and 100 µM AA at 47.5 mV (Fig. 5-41 (a)). However, the AA has a large signal potential range from −78.43 mV to 817.87 mV, which cover the signals of DA and 5-HT oxidation potential. Therefore, after the mix of 1 µM DA, 1 µM 5-HT, and 100 µM AA (Fig. 5-41 (d)), it resulted in a mixed signal which is unable to distinguish the three different species. Comparing the nafion-BDUNCD to the BDUNCD electrode, the nafion layer was successfully refused the AA signals and had the oxidation potential of 1 µM DA at 7.93 mV and 1 µM 5-HT at 77.66 mV (Fig. 5-41 (b)). AA, normally in the human brain, has higher concentration compared to DA and 5-HT, while we are more interested in DA and 5-HT signals; it is related to brain disease.

However, after the mix of 1 µM DA, 1 µM 5-HT, and 100 µM AA, it blocked the AA signals very well, but it was unable to distinguish between the two different species. This is due to the large DA oxidation potential range from −164.03 mV to 274.04 mV, which covered the 5-HT signals. Therefore, after mixing the solution, it resulted in a superimposed signal (Fig. 5-41 (e)). Different to those two electrodes, the nafion-MWCNT-BDUNCD has the oxidation potential of 1 µM DA at −83.47 mV and 1 µM 5-HT at 62.56 mV, which have clear separate signals of DA and 5-HT.
Therefore, after the mix solution, it had two oxidation signals which had
an oxidation potential of 1 μM DA at -18.00 mV and 1 μM 5-HT at 173.34 mV
(Fig. 5-41 (f)). The nafion-BDUNCD electrode oxidation current was increased 5.5 times
(0.11 nA) than BDUNCD (0.02 nA) in 1μM DA, and increased 3.3 times (0.46 nA) than
BDUNCD (0.14 nA) in 1μM 5-HT (Table 5-10). However, the nafion-MWCNT-
BDUNCD electrode of MWCNT layer was used to increase the sensitivity and
selectivity. It increased 165.5 times (3.31 nA) than BDUNCD (0.02 nA) in 1μM DA and
increased 15.9 times (2.23 nA) than BDUNCD (0.14 nA) in 1μM 5-HT. The nafion layer
was working properly in both electrodes to block AA signals and enhanced the
sensitivity.

Fig. 5-41. (a) DPV plots of individual of 10 μM DA, 3 μM 5-HT, and 100 μM AA with
BDUNCD microelectrode. (b) DPV plots of individual of 10 μM DA, 3 μM 5-HT, and
100 μM AA with nafion-BDUNCD microelectrode. (c) DPV plots of individual of 1 μM
DA, 1 μM 5-HT, and 100 μM AA with nafion-MWCNT-BDUNCD microelectrode. (d)
DPV plots of a mixture of 10 μM DA, 3 μM 5-HT, and 100 μM AA with BDUNCD
microelectrode. (e) DPV plots of a mixture of 1 μM DA, 1 μM 5-
HT, and 100 μM AA with nafion-BDUNCD microelectrode. (f) DPV plots of a mixture of 1 μM DA, 1 μM 5-
HT, and 100 μM AA with nafion-MWCNT-BDUNCD microelectrode.
Table 5-9. Comparison of DPV plots peak potentials on an individual of 1 µM DA, 1 µM 5-HT, and 100 µM AA.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>DA Potential (mV)</th>
<th>5-HT Potential (mV)</th>
<th>AA Potential (mV)</th>
<th>$E_{5-HT} - E_{DA} = \Delta E$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDUNCD</td>
<td>52.49 ± 0.2%</td>
<td>82.70 ± 0.2%</td>
<td>47.5 ± 0.2%</td>
<td>30.21</td>
</tr>
<tr>
<td>Nafion-BDUNCD</td>
<td>-7.93 ± 0.2%</td>
<td>77.66 ± 0.2%</td>
<td>0</td>
<td>85.59</td>
</tr>
<tr>
<td>Nafion-MWCNT-BDUNCD</td>
<td>-83.47 ± 0.2%</td>
<td>62.56 ± 0.2%</td>
<td>0</td>
<td>146.03</td>
</tr>
</tbody>
</table>

Table 5-10. Comparison of DPV plots peak currents on an individual of 1 µM DA, 1 µM 5-HT, and 100 µM AA.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>DA Current (nA) (1 µM)</th>
<th>5-HT Current (nA) (1 µM)</th>
<th>AA Current (nA) (100 µM)</th>
<th>Sensitivity Value µA µM cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDUNCD</td>
<td>0.02 ± 0.2%</td>
<td>0.14 ± 0.2%</td>
<td>0.17 ± 0.2%</td>
<td>0.04</td>
</tr>
<tr>
<td>Nafion-BDUNCD</td>
<td>0.11 ± 0.2%</td>
<td>0.46 ± 0.2%</td>
<td>0</td>
<td>0.22</td>
</tr>
<tr>
<td>Nafion-MWCNT-BDUNCD</td>
<td>3.31 ± 0.2%</td>
<td>2.23 ± 0.2%</td>
<td>0</td>
<td>6.75</td>
</tr>
</tbody>
</table>

In the addition of selectivity of the DA, the nafion-MWCNT-BDUNCD was used to investigate the mix solution of DA, 5-HT, and AA; it fixed the concentration of 1 µM 5-HT and 100 µM AA and changed the concentration of DA from 0.5 µM to 10 µM (Fig. 5-42). After increasing the DA concentration, both DA and 5-HT shifted the oxidation potential, which is affected by the mass transfer effect. The mass transfer effect includes diffusion, migration, and convection, which vary by concentration. Therefore, after the DA concentration increased from 0.5 µM to 10 µM, the oxidation potential of DA shifted from -18.00 mV to 17.24 mV, and 5-HT shifted from 168.30 mV to 208.59 mV; the total shift is 35.24 mV for DA oxidation potential and the shift of 40.29 mV for 5-HT oxidation potential (Table 5-11). However, the $\Delta E$ of DA oxidation potential to
5-HT oxidation potential is shifted from 186.3 mV to 191.35 mV, which is a total shift of 5.05 mV.

In the various concentrations of selectivity study for nafion-MWCNT-BDUNCD, the oxidation currents were increased linearly in the mix solution from 0.5 μM of DA (1.57 nA) to 5 μM of DA (9.75 nA) (Table 5-11). However, the shifting oxidation potentials of 10 μM DA (35.24 mV) and 1 μM 5-HT (40.29 mV) are affected by the mass transfer effect, but the ΔE=ΔE_{5-HT}−ΔE_{DA} is only shifted by 5.05 mV. Therefore, the mass transfer effect has increased the same oxidation potential of both DA and 5-HT with increasing DA concentration from 0.5 μM to 10 μM. The excellent selectivity of the linear property can be used to study multiple neurochemicals at the same time.

Fig. 5-42. DPV plots of a mixture of 1 μM 5-HT, 100 μM AA, and DA (0.5 μM, 1 μM, 2.5 μM, 5 μM, and 10 μM) with nafion-MWCNT-BDUNCD microelectrode.
Table 5-11. The signals analyzed of DPV plots of a mixture of 1 μM 5-HT, 100 μM AA, and DA (0.5 μM, 1 μM, 2.5 μM, 5 μM, and 10 μM) with nafion-MWCNT-BDUNCD microelectrode.

<table>
<thead>
<tr>
<th>DA</th>
<th>Oxidation Potential (mV)</th>
<th>Peak Current (nA)</th>
<th>5-HT</th>
<th>Oxidation Potential (mV)</th>
<th>Peak Current (nA)</th>
<th>ΔE_{5-HT} − ΔE_{DA}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 μM</td>
<td>-18.00 ± 0.2%</td>
<td>1.57 ± 0.2%</td>
<td>1 μM</td>
<td>168.30 ± 0.2%</td>
<td>3.56 ± 0.2%</td>
<td>186.3</td>
</tr>
<tr>
<td>1 μM</td>
<td>-12.97 ± 0.2%</td>
<td>2.44 ± 0.2%</td>
<td>1 μM</td>
<td>173.34 ± 0.2%</td>
<td>3.44 ± 0.2%</td>
<td>186.31</td>
</tr>
<tr>
<td>2.5 μM</td>
<td>-7.93 ± 0.2%</td>
<td>5.89 ± 0.2%</td>
<td>1 μM</td>
<td>183.41 ± 0.2%</td>
<td>3.32 ± 0.2%</td>
<td>191.34</td>
</tr>
<tr>
<td>5 μM</td>
<td>2.14 ± 0.2%</td>
<td>9.75 ± 0.2%</td>
<td>1 μM</td>
<td>193.48 ± 0.2%</td>
<td>3.12 ± 0.2%</td>
<td>191.34</td>
</tr>
<tr>
<td>10 μM</td>
<td>17.24 ± 0.2%</td>
<td>13.42 ± 0.2%</td>
<td>1 μM</td>
<td>208.59 ± 0.2%</td>
<td>2.92 ± 0.2%</td>
<td>191.35</td>
</tr>
</tbody>
</table>

5.3.4.2 Additional Selectivity of Nafion-MWCNT-BDUNCD

The additional selectivity study of nafion-MWCNT-BDUNCD studies sensitivity affected by the electrode oxidation (Fig. 5-43 (a)). During the droplets sensing, it has the droplets volume 0.02 ml, a background flow rate of 0.1 ml/min, and a potential of +0.35 V with the frequency of employing one droplet every 2 minutes for a total of 9 hours. The DPV scans at 0, 3, 6, and 9 hours with a mixture of 1 μM 5-HT, 100 μM AA, and 1 μM DA. Before DPV scanning, the electrode was cleaned by 1x PBS solution with a flow rate of 0.1 ml/min for 20 minutes to remove the DA remaining on the electrode’s surface (Fig. 5-43 (b)). By depositing the nafion layer, it refuses the polydopamine fouling and results in only electrode oxidation to affect the electrode’s sensitivity and selectivity in the additional selectivity study.

The electrode has the initial sensitivity of oxidation current DA (0.58 nA) and 5-HT (1.03 nA) with a sensitivity value of DA 1.18 μA μM⁻¹ cm⁻² and 5-HT 2.09 μA μM⁻¹ cm⁻² (Table 5-12). After 3 hours oxidation, the sensitivity of oxidation current reduced to 28% of DA (0.42 nA) and 0.06% of 5-HT (0.97 nA). During the oxidation, it reduced the functional group of (COOH) and resulted in the reduction of
electrode selectivity. However, after 9 hours, the DA oxidation current reduced 47% (0.31 nA) with $\Delta I_{DA} = 0.27$ nA and the 5-HT oxidation current reduced 26% (0.76 nA) with $\Delta I_{5-HT} = 0.26$ nA, and the sensitivity value of DA 0.63 $\mu$A $\mu$M$^{-1}$ cm$^{-2}$ and 5-HT 1.55 $\mu$A $\mu$M$^{-1}$ cm$^{-2}$. Therefore, during the 9 hours electrode oxidation, both DA and 5-HT reduced sensitivity by 47% and 26% by reducing a functional group of (COOH) on the MWCNT. Thus, nafion-MWCNT-BDUNCD has excellent selectivity with cleanly separated oxidation peaks of a mixture of 1 $\mu$M 5-HT, 100 $\mu$M AA, and 1 $\mu$M DA solution after 9 hours electrode oxidation.

Fig. 5-43. The signals analyzed of DPV plots of a mixture of 1 $\mu$M 5-HT, 100 $\mu$M AA, and 1 $\mu$M DA with nafion-MECNT-BDUNCD microelectrode. (a) The DPV scans at 0, 3, 6, and 9 hours during droplets sensing with a flow rate of 0.1 ml/ min; during droplets sensing, it has the droplets volume 0.02 ml, a background flow rate of 0.1 ml/min, and a potential 0.35 V with the frequency of employing one droplet every 2 minutes for a total of 9 hours. (b) every 3 hours, applied 20 minutes 1x PBS solution to the clean electrode’s surface.
Table 5-12. The DPV scans at 0, 3, 6, and 9 hours of a mixture of 1 μM 5-HT, 100 μM AA, and 1 μM DA with nafion-MECNT-BDUNCD microelectrode.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>DA Oxidation Potential (mV)</th>
<th>DA Peak current (nA)</th>
<th>5-HT Oxidation Potential (mV)</th>
<th>5-HT Peak current (nA)</th>
<th>ΔE_{5-HT} - ηΔE_{DA}</th>
<th>DA Sensitivity Value μA μM^{-1} cm^{-2}</th>
<th>5-HT Sensitivity Value μA μM^{-1} cm^{-2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.45 ± 0.2%</td>
<td>0.58 ± 0.2%</td>
<td>213.62 ± 0.2%</td>
<td>1.03 ± 0.2%</td>
<td>166.17</td>
<td>1.18</td>
<td>2.09</td>
</tr>
<tr>
<td>3</td>
<td>32.35 ± 0.2%</td>
<td>0.42 ± 0.2%</td>
<td>198.52 ± 0.2%</td>
<td>0.97 ± 0.2%</td>
<td>166.17</td>
<td>0.85</td>
<td>1.98</td>
</tr>
<tr>
<td>6</td>
<td>7.17 ± 0.2%</td>
<td>0.33 ± 0.2%</td>
<td>188.45 ± 0.2%</td>
<td>0.78 ± 0.2%</td>
<td>181.27</td>
<td>0.67</td>
<td>1.59</td>
</tr>
<tr>
<td>9</td>
<td>7.17 ± 0.2%</td>
<td>0.31 ± 0.2%</td>
<td>188.45 ± 0.2%</td>
<td>0.76 ± 0.2%</td>
<td>181.27</td>
<td>0.63</td>
<td>1.55</td>
</tr>
</tbody>
</table>

ΔE_{DA} = 40.28317    Δl_{DA} = 0.27    ΔE_{5-HT} = 25.177    Δl_{5-HT} = 0.26

5.3.4.3 Limit of Detection

The limit of detection (LOD) is the lowest concentration detection that has a reliable analysis, usually higher than the lowest detected concentration. For example, the lowest detection concentration of nafion-MWCNT-BDUNCD is 1 nM DA with a peak current of 0.063 nA, but the LOD is 5.4 nM. The LOD was calculated by Equation (5), which is three times the standard deviation divided by the slope. The slope is the trend line of the varying current with concentrations from 1nM to 100 nM DA and the slope is 0.0014 from the equation of the trend line.

\[
\text{Standard Deviation} \times 3 = \text{LOD}
\]

BDUNCD has the LOD of 2.58 μM DA with nafion layer reducing the LOD to 1.4 μM DA (in ranges of 0.01-0.1 mM in FSCV) (Table 5-13). However, both BDUNCD and nafion-BDUNCD have the lowest detected concentration of 50 nM DA, but the current signals were not reliable analyses which did not linearly increase with a change in concentration. The LOD varied by the different techniques, BDUNCD has LOD of FSCV 20.25 ± 2% μM (correlation coefficient 0.9854) (Fig. 5-44 (a)), and AM 2.58 ± 2% μM.
(correlation coefficient 0.9999) (Fig. 5-44 (C)), the nafion-BDUNCD has LOD of FSCV 3.95 ± 2% μM (correlation coefficient 0.9995) (Fig. 5-44 (a)), and AM 1.40 ± 2% μM (correlation coefficient 0.9999) (Fig. 5-44 (C)). However, after concentration increasing more than 0.1 mM DA, there is second linearly increase of concentrations to currents, and BDUNCD has higher signals compared to nafion-BDUNCD in higher concentration of DA (Fig. 5-44 (d)). Therefore, compared with nafion-MWCNT-BDUNCD (Fig. 5-45 (a)), the MWCNT has the functional group of (COOH) which reduced the LOD to 5.4 nM DA (Table 5-14) with a linearly increase of concentrations to currents from 1 nM to 100 nM DA (correlation coefficient 0.9985) (Fig. 5-45 (b)), and with 5 minutes nitric acid treatment, it enhanced the functional group of (COOH) by reducing the LOD of FSCV 1.78 ± 2% nM (correlation coefficient 0.9997) (Fig. 5-44 (b)). However, both have the lowest detected concentration of 1 nM DA.
Fig. 5-44. (a) FSCV scans of BDUNCD and nafion-BDUNCD electrodes with DA concentrations 0.001 mM, 0.01 mM, 0.05 mM, and 0.1 mM and trend line equation. (b) FSCV scans of nafion-MWCNT-BDUNCD electrodes with DA concentrations 1 nM, 10 nM, and 50 nM and the trend line equation. (c) AM of BDUNCD and nafion-BDUNCD electrodes with DA concentrations 0.01 mM, 0.05 mM, and 0.1 mM and trend line equation. (d) AM of BDUNCD and nafion-BDUNCD electrodes with DA concentrations from 0.01 mM to 1 mM.

Table 5-13. Comparison of LOD and LOQ on BDUNCD, nafion-BDUNCD, and nafion-MWCNT-BDUNCD electrodes.

<table>
<thead>
<tr>
<th></th>
<th>BDUNCD</th>
<th>Nafion-BDUNCD</th>
<th>BDUNCD</th>
<th>Nafion-BDUNCD</th>
<th>Nafion-MWCNT-BDUNCD (with Nitric Acid Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In ranges of concentration</td>
<td>(1-100 µM) (FSCV)</td>
<td>(1-100 µM) (FSCV)</td>
<td>(0.01-0.1 mM) (AM)</td>
<td>(0.01-0.1 mM) (AM)</td>
<td>(1-100 nM) (DPV) (nM)</td>
</tr>
<tr>
<td>LOD</td>
<td>20.25 ± 2%</td>
<td>3.95 ± 2%</td>
<td>2.58 ± 2% µM</td>
<td>1.40 ± 2%</td>
<td>5.4 ± 2%</td>
</tr>
<tr>
<td>LOQ</td>
<td>67.49 ± 2%</td>
<td>13.16 ± 2%</td>
<td>8.60 ± 2% µM</td>
<td>4.68 ± 2%</td>
<td>18.9 ± 2%</td>
</tr>
</tbody>
</table>
Fig. 5-45. (a) DPV plots of DA (1 nM, 5 nM, 10 nM, 50 nM, and 100 nM) with nafion-MECNT-BDUNCD microelectrode. (b) DPV plots of nafion-MWCNT-BDUNCD electrodes with DA concentrations 1 nM, 5 nM, 10 nM, and 50 nM and the trend line equation.

Table 5-14. LOD and LOQ of nafion-MWCNT-BDUNCD electrodes.

<table>
<thead>
<tr>
<th>DA</th>
<th>Peak Current</th>
<th>Nafion-MWCNT-BDUNCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 nM</td>
<td>0.063 ± 0.2% nA</td>
<td>(in ranges of 1-100 nM)</td>
</tr>
<tr>
<td>5 nM</td>
<td>0.073 ± 0.2% nA</td>
<td>LOD</td>
</tr>
<tr>
<td>10 nM</td>
<td>0.081 ± 0.2% nA</td>
<td>5.4 ± 0.2% nM</td>
</tr>
<tr>
<td>50 nM</td>
<td>0.133 ± 0.2% nA</td>
<td>LOQ</td>
</tr>
<tr>
<td>100 nM</td>
<td>0.200 ± 0.2% nA</td>
<td>18.9 ± 0.2% nM</td>
</tr>
</tbody>
</table>

5.3.4.4 Limit of Detection of Nitric Acid Treatment on MWCNT

A process of nitric acid treatment has been employed in multiwall carbon nanotubes (MWCNTs) to purify and oxidize the sandwich electrodes of the CNT layer to achieve the low concentration of chemical detection to 1 nM. The original and the treated MWCNT are studied using SEM, XPS, CV, and FSCV. The as-prepared MWCNT have impurities of amorphous carbon and metallic catalysts which we used in the production of MWCNT. Therefore, nitric acid treatment has been reported that it can purify and
change the physico-chemical properties of Carbon Nanotubes, and change their hydrophobic nature to hydrophilic. MWCNT-BDUNCD electrodes have a high percentage (60-70%) of electrodes which resulted in noise signals such as multi-peak, high current charge, and unclear-peak. It was due to the non-uniform CNT deposition, and impurity of metallic catalysts. After nitric acid treatment, the CNT has completed the removal of amorphous carbon and metallic catalysts impurities. However, nitric acid can also oxidize the CNT to create amorphous carbon on the surface and it can cover up to 90% of the CNT’s surface when exposed for 48 hours [136]. Therefore, the protocol has a set up of 5 minutes treatment time at concentration 10 M, then rinsed twice with DI water. The treatment MWCNT-BDUNCD has changed physico-chemical properties of Carbon Nanotubes which enhanced sensitivity two times and resulted in clear-peak signals. However, MWCNT-BDUNCD electrodes only achieved the low concentration of chemical detection to 30 nM DA.

The design of sandwich electrode nafion-MWCNT-BDUNCD electrode has been introduced to achieve the low concentration of chemical detection to 1 nM DA. The protocol of nafion deposition has been developed by 2 minutes, electrophoretic deposition (EPD) at +1.5 V and carefully rinsed with DI water with a final bake time of 10 minutes at 75°C. The sandwiched electrodes of the nafion layer have advantages of chemical resistant and sensitivity enhanced about two to three times.

The comparison of nitric acid treatment on MWCNT layer in sandwiched electrode nafion-MWCNT-BDUNCD has successfully increased low concentration detection limited to 1.78 nM with the lowest signals detected from 1 nM. However,
without the nitric acid treatment of the sandwich, electrodes have the lowest
congestion detection limited to 5.4 nM with the lowest signals detected from 1 nM.

The advantages of the thin nafion layer have CNT layer protection, increased
sensitivity, chemical resistant, long-lasting electrodes, and enhanced the electrodes’
chemical properties. However, without the thin nafion layer, CNT layer has the
disadvantage of losing materials and reducing the sensitivity. Therefore, the CNT layer’s
lifetime is about 3 hours. However, after nafion deposition, the nafion-CNT-UNCD
electrodes have a lifetime of more than 10 hours in amperometry with no materials lost
and sensitivity reduced.

In the EPD of the nafion deposition, the CNT materials are lost during this time.
It was observed by using the optical microscopy, confocal microscopy, and SEM. In the
optical image, after EPD deposition, CNT materials were lost with the surface change
from dark to light color. However, in the SEM image, the light color area has a thin layer
of CNT cover on the UNCD surface which cannot be observed by optical microscopy or
confocal microscopy.
CHAPTER 6

CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

At boron-doped UNCD microelectrodes, the surface fouling due to dopamine oxidation occurs at a steady rate until the surface becomes completely inactive. It takes about 4 hours for a 250-μm UNCD microelectrode to foul completely in amperometry and takes about 6.5 hours to foul 50% in fast scan cyclic voltammetry. I demonstrated that by employing a 200 seconds silver particles deposition (3 μM AgNO₃ with 0.01 M per chloric acid solution) to arrange the electrode’s active surface. During the electrode’s fouling and oxidation, the BDUNCD microelectrode’s surface losing electrode conductivity and reducing oxidation current signals, it also results in decreasing Ag particles deposition area and vary Ag particle’s dimension. At initial first 2 hours, the chemical intermediates of DA which have negative charged attract on the positive charged of BDUNCD surface employing +0.3 V. Therefore, it generated polydopamine film and slowly covering on the electrode’s surface. The negative charged of thin polydopamine film enhanced DA signals in first 2 hours (from 0.95 μA to 1.15 μA in FSCV), and resulted in flower-like silver nanoparticles which increased particle’s dimension from 500 ± 18% nm to about 1 ± 20% μm. It due to the polydopamine film reducing electrode’s electro conductive surface which caused the higher current charge on the exposure BDUNCD surface in order to enhance deposition rate (flower-like silver...
nanoparticles generated at longer deposition time or higher silver nitric concentration). However, BDUNCD electrode losing electro conductive with DA signals reduced 90% (0.1 μA in FSCV) after 4 hours. The polydopamine film thickness increased which enhance the difficult of DA diffusion through the polydopamine film (the same result as thick nafion layer), and melanin generated both reducing the electrode’s electro conductive. After 2 hours, The DA signals reduced during the enhancing of polydopamine film thickness, and flower-like silver nanoparticles reduced during electrode’s surface losing electro conductive. Finally, BDUNCD loses electro conductive after 4 hours with a few silver nanoparticles (50 nm) and it is 1/10 dimension of fresh surface silver nanoparticles (500 nm).

We developed the 5 minutes electrochemical FSCV cleaning in a PBS buffer, one can restore the microelectrode sensitivity and achieve a stable dopamine current signal. The FSCV cleaning was optimized so that the UNCD microelectrode can monitor a 100 μM of dopamine solution for up to 28 hours with intermittent cleaning of the electrode for every 30 minutes of detection. However, the FSCV cleaning process was found to be insufficient when the voltage window employed to reactivate the fouled UNCD electrode was narrow (−0.5 V to +0.8 V) or extended (−0.5 V to +1.2 V). The −0.5 V to +0.8 V potential window not cleaned properly lead to polydopamine film remaining and DA signals reducing. It also resulted in silver particles non-deposition on fouling area. On other hand, the −0.5 V to +1.2 V potential window oxidized electrode’s surface and reducing electro conductive which cause oxidation potential increased in order to enhance oxidation current but it enhances for short period of time. The silver particles deposition was shown that it cleaned the electrode’s surface, but it also loses the
electro conductive with reducing active area (silver particles deposition area reduced). Finally, we found the best reactivation window -0.4 V to +1.0 V which has maintained DA signals in 6.5 hours, it has lower oxidation potential increased, and more active area (silver particles on 90% of area) with clean electrode’s surface. It successfully extended the lifetime from 6.5 hours to 28 hours in FSCV monitoring.

In the droplets’ sensing, it developed a protocol for Electrophoretic deposition (EPD) of nafion for BDUNCD, which is 2 minutes with a potential at +1.5 V and thickness 50 nm. Nafion has a deposited negative charge film on the BDUNCD’s surface which enhanced the sensitivity two to three times of BDUNCD, and limited poly-DA fouling electrodes which resulted in high accuracy. However, increasing the thickness of nafion layer will enhance the difficult of DA diffusion through the polydopamine film, the 6 minutes nafion deposition layer have no DA signals enhancement. The 10 minutes of MWCNT deposition layer was used to increase the electrode’s sensitivity, but MWCNT-BDUNCD has an injury due to MWCNT deposited loss by the time. Therefore, 50 nm of nafion layer has addressed the problem and enhanced MWCNT-BDUNCD signals about three to four times with a total of 10 times to compare with BDUNCD.

In the DA droplet’s sensing, the effect factors were study individually in three electrodes, the background flow rate, the Signal to noise ratio, and the droplet’s volume to develop a protocol for three electrodes. The best protocol has the background flow rate 0.1 ml/min with droplet’s volume 0.02 ml at potential +0.35 V.

BDUNCD has resulted in the background currents tending to move toward a negative potential (-2.51 nA with total 270 droplets, -6.20 nA with a total of 720 droplets) due to a fouling surface in 9 hours of droplet’s monitoring. However, after
9 hours, both nafion-BDUNCD and nafion-MWCNT-BDUNCD are not fouled and sensitivity has no significantly reducing due to the nafion deposited surface has DA inertness. In comparison, the nafion multi wall carbon nanotube modified BDUNCD electrode which has highest sensitivity of 43.7 nA (100 µM DA), quick response time (2 ± 8% s), and nafion-BDUNCD enhances sensitivity (18.1 nA) and has stable signals in 9 hours of droplet’s monitoring. The BDUNCD has the lowest signals current (7.75 nA) and fouling surface in 9 hours of droplet’s monitoring. However, the lower clearance rate of nafion-MWCNT-BDUNCD was caused by higher sensitivity and surface roughness on the electrodes’ surface, which increased the DA to remain on the electrodes’ surface.

In the selectivity study, UNCD has the oxidation potential about +52.49 mV (DA), +82.7 mV (5-HT), and +47.5 mV (AA). The DA signals have a wide oxidation potential window, which covered from −0.1 V to +0.5 V, and AA has covered both DA and 5-HT signals from −0.1 V to +0.83 V. There is very close oxidation potential between DA and 5-HT. However, Nafion-UNCD has the oxidation potential of DA (−7.93 mV) and 5-HT (77.66 mV) and the DA signals has coved the 5-HT signals; the advantage is the nafion layer successfully blocking the AA signal, and also increasing the signal of DA (0.11 nA) and 5-HT (0.46 nA) compare to BDUNCD of DA (0.02 nA) and 5-HT (0.14 nA).

The nafion multi wall carbon nanotube modified BDUNCD electrode has very good sensitivity in DA (3.31 nA) and 5-HT (2.23 nA), and blocks the AA signals. It has the oxidation potential −83.47 mV (DA) and +62.56 mV (5-HT) with two clean separated signals. In the vary DA Concentration selectivity study, it changes the DA concentration from 0.5 µM to 10 µM with a fix of 100 µM AA and 1 µM 5-HT. it performs an excellent
selectivity property with a linear increase of DA concentration to oxidation current signals with a 5-HT oxidation current $3.56 \pm 7\%$ nA. The DA concentration increasing shifting oxidation potential but the $\Delta E (\Delta E = \Delta E_{5-HT} - \Delta E_{DA})$ is not varied by the DA concentration, it total shifted 5 mV on $\Delta E$ comparing to shift 35.24 mV DA and 40.29 mV 5-HT from 0.5 $\mu$M to 10 $\mu$M.

In the additional selectivity study in 9 hours of monitoring, the nafion multi wall carbon nanotube modified BDUNCD electrode has the initial sensitivity of oxidation current DA (0.58 nA) and 5-HT (1.03 nA) with a sensitivity value of DA 1.18 $\mu$A $\mu$M$^{-1}$ cm$^{-2}$ and 5-HT 2.09 $\mu$A $\mu$M$^{-1}$ cm$^{-2}$. After 9 hours, it has DA (0.31 nA) and 5-HT (0.76 nA) separately oxidation signals with a sensitivity value of DA 0.63 $\mu$A $\mu$M$^{-1}$ cm$^{-2}$ and 5-HT 1.55 $\mu$A $\mu$M$^{-1}$ cm$^{-2}$. The signals current reduced by oxidizing the functional group of COOH on MWCNT not the DA fouling, both DA and 5-HT reduced sensitivity by 47% and 26%. Thus, nafion-MWCNT-BDUNCD has excellent selectivity with cleanly separated oxidation peaks of a mixture of 1 $\mu$M 5-HT, 100 $\mu$M AA, and 1 $\mu$M DA solution after 9 hours electrode oxidation. By 5 minutes nitric acid treatment, it increases the functional group of (COOH) on MWCNT in order to increase the selectivity and also reduce the limit of detection.

The other advantage of multi-wall carbon nanotube modified BDUNCD electrode it has the lowest concentration detection of 1 nM (DA) and LOD of 5.4 nM (DA). By the nitric acid treatment, the LOD can be reduced to 1.78 nM (DA). The 10 M nitric acid treatment can increase the functional group of (COOH) on multi-wall carbon nanotube layer to reduce the limit of detection.
Finally, the developing protocol of microfluidic droplets sensor successfully evaluates the three different electrode’s properties of sensitivity, response time, clearance rate, selectivity and LOD. The best electrode is nafion multi wall carbon nanotube modified BDUNCD electrode which has highest sensitivity of FSCV 15.01 μA (100 μM DA) and AM 43.7 nA (100 μM DA), quick response time (2 ± 8% s), and excellent selectivity of DA Sensitivity Value 1.18 μA μM⁻¹ cm⁻² and 5-HT Sensitivity Value 2.09 μA μM⁻¹ cm⁻² for long-lasting sensing up to 9 hours in AM with no DA fouling.

6.2 Future Work

In future work of BDUNCD electrode’s fouling mechanism, I’m more interested in different scan rate of reactivation, which I fix at 400 V/s; it may vary from 200 V/s to 2000 V/s. The fast scan rate may increase the clean rate and reduce the reactivation time with longer monitoring time, but higher scan rate may have oxidized electrode’s surface. Therefore, finding a suitable scan rate of reactivation is an important improvement. It also can study the fouling rate in a mix of different concentration solution condition such as DA, 5-HT, and AA mix solution which is closer to the brain condition; the reactivation time may vary by the different fouling conditions, it may take longer time to clean the electrode’s fouling.

At droplet’s sensing, it can compare BDUNCD to other material electrodes such as gold or different modify electrode’s surfaces to evaluate different material electrodes and discover electrode’s properties for neurochemical sensing. The roughness of the multi-wall carbon nanotube modified BDUNCD electrode can be improved to improve the clearance rate to reduce the remaining DA effect. Therefore, it can study the different thickness of multi-wall carbon nanotube layer affect the selectivity and sensitivity
REFERENCES


