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affiliée à l'Université de Montréal

Sepia Melanin Bio-Inks for the Electrochemical Detection of Neurotransmitters

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Département de génie physique

Mémoire présenté en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*

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Sepia Melanin Bio-Inks for the Electrochemical Detection of Neurotransmitters

présenté par **Oscar Joaquin ISASMENDI RAMIREZ**

en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*

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DEDICATION

See you space cowboy...

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Gracias a mis padres, Oscar Osvaldo y Maria Elisa. Gracias a mi hermana, Luciana. Gracias a Mak.

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RÉSUMÉ

Ce mémoire porte sur l'étude de la détection électrochimique de la dopamine à l'aide d'électrodes modifiées par un revêtement de mélanine de seiche. Deux types d'électrodes sont étudiées: des électrodes planaires en or, ainsi que des électrodes en papier graphitisé. La dopamine est un neurotransmetteur largement étudié, surtout en électrochimie. La mélanine de seiche, elle, est une eumélanine biosourcée, composée de nanoparticules sphériques, qui possède de l'activité redox intéressante à étudier dans un contexte d'électrochimie.

L'objectif de ce travail est d'évaluer l'influence de la mélanine de seiche sur la réponse électrochimique d'électrodes pour la détection électrochimique de dopamine. L'hypothèse est que les nanoparticules de mélanine adsorbées à la surface des électrodes favorisent l'oxidoréduction de la dopamine de par la similarité des structures chimiques des monomères de mélanine avec ceux de la polydopamine.

D'abord, l'utilisation d'électrodes à disque planaires ont été étudiées avec et sans modification par la bioencre de mélanine. Pour cela, des mesures de voltammétrie cyclique ont été faites sur des solutions de ruthénium hexaamine, de dopamine, et d'acide ascorbique. Les résultats obtenus sur électrodes en or montrent que la modification par la mélanine entraîne une augmentation des courants faradiques et capacitifs lors de la détection de ruthénium hexaamine et de la dopamine, mais pas pour l'acide ascorbique. Notre hypothèse est que le phénomène de *redox cycling* survient à l'interface entre l'électrode et les nanoparticules de mélanine.

Ensuite, des électrodes en papier graphitisé par laser ont aussi été modifiées avec de la mélanine de seiche, puis utilisées pour faire des mesures en voltammétrie cyclique de solutions de ruthénium hexaamine et de dopamine. Des tendances similaires sont observées sur les électrodes en papier, où la présence de mélanine conduit également à une augmentation des courants mesurés pour le ruthénium hexaamine et la dopamine.

En conclusion, ce travail montre que la mélanine de seiche augmente les courants faradiques et capacitifs pour la détection de ruthénium hexaamine et de dopamine pour des électrodes en or et sur papier. Ces résultats pourraient être l'objet de nouvelles études pour optimiser la concentration de nanoparticules pour la modification d'électrodes, ainsi que l'utilisation de la mélanine de seiche dans des applications de voltammétrie cyclique à balayage rapide, ou pour l'étude de concentration de neurotransmetteurs dans des cultures cellulaires.

ABSTRACT

This thesis focuses on the electrochemical detection of dopamine using electrodes modified with a sepia melanin coating. Two types of electrode are investigated: planar gold disk electrodes and graphitized paper electrodes. Dopamine is a widely studied neurotransmitter, particularly in electrochemistry. Sepia melanin, meanwhile, is a bio-sourced eumelanin, composed of spherical nanoparticles, which possesses redox activity of interest for study in an electrochemical context.

The aim of this study is to evaluate the effects of sepia melanin on the electrochemical response of electrodes used for the electrochemical detection of dopamine. The hypothesis is that melanin nanoparticles adsorbed onto the surface of the electrodes promote the redox reaction of dopamine due to the similarity of the chemical structures of melanin monomers to those of polydopamine.

Firstly, the use of planar disk electrodes was investigated with and without modification using melanin bio-ink. To this end, cyclic voltammetry measurements were performed on solutions of ruthenium hexaamine, dopamine, and ascorbic acid. The results obtained on gold electrodes show that modification with melanin leads to an increase in faradic and capacitive currents during the detection of ruthenium hexaamine and dopamine, but not for ascorbic acid. Our hypothesis is that redox cycling occurs at the interface between the electrode and the melanin nanoparticles.

Secondly, laser-graphitized paper electrodes were also modified with sepia melanin and then used to perform cyclic voltammetry measurements on solutions of ruthenium hexaamine and dopamine. Similar trends were observed on the paper electrodes, where the presence of melanin also led to an increase in the currents measured for ruthenium hexaamine and dopamine.

In conclusion, this work demonstrates that sepia melanin increases the faradic and capacitive currents for the detection of ruthenium hexaamine and dopamine on both gold and paper electrodes. These results could form the basis for further studies to optimise the concentration of nanoparticles for electrode modification, as well as the use of sepia melanin in rapid-scan cyclic voltammetry applications, or for the study of neurotransmitter concentrations in cell cultures.

TABLE OF CONTENTS

DEDICATION	iii
ACKNOWLEDGEMENTS	iv
RÉSUMÉ	v
ABSTRACT	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF SYMBOLS AND ACRONYMS	xiv
LIST OF APPENDICES	xvi
CHAPTER 1 INTRODUCTION	1
1.1 Context	1
1.2 Research Objectives	2
1.3 Thesis Outline	2
CHAPTER 2 LITERATURE REVIEW	4
2.1 Fundamentals of Neural Communication	4
2.2 Dopamine: A Key Neurotransmitter	5
2.3 Electrochemical Detection of Neurotransmitters	6
2.3.1 Analytical Electrochemistry	6
2.3.2 Cyclic Voltammetry	7
2.3.3 Fast-Scan Cyclic Voltammetry and Amperometry	10
2.4 Electrode Modifications	11
2.4.1 Engineered materials	11
2.4.2 Conducting Polymers	12
2.4.3 Biomimetic Materials	12
2.5 Melanin	12
2.6 Electrochemical Dopamine Sensors	13

CHAPTER 3	MATERIALS AND METHODS	15
3.1	Reactants & Solutions	15
3.2	Extraction and Preparation of the Sepia Melanin Aqueous Samples	15
3.3	Electrode Preparation and Modification	15
3.3.1	Gold Disc Electrodes	15
3.3.2	Paper Electrodes	16
3.4	Optical Microscopy	16
3.5	Scanning Electron Microscopy	17
3.6	Energy Dispersive Spectroscopy	17
3.7	Electrochemical Equipment	17
3.8	Cyclic Voltammetry	17
3.9	Data Analysis & Statistics	18
3.9.1	Voltammograms	19
CHAPTER 4	RESULTS AND DISCUSSION	21
4.1	Sepia Melanin Ink Morphology and Composition	21
4.1.1	Spectroscopy	21
4.1.2	Imaging	26
4.1.3	Discussion	29
4.2	Electrochemistry on Gold Electrodes	30
4.2.1	Ruthenium Hexaamine	30
4.2.2	Dopamine	35
4.2.3	Ascorbic Acid	40
4.2.4	Discussion	42
4.3	Electrochemistry on Paper Electrodes	46
4.3.1	Ruthenium Hexaamine	46
4.3.2	Dopamine	51
4.3.3	Exploratory Results at High Scan Rates	55
4.3.4	Discussion	57
CHAPTER 5	CONCLUSION	59
5.1	Summary of Works	59
5.2	Limitations	59
5.3	Future Research	60
REFERENCES		61

APPENDICES	69
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LIST OF TABLES

Table 2.1	A comparison of typical electrochemical dopamine sensors	14
Table 3.1	Summary of electrochemical experiments and number of independently fabricated electrodes for each electrode type, analyte, and SM concen- tration.	18
Table 4.1	Atomic composition of different samples	23

LIST OF FIGURES

Figure 2.1	Simplified representation of a chemical synapse	4
Figure 2.2	Chemical structure of dopamine [1]	5
Figure 2.3	Initial steps of dopamine polymerization	6
Figure 2.4	Three-electrode electrochemistry setup	8
Figure 2.5	Chemical structures of Sepia Melanin Monomers	13
Figure 3.1	Paper electrode fabrication process	16
Figure 3.2	Example of an analyzed cyclic voltammogram	19
Figure 4.1	UV-Vis absorbance spectrum of 10mg mL^{-1} SM in DIW ink formulation.	22
Figure 4.2	Raman spectrum of 0.5 mg mL^{-1} SM-LIG and LIG electrodes	22
Figure 4.3	EDX imaging results of an unrinsed sample. (a) SEM image where data was acquired. (b) Elemental maps of edx results.	24
Figure 4.4	EDX imaging results of a DIW rinsed sample. (a) SEM image where data was acquired. (b) Elemental maps of EDX results.	25
Figure 4.5	Gold disc electrodes (3 mm diameter) (a) before deposition, (b-c) after deposition (left), and after rinsing with DIW (right).	26
Figure 4.6	Scanning Electron Micrograph of <i>sepia melanin</i> on a gold substrate	27
Figure 4.7	Paper Electrodes	28
Figure 4.8	SEM of LIG electrode at (a) 400X and (b) 8000X.	28
Figure 4.9	SEM of 5 mg mL^{-1} SM-LIG electrode at (a) 400X and (b) 8000X.	29
Figure 4.10	Representative CVs of 1 mM Ruthenium Hexamine in 1 M KCl with varying MNP concentration working electrodes at different scan rates (5, 10, 25, 50, 75, and 100 mV s^{-1}).	31
Figure 4.11	Peak redox currents as a function of the square root of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.	32
Figure 4.12	Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, and 100 mV s^{-1})	32
Figure 4.13	Electrode capacitance at $E_c = 200\text{ mV}$ as a function of the scan rate.	33
Figure 4.14	Halfway peaks as a function of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.	33
Figure 4.15	Redox peak separation as a function of the scan rate.	34
Figure 4.16	Amplification factor γ as a function of the square root of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.	34

Figure 4.17	Representative CVs of 250 μM Dopamine Hydrochloride in 0.01 M PBS with varying MNP concentration working electrodes at different scan rates (5-100 mV s^{-1}).	36
Figure 4.18	Peak redox currents as a function of the square root of the scan rate for 250 μM Dopamine Hydrochloride in 0.01 M PBS.	36
Figure 4.19	Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, and 100 mV s^{-1})	37
Figure 4.20	Electrode capacitance at $E_c = 0$ mV as a function of the scan rate.	37
Figure 4.21	Redox peak separation as a function of the scan rate.	38
Figure 4.22	Halfway peaks as a function of the scan rate for 250 μM Dopamine Hydrochloride in 0.01 M PBS.	38
Figure 4.23	Amplification factor γ as a function of the square root of the scan rate for 250 μM Dopamine Hydrochloride in 0.01 M PBS.	39
Figure 4.24	Representative CVs of 250 μM L-Ascorbic Acid in 0.01 M PBS with 5.0 mg mL^{-1} MNP concentration working electrodes at different scan rates (5-100 mV s^{-1}).	41
Figure 4.25	Peak redox currents as a function of the square root of the scan rate for 250 μM L-Ascorbic Acid in 0.01 M PBS.	41
Figure 4.26	Electrode capacitance at $E_c = 0$ mV as a function of the scan rate.	42
Figure 4.27	Amplification factor γ as a function of the square root of the scan rate for 250 μM L-Ascorbic Acid in 0.01 M PBS.	42
Figure 4.28	Proposed working principle of redox-cycling with a nanoparticle. $E_{\text{electrode}}$ and $E_{\text{nanoparticle}}$ are the applied potential at the electrode and the real potential at the nanoparticle respectively.	45
Figure 4.29	Representative CVs of 1 mM Ruthenium Hexamine in 1 M KCl with varying MNP concentration working electrodes at different scan rates (5, 10, 25, 50, 75, 100, 125, 150, 200 mV s^{-1}).	46
Figure 4.30	Peak redox currents as a function of the square root of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.	47
Figure 4.31	Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, 100, 125, 150, 200 mV s^{-1})	48
Figure 4.32	Electrode capacitance at $E_c = 200$ mV as a function of the scan rate.	48
Figure 4.33	Redox peak separation as a function of the scan rate.	49
Figure 4.34	Halfway peaks as a function of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.	50

Figure 4.35	Amplification factor γ as a function of the square root of the scan rate for 1 mM Ruthenium Hexaamine in 1 M KCl.	50
Figure 4.36	Representative CVs of 250 μ M Dopamine Hydrochloride in 0.01 M PBS with varying MNP concentration working electrodes at different scan rates (5, 10, 25, 50, 75, 100, 125, 150, 200 mV s^{-1}).	51
Figure 4.37	Peak redox currents as a function of the square root of the scan rate for 250 μ M Dopamine Hydrochloride in 0.01 M PBS.	52
Figure 4.38	Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, and 100 mV s^{-1})	52
Figure 4.39	Electrode capacitance at $E_c = 0$ mV as a function of the scan rate.	53
Figure 4.40	Redox peak separation as a function of the scan rate.	54
Figure 4.41	Halfway peaks as a function of the scan rate for 250 μ M Dopamine Hydrochloride in 0.01 M PBS.	54
Figure 4.42	Amplification factor γ as a function of the square root of the scan rate for 250 μ M Dopamine Hydrochloride in 0.01 M PBS.	55
Figure 4.43	Representative CVs of (a) 1 mM Ruthenium Hexaamine in 1 M KCl and (b) 250 μ M Dopamine Hydrochloride in 0.01 M PBS with varying MNP concentration working electrodes at different scan rates (500 mV s^{-1} , 1 V s^{-1} , 5 V s^{-1}).	56
Figure .1	Low Concentration - 0.1 mg/mL of Sepia Melanin in Deionized Water	69
Figure .2	Medium Concentration - 0.1 mg/mL of Sepia Melanin in Deionized Water	69
Figure .3	High Concentration - 5.0 mg/mL of Sepia Melanin in Deionized Water	70
Figure .4	Low Concentration - 0.1 mg/mL	70
Figure .5	Medium Concentration - 2.5 mg/mL	70
Figure .6	High Concentration - 5.0 mg/mL	71
Figure .7	Low Concentration - 0.1 mg/mL	71
Figure .8	Medium Concentration - 2.5 mg/mL	71
Figure .9	High Concentration - 5.0 mg/mL	72

LIST OF SYMBOLS AND ACRONYMS

CE Counter Electrode. 7, 10

CV Cyclic Voltammetry. xi–xiii, 1–3, 5–10, 31, 36, 41, 46, 51, 55, 56, 58, 59

DA Dopamine. 1, 2, 5–7, 13, 15, 59

DHI 5,6-Dihydroxyindole. 2, 6, 12

DHICA 5,6-Dihydroxyindole-2-carboxylic acid. 2, 12

DIW Deionized Water. xi, 15–17, 21, 22, 25–27

EDL Electrical Double Layer. 8, 9, 57

EDX Energy Dispersive X-ray Spectroscopy. xi, 17, 21, 23–25, 29, 59

FSCV Fast-Scan Cyclic Voltammetry. 6, 10, 13, 14, 55, 60

GE Gold Electrode. 15, 20, 26, 30, 31, 35, 36, 40, 44

IPA Isopropanol. 17

LIG Laser-Induced Graphitic Electrodes. xi, 2, 3, 18, 22, 28, 46–49, 51, 53–57, 59, 60

MNP Melanin Nanoparticles. xi–xiii, 2, 20, 26–31, 36, 41, 44, 46, 51, 56, 58, 59

NP Nanoparticles. 12, 13, 30

PBS Phosphate Buffered Saline. xii, xiii, 15, 18, 20, 21, 30, 36, 38–43, 46, 51, 52, 54–56, 59

PDA Polydopamine. 1, 2, 6, 13

PDMS Polydimethylsiloxane. 27

PEDOT Poly(3,4-ethylenedioxythiophene). 12

RE Reference Electrode. 7, 10

SEM Scanning Electron Microscopy. xi, 17, 21, 23–25, 27–30, 59, 69

SM Sepia Melanin. x–xiii, 2, 3, 13, 15–18, 21, 22, 26–32, 35–37, 40, 42–44, 47, 48, 50, 52, 53, 56–60, 69

SM-LIG Sepia Melanin-Modified Laser-Induced Graphitic Electrodes. xi, 3, 22, 28, 29, 46–49, 51, 53–60

SME Sepia Melanin on Gold Electrode. 3, 15, 26, 30, 31, 35, 40, 43, 44, 57, 59

UV-Vis Ultraviolet-Visible Spectroscopy. xi, 21, 22, 29, 59

WE Working Electrode. 6–8, 10, 11, 27

LIST OF APPENDICES

SCANNING ELECTRON MICROGRAPHS	69
1. Sepia Melanin on Gold	69
1.1 Sepia Melanin on Gold	69
1.2 3 drop-casts	70
1.3 3 drop-casts and Rinsed	71

CHAPTER 1 INTRODUCTION

1.1 Context

The brain is made up of an intricate network of specialized cells called neurons. The latter communicate with each other through electrical and chemical signals [2]. These chemical signals are a process that involves the release and capture of molecules known commonly as neurotransmitters. There are different types of neurotransmitters, each of which has different functions. Among them, one of the most interesting and studied is Dopamine (DA), because it is directly or indirectly involved in most physiological functions of the central nervous system [3]. DA, whose chemical structure is illustrated in Figure 2.2, is associated with substance use disorder, schizophrenia, and Parkinson's disease [3]. So, DA detection is needed to deepen our understanding of neurological conditions that affect a large part of the population.

To study the release of neurotransmitters, many different methods were developed. The first techniques were push-pull perfusion, which eventually evolved into microdialysis. However, the time scale of microdialysis is at best multiple seconds, enough to allow a sufficient volume to be collected. Eventually, electrochemical methods, such as Cyclic Voltammetry (CV), became prevalent, as they have been shown to have sufficient sensitivity and time resolution to reliably provide quantifiable data [4, 5].

The most common issue that plagues CV measurements is the passivation of the sensor's surface due to biofouling [6]. This occurs when the reaction of the detected chemical species forms an electrically resistive film on the active surface of the sensing electrode, which in turn decreases the detected signal, thus reducing the performances of the sensor [7]. This issue is very common with DA, as its oxidation can lead to the formation of Polydopamine (PDA), an insulating polymer [8].

To mitigate electrochemical fouling, antifouling coatings have been the subject of promising research [9, 10]. The main objective for these coatings is to minimize electrode passivating molecule adsorption, all while maintaining as much sensitivity as possible. Although some coatings are based on more complex materials such as composite nanomaterials, polymeric nanomaterials, or metal-based nanomaterials [11, 12], this work will focus on the use of melanin pigment, a bio-sourced nanomaterial, as an electrode coating.

Many research groups have shown that melanin, synthesized from DA, can modify the signal from an electrochemical sensors in different ways [13]. These PDA coatings, which are often

called melanin-like, have been known to show promise as an electrode-coating for surface functionalization [14–17]. While most studies focus on synthetic melanin, biosourced melanin-based materials could also be used for the same purposes.

Sepia Melanin (SM) is a form of eumelanin extracted from the cuttlefish *Sepia Officinalis*. As SM is a natural compound, its chemical make-up is complex, including small Melanin Nanoparticles (MNP) spheres that vary in shape from approximately 150 *nm* to 300 *nm* diameter spheres [18]. The principal monomers of SM are 5,6-Dihydroxyindole (DHI) and 5,6-Dihydroxyindole-2-carboxylic acid (DHICA). MNP have been shown to be conductive on a nanometric [19] and micrometric [20] scale and have been used in biodegradable conductive inks [20, 21].

We hypothesize that the structural similarities between the SM and PDA monomers will create an affinity for DA sensing using MNP-modified gold disc electrodes as well as Laser-Induced Graphitic Electrodes (LIG) on paper for CV. This increased affinity could potentially reduce electrochemical fouling by facilitating the reduction of DA, and therefore reducing the polymerization rate.

1.2 Research Objectives

In this work, we investigate how the modification of gold disc electrodes, and paper-based electrodes, by drop-casting or dip-coating SM affects the electrochemical response of different redox active analytes. By varying the density of SM nanoparticles on the electrode surface and using CV, we show that the sepia melanin modified electrodes show an increase in faradic current, and therefore an increased DA detection.

1.3 Thesis Outline

This thesis is structured as follows. Chapter 2 provides a review of the literature. It begins with section 2.1, an overview of the role of the fundamentals of neural communication. Section 2.2 describes the importance of DA as a key neurotransmitter. Section 2.3 explores the concepts behind the electrochemical detection of neurotransmitters. Section 2.4 details the state of the art of electrode modifications in electrochemistry. section 2.5 describes the state of the art as well as the material properties of SM. Chapter 3 describes the materials and the experimental methods that were used to accomplish the research objectives, including electrode fabrication and characterization, electrochemical techniques and data analysis. Chapter 4 presents and discusses the results obtained for the bulk of this work. Firstly, Section 4.1 describes the results of SM ink morphology and composition. Section 4.2 then shows

the electrochemical results obtained using Sepia Melanin on Gold Electrode (SME) for different analytes. Section 4.3 extends the study to paper-based, LIG electrodes. It discusses the results of CV using Sepia Melanin-Modified Laser-Induced Graphitic Electrodes (SM-LIG) for the electrochemical detection of different analytes. Finally, Chapter 5 summarizes the main findings of this work, and proposes future perspectives for SM-based electrochemical sensors.

CHAPTER 2 LITERATURE REVIEW

2.1 Fundamentals of Neural Communication

The brain is a very complex organ, made up of more than 100 billion individual neural cells [2]. Around 10% of the cells that make up these neural circuits are responsible for performing computations, and those cells are called neurons. Neurons are able to communicate with other neurons through synapses, using different chemical and electrical signals [4].

There are 2 types of synapses: electrical and chemical. Both involve a presynaptic neuron, and a postsynaptic neuron, but their working mechanisms are vastly different. In an electrical synapse, both neurons are physically connected, and exchange ions directly through a gap junction [22], whereas, in chemical synapses, both neurons are separated by a gap, or synaptic cleft. Although electrical signals also have a primordial role in chemical synapses, neurons communicate through the synaptic cleft using molecules called neurotransmitters. These chemical messengers are stored inside vesicles contained within the presynaptic neuron. When the appropriate electrical signal reaches the presynaptic neuron, they are released into the synaptic gap and diffuse across it, where they are then bound to receptors on the postsynaptic neuron. These receptors allow the flow of ions into the postsynaptic neuron, which then creates a postsynaptic electrical signal that flows through the neuron. Figure 2.1 illustrates the simplified mechanism of a chemical synapse. [2, 4, 22, 23]

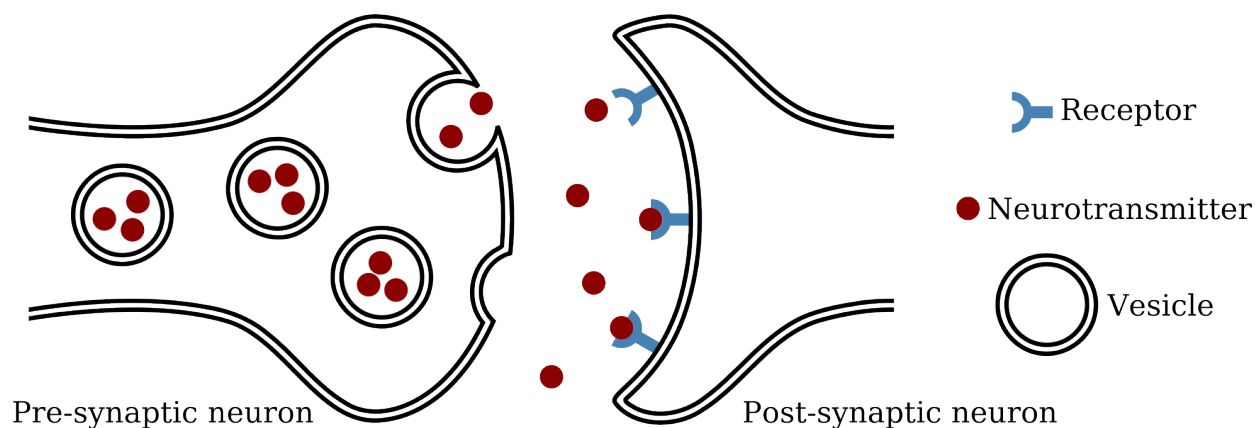


Figure 2.1 Simplified representation of a chemical synapse

In humans, most synapses are chemical [23], making neurotransmitters responsible for many functions. Indeed, the basic operations of our nervous systems are governed by neurotransmit-

ters. Neurotransmitter dysregulations have been linked to degenerative diseases like Parkinson's, disorders like schizophrenia and substance abuse, and basic functions like motor control [2, 3]. It is therefore crucial to the understanding of these pathologies, for us to be able to measure fluctuating levels of neurotransmitters.

2.2 Dopamine: A Key Neurotransmitter

Dopamine, or 4-(2-aminoethyl)benzene-1,2-diol, is one of the most studied neurotransmitters due to its importance in multiple physiological processes, like movement, motivation, reward, and neurodegeneration [24]. Abnormal DA levels have been linked to a multitude of neurological and psychiatric disorders, including but not limited to Parkinson's disease, schizophrenia, depression, and addiction [25, 26]. For example, in Parkinson's disease, the degeneration of dopaminergic neurons in the *substantia nigra* leads to low DA activity, which leads to motor function impairment or dyskinesia [2]. On the other hand, in schizophrenia, it is theorized that dopamine overactivity, among other things, is the cause of the psychotic symptoms associated with the disorder [27].

DA is an electroactive catecholamine that is synthesized by multiple enzymes from its precursor, tyrosine, in dopaminergic neurons [2]. Its structure, seen here in fig. 2.2, is composed of a benzene ring with two hydroxyl substituents at positions 1 and 2 (also known as a catechol), with an aminoethyl side chain at position 4 [1]. It is a redox

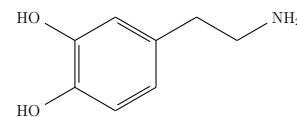


Figure 2.2 Chemical structure of dopamine [1]

active molecule whose electrochemical behaviour is characterized by a reversible oxidation of the catechol moiety into the corresponding quinone. During CV, this process exhibits a quasi-reversible two-electron ($2e^-$) two-proton ($2H^+$) redox couple, where oxidation of DA produces dopamine-o-quinone, and reduction of the latter regenerates the former [28]. Although *pH* greatly affects the kinetics of these redox reactions, we can focus on a *pH* of 7.4, which is the physiological *pH* of extracellular fluids [29, 30]. At that *pH*, previous studies have demonstrated that the oxidation potential is close to 0.2 V vs Ag|AgCl, and the reduction potential is around 0.1 V [8, 15].

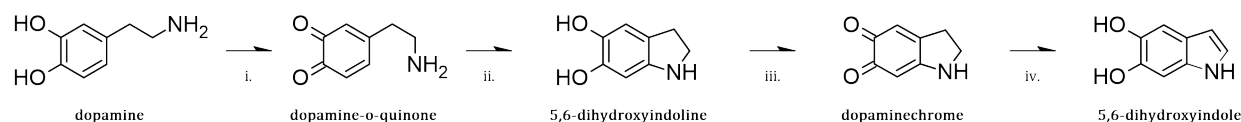


Figure 2.3 Initial steps of DA polymerization. i) DA oxidation into dopamine-o-quinone. ii) dopamine-o-quinone cyclization into 5,6-dihydroxyindoline. iii) 5,6-dihydroxyindoline oxidation into dopaminechrome. iv) dopaminechrome isomerization into DHI. [31]

One interesting property of DA, is that it can polymerize into PDA [31]. In aqueous solutions, DA is unstable, and can undergo intramolecular cyclization and polymerization [14,31]. This self-polymerization, shown in fig. 3.1, is one of the biggest issues when trying to detect dopamine via electrochemical methods. Evidently, the resulting polymer, PDA, is an insulating polymer, and when it is formed at the sensing surface, it decreases signal quality and electrode stability [8, 17].

2.3 Electrochemical Detection of Neurotransmitters

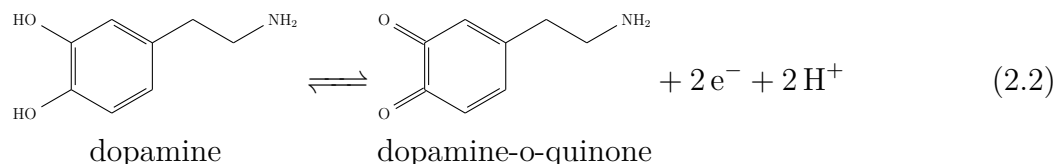
Dopamine is not the only redox-active molecule. In fact, many neurotransmitters are redox-active molecules, and their concentrations are usually within the nanomolar to the micromolar range [2]. Quantifying neurotransmitters accurately, whether *in vivo* or *in vitro*, is of primordial importance to understand the phenomena described in section 2.1, and therefore understanding the associated pathologies. Although many methods have been developed, electrochemical techniques are popular for detecting, monitoring and measuring neurotransmitters, due to their high sensitivity, and fast timescale [32, 33]. There exist a multitude of electrochemical techniques, but the staples that have been used to probe brain chemistry for the past 20 years are CV, Fast-Scan Cyclic Voltammetry (FSCV), and amperometry [28, 32, 34]. Although each technique has its own specificities and applications, they are all based on the same functional principles.

2.3.1 Analytical Electrochemistry

Analytical electrochemistry relies on the oxidation or reduction of the molecule of interest, or analyte, at the surface of a Working Electrode (WE) [35]. The redox reactions that occur at the surface of said WE involve the exchange of n electrons between the analyte and the WE, as shown in eq. (2.1):



where Ox and Red denote the oxidized and reduced forms of the analyte, respectively. These reactions can be seen at specific redox potentials (E_{red} , E_{ox}) associated with a specific analyte's reactions. In the case of DA, it is the quasi-reversible redox couple DA / dopamine-o-quinone. This redox reaction, shown in eq. (2.2), as mentioned before, involves the exchange of $2e^-$ and $2H^+$, and therefore $n = 2$, and can be easily probed by electrochemical techniques.



2.3.2 Cyclic Voltammetry

Cyclic voltammetry (CV) is the basis of many electrochemical studies. It involves using a potentiostat to apply a linearly varying potential to an electrode within a defined potential window. The resulting electrical currents are measured as a function of potential. The potential window is scanned multiple times, and each repetition dubbed a single cycle for each voltammogram. Each cycle can be divided into two separate parts: the forward and backwards scans. For a potential window consisting of two vertices E_1 and E_2 , the forward scan is measured when the potential goes from vertex E_1 to E_2 , and the backwards scan when the potential goes from E_2 to E_1 .

Three-Electrode Electrochemical Cell

Electrochemical measurements are taken inside an electrochemical cell, comprising several electrodes. The most common electrochemical cell setup is a 3-electrode cell: a WE, a Reference Electrode (RE), and a Counter Electrode (CE), as illustrated in fig. 2.4b. The three electrodes are defined as follows:

- The **WE** is the one where the analyte will react.
- The **RE** maintains a stable, known potential inside the cell at all times, which allows for accurate control of the WE potential. Ideally, no current flows through the RE.
- The **CE** completes the circuit and allows the potentiostat to balance the charges exchanged at the WE.

The potentiostat typically controls the potential applied between WE and RE, and simultaneously measures the current flowing between WE and CE. This electrode configuration

allows for the measurement of faradaic and capacitive currents inside an aqueous solution with dissolved ions called an electrolyte. The dissolved ions help increase the conductivity of the solution.

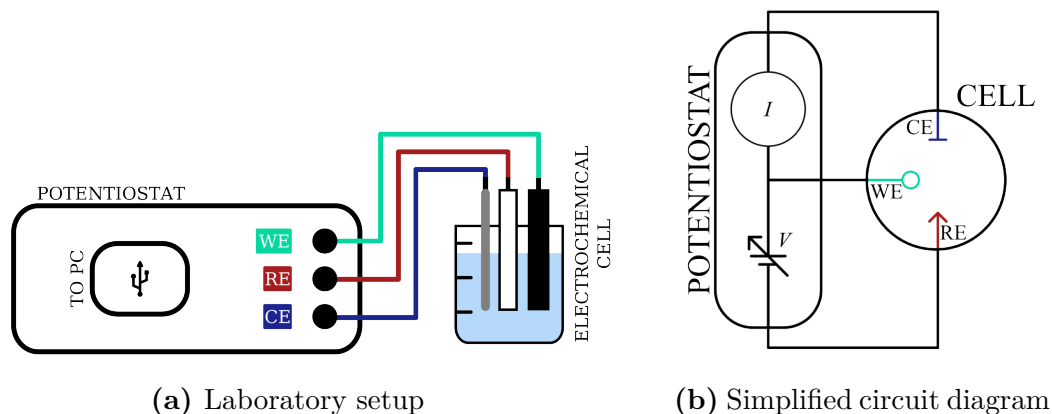


Figure 2.4 Three-electrode system electrochemistry setup with working electrode (WE), reference electrode (RE), and counter-electrode (CE)

Diffusion at the Electrode Interface

The electrochemical response in a CV curve (or voltammogram) depends on the mass transport of electroactive species to the WE surface. In solution, ions and neutral molecules move mostly by diffusion, driven by concentration gradients. At the surface of the WE, the concentration gradients established by the redox reactions give rise to a diffusion layer, where concentrations therefore differ from that of the bulk of the solution. This diffusion follows Fick's first law eq. (2.3).

$$J = -D \cdot \nabla c \quad (2.3)$$

where J is the diffusion current density of a given diffusive species, D its diffusion coefficient, and c its concentration.

In addition, the potential applied to the WE generates an electric field close to its surface, forming an Electrical Double Layer (EDL) at the electrode-electrolyte interface, due to the immobilization of charged species. The EDL behaves much like a capacitor, composed of adsorbed ions and solvated counter-ions. It affects the kinetics of charge transfer and generates a capacitive background current during CV measurements.

Capacitive and Faradic currents

Although a single current is measured by the potentiostat, it has two separate contributions: a capacitive contribution, and a faradic contribution. As such, the resulting measured current during CV experiments can be defined by the following equation:

$$i = i_C + i_F \quad (2.4)$$

where i_C is the capacitive current, and i_F is the faradic current.

The capacitive current is the current the EDL generates, and is defined in equation eq. (2.5):

$$i_C = C_e \cdot \frac{dE}{dt} \quad (2.5)$$

where C_e is the electrode capacitance due to the EDL, and dE/dt is the temporal variation of the electric field. In the case of CV measurements, dE/dt is the scan rate ν .

The faradic current is the current generated by the charge transfers from the redox-active species that are adsorbed at the electrode surface. In other words, the faradic current is the current generated by redox reactions. For planar disc macroelectrodes, which are diffusion controlled systems, it is described by the Randles-Ševčík equation:

$$i_p = 0.4463 n^{3/2} F^{3/2} S_c \left(\frac{\nu D}{RT} \right)^{1/2} \quad (2.6)$$

Voltammetric Data

The resulting voltammogram gives a lot of information about the behaviour of the electrochemical system, by identifying the important regions; the reduction and oxidation peaks, and the capacitive regions. The reduction and oxidation peaks are regions where the current increases and then decreases rapidly, forming a gaussian-like peak if the reaction is diffusion controlled. The capacitive regions are regions where the measured current seems fairly linear, and is most likely due to the charging or discharging of the EDL. The parameters that can be extracted or calculated from a voltammogram are as follows:

- **Baseline currents** (i_C), which is the capacitive component of the measured current. It is calculated using a linear fit over the measured current before any redox peaks, as $i_C \propto \nu$.
- **Peak currents** (i_p), which is the faradic component of the measured current. It can be calculated using eq. (2.4). Once all other variables from eq. (2.6) are known, i_p can

give the exact concentration in solution. Moreover, if $i_{red} \neq i_{ox}$, then the reaction is not perfectly reversible.

- **Peak potentials** (E_p) indicate the potentials at which a redox reaction happens at the surface of the electrode. It gives insight into the energy that is necessary for a given reaction to occur.
- **Capacitance** (C), which can be calculated from i_C by extracting the value from purely capacitive regions. It shows how much charge the electrode can accumulate at its surface.
- **Peak separation** (ΔE), which is the separation between the reduction potential (E_{red}) and oxidation potential (E_{ox}). This parameter indicates the reversibility of a redox reaction, where a perfectly reversible reaction should have a peak separation of $\Delta E = 59$ mV at 25°C.
- **Halfway peak** ($E_{1/2}$), which is the halfway point between E_{red} and E_{ox} . It is the point at which the concentrations of the reduced and oxidized analyte is the same, at the surface of the WE.

2.3.3 Fast-Scan Cyclic Voltammetry and Amperometry

Typical CV last several tens of seconds, which is not compatible the fast neurochemical transients. Since synaptic events can last on the order of 10 to 100 ms [36], faster techniques are used for *in vivo* measurements.

Fast-Scan Cyclic Voltammetry

Entirely based on the same principles of CV, FSCV is exactly like its name suggest: CV performed at very fast scan rates. While CVs are usually performed with scan rates in the mV s^{-1} range, FSCV experiments can reach the 100 V s^{-1} up to 1000 V s^{-1} [28]. To reach these speeds, the electrode geometries are much different. The standard electrodes used in neuroscience are $7\mu\text{m}$ diameter carbon fiber microelectrodes [28,37]. At such high scan rates, the background charging currents dominate most of the signal, and the currents are so small that the RE can be used as both the RE and the CE. To mitigate this, the background signal is subtracted, making this a differential technique; this means that, unlike CV, FSCV cannot quantitatively measure absolute concentrations. It can, however, accurately measure changes in concentration, and give detailed temporal data on neurotransmitter dynamics.

This technique can further increase its selectivity by applying a fingerprint-like signal for different analytes [28,32].

Amperometry

Amperometry is another powerful technique used to quantify the vesicular release of neurotransmitters [32,34]. Unlike the other two methods, this technique applies a single stable potential, at which the chosen analyte oxidizes or reduces. Carbon fiber microelectrodes are also the WE of choice for this technique, as can be less than 10 μm in size, allowing for very specific placement. The current from the WE is monitored as a function of time, with individual spikes in current being associated with distinct oxidation/reduction events [32,34]. Since the applied potential signal is constant, amperometry can reach sub-millisecond temporal resolutions, allowing for quantitative analysis of neurotransmitter release kinetics. However, it lacks selectivity, because any molecule whose reduction or oxidation potential is below the applied potential will react and contribute to the measured current. Furthermore, this increases the rate of electrode fouling when compared to other techniques [32,34].

Together, these three techniques form the foundation of neurotransmitter detection using electrochemistry. However, all techniques' performances are limited by electrode fouling, overlapping redox potentials, and limiting charge transfer kinetics. To overcome these challenges, many studies have focused on modifying the surface of the WE to enhance its performance.

2.4 Electrode Modifications

There are many ways to modify the physicochemical properties of electrodes, and a multitude of properties that can be modified to tailor an electrode for very a specific application. By altering surface chemistry, morphology, or conductivity, it is possible to improve charge transfer kinetics, enhance signal acquisition, or increase selectivity. As far as neurotransmitters are concerned, electrode modification to mitigate electrode fouling all while increasing sensitivity is particularly important, especially when detecting dopamine [11].

2.4.1 Engineered materials

A common approach to improve the performance of an electrode is the use of engineered nanomaterials. For example, carbon-based materials like graphene, carbon nano-tubes, and carbon micro/nano-fibers have a high surface area, good conductivity, and have tunable surface functionality [6,11,37]. Another example would be the addition of metallic nanoparticles

to a pre-established electrode, like gold, platinum or metal-oxide nanoparticles (Nanoparticle-less (NPs)). The addition of NPs can increase the electroactive surface area, catalyze certain redox reactions, or enhance charge transfer [11].

2.4.2 Conducting Polymers

Conductive polymers such as poly(3,4-ethylenedioxythiophene) (Poly(3,4-ethylenedioxythiophene) (PEDOT)), polyaniline, polypyrrole and many others have been studied extensively for electrode coatings, as they have high conductivities (either electronic or ionic), are highly stable, and are easily processable [11,12]. Their redox activity can also facilitate charge transfer between the analyte and the electrode material, while their porosity and flexibility often makes for a biocompatible interface. Their ease of processability makes it easy to combine them with the aforementioned engineered materials and NPs to achieve even better performance or expand an electrode's functionalities [38].

2.4.3 Biomimetic Materials

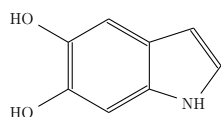
More recently, research has shifted towards biomimetic and bio-sourced materials for electrode modification and functionalization. Some examples include proteins (e.g.:chitosan [9]), cellulose [39], and melanin [13]. These materials form stable films under mild conditions, and offer improved biocompatibility and sustainability compared to synthetic alternatives. Among said biomaterials, melanin and melanin-like polymers have emerged as an alternative, due to their redox activity, chemical versatility, and multiple uses in many different fields.

2.5 Melanin

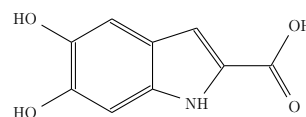
Melanin is a ubiquitous biopigment split into multiple types: eumelanin, pheomelanin, neuromelanin, allomelanin and pyromelanin. Of these types, the most common one is eumelanin, a black or brown pigment. It is found in all kingdoms of life: from large animals, to microscopic-sized microbes [40–43]. This ubiquity underlines the pigment's potential as a functional material that goes beyond pigmentation. It and its synthetic analogues are subject to much research for conductive, bio-compatible sensing applications [13,20,44]. In the literature, eumelanin and some melanin-like coatings, have been explored as adhesives [45], functional coatings for sensors [13], protection against free radicals [46].

Its structure is quite complex. At the molecular level, it is a biopolymer largely composed of two monomers whose structures are shown in fig. 2.5, DHI and DHICA, and their oxidized/quinone derivatives [47]. Said monomers polymerize into oligomeric or amorphous

structures, all of which vary greatly with each source, processing methods, and extraction protocols [48]. Many papers have synthesized melanin by polymerizing dopamine in solution, resulting in PDA NPs of around 100 – 200 nm in diameter [49, 50]. But, there are also biosourced alternatives, like SM, who have shown interesting physical and chemical properties. SM has been used to make conductive inks, printed films, and various sensors for the environment and for medical applications [13, 13, 19–21, 50, 51].



5,6-dihydroxyindole



5,6-dihydroxyindole-2-carboxylic acid

Figure 2.5 Chemical structures of Sepia Melanin monomers

Biosourced eumelanins have attracted significant research interest due to their unique physicochemical properties. In particular, SM extracted from cuttlefish ink is easily extracted using simple protocols [52, 53] and possesses interesting electrical and chemical characteristics. The quinone groups in SM give it reversible redox activity [47, 54] and a humidity-dependent electrical response [20].

Neuromelanin is similar, but different from SM. It is a combination of pheomelanin and eumelanin, and is found in the *substantia nigra* in human brains. It is said to protect some neural cells from oxidative stress [55].

2.6 Electrochemical Dopamine Sensors

Modern electrochemical DA sensors rely predominantly on carbon-based electrodes, mainly due to their biocompatibility and favourable electrochemical properties []. Among all other sensors, carbon fibre microelectrodes, used with FSCV, are considered the gold standard for *in vivo* sensing of dopamine (and other neurotransmitters) because of their high temporal resolution and sensitivity. These sensors have very nanomolar sensitivity and sub-second temporal resolution that arise from their very small dimensions. [28]

In parallel, graphene-based and nanostructured carbon-based electrodes have been developed for enhanced sensitivity in analytical dopamine sensing. They have high electroactive surface areas, tunable surface properties, and often show improved charge-transfer properties that enable sensitivities ranging from nanomolar to micromolar, depending on the fabrication methods. [56]

More recent advances in dopamine detection have brought more focus to paper-based electrochemical sensors as a low-cost, scalable alternative. These systems typically involve screen-printing of conductive inks or pyrolyzed carbon substrates. This results in flexible, disposable sensing electrodes that are generally capable of reaching micromolar sensitivities. While these electrodes are less sensitive than carbon fibre microelectrodes, they offer advantages in simple fabrication and accessibility. [39,57]

Table 2.1 shows a summary of the different characteristics of these state of the art electrochemical dopamine sensors:

Table 2.1 A comparison of typical electrochemical dopamine sensors

Sensor type	Scale	Sensitivity	Main Application
Carbon fibre microelectrodes	$\sim 10\ \mu\text{m}$	nM	<i>In vivo</i> detection using FSCV
Graphene/nanostructured carbon	μm -mm	nM- μM	High-sensitivity analytical electrochemistry
Paper-based	mm	μM	Low-cost, flexible, disposable sensing

CHAPTER 3 MATERIALS AND METHODS

3.1 Reactants & Solutions

All the reactants were purchased from Millipore Sigma (Canada) unless otherwise stated. Deionized Water (DIW) from an ultra-purification system (resistivity $> 18 \text{ M}\Omega \text{ m}$) was used throughout the tests for solution preparation and rinsing.

Ruthenium hexamine solutions were prepared by adding 1 mM hexaammineruthenium(III) chloride in 1 M KCl. The DA and ascorbic acid solutions were prepared by adding 250 μM dopamine hydrochloride or l-ascorbic acid to pH 7.4, 0.01 M Phosphate Buffered Saline (PBS).

3.2 Extraction and Preparation of the Sepia Melanin Aqueous Samples

The extraction protocol was based on a previously established technique for the extraction of melanin from cuttlefish ink [52]. To start, 300 g of *Sepia Officinalis* ink were purchased from the local fish market. Then, all 300 g were added to 600 ml of DIW, and left to stir for 24 h. Subsequently, the mixture was centrifuged at 10 000 RPM for 30 min for a total of 6 times. After each step, the supernatant liquid was discarded and the pellet was dried in a vacuum oven for 3 h at 60°C. The resulting powder was then ground using a glass mortar and pestle and 3 different suspensions in DIW were made (0.1 mg mL^{-1} , 2.5 mg mL^{-1} , and 5.0 mg mL^{-1}) and stored in the fridge. All aqueous suspensions were put in an ultrasonic cleaner (SPER SCIENTIFIC) for 180 s before use to homogenize as best as possible.

3.3 Electrode Preparation and Modification

3.3.1 Gold Disc Electrodes

The gold disc electrodes (3 mm-diameter, BASInc, IN, USA) were cleaned by polishing with multiple alumina slurries by descending order by size: 15 μm , 3 μm , 1 μm , 0.3 μm , and 0.05 μm . The electrodes were then sonicated in water for 180 seconds. After sonication, 5 μL of SM in DIW (0.1, 2.5, or 5 mg mL^{-1}) were drop-cast onto the Gold Electrode (GE) surface, and dried in an oven at 80 degC for 10 minutes. The drop-casting and drying steps were repeated three times in total. The SME were then rinsed with DIW to remove any material that had not adhered well to the surface of the disc electrodes.

3.3.2 Paper Electrodes

Paper electrodes were fabricated according to the protocol described in reference [58]. Whatman filter papers were first treated with a flame-retardant ammonium sulfamate solution, and then air-dried whilst weighed down. The electrode patterns were then pyrolyzed onto the substrates using a CO_2 laser engraver (Trotec Rayjet), followed by rinsing with DIW and a second air-drying step. Once dry, each electrode was cut out individually. A polydimethylsiloxane layer was subsequently applied as a hydrophobic sealant to prevent solutions wicking to the electrical connection, and copper tape was attached to for ease of use with electrical connections. Finally, the electrodes were functionalized by immersion in SM in DIW solutions (5.0 mg mL^{-1} or 0.1 mg mL^{-1}), followed by drying in an oven at 60°C for 10 minutes; the coating step was repeated three times to ensure complete surface coverage.

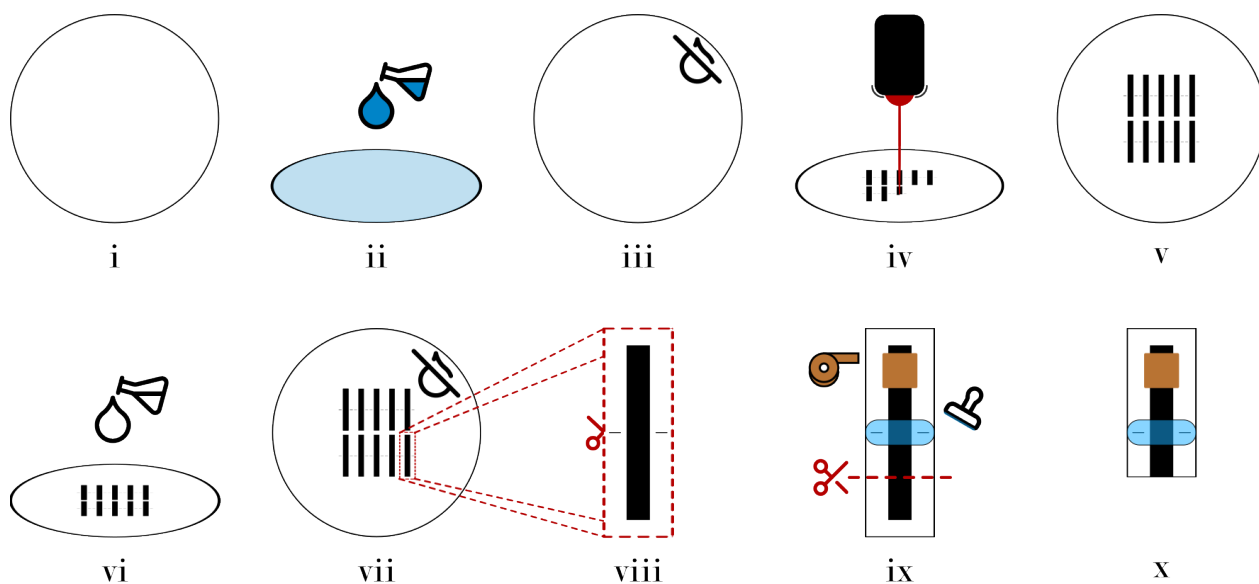


Figure 3.1 Paper electrode fabrication process

3.4 Optical Microscopy

The images were taken with a stereo zoom microscope (Nikon SMZ 745 T). White-balance and exposure-time using an attached camera (Moticam X). All images were taken with automatic white-balance, $500 \mu\text{s}$ exposure-time, and 7.5X magnification. The resulting images were then cropped. All post-processing was done with the Python Open-CV library to ensure consistency across all samples.

3.5 Scanning Electron Microscopy

The imaging was done with a JEOL JSM-7600F scanning electron microscope with a 5 kV accelerating voltage and a 6.3 mm working distance, using the low angle backscattered electrons (LBE) detector. Samples that were imaged using Scanning Electron Microscopy (SEM) were prepared on 1 cm x 1 cm gold substrates. These substrates were prepared by electron-beam evaporation of a 45 nm layer of Au on a 5 nm Ti adhesion layer, on top of a 4 inch oxide Si wafer (500 μ m, Addison Semiconductor Materials, CA, USA). The wafer was then diced into 1 cm x 1 cm squares and cleaned by sonicating for 15 minutes in acetone, Isopropanol (IPA), acetone, and ending with DIW.

Sepia Melanin on Gold Substrates

3 different concentrations of SM in DIW were prepared as detailed in 3.2, and deposited: 0.1 mg/mL, 2.5 mg/mL, and 5.0 mg/mL.

All samples were prepared as if they were gold disc electrodes, but the process was stopped at different steps of the deposition process. The first sample stopped after 1 drop-cast. The second sample was stopped after 3 drop-casts, and the third sample was prepared fully (rinsed with deionized water after 3 drop-casts).

3.6 Energy Dispersive Spectroscopy

Energy Dispersive X-ray Spectroscopy (EDX) was performed on the SEM samples with a Tmax Oxford Instruments spectrometer.

3.7 Electrochemical Equipment

All the electrochemical experiments were performed with a CHI 660 potentiostat (CHInstruments, TX, USA). The SM-modified electrodes were integrated into a 3-electrode system, where the reference electrode was an Ag|AgCl (3M NaCl) electrode, and the counter electrode was a Pt wire. All potentials are reported *vs.* Ag|AgCl (3M NaCl). Figure 2.4b shows the typical setup used for all electrochemical measurements.

3.8 Cyclic Voltammetry

Cyclic voltammograms were taken in decreasing order of scan rates: 100, 75, 50, 25, 10, and 5 mV/s, with electrochemical windows adapted for each analyte. The electrochemical windows

were $[-600\text{mV}, 300\text{mV}]$ for 1 mM Ruthenium Hexamine in 1 M KCl, and $[-100\text{mV}, 700\text{mV}]$ for 250 μM Dopamine Hydrochloride in 0.01 M PBS and 250 μM L-Ascorbic Acid in 0.01 M PBS. Each working electrode was used to measure 3 full cycles for each scan rate, first in the buffer solution alone, then in the buffer in solution with the analyte. Ruthenium hexamine was measured first and then dopamine. Ascorbic acid was measured using electrodes that had not been used prior. All electrodes (working, reference, and counter) were rinsed before and after being inserted into any electrolytic solution.

Table 3.1 Summary of electrochemical experiments and number of independently fabricated electrodes for each electrode type, analyte, and SM concentration.

Type	Electrode	Analyte	n (independent electrodes)
Gold electrodes	Bare gold	RuHex	6
	2.5 mg/mL SM	RuHex	6
	5 mg/mL SM	RuHex	12
	Bare gold	L-ascorbic acid	3
	5 mg/mL SM	L-ascorbic acid	3
Paper electrodes	LIG	RuHex	12
	0.1 mg/mL SM	RuHex	12
	5 mg/mL SM	RuHex	12
	LIG	Dopamine	12
	0.1 mg/mL SM	Dopamine	12
	5 mg/mL SM	Dopamine	12

3.9 Data Analysis & Statistics

In all figures, the shaded areas indicate the standard error around the data points, and the solid curves represent the mean.

3.9.1 Voltammograms

Cyclic voltammograms of 3 to 12 independent electrodes of each type were analyzed using a custom Python library to extract the measured capacitive current (i_c), redox peak currents (i_{red} , i_{ox}) and redox potentials (E_{red} , E_{ox}). The raw voltammogram data were filtered using a Savitzky-Golay filter to remove any digital noise.

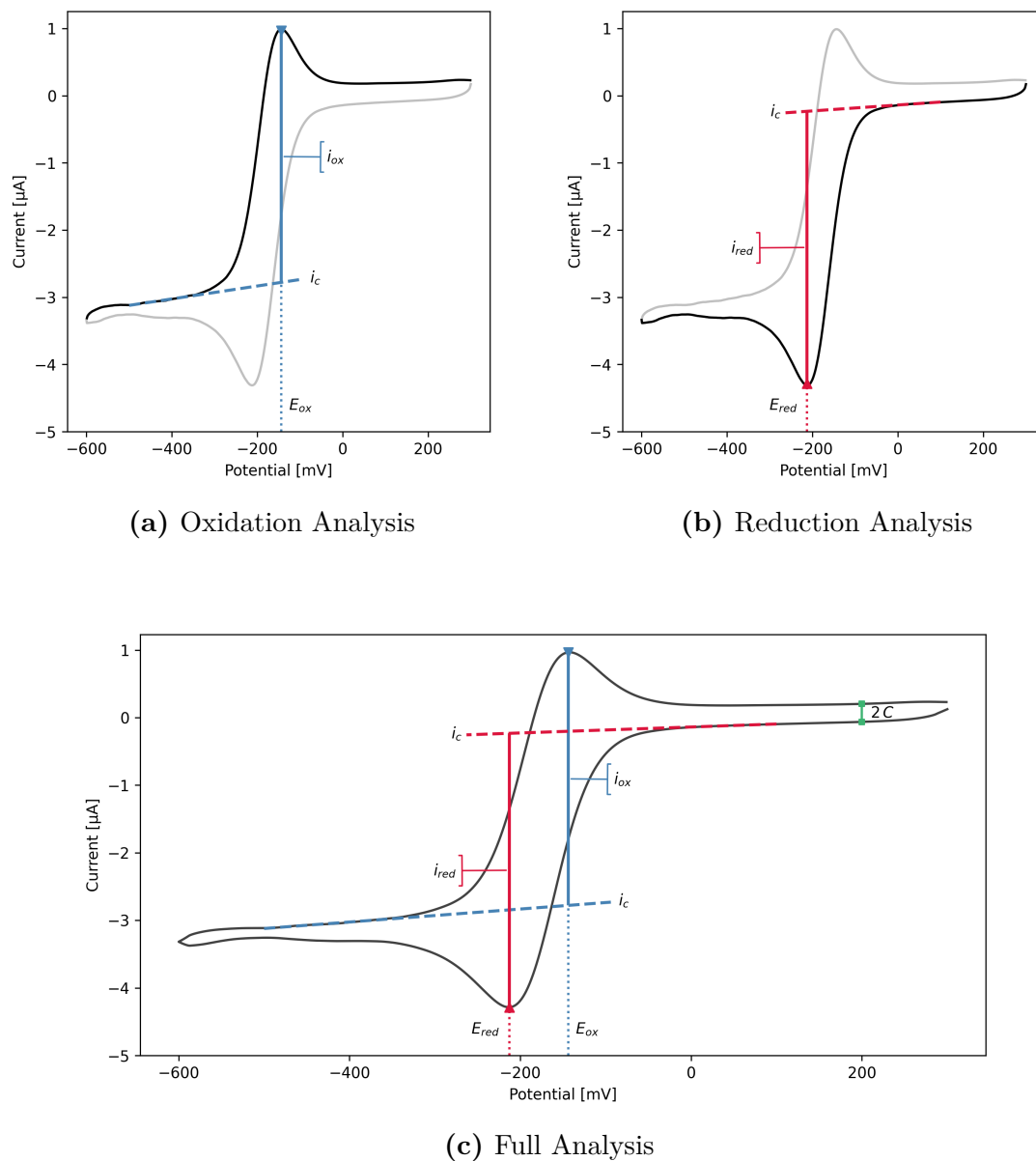


Figure 3.2 Example of an analyzed CV curve using 1 mM Ruthenium Hexaamine in 1 M KCl on a gold disc electrode, at 5 mV s^{-1} .

Electrode capacitance was calculated at a single potential (E_C) where a mostly capacitive current is observed, as illustrated in fig. 3.2c, using the following equation:

$$C = \frac{|I_{forward} - I_{reverse}|}{2 \cdot \nu} \quad (3.1)$$

where $I_{forward}$ and $I_{reverse}$ are the measured forward or reverse scan currents, ν is the scan rate, and $E_C = 200 \text{ mV s}^{-1}$ for 1 mM Ruthenium Hexaamine in 1 M KCl, $E_C = 600 \text{ mV s}^{-1}$ for , and $E_C = 0 \text{ V}$ for 250 μM L-Ascorbic Acid in 0.01 M PBS .

Peak redox currents (i_{red} , i_{ox}) were extracted by subtracting the capacitive current from each half-cycle and finding the inflection points of the derivative of the current ($\frac{di}{dE} = 0$). The capacitive current itself was calculated using a RANSAC linear model estimator (RANDOM Sample Consensus). [59]

Peak separation (ΔE) was calculated with the following equation:

$$\Delta E = |E_{red} - E_{ox}| \quad (3.2)$$

where E_{red} and E_{ox} are the reduction and oxidation potentials, respectively.

Peak shifts (δE_p) are defined as the displacement of a redox peak for a melanin-modified electrode with respect to that same peak for a non-modified gold electrode. They were calculated using the following equation:

$$\delta E_p = |E_p^{modified} - E_p^{Au}| \quad (3.3)$$

where $p \in [red, ox]$.

The halfway potential ($E_{1/2}$) is given by the average potential between the reduction and oxidation potentials:

$$E_{1/2} = (E_{re} + E_{ox})/2 \quad (3.4)$$

To compare the effects of the MNP-modified electrodes to the bare GE, we defined the amplification factor γ , for a given scan rate and electrode k , as follows:

$$\gamma_p = i_p^k / i_{red}^{Au} \quad (3.5)$$

where $p \in [red, ox]$.

CHAPTER 4 RESULTS AND DISCUSSION

The following chapter presents and discusses the results obtained by modifying gold disk electrodes with sepia melanin. All data are first presented in their respective sub-sections. This is followed by a general discussion of the analysis of the results that highlights key trends and why these results are relevant.

To start, the surface of the modified electrodes was probed. First, SM ink morphology and composition was studied using Ultraviolet-Visible Spectroscopy (UV-Vis), Raman spectroscopy, EDX, and SEM. Then, electrodes were imaged with optical and electron microscopes. Finally, the modified and unmodified electrodes were used to perform electrochemistry on different solutions: 1 mM Ruthenium Hexamine in 1 M KCl, 250 μ M Dopamine Hydrochloride in 0.01 M PBS, and 250 μ M L-Ascorbic Acid in 0.01 M PBS.

4.1 Sepia Melanin Ink Morphology and Composition

This section shows the results of SM characterization using spectroscopy methods for the material characteristics, and optical and electron microscopy for the morphology after deposition on gold and paper substrates to best simulate the surfaces of the electrodes used for the electrochemical measurements.

4.1.1 Spectroscopy

UV-Vis Absorbance

The UV-Vis spectrum of the SM in DIW suspension can be seen in fig. 4.1, and shows a broadband absorption throughout the spectrum, from 200 nm to 1000 nm, with a higher absorbance in the ultraviolet range (below 400 nm). This is consistent with what is visible to the naked eye: a greyish black semi-transparent liquid.

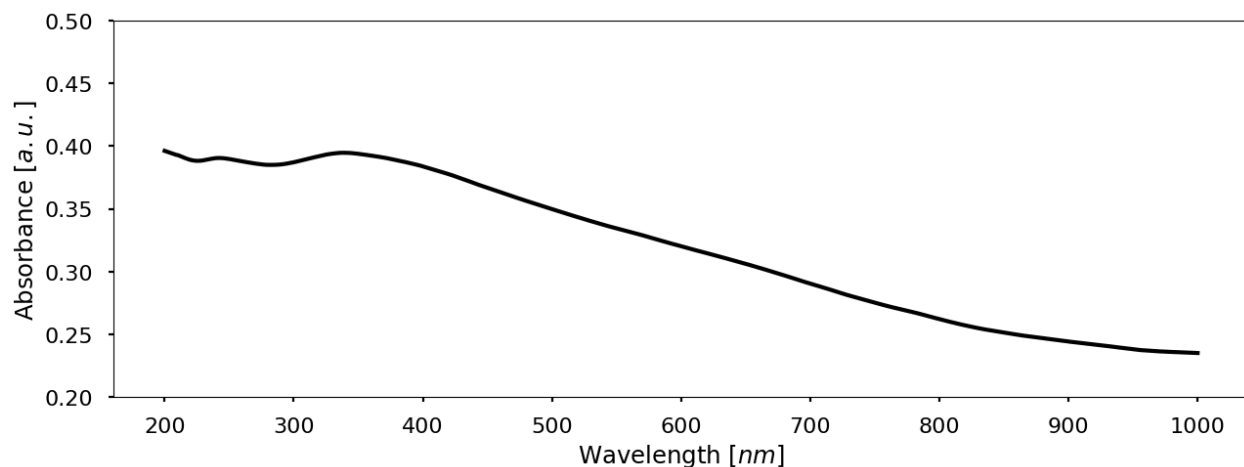


Figure 4.1 UV-Vis absorbance spectrum of 10 mg mL^{-1} SM in DIW ink formulation.

Raman

Figure 4.2 shows the Raman spectra of different parts of a single SM-LIG electrode: bare paper, 0.5 mg mL^{-1} SM on paper, LIG, and 0.5 mg mL^{-1} SM on LIG. The melanin-covered spectra are nearly identical, even though the spectra of the underlying substrates are quite different.

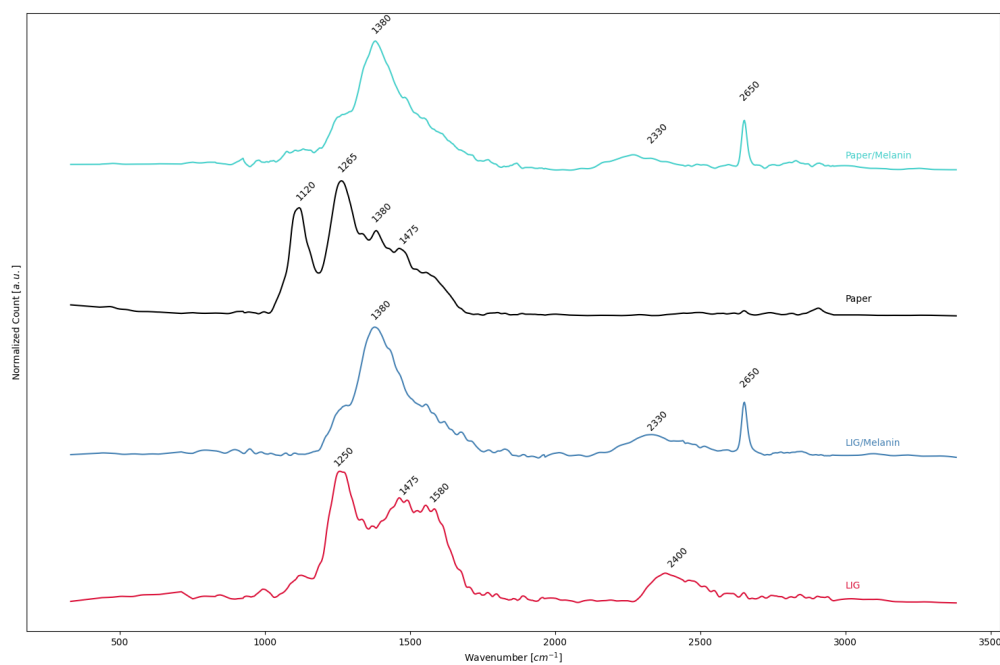


Figure 4.2 Raman spectrum of 0.5 mg mL^{-1} SM-LIG and LIG electrodes

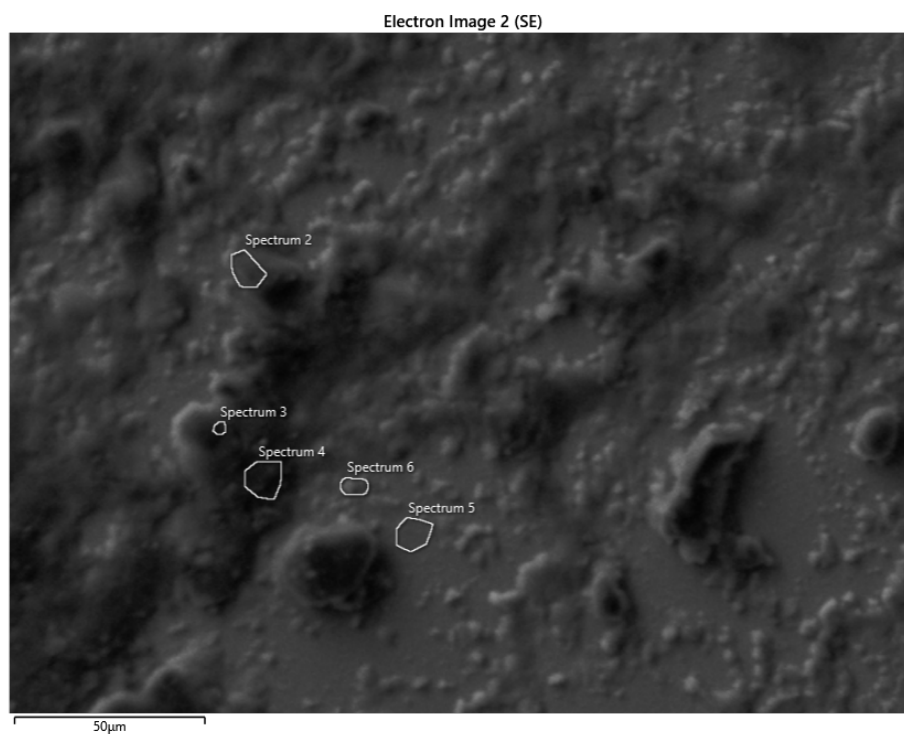
Energy Dispersive X-Ray Spectroscopy

The results of the EDX analysis seen in Table 4.1, show that the samples are made up of $\sim 50\%$ carbon and $\sim 45\%$ substrate metals (silicon, titanium, and gold), with $\sim 5\%$ oxygen and $< 1\%$ other metals (sodium, magnesium, and calcium). The proportions of substrate metals remain relatively constant before and after rinsing: around 20% for Si, 0.3% for Ti, and 20% for Au. As for the carbon and oxygen content, After rinsing, sodium and magnesium are undetectable and the amount of calcium is considerably reduced with each fabrication step.

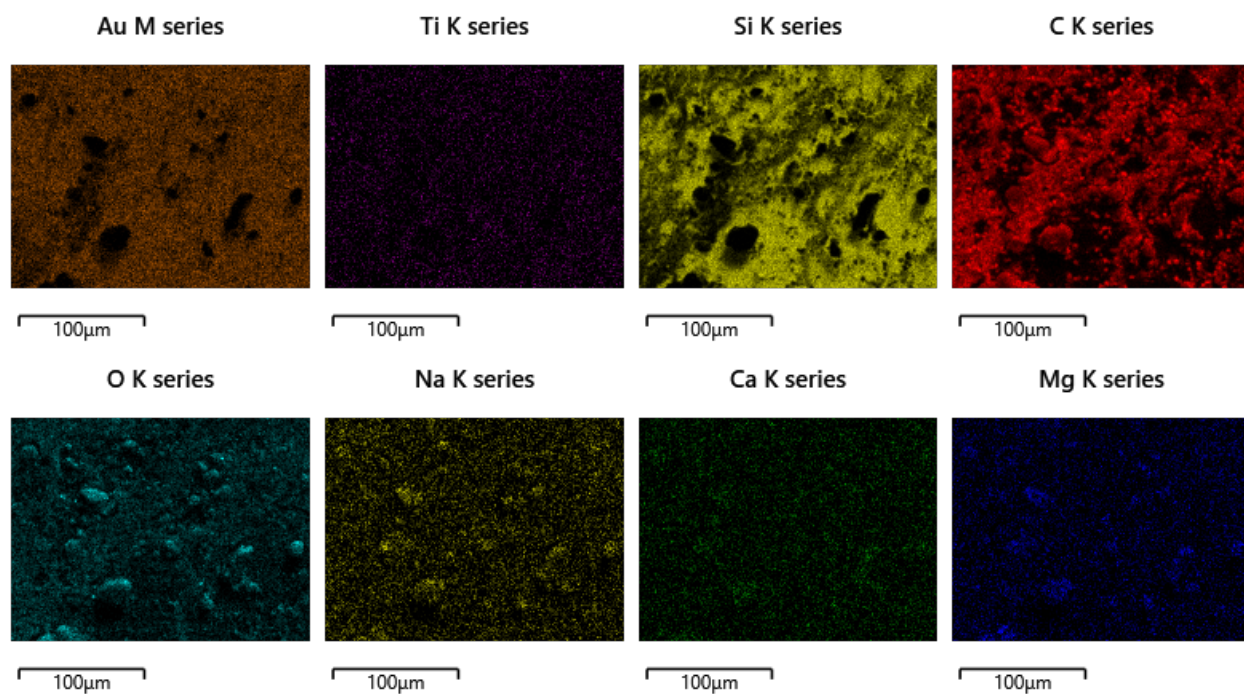
Table 4.1 Atomic composition of different samples

Sample	C (%)	O (%)	Na (%)	Mg (%)	Ca (%)	Si (%)	Ti (%)	Au (%)
1 drop-cast	59.68	7.83	0.28	0.17	0.22	14.61	0.25	16.96
3 drop-casts	47.72	6.52	0.14	0.1	0.16	23.31	0.32	21.72
3 drop-casts, rinsed	46.21	5.5	-	-	0.07	25.51	0.33	22.38

Figures 4.3 and 4.4 show the SEM image and the EDX elemental mappings to the aforementioned SEM image of the 3 drop-casts, and 3 drop-casts, rinsed samples from table 4.1. The metals that are seen in fig. 4.3 but not in fig. 4.4 are the same as mentioned in the previous paragraph: Na, Mg, and Ca. From the elemental maps seen in fig. 4.3b and fig. 4.4b, the aforementioned metals are present all over the sample, as opposed to the carbon and oxygen that coincide with where the SEM images show material deposits (fig. 4.3a and fig. 4.4a).

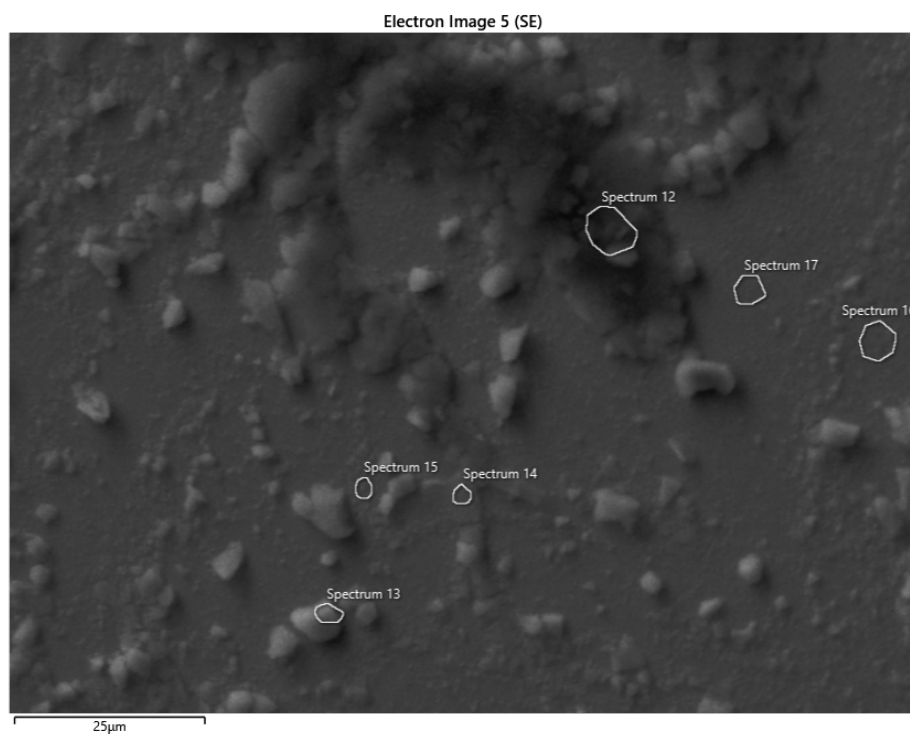


(a) SEM image

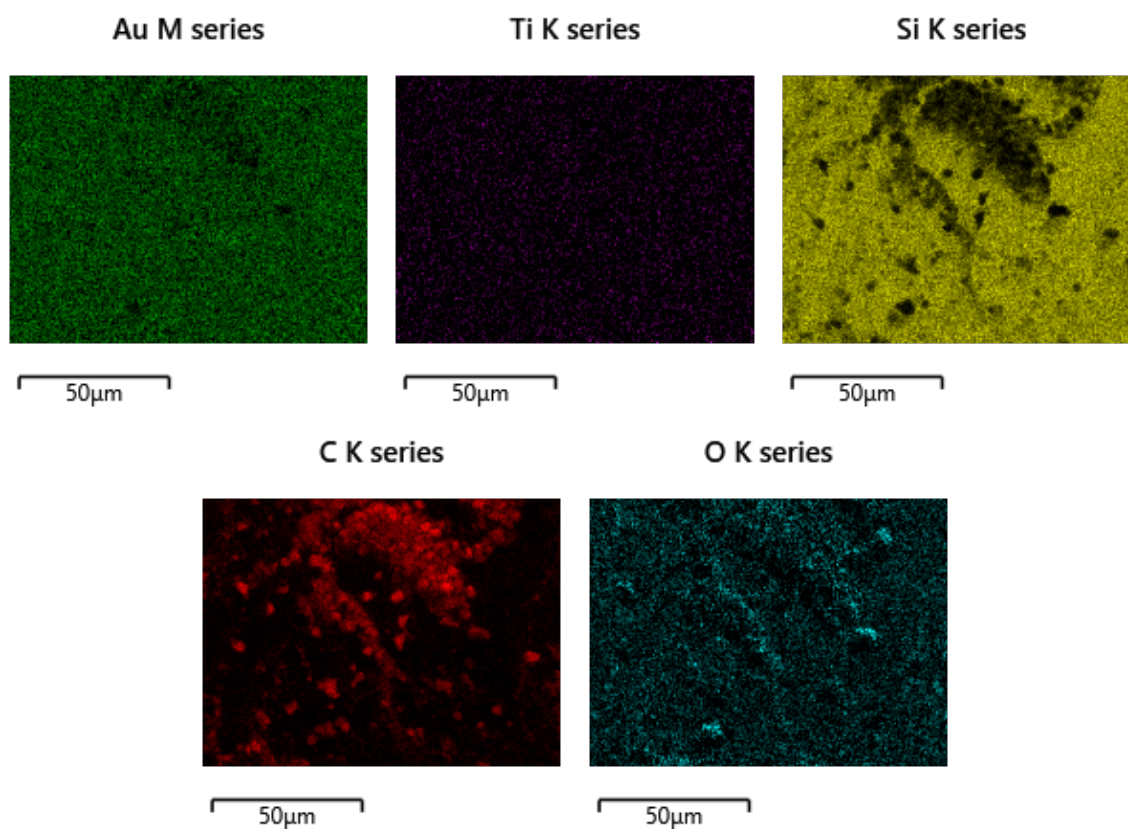


(b) Elemental mappings

Figure 4.3 EDX imaging results of an unrinsed sample. (a) SEM image where data was acquired. (b) Elemental maps of edx results.



(a) SEM image



(b) Elemental mappings

Figure 4.4 EDX imaging results of a DIW rinsed sample. (a) SEM image where data was acquired. (b) Elemental maps of EDX results.

4.1.2 Imaging

Sepia Melanin on Gold

Figure 4.5 shows a microscopic view of the electrode surface before and after depositing different concentrations of SM in DIW, as well as before and after rinsing with DIW.

Figures 4.5b and 4.5c show that there is a visible difference before and after the deposition of MNP on the surface. The resulting electrode surface coating is heterogeneous, but both samples are mostly covered in black (SM). There are some areas where the gold surface can be seen, and the amount of uncovered surface area varies with the concentration of SM: electrode coverage increases with concentration. On the outer perimeter, cracks can be seen in both samples. Although a slight coffee ring effect is observed in Figure 4.5b, there is no visible ring at the higher concentration shown in Figure 4.5c. The most noticeable difference is observed after rinsing the samples with DIW. The rinsed samples have much less surface area covered in SM, and almost all of the mass that was in the outer perimeter has been rinsed off.

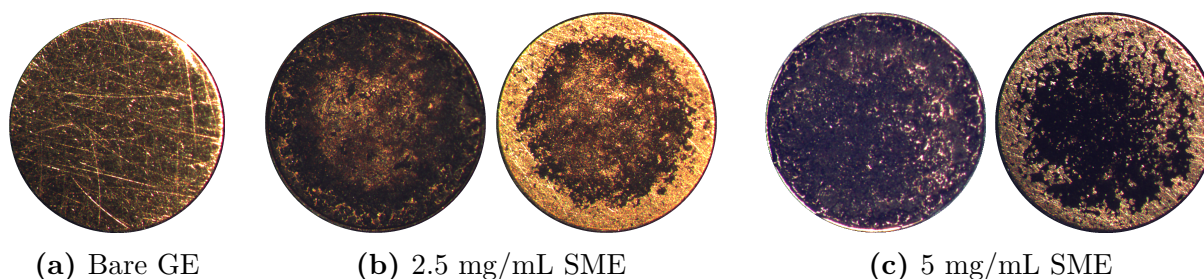


Figure 4.5 Gold disc electrodes (3 mm diameter) (a) before deposition, (b-c) after deposition (left), and after rinsing with DIW (right).

Figure 4.6 shows the surface of a Au/Ti/SiO₂/Si substrate prepared as described in section 3.5. Both fig. 4.6a and fig. 4.6a show a partial coverage of the gold surface by the MNP. Two different types of coverage can be seen: a spattering of differently sized spherical particles, and large masses of irregular shapes. The former are the MNP, more visible in fig. 4.6b, whose diameters are between 150 nm and 500 nm, while the bigger aggregates have yet to be definitely indentified, they are most likely a result of poor purification.

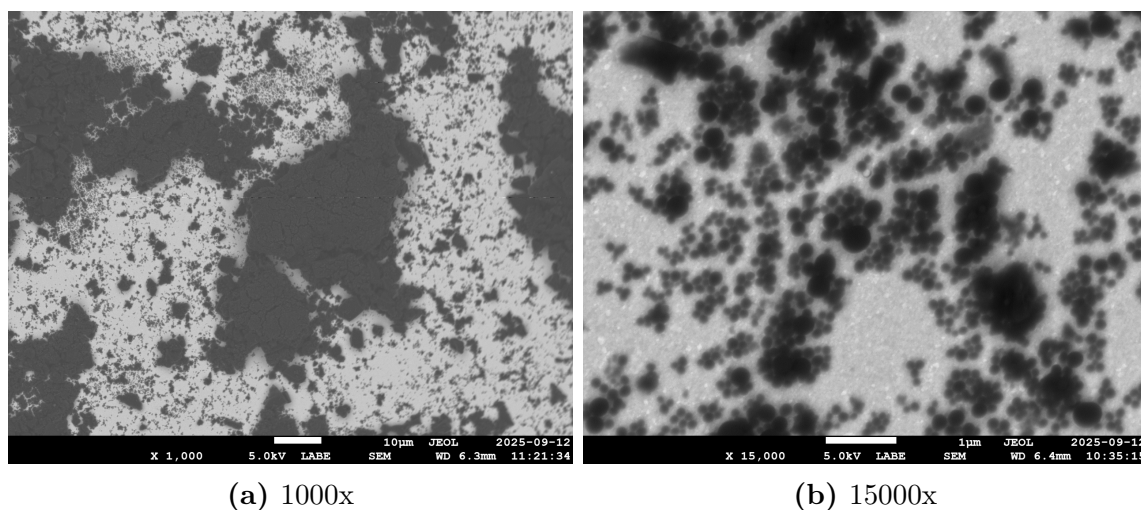


Figure 4.6 SEM of 3 drop-casts of 5.0 mg mL^{-1} MNP on gold after rinsing with DIW (for more SEM micrographs, see Annex A 5.3)

Sepia Melanin on Graphitized Paper

Figure fig. 4.7 shows a microscopic view of the electrode surface before and after depositing different concentrations of SM in DIW, as described by section 3.3.

Figures fig. 4.7c and fig. 4.7a show that there is a very visible difference before and after the deposition of 5 mg mL^{-1} SM in DIW. However, when comparing fig. 4.7b and fig. 4.7a, there is no visible difference to the naked eye before or after deposition of 0.1 mg mL^{-1} SM in DIW. For the 5 mg mL^{-1} SM in DIW electrode, fig. 4.7c, the active part of the WE looks homogeneously covered in black pigment (SM), and the pigment stops at the interface where the Polydimethylsiloxane (PDMS) begins.

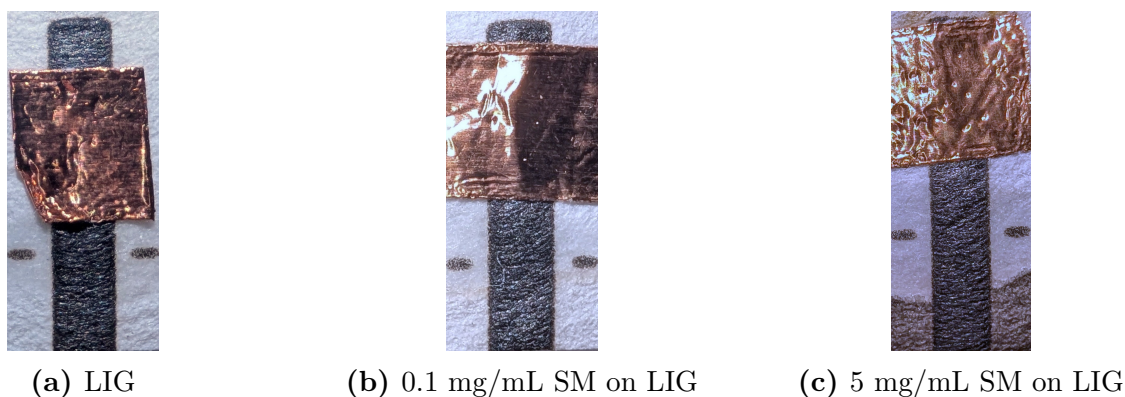


Figure 4.7 Paper Electrodes

Figure 4.8 shows SEM images of the active area of the LIG paper electrodes. The now graphitized (or carbonized) paper fiber network is still very visible as seen in fig. 4.8a. Some fibers are cracked and broken, but the overall structure is still present. fig. 4.8b shows a close-up of the graphitized surface, which seems mostly smooth, with some pores likely where some of the particles present in the paper underwent significant heating during the laser-induced graphitization process.

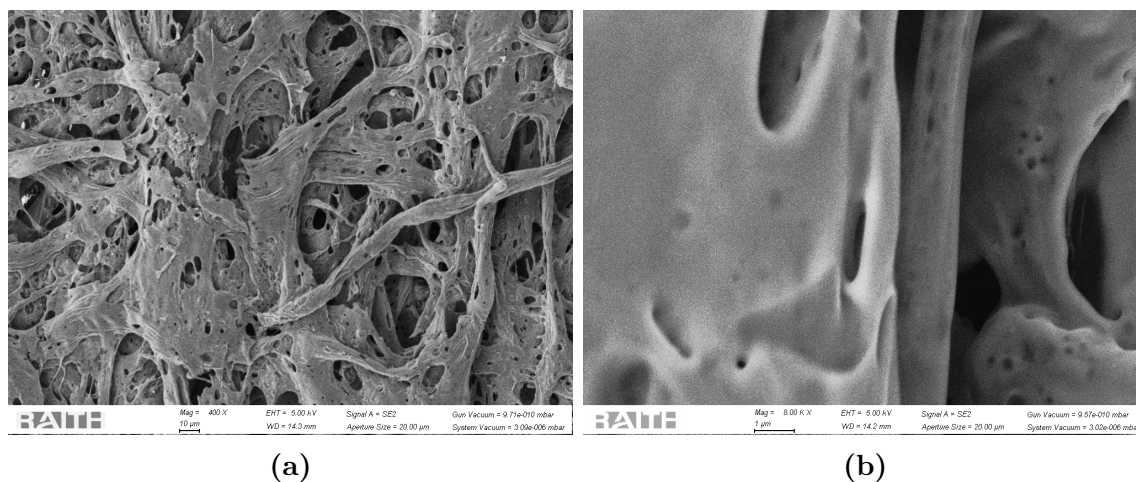


Figure 4.8 SEM of LIG electrode at (a) 400X and (b) 8000X.

Figure 4.9 shows SEM images of the active area of the SM-LIG paper electrodes after deposition of 5 mg mL^{-1} . Just like in fig. 4.8a, fig. 4.9a shows the graphitized paper fiber network, also damaged by the graphitization process. However, the fibers have a grainier texture. That is explained by fig. 4.9b, where tightly packed MNP can be seen at the surface of the

fibers. In this case, we also only have partial coverage of the surface by the MNP of various diameters (150 nm to 500 nm, as well as a few non-spherical particles (seen in the top left quadrant)).

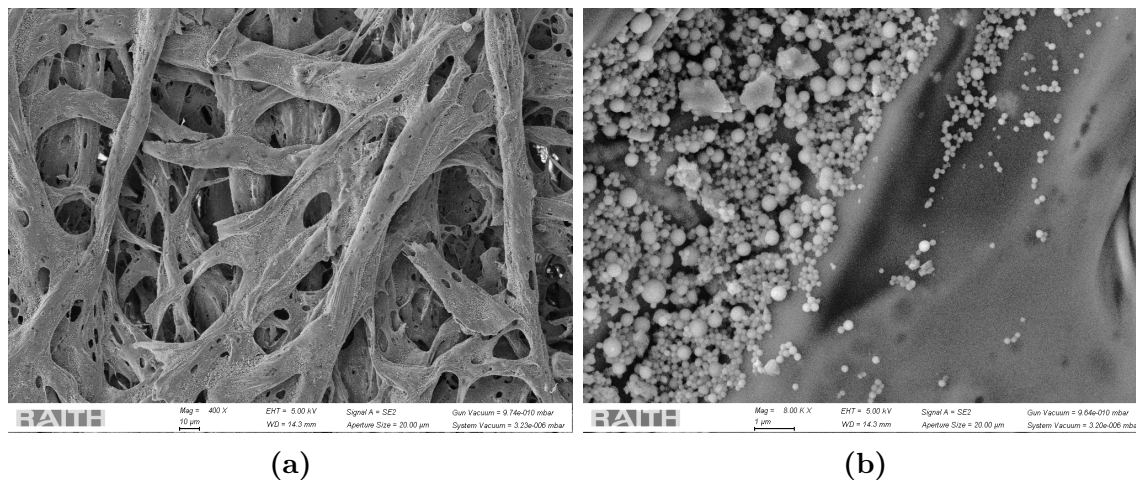


Figure 4.9 SEM of 5 mg mL⁻¹ SM-LIG electrode at (a) 400X and (b) 8000X.

4.1.3 Discussion

To characterize the SM ink composition and morphology, we performed UV-Vis absorption, Raman, and EDX spectroscopies, followed by optical and SEM imaging of the electrode surfaces before and after depositing SM.

The spectroscopy results shown in fig. 4.1 and fig. 4.2 confirm that our material is melanin, as the spectra resemble what is found in the literature [60,61].

The EDX results (section 4.1.1) show that the biomaterial is not purely melanin, as there are metallic residues present in the samples, as well as non-spherical carbon-based structures present in the EDX and SEM images (see figs. 4.3a, 4.4a, 4.6a and 4.6b).

The SEM and optical micrographs show that the SM bio-ink modifies both types of electrode surfaces. The gold disk electrodes micrographs (as seen in fig. 4.5) show the formation of a discontinuous black layer on top of the gold surface. After rinsing, some material is lost, especially around the outer edges of gold. The paper electrodes micrographs (as seen in fig. 4.7) show a slight darkening of the paper around the active area of the electrode, confirming the surface modification by the SM bio-ink. The micrographs of both electrode types confirm that SM is adhering to both surface types and modifying the active area of the working electrodes. As for the SEM micrographs (as seen in figs. 4.6 and 4.8), both electrode

types show spherical NPs of varying diameters that are consistent with the diameters found in the literature for SM (150 - 200 nm) [?, 18]. In addition to the latter, some irregularly shaped inclusions can be seen in both electrode types. However, the aforementioned non-spherical organic inclusions seem to be much less prominent in the SEM micrographs of the paper electrodes (fig. 4.9), which could indicate that these inclusions have vastly different surface interactions with gold than with paper. It is possible that these inclusions are more water-soluble than MNP, and this is the material that is removed during rinsing, as shown in the optical images of the gold disk electrodes (fig. 4.5). This would also explain why they are less present on paper substrates. As water dries along the paper's fibres, it recedes along the fibres' edges, until it reaches a point where multiple fibres intersect (see section .0 for more micrographs).

4.2 Electrochemistry on Gold Electrodes

This section details the results obtained for the electrochemical measurements of 1 mM Ruthenium Hexamine in 1 M KCl (section 4.2.1), 250 μ M Dopamine Hydrochloride in 0.01 M PBS (section 4.2.2), and 250 μ M L-Ascorbic Acid in 0.01 M PBS (section 4.2.3) that were performed using gold disk electrodes as a base. All calculations for this section are detailed in section 3.8.

4.2.1 Ruthenium Hexamine

The cyclic voltammograms obtained for the ruthenium hexamine redox couple for GE and SME are shown in fig. 4.10. Well-defined redox peaks can be observed for all types of electrodes and scan rates. Using data from these voltammograms, several parameters were calculated and are shown in figs. 4.11 to 4.16.

At all scan rates, both modified and unmodified electrodes exhibit the characteristic reversible redox couple of ruthenium hexamine, with peak currents increasing linearly with the square root of the scan rate, as shown in fig. 4.11. SM-modified electrodes exhibit significantly higher reduction currents compared to oxidation currents for all scan rates.

The dependence of redox currents with respect to SM concentration, shown in fig. 4.12, is increasingly non-linear as the scan rates increase, but non-linearity is much more important at the reduction of ruthenium hexamine than at its oxidation.

Figure 4.13 shows the electrode capacitance, calculated by eq. (3.1), which decreases progressively with scan rate. The higher concentration of SM, the higher the capacitance.

Figure 4.14 shows the halfway potentials $E_{1/2}$ for each type of electrode. For 5 mg mL⁻¹ SME, the halfway potential stays constant around -209 mV for all scan rates. For GE, $E_{1/2}$ also stays constant for all scan rates, but at a lower potential around -172 mV. For 2.5 mg mL⁻¹ SME, the halfway potential at 5 mV s⁻¹ is similar to the GE, at 176 mV, but decreases rapidly to reach the same halfway potential as the 5 mg mL⁻¹ SME around -209 mV at 100 mV s⁻¹.

Peak separation as a function of scan rate, shown in fig. 4.15, behaves differently for each electrode type. For GE, it stays around 60 mV for scan rates higher than 5 mV s⁻¹, where it goes up to 64 mV. For 5.0 mg mL⁻¹ SME, ΔE increases steadily from 58 mV at 5 mV s⁻¹ to 71 mV at 100 mV s⁻¹. Lastly, for 2.5 mg mL⁻¹ SME, ΔE starts very high at 84 mV at 5 mV s⁻¹, and quickly drops to 64 mV at 10 mV s⁻¹ and stays around 60 mV at scan rates above 25 mV s⁻¹.

The amplification factor γ , shown in fig. 4.16, increases with scan rate, and with SM concentration. A higher γ_{red} than γ_{ox} is observable for all electrode types. However, for 2.5 mg mL⁻¹ SM, the oxidation amplification is much lower than the reduction amplification.

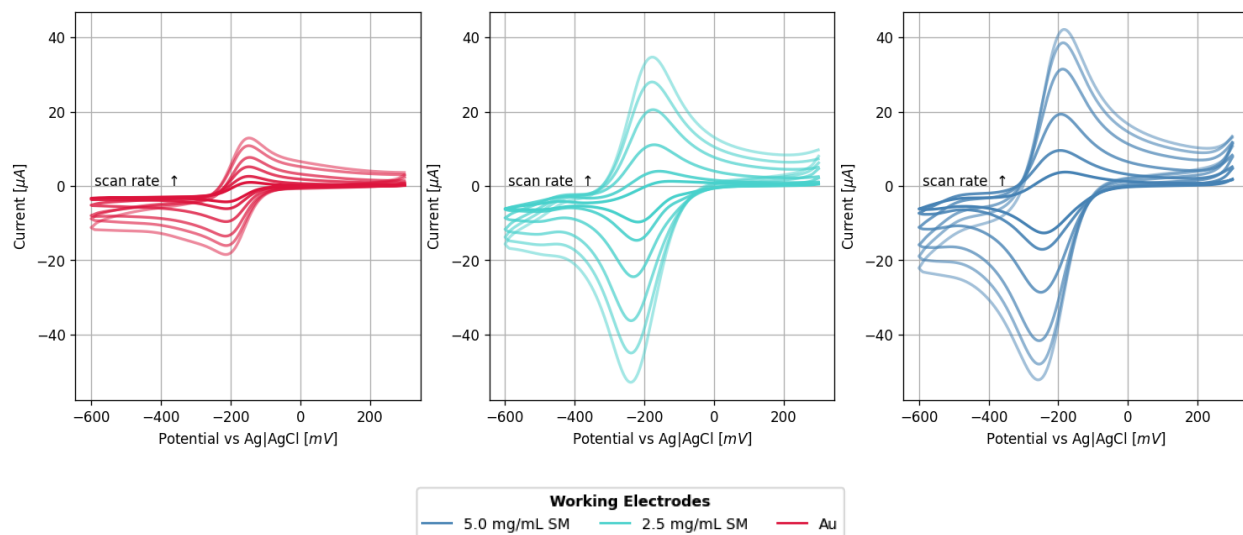


Figure 4.10 Representative CVs of 1 mM Ruthenium Hexamine in 1 M KCl with varying MNP concentration working electrodes at different scan rates (5, 10, 25, 50, 75, and 100 mV s⁻¹).

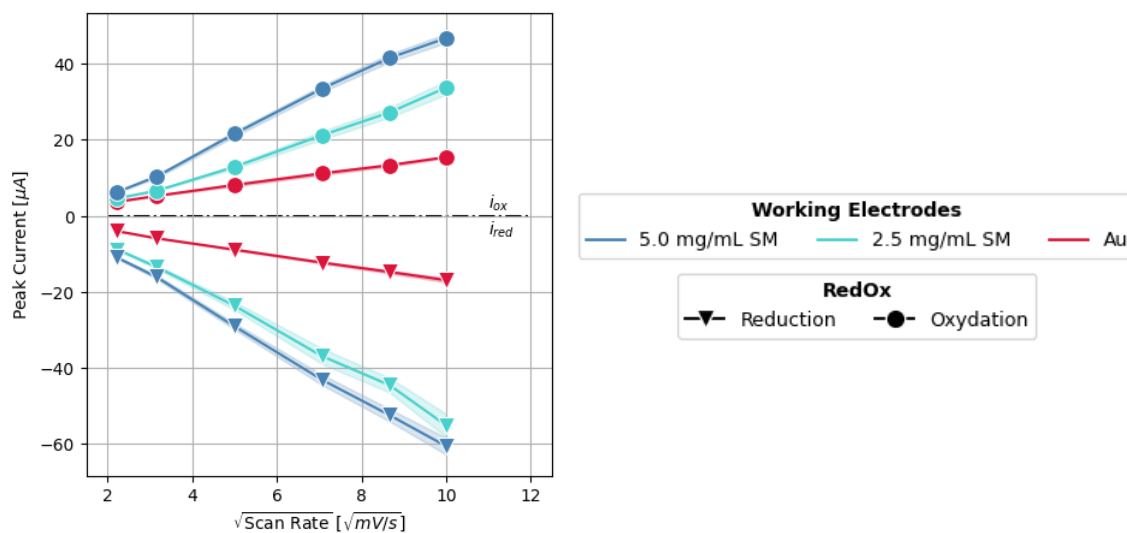


Figure 4.11 Peak redox currents as a function of the square root of the scan rate for 1 mM Ruthenium Hexaamine in 1 M KCl.

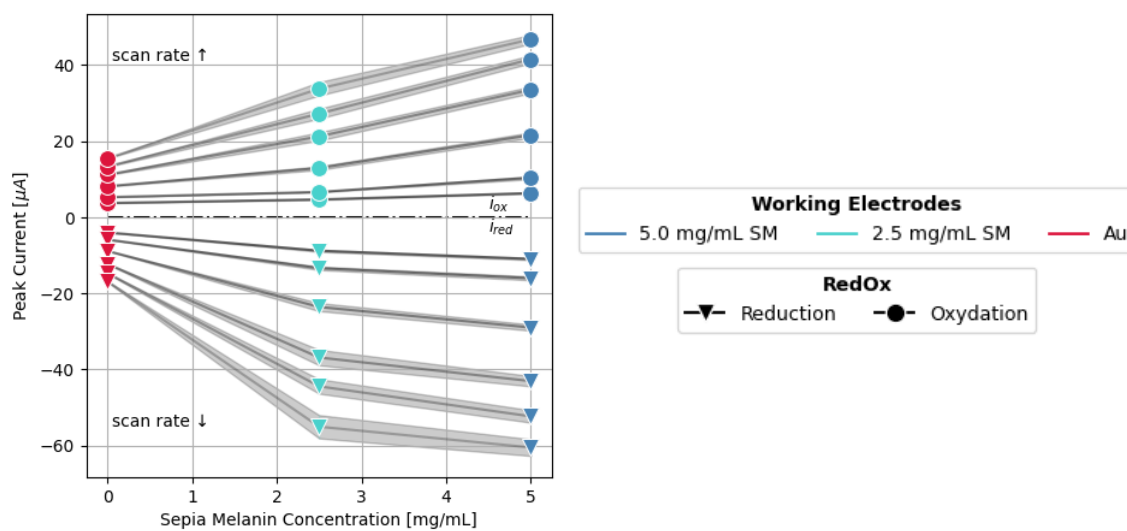


Figure 4.12 Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, and 100 mV s^{-1})

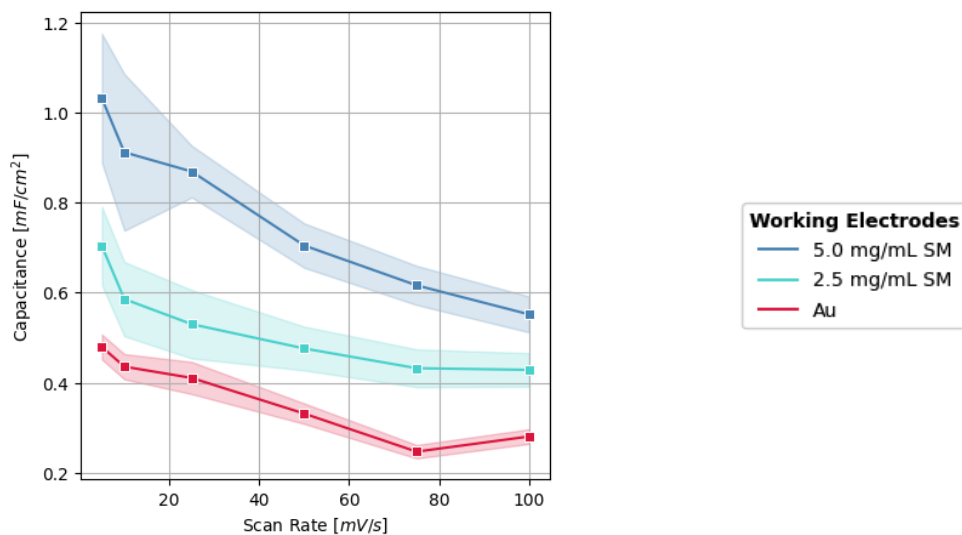


Figure 4.13 Electrode capacitance at $E_c = 200$ mV as a function of the scan rate.

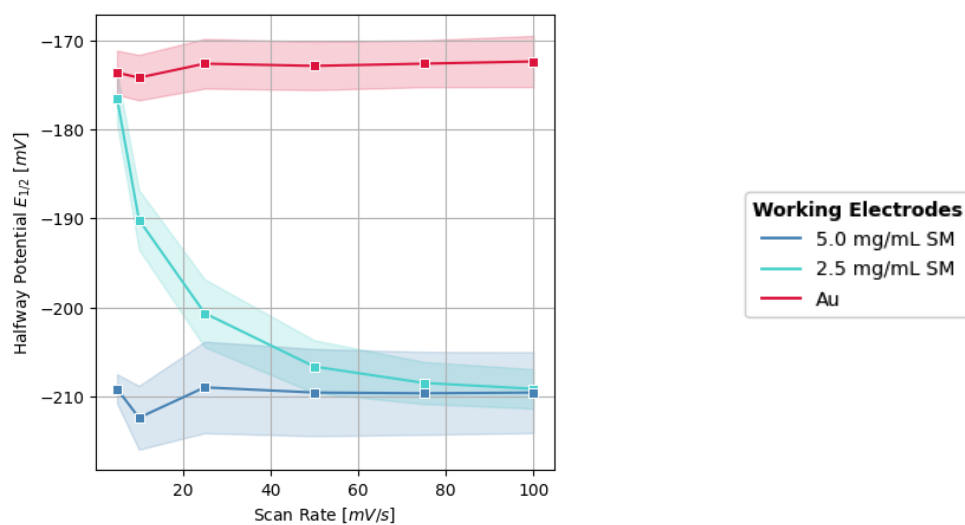


Figure 4.14 Halfway peaks as a function of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.

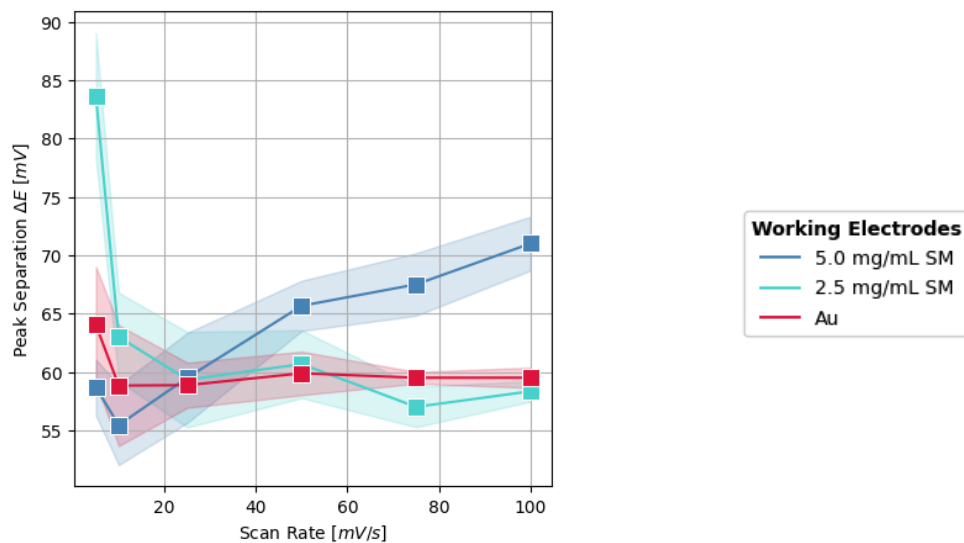


Figure 4.15 Redox peak separation as a function of the scan rate.

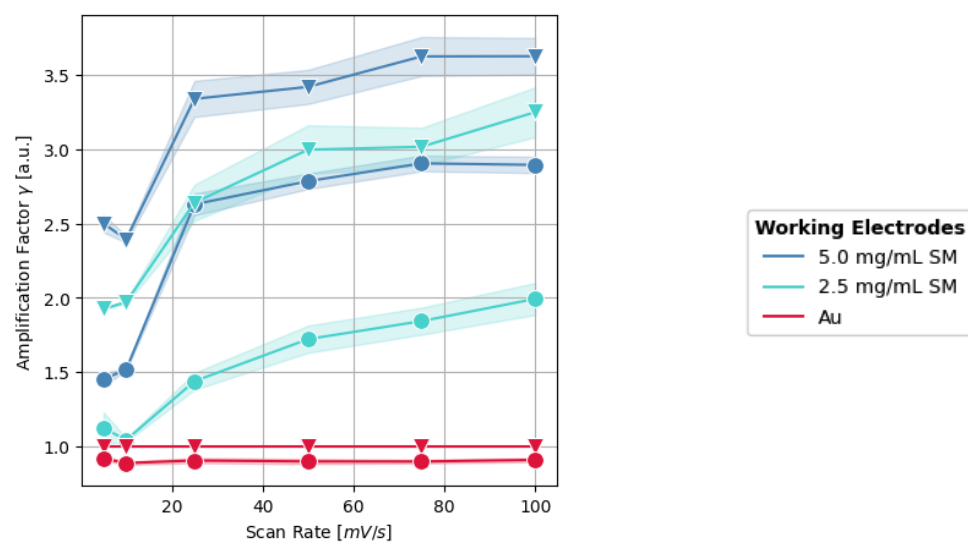


Figure 4.16 Amplification factor γ as a function of the square root of the scan rate for 1 mM Ruthenium Hexaamine in 1 M KCl.

4.2.2 Dopamine

The cyclic voltammograms obtained for the dopamine redox couple for GE and SME are shown in fig. 4.17. Well-defined redox peaks can be observed for all types of electrodes and scan rates. Using data from these voltammograms, several parameters were calculated and are shown in figs. 4.18 to 4.23.

At most scan rates, both modified and unmodified electrodes exhibit the characteristic quasi-reversible redox couple of dopamine, where the oxidation peaks are more prominent than the reduction peaks, with peak currents also increasing linearly with the square root of the scan rate, as shown in fig. 4.18. Although 2.5 mg mL^{-1} SME currents are comparable to those of the unmodified GE, currents that are greater by one order of magnitude are observed when using the 5.0 mg mL^{-1} SME. In addition to the increased currents, the measured redox peaks of SM-modified electrodes are much broader than those of its unmodified counterpart.

The dependence of redox currents with respect to SM concentration, shown in fig. 4.19, is non-linear for oxidation currents for all scan rates, while the reduction currents are more linear at low scan rates and become non-linear at scan rates above 10 mV s^{-1} . To add, the dependence becomes increasingly superlinear as the scan rates get higher.

Figure 4.20 shows the electrode capacitance, calculated by eq. (3.1), which decreases progressively with scan rate. The higher concentration of SM, the higher the capacitance. The capacitance of the 2.5 mg mL^{-1} SME is much closer to that of the GE than the 5.0 mg mL^{-1} SME, which indicates a non-linear dependence of capacitance as a function of SM concentration.

Peak separation as a function of scan rate, shown in fig. 4.21, behaves differently for each electrode type. For GE, it stays around 64 mV for scan rates slower than 75 mV s^{-1} , where it goes down to 59 mV . For 5.0 mg mL^{-1} SME, ΔE increases steadily from 58 mV at 5 mV s^{-1} to 71 mV at 100 mV s^{-1} . Lastly, for 2.5 mg mL^{-1} SME, ΔE starts very high at 84 mV at 5 mV s^{-1} , and quickly drops to 64 mV at 10 mV s^{-1} and stays around 60 mV at scan rates above 25 mV s^{-1} .

Figure 4.22 shows the halfway potentials $E_{1/2}$ for each type of electrode. For 5 mg mL^{-1} SME, the halfway potential fluctuates around 240 mV for all scan rates except 5 mV s^{-1} . The extremely high halfway potential at 5 mV s^{-1} is likely due to the algorithm having difficulty finding the real peak due to the extreme peak broadening that happens at such a slow scan rate with this high concentration of SM. For GE, $E_{1/2}$ also stays constant for all scan rates, but at a lower potential around 170 mV . For 2.5 mg mL^{-1} SME, the halfway potential starts even lower at 150 mV for 5 mV s^{-1} and stops increasing at around 164 mV

from 50 mV s^{-1} to 100 mV s^{-1} , which is close to the GE halway potential.

The amplification factor γ , shown in fig. 4.23, increases with scan rate, and with SM concentration. A higher γ_{red} than γ_{ox} is observable for all electrode types. However, for 2.5 mg mL^{-1} SM, the oxidation amplification is much lower than the reduction amplification.

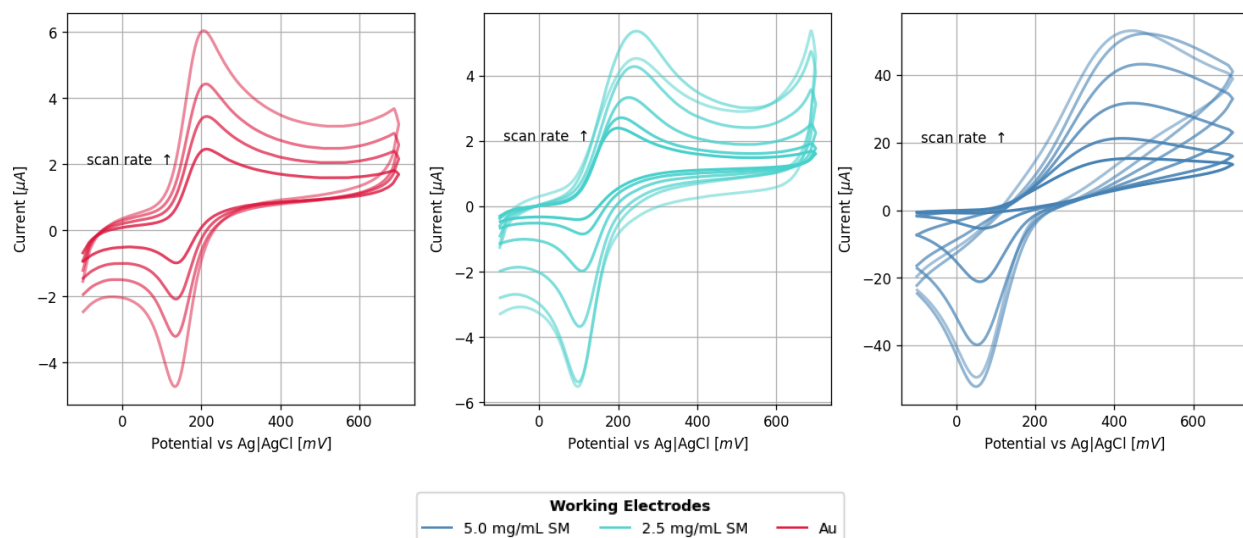


Figure 4.17 Representative CVs of $250 \text{ } \mu\text{M}$ Dopamine Hydrochloride in 0.01 M PBS with varying MNP concentration working electrodes at different scan rates ($5\text{-}100 \text{ mV s}^{-1}$).

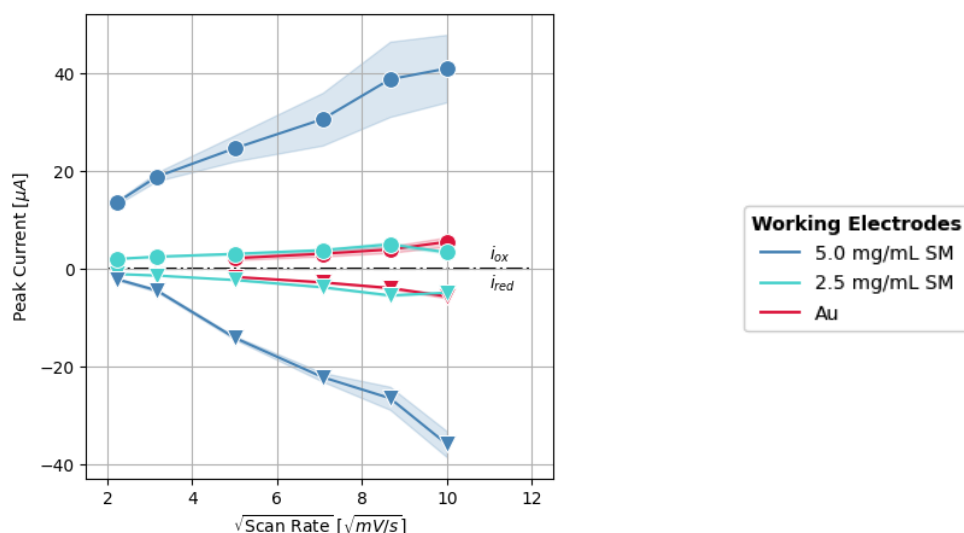


Figure 4.18 Peak redox currents as a function of the square root of the scan rate for $250 \text{ } \mu\text{M}$ Dopamine Hydrochloride in 0.01 M PBS.

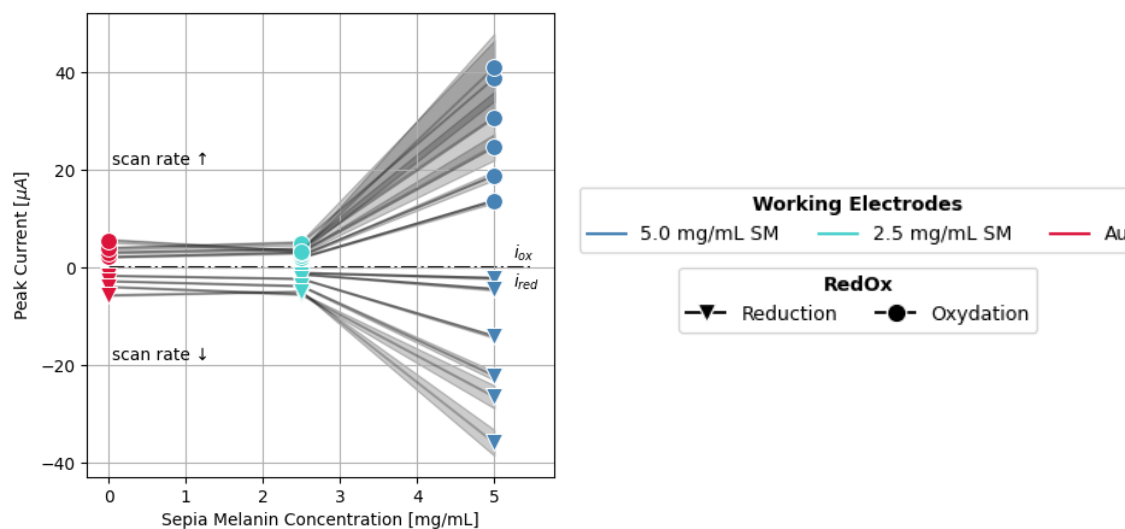


Figure 4.19 Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, and 100 mV s^{-1})

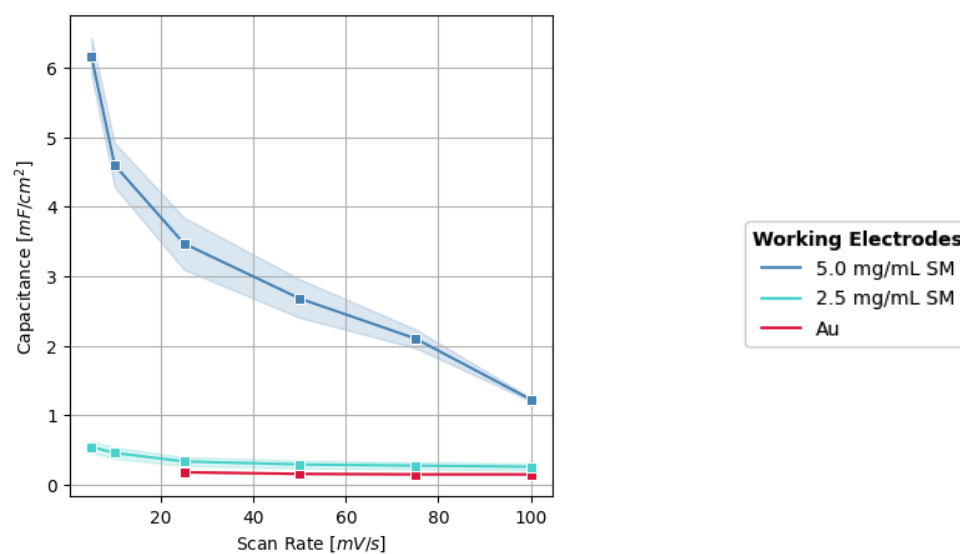


Figure 4.20 Electrode capacitance at $E_c = 0 \text{ mV}$ as a function of the scan rate.

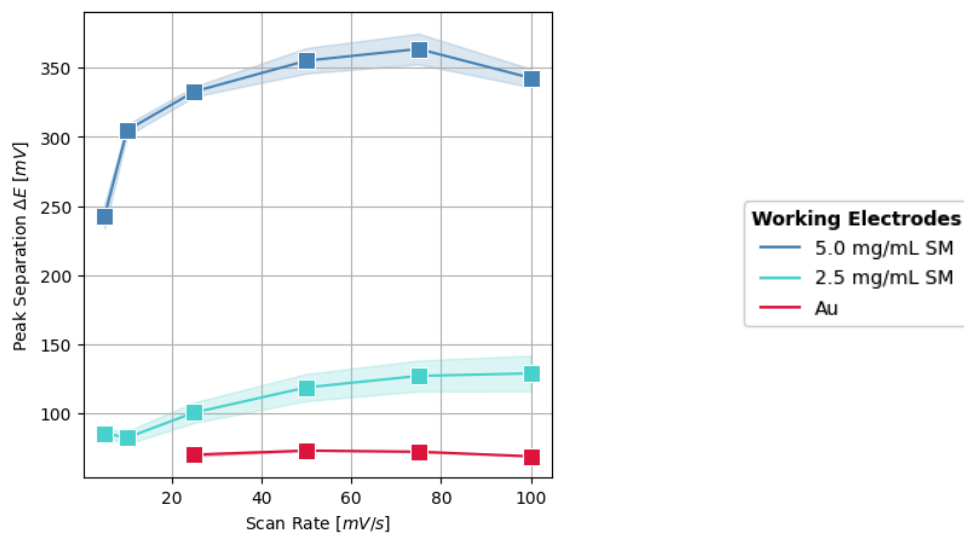


Figure 4.21 Redox peak separation as a function of the scan rate.

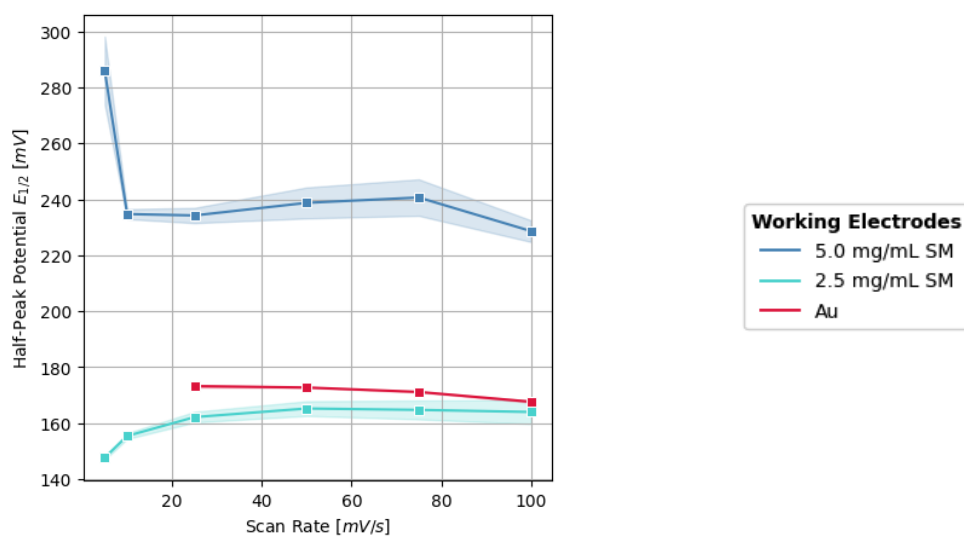


Figure 4.22 Halfway peaks as a function of the scan rate for 250 μM Dopamine Hydrochloride in 0.01 M PBS.

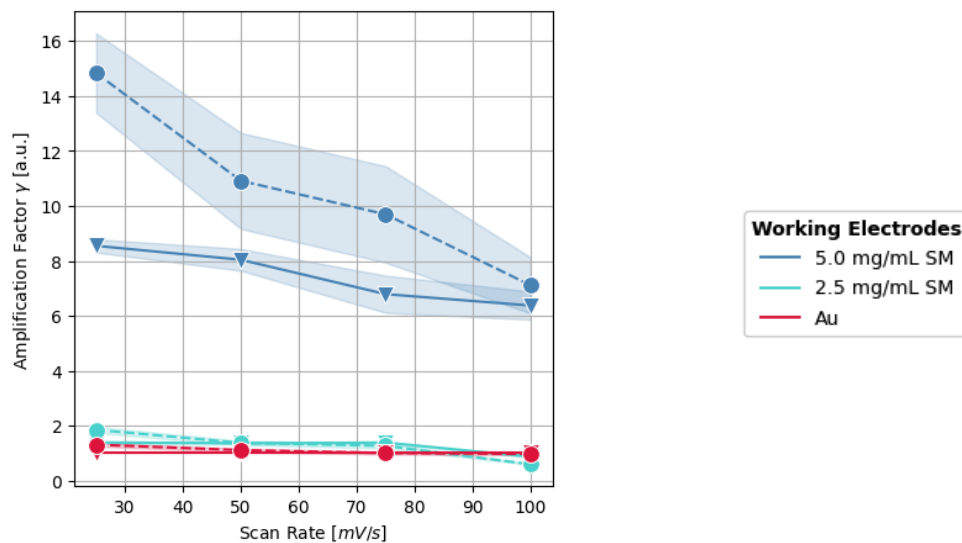


Figure 4.23 Amplification factor γ as a function of the square root of the scan rate for 250 μM Dopamine Hydrochloride in 0.01 M PBS.

4.2.3 Ascorbic Acid

The cyclic voltammograms obtained for the 250 μM L-Ascorbic Acid in 0.01 M PBS redox couple for GE and SME are shown in fig. 4.24. For this irreversible redox reaction, oxidation peaks can be clearly observed for both types of electrodes and all scan rates, but reduction is much less present and mostly undetectable by the algorithm. Using data from these voltammograms, several parameters were calculated and are shown in figs. 4.25 to 4.27.

At all scan rates, both modified and unmodified electrodes exhibit the characteristic irreversible redox couple of l-ascorbic acid, where the oxidation peaks are prominent, and the reduction peaks are weak if present at all, with the peak oxidation currents increasing with the square root of the scan rate, as shown in fig. 4.18. Although 5.0 mg mL^{-1} SME currents are smaller than those of the unmodified GE, the dependence of the current to the square root of the scan rate is non-linear for the modified electrodes. The measured redox peaks of SM-modified electrodes are also much broader than those of the GE.

Figure 4.26 shows the electrode capacitance, calculated by eq. (3.1), which decreases progressively with scan rate. The SM-modified electrodes exhibit a higher capacitance than that of their unmodified counterparts.

The amplification factor γ , shown in fig. 4.27, for all scan rates, is smaller than unity, meaning the peak currents of the GE are greater than those of the SME. γ increases slightly from 5 to 25 mV s^{-1} , but decreases for scan rates of 50 mV s^{-1} and above.

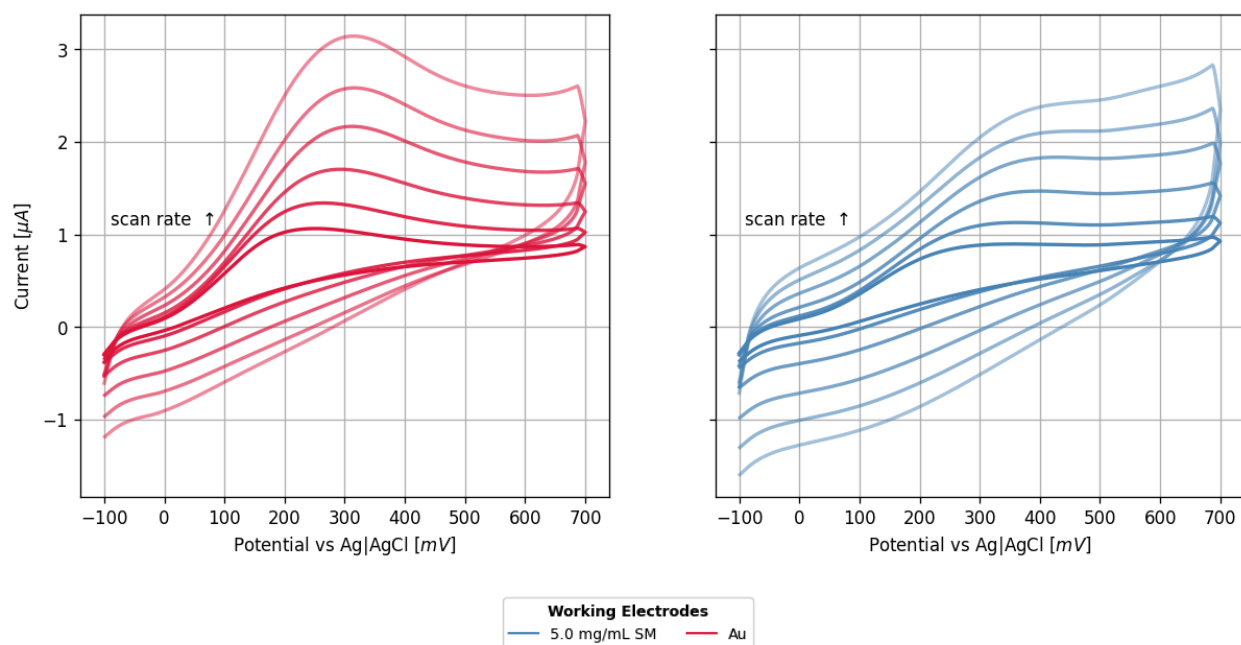


Figure 4.24 Representative CVs of 250 μM L-Ascorbic Acid in 0.01 M PBS with 5.0 mg mL^{-1} MNP concentration working electrodes at different scan rates (5-100 mV s^{-1}).

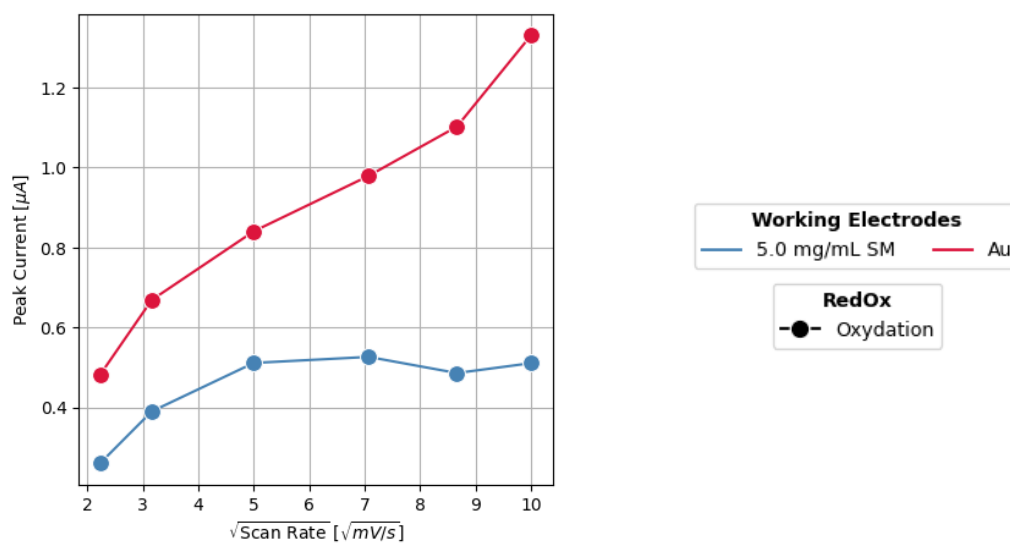


Figure 4.25 Peak redox currents as a function of the square root of the scan rate for 250 μM L-Ascorbic Acid in 0.01 M PBS.

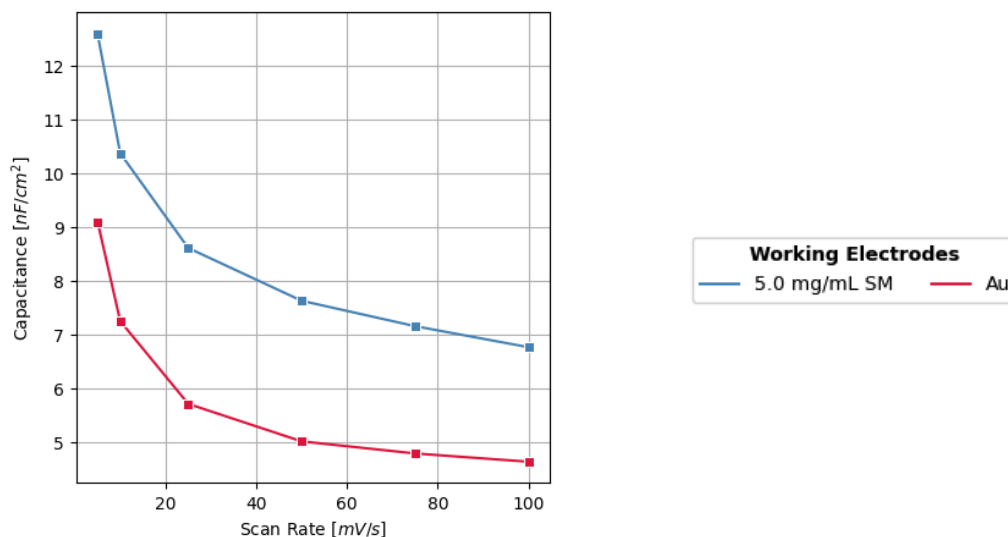


Figure 4.26 Electrode capacitance at $E_c = 0$ mV as a function of the scan rate.

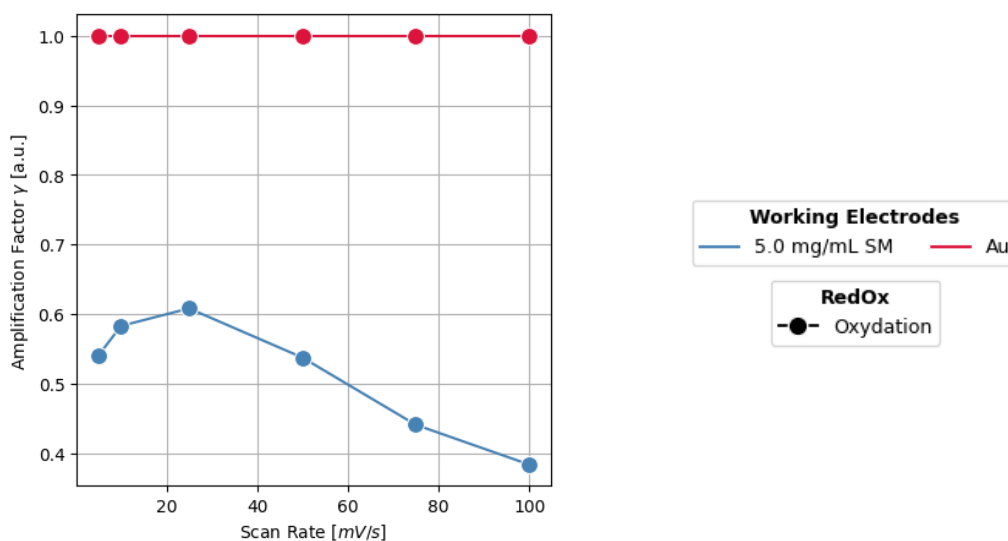


Figure 4.27 Amplification factor γ as a function of the square root of the scan rate for 250 μ M L-Ascorbic Acid in 0.01 M PBS.

4.2.4 Discussion

The results shown in this section (section 4.2) show that the surface modification of a gold disk electrode using SM will increase sensitivity to both ruthenium hexaamine and dopamine, but not to L-ascorbic acid, and that the increase in faradic current is dependent on the concentration of SM ink that is deposited onto the electrode surface.

Let us start with ruthenium hexamine detection. The peak reduction currents i_{red} being larger in magnitude than the peak oxidation currents i_{ox} as seen in fig. 4.11 indicate that ruthenium hexamine is easier to reduce than oxidize, which is consistent with the literature [62], and can be seen in fig. 4.16, where $\gamma_{red} > \gamma_{ox}$ for all electrodes at all scan rates. An increase in non-linearity with scan rate of the dependence of peak currents as a function of concentration, as shown in fig. 4.12. Ruthenium hexamine is an outer-sphere redox couple, which means the electron transfer between the analyte and the electrode is fast because it does not need to adsorb or bond to the electrode for it to happen [62]. As we add more matter (in this case SM) to the electrode surface, the resistivity of the electrode increases. With an increase in scan rate, we have an increase in diffusion, and so the RC characteristics of the SM have a greater impact on charge transfer. This is further supported by fig. 4.15, where the peak separation is fairly equal for all electrodes, but as the scan rate goes above 25 mV s^{-1} , the peak separation increases with the scan rate for the electrodes with the highest concentration of SM (5.0 mg mL^{-1}). As fig. 4.13 shows, as we add more SM, the electrode capacitance increases, which is the expected outcome, as more charge can be accumulated in more material. Figure 4.14 shows that the halfway potential $E_{1/2}$ is constant for the bare gold electrodes and the 5.0 mg mL^{-1} SME, but the 2.5 mg mL^{-1} SME exhibit some interesting properties. At 5 mV s^{-1} , its $E_{1/2}$ is close to that of the gold electrodes (-175 mV), but it rapidly decreases as the scan rate increases to reach the same value as the high concentration (5.0 mg mL^{-1}) SME (-210 mV). This indicates that the scan rate ν

In terms of dopamine detection, the oxidation peaks are greater than the reduction peaks, as seen in fig. 4.18, which is consistent with the literature [63]. Redox peak currents are greatly amplified with the concentration of SM present on the modified electrodes. This effect is much greater with 5 mg mL^{-1} SM than 2.5 mg mL^{-1} , as can be seen in fig. 4.19 and fig. 4.23, where γ is less than 2 for the 2.5 mg mL^{-1} SM electrodes, while it is greater than 6 for 5 mg mL^{-1} SM, even going as high as ~ 15 for the oxidation peaks at 5 mV s^{-1} . However, the high concentration in SM also leads to extreme peak broadening, as well as an increase in $E_{1/2}$, and a ΔE that is more than two times greater for 5.0 mg mL^{-1} SM than 2.5 mg mL^{-1} . In addition to the increase in faradic currents, the capacitance of the electrode also increased greatly with SM concentration, as shown in fig. 4.20.

To determine whether the increase in peak currents during cyclic voltammogram acquisition was due to the increase in the effective electrode surface area, l-ascorbic acid was studied using the same electrode types. The main difference between l-ascorbic acid and the other two analytes is that l-ascorbic acid is an almost irreversible oxydative reaction. This means that once ascorbic acid oxydizes with the electrode surface, it does not easily reduce [16]. As can be seen in fig. 4.24, the reduction peaks for $250 \text{ }\mu\text{M}$ L-Ascorbic Acid in 0.01 M PBS are so

small that they are almost undetectable by eye and very unreliably detected by the python algorithm. Unlike the detection of the other two analytes that were studied, as shown in fig. 4.25 and fig. 4.27, the SME performed worse than the unmodified GE.

The non-amplification of l-ascorbic acid means that the increase in the electrode's effective surface area due to the increased roughness after the addition of the SM-based bioink is not the reason for faradic current amplification. This behaviour is more consistent with bipolar electrode systems that exploit redox cycling, which is a process in which a molecule repeatedly cycles through reduction and oxidation [64]. This process requires a reversible redox pair to work because if the process is not reversible, the analyte cannot cycle between both states. Repetition of the same molecule oxidoreduction over and over means that the amount of exchanged electrons increases because, instead of reacting once, it continues to react, generating a greater flow of electrons. Since MNPs would be responsible for this cycling, then their concentration directly affects γ . Figure 4.28 shows an illustration of the hypothesised redox cycling working principle behind current amplification. At the electrode surface, the gold electrode is shown at potential $E_{electrode}$, and an adsorbed MNP. Because an MNP can be represented by an equivalent circuit of a parallel Resistor-Capacitor circuit [19], the potential at the SM granule's surface is $|E_{nanoparticle}| < |E_{electrode}|$, functioning like a passive bipolar electrode [64]. As granules stack on top of each other, their sum could potentially be equated to a series of parallel RC circuits.

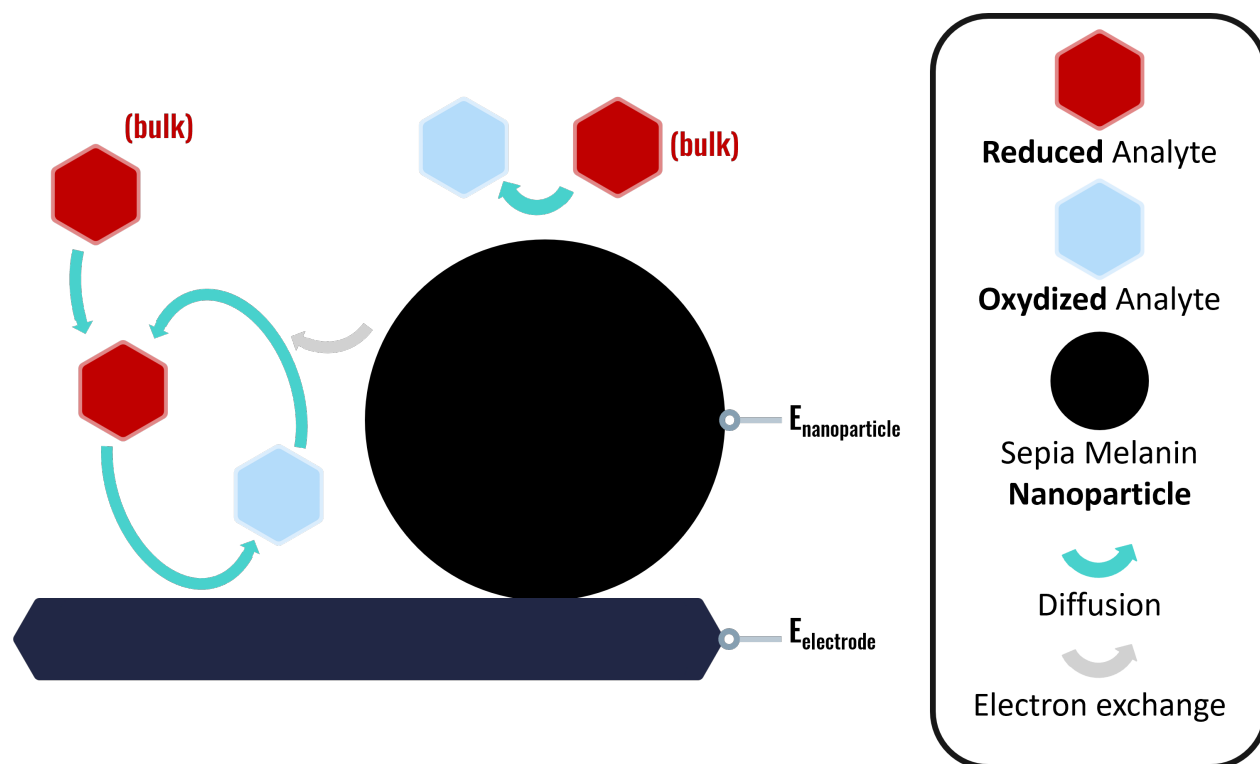


Figure 4.28 Proposed working principle of redox-cycling with a nanoparticle. $E_{\text{electrode}}$ and $E_{\text{nanoparticle}}$ are the applied potential at the electrode and the real potential at the nanoparticle respectively.

4.3 Electrochemistry on Paper Electrodes

This section details the results obtained for the electrochemical measurements of 1 mM Ruthenium Hexaamine in 1 M KCl (section 4.3.1), and 250 μM Dopamine Hydrochloride in 0.01 M PBS (section 4.3.2) that were performed using paper based LIG electrodes as a base. All calculations for this section are detailed in section 3.8.

4.3.1 Ruthenium Hexaamine

The cyclic voltammograms obtained for the ruthenium hexamine redox couple for LIG and SM-LIG are shown in fig. 4.29. Well-defined redox peaks can be observed for all types of electrodes and scan rates, except for the reduction peaks with 5.0 mg mL^{-1} SM-LIG. Using data from these voltammograms, several parameters were calculated and are shown in figs. 4.30 to 4.35.

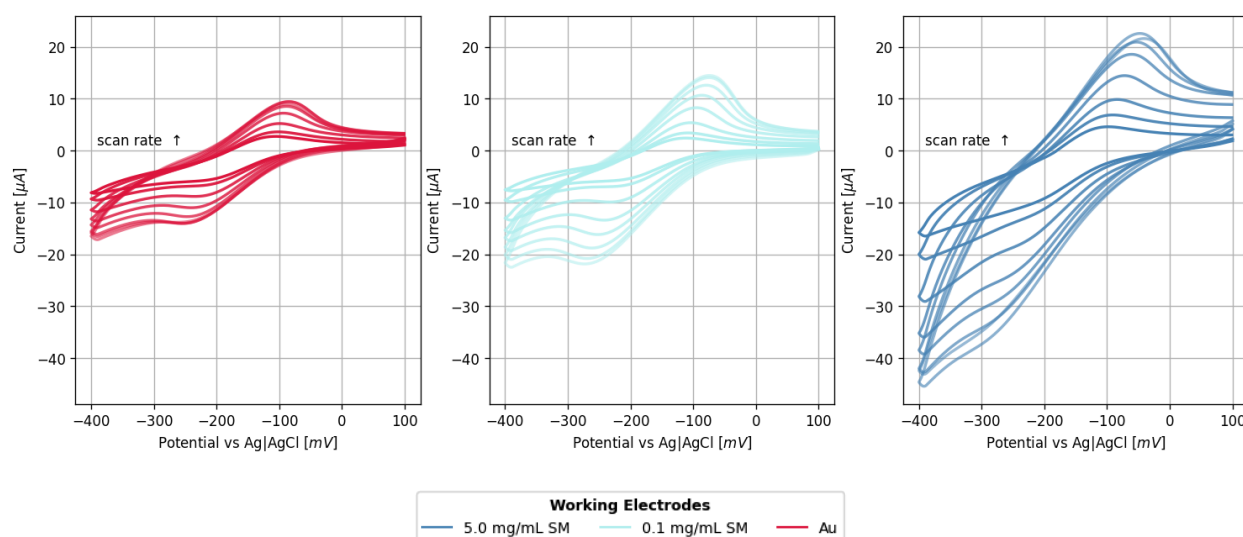


Figure 4.29 Representative CVs of 1 mM Ruthenium Hexaamine in 1 M KCl with varying MNP concentration working electrodes at different scan rates (5, 10, 25, 50, 75, 100, 125, 150, 200 mV s^{-1}).

Figure 4.30 shows the dependence of peak currents as a function of the square root of the scan rate. At all scan rates, both modified and unmodified electrodes exhibit the characteristic reversible redox couple of ruthenium hexamine. However, the capacitive currents have a more aggressive curve as the scan rate increases for all electrodes, giving the Python algorithm trouble when detecting a baseline, which leads to erratic oxidation currents. This issue is

less present for the reduction peaks, as the capacitive current is fairly linear, showing a linear dependance with the square root of the scan rate.

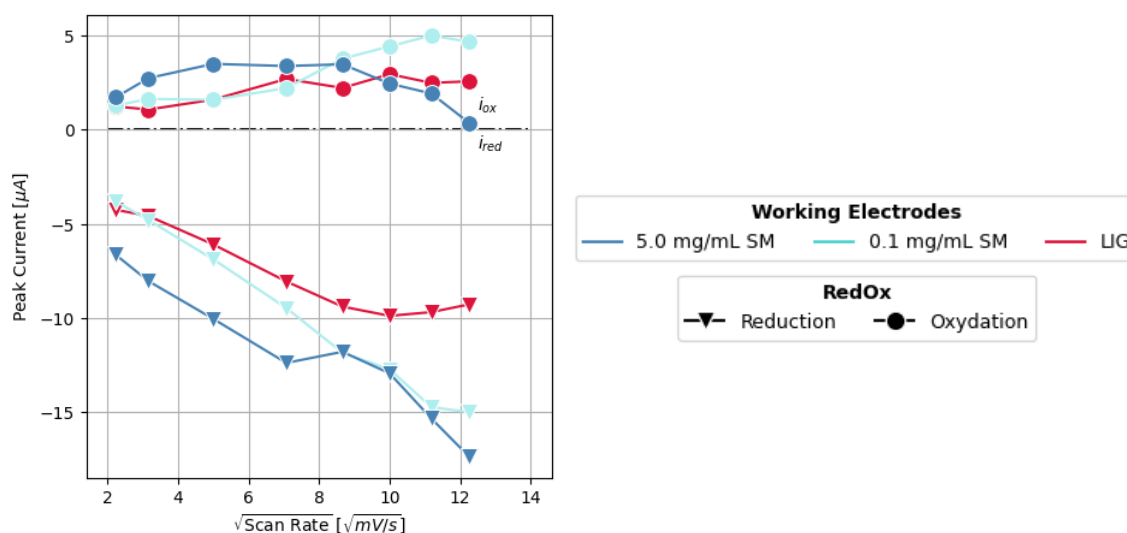


Figure 4.30 Peak redox currents as a function of the square root of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.

Figure 4.31 shows the peak redox currents as a function of SM concentration for different scan rates. As like mentioned previously, the peak detection is not as accurate as it needs to be. This makes the peak oxidation currents erratic. As for the peak reduction currents, the dependance on SM concentration seems to be non-linear, increasing faster from the LIG to 0.01 mg mL⁻¹ SM-LIG than 0.01 mg mL⁻¹ SM-LIG to 5.0 mg mL⁻¹ SM-LIG.

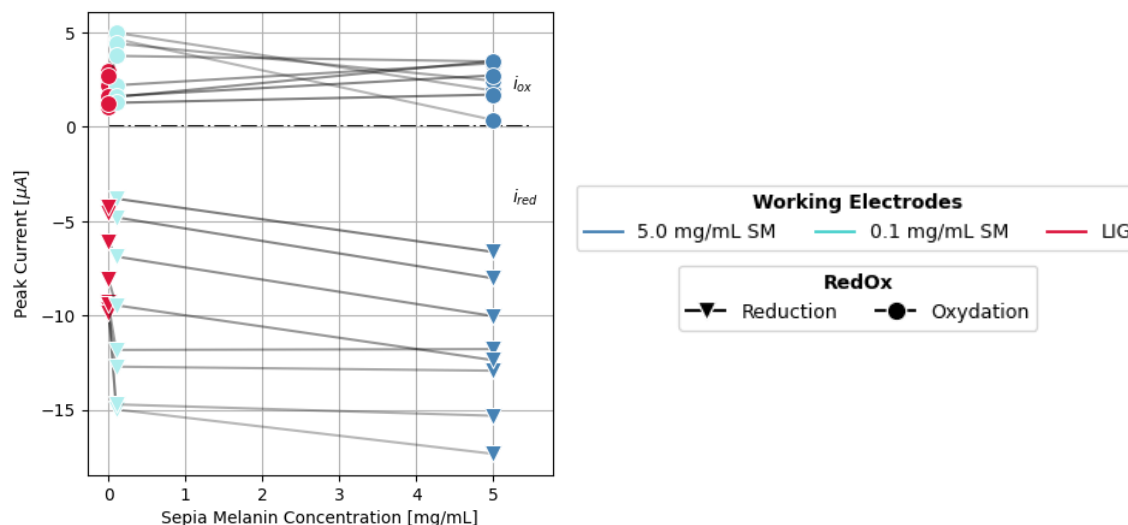


Figure 4.31 Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, 100, 125, 150, 200 mV s^{-1})

Figure 4.32 shows the electrode capacitance, calculated by eq. (3.1), which decreases progressively with scan rate. The 0.1 mg mL^{-1} SM-LIG has a very similar capacitance to the unmodified LIG, while the 5.0 mg mL^{-1} SM-LIG has the highest capacitance of the three.

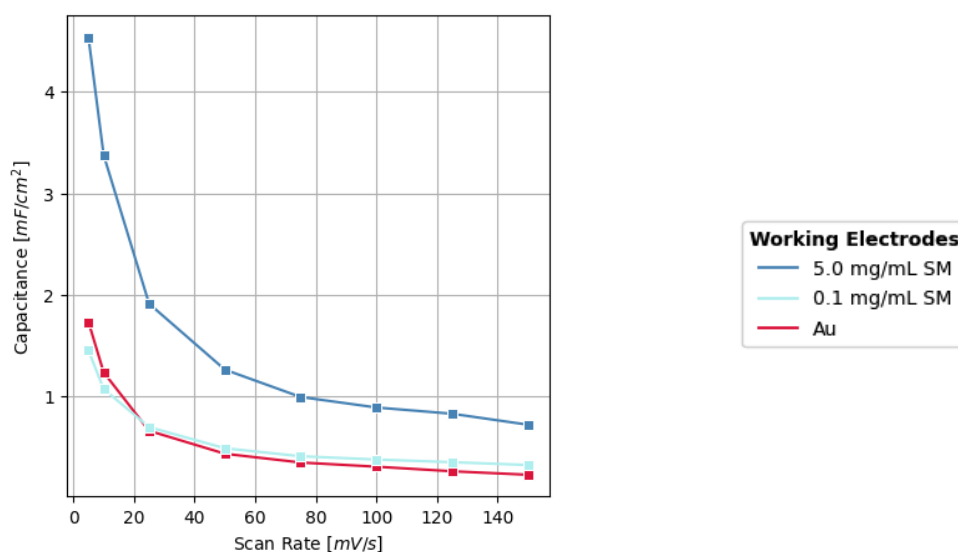


Figure 4.32 Electrode capacitance at $E_c = 200 \text{ mV}$ as a function of the scan rate.

Figure 4.33 shows peak separation as a function of scan rate. All three electrodes exhibit the same relation between peak separation and scan rate: a mostly linear increase of peak separation

ration as the scan rate increases. The 5.0 mg mL^{-1} SM-LIG has the highest peak separation, followed by the 0.1 mg mL^{-1} SM-LIG, and the unmodified LIG has the lowest. However, the LIG and the 0.1 mg mL^{-1} SM-LIG peak separations are similar from 5 up to 50 mV s^{-1} , but the rate at which the peak separation increases slows when comparing the LIG to the 0.1 mg mL^{-1} SM-LIG.

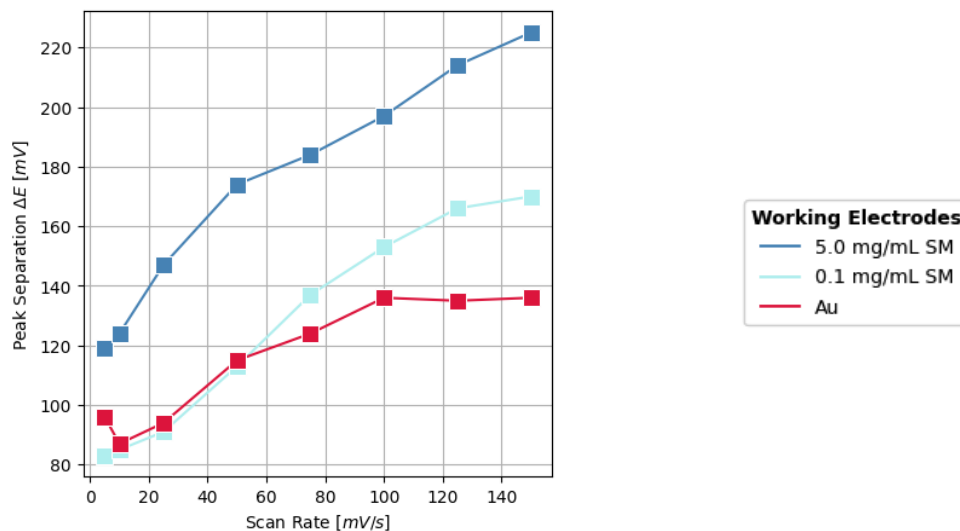


Figure 4.33 Redox peak separation as a function of the scan rate.

Figure 4.34 shows the halfway potentials $E_{1/2}$ for each type of electrode. For 0.1 mg mL^{-1} SM-LIG and the unmodified LIG, the halfway potential stays more or less around -175 mV for all scan rates above 10 mV s^{-1} . For 5.0 mg mL^{-1} SM-LIG, $E_{1/2}$ is inversely proportionate to scan rates, starting around -190 mV at 5 mV s^{-1} and -218 mV at 150 mV s^{-1} .

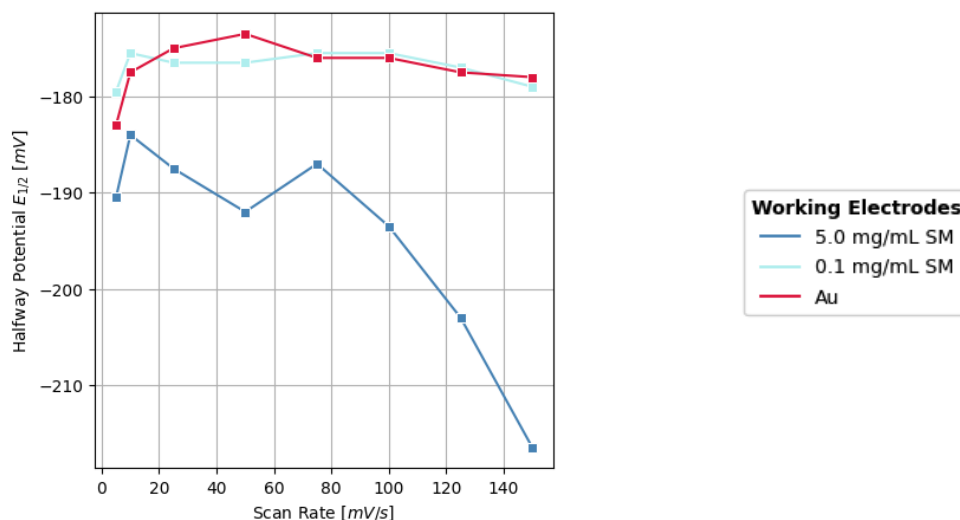


Figure 4.34 Halfway peaks as a function of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.

The amplification factor γ , shown in fig. 4.35, increases with scan rate, and with SM concentration for the oxidation peaks, but changes little for the oxidation peaks. A higher γ_{red} than γ_{ox} is observable for all electrode types.

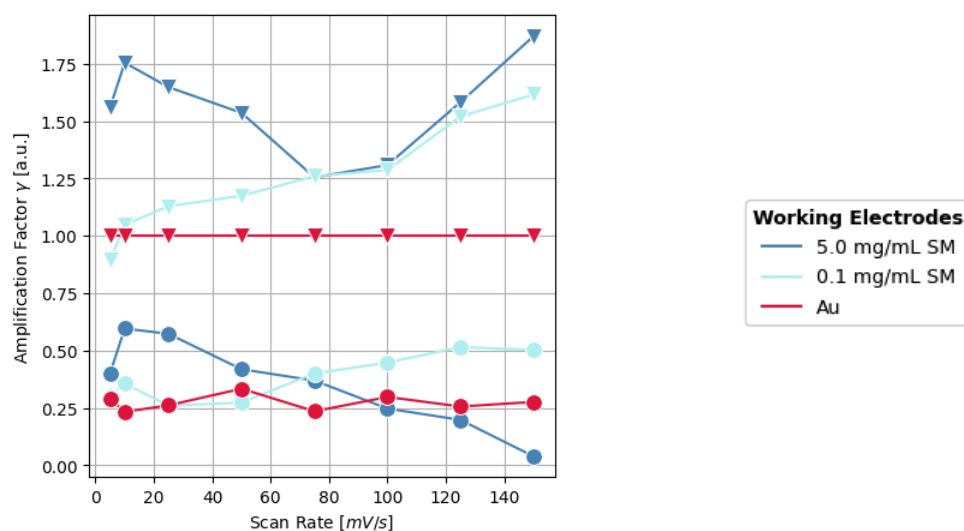


Figure 4.35 Amplification factor γ as a function of the square root of the scan rate for 1 mM Ruthenium Hexamine in 1 M KCl.

4.3.2 Dopamine

The cyclic voltammograms obtained for the dopamine redox couple for LIG and SM-LIG are shown in fig. 4.36. Well-defined redox peaks can be observed for all types of electrodes and scan rates. Using data from these voltammograms, several parameters were calculated and are shown in figs. 4.37 to 4.42.

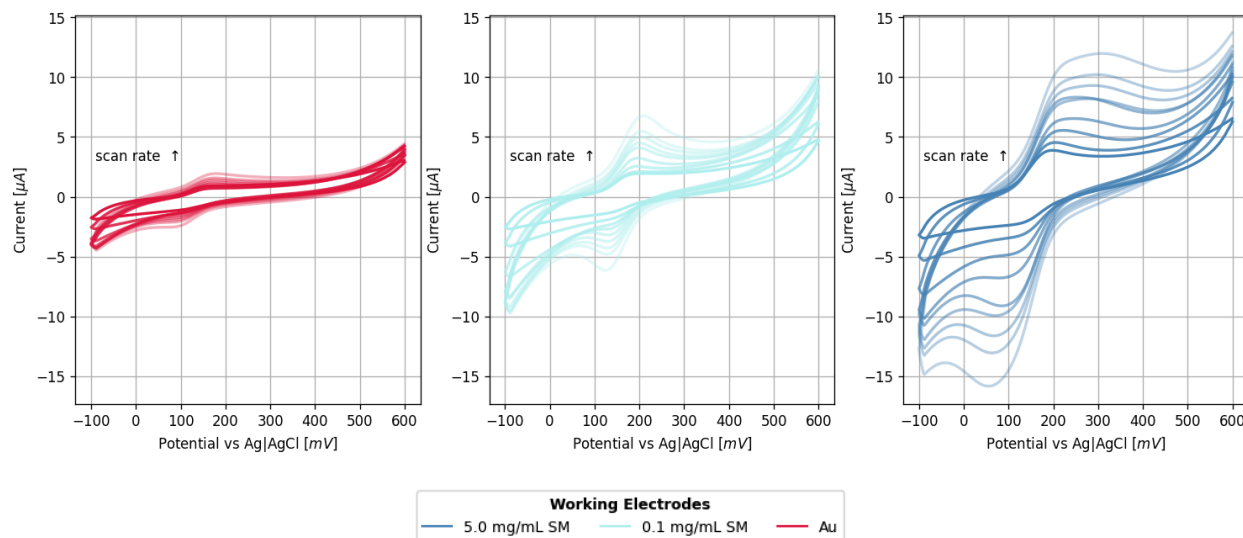


Figure 4.36 Representative CVs of 250 μM Dopamine Hydrochloride in 0.01 M PBS with varying MNP concentration working electrodes at different scan rates (5, 10, 25, 50, 75, 100, 125, 150, 200 mV s^{-1}).

At most scan rates, both modified and unmodified electrodes exhibit the characteristic quasi-reversible redox couple of dopamine, where the oxidation peaks seem more prominent than the reduction peaks, with peak currents also increasing linearly with the square root of the scan rate, as shown in fig. 4.18. The 5.0 mg mL^{-1} SM-LIG peak currents are greater than the 0.1 mg mL^{-1} SM-LIG, which are greater than the unmodified LIG currents. In addition to the increased currents, the measured redox peaks of the 5.0 mg mL^{-1} SM-LIG are much broader than those of its unmodified counterpart as well as the lower concentration 0.1 mg mL^{-1} SM-LIG, whose peak widths are comparable to those of the unmodified LIG.

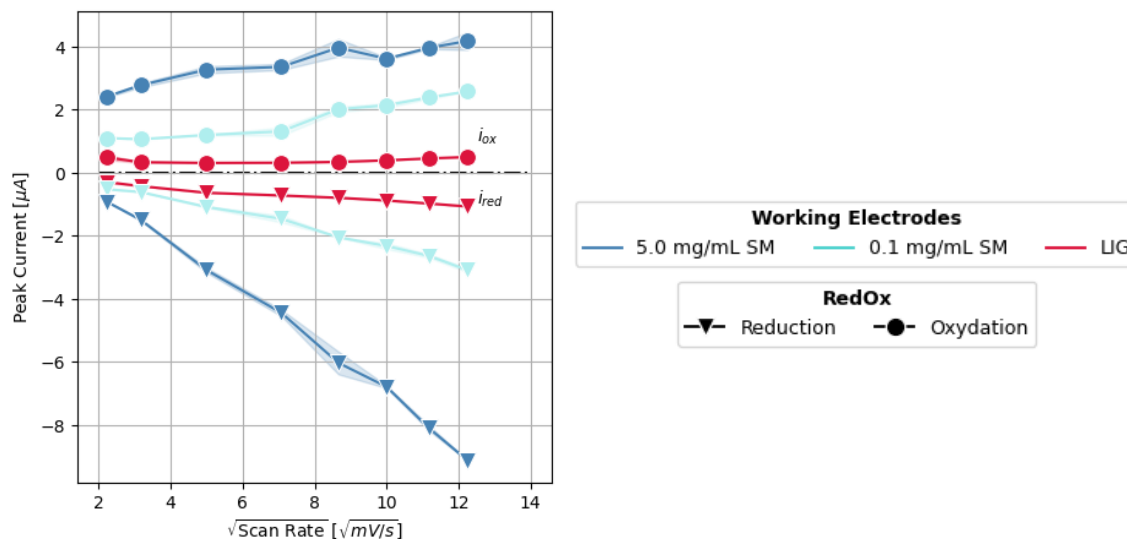


Figure 4.37 Peak redox currents as a function of the square root of the scan rate for 250 μM Dopamine Hydrochloride in 0.01 M PBS.

The dependence of redox currents with respect to SM concentration, shown in fig. 4.19, is non-linear for all scan rates, with the reduction currents being more sensitive to the scan rate.

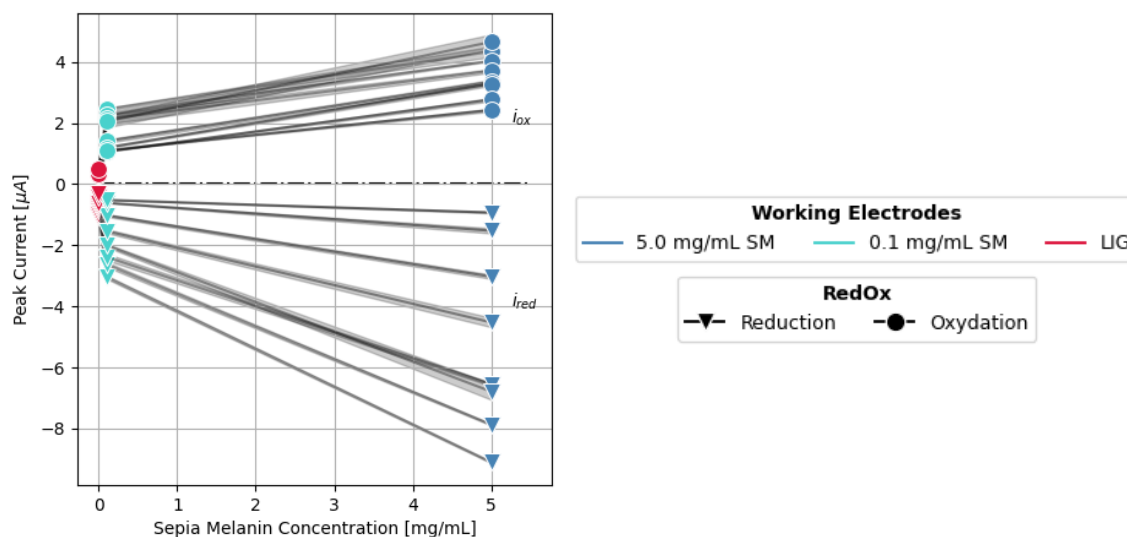


Figure 4.38 Peak redox currents as a function of SM concentration for different scan rates (5, 10, 25, 50, 75, and 100 mV s^{-1})

Figure 4.39 shows the electrode capacitance, which decreases progressively with scan rate.

The higher concentration of SM, the higher the capacitance. In this case, all three electrode types have similar capacitance.

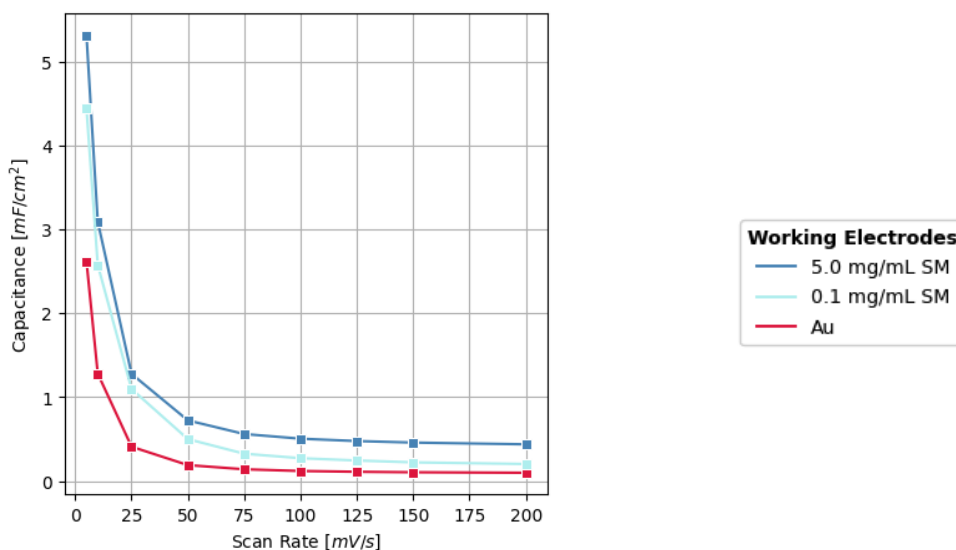


Figure 4.39 Electrode capacitance at $E_c = 0$ mV as a function of the scan rate.

Figure 4.40 shows peak separation as a function of scan rate for each electrode type. For the unmodified LIG, it starts around 73 mV at 5 mV s^{-1} , dips slightly to reach 61 mV at 25 mV s^{-1} and slowly rises until it reaches 65 mV at 200 mV s^{-1} . The 0.1 mg mL^{-1} SM-LIG behaves similarly to the unmodified electrodes, ΔE starting at 61 mV at 5 mV s^{-1} and dipping to a minimum of 43 mV at 50 mV s^{-1} , and then rises back up to 61 mV at 200 mV s^{-1} . For 5.0 mg mL^{-1} SM-LIG, ΔE increases steadily from 59 mV at 5 mV s^{-1} to 138 mV at 200 mV s^{-1} .

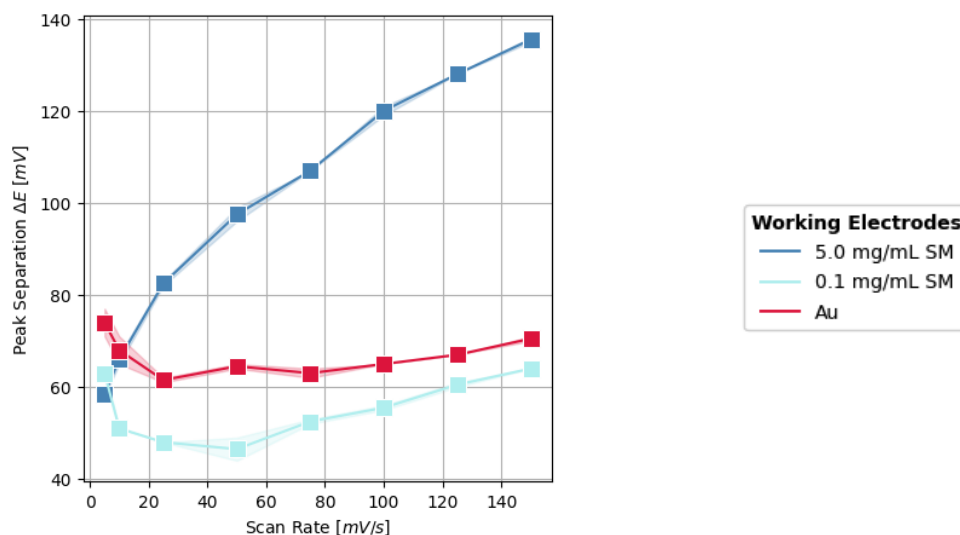


Figure 4.40 Redox peak separation as a function of the scan rate.

Figure 4.41 shows the halfway potentials $E_{1/2}$ for each type of electrode. For 5 mg mL⁻¹ SM-LIG, the halfway potential starts at 160 mV at 5 mV s⁻¹, and decreases linearly to 147 mV at 200 mV s⁻¹. For 0.1 mg mL⁻¹ SM-LIG, $E_{1/2}$ increases with the scan rate, from 155 mV at 5 mV s⁻¹ to 166 mV at 200 mV s⁻¹. For LIG, $E_{1/2}$ also increases slightly, from 119 mV at 5 mV s⁻¹ to 124 mV at 200 mV s⁻¹.

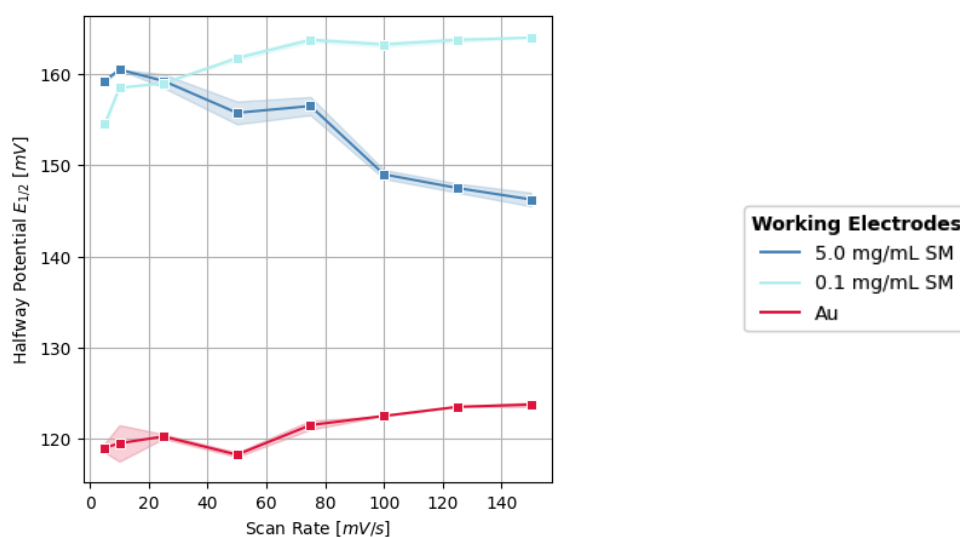


Figure 4.41 Halfway peaks as a function of the scan rate for 250 μM Dopamine Hydrochloride in 0.01 M PBS.

The amplification factor γ , shown in fig. 4.42, exhibits different behaviours for all three electrode types. The 5.0 mg mL⁻¹ SM-LIG electrodes show a higher γ_{ox} than γ_{red} at 5 mV s⁻¹ until an inflection point at 25 mV s⁻¹, where they are more or less equal, and at higher scan rates (50 mV s⁻¹ up to 200 mV s⁻¹) γ_{red} is greater than γ_{ox} . The 0.1 mg mL⁻¹ SM-LIG electrodes show a higher γ_{ox} than γ_{red} at 5 mV s⁻¹, with a decreasing γ_{ox} and increasing γ_{red} that meet around 25 mV s⁻¹ and increase almost linearly from then up to 200 mV s⁻¹. The unmodified LIG electrodes have a greater γ_{ox} than γ_{red} at 5 mV s⁻¹, but γ_{ox} decreases below γ_{red} at 10 mV s⁻¹ and stays around 0.5 from 25 mV s⁻¹ up to 200 mV s⁻¹.

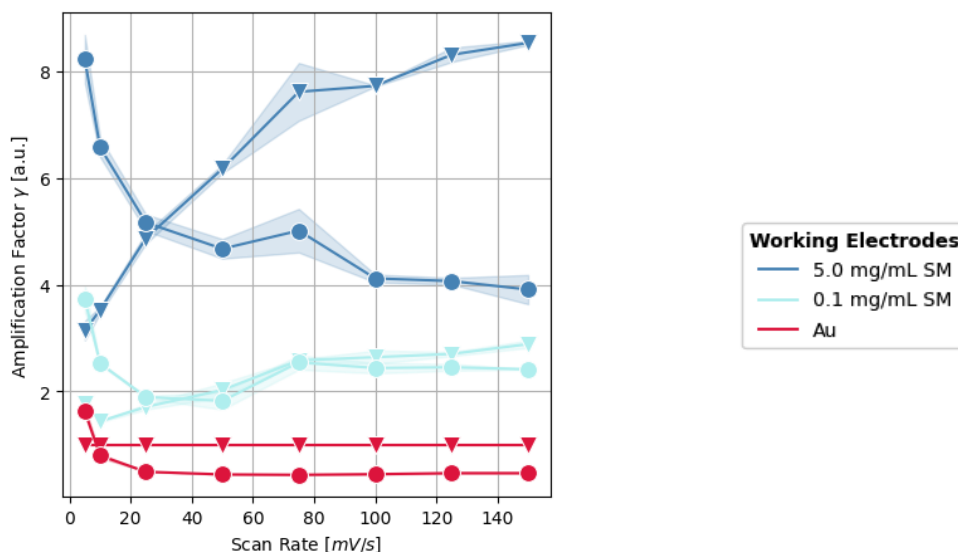


Figure 4.42 Amplification factor γ as a function of the square root of the scan rate for 250 μ M Dopamine Hydrochloride in 0.01 M PBS.

4.3.3 Exploratory Results at High Scan Rates

To explore the feasibility of using these electrodes with FSCV, faster scan rates than are commonly used in more classical CVs were explored (500 mV s⁻¹, 1000 mV s⁻¹ and 5000 mV s⁻¹). Figure 4.43 shows cyclic voltammograms obtained for dopamine redox couple for LIG and SM-LIG. All three electrodes exhibit large peak separations to the point they have difficulty detecting ruthenium hexaamine within the same electrochemical window used in section 4.3.1. This is especially true for the 5.0 mg mL⁻¹ SM-LIG electrodes, for which there is no reduction peak. For all three electrode types, the oxidation peak disappears above 100 mV at 5000 mV s⁻¹. For the unmodified LIG and the 0.1 mg mL⁻¹ SM-LIG electrodes, oxidation and reduction peaks are present at 500 mV s⁻¹ and 1000 mV s⁻¹.

At all scan rates, both modified and unmodified electrodes exhibit the characteristic quasi-

reversible redox couple of dopamine; however, the reduction and oxidation peaks seem fairly equal. The voltammograms obtained using 5.0 mg mL^{-1} SM-LIG have the highest measured currents, followed by 0.1 mg mL^{-1} SM-LIG, and then the unmodified LIG electrodes. In addition to the increased currents, there is an observable increase in capacitance, as well as an increase in peak broadness with SM concentration.

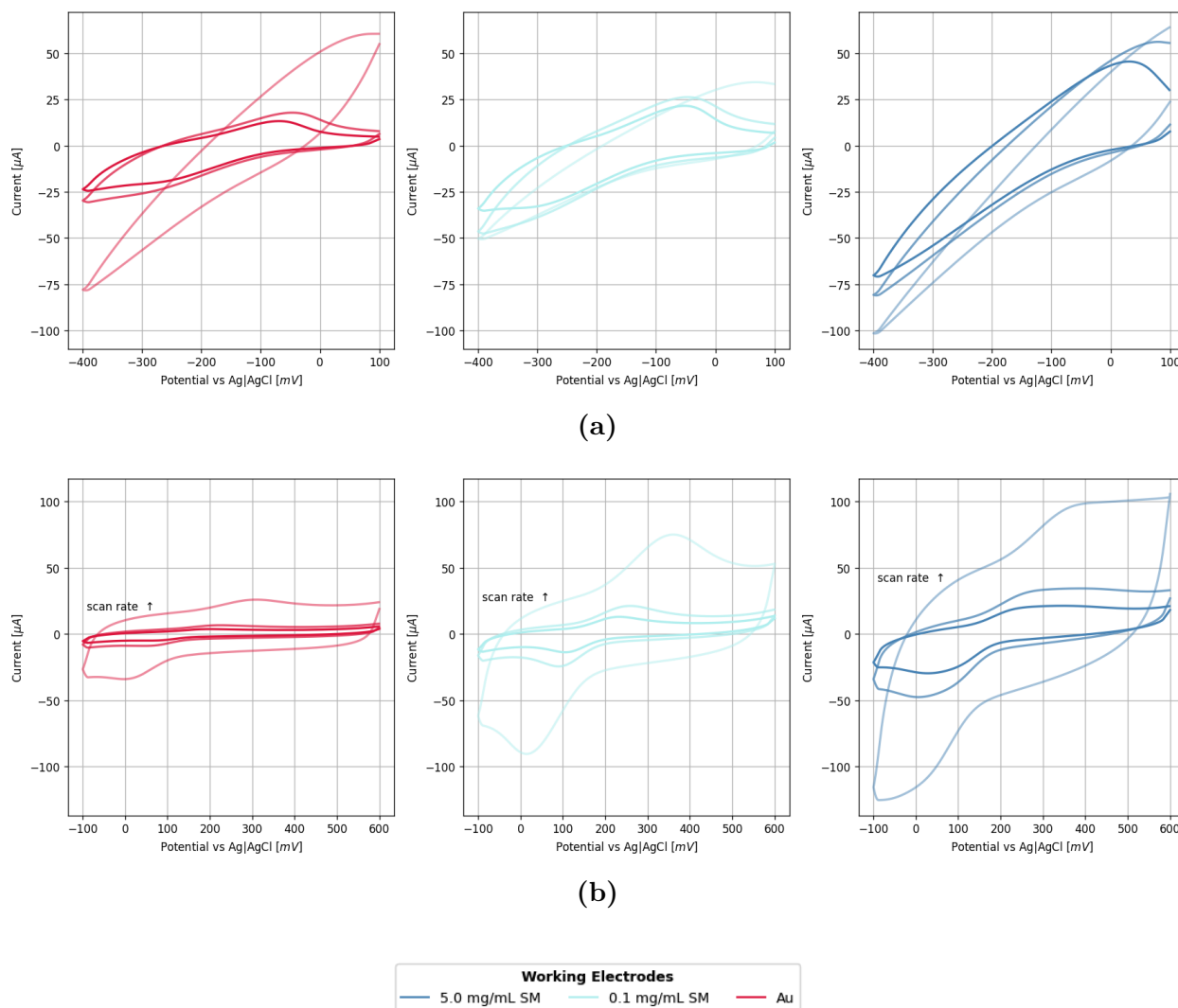


Figure 4.43 Representative CVs of (a) 1 mM Ruthenium Hexaamine in 1 M KCl and (b) 250 μM Dopamine Hydrochloride in 0.01 M PBS with varying MNP concentration working electrodes at different scan rates (500 mV s^{-1} , 1 V s^{-1} , 5 V s^{-1}).

4.3.4 Discussion

The results shown in this section (section 4.3) show that the surface modification of a LIG paper electrode using SM will increase sensitivity to both ruthenium hexaamine and dopamine, and that the increase in faradic current is dependent on the concentration of SM ink that is deposited onto the electrode surface.

With the detection of ruthenium hexaamine, much of the same effects seen in fig. 4.29 are the same as those found in section 4.2.1. However, there is a notable difference: the capacitive currents are much less linear than with gold electrodes. These capacitive effects make the approximation we stipulated in section 3.9 less than ideal, and leads to poor peak current detection, as the tiniest change in curvature leads to a large change in slope for i_c . Although i_{ox} was very unreliably detected by the python algorithm, i_{red} was more reliably calculated, and is the reaction of interest for this analyte. In addition to the increased capacitance, seen in fig. 4.32, fig. 4.33 shows an increased ΔE for the SM-LIG, where SM concentration increases the peak separation. Furthermore, $E_{1/2}$ is approximately equal for 0.1 mg mL⁻¹ SM-LIG, whereas $E_{1/2}$ is more electronegative for the higher concentration of 5.0 mg mL⁻¹ SM-LIG. All of these results are most likely due to the fact that the LIG paper electrodes have a much more complex three-dimensional morphology, which leads to a large increase in capacitance and affects the diffusion of species from the bulk of the solution to the inner pores of the electrode, all of which makes diffusion and subsequently the formation of an EDL more energetically costly [39]. A notable result is that γ seems unstable at a high SM concentration, but is non-zero for as little as 0.1 mg mL⁻¹ of SM.

As for dopamine detection, although the differences are not as drastic as seen in section 4.2.2 with gold electrodes, the results are similar, there is an increase in peak and capacitive currents as SM content increases, as can be seen in figs. 4.36 and 4.37. Figure 4.37 shows that $i_p \propto \sqrt{\nu}$, which means the system is diffusion controlled, according to eq. (2.6). Unlike the gold electrodes, peak currents increase non-linearly with SM concentration, but the increase is faster and tapers off. Another difference when comparing SM-LIG to SME is that reduction currents are greater than oxidation currents for 5.0 mg mL⁻¹ SM-LIG at higher scan rates, as shown in fig. 4.37. Figure 4.42 also shows that $\gamma_{red} > \gamma_{ox}$ at $\nu > 25$ mV s⁻¹ for 5.0 mg mL⁻¹ SM-LIG, and $\nu > 5$ for LIG, whereas $\gamma_{red} \approx \gamma_{ox}$ for 0.1 mg mL⁻¹ SM-LIG at $\nu > 25$ mV s⁻¹. This increase in reduction currents is quite stable for the unmodified LIG, but increases with scan rate for both SM-LIG electrodes, meaning a part of this increase is due to the paper electrode, and another part is due to the SM-modified electrode surface.

For the exploratory high scan rate results, it is evident that, at these speeds, ruthenium hexaamine detection needs to be tweaked, as the RC effects discussed previously are so

overwhelming, almost all distinctive CV features are lost at the same electrochemical window used previously. However, for dopamine, even 0.1 mg mL^{-1} SM-LIG is enough to increase the peak currents significantly. And, even though the peaks are shifting farther apart as the scan rate increases, the voltammograms are still viable for dopamine detection. It should be noted that, for the 5.0 mg mL^{-1} SM-LIG electrodes, the peaks are so broad, the voltammogram starts to resemble a sigmoid. This could mean that the packing of MNPs on the surface of the paper fibers could be optimized by maximizing the amplification and minimizing the capacitive and resistive effects tied to the concentration of SM on the surface.

CHAPTER 5 CONCLUSION

In this work, we evaluated how modifying gold disc electrodes, and paper-based electrodes, by drop-casting or dip-coating SM affects the electrochemical response of different redox active analytes, maintaining a focus on DA. By varying the density of SM nanoparticles on the electrode surface and using CV, we showed that the sepia melanin modified electrodes increased the faradic current, and therefore an increased DA detection.

5.1 Summary of Works

Firstly, the composition and morphology of the SM-based bioinks using UV-Vis, Raman, EDX, SEM and optical microscopy. The spectroscopy showed that the results of the SM extraction from cuttlefish ink was indeed SM, but it also contained many unknown organic impurities. Secondly, electrochemical testing on gold disk electrodes showed that the SME had increased the faradic and capacitive currents measured during the detection of 1 mM Ruthenium Hexaamine in 1 M KCl, and 250 μ M Dopamine Hydrochloride in 0.01 M PBS, but not 250 μ M L-Ascorbic Acid in 0.01 M PBS, due to redox cycling at the electrode-MNP interface. Lastly, electrochemical testing on paper electrodes showed that the SM-LIG also increased both the faradic and capacitive currents measured during the detection of 1 mM Ruthenium Hexaamine in 1 M KCl, and 250 μ M Dopamine Hydrochloride in 0.01 M PBS.

5.2 Limitations

There are many limitations that were not explored in the scope of this project. For the bioink composition, the melanin extraction protocol needs refinement, as the resulting material is not as pure as expected at the start of this endeavour. Since SM is biosourced, the extraction and purification protocol is crucial for the uniformity and reproducibility of samples, as the cuttlefish ink composition can vary with fishing seasons and many other uncontrollable factors [65]. As for the paper electrodes, there are multiple limitations. The large increase in capacitance associated with the SM-modified electrodes could hinder the electrode performance at higher scan rates, as can be observed in section 4.3.3 for 1 mM Ruthenium Hexaamine in 1 M KCl. The LIG electrodes' shape was kept as simple as possible to minimize the errors that could come from their delicate fabrication, but it could be optimised for better performance at high scan rates. Another limitation is electrode fragility. The graphitized paper is very frail, and can become damaged if not handled with extreme

care.

5.3 Future Research

Building upon the findings and limitations of this project, there are many potential avenues for future work. First, since FSCV is mostly done with carbon fiber microelectrodes, performing a similar study using the aforementioned electrode type instead of paper electrodes could provide higher sensitivity and more longevity to them. Secondly, extending the study to other commonly studied neurotransmitters, such as serotonin or estrogen, could help in evaluating the selectivity of SM-modified electrodes, as well as their applicability for broader electrochemistry. Finally, the LIG and SM-LIG electrodes could be used with tissue cultures to verify their biocompatibility and usability for pharmacological tests.

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SCANNING ELECTRON MICROGRAPHS

The following annex contains all SEM images taken of SM drop-cast on gold substrates, as well as SM-modified paper electrodes.

.1 Sepia Melanin on Gold

.1.1 1 drop-cast

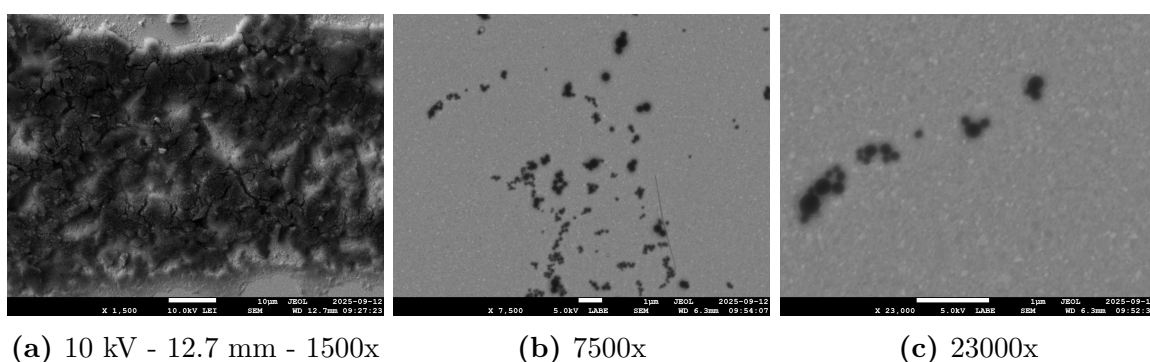


Figure .1 Low Concentration - 0.1 mg/mL of Sepia Melanin in Deionized Water

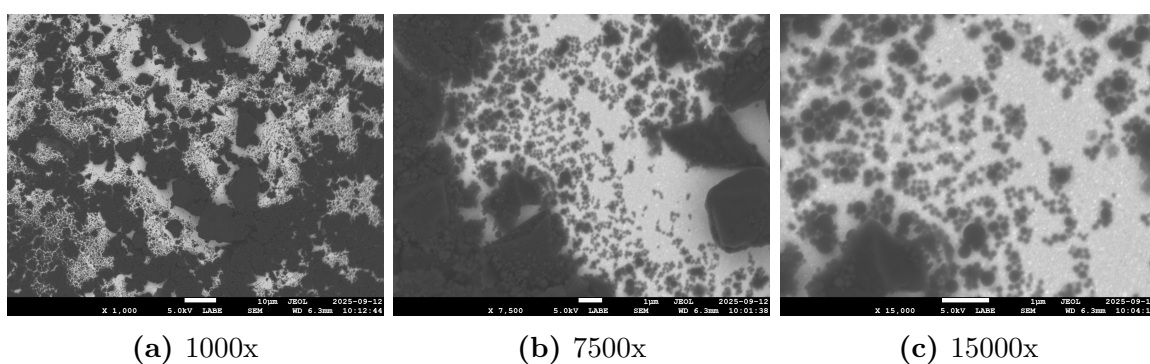


Figure .2 Medium Concentration - 0.1 mg/mL of Sepia Melanin in Deionized Water

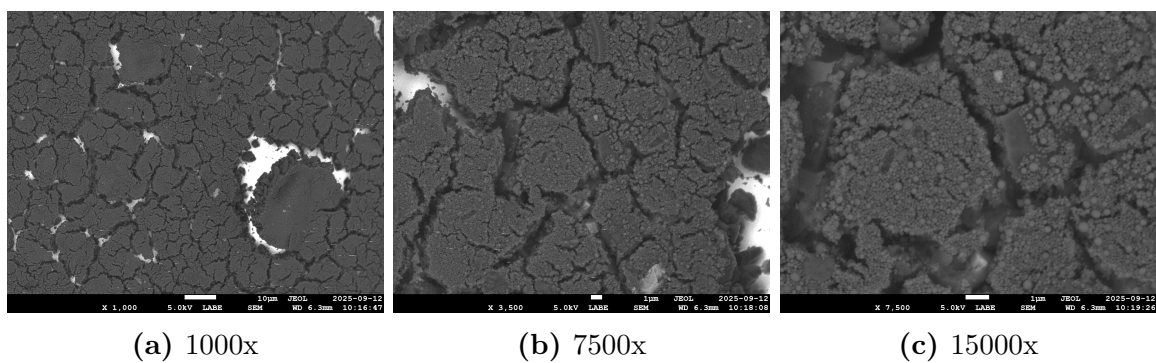


Figure .3 High Concentration - 5.0 mg/mL of Sepia Melanin in Deionized Water

.1.2 3 drop-casts

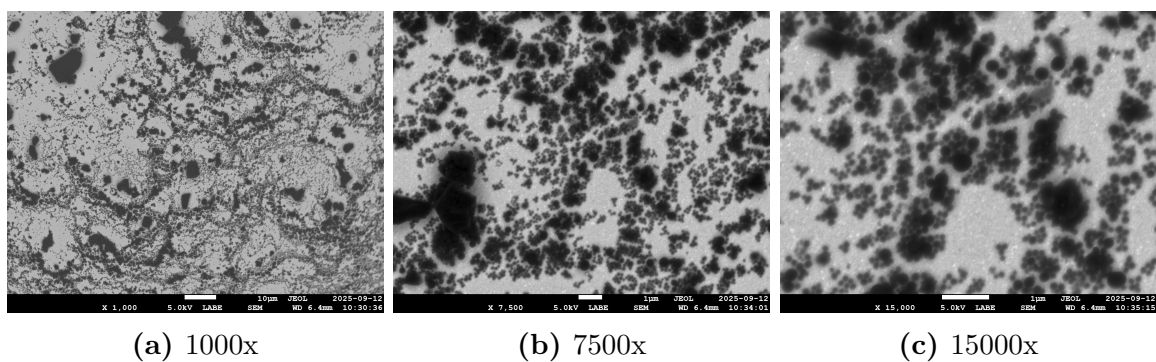


Figure .4 Low Concentration - 0.1 mg/mL

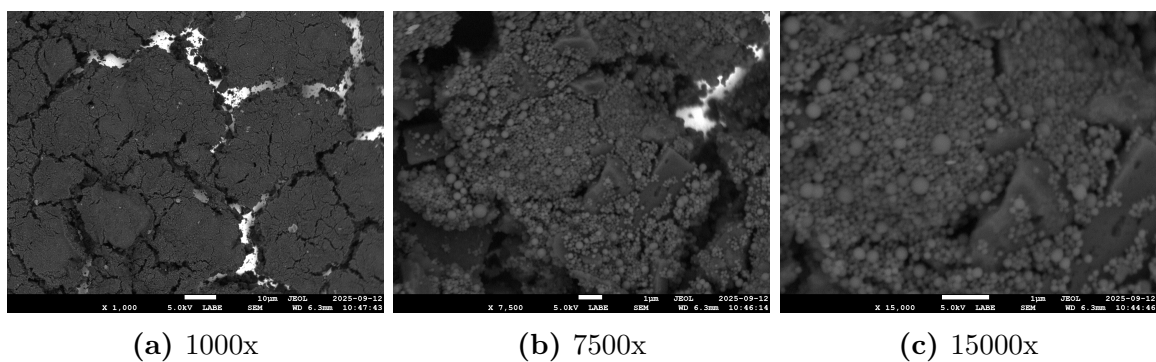


Figure .5 Medium Concentration - 2.5 mg/mL

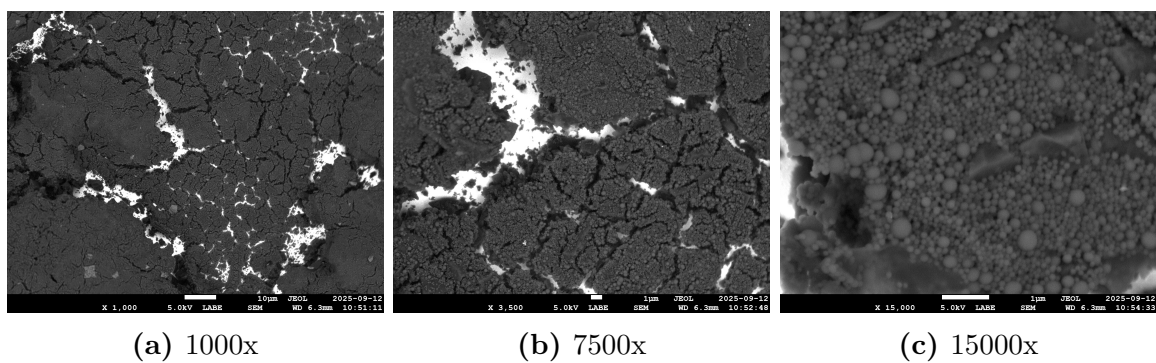


Figure .6 High Concentration - 5.0 mg/mL

.1.3 3 drop-casts and Rinsed

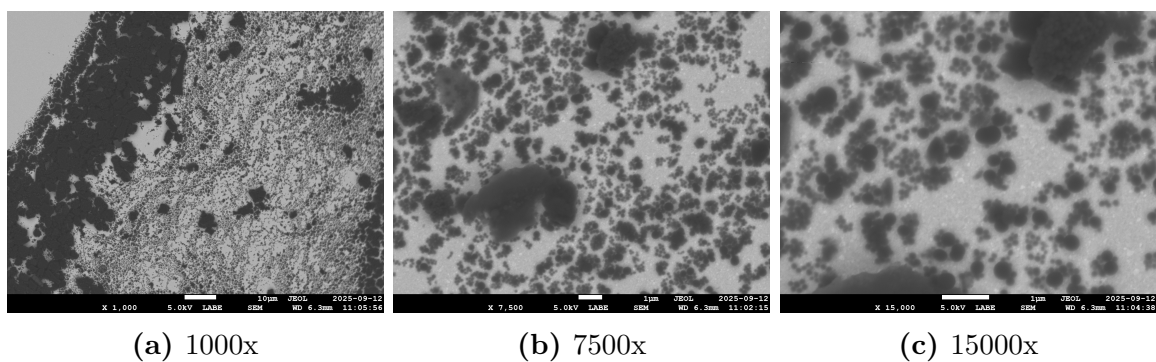


Figure .7 Low Concentration - 0.1 mg/mL

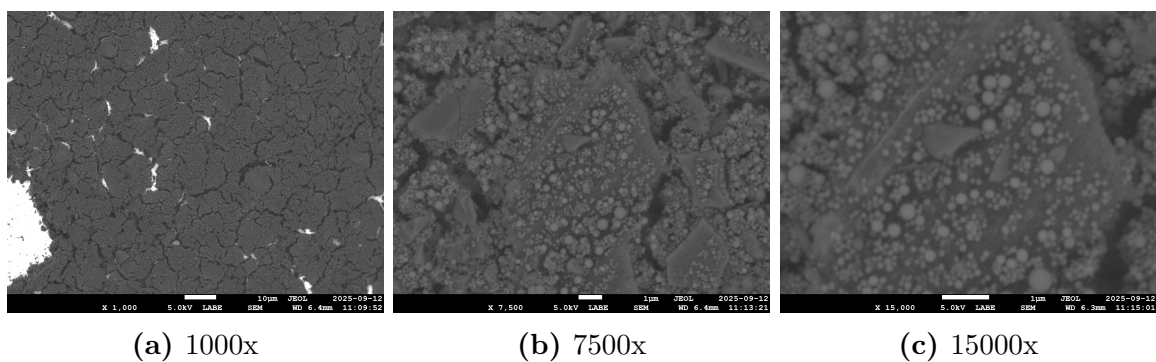


Figure .8 Medium Concentration - 2.5 mg/mL

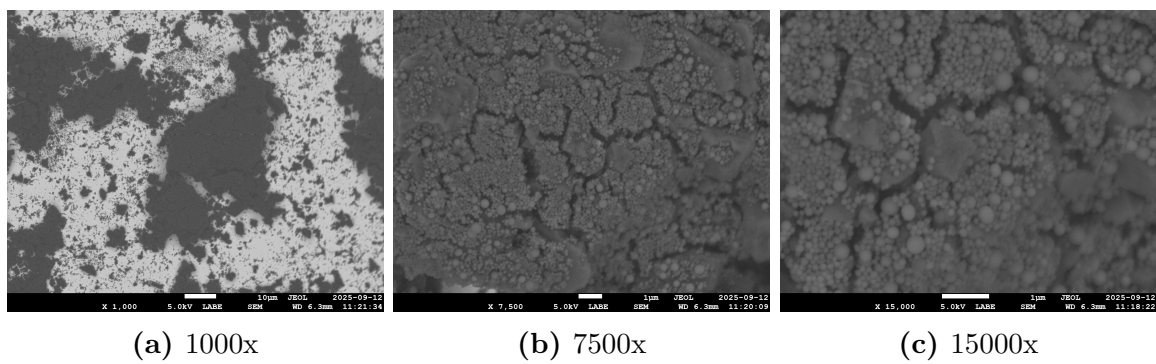


Figure .9 High Concentration - 5.0 mg/mL