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affiliée à l'Université de Montréal

Detection of Infection on Carbonized Paper

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Institut de génie biomédical

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Ce mémoire intitulé :

Detection of Infection on Carbonized Paper

présenté par **Sabrina ZOSO**

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

a été dûment accepté par le jury d'examen constitué de :

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DEDICATION

*To my dear grandparents, Danilo Francesco Zoso and Yolande Drouin
who have supported me since day 1 . . .*

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I would like to express my deepest gratitude to my supervisor Raphaël Trouillon for his unwavering support and guidance throughout my learning journey. His patience, expertise, and ability to explain complex concepts with clarity made the process feel effortless and enjoyable. I am truly grateful for the time and effort he dedicated to my growth.

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Audrey, my sister and best friend.

Zoë, my best friend and practically sister, for being there through thick and thin.

Thomas, le plus patient, et celui qui me soutient jour et nuit sans problème.

Merci !

RÉSUMÉ

Ce mémoire présente le développement et la caractérisation analytique de capteurs électrochimiques à base de papier pour la détection de molécules rédox-actives, en particulier le pyocyanine (PYO), un facteur de virulence clé sécrété par *Pseudomonas aeruginosa*. Le projet s'inscrit dans le contexte d'un besoin croissant en dispositifs diagnostiques portables, abordables et sensibles, utilisables en milieux à ressources limitées et sans équipement spécialisé.

L'objectif principal de cette recherche est d'optimiser la fabrication d'électrodes à partir de papier pyrolysé, afin de générer des structures conductrices de type graphène sans recours à des encres conductrices ni à l'impression sérigraphique. Une stratégie d'encapsulation a été mise en œuvre pour améliorer la rétention des analytes et prolonger le temps de mesure, permettant ainsi une analyse plus stable dans le temps. Les objectifs spécifiques incluent l'évaluation des performances électrochimiques des électrodes, la stabilité du signal, ainsi que la détermination de la limite de détection de la pyocyanine dans des conditions modifiées et encapsulées.

Les mesures électrochimiques ont été réalisées à l'aide de la voltampérométrie cyclique (CV) et de la voltampérométrie à onde carrée (SWV). Les données CV ont été traitées pour extraire les pics de courants et les potentiels rédox, la capacité et la séparation de pics. Les séries SWV ont permis de construire des courbes d'étalonnage, d'ajuster un modèle d'isotherme de Langmuir et de calculer les limites de détection.

Les résultats montrent que l'encapsulation améliore la stabilité et la rétention du signal, tandis que les électrodes modifiées offrent une sensibilité accrue. La courbe d'étalonnage des capteurs encapsulés présentent un comportement de liaison de type Langmuir avec une bonne linéarité à faibles concentrations.

Cette recherche ouvre la voie au développement de capteurs papier rapides, sensibles et reproductibles, adaptés aux contextes de diagnostic en temps réel et en dehors des laboratoires. Elle contribue également au domaine des biosenseurs en mettant en évidence l'influence des propriétés des matériaux, des modifications de surface et de la gestion des fluides sur la réponse électrochimique.

ABSTRACT

This thesis presents the development and analytical characterization of paper-based electrochemical sensors for the detection of redox-active molecules, with a particular focus on pyocyanin (PYO), a key virulence factor secreted by *Pseudomonas aeruginosa*. The work addresses the growing need for low-cost, portable, and sensitive diagnostics that can operate without the need for specialized equipment or technical expertise.

The research aims to optimize paper electrode fabrication using pyrolyzed cellulose to produce graphene-like conductive structures without requiring conductive inks or screen printing. An encapsulation strategy was employed to enhance analyte retention and extend the sensing time, enabling real-time measurements. The primary objectives included evaluating the electrode's electrochemical performance, determining the signal stability over time, and quantifying the limit of detection for PYO under both modified and encapsulated conditions.

Electrochemical measurements were performed using cyclic voltammetry (CV) and square wave voltammetry (SWV). CV data were analyzed to extract peak currents, redox peak potentials, capacitance, and peak separation. SWV datasets were used to build calibration curves, calculate Langmuir isotherm fits, and determine detection limits.

The results indicate that encapsulation enhances signal stability and retention over time, while modified electrodes improve sensitivity. The encapsulated sensors exhibited Langmuir-like binding behavior with improved linearity at low concentrations.

This work demonstrates a promising route toward rapid, sensitive, and scalable paper-based sensors suitable for point-of-care diagnostics and low-resource settings. The findings contribute to the broader field of biosensor design by highlighting the interplay between electrode material properties, surface modification, and fluid handling in optimizing electrochemical response.

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LIST OF SYMBOLS AND ACRONYMS

Biological Terms

- PA** *Pseudomonas aeruginosa* — a gram-negative opportunistic bacterium responsible for hospital-acquired and chronic infections.
- PYO** Pyocyanin — a redox-active phenazine uniquely secreted by *P. aeruginosa*, used as an electrochemical biomarker.

Chemical Reagents and Materials

- RuHex** Ruthenium Hexamine — a well-defined redox molecule used for benchmarking electrochemical behavior.
- PBS** Phosphate-Buffered Saline — an aqueous solution used to maintain pH of 7.4 and ionic strength during electrochemical experiments.
- FC-40** Perfluorinated Fluorocarbon 40 (Fluorinert) — a hydrophobic, biocompatible liquid used to encapsulate samples and prevent evaporation in paper-based devices.
- EG** Ethylene Glycol — a secondary dopant used to improve PEDOT:PSS conductivity.
- DMSO** Dimethyl Sulfoxide — a co-solvent used to enhance film homogeneity and adhesion of PEDOT:PSS to substrates.
- PDMS** Polydimethylsiloxane — a silicone elastomer used to create hydrophobic barriers and microfluidic chambers on paper electrodes.

Electrochemical Techniques

- CV** Cyclic Voltammetry — an electrochemical method involving linear potential sweeps to study redox behavior.
- SWV** Square Wave Voltammetry — a highly sensitive electrochemical technique using square-wave pulses for detection.
- GCE** Glassy Carbon Electrode — a commercial inert electrode used as a benchmark for comparison.

Voltammetric Parameters

- I_p Peak current — the maximum current magnitude observed during a redox event.

Cyclic Voltammetry (CV)

- $I_{p,red}$ Reduction peak current — cathodic peak associated with electron gain.
- $I_{p,ox}$ Oxidation peak current — anodic peak associated with electron loss.

I_{cap}	Capacitive current — current associated with double-layer charging rather than faradaic processes.
ΔE	Peak-to-peak separation — voltage difference between oxidation and reduction peak potentials.
$j_{p,red}$	Reduction peak current density — cathodic peak normalized by electrode area.
$j_{p,ox}$	Oxidation peak current density — anodic peak normalized by electrode area.
j_{cap}	Capacitive current density — non-faradaic charging current normalized by electrode area.
<i>Square Wave Voltammetry (SWV)</i>	
I_p	Main peak current — the highest current magnitude of the primary differential redox peak.
E_p	Main peak potential — the potential at which the primary differential redox peak occurs.
I_{p2}	Secondary peak current — the current magnitude of the secondary differential redox peak.
E_{p2}	Secondary peak potential — the potential at which the secondary differential redox peak occurs.
J_{peak}	SWV peak current density — maximum current per unit area at the main peak.
E_{peak}	SWV peak potential — potential at which the peak current density occurs.

Statistical Terms

LOD	Limit of Detection — the lowest concentration of analyte detectable above background noise.
AUC	Area Under the Curve — used to quantify cumulative effects such as signal decay or water loss.
R^2	Coefficient of determination — a measure of goodness-of-fit in regression analysis.
Langmuir	Adsorption isotherm model used to describe surface-limited analyte binding for LOD quantification.
I_{max}	Maximum current in Langmuir model — indicates saturated redox response.
K	Langmuir affinity constant — binding constant describing analyte–surface interaction strength (1/mM).

Device and Sensor Components

- LIG** Laser-Induced Graphene — a porous, conductive carbon network produced via laser pyrolysis on carbon-rich substrates.
- POC** Point-of-Care — diagnostic testing performed near the patient without centralized lab equipment.

CHAPTER 1 INTRODUCTION

Pseudomonas aeruginosa is a clinically significant pathogen whose early detection is critical, particularly in patients with hospital-acquired infections or cystic fibrosis. Traditional diagnostic techniques such as culturing, PCR, and chromatographic methods are either time-consuming, resource-intensive, or require centralized laboratories, limiting their utility for point-of-care (POC) applications. PYO, a redox-active phenazine secreted uniquely by PA, presents a promising electrochemical biomarker for rapid detection [2]. Electrochemical techniques such as cyclic voltammetry (CV) and square wave voltammetry (SWV) have enabled selective pyocyanin (PYO) detection in complex samples, and a variety of sensors, including screen-printed and graphene-based platforms, have been developed to support this. However, these sensors often suffer from limited shelf life and evaporation-driven signal loss when used in open systems.

To address this, we designed a paper-based electrochemical sensor integrating a three-layer structure and hydrophobic encapsulation using FC-40, a well-established biocompatible fluorocarbon [5]. Inspired by oil–water separation strategies on wax- or polymer-treated paper, this encapsulation significantly slows sample evaporation and preserves electrochemical signals. Carbonized electrodes were fabricated directly on cellulose paper via pyrolysis, forming a conductive, porous platform for PYO detection. This work introduces a low-cost, field-deployable sensor capable of stable PYO detection, advancing diagnostic options for PA in decentralized settings.

1.1 Dissertation Plan

This dissertation documents the research conducted to optimize a paper-based POC device for the voltammetric detection of PYO. The project was carried out at the BASyL laboratory of Polytechnique Montréal under the supervision of Raphaël Trouillon.

Chapter 1: Introduction This chapter includes a brief introduction, including a dissertation plan.

Chapter 2: Definitions and Key Concepts

Chapter 3: Literature Review and Background

This chapter presents a comprehensive literature review, beginning with an overview on the biology of PA and PYO, its societal burden, followed by existing detection methods before focusing on the electrochemical detection. It explores various materials for sensor fabrication, emphasizing the advantages of paper substrates in POC applications. Additionally, it discusses hydrophobic encapsulation strategies used in water-diesel separation, which served as inspiration for the device design.

Chapter 4: Problem Statement and Research Objectives

This chapter formally defines the research problem and outlines the specific objectives guiding this study.

Chapter 5: Materials and Experimental Methodology

This chapter details the chemicals and materials used, along with the experimental procedures employed, including electrode fabrication and characterization techniques.

Chapter 6: Results and Discussion

This chapter presents the experimental results obtained from the characterization and performance evaluation of paper-based sensors. To evaluate their significance, an in-depth analysis and interpretation of the results.

Chapter 7: Conclusion and Future Prospects

This final chapter summarizes the key findings and limitations of the research and proposes future directions for developing the paper-based POC device.

CHAPTER 2 DEFINITIONS AND KEY CONCEPTS

This section outlines the key terms and acronyms used throughout this thesis to ensure clarity and consistency.

2.0.1 Biological Terms

***Pseudomonas aeruginosa* (PA)** is a gram-negative opportunistic pathogen frequently associated with hospital-acquired infections, chronic respiratory conditions, and immunocompromised patients. Its early detection is critical for clinical decision-making and effective treatment.

Pyocyanin (PYO) is a redox-active phenazine compound uniquely secreted by PA. It undergoes reversible redox cycling, contributing to oxidative stress in host tissues. Its distinct electrochemical behavior, characterized by well-defined redox peaks, makes it a reliable biomarker for electrochemical detection of PA infections.

Point-of-Care (POC) diagnostics refer to medical tests conducted at or near the site of patient care, without the need for centralized laboratory infrastructure. [2] POC technologies aim to be rapid, accessible, and easy to operate.

2.0.2 Materials and Device Terminology

Laser-Induced Graphene (LIG) is a conductive carbon-based nanomaterial formed by laser pyrolysis of carbon-rich substrates such as paper or polyimide in an inert or controlled atmosphere. LIG is known for its high electrical conductivity, surface area, and electrochemical activity, making it well-suited for biosensing applications. In this thesis, the term “LIG” is used only when referring to prior work in the literature where inert atmospheres were employed.

Carbonized Paper Electrodes refer to porous, conductive structures formed by laser pyrolysis of cellulose paper under ambient air conditions. Though similar in appearance and fabrication method to LIG, these structures are less graphitized and are referred to as “carbonized” to reflect their open-air synthesis conditions.

FC-40 (Perfluorinated Fluorocarbon 40) is a chemically inert, hydrophobic, and biocompatible oil used in microfluidic systems [5]. In this work, FC-40 is used to encapsulate aqueous samples on paper electrodes, forming a vapor barrier that reduces evaporation and improves signal retention.

Scanning Electron Microscopy (SEM) is a high-resolution imaging technique that uses focused electron beams to visualize surface morphology. SEM is employed to characterize the microstructure and surface roughness of carbonized electrodes.

2.0.3 Electrochemical Concepts

Voltammetry is a family of electrochemical techniques where current is measured as a function of an applied potential. A typical setup includes a three-electrode system:

- **Working Electrode (WE):** Site of the redox reaction. In this study, WE usually refers to a paper-based electrode either carbonized or modified with conductive coatings.
- **Reference Electrode (RE):** Provides a known and stable potential. Ag|AgCl is used as the reference electrode in all tests.
- **Counter Electrode (CE):** Completes the electrical circuit; typically inert (e.g., carbon or platinum).

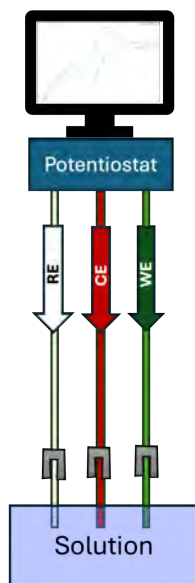


Figure 2.1 Voltammetry Set-up.

Cyclic Voltammetry (CV) is a voltammetric technique where the applied potential is swept linearly in forward and reverse directions, forming a triangular waveform. Key outputs include oxidation and reduction peak currents (I_p), peak potentials (E_p), and the separation between them (ΔE). CV provides insights into redox kinetics and reversibility.

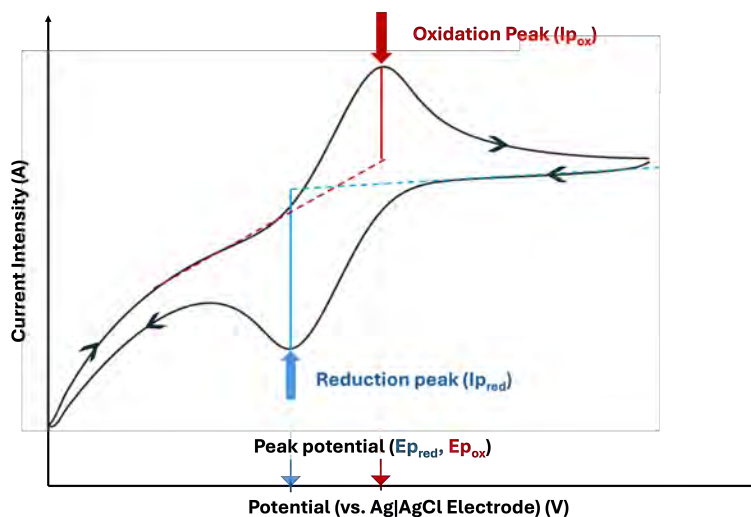


Figure 2.2 Cyclic Voltammetry Analysis Diagram.

Square Wave Voltammetry (SWV) is a pulse technique that applies a square wave superimposed on a staircase potential. It measures the difference current (ΔI) between forward and reverse pulses. SWV enhances sensitivity and is used here for constructing calibration curves and determining the limit of detection (LOD) for PYO.

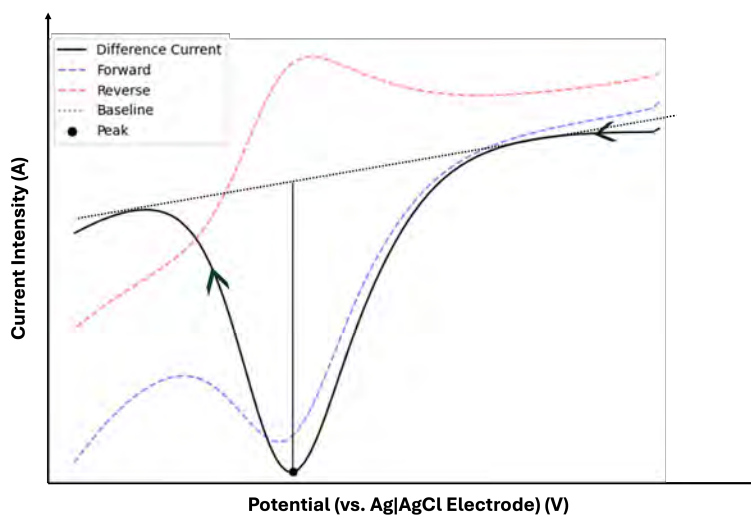


Figure 2.3 Square Wave Voltammetry Analysis Diagram.

2.0.4 Performance Metrics for Electrochemical Sensors

The performance of paper-based electrochemical sensors in this thesis is evaluated based on:

- **Sensitivity:** The ability to detect low concentrations of PYO with high signal-to-noise ratio.
- **Stability:** Preservation of electrochemical signal over time, particularly against evaporation and degradation.
- **Reproducibility:** Consistency of results across multiple sensors or repeated measurements.

2.0.5 Additional Electrochemical and Analytical Concepts

Limit of Detection (LOD) refers to the lowest concentration of analyte that can be reliably distinguished from background noise. It is typically defined as:

$$\text{LOD} = \frac{3\sigma}{m}$$

where σ is the standard deviation of the blank signal, and m is the slope of the calibration curve in a linear model. However, in electrochemical responses influenced by adsorption phenomena, Langmuir adsorption isotherm more appropriately describes slope at smaller concentrations.

Langmuire Isotherm Absorption Model It is a theorem used to more effectively approximate the slope of the calibration curve used for LOD calculation when adsorption effects take place.

$$I(C) = \frac{I_{\max} \cdot K \cdot C}{1 + K \cdot C} \quad (2.1)$$

In this expression, $I(C)$ is the observed peak current at analyte concentration C , $I_{\max}$ is the saturation current corresponding to full monolayer coverage of electroactive sites, and K is the Langmuir binding constant (with units of inverse concentration, e.g., mM^{-1}). The model assumes reversible adsorption to a finite number of non-interacting binding sites, with no cooperative effects [6]. As such, it captures the saturation behavior typical of surface-bound electron transfer reactions, where increases in concentration no longer yield proportional increases in signal.

Importantly, the Langmuir model allows for analytical derivation of the initial response slope as $C \rightarrow 0$, yielding:

$$\left. \frac{dI}{dC} \right|_{C \rightarrow 0} = I_{\max} \cdot K \quad (2.2)$$

This term replaces the conventional linear slope in the LOD equation, yielding a generalized form:

$$\text{LOD} = \frac{3\sigma}{I_{\max} \cdot K} \quad (2.3)$$

Faradaic vs. Non-Faradaic Processes:

- **Faradaic Processes** involve electron transfer reactions, such as the redox activity of PYO, leading to current peaks in voltammetry.
- **Non-Faradaic Processes** arise from the charging and discharging of the electrical double layer, producing background capacitive current without net electron transfer.

Capacitive Current (I_{cap}) is the non-faradaic current generated by charging the double-layer capacitance at the electrode–electrolyte interface. It is proportional to the scan rate and can obscure faradaic signals if not accounted for.

Scan Rate (SR) is the speed at which the potential is linearly swept during cyclic voltammetry, typically expressed in mV/s. Higher scan rates increase capacitive currents and shift redox peak positions depending on reaction kinetics.

Redox Peak Potential (E_p) is the potential at which the maximum current for oxidation or reduction occurs during voltammetric analysis. The difference between anodic and cathodic peak potentials, $\Delta E = |E_{\text{pa}} - E_{\text{pc}}|$, provides insight into redox reversibility.

Diffusion-Controlled Process: In this regime, the rate-limiting step is the diffusion of analyte from the bulk solution to the electrode surface. In CV, this is indicated by a linear relationship between the peak current (I_p) and the square root of the scan rate ($\sqrt{SR}$).

Surface-Confined Process: In this regime, redox-active species are immobilized on the electrode surface, and the peak current varies linearly with the SR, rather than $\sqrt{SR}$.

Electroactive Surface Area (ECSA) is the effective area of an electrode that participates in charge transfer reactions. It can be estimated using CV in a standard redox system by applying the Randles–Ševčík equation:

$$I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C SR^{1/2}$$

where I_p is the peak current, n is the number of electrons transferred, A is the electroactive surface area, D is the diffusion coefficient, C is the analyte concentration, and SR is the scan rate.

Pseudocapacitance arises from fast, reversible redox reactions that mimic capacitive behavior. Unlike true double-layer capacitance, pseudocapacitance involves charge transfer and is often observed in surface-modified or redox-active materials.

Proton-Coupled Electron Transfer (PCET). A redox process in which electron transfer is directly linked to the transfer of one or more protons. PCET mechanisms are important in understanding the pH-dependent electrochemical behavior of redox-active species like PYO.

Thin-Layer Diffusion: A mass transport regime in which the diffusion layer thickness is comparable to the physical thickness of the electrolyte volume (e.g., a confined droplet), leading to rapid depletion of electroactive species and often resulting in steady-state or peak-shaped voltammetric signals.

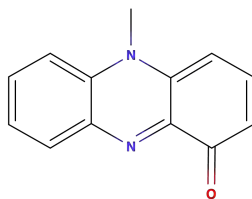
Semi-Infinite Diffusion: A classical diffusion regime in which the electrolyte volume is effectively infinite relative to the diffusion layer. Under this condition, concentration gradients extend indefinitely from the electrode surface, leading to well-defined, SR-dependent peak currents in CV.

Droplet Microfluidics: A microfluidic approach where discrete, microliter- to nanoliter-sized droplets are used as reaction compartments. In electrochemical sensing, droplets are often deposited on paper or microstructured surfaces to localize analytes, minimize sample volumes, and control diffusion geometry.

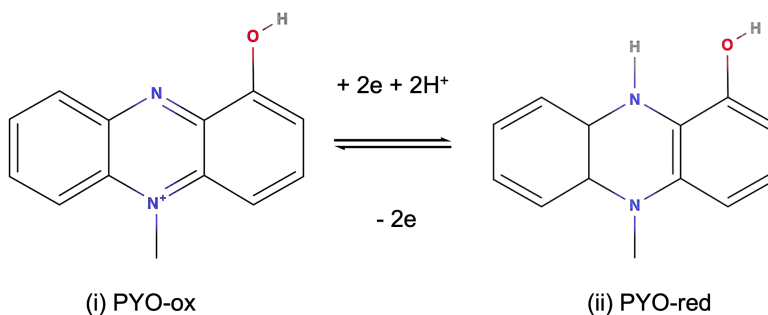
CHAPTER 3 LITERATURE REVIEW

3.1 Biology of *Pseudomonas aeruginosa* and Pyocyanin

PYO plays a critical role in the bacterium's physiology, contributing to oxidative stress and cellular damage in host tissues [7]. Its distinct electrochemical behavior, characterized by well-defined redox peaks, making it an ideal target for electrochemical sensing. As a result, PYO serves as a reliable biomarker for the early and selective detection of PA.



(a) PYO in neutral state.



(b) Redox interconversion between oxidized (i) and reduced (ii) forms of PYO at physiological pH (7.4).

Figure 3.1 PYO's redox reaction in controlled pH. Adapted from [1, 2]

Under ambient laboratory conditions and in the presence of atmospheric oxygen, PYO predominantly exists in its oxidized form (Figure 3.1bA). However, its redox state is known to shift depending on environmental pH, enabling the formation of additional protonated or deprotonated intermediates [3]. This pH dependence can significantly influence both its electrochemical signature and biological reactivity.

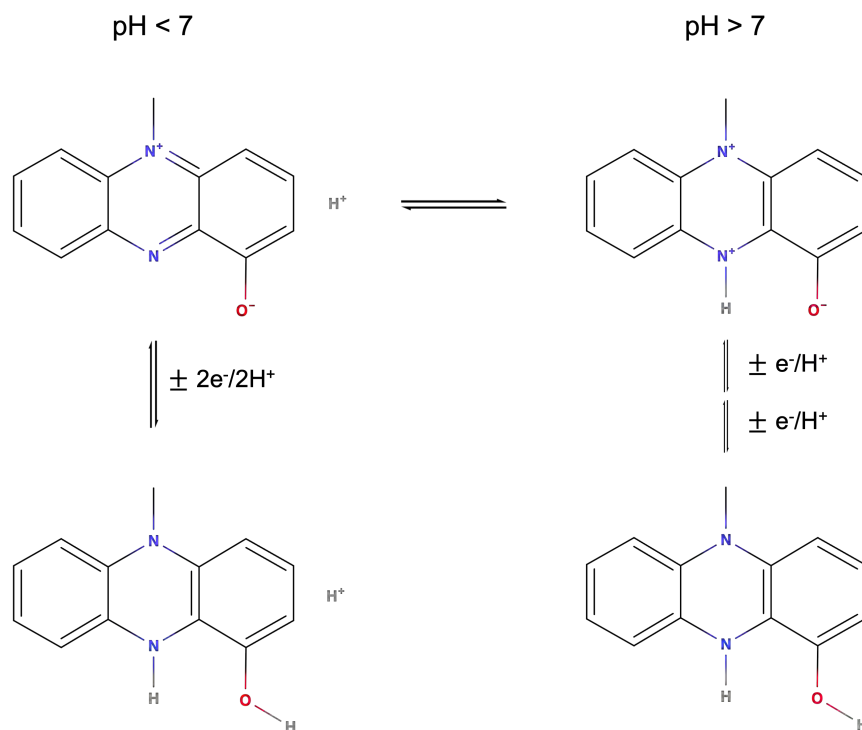


Figure 3.2 pH-dependent redox behavior of PYO and possible intermediate species under acidic and basic conditions, adapted from Do *et al.* [3].

3.2 Epidemiology and Societal Burden of *Pseudomonas aeruginosa*

Hospital-acquired infections (HAIs) are a growing global concern, with a prevalence estimated by the World Health Organization (WHO) at 15% and an annual increase of 0.06% worldwide [8,9]. This upward trend underscores the urgent need for effective prevention and control strategies to mitigate the impact of HAIs on healthcare systems worldwide. PA, an opportunist gram-negative bacterium, is the leading cause of HAI due to its high prevalence and pathogenicity of both its drug-sensitive and drug-resistant strains [10]. It is a key contributor for severe infections in various body systems such as the respiratory and urinary tracts,

vascular system, and central nervous system [11]. Indeed, a recent international study has found that the overall prevalence of confirmed PA in patient blood was 14.5% (N=153), and the overall prevalence of PA with at least one drug-resistant mechanism was 5.1% (N=55) for patients with ICU-related respiratory infections [12]. Among cystic fibrosis patients, 80% will suffer from PA infection at one point in their lives, and due to severe inflammation build-up, PA will lead to death in 90% of infected patients [13].

Healthcare costs for patients dramatically rise once affected by PA. Two studies examined the impact of PA on resource utilization and costs in patients with exacerbated non-cystic fibrosis bronchiectasis (NCFB). Blanchette *et al.* from 2017 found that patients diagnosed with PA experience a significant increase in healthcare costs, rising from an average of \$36,213 before diagnosis to \$67,764 after, an increase of over \$31,000. Inpatient costs represented the largest proportion of total healthcare costs post-PA (54%), with an increase of four hospitalizations per patient ($p < 0.0001$) [14].

A large-scale analysis of U.S. insurance claims revealed that patients with NCFB who experienced exacerbations due to PA (PA⁺) faced dramatically higher direct medical costs and healthcare utilization. On average, PA⁺ patients incurred \$204,791 per year, compared to \$74,453 for PA⁻ patients, a difference of \$130,338 annually, with inpatient services accounting for 69.4% of total PA⁺ costs [15]. In contrast, NCFB patients with no exacerbation or PA infections cost \$25,587 annually, demonstrating a \$179,000+ gap in total costs compared to PA⁺ individuals [15].

3.3 Current Diagnostic Methods

3.3.1 Microbiological Culture

Currently, PA is diagnosed by culturing patient samples on selective agar plates. After 2–5 days of incubation, plates are visually assessed for colony morphology and pigment characteristic of PA [1]. While widely used, this method is time-consuming, labor-intensive, and depends heavily on the initial bacterial load. Low cell numbers may result in false negatives. Additionally, overgrowth by other microorganisms in the sample can obscure PA colonies, further reducing sensitivity [16]. Despite these limitations, culture remains the standard due to the lack of simple alternatives [1, 17, 18].

3.3.2 PCR Detection

Polymerase chain reaction (PCR) targeting 16S rDNA offers a more specific diagnostic approach but requires DNA extraction, sample purification, expensive reagents, and access to centralized facilities with trained personnel, which limits its accessibility [19]. Furthermore, PCR data processing requires a high degree of expertise and can take several hours, making it inadequate for POC testing [20]. In contrast, detecting PYO as a specific biomarker presents a promising alternative for earlier and more accessible PA diagnosis.

3.3.3 Chromatography, Spectroscopy, and Spectrometry

PYO has previously been quantified by high-performance liquid chromatography (HPLC) or spectrophotometry [21, 22]. For instance, HPLC allows for the separation and quantification of PYO with high precision, often utilizing UV detection at specific wavelengths corresponding to PYO's absorbance maxima. Spectrophotometric methods typically measure absorbance at 520 nm after acidification, correlating to PYO concentration. However, these methods are time-consuming and costly due to the need for pre-purification and bacterial culturing steps [2].

Liquid chromatography-mass spectrometry (LC-MS) offers enhanced specificity and sensitivity for PYO detection, capable of identifying and quantifying PYO even in complex biological matrices. Despite its advantages, LC-MS also demands rigorous sample preparation, including extraction and purification, and relies on sophisticated instrumentation and specialized expertise [2, 23].

Surface-enhanced Raman spectroscopy (SERS) has also been applied to detect PYO, offering high molecular specificity and sensitivity through the enhancement of Raman scattering near nanostructured metallic surfaces [24, 25]. However, this method typically requires extensive sample preparation, including analyte extraction to reduce biological matrix interference, pH adjustment to promote optimal adsorption of PYO onto the metallic substrate, and the fabrication or tuning of reproducible nanostructured surfaces, often based on silver or gold nanoparticles, to maximize enhancement. These steps are critical to minimize signal variability and background noise but limit the feasibility of SERS for routine or point-of-care use.

3.3.4 Other Detection Methods: Colorimetric Assays, Fluorescence-Based Probes, and Biosensors

In addition to electrochemical and chromatographic techniques, other detection methods for PYO have been explored. Colorimetric assays leverage the pH-dependent color changes of PYO (blue in alkaline solutions and reddish-pink in acidic conditions) and allow for simple, rapid detection through visual inspection or spectrophotometric measurement, typically around 520 nm [26]. While accessible and low-cost, these assays often lack the selectivity needed for complex biological matrices.

Fluorescence-based probes provide another promising avenue by enabling spatially resolved detection and offering molecular-level sensitivity [27]. However, these techniques typically require specialized optical equipment and can suffer from interference by other endogenous fluorescent species. Consequently, despite their potential, fluorescence-based detection methods still require further development and standardization before they can be reliably integrated into clinical workflows.

Biosensors, especially those incorporating enzymes, antibodies, or whole-cell systems, have also been investigated for their ability to transduce PYO's biochemical effects into measurable signals [7, 28–30]. These systems can offer high specificity, but their practical implementation is challenged by issues such as signal stability, shelf life, and fabrication complexity. Consequently, while all these methods contribute valuable insights, further development and standardization are needed before they can be reliably adopted in routine or POC settings.

3.4 Electrochemical Background

3.4.1 Electrochemistry of Pyocyanin

PYO is the most abundant and electrochemically active phenazine secreted by PA, offering a distinct and selective redox signature for monitoring infections [1]. As a redox-active small molecule, PYO readily undergoes reversible electron transfer reactions at physiological pH (as seen in Figure 3.1), which can be detected using electrochemical techniques such as cyclic voltammetry (CV) and square wave voltammetry (SWV).

PYO typically exhibits a well-defined redox couple, with an oxidation peak around -0.25 to -0.30 V and a corresponding reduction peak between -0.40 and -0.45 V vs. Ag|AgCl, though these values can shift depending on electrode material and solution conditions [31, 32]. This redox behavior enables its selective detection in biological fluids, even without extensive sample preparation, and distinguishes it from other electro-active components.

The sensitivity of PYO electrochemical detection is further enhanced by its stability and consistent redox behavior across different sensing platforms. Moreover, since PYO is uniquely produced by PA, its electrochemical detection provides a direct and rapid means of identifying the pathogen, without relying on time-consuming culturing or complex sample processing. These characteristics make PYO an ideal biomarker for the development of POC diagnostic tools based on electrochemical sensing.

Notably, a study employing commercially available screen-printed electrodes demonstrated that CV enables selective detection of PYO within a well-defined potential window between 0.58 V and 0.82 V with a LOD of 2 μ M [31]. Using sputum samples mixed with saliva from patients with respiratory tract infections, researchers demonstrated that within this window, PYO can be detected without interference from other redox-active biomolecules, reinforcing the suitability of electrochemical detection for this application [19]. Furthermore, a separate study confirmed that all clinically relevant strains of PA associated with HAIs or cystic fibrosis produce a measurable electrochemical signal for PYO, supporting its use as a reliable diagnostic biomarker [33]. To accurately quantify low concentrations of PYO, especially in the nonlinear response regime typical of surface-bound electrochemical interactions, it is important to consider models beyond linear calibration. The Langmuir adsorption isotherm (Eq. 2.1) provides a theoretical framework that better reflects the sensor's behavior under such conditions. A detailed discussion of this model and its role in estimating the limit of detection is presented below.

3.4.2 Theoretical Basis for Pyocyanin Detection and Limit of Detection Estimation

The accurate quantification of low-concentration analytes is a central requirement for the development of sensitive biosensors. In electrochemical platforms, the limit of detection (LOD) is defined as the lowest concentration of analyte that produces a signal distinguishable from baseline noise, typically estimated by the 3σ criterion, where σ is the standard deviation of the blank signal and the denominator is the calibration slope near zero concentration.

A purely linear calibration,

$$I(C) \approx S_0 C,$$

with S_0 obtained by regression over a finite low-concentration range, can misrepresent systems governed by surface adsorption. For PYO, adsorption onto the electrode modifies the current–concentration relationship such that the response saturates at high C . This behavior

is captured mechanistically by the Langmuir isotherm in its two equivalent forms:

$$I(C) = \frac{I_{\max} K C}{1 + K C}, \quad (\text{affinity form})$$

$$I(C) = \frac{I_{\max} C}{C + C_0}, \quad C_0 = \frac{1}{K}, \quad (\text{half-saturation form})$$

where $I_{\max}$ is the saturation current at full monolayer coverage, K the Langmuir (association) constant, and C_0 the concentration yielding half of $I_{\max}$ (i.e. $I(C_0) = I_{\max}/2$) [34, 35]. Equation (*affinity form*) emphasizes binding affinity (K), whereas (*half-saturation form*) highlights the mid-point concentration C_0 familiar from Michaelis–Menten analysis [36].

Linearizing either form around $C \rightarrow 0$ yields the true initial sensitivity,

$$\left. \frac{dI}{dC} \right|_0 = I_{\max} K = \frac{I_{\max}}{C_0}. \quad (3.1)$$

Substituting (3.1) into the 3σ LOD formula gives a mechanistically faithful expression:

$$\text{LOD} = \frac{3\sigma}{I_{\max} K} = \frac{3\sigma C_0}{I_{\max}}. \quad (3.2)$$

This formulation uses the adsorption-derived slope at zero concentration, avoiding biases introduced by curvature at higher C . By fitting the full calibration curve to (*affinity form*) or (*half-saturation form*), one obtains $I_{\max}$ and K (or C_0) directly, ensuring that the LOD reflects the true surface-limited sensitivity of the PYO sensor [37].

3.4.3 Voltammetric Techniques

Voltammetric techniques such as CV and SWV are commonly employed to evaluate sensor performance and detect redox-active biomarkers like PYO. CV provides qualitative insight into redox behavior, including reversibility and electron transfer kinetics, making it useful for initial sensor characterization. SWV, on the other hand, offers higher sensitivity and lower detection limits due to its pulsed waveform and effective background subtraction, making it particularly suitable for quantitative analysis [38, 39]. Both techniques have been widely used in PYO detection, especially in biological fluids, where distinguishing the signal from background noise is critical [2, 19, 31, 33, 40–43].

Electrochemical monitoring of PYO is therefore an active area of research, with ongoing efforts to enhance sensor performance and device integration. For instance, an early study demonstrated the use of carbon fiber sensors to detect clinically relevant PYO concentrations

ranging from 1 μM to 100 μM [40]. Since then, screen-printed electrodes have emerged as a promising low-cost, high-throughput alternative. One study using a screen-printed carbon electrode achieved detection of PYO directly from wound exudate samples with a 71% sensitivity and 57% specificity [20]. In parallel, various conductive inks, such as those incorporating gold or graphene in the form of nanoparticles, are being explored to further improve analytical performance. However, these materials can present challenges, including gold amalgam formation and the need for time-consuming and hazardous electrode cleaning procedures [2, 44, 45]. More broadly, graphene- and graphite-based electrodes have been gaining interest due to their lower costs and greater potential range compared to metallic surfaces such as gold and silver [43–45]. Among the many strategies under investigation, paper-based devices stand out as particularly attractive for POC settings.

3.5 Paper-Based Devices

Paper-based sensors have been successfully applied for PYO detection [32]. Other studies have highlighted paper as a promising electrode substrate for rapid and precise electrochemical sensing [4, 46–50]. Paper offers several key advantages: it is low-cost, easy to fabricate and modify, and both disposable and biodegradable. Its inherent capillarity also facilitates passive fluid transport, which can streamline sample handling and make assaying more efficient. An additional and remarkable advantage of paper is its biocompatibility which makes it suitable for cell culture [51].

Seminal work by Dungchai, Chailapakul, and Henry [46] demonstrated the first integration of electrochemical detection with paper microfluidics. They used photolithography to define microfluidic channels on filter paper, then screen-printed carbon working and counter electrodes and Ag|AgCl reference electrodes directly on the paper. The system successfully quantified glucose, lactate, and uric acid in serum via chronoamperometry at 0V vs. on-chip Ag|AgCl, achieving results comparable to standard assays [46].

Alatraktchi *et al.* developed a novel paper-based sensor capable of detecting PYO concentrations ranging from 1 to 40 μM in lysogeny broth (LB) without the need for complex sample preparation, demonstrating the sensor’s practicality for rapid diagnostics [32].

Recent studies have also focused on tuning the properties of paper-based devices to optimize their performance. Pelletier and Trouillon investigated the modification of paper electrodes for neurochemical analysis, emphasizing the importance of electrode surface properties in achieving reliable and reproducible measurements [4, 48]. Moreover, Anushka *et al.* emphasize the importance of choosing appropriate paper substrates and conductive inks. They indicate

how the porosity, thickness, and surface chemistry of paper can influence fluid flow and sensor performance [49].

Hamedi *et al.* pioneered integrated electrofluidic circuits by wicking conductive aqueous ink PEDOT:PSS (poly(3,4-ethylenedioxythiophene) polystyrene sulfonate) into paper microchannels, creating both fluidic pathways and conductive electrodes in a single fabrication step. [52] They report a sheet resistance of $\sim 300 \Omega \text{sq}^{-1}$, sufficient not only for sensing applications but also for powering a paper-based battery. Their work demonstrates the feasibility of monolithically integrating conductive ink and microfluidics, supporting the use of PEDOT:PSS in paper-based electrochemical devices for PYO detection.

3.5.1 Conductive Inks for Paper Sensors

Conductive inks play a central role in enhancing the electrochemical performance of paper-based sensors by reducing sheet resistance and promoting efficient charge transport. Among these, PEDOT:PSS is one of the most widely used organic conductors due to its aqueous processability, film-forming properties, and environmental stability. PEDOT provides the conjugated polymer backbone responsible for electronic conductivity, while PSS serves as a polyelectrolyte dopant that enhances solubility and processability in water [53]. However, PEDOT:PSS films typically exhibit limited conductivity due to excess insulating PSS content and phase separation between the two components [54].

To overcome this limitation, secondary dopants such as ethylene glycol (EG) and dimethyl sulfoxide (DMSO) are commonly introduced to improve charge carrier mobility and film morphology (refer to Figure 3.3). These additives induce conformational changes in the PEDOT chains, promoting a transition from a coiled to a more linear and expanded structure, which facilitates π - π stacking and interchain charge transport [55]. Simultaneously, they help to reorganize the film microstructure by selectively removing excess PSS or inducing phase segregation, resulting in enhanced electrical conductivity by up to three orders of magnitude [56]. EG is particularly effective in improving film homogeneity and reducing surface roughness, while DMSO has been shown to enhance long-range ordering and crystallinity of the PEDOT phase [55, 57].

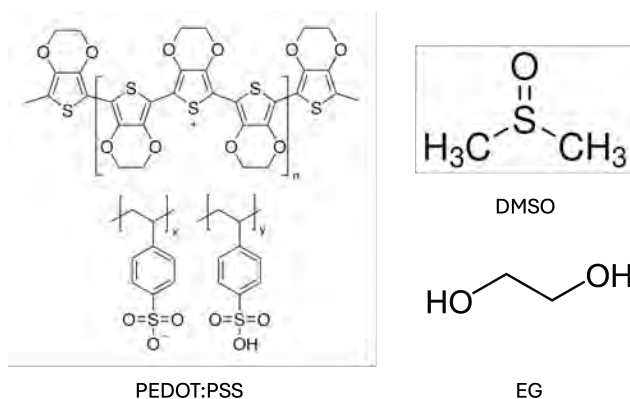


Figure 3.3 Molecular depiction of conductive polymers, PEDOT:PSS, DMSO and EG. PEDOT:PSS figures obtained with permission from Pelletier *et al.* [4]

Oziat *et al.* validated the advantages of PEDOT:PSS by demonstrating amplified electrochemical signals for PYO, PQS, and 2-aminoacetophenone when compared to unmodified glassy carbon electrodes [58]. The authors also emphasize the importance of film structuration to improve electrode/electrolyte interface properties, underlining the dual role of PEDOT:PSS as both a conductor and a surface morphology modifier.

In the context of paper-based electrodes, PEDOT:PSS blends with EG and DMSO offer a versatile platform for printable and flexible conductive coatings that maintain electrochemical stability across repeated voltammetric cycles. Their compatibility with cellulose substrates, combined with tunable viscosity and drying characteristics, makes them well-suited for applications in low-cost biosensors and microfluidic electrochemical devices.

3.5.2 Hydrophobic Encapsulation Strategies on Paper

Hydrophobic coatings on paper have been widely explored as a means to control liquid transport, separate immiscible phases, and protect sensitive samples. Materials such as waxes [59], silanes [60,61], and fluorocarbons [5] have been applied to create barriers that inhibit water penetration while allowing selective passage or retention of nonpolar compounds. Wang *et al.* demonstrated that filter paper coated with carnauba wax and TiO₂ nanoparticles achieved water contact angles above 140°, enabling effective water/diesel separation and drastically reducing water content in the collected phase [62]. In a related approach, a mixed wax emulsion produced superhydrophobic paper surfaces with contact angles near 151°, allowing over 99% oil/water separation efficiency [59]. Others have utilized PMHS/silica coatings or polymer-modified cellulose aerogels to achieve selective oil uptake, demonstrating the feasibility of using hydrophobic paper matrices to trap or preserve nonpolar compounds [60,61]. These

approaches provide compelling evidence that paper can serve not only as a sensing substrate, but also as an effective encapsulation medium for hydrophobic or amphiphilic analytes.

3.6 Laser-Induced Graphene (LIG) and Carbonized Paper Electrodes

3.6.1 Laser-Induced Graphene in Literature

Conventional fabrication methods for paper-based electrodes, such as screen printing, inkjet printing, metal sputtering, thermoplastic forming, and pencil drawing, often involve multiple processing steps, require specialized materials, and can hinder capillary flow due to the hydrophobicity of deposited inks [63]. These limitations can restrict the design flexibility and performance of microfluidic paper-based analytical devices (μ PADs).

In contrast, LIG offers a rapid, mask-free approach by directly converting cellulose paper into conductive, porous electrodes through localized laser irradiation. This process creates laser-induced graphene electrodes (LIG-E) that preserve the inherent porosity and hydrophilicity of the paper, allowing for efficient capillary-driven fluid transport. The resulting structures exhibit high electrical conductivity and electrochemical activity, making them suitable for sensitive biosensing applications. The properties of LIG can be finely tuned by adjusting laser parameters such as power, scan speed, and wavelength, as well as by selecting appropriate precursor materials [64]. These adjustments influence the morphology, porosity, and defect density of the resulting graphene, thereby affecting its electrical conductivity and surface area. Such control enables the customization of LIG for specific applications, including sensors, energy storage devices, and flexible electronics.

LIG has emerged as a highly versatile material for flexible electronic devices due to its excellent electrical conductivity, mechanical flexibility, and tunable porosity. In their comprehensive review, Hu *et al.* summarize recent advancements in LIG fabrication and applications across a broad range of wearable and adaptable technologies [65]. LIG can be directly synthesized on various carbon-rich substrates, including polyimide, paper, textiles, and even natural materials, through rapid, mask-free laser irradiation. These properties have enabled its use in diverse sensor modalities, including strain, temperature, humidity, electrochemical, and electrophysiological sensors, as well as in heaters and actuators [65]. While not yet applied to bacterial sensing, the material's favorable electrochemical characteristics and compatibility with flexible substrates make it a strong candidate for developing biosensors, such as those aimed at detecting PYO from PA.

Bezing *et al.* demonstrated the versatility of LIG-E by integrating them into both lateral and vertical flow μ PADs [63]. These platforms enabled a range of electrochemical bioassays,

including the detection of alkaline phosphatase in serum and the quantification based on CRISPR of HPV16 [63]. The ability to produce electrodes that are simultaneously conductive and permeable enables the development of compact, low-cost diagnostics that combine the simplicity of lateral flow devices with the quantification capability of electrochemical sensors.

Prakash *et al.* developed a highly conductive LIG–silver nanoparticle compound that exhibited excellent electrochemical performance as a supercapacitor electrode, along with notable antifungal properties [66]. This study highlights the versatility of LIG and its suitability for biomedical and sensing applications.

3.6.2 From LIG to Carbonized Paper Electrodes

While LIG methods discussed above rely on laser pyrolysis techniques, often performed in ambient air, many studies clarify that the atmospheric conditions strongly influence the structure of the resulting carbon material. For example, studies note that inert atmospheres (*e.g.*, Ar, N_2) during laser-induced carbonization yield more highly graphitized materials with well-defined pore structures [64, 65]. Conversely, laser-assisted carbonization in air produces a more disordered, porous carbon, which retains useful electrical conductivity but lacks the turbostratic graphene-like domains seen in true LIG [67]. Consequently, we adopt the term carbonized paper electrodes to accurately reflect the composition and fabrication environment.

3.6.3 Carbonized Paper Electrodes for PA Detection

Building on recent advancements in hydrophobic encapsulation and carbon-based paper microdevices, we introduce a novel paper-based electrochemical platform for the detection of PA through its unique redox biomarker, PYO. Our system consists of a three-layer cellulose structure, with carbonized electrodes directly patterned onto paper via thermal energy delivery from the laser in ambient air, offering a low-cost, flexible, and conductive sensing interface.

To address the challenge of rapid analyte evaporation, a limitation in many open-paper systems, the device incorporates a final encapsulation step using FC-40, a hydrophobic and biocompatible fluorocarbon [5]. This encapsulation forms a barrier that helps preserve the integrity of the absorbed sample, thereby extending the detection window and improving signal stability.

Designed with POC applications in mind, this platform enables rapid, equipment-free detection of PYO in small volumes of biological fluid. Its potential impact includes faster clinical

decision-making, reduced hospital stays and antibiotic usage, and ultimately, improved outcomes for patients with PA infections. The following section describes the fabrication process, encapsulation strategy, and electrochemical testing protocol used to validate the device.

CHAPTER 4 PROBLEM AND RESEARCH OBJECTIVES

4.1 Problem Statement

PA is a multidrug-resistant, opportunistic pathogen associated with chronic respiratory infections and hospital-acquired conditions. Early detection is critical to prevent progression and reduce mortality, but current diagnostic methods, such as bacterial culture, PCR, or chromatography, are time-consuming, require specialized infrastructure, and are impractical for rapid screening or use in low-resource settings.

PYO, a redox-active metabolite uniquely secreted by PA, offers a promising electrochemical biomarker for direct detection. However, existing electrochemical platforms often suffer from short sample retention times, poor integration into POC formats, and the need for costly fabrication techniques. Moreover, evaporation of small liquid samples on paper-based electrodes leads to signal instability and hinders repeatable measurements.

Therefore, there is a need for a robust, low-cost, and field-deployable sensing system that enables the sensitive and stable detection of PYO. This work addresses these challenges by developing a paper-based electrode platform that combines laser-induced carbonization and hydrophobic encapsulation to improve performance, extend sample lifetime, and simplify implementation at the POC.

4.2 Research Objectives

To guide the development of a paper-based electrochemical detection system for PA, four main research objectives were defined:

1. **Evaluate the effect of conductive ink modifications and application on electrode sensitivity.** PEDOT:PSS, EG, and DMSO were applied to improve electrode performance. Various deposition strategies (drop-casting, dipping) and varying PEDOT:PSS concentrations (1:3, 1:30) were tested and compared to determine ideal coating conditions for optimal detection capacity.
2. **Characterize the electrochemical response of PYO using CV and SWV.** Electrochemical performance was assessed by analyzing peak current, peak position, and response reproducibility. These metrics were extracted from CV and SWV data across multiple sensors to determine analytical reliability and sensitivity in first a well-known,

simple redox molecule, ruthenium hexamine (RuHex), and then for the detection of the target molecule PYO.

3. **Design and validate encapsulating paper design for sample confinement.** A folded, multilayer paper geometry was implemented to localize fluid introduction and retain the sample within the active zone. This configuration was coated with FC-40 to encapsulate the sensing region, aimed to improve usability and reproducibility by confining the analyte to this defined region. The effectiveness of this encapsulation was assessed by monitoring signal retention over time and comparing water loss percentages between treated and untreated electrodes.
4. **Implement a local detection system for PYO under laboratory conditions.** A functional testing setup was developed using the fabricated paper-based electrodes with a standard potentiostat to detect PYO under controlled lab conditions. This included designing an accessible three-electrode configuration and adapting CV and SWV protocols.

CHAPTER 5 MATERIALS AND EXPERIMENTAL METHODOLOGY

5.1 Reactants and Solutions

All reagents were obtained from Millipore Sigma (Canada), unless stated otherwise. Aqueous solutions were prepared using deionized water with a resistivity greater than $18 \text{ M}\Omega\cdot\text{cm}$.

Two formulations of PEDOT:PSS (Ref:655201) (3% in water) were prepared by dilution in deionized water: one at 1:3 v/v and another at 1:30 v/v. Both were supplemented with 5% v/v ethylene glycol (Ref:102466) in water (EG) to enhance film formation and conductivity [4]. In parallel, dimethyl sulfoxide (DMSO; Fisher BioReagents), containing 5% water, was applied to further improve PEDOT:PSS conductivity [4,57].

Polydimethylsiloxane (PDMS) SYLGARDTM 184 Silicone Elastomer Kit (Dow, U.S.A.) was used as a top-layer coating to prevent solution spreading. It was prepared at a 10:1 base-to-curing-agent ratio and applied to paper device before thermal curing.

To construct the reference electrode for encapsulated paper devices, Silver/silver chloride (60/40) paste for screen printing (Ref:901773) was applied with a fine brush (more details to follow in the encapsulated electrode assembly section).

PYO stock solutions were prepared in phosphate-buffered saline (PBS, at pH 7.4) for all voltammetry tests. Fluorinated oil (3MTM FluorinertTM Electronic Liquid FC-40, 3M, U.S.A.) was added by drop in excess onto the electrode surface after sample application for encapsulation purposes.

5.2 Paper combustion by Laser-Induced Carbonization

Whatman Grade 2 filter paper (150 μm thick, 11 μm particle retention size) was pretreated by immersion in a solution of ammonium sulfamate ($\text{NH}_4\text{SO}_3\text{NH}_2$, >98%, Sigma) and allowed to dry overnight. Once dried, the papers were pressed under weight to ensure flatness and consistency prior to laser processing.

Carbonization (see Figure 5.1) was performed using a Trotec Rayjet CO_2 laser engraver (power setting: 9/100; estimated power: 400 mW). Prior to laser treatment, the focal point of the CO_2 laser was defocused by 10mm, as done in previous literature [63]. This controlled defocus minimized thermal degradation of the cellulose substrate and improved both the reproducibility and conductivity of the resulting carbonized electrode tracks, as localized laser-induced thermal energy delivery promotes the conversion of cellulose into a porous

conductive carbon network [68]. Following laser treatment, the samples were rinsed with deionized water to remove residual particulates and dried overnight before use.

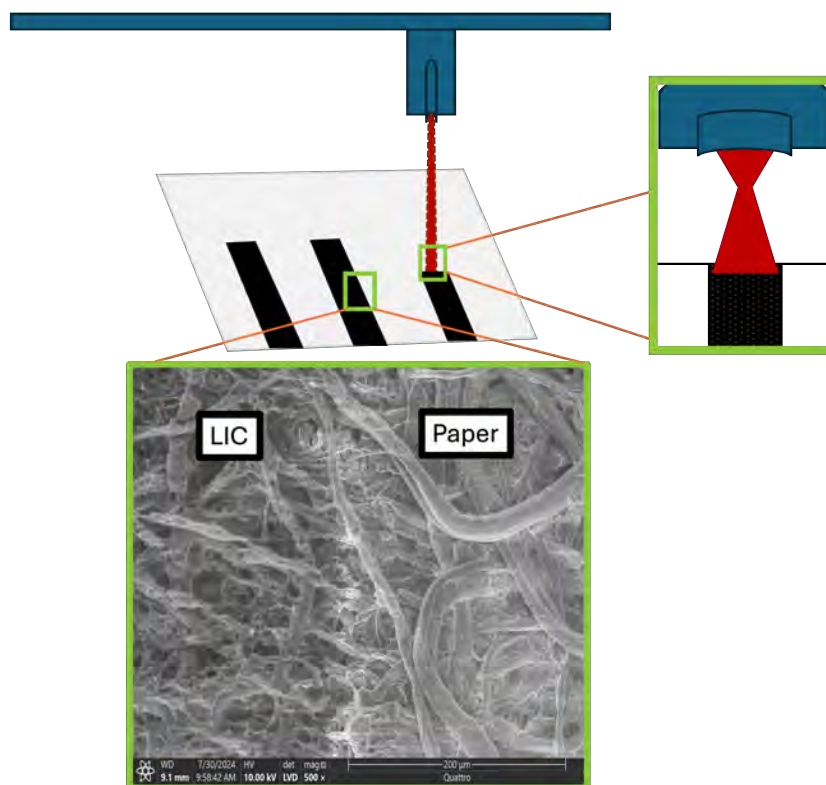


Figure 5.1 Laser-induced carbonization (LIC) of cellulose paper, as visualized by scanning electron microscopy (SEM). The left side of the image corresponds to the carbonized region, exhibiting morphological changes and increased surface roughness, while the right side remains unmodified, retaining the original fibrous paper structure. Zoom on laser demonstrates laser defocusing of 10mm.

5.3 Simple Electrode Assembly

After laser induced carbonization and cutting of the paper, a thin central layer of PDMS was applied using the edge of a microscope slide and cured overnight at room temperature or for 1 hour at 50°C. A group of electrodes were fully immersed (dipped) in a PEDOT:PSS solution, and for a second group PEDOT:PSS was drop-cast in three successive 2 μL layers, each allowed to dry between applications. The films were then drop-cast (2 μL) with DMSO and baked at 80°C until fully dry (about 20 minutes). The active area was standardized by cutting the tip of the electrode to a 2 mm-long active area. Copper tape was adhered to the top of the electrode to provide electrical contact. Refer to Figures 5.2 for steps and 5.3 for physical examples of resulting electrodes.

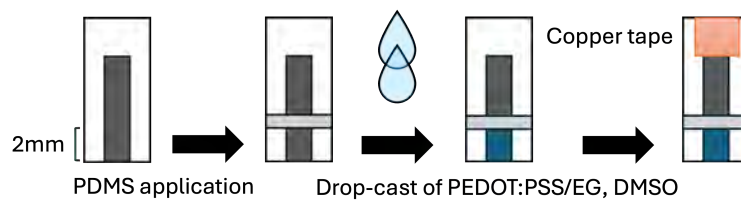


Figure 5.2 Simple electrode fabrication by drop casting conductive polymers.

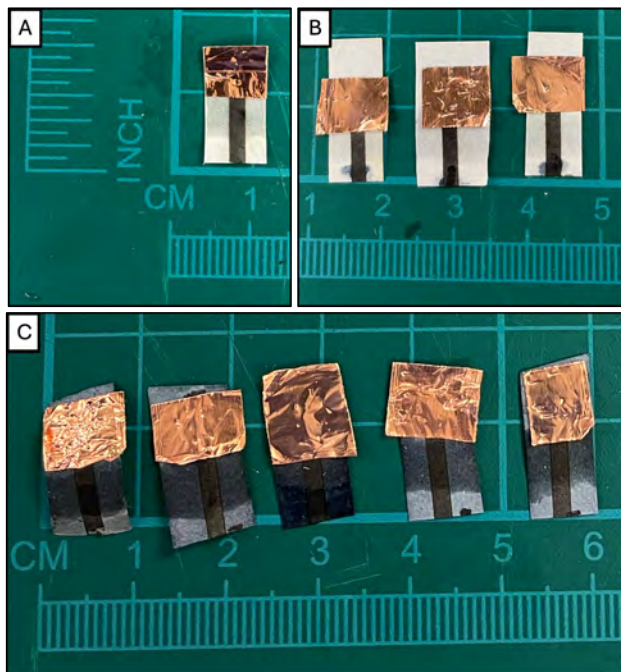


Figure 5.3 Simple electrode fabrication by (A) selective PDMS coating to define the detection area, (B) localized drop-casting of PEDOT:PSS at the sensing zone, and (C) full immersion of the carbonized electrode in PEDOT:PSS solution.

5.4 Scanning Electron Microscopy (SEM)

SEM was used to characterize the morphology of the paper-based electrodes after laser carbonization and subsequent surface modifications. Samples included (i) carbonized electrodes without additional treatment, (ii) carbonized electrodes coated with PDMS, and (iii) electrodes modified with PEDOT:PSS at 1:3 and 1:30 dilution ratios. Micrographs were acquired at both low and high magnification to visualize the overall architecture, the delineation between carbonized and untreated regions, and the distribution of PDMS and PEDOT:PSS coatings across the fibrous network. Imaging was performed on a Quattro environmental SEM operated at an accelerating voltage of 10.0 kV, with a beam current of 0.10 nA and a

working distance of 10.5–10.7 mm. Images were collected using the LVD detector, with magnifications ranging from $156\times$ (HFW 1.33 mm) to $500\times$ (HFW 414 μm). Samples were imaged directly without sputter coating, and all measurements were performed in high-vacuum mode.

5.5 PDMS Application Optimization for Encapsulated Electrode Fabrication

Initial attempts to apply PDMS using an AxiDraw plotter were found to be inefficient and challenging in terms of both alignment and flow control. As illustrated in Figure 5.5, this method frequently resulted in uneven deposition of PDMS, particularly along the edges of the carbonized working electrode. Due to the low viscosity and slow curing time of PDMS, multiple application cycles were often required to achieve a sufficiently thick and continuous layer.

To improve flow properties, PDMS was diluted with hexane in a 1:3 ratio. This initially reduced viscosity and allowed for smoother deposition through the syringe. However, over time, PDMS began to partially cure within the syringe, likely due to the limited pot life of the pre-mixed solution and prolonged room-temperature exposure resulting in hexane evaporating, and possibly increasing local concentration of reactive species. This clogging disrupted application mid-process and reduced reproducibility.

To overcome these challenges and enhance fabrication throughput, a custom stamp was developed (represented in Figures 5.6 and 5.9a). This stamping method enabled rapid and spatially controlled deposition over targeted regions of the paper substrate, eliminating the need for mechanical alignment and significantly improving coating uniformity.

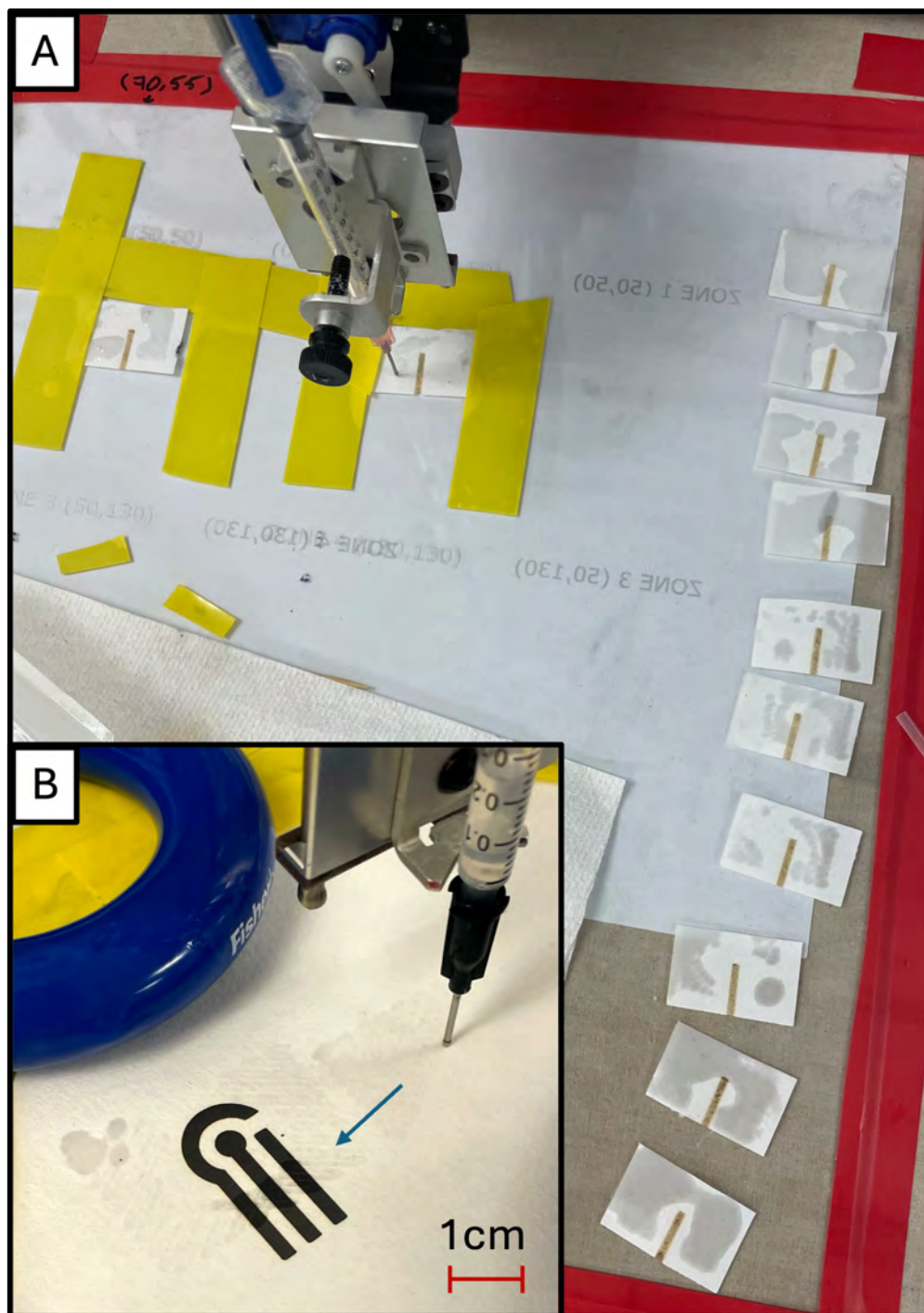


Figure 5.4 Optimization workflow using AxiDraw for PDMS application. (A) Electrodes were manually aligned and fixed in place for plotting, with PDMS applied around the central carbonized WE. Initially, only the WE was included for simplicity; RE and CE were added separately during testing. Multiple deposition attempts were required to achieve uniform PDMS coverage. (B) In some cases, premature polymerization within the syringe led to incomplete or uneven application, resulting in an insufficient barrier to retain the liquid sample.

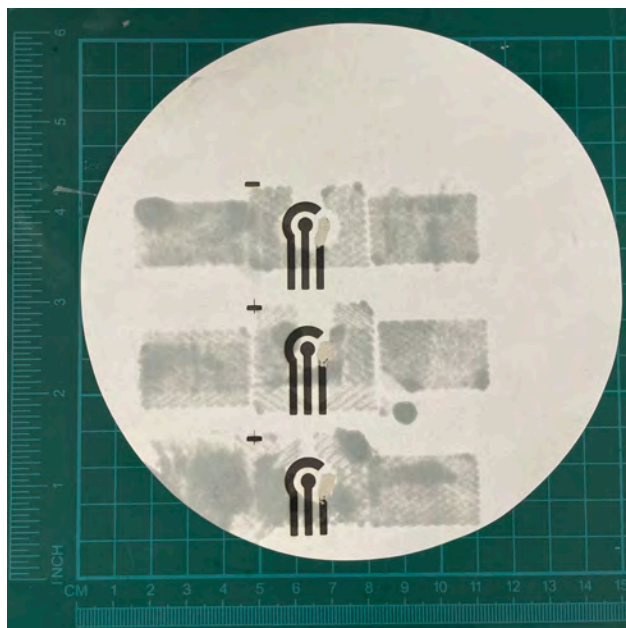


Figure 5.5 Example of misalignment and uneven PDMS deposition using the AxiDraw plotter.

5.5.1 PDMS Stamp Fabrication for Electrode Patterning

Replica Molding of PDMS Stamp from 3D-Printed PLA Master

A custom polydimethylsiloxane (PDMS) stamp was fabricated using replica molding from a 3D-printed polylactic acid (PLA) master mold (refer to Figure 5.6 for design). The mold was designed in *PrusaSlicer* MK4 and printed with a fused deposition modeling (Prusa MK4) 3D printer using PLA filament (Elegoo).

The master mold measured 105 mm in length, 40 mm in width, and 18 mm in height (seen below in Figure 5.7b). It was used to define the stamping surface dimensions of the PDMS, which resulted in a flat cured elastomer of approximately 102.77 mm $\times$ 37.79 mm with a thickness of 1.0 cm.

The PDMS pre-polymer (Sylgard 184, mixed at a 10:1 base-to-curing agent ratio) was poured into the PLA mold (Figure 5.7a) and degassed under vacuum for 10 minutes to remove air bubbles. It was then thermally cured at 50 °C for 12 h to ensure full cross-linking.

After curing, the PDMS sheet was bonded to a separate PLA handle (110 mm $\times$ 45 mm $\times$ 55 mm) using UV-curable resin (ABS-like Resin V3.0 Colored, Elegoo). This rigid handle allowed for consistent manual stamping and alignment.

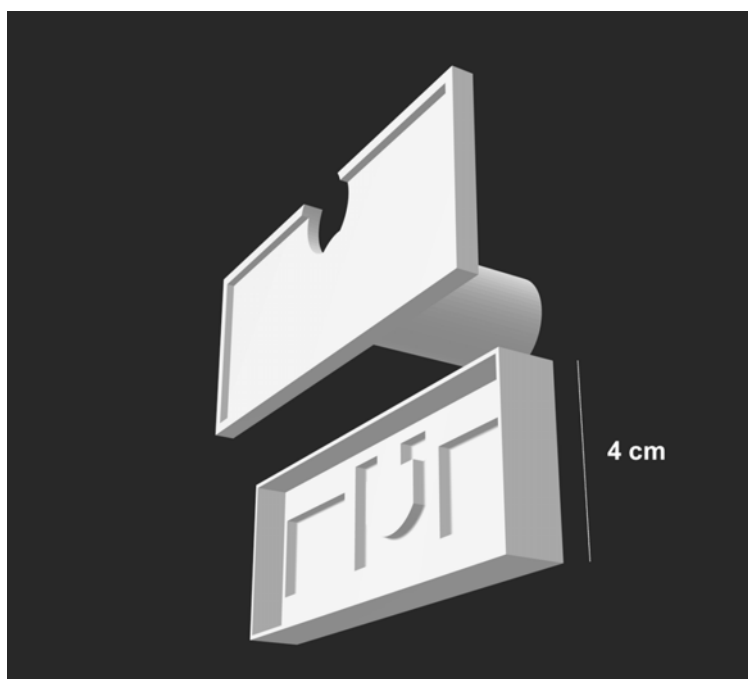
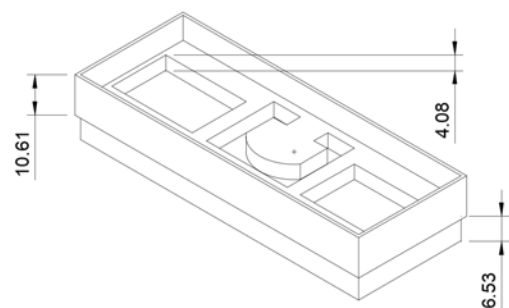


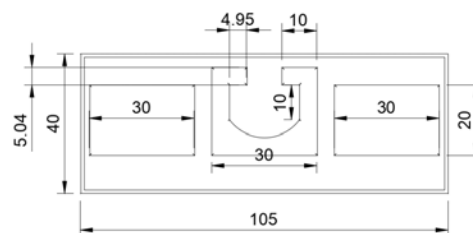
Figure 5.6 Design and fabrication of the PDMS stamping tool. The lower PLA mold defines the pattern into which PDMS is poured and cured. A PLA support handle is bonded to the cured PDMS using UV-curable resin, allowing manual stamping. The patterned surface enables reproducible PDMS transfer for hydrophobic barrier formation.

Application of the PDMS Stamp for Selective Coating

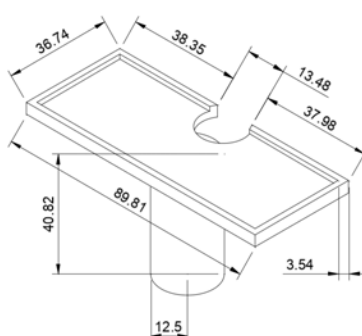
The PDMS stamp was used to selectively transfer thin layers of PDMS to specific regions on the carbonized paper electrodes. Manual stamping was performed by aligning the handle and gently pressing the elastomeric pad against the paper's surface to form well-defined hydrophobic barriers. This process prevented undesired fluid spreading while standardizing the exposed sample zone geometry. The stamp dimensions were matched to the electrode width to ensure full coverage and reproducibility. Compared to manual drop-casting or AxiDraw-assisted PDMS application, the stamping method offered faster throughput, improved alignment, and greater inter-device consistency.



(a) Side view of PLA mold.



(b) Top view of master mold tray.



(c) Technical drawing of PLA handle.

Figure 5.7 Technical views of the PDMS mold and stamping components. Units for measurements are in millimeters.

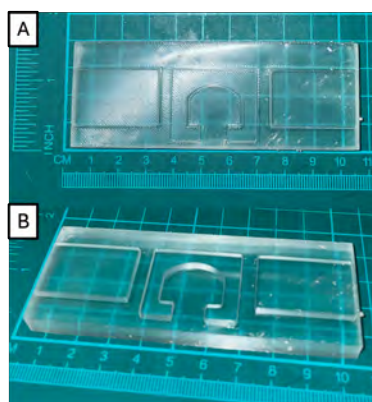
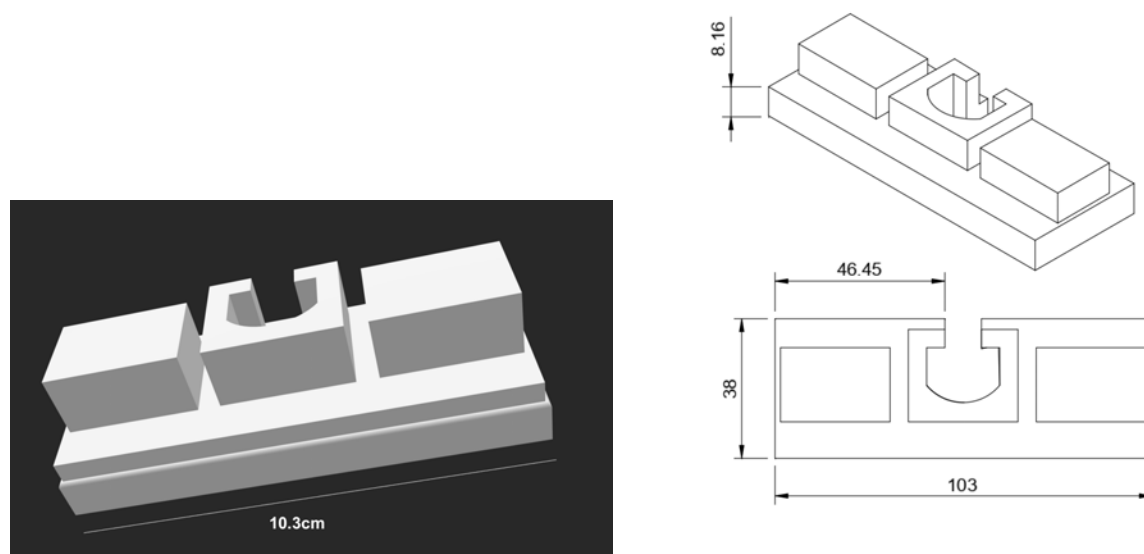


Figure 5.8 (A) Top view of resulting PDMS stamp along with (B) Side-view

Direct PDMS-Coated PLA Stamp

As an alternative approach, as demonstrated in Figure 5.9, a stamp was fabricated by directly coating a 3D-printed PLA structure, identical in geometry to the replica-molded PDMS stamp, with uncured PDMS pre-polymer. To fabricate the PDMS stamping surface, uncured PDMS (prepared at a 10:1 base-to-curing-agent ratio) was poured over a 3D-printed polylactic acid (PLA) mold. Excess PDMS was removed using a controlled wiping method to achieve a uniform surface layer and allowed to cure at room temperature. This process generated a negative-relief PDMS replica of the mold, providing a flexible and conformal surface for consistent PDMS deposition during electrode encapsulation. The coated structure was then thermally cured at 50 °C for 12 h, yielding a rigid stamp with a conformal PDMS surface that mimics the flexibility of the molded version while simplifying the fabrication process.



(a) 3D render of the PDMS-coated PLA stamp.

(b) Technical diagram with dimensions.

Figure 5.9 Design of the alternative stamp fabricated by directly coating PLA with PDMS. This method integrates mechanical rigidity and surface flexibility in a single fabrication step. Measurement values for the technical diagram (b) in millimeters.



Figure 5.10 Top view (A) and side-view (B) of resulting PLA stamp coating by PDMS by pouring over PDMS.

Final Selection of Stamping Method: PDMS Stamp vs. PDMS-Coated PLA

Based on the comparative analysis presented in the results section (Figure 6.19), the PDMS stamp was selected as the preferred method for applying the hydrophobic PDMS barrier. This choice was motivated by its ability to consistently produce cleaner and more uniform PDMS layers on the paper substrate. Additionally, the optical transparency of PDMS facilitated precise alignment with the underlying carbonized electrode pattern, improving reproducibility and fabrication accuracy. To fully leverage this advantage, the stamp was used without the 3D printed handle, allowing for direct visual guidance during placement.

5.6 Encapsulated Electrode Assembly

Following laser-treatment and paper washing steps, PDMS was applied as previously described to specific regions of the paper and allowed to partially absorb into the substrate for approximately 5 minutes. This pre-treatment helped define hydrophobic zones and limit lateral wicking. Ag|AgCl ink was then applied to the terminal end of the carbonized strip for the RE using a fine brush. This Ag|AgCl coating served as a quasi-reference electrode, enabling stable and reproducible potential measurements during voltammetric testing.

The same procedure as used in the simple electrode assembly was followed for modifying the WE: PEDOT:PSS diluted with EG was drop-cast at the circular sensing area located at the center of the WE region. After drying, DMSO was drop-casted to enhance conductivity as per previously elaborated for simple electrodes.

The paper was then folded to create a chamber structure. Figure 5.11 and 5.12 demonstrate the fabrication steps for the encapsulated sensor assembly. One side of the paper was folded forward, and the opposite side folded backward, aligning the three carbonized electrode strips (WE, CE, and RE) into a layered sandwich configuration. The folded assembly was secured using mechanical clips and oven-cured at 50 °C for 30 minutes to allow PDMS cross-linking and Ag|AgCl ink setting as demonstrated in Figure 5.12.

After curing, copper tape was affixed to each exposed end of the carbonized paper corresponding to the working, counter, and reference electrodes. Care was taken to ensure that each tape segment remained electrically isolated to prevent short-circuiting between adjacent electrodes. These conductive pads provided external electrical connections to the potentiostat. The resulting structure formed a self-contained electrochemical cell with a central chamber capable of capillary-driven sample uptake.

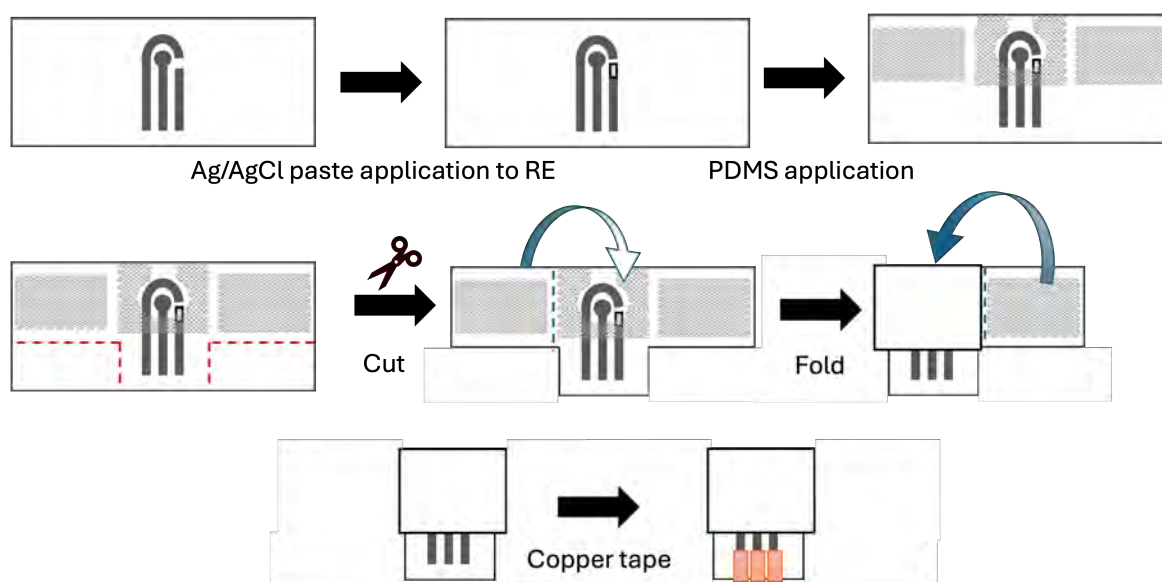


Figure 5.11 Encapsulating electrode fabrication steps.

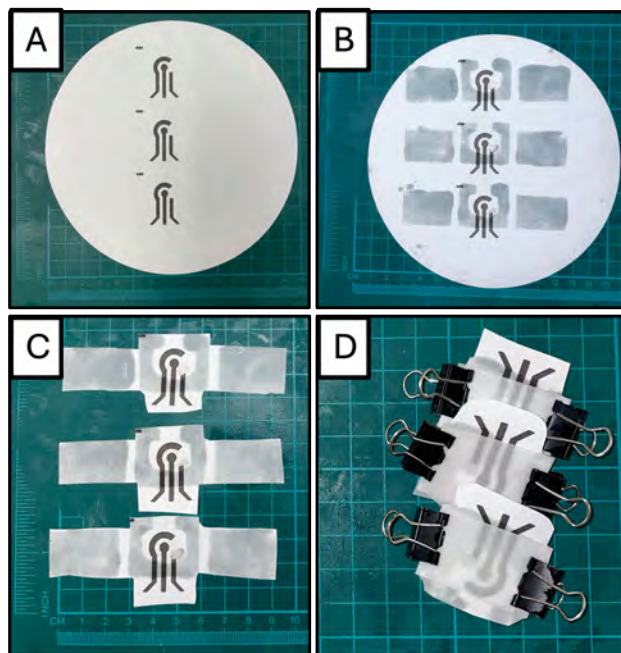


Figure 5.12 Encapsulated electrode assembly steps including (A) Carbonization pattern of electrode on Whatman paper, (B) Application of PDMS (by PDMS stamp) along with Ag|AgCl paste onto WE carbonized strip, (C) cutout of paper including a front and back covering flap and (D) clipping on ends to stabilize sandwich structure for oven curing

5.7 Electrochemical Detection of Pyocyanin

Electrochemical detection of PYO was carried out using a three-electrode setup connected to a benchtop potentiostat (CH Instruments, Austin, Texas). CV protocols were adapted from Pelletier *et al* [4]. The CV scan range was limited to -0.4 V to 0 V to prevent the onset of PYO polymerization, which can occur at more positive potentials. This window selectively captures the reversible redox transformations near -0.18 V and -0.30 V , while deliberately excluding the irreversible oxidation process typically observed around $+0.85\text{ V}$ [40]. While for SWV measurement parameters were as follows: step potential = 4 mV , pulse amplitude = 25 mV , frequency = 5 Hz , init $E = 0\text{ V}$, final $E = -0.6\text{ V}$, quiet time = 10 sec and sensitivity (A/V) = 1×10^{-4} . This positive-to-negative scan direction was selected to target the cathodic peak of PYO, which typically appears between -0.2 to -0.3 V vs Ag|AgCl. While many studies emphasize anodic detection of PYO, cathodic detection by SWV has also been reported in the literature and provides a reliable signal corresponding to the reversible redox couple of PYO [40, 69]. Measurements were performed using paper-based sensors in phosphate-buffered saline (PBS) to evaluate the redox response of PYO. Comparisons were made between unmodified electrodes and those modified with PEDOT:PSS, EG, and DMSO.

5.7.1 Electrode Connection and Measurement Process

For unencapsulated, simple electrodes, only the WE was integrated into the paper substrate (Figure 5.13). This electrode was immersed in a measurement vial along with a separate platinum wire serving as the CE and a commercial Ag|AgCl as the RE.



Figure 5.13 Voltammetry set-up for simple paper electrode including three separate electrodes (RE, WE (paper chip), CE) in the beaker containing the analyte (here, PYO solution, naturally blue)

Encapsulated electrodes, by contrast, contained all three electrodes (WE, CE, and RE) embedded within the carbonized paper (Figure 5.14). These were suspended horizontally and connected to the potentiostat via copper tape affixed to the conductive ends of each strip. (Figure 5.15) A single droplet (0.5 mL) of analyte solution was introduced at the bottom opening of the folded electrode chamber, where capillary action transported the liquid toward the central sensing zone within approximately one minute. Between successive concentrations, any residual solution near the inlet was removed by gently blotting with a paper towel to avoid dilution effects.

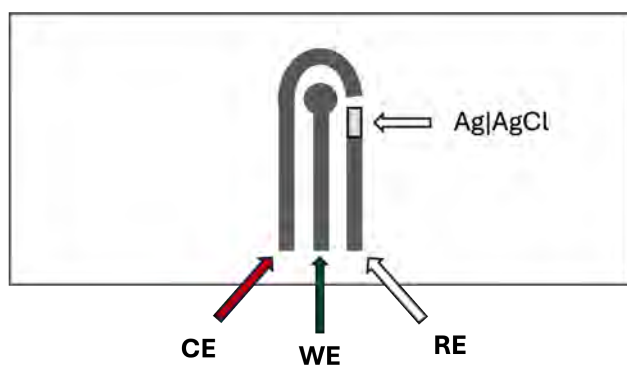


Figure 5.14 Counter, working and reference electrode included in encapsulated paper sensor. For this design, WE is 4mm in diameter.

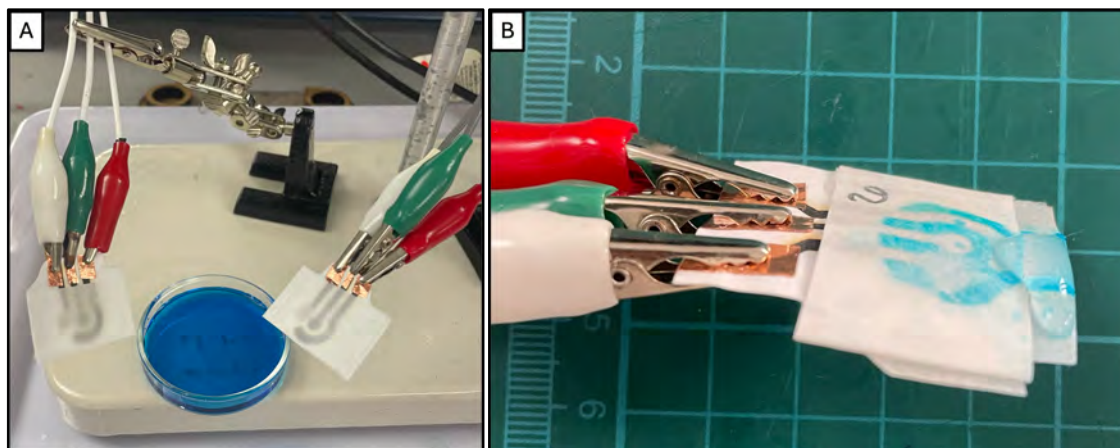


Figure 5.15 (A) Top view of a horizontally clipped encapsulated electrode, showing individual connections to the carbonized reference (RE, white), working (WE, green), and counter (CE, red) electrodes prior to measurement. (B) Side view of the same electrode after measurement, illustrating the liquid-filled sensing chamber. The entry point for sample introduction is visible, where solution droplets were instilled to form a reservoir. For visualization purposes, three drops of blue dye were added to highlight fluid diffusion through the inlet and into the main sensing zone of the paper-based device.

5.7.2 Electrochemical Testing in Oxygen-Depleted and Ambient Conditions

To assess the effect of dissolved oxygen on electrochemical response, measurements were performed on simple (non-encapsulated) electrodes in 1,M PBS under two conditions: ambient air and oxygen-depleted solution. Oxygen removal was achieved by bubbling nitrogen gas into the PBS for 15 minutes prior to testing. CV was conducted at a scan rate of 10 mV/s, and SWV was performed at 5 Hz. Each electrode was tested immediately after purging, al-

lowing direct comparison of voltammograms recorded in the presence and absence of dissolved oxygen.

5.7.3 Pyocyanin LOD Estimation

SWV calibration was performed with PYO standards from 0.1 to 1000 μM , measuring peak current I_p sequentially from lowest to highest concentration on the same electrode. The resulting current–concentration data were fitted to the Langmuir model (Eq. *affinity form*):

$$I(C) = \frac{I_{\max} K C}{1 + K C}$$

where $I_{\max}$ and K are the saturation current and adsorption constant, respectively (see Sec 2.1) for notation and the equivalent half-saturation form, Eq. *half-saturation form*). Nonlinear least-squares regression was carried out in Python (SciPy 1.11.1, `curve_fit`), with initial guesses $I_{\max} = \max(I_p)$ and $K = 1/\text{median}(C)$ to ensure stable convergence.

From the fitted parameters we computed the initial slope,

$$\left. \frac{dI}{dC} \right|_0 = I_{\max} K,$$

and applied the 3σ criterion to obtain

$$\text{LOD} = \frac{3\sigma}{I_{\max} K},$$

where σ is the standard deviation of blank responses measured in PBS on uncoated control electrodes (Eq. 3.2). This approach employs the slope obtained from the full Langmuir fit, avoiding biases introduced by fitting only a limited linear segment of the calibration curve.

5.8 Evaporation Time Measurements

5.8.1 Water Weight Loss Method

The drying kinetics of the electrodes were characterized using a gravimetric method. Electrodes were first weighed in their dry state, then dipped in distilled water and weighed again to determine wet mass. Samples were placed on a balance, and the weight was recorded every 15 minutes. The percentage of water loss was calculated over time to generate evaporation curves. Results from electrodes treated with FC-40 were compared to untreated controls to assess the effect of encapsulation on evaporation rate.

5.8.2 Signal Intensity Loss Over Time

To evaluate signal retention under conditions of prolonged exposure, CV measurements were recorded at 15 minute intervals. Prior to each measurement, the paper electrode was saturated by dipping it in a 1 mM RuHex solution. Immediately afterward, an excess of FC-40 fluorinated oil was gently applied to the surface of the sensor to create a vapor barrier. Care was taken to avoid contact between the FC-40 and the copper tape at the top of the electrode to prevent loss of electrical connectivity at the clip interfaces.

For clarity, in this section, electrodes treated with FC-40 are referred to as “encapsulated,” while those without FC-40 are designated “non-encapsulated.” Both configurations share the same multilayer paper structure and PDMS chamber design, and include integrated working, counter, and reference electrodes.

This time-course study was conducted on both encapsulated and non-encapsulated paper sensors to assess the effectiveness of FC-40 in preserving electrochemical signal stability. The peak currents were monitored over time and used as a direct proxy for analyte availability and electrode hydration.

This protocol allowed for a systematic comparison of temporal signal degradation between device configurations, forming the basis for subsequent analysis of the encapsulation strategy’s performance in preserving sensor functionality.

CHAPTER 6 RESULTS AND DISCUSSION

6.1 Laser-Induced Graphene Synthesis and Translation to Carbonized Paper Electrodes

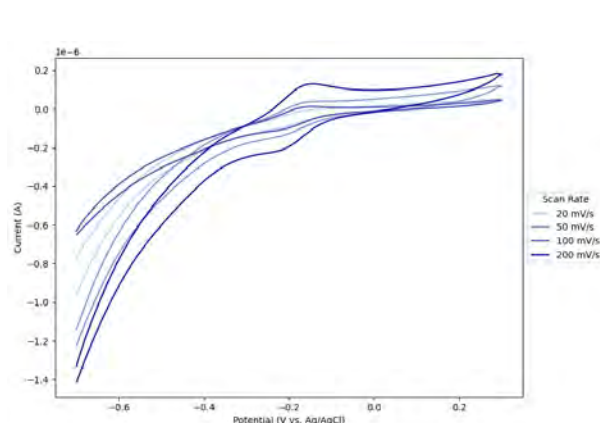
LIG is synthesized by focusing a CO₂ laser onto carbon-rich substrates such as polyimide, wood, or paper, inducing a localized photothermal conversion of sp³-hybridized carbon into sp² domains [70]. This rapid, mask-free process produces a porous, electrically conductive network under ambient conditions, with the resulting structure exhibiting high surface area, mechanical flexibility, and tunable electrical conductivity [64].

Although optimal LIG synthesis typically occurs under inert gas to promote graphene crystallinity, the same laser-substrate interaction can be applied in air to yield amorphous yet conductive carbon structures. Inspired by these principles, we adapted the LIG methodology to fabricate our own carbonized paper electrodes using cellulose substrates under ambient conditions. Prior work guided the selection of laser parameters (power, scan speed) to ensure reproducible carbonization and sufficient surface conductivity to support electrochemical detection [71].

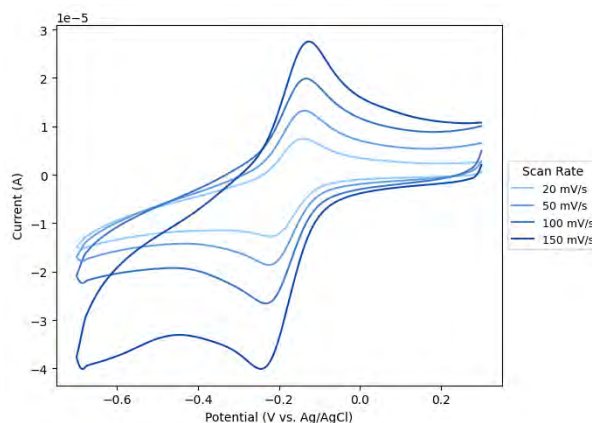
6.1.1 Characterization of Simple Carbonized Electrodes in RuHex

As an initial validation step, the carbonized paper electrodes were characterized using 1 mM RuHex, a widely employed outer-sphere redox probe [72–74]. RuHex undergoes a single-electron transfer without forming any intermediate chemical species or adsorbing to the electrode surface, making it an ideal standard for evaluating fundamental electron transfer behavior. Without a chemical transformation, product formation, or specific surface interaction involved, the redox process is thermodynamically reversible and entropy-neutral. These characteristics eliminate complicating factors such as fouling, catalysis, or surface binding, thereby allowing the electrochemical response to be attributed solely to the electronic properties of the electrode.

CV was performed across a range of SR and compared to a commercial glassy carbon electrode (GCE).



(a) Carbonized paper electrode.



(b) Commercial glassy carbon electrode.

Figure 6.1 CV of 1 mM RuHex in 1 M KCl up to 200 mV/s using (A) a carbonized paper electrode and (B) a commercial glassy carbon electrode. Both electrodes exhibit the characteristic reversible redox couple of RuHex, validating the electrochemical functionality of the carbonized substrate.

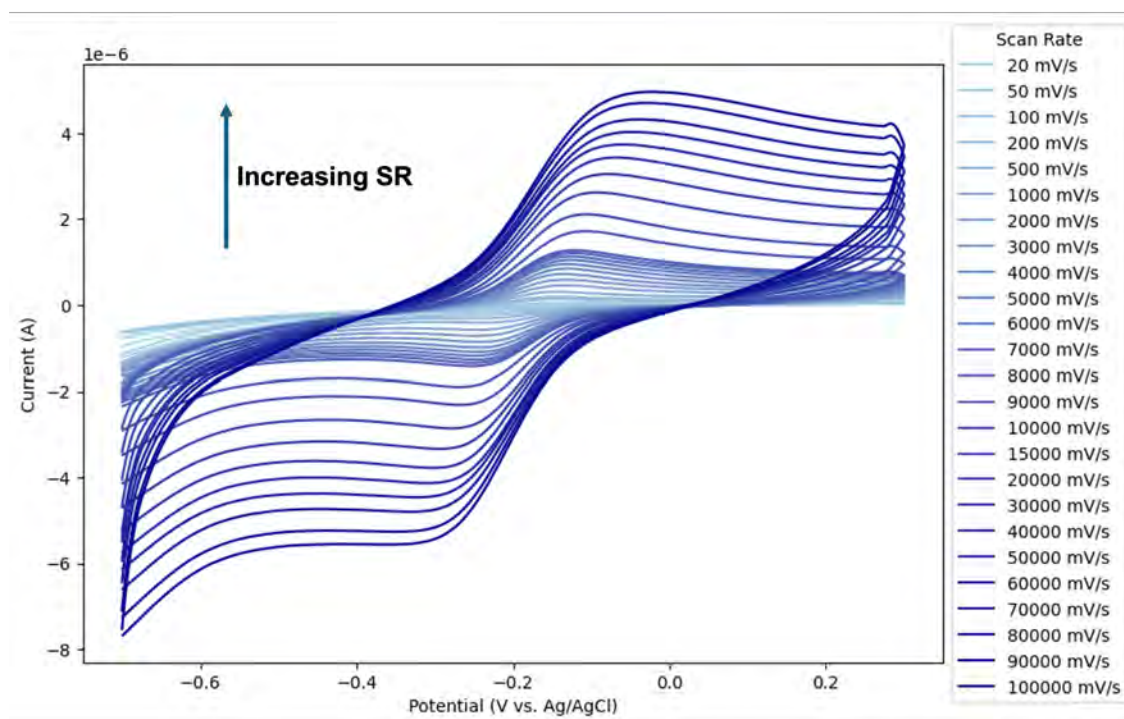


Figure 6.2 CV of 1 mM RuHex in 1 M KCl using a carbonized paper electrode across a wide range of SRs (20–100,000 mV/s). Peak currents increase linearly with SR while maintaining a symmetric redox profile, confirming the stability and responsiveness of the simple carbonized electrode.

6.1.2 SR dependence of carbonized-paper electrodes versus GCE in 1 mM RuHex

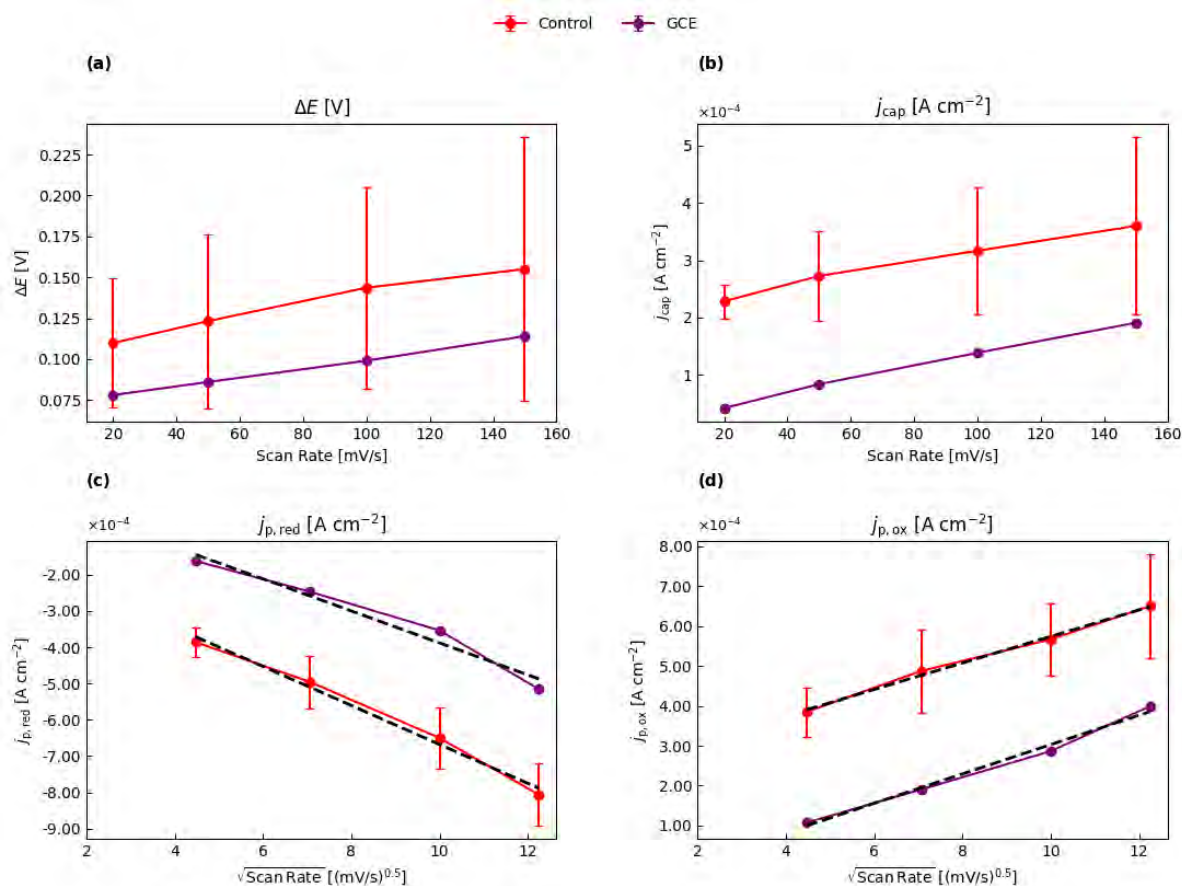


Figure 6.3 SR Analysis of ΔE , peak current densities ($j_{p,red}$ and $j_{p,ox}$) and capacitive current densities ($j_{p,cap}$) of simple carbonized electrodes (control) in comparison to commercial GCE in 1 mM RuHex.

Carbonized paper electrodes (“Control”) were benchmarked in 1 mM RuHex against a commercial GCE, with all currents normalized to geometric area (Control: $2 \times 2\text{ mm}$; GCE: $3\text{ mm } \varnothing$). Across $SR = 20\text{--}150\text{ mV s}^{-1}$ the voltammograms show the expected, symmetric redox couple for an outer-sphere probe.

Peak separation (ΔE). For Control, ΔE increases with SR, more rapidly than with GCE. This pattern is consistent with near-reversible electron transfer on both electrodes, with GCE exhibiting marginally faster apparent kinetics and/or lower ohmic drop. No signatures of sluggish or surface-limited behavior are observed.

Peak current scaling and SR linearity (j_p vs $\sqrt{\text{SR}}$). For both Control and GCE, the oxidation and reduction peak current densities increase linearly with $\sqrt{\text{SR}}$, the signature of diffusion control in RuHex. Peak magnitudes remain balanced between anodic and cathodic branches, consistent with reversible behavior. Regressions on the mean points across the four SR values (including 150 mV s^{-1}) are near-ideal for both substrates (Control: $R^2=0.992 \pm 0.004$; GCE: 0.977 ± 0.017), with slopes of the expected sign (negative for reduction, positive for oxidation) and comparable magnitude. On a per-area basis, Control often exhibits larger j_p than GCE, indicating a higher electrochemically active surface area rather than fundamentally different kinetics.

Capacitive background. The capacitance-like current density increases with SR on both electrodes, as expected for double-layer charging. Control shows a consistently larger capacitive baseline than GCE, pointing to greater surface roughness/porosity. Importantly, this elevated background does not disrupt the diffusion-controlled scaling of the faradaic peaks.

Area-normalized CVs in RuHex confirm that the Control electrodes behave as nearly reversible, diffusion-limited systems with signals comparable to a GCE. Slightly larger ΔE alongside stronger j_p per area indicates that the carbonized paper offers more interfacial area while maintaining outer-sphere kinetics close to the GCE benchmark.

6.1.3 Voltammetric Characterization of Simple Carbonized Electrodes in PYO

6.1.4 Characterization by CV

Following these preliminary tests, the electrodes were evaluated in 1 mM PYO to establish their suitability for sensing applications. CV was conducted using unmodified carbonized paper electrodes across a broad SR range (10–8000 mV/s), as shown in Figure 6.4. This experiment allowed characterization of key electrochemical features of PYO on the native surface, including peak current evolution and widening of peak-to-peak separation (ΔE) at higher SRs. Compared with RuHex, ΔE is more pronounced, especially at SR above 200 mV/s, possibly attributable to slower heterogeneous electron transfer kinetics, partial surface adsorption, and possible proton-coupled or chemically coupled redox mechanisms, resulting in a more sluggish response [75]. Overall, these foundational measurements confirm the chemically reversible redox behavior of PYO on carbonized paper and serve as a benchmark for assessing enhancements introduced by conductive polymer coatings. Data representing CV parametrics are tabulated in Table 6.1 where simple electrodes were compared to those modified with PEDOT:PSS ink.

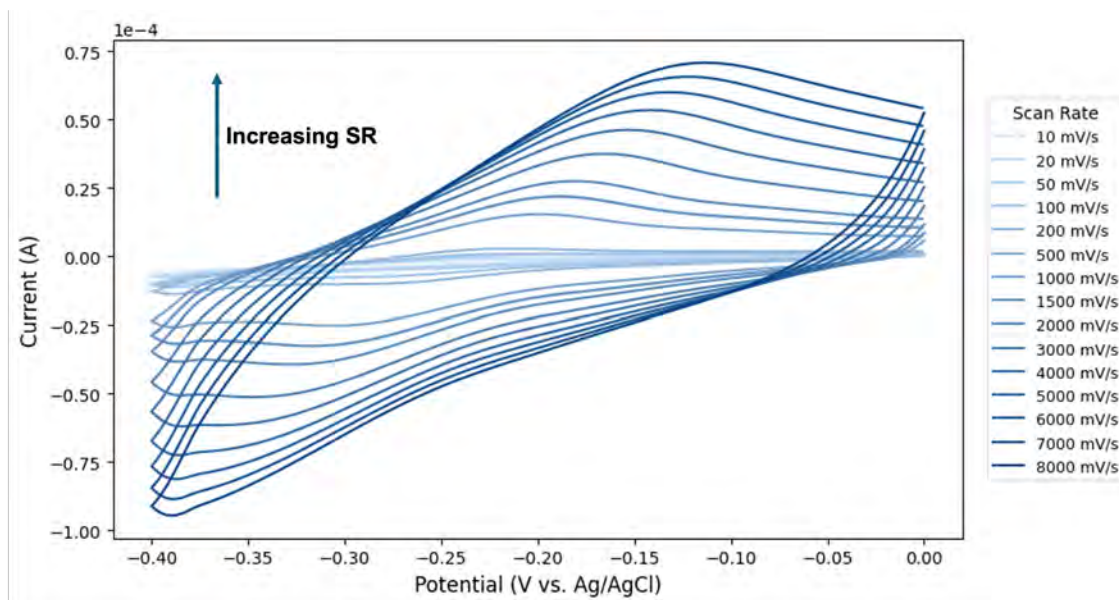


Figure 6.4 CV of 1 mM PYO on a carbonized paper electrode at SR ranging from 10 to 8000 mV/s.

6.1.5 SR dependence of carbonized-paper electrodes versus GCE in 1 mM PYO

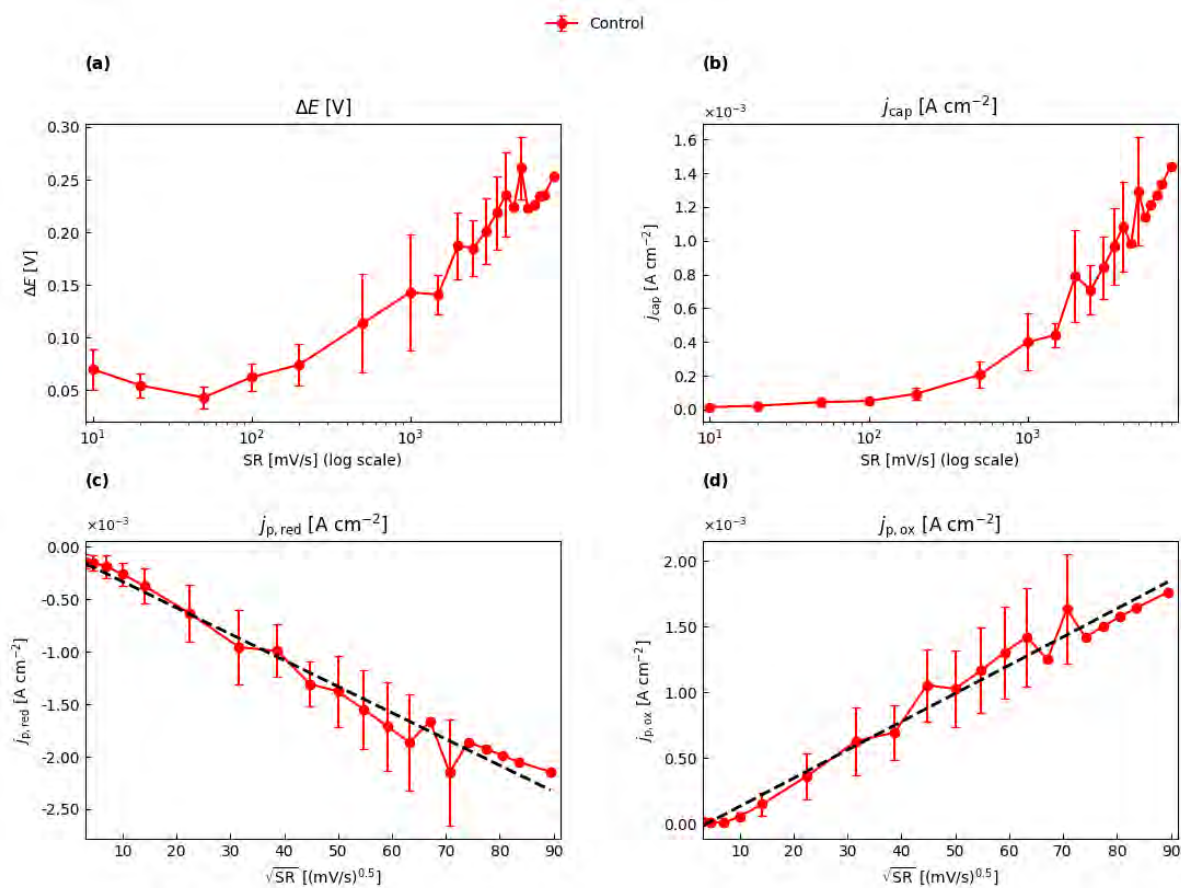


Figure 6.5 SR Analysis of simple carbonized electrodes (control) in 1 mM PYO.

Peak separation (ΔE). The peak-to-peak separation increases with SR, consistent with quasi-reversible electron transfer in PYO. Values remain below 300 mV even at the highest rates, without signs of irreversible behavior or electron transfer limitations. The trend indicates a diffusion-limited regime with moderate kinetic contributions, as expected for redox-active phenazines.

Peak current density scaling and SR linearity (j_p vs $\sqrt{SR}$). The peak current densities for both oxidation and reduction scale linearly with $\sqrt{SR}$, confirming that PYO detection on the paper electrodes remains governed by semi-infinite diffusion. The correlation remains strong across a wide range of scan rates, with mean $R^2 = 0.975 \pm 0.003$ for reductive and oxidative peaks. This mirrors the behavior observed in RuHex and further supports that faradaic response is not surface-confined.

Capacitive background. The capacitive current density (j_{cap}) grows with increasing SR, in line with double-layer charging. The magnitude of j_{cap} rises monotonically but does not distort the faradaic response, even at elevated scan rates. This indicates that background charging remains linear and additive, rather than interfering with peak detection.

Cyclic voltammetry of PYO on carbonized paper electrodes yields peak currents that scale with $\sqrt{\text{SR}}$, confirming diffusion-controlled transport. The electrodes maintain this linearity over nearly three orders of magnitude in SR, with peak separation and capacitive trends that match expectations for outer-sphere redox couples.

6.1.6 Characterization by SWV

Optimization of Frequency for SWV Measurements

Before measuring LOD, various SWV frequencies were evaluated to determine the optimal condition for electrode response to PYO. Unmodified carbonized electrodes were tested at frequencies of 1, 2, 5, 10, 15, and 30 Hz to assess both differential peak current intensity (I_p) and reproducibility. Representative voltammograms for one electrode (three replicates) are shown in Figure 6.6; shaded regions indicate the standard error of the mean (SEM). The mean anodic peak currents for control electrodes as a function of frequency (Figure 6.7), with error bars denoting SEM ($n = 3$) is depicted below.

As frequency increased, I_p rose sharply up to 15 Hz. However, the peak magnitudes became more comparable between electrodes (refer to the red arrow on Fig. 6.7) at 5 Hz. Furthermore, each SWV scan at 5 Hz required only one-fifth of the acquisition time compared to 1 Hz, markedly improving throughput without sacrificing signal fidelity. Accordingly, 5 Hz was selected as the optimum frequency for all subsequent calibration investigations.

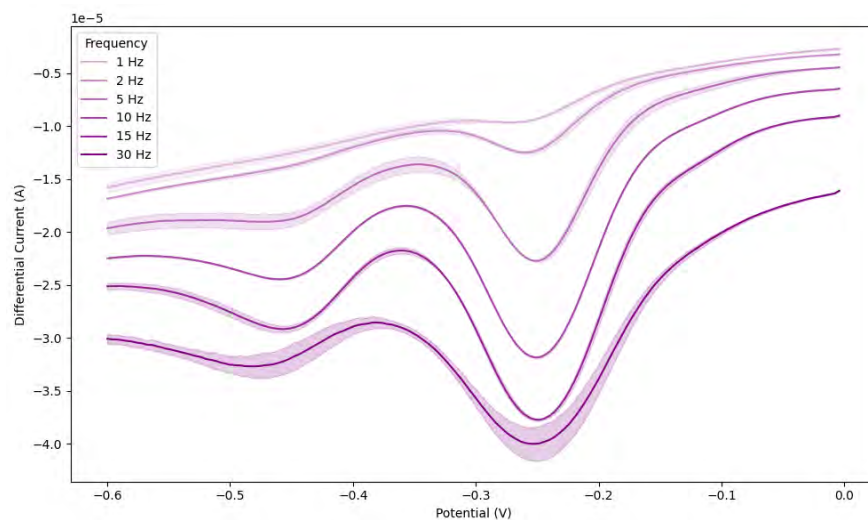


Figure 6.6 Representative SWV curves for a control electrode at 1, 2, 5, 10, 15, and 30 Hz in 1mM PYO solution. Shaded regions represent SEM ($n = 3$).

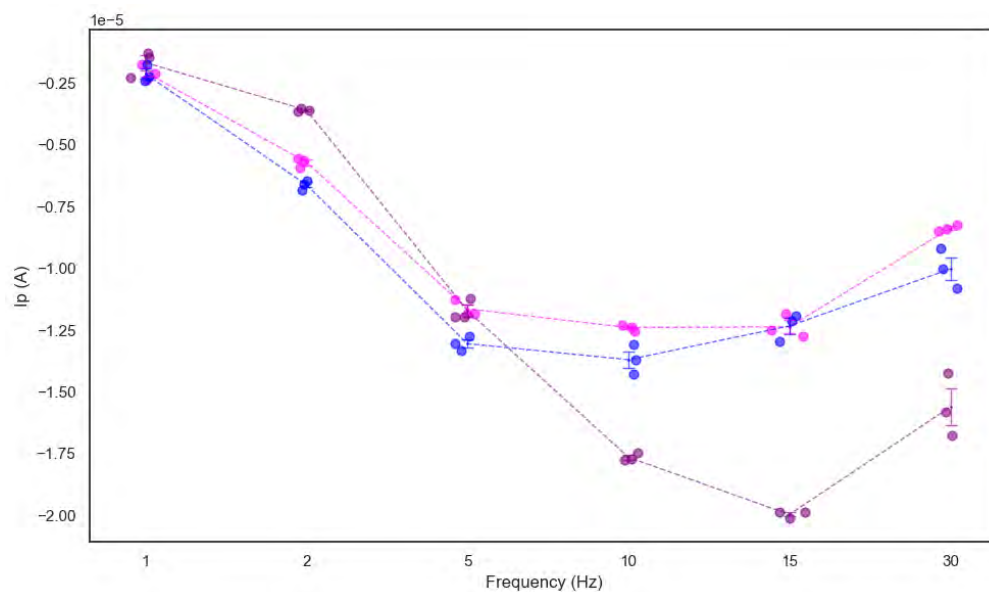


Figure 6.7 Anodic peak current (I_p) versus frequency for three control electrodes in 1mM PYO solution, with error bars denoting SEM ($n = 3$).

SWV Frequency Effects on Current Peak

In the SWV figure depicted above (Figure 6.7) a secondary peak current appears, seeming to grow with frequency. To further investigate the effects of square wave frequency on the electrochemical behavior of the electrodes, both the primary and secondary peak responses

were analyzed as a function of frequency for all control and PEDOT:PSS-modified electrodes. Figure 6.8 presents a comparative overview of anodic peak current (I_p), peak potential (E_p), and the secondary peak features (I_{p2} and E_{p2}) across the frequency range tested.

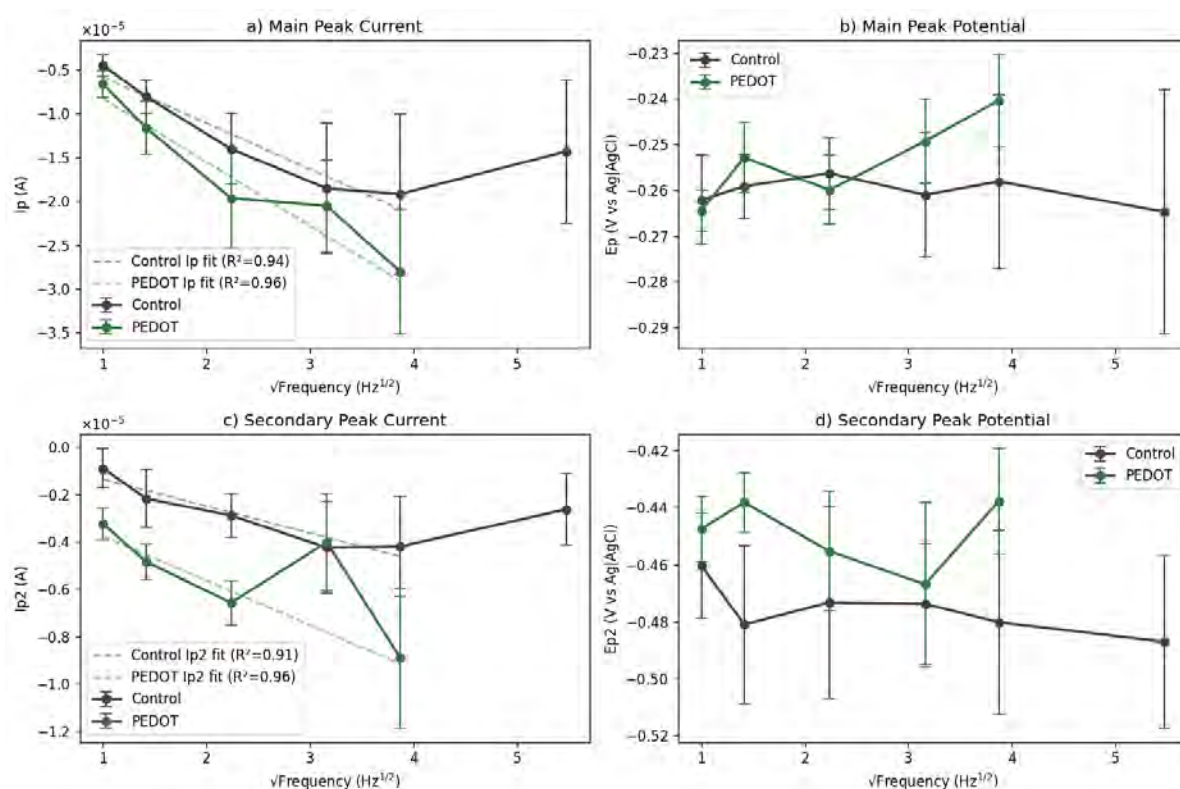


Figure 6.8 Group-level mean and standard deviation of SWV peak parameters versus frequency for control (gray) and PEDOT:PSS-modified (green) electrodes in 1 mM PYO. Top row: main peak current (I_p) and peak potential (E_p). Bottom row: secondary peak current (I_{p2}) and potential (E_{p2}). Error bars denote standard deviation across electrodes ($n = 3$ per electrode).

Main peak current (I_p). Both control and PEDOT-modified electrodes exhibited a non-monotonic response: $|I_p|$ increased between 1 and 5 Hz, then plateaued or declined at higher frequencies. This is consistent with reduced faradaic efficiency under rapid perturbation, as the charging current increases and mass transport becomes limiting. PEDOT-modified electrodes showed greater variability at elevated frequencies, likely due to enhanced capacitive contributions from the conductive polymer coating.

Linear regression analysis in the low-frequency regime revealed strong correlations between $|I_p|$ and $\sqrt{f}$ for both groups (Control: $R^2 = 0.94$, PEDOT: $R^2 = 0.96$), consistent with diffusion-controlled redox processes. The magnitude of $|I_p|$ increased from approximately

6×10^{-6} A at 1 Hz to 1.6×10^{-5} A at 5 Hz and $2.7\text{--}3.0 \times 10^{-5}$ A at 10–15 Hz, exceeding $\sqrt{f}$ scaling but remaining below linear, suggesting a mixed diffusion–adsorption regime [76]. Beyond 10 Hz, both groups deviate from linearity, likely due to increased phase lag, interfacial capacitance, and kinetic limitations in electron transfer.

Main peak potential (E_p). The corresponding peak potentials showed heterogeneous trends. Some control electrodes exhibited a clear negative shift with increasing frequency (e.g., -0.26 V at 1 Hz to -0.292 V at 15 Hz and -0.328 V at 30 Hz), consistent with quasi-reversible kinetics and an apparent slope of approximately -46 mV/decade. Other control electrodes, however, showed minimal shift (0–10 mV), likely reflecting heterogeneity in the carbonized surface. In PEDOT:PSS electrodes, E_p remained relatively stable across frequency, occasionally shifting slightly positive, indicative of more reversible electron transfer at the conductive interface.

Secondary peak current (I_{p2}). A secondary peak was consistently detected in PEDOT-modified electrodes, even at 1 Hz, whereas control electrodes often lacked a defined second peak at low frequency. This may reflect both faster kinetics and heightened background sensitivity in PEDOT interfaces, revealing low-level contributions from background charging or oxygen reduction.

For both groups, I_{p2} increased between 1 and 5 Hz. Regression analysis up to 5 Hz yielded high coefficients of determination (Control: $R^2 = 0.91$, PEDOT: $R^2 = 0.96$), confirming a reproducible trend in this range. However, only PEDOT-modified electrodes showed a sharp decline in I_{p2} at 10 Hz, followed by recovery at 15 Hz. This non-monotonic response may represent a transient frequency regime in which capacitive or interfacial effects suppress the weaker faradaic signal. Control electrodes, in contrast, exhibited a smoother progression.

Secondary peak potential (E_{p2}). Most E_{p2} values were frequency-independent, suggesting that the secondary process is not limited by charge-transfer kinetics. However, one electrode series displayed a clear shift from -0.512 V (2 Hz) to -0.552 V (15 Hz) and -0.572 V (30 Hz), corresponding to approximately -50 mV/decade. This is consistent with a quasi-reversible redox process or partial oxygen reduction. Since the measurements were performed in ambient air, the secondary peak may include ORR contributions that intensify or broaden with increasing phase lag and perturbation speed.

For the regression lines of both E_p and I_{p2} , the data point at 10 Hz was excluded due to its abrupt deviation from the surrounding trend. Including it would artificially reduce the

quality of the linear fit and obscure the otherwise consistent relationship with $\sqrt{\text{frequency}}$, particularly given that this outlier likely arises from transient suppression mechanisms not representative of the dominant electrochemical behavior.

At higher frequencies, overlapping capacitive and background currents increasingly distort both I_p and I_{p2} , reducing resolution and increasing variability particularly in PEDOT-coated systems. The selection of 5 Hz as the working frequency balances signal intensity, acquisition time, and reproducibility, making it optimal for quantitative sensing across both control and modified electrodes.

CV for Evaluation of Oxygen Effects on Control and PEDOT:PSS-Modified Electrodes

To evaluate whether, and to what extent, dissolved oxygen in ambient air contributes to the formation of a second voltammetric peak, CV and SWV measurements were conducted on both control and PEDOT:PSS-modified electrodes in PBS under two conditions: ambient air and after purging with nitrogen gas for 15 minutes. By comparing the voltammograms obtained in oxygen-rich versus oxygen-depleted environments, the influence of oxygen on the electrochemical signature could be assessed.

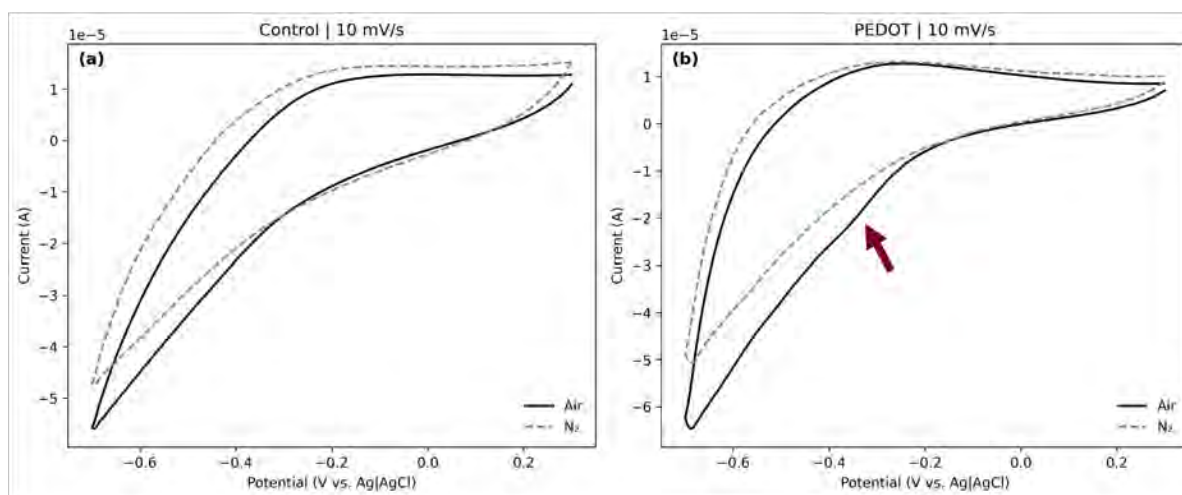
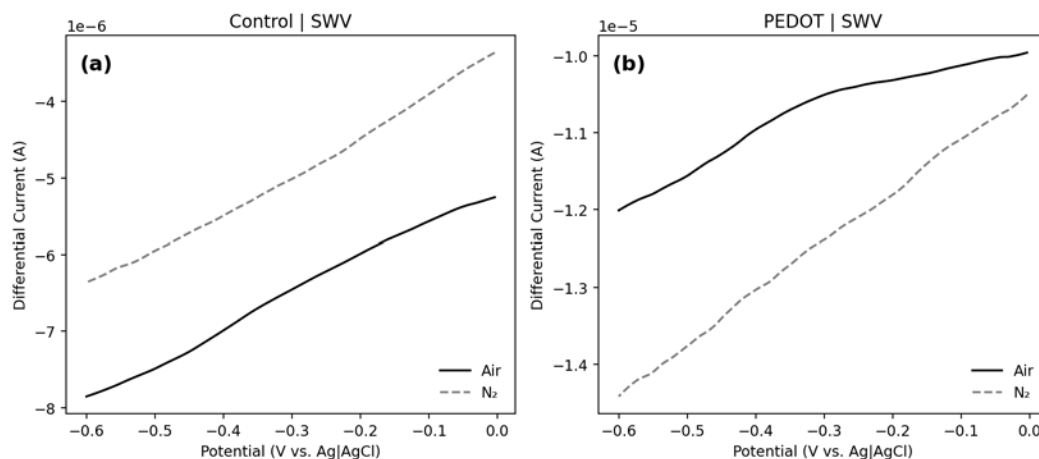


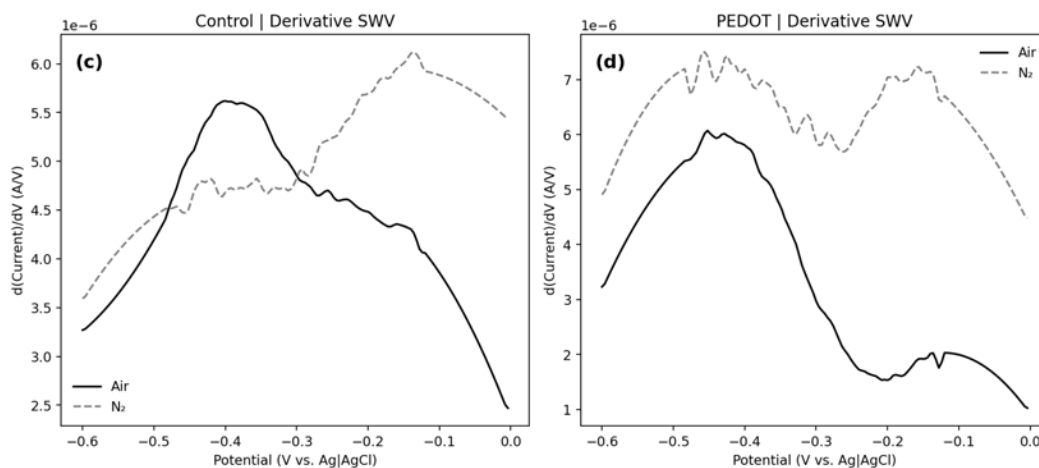
Figure 6.9 CV comparison of (a) control and (b) PEDOT:PSS-modified electrodes in PBS at 10 mV/s under ambient air and N_2 -purged conditions. Red arrow suggests rise in oxygen-peak in ambient conditions due to greater sensitivity in PEDOT-modified electrodes.

CV Comparison in Ambient Conditions Versus Nitrogen-Purged Voltammograms of electrodes in PBS reveal subtle yet reproducible changes when oxygen is removed. In con-

trol electrodes, the voltammetric profiles remain largely unchanged, though a minor suppression of the anodic current is observed under N_2 . In contrast, PEDOT:PSS-modified electrodes display notable changes: the cathodic current broadens in air and becomes more symmetric and featureless under N_2 . Notably, the redox peak observed in ambient conditions is suppressed in the nitrogen-purged case, supporting the hypothesis that this secondary feature is oxygen-dependent. This behavior may reflect either direct oxygen reduction processes or altered interfacial kinetics facilitated by the presence of dissolved O_2 at the PEDOT-modified surface.



(a) SWV of Control and PEDOT electrodes measured in Air and N_2 .



(b) First derivative of the SWV curves for Control and PEDOT electrodes.

Figure 6.10 Comparison of SWV responses at 5Hz of (a) Control and (b) PEDOT electrodes under oxygenated (Air) and deoxygenated (N_2) conditions, shown as SWV curves and (c), (d) their derivatives.

SWV Comparison in Ambient Conditions Versus Nitrogen-Purged SWV traces exhibited subtle differences between ambient and deoxygenated conditions. To enhance resolution of potential redox features, the first derivative of the current with respect to potential was computed.

In ambient air, both control and PEDOT:PSS electrodes display a broad derivative peak between -0.50 and -0.3 V vs. Ag|AgCl, consistent with a redox-active species, potentially oxygen or an oxygen-related intermediate. Under N_2 conditions, this feature becomes less pronounced in control electrodes and shifts in shape, suggesting a reduction in faradaic activity or altered electron transfer dynamics.

In PEDOT-modified electrodes, derivative peaks remain sharper under both conditions, but their amplitude is reduced under N_2 . The preservation of these features, albeit at lower intensity, may reflect residual surface-bound redox activity or capacitive behavior distinct from dissolved oxygen contributions. This again supports the role of oxygen in amplifying or enabling the secondary peak observed in PEDOT-modified systems.

Such behavior is consistent with previous reports, where oxygen-related processes have been observed at comparable potentials across various electrode materials. CV studies have highlighted oxygen effects on GCEs [77], bulk silver electrodes [78], and titanium-based sensors [79], while SWV work has also demonstrated small currents likely arising from oxygen reduction near -0.3 V vs. Ag|AgCl [80].

6.1.7 Effect of PEDOT:PSS Formulation on Simple Paper Sensor’s Electrochemical Performance

Optimizing the conductive layer is a critical step in the development of paper-based electrochemical sensors, as it directly impacts sensitivity, signal stability, and redox efficiency. Among commonly used conductive materials, PEDOT:PSS offers a promising balance of processability and electrochemical performance [58]. However, its conductivity and interfacial properties can vary significantly depending on formulation, particularly dilution ratios and the inclusion of secondary dopants. In this section, PEDOT:PSS formulations (1:3 and 1:30 dilution in water, equally supplemented with 5% v/v EG) were assessed to evaluate voltammetric behavior of simple carbonized paper electrodes.

EG was added as a secondary dopant to enhance the electrical performance of PEDOT:PSS. It promotes phase separation between the conductive PEDOT and the insulating PSS components, facilitating the formation of interconnected PEDOT-rich domains [81–83]. This microstructural reorganization significantly improves charge carrier mobility and reduces the

overall resistance of the resulting film. Prior studies have shown that an EG concentration of 5%v/v yields optimal conductivity enhancement [81].

To investigate the effect of conductive ink composition on electrochemical behavior, CV was performed in 1 mM PYO using paper-based electrodes modified with PEDOT:PSS at two formulation ratios (1:3 and 1:30), alongside unmodified controls (carbonized paper with only PDMS applied). Four key electrochemical parameters were analyzed as a function of SR: peak-to-peak voltage separation (ΔE), reduction peak current ($I_{p,\text{red}}$), oxidation peak current ($I_{p,\text{ox}}$), and capacitive current (I_{cap}).

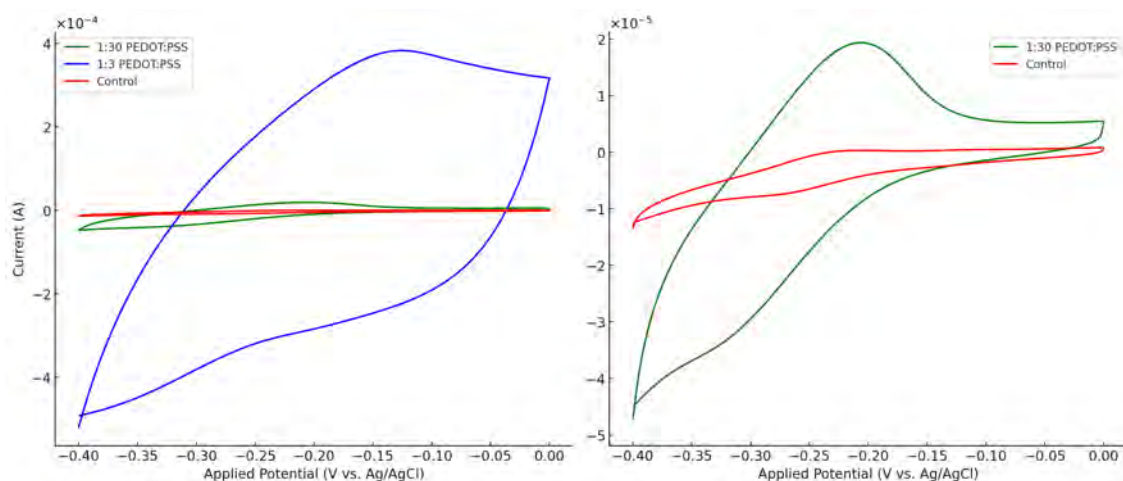


Figure 6.11 Cyclic voltammograms comparing control, 1:3 PEDOT:PSS, and 1:30 PEDOT:PSS-coated electrodes in 1 mM PYO and 1 M PBS at 100 mV/s. Left: Overlay of two CV segments for each condition. Right: Zoomed-in view comparing 1:30 PEDOT:PSS and control electrodes. The applied potential is referenced against commercial Ag|AgCl electrode.

Table 6.1 Electrochemical parameters measured at various scan rates for control, PEDOT:PSS 1:3, and PEDOT:PSS 1:30 electrodes in 1 mM PYO. Values are expressed as mean $\pm$ standard deviation (N=3).

SR (mV/s)	Method	ΔE [V]	$I_{p,red}$ [A]	$I_{p,ox}$ [A]	I_{cap} [A]
10	Control	0.065 ± 0.018	$(-2.33 \pm 0.99) \times 10^{-6}$	$(1.67 \pm 0.46) \times 10^{-7}$	$(2.00 \pm 2.90) \times 10^{-6}$
	PEDOT 1:3	0.062 ± 0.010	$(-3.61 \pm 0.90) \times 10^{-5}$	$(8.68 \pm 2.10) \times 10^{-6}$	$(8.03 \pm 1.70) \times 10^{-5}$
	PEDOT 1:30	0.062 ± 0.005	$(-6.41 \pm 1.30) \times 10^{-6}$	$(2.36 \pm 0.75) \times 10^{-6}$	$(2.31 \pm 0.34) \times 10^{-6}$
20	Control	0.076 ± 0.027	$(-2.68 \pm 1.50) \times 10^{-6}$	$(3.66 \pm 1.60) \times 10^{-7}$	$(1.24 \pm 0.57) \times 10^{-6}$
	PEDOT 1:3	0.075 ± 0.002	$(-3.83 \pm 0.72) \times 10^{-5}$	$(2.09 \pm 0.68) \times 10^{-5}$	$(1.52 \pm 0.36) \times 10^{-4}$
	PEDOT 1:30	0.071 ± 0.004	$(-6.92 \pm 1.60) \times 10^{-6}$	$(3.54 \pm 1.50) \times 10^{-6}$	$(3.84 \pm 0.65) \times 10^{-6}$
50	Control	0.085 ± 0.035	$(-3.70 \pm 1.40) \times 10^{-6}$	$(3.30 \pm 1.80) \times 10^{-7}$	$(2.21 \pm 0.89) \times 10^{-6}$
	PEDOT 1:3	0.131 ± 0.021	$(-3.14 \pm 1.40) \times 10^{-5}$	$(3.54 \pm 0.80) \times 10^{-5}$	$(3.22 \pm 0.75) \times 10^{-4}$
	PEDOT 1:30	0.086 ± 0.009	$(-8.03 \pm 2.00) \times 10^{-6}$	$(6.31 \pm 1.80) \times 10^{-6}$	$(7.49 \pm 1.80) \times 10^{-6}$
100	Control	0.101 ± 0.047	$(-5.21 \pm 3.20) \times 10^{-6}$	$(1.32 \pm 0.80) \times 10^{-6}$	$(2.82 \pm 0.63) \times 10^{-6}$
	PEDOT 1:3	0.215 ± 0.003	$(-6.35 \pm 0.27) \times 10^{-5}$	$(2.34 \pm 0.24) \times 10^{-5}$	$(5.68 \pm 1.40) \times 10^{-4}$
	PEDOT 1:30	0.113 ± 0.008	$(-1.06 \pm 0.31) \times 10^{-5}$	$(9.94 \pm 1.40) \times 10^{-6}$	$(1.20 \pm 0.35) \times 10^{-5}$

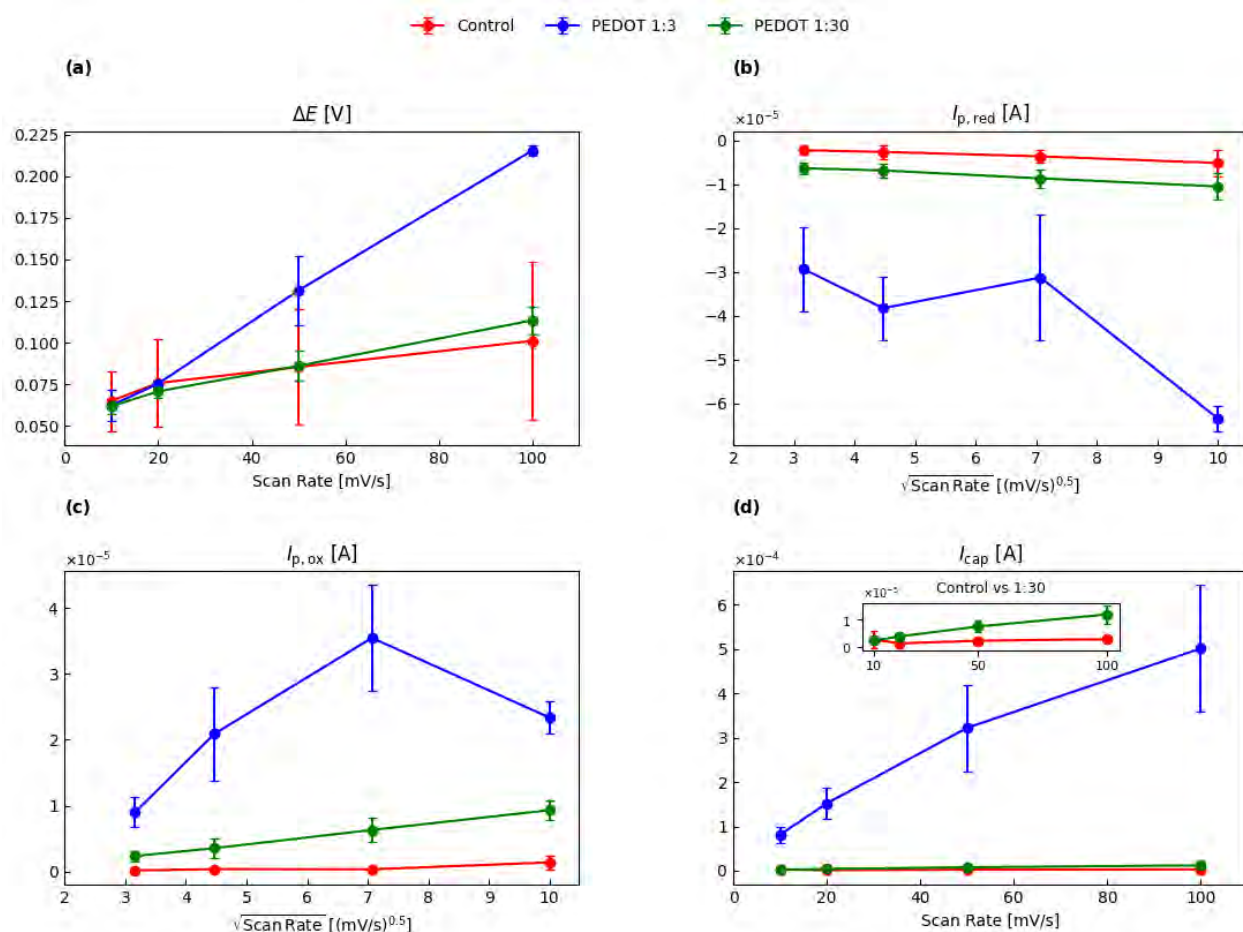


Figure 6.12 Electrochemical performance of paper-based electrodes modified with different PEDOT:PSS formulations contrasted with unmodified controls, measured in 1 mM PYO in PBS. (a) peak-to-peak voltage separation ΔE vs. SR, (b) reduction peak current $I_{p,red}$ vs. $\sqrt{\text{SR}}$, (c) oxidation peak current $I_{p,ox}$ vs. $\sqrt{\text{SR}}$, and (d) capacitive current I_{cap} vs. SR and subplot to better visualize the difference in I_{cap} for control and PEDOT:PSS 1:3 electrodes. Error bars represent standard deviation (N=3 for all electrodes).

Both PEDOT:PSS-modified formulations (1:3 and 1:30 dilutions) demonstrated increased peak currents compared to the unmodified control, indicating enhanced charge transfer at the electrode–electrolyte interface. This aligns with previous findings on PEDOT:PSS-based electrochemical enhancements [58]. Among the tested conditions, the 1:3 PEDOT:PSS formulation exhibited the highest peak currents ($I_{p,red}$, $I_{p,ox}$), particularly at elevated SR, consistent with greater conductivity and a larger electroactive surface area.

However, this gain in signal intensity was accompanied by broader redox peaks and a larger peak-to-peak separation (ΔE), reaching values up to 0.215 V. Such features are indicative of less reversible electron transfer kinetics, which may arise from over-thick and/or non-uniform

film formation due to the higher viscosity of the 1:3 formulation. Additionally, the increased capacitance of the thicker PEDOT:PSS layer likely contributes to the electrochemical sluggishness observed at high SR. In physical terms, the high resistance–capacitance (RC) time constant ($\tau = R \cdot C$) introduces a low-pass filtering effect, limiting the temporal resolution of the system [84, 85]. Overall, at high SR, the electrochemical interface struggles to keep pace with the rapidly changing potential, leading to delayed response, peak smearing, and distortion of the voltammetric profile.

This interpretation is further supported by the greater variability observed across 1:3-coated electrodes, as reflected in the wider error bars than both the unmodified control and the 1:30 formulation. These fluctuations may reflect inconsistencies in film thickness and wetting behavior, which would affect capacitive charging and redox site accessibility.

In contrast, the 1:30 dilution offered a more favorable balance: while peak currents were slightly smaller in magnitude than in the 1:3 formulation, the signals were better defined, with reduced background capacitance and tighter peak separations. Specifically, ΔE values for PEDOT:PSS 1:30 electrodes ranged from 0.06 V to 0.11 V across scan rates of 10 to 100 mV/s, values close to the theoretical limit for a reversible redox process. This narrow and gradually increasing peak separation reflects efficient electron transfer and well-controlled diffusion, even at elevated SR. These values are also consistent with previously reported results on GCE, where a peak-to-peak separation of 0.03 V was observed for PYO under optimized conditions [86].

Statistical Analysis to Compare PEDOT:PSS Ink Formulation Effects

To validate the significance of PEDOT:PSS coatings effects on CV responses, a one-way ANOVA was conducted on each metric (ΔE), faradaic peak currents and capacitive current) at four SR (10, 20, 50 and 100 mV/s). Where the ANOVA indicated a significant overall effect ($p < 0.05$), then a comparison between control and PEDOT:PSS 1:3 and between control and PEDOT:PSS 1:30 was performed using Welch’s two-sample t-test. The p-values for these specific comparisons are presented alongside the ANOVA results in Table 6.2, allowing direct evaluation of how the dilute coating modifies each electrochemical parameter.

Table 6.2 ANOVA and two-sample p -values for Control (C) vs PEDOT:PSS coatings at four SRs (1mM, $N = 3$).

SR (mV/s)	Param.	p_{ANOVA}	C vs 1:3 p	C vs 1:30 p
10	ΔE	0.9395	0.8326	0.7870
	$I_{p,\text{red}}$	0.0023	0.0388	0.0157
	$I_{p,\text{ox}}$	0.0006	0.0219	0.0387
	I_{cap}	0.0002	0.0161	0.8568
20	ΔE	0.9065	0.9844	0.7733
	$I_{p,\text{red}}$	0.0001	0.0108	0.0296
	$I_{p,\text{ox}}$	0.0019	0.0373	0.0647
	I_{cap}	0.0002	0.0179	0.0076
50	ΔE	0.0915	0.1355	0.9769
	$I_{p,\text{red}}$	0.0145	0.0770	0.0323
	$I_{p,\text{ox}}$	0.0002	0.0168	0.0300
	I_{cap}	0.0006	0.0295	0.0382
100	ΔE	0.0045	0.0522	0.6988
	$I_{p,\text{red}}$	<0.0001	<0.0001	0.0973
	$I_{p,\text{ox}}$	<0.0001	0.0013	0.0023
	I_{cap}	0.0005	0.0263	0.0396

ANOVA revealed that for all SRs (10–100 mV/s) the faradaic peak currents ($I_{p,\text{red}}$, $I_{p,\text{ox}}$) and the capacitive current significantly differed among the three coatings (all $p < 0.05$), whereas ΔE only showed a significant effect at the slowest SR (10 and 20 mV/s).

The two-sample T-tests for Control vs. PEDOT:PSS 1:3 show that the 1:3 coating markedly amplifies both faradaic currents and capacitance at nearly all SR, while leaving peak separation mostly unchanged, except at a greatest SR 100mv/s. Specifically, the reduction peak current ($I_{p,\text{red}}$) is significantly higher for 1:3 at 10 mV/s ($p = 0.0388$), 20 mV/s ($p = 0.0108$) and 100 mV/s ($p < 0.0001$), with a non-significant trend at 50 mV/s ($p = 0.0770$). The oxidative peak current ($I_{p,\text{ox}}$) is elevated at all rates ($p = 0.0219$ to 0.0013), and the capacitive current likewise increases significantly at every rate ($p = 0.0161$ to 0.0263). Peak separation (ΔE) remains statistically unchanged ($p > 0.05$ for all SRs), indicating that reversibility is preserved even as signal amplitudes rise.

PEDOT:PSS 1:3 delivers strong signal enhancement, but at the expense of higher background capacitance, motivating the subsequent dilution to 1:30.

Contrasting Control vs PEDOT:PSS 1:30, the reduction peak current was significantly stronger at 10 mV/s ($p = 0.0157$), 20 mV/s ($p = 0.0296$) and 50 mV/s ($p = 0.0323$) but not at 100 mV/s ($p = 0.0973$), while the oxidative peak current was significantly increased at 10 mV/s ($p = 0.0387$), 50 mV/s ($p = 0.0300$) and 100 mV/s ($p = 0.0023$) but not at 20 mV/s

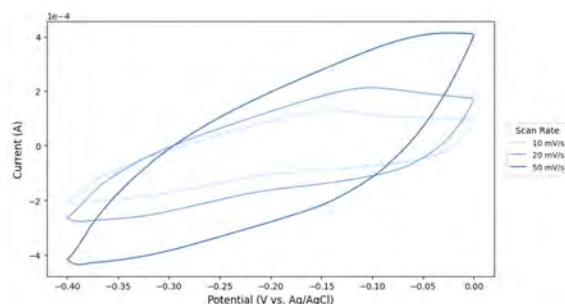
($p = 0.0647$). Peak separation (ΔE) remained non-significant at all rates ($p = 0.7870$ – 0.9769), and capacitive current did not differ at 10 mV/s ($p = 0.8568$) but was significantly higher at 20 mV/s ($p = 0.0076$), 50 mV/s ($p = 0.0382$) and 100 mV/s ($p = 0.0396$).

In addition to statistical significance, the t-test p -values correspond to substantial, practical enhancements in peak currents with the PEDOT:PSS 1:30 film. For example, at 20 mV s^{−1} the mean reductive current increases from $-2.68 \pm 1.53 \mu\text{A}$ (Control) to $-6.92 \pm 1.59 \mu\text{A}$ (1:30), a ~ 2.6 -fold gain, while the oxidative current jumps from $0.37 \pm 0.19 \mu\text{A}$ to $3.54 \pm 1.49 \mu\text{A}$ (~ 9.7 -fold). Despite these large signal boosts, ΔE remains unchanged at all rates ($p = 0.787$ – 0.977), suggesting that electron-transfer kinetics are preserved. The capacitive current shows a rise only at $\geq 20 \text{ mV s}^{-1}$ ($p = 0.0076$ – 0.0396), indicating that dilution to 1:30 substantially mitigates the charging background introduced by the 1:3 film. The lack of a significant $I_{p,\text{red}}$ increase at 100 mV s^{−1} ($p = 0.0973$) further suggests a kinetic or ohmic limit under very fast sweeps.

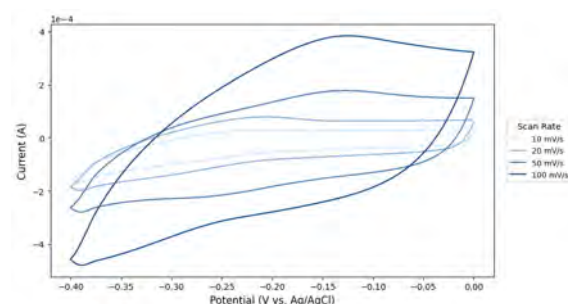
PEDOT:PSS 1:30 delivers pronounced faradaic amplification with minimal capacitive penalty and loss of reversibility, making it the optimal coating for quantitative sensing. PEDOT:PSS 1:30 was therefore selected for all subsequent experiments.

6.2 Deposition Method for PEDOT:PSS

To assess the influence of ink application method on electrode performance, PEDOT:PSS 1:30-coated electrodes fabricated by dipping and drop-casting were evaluated via CV in 1 mM PYO solution, as shown in Figure 6.14. For supplementary evaluation, electrodes dipped and drop-casted with PEDOT:PSS 1:3 formulation were also contrasted (Figure 6.13).

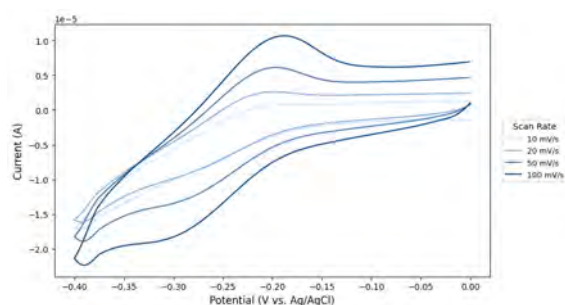


(a) CV of simple electrode (PEDOT:PSS 1:3, dipped) at various SR.

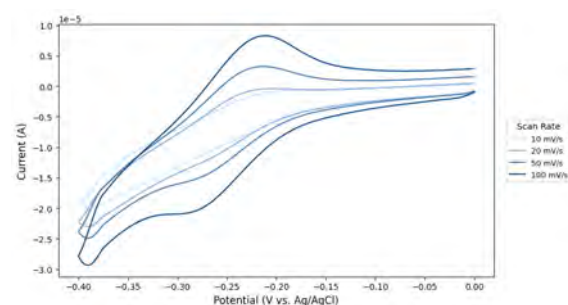


(b) CV of simple electrode (PEDOT:PSS 1:3, drop-casted) at various SR.

Figure 6.13 Comparison of CV profiles for PEDOT:PSS 1:3-coated electrodes prepared by (a) dipping and (b) drop-casting. CVs recorded in 1 mM PYO with 1 M PBS at SR ranging from 10 to 100 mV/s.



(a) CV of simple electrode (PEDOT:PSS 1:30, dipped) at various SR.



(b) CV of simple electrode (PEDOT:PSS 1:30, drop-casted) at various SR.

Figure 6.14 Comparison of CV profiles for PEDOT:PSS 1:30-coated electrodes prepared by (a) dipping and (b) drop-casting. CVs recorded in 1 mM PYO with 1 M PBS at SR ranging from 10 to 100 mV/s.

6.2.1 Evaluation of PEDOT:PSS Deposition Method

Table 6.3 summarizes the electrochemical performance of PEDOT:PSS 1:30-coated electrodes prepared by dipping or drop-casting across a range of SR. Several key distinctions emerge from the dataset, particularly regarding peak-to-peak separation (ΔE), capacitive current, and the SR dependence of the oxidation peak current ($I_{p,ox}$).

Table 6.3 Electrochemical performance of electrodes fabricated by dipping and drop-casting methods at various scan rates (SR). Values are expressed as mean $\pm$ SD in grouped scientific notation ($N = 3$).

SR (mV/s)	Method	ΔE [V]	$I_{p,red}$ [A]	$I_{p,ox}$ [A]	I_{cap} [A]
10	Dipped	0.062 ± 0.005	$(-6.41 \pm 1.34) \times 10^{-6}$	$(2.36 \pm 0.77) \times 10^{-6}$	$(2.31 \pm 0.34) \times 10^{-6}$
	Drop-Casted	0.033 ± 0.012	$(-4.22 \pm 1.14) \times 10^{-6}$	$(1.64 \pm 0.40) \times 10^{-6}$	$(0.86 \pm 0.18) \times 10^{-6}$
20	Dipped	0.071 ± 0.004	$(-6.92 \pm 1.59) \times 10^{-6}$	$(3.54 \pm 1.50) \times 10^{-6}$	$(3.83 \pm 0.68) \times 10^{-6}$
	Drop-Casted	0.034 ± 0.004	$(-4.58 \pm 1.31) \times 10^{-6}$	$(3.05 \pm 0.90) \times 10^{-6}$	$(1.43 \pm 0.34) \times 10^{-6}$
50	Dipped	0.086 ± 0.009	$(-8.72 \pm 2.08) \times 10^{-6}$	$(6.31 \pm 1.87) \times 10^{-6}$	$(7.49 \pm 2.19) \times 10^{-6}$
	Drop-Casted	0.057 ± 0.005	$(-7.15 \pm 1.90) \times 10^{-6}$	$(5.62 \pm 1.43) \times 10^{-6}$	$(2.95 \pm 0.63) \times 10^{-6}$
100	Dipped	0.113 ± 0.009	$(-10.6 \pm 3.05) \times 10^{-6}$	$(9.30 \pm 1.42) \times 10^{-6}$	$(12.0 \pm 3.50) \times 10^{-6}$
	Drop-Casted	0.070 ± 0.013	$(-10.5 \pm 3.00) \times 10^{-6}$	$(8.56 \pm 1.46) \times 10^{-6}$	$(5.30 \pm 1.08) \times 10^{-6}$
200	Dipped	0.145 ± 0.019	$(-13.7 \pm 2.44) \times 10^{-6}$	$(12.0 \pm 1.39) \times 10^{-6}$	$(22.0 \pm 6.94) \times 10^{-6}$
	Drop-Casted	0.098 ± 0.015	$(-16.2 \pm 3.96) \times 10^{-6}$	$(13.5 \pm 3.76) \times 10^{-6}$	$(10.1 \pm 2.30) \times 10^{-6}$
500	Dipped	0.223 ± 0.036	$(-18.1 \pm 2.82) \times 10^{-6}$	$(19.1 \pm 2.75) \times 10^{-6}$	$(49.0 \pm 9.79) \times 10^{-6}$
	Drop-Casted	0.151 ± 0.028	$(-26.6 \pm 6.45) \times 10^{-6}$	$(22.7 \pm 5.47) \times 10^{-6}$	$(26.5 \pm 6.84) \times 10^{-6}$
1000	Dipped	0.267 ± 0.034	$(-17.5 \pm 2.60) \times 10^{-6}$	$(23.3 \pm 1.80) \times 10^{-6}$	$(72.3 \pm 10.4) \times 10^{-6}$
	Drop-Casted	0.215 ± 0.046	$(-33.6 \pm 5.76) \times 10^{-6}$	$(35.7 \pm 5.43) \times 10^{-6}$	$(61.4 \pm 16.9) \times 10^{-6}$

At lower SR (10–100 mV/s), dipped electrodes exhibited moderately higher $I_{p,red}$ and $I_{p,ox}$ than drop-casted ones. For example, at 50 mV/s, $I_{p,ox}$ was 6.31×10^{-6} A for dipped electrodes vs. 5.62×10^{-6} A for drop-casted. However, this performance advantage diminishes at 100 mV/s and is reversed at 200 mV/s, where drop-casted electrodes show slightly higher oxidation peak currents (1.35×10^{-5} A vs. 1.20×10^{-5} A), and becomes more pronounced at 500 and 1000 mV/s. At 1000 mV/s, drop-casted electrodes yielded the highest faradaic response overall, with $I_{p,ox} = 3.57 \times 10^{-5}$ A, compared to 2.33×10^{-5} A for dipped electrodes.

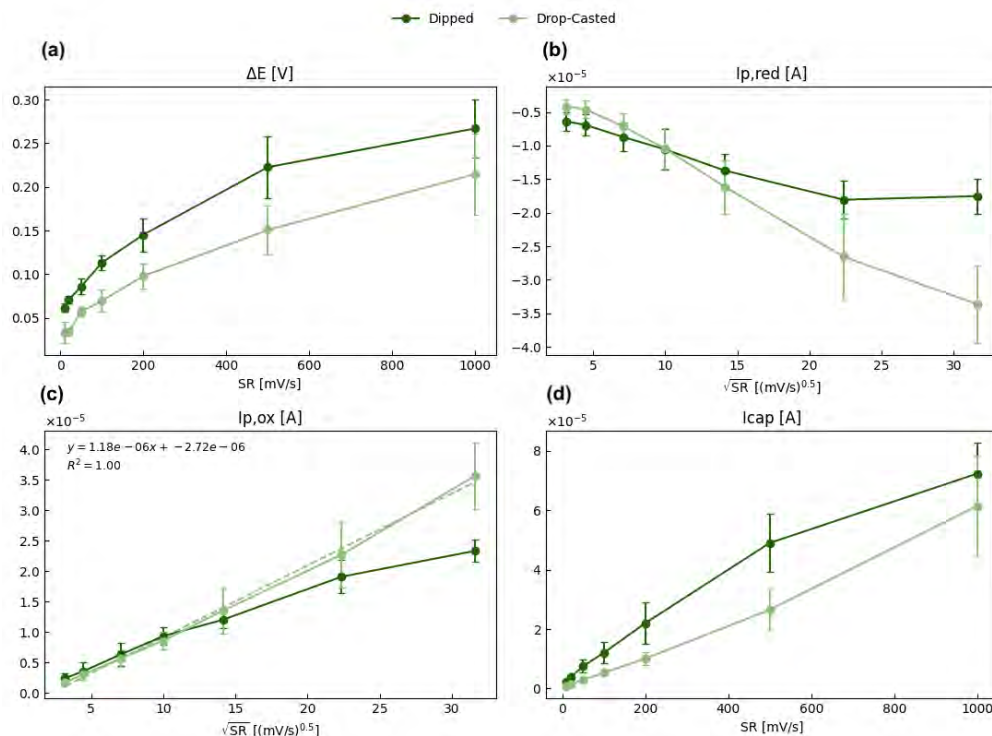


Figure 6.15 Comparison of PEDOT:PSS 1:30-coated electrodes fabricated by dipping (N=3) and drop-casting (N=3) in 1 mM PYO. Shown are (a) peak-to-peak voltage separation ΔE vs. SR, (b) reduction peak current $I_{p,red}$ vs. $\sqrt{SR}$, (c) oxidation peak current $I_{p,ox}$ vs. $\sqrt{SR}$, and (d) capacitive current I_{cap} vs. SR.

This reversal at higher SR likely reflects the difference in capacitive behavior between the two methods. Dipped electrodes exhibit significantly greater capacitive currents as SR increases, suggesting higher capacitance and thus a larger RC time constant. For instance, I_{cap} reaches 7.23×10^{-5} A at 1000 mV/s for dipped electrodes versus 6.14×10^{-5} A for drop-casted ones. This supports the interpretation (as described in our PEDOT 1:3 vs. PEDOT 1:30 comparison previously) that dipped films act as low-pass filters due to their higher capacitive loading, slowing system response at high frequencies and degrading peak sharpness.

The advantage of drop-casting is further denoted by the smaller and more stable ΔE values observed across SR. Drop-casted electrodes maintain $\Delta E < 0.1$ V up to 200 mV/s, whereas dipped electrodes show more rapid increases, reaching 0.223 ± 0.036 V at 500 mV/s and 0.267 ± 0.034 V at 1000 mV/s. These larger peak separations indicate a loss of redox reversibility in the dipped films, likely due to kinetic limitations imposed by thicker, more resistive coatings.

Importantly, the oxidation peak current ($I_{p,ox}$) for drop-casted electrodes follows a highly linear trend with respect to $\sqrt{SR}$, as shown in Figure 6.15c. A linear regression line yielded

$R^2 = 1.000$, indicating diffusion-controlled behavior. This confirms that drop-casting yields electrodes with predictable electrochemical dynamics, crucial for quantitative sensing applications.

Regarding reproducibility, drop-casted electrodes exhibited lower standard deviations at SR rates (10–50 mV/s). For instance, the standard deviation on $I_{p,ox}$ at 20 mV/s was 9.03×10^{-7} A for drop-casted vs. 1.49×10^{-6} A for dipped electrodes. However, this trend reverses at higher SRs: from 200 mV/s upward, dipped electrodes consistently show tighter variability, with lower standard deviations on peak currents. This shift may reflect greater signal instability in thinner, drop-cast films when exposed to high-frequency potential sweeps.

Dipped electrodes perform comparably at low SR but become kinetically limited as SR increases, likely due to excessive film thickness and capacitive load. Drop-casted electrodes, in contrast, maintain superior redox reversibility, linear peak current scaling, and more favorable peak separation at high SR, making them better suited for fast, quantitative electroanalysis.

6.2.2 Statistical Comparison of Electrode Coatings and Deposition Methods

To evaluate the impact of PEDOT:PSS deposition method on electrochemical performance, a statistical analysis was conducted.

The one-way ANOVA and Tukey HSD comparisons (Table 6.4) demonstrate that drop-casting and dipping yield statistically distinct behaviors for peak separation and capacitive currents across most SRs. In particular, ΔE is significantly lower for drop-casted films at 10–200 mV/s (all $p < 0.05$), indicating more reversible electron-transfer kinetics. Similarly, I_{cap} is consistently reduced in drop-casted electrodes ($p < 0.05$ at 10–500 mV/s), reflecting a lower background charging current. In contrast, the faradaic peak currents ($I_{p,red}$ and $I_{p,ox}$) show no significant differences at most SR, except for effects at higher SR (1000 mV/s). These results confirm that drop-casting affords better baseline stability and kinetic reversibility without sacrificing redox sensitivity, supporting its selection for further sensor development.

Table 6.4 One-way ANOVA and Tukey HSD post-hoc comparing dipped and drop-casted electrode results for each electrochemical parameter at different SRs (N=3) to evaluate if null hypothesis is rejected.

SR (mV/s)	Parameter	F	p (ANOVA)	Mean diff.	95% CI	Reject
10	ΔE [V]	14.805	0.018	-2.83×10^{-2}	$[-4.88 \times 10^{-2}, -7.89 \times 10^{-3}]$	Yes
	$I_{p,red}$ [A]	4.668	0.097	2.19×10^{-6}	$[-6.25 \times 10^{-7}, 5.01 \times 10^{-6}]$	No
	$I_{p,ox}$ [A]	2.007	0.230	-7.13×10^{-7}	$[-2.11 \times 10^{-6}, 6.85 \times 10^{-7}]$	No
	I_{cap} [A]	42.268	0.003	-1.44×10^{-6}	$[-2.06 \times 10^{-6}, -8.28 \times 10^{-7}]$	Yes
20	ΔE [V]	138.151	< 0.001	-3.63×10^{-2}	$[-4.49 \times 10^{-2}, -2.78 \times 10^{-2}]$	Yes
	$I_{p,red}$ [A]	3.851	0.121	2.34×10^{-6}	$[-9.69 \times 10^{-7}, 5.64 \times 10^{-6}]$	No
	$I_{p,ox}$ [A]	0.239	0.650	-4.93×10^{-7}	$[-3.29 \times 10^{-6}, 2.31 \times 10^{-6}]$	No
	I_{cap} [A]	30.169	0.005	-2.41×10^{-6}	$[-3.62 \times 10^{-6}, -1.19 \times 10^{-6}]$	Yes
50	ΔE [V]	22.527	0.009	-2.90×10^{-2}	$[-4.60 \times 10^{-2}, -1.20 \times 10^{-2}]$	Yes
	$I_{p,red}$ [A]	0.935	0.388	1.57×10^{-6}	$[-2.94 \times 10^{-6}, 6.09 \times 10^{-6}]$	No
	$I_{p,ox}$ [A]	0.261	0.636	-6.93×10^{-7}	$[-4.46 \times 10^{-6}, 3.08 \times 10^{-6}]$	No
	I_{cap} [A]	11.960	0.026	-4.55×10^{-6}	$[-8.20 \times 10^{-6}, -8.96 \times 10^{-7}]$	Yes
100	ΔE [V]	25.016	0.007	-4.37×10^{-2}	$[-6.79 \times 10^{-2}, -1.94 \times 10^{-2}]$	Yes
	$I_{p,red}$ [A]	0.002	0.966	1.13×10^{-7}	$[-6.75 \times 10^{-6}, 6.97 \times 10^{-6}]$	No
	$I_{p,ox}$ [A]	0.394	0.564	-7.37×10^{-7}	$[-4.00 \times 10^{-6}, 2.52 \times 10^{-6}]$	No
	I_{cap} [A]	10.150	0.033	-6.73×10^{-6}	$[-1.26 \times 10^{-5}, -8.65 \times 10^{-7}]$	Yes
200	ΔE [V]	11.723	0.027	-4.73×10^{-2}	$[-8.57 \times 10^{-2}, -8.95 \times 10^{-3}]$	Yes
	$I_{p,red}$ [A]	0.822	0.416	-2.43×10^{-6}	$[-9.89 \times 10^{-6}, 5.02 \times 10^{-6}]$	No
	$I_{p,ox}$ [A]	0.415	0.555	1.49×10^{-6}	$[-4.93 \times 10^{-6}, 7.91 \times 10^{-6}]$	No
	I_{cap} [A]	7.974	0.048	-1.19×10^{-5}	$[-2.36 \times 10^{-5}, -2.00 \times 10^{-7}]$	Yes
500	ΔE [V]	7.606	0.051	-7.20×10^{-2}	$[-1.44 \times 10^{-1}, 4.84 \times 10^{-4}]$	No
	$I_{p,red}$ [A]	4.373	0.105	-8.50×10^{-6}	$[-1.98 \times 10^{-5}, 2.79 \times 10^{-6}]$	No
	$I_{p,ox}$ [A]	1.058	0.362	3.63×10^{-6}	$[-6.17 \times 10^{-6}, 1.34 \times 10^{-5}]$	No
	I_{cap} [A]	10.651	0.031	-2.25×10^{-5}	$[-4.16 \times 10^{-5}, -3.36 \times 10^{-6}]$	Yes
1000	ΔE [V]	2.523	0.187	-5.23×10^{-2}	$[-1.44 \times 10^{-1}, 3.91 \times 10^{-2}]$	No
	$I_{p,red}$ [A]	19.457	0.012	-1.61×10^{-5}	$[-2.62 \times 10^{-5}, -5.97 \times 10^{-6}]$	Yes
	$I_{p,ox}$ [A]	13.940	0.020	1.23×10^{-5}	$[3.16 \times 10^{-6}, 2.15 \times 10^{-5}]$	Yes
	I_{cap} [A]	0.901	0.396	-1.09×10^{-5}	$[-4.27 \times 10^{-5}, 2.09 \times 10^{-5}]$	No

6.2.3 Characterization by SEM

Scanning electron microscopy (SEM) was employed to examine the morphology of the laser-carbonized paper electrodes prior to electrochemical testing. The morphology of the simple carbonized electrode without further modification was first examined. No polymeric coatings were applied at this stage, so the observed morphology represents the baseline microstructure of the carbonized paper substrate. These SEM observations provide a structural rationale for the subsequent electrochemical behavior in RuHex, namely the balance between accessible surface area and resistive pathways arising from non-uniform carbonization. As shown in Figure 6.16, the laser-treated region is readily distinguishable from the untreated cellulose due to its rougher and more fibrous microstructure.

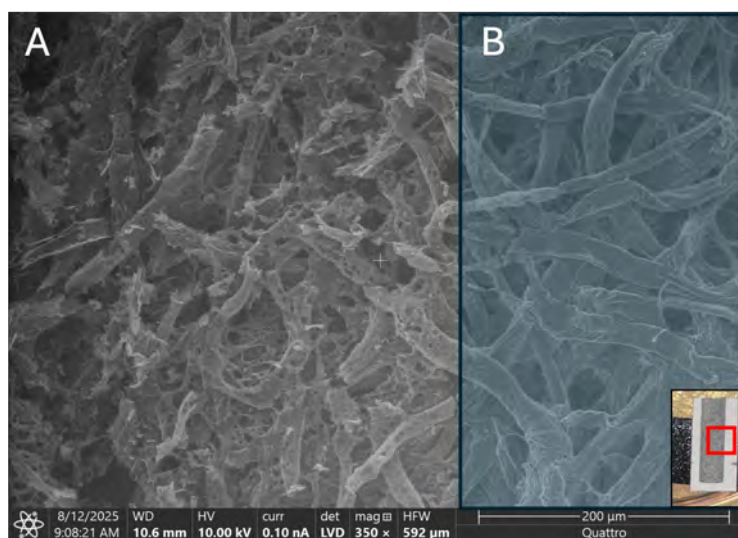


Figure 6.16 Delineation between laser-treated carbonized region (A) and untreated cellulose (B). The representative region of the electrode is highlighted.

The effect of PDMS treatment on the electrode morphology was then investigated. As shown in Figure 6.17, PDMS penetrated both the carbonized and untreated regions, forming a continuous coverage across the paper substrate. The laser-treated areas coated with PDMS appeared more textured, which is consistent with the rougher surface produced by carbonization. This homogeneous distribution confirms that PDMS permeates the cellulose matrix, acting as a hydrophobic barrier layer while conforming to the underlying topography.

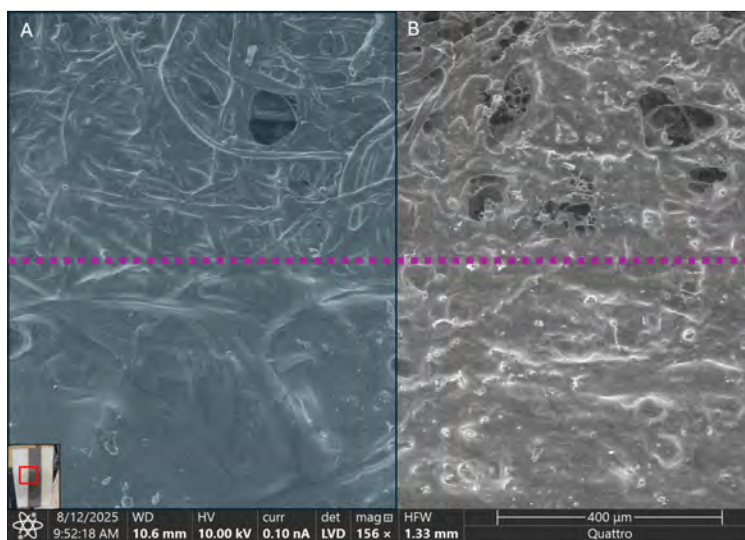


Figure 6.17 SEM image of PDMS applied at the center of the electrode (purple dashed line), showing absorption into bare Whatman paper (A) and laser-treated electrode region (B).

Finally, electrodes modified with PEDOT:PSS at 1:3 and 1:30 dilution ratios were compared (Figure 6.18). Both treatments preserved the overall geometry of the paper microstructure, with conductive polymer coating the underlying fibers without altering their arrangement.

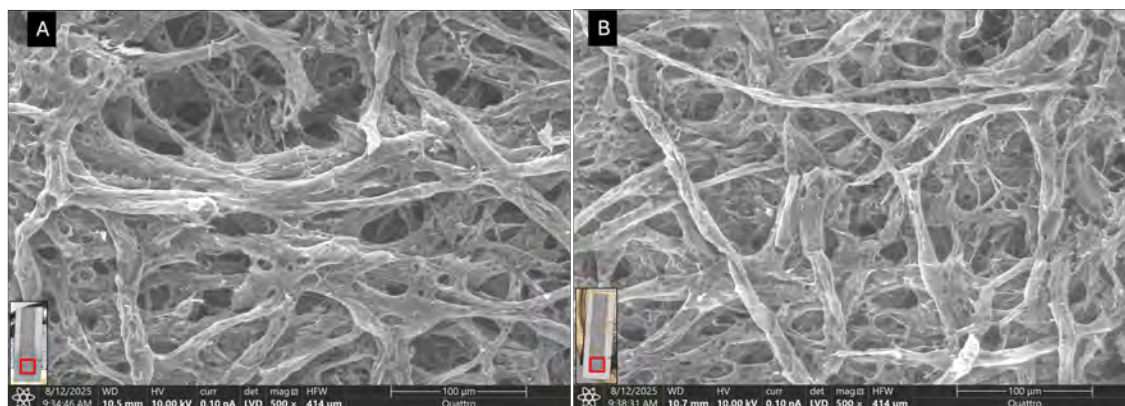


Figure 6.18 Comparison of electrodes treated with PEDOT:PSS at 1:3 and 1:30 dilution ratios.

The carbonized regions displayed a heterogeneous fibrous network typical of the underlying cellulose scaffold, but with added surface roughening caused by the laser's thermal effects. The interconnected fiber structure retained, ensuring electrical contact while providing a porous matrix for electrolyte infiltration. Between carbonized fibers to untreated Whatman paper, the morphological variation of the former was apparent: surface corrugations, micro-cracks, and localized densification appeared post-laser treatment; these are consistent with the partial graphitization of the surface of the fibers. Such nanoscale textures increase the effective electroactive surface area and are assumed to facilitate charge transfer processes. Occasionally, open spaces were seen among adjacent fibers, reflecting the network's porosity and providing possible sites for enhanced diffusion.

These findings show that laser treatment produces a conductive and porous carbonized scaffold, PDMS provides a conformal hydrophobic layer, and PEDOT:PSS disperses consistently into the fibers, regardless the concentration, demonstrating how both polymers improve the overall sensors functionality through distinct yet complementary contributions.

6.3 Encapsulation

6.3.1 Development of Encapsulated Electrode Design by Optimizing PDMS Application Method - PLA vs PDMS Stamp Comparison

Optimizing PDMS application for sample encapsulation was a foundational step in the development of our sensor platform, as it directly influenced the reliability and consistency of all subsequent electrochemical measurements. Several methods were explored to apply PDMS patterns onto the electrode surface. These included using an AxiDraw plotter with a syringe and gravity flow, creating a PLA stamp that was dipped in PDMS, and fabricating a PLA mold to cure PDMS into a structured stamp.

To compare the effectiveness and resolution of these methods, a greyscale intensity analysis was conducted using a resolution test pattern. Both a PDMS mold and a PLA stamp were fabricated with rectangular features of varying widths (0.5 mm, 1 mm, 2 mm, 3 mm, and 4 mm) and equal spacing. After stamping onto paper, the resulting patterns were imaged and converted to 8-bit grayscale. A linear intensity profile was extracted across the features to assess the printing fidelity.

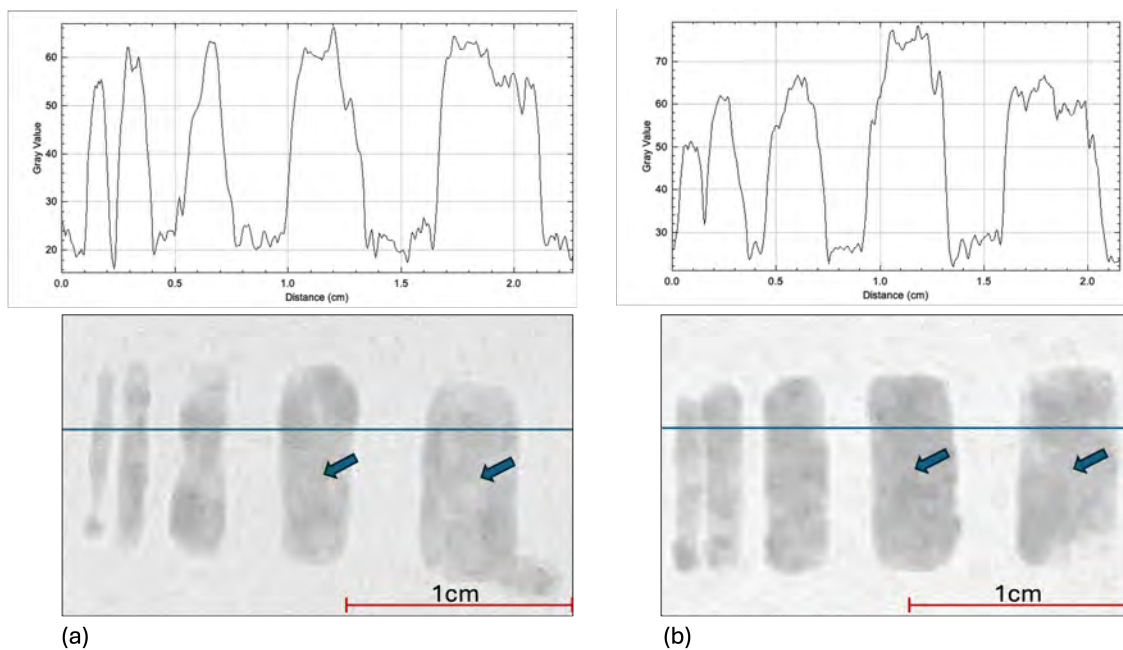


Figure 6.19 Line intensity profiles across stamped features used to compare resolution fidelity of (a) PLA and (b) PDMS stamps. Features range in width from 0.5 to 4 mm. Comparable patterning denoted by arrows.

The PDMS stamp was ultimately selected as the preferred method due to its flexibility and transparency, which facilitated better contact with the paper substrate and allowed for easier visual alignment during stamping. In contrast, while the PLA stamp yielded acceptable resolution, it was difficult to achieve uniform PDMS coverage on its surface. PDMS tended to accumulate unevenly at the edges when the stamp was dipped and left to dry, resulting in inconsistent transfer.

The PDMS stamp could be reused up to five times before degradation of print quality due to PDMS build-up and surface distortion. To extend its usability, it was essential to remove excess PDMS after each use to prevent unwanted polymerization and surface roughening over time.

Both stamping methods yielded comparable results in terms of PDMS absorption into the paper and pattern resolution. This suggests that further optimization of electrode performance can focus on other design parameters beyond the choice of stamping technique.

6.3.2 Water Contact Angle Measurement of FC-40-Treated Encapsulated Electrodes

Sessile-drop experiments were performed to assess the wettability of paper electrodes encapsulated in FC-40. A 3 μL water droplet was deposited onto each electrode surface using a micropipette to minimize gravitational distortion. Side-view images were acquired with a digital camera, converted to 8-bit TIFF format, and analyzed in ImageJ using the LB-ADSA plugin. Static contact angles were extracted via the “Canvas” fitting mode.

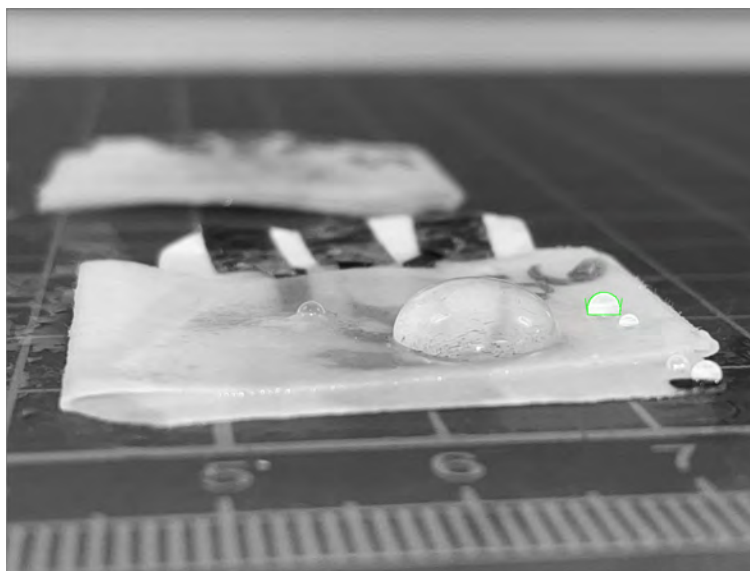
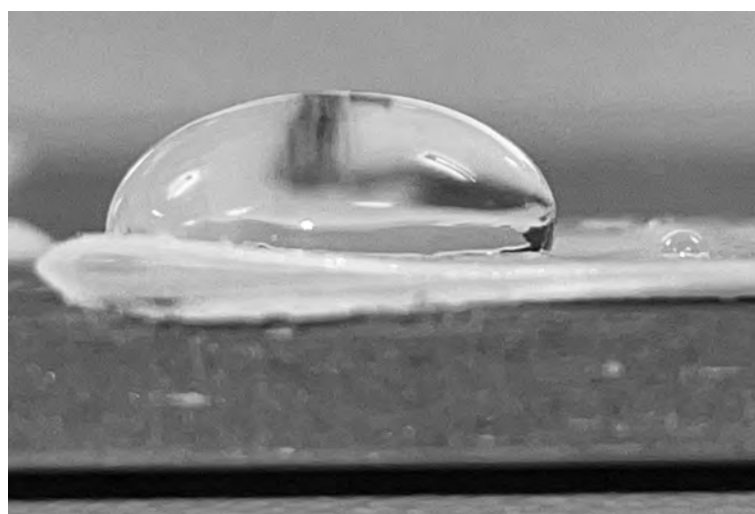


Figure 6.20 Representative side-view image of a water droplet on an FC-40-treated electrode with the LB-ADSA fit overlaid in green. Ruler included in figure in cm.



(a) Full droplet profiles



(b) Zoomed-in view at the contact line

Figure 6.21 Contact angle measurement on an FC-40-treated electrode. (a) Side-view profiles of a large (left) and a small (right) sessile drop; the smaller droplet, being less affected by gravitational flattening, exhibits a higher static contact angle. (b) Detailed view of the solid–liquid–air interface for the small droplet, highlighting the fitted contact line.

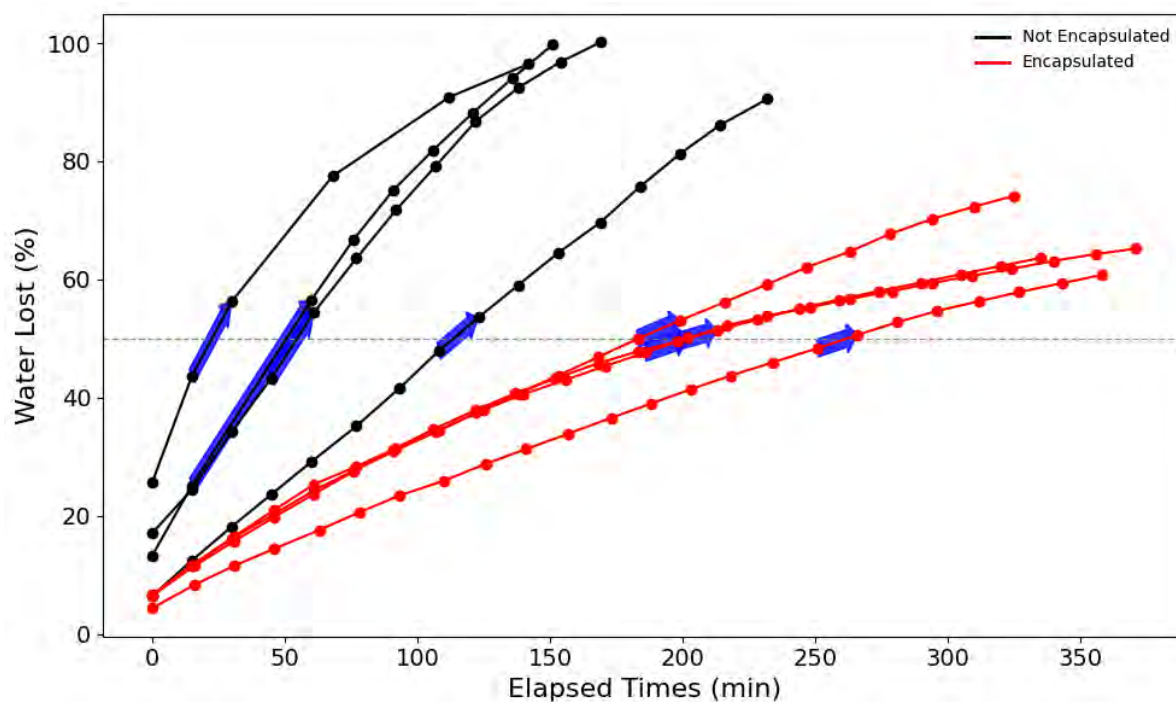
Table 6.5 Static water contact angles measured on PDMS + FC-40 coated electrodes (LB-ADSA, Canvas fit).

Drop #	Contact angle (°)
1	101.4
2	103.5
3	99.6
4	101.3
Mean $\pm$ SD (n=4)	101.5 $\pm$ 1.6

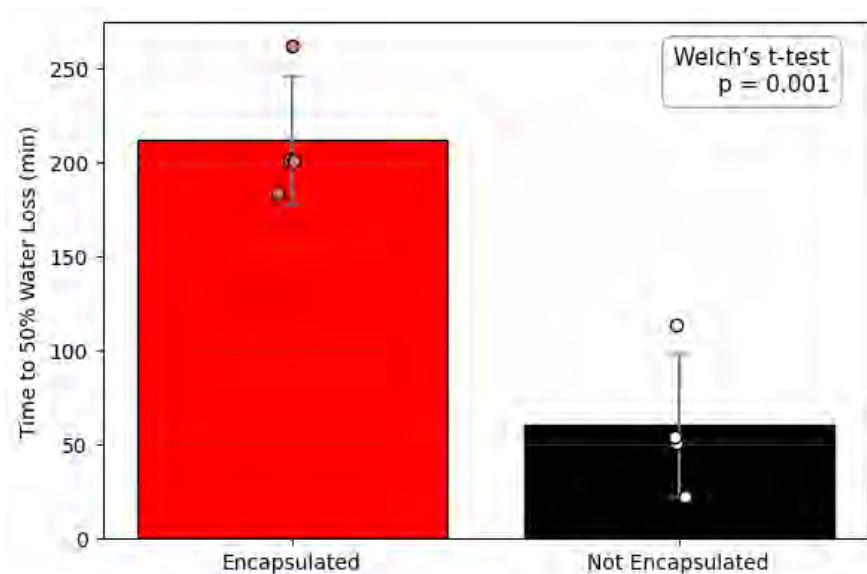
The mean static contact angle of $101.5 \pm 1.6^\circ$ demonstrates that the FC-40 encapsulation imparts strong hydrophobicity to the paper electrode surface, in line with reported values for PDMS and fluorocarbon-coated substrates [87,88]. The low standard deviation indicates high reproducibility across multiple droplets. Larger droplets exhibited reduced angles due to gravitational flattening, volumes below 5 μL were therefore measured to maintain a near-spherical cap. These results suggest that the FC-40 treatment effectively minimizes water spreading, which is critical to preserving sample integrity and preventing evaporation in electrochemical measurements.

6.3.3 Drying Behavior and Moisture Retention Analysis

Encapsulation with FC-40 fluorocarbon oil was hypothesized to slow sample evaporation by forming a hydrophobic barrier, thereby extending the hydration window and functional lifetime of the paper-based electrode. To quantify this, the mass loss over time was monitored for both encapsulated (treated with FC-40) and non-encapsulated (not treated with FC-40) devices under ambient drying conditions. The resulting drying curves, shown in Figure 6.22a, reveal clear differences between the two groups, with electrodes encapsulated in FC-40 exhibiting markedly slower and more gradual weight reduction.



(a) Fractional water loss (percent of initial water content) plotted against elapsed time for encapsulated (red markers, $N = 4$) and non-encapsulated (black markers, $N = 4$) sensors. Blue arrows indicate estimated slopes near the 50% water-loss point.



(b) Time taken to reach 50% water loss for each electrode.

Figure 6.22 Water-loss behavior of encapsulated versus non-encapsulated paper electrodes. (b) Water-loss half-lifetimes $T_{50,\text{water}}$ (time to 50% water loss), showing group means (bars ± 1 SD) and individual electrodes (jittered dots: white for non-encapsulated, light-coral for encapsulated). The unpaired Welch's t -test p -value comparing the two groups is indicated above.

6.3.4 Statistical Analysis of Water Loss Over Time

To examine the impact of encapsulation on drying kinetics, the water-loss half-lifetimes ($T_{50,\text{water}}$) of encapsulated and non-encapsulated paper electrodes were compared using a one-way ANOVA. The analysis revealed a significant effect of encapsulation on $T_{50,\text{water}}$ ($F(1, 6) = 34.90$, $p = 0.0010$; Table 6.6). A Tukey's HSD post-hoc test confirmed that encapsulated electrodes exhibited a mean $T_{50,\text{water}}$ approximately 152 min longer than non-encapsulated electrodes (mean difference = 151.75 min, 95% CI [88.89, 214.61], $p_{\text{adj}} = 0.0010$). These results demonstrate that the encapsulation layer significantly slows water evaporation from the paper matrix, extending its half-life by over 2.5 hours.

Table 6.6 Statistical analysis of 50% water-loss half-life ($T_{50,\text{water}}$) by encapsulation.

ANOVA	Sum of Squares	df	F	p-value
Encapsulation	46056.78	1	34.90	0.0010
Residual	7919.13	6		

Table 6.7 Tukey HSD post-hoc comparisons (Encapsulated vs. Not Encapsulated) at each timepoint, where a sufficient number of electrodes were available in both groups for statistical comparison.

Time (min)	Mean Diff	p -adj	Lower 95% CI	Upper 95% CI	Significant
0	9.63	0.0548	-0.27	19.52	No
20	15.63	0.0529	-0.26	31.53	No
30	21.24	0.0719	-2.75	45.23	No
60	23.97	0.0258	4.32	43.61	Yes
80	29.14	0.0201	6.84	51.44	Yes
90	33.55	0.0151	9.79	57.32	Yes
110	42.66	0.0044	19.16	66.16	Yes
120	38.42	0.0274	6.98	69.85	Yes
140	47.18	0.0021	24.74	69.61	Yes
150	43.52	0.0585	-2.94	89.97	No
170	41.26	0.0145	13.54	68.97	Yes
180	26.83	0.0435	3.51	50.15	Yes
200	32.74	0.0099	14.98	50.49	Yes
230	37.48	0.0086	18.12	56.84	Yes

To further delineate the onset and persistence of encapsulation's protective effect, Tukey's HSD comparison was performed at each timepoint (Table 6.7). Significant reductions in percent water loss between encapsulated and non-encapsulated electrodes first appeared at 60min ($p = 0.0258$) and remained so through most subsequent intervals. Notably, several

timepoints (e.g. 20 min, 30 min and 150min) yielded p -values between 0.05 and 0.07, reflecting little variability across individual electrodes, however, the overall pattern confirms that encapsulation consistently slows drying relative to unprotected sensors.

6.4 Electrochemical Stability of Encapsulated Electrodes Over Time

This section evaluates the temporal stability of the electrochemical signal under drying conditions. CV scans were recorded every 15 minutes in a 1mM RuHex solution with 1M KCl as the supporting electrolyte. Key electrochemical parameters: reduction and oxidation peak currents, peak-to-peak voltage separation (ΔE), and capacitance, were extracted at each timepoint and normalized to the corresponding capacitive current. RuHex was selected over PYO for this stability test due to its well-defined redox peaks and broader documentation in the electrochemical literature [72,73]. These measurements were used to assess how signal quality deteriorates over time and whether encapsulation with FC-40 improves performance stability.

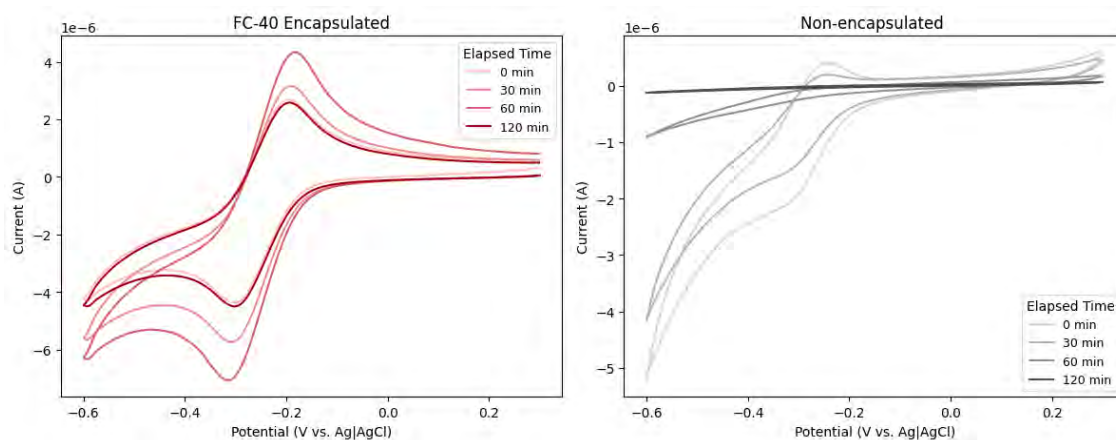


Figure 6.23 Cyclic voltammograms over time for encapsulated (left) and non-encapsulated (right) electrodes exposed 1mM RuHex in 1M KCl. The encapsulated electrode shows sustained redox signal up to 120 minutes. In contrast, the non-encapsulated electrode exhibits a complete loss of signal by 120 minutes.

To visualize the signal retention trends observed in our quantitative I_p data (see Table 6.8), time-resolved CV responses for encapsulated and non-encapsulated electrodes were contrasted. Figure 6.23 shows representative overlays of CV curves for both conditions. At 120 minutes, the encapsulated electrode, protected with FC-40 (red), still produces a distinguishable faradaic signal, while the non-encapsulated counterpart (grey) exhibits a featureless baseline.

This CV comparison demonstrates that FC-40 encapsulation preserves electrochemical activity over time, consistent with the enhanced I_p retention quantified later in this study.

6.4.1 Relative Current Peak Analysis

As depicted in Figure 6.24 a-b), we define the relative peak current at time t by

$$R(t) = \frac{I(t)}{I_0} \left[R_{p,\text{red}}(t) = \frac{I_{p,\text{red}}(t)}{I_{p,\text{red},0}} \quad \text{and} \quad R_{p,\text{ox}}(t) = \frac{I_{p,\text{ox}}(t)}{I_{p,\text{ox},0}} \right] \quad (6.1)$$

where I_0 is the peak current measured at $t = 0$ min. Encapsulation clearly slows the decrease in both reduction and oxidation signals: after 75 min the encapsulated sensors retain roughly 95 % of their initial currents, whereas the unencapsulated devices drop below 30 %.

In Equation (6.1), a value of $R < 1$ indicates signal decay, while $R > 1$ (as occasionally observed at longer times) may arise from improved wetting or redistribution of analyte within the PDMS–FC-40 chamber, momentarily enhancing the electroactive surface area before further decay sets in. Specifically, as evaporation progresses, the remaining aqueous microdroplets may spread or re-wet previously dry electrode surfaces, temporarily increasing the electroactive surface area and thus the measured current before further decay ensues. Similar behavior has been reported in droplet microfluidics co-encapsulated in oil phases: oil-layer isolation preserves droplet geometry and internal recirculation, and the oil–droplet interface can promote transient increases in electrode contact area and signal stability [89, 90].

Table 6.8 Relative peak currents (mean $\pm$ SD) over time for encapsulated and non-encapsulated electrodes normalized to their respective initial values (I/I_0).

Time (min)	Encapsulated		Not Encapsulated	
	$I_{p,\text{red}}$	$I_{p,\text{ox}}$	$I_{p,\text{red}}$	$I_{p,\text{ox}}$
0	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
15	1.14 $\pm$ 0.38	0.93 $\pm$ 0.24	0.79 $\pm$ 0.25	0.34 $\pm$ 0.71
30	1.16 $\pm$ 0.59	0.89 $\pm$ 0.39	0.45 $\pm$ 0.33	0.29 $\pm$ 0.31
45	1.16 $\pm$ 0.64	0.94 $\pm$ 0.45	0.25 $\pm$ 0.30	0.11 $\pm$ 0.20
60	1.25 $\pm$ 0.17	1.03 $\pm$ 0.42	0.20 $\pm$ 0.33	0.08 $\pm$ 0.15
75	0.99 $\pm$ 0.41	0.87 $\pm$ 0.58	0.25 $\pm$ 0.33	0.07 $\pm$ 0.12
90	0.67 $\pm$ 0.45	0.58 $\pm$ 0.48	0.01 $\pm$ N/A	0.00 $\pm$ N/A
105	0.15 $\pm$ 0.13	0.07 $\pm$ 0.09	—	—
120	0.04 $\pm$ 0.05	0.01 $\pm$ 0.01	—	—
135	0.02 $\pm$ 0.02	0.00 $\pm$ 0.00	—	—

6.4.2 Statistical Comparison of Relative Reduction Current Over Time for Encapsulated Electrodes

Table 6.9 Unpaired t -test comparison of normalized redox peak currents (I_p/I_0) between encapsulated and non-encapsulated electrodes at selected times (valid tests only). Significant if $p < 0.05$.

Time (min)	n_{encap}	n_{non}	t_{red}	p_{red}	Sig. red ($p < 0.05$)	t_{ox}	p_{ox}	Sig. ox ($p < 0.05$)
15	7	6	1.976	0.0753	No	1.929	0.1022	No
30	7	6	2.686	0.0235	Yes	3.120	0.0098	Yes
45	7	6	3.357	0.0087	Yes	4.372	0.0020	Yes
60	6	4	5.864	0.0041	Yes	5.126	0.0017	Yes
75	6	2	2.565	0.1140	No	3.217	0.0191	Yes

To evaluate whether encapsulation significantly improves signal retention over time, unpaired t -tests were conducted at each time point comparing the relative reduction current between encapsulated and non-encapsulated electrodes.

As shown in Table 6.9, encapsulated electrodes exhibited a consistent trend toward higher relative reduction current (I_p/I_0). After 30 minutes, the difference in relative peak intensity becomes significant. Between 45 to 60 minutes, the encapsulated group showed a significantly larger retained signal, indicating enhanced electrochemical stability at this intermediate stage. Beyond 75 minutes, group sizes reduced, especially in the non-encapsulated condition due to electrode dropout, limiting the power of comparison to 75 minutes. At this time, only $I_{p,\text{ox}}/I_0$ was significantly greater in encapsulated electrodes.

Given the reduced number of significant results across discrete timepoints and the increasing variability caused by electrode loss, this approach was complemented with a more integrative analysis: the T_{50} time, defined as the duration required for each electrode's signal to decay to 50% of its initial value, enabling a clearer, quantitative comparison of signal retention performance across conditions.

6.4.3 Evaluating Water Loss and Signal Loss Over Time by T_{50}

To validate the protective effect of encapsulation on electrode performance, an analysis comparing the time-dependent decline of both water content and electrochemical signal strength was carried out. This analysis involved calculating the time to 50% loss (T_{50}) for both water evaporation and signal decay, providing quantitative metrics to assess electrode longevity.

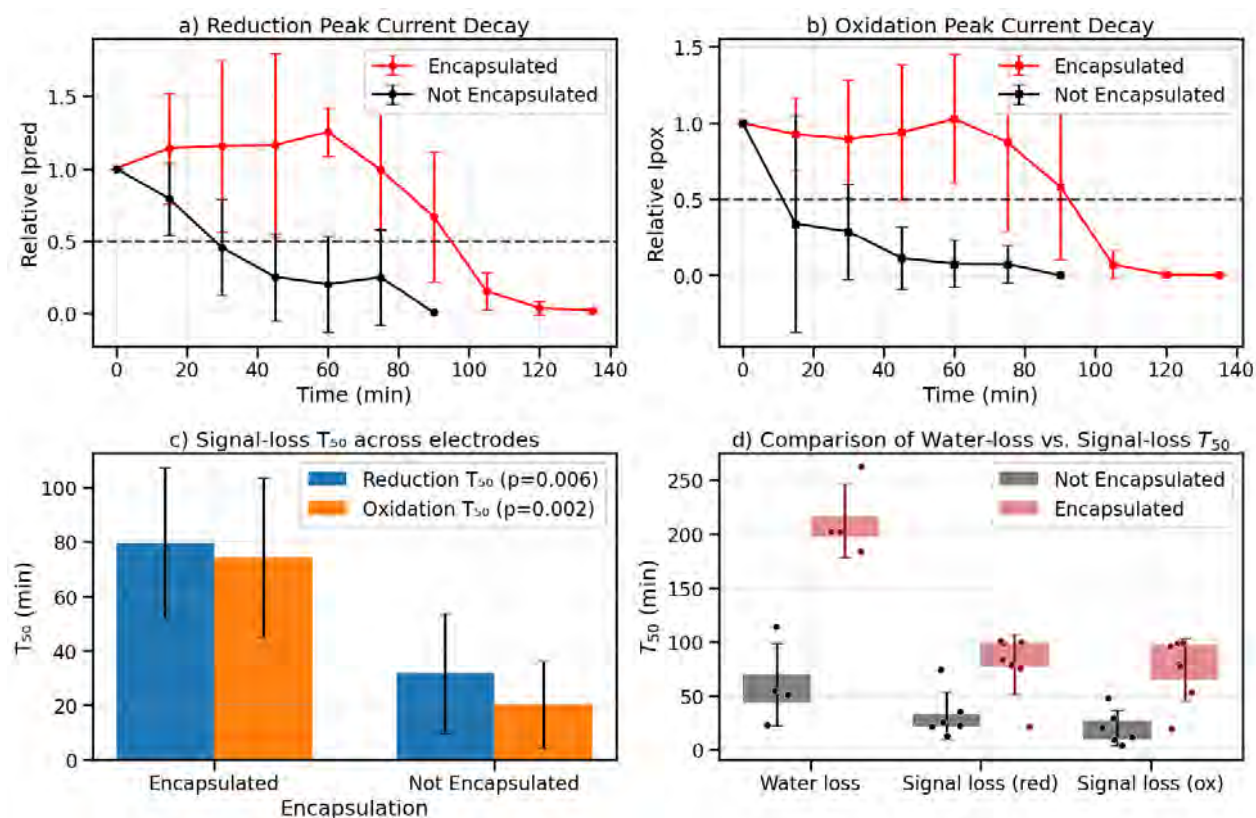


Figure 6.24 (a–c) Time-dependent decay of the normalized peak currents and their associated T_{50} metrics. Solid lines represent group means; error bars denote standard deviations. The horizontal dashed line marks the 50% signal retention threshold (Encaps., $N = 7$; non-encaps., $N = 6$). (d) Compares with water-loss T_{50} , with (Encaps., $N = 4$; non-encaps., $N = 4$)

Currents were normalized to their initial value at $t = 0$ min, yielding a dimensionless relative signal $R(t) = I(t)/I_0$ (Equation 6.1). The 50% threshold was used to define a signal half-life $T_{50, \text{signal}}$, calculated for each electrode and summarized by group (Figure 6.24c). In the absence of FC-40 encapsulation, both reduction (I_{pred} , panel a) and oxidation (I_{pox} , panel b) currents decay rapidly within the first 30–60 min, consistent with progressive fouling and depletion of analyte in the open chamber.

Encapsulated sensors showed significantly longer signal half-lives in comparison to non-encapsulated ones for both reduction and oxidation peaks ($p < 0.02$, Welch's t-test). In Figure 6.24d, mean T_{50} values were compared for water loss and signal loss.

To further evaluate the relationship between hydration retention and signal longevity, the time at which electrodes reached 50% water loss ($T_{50, \text{water}}$) was paralleled with the time at which they reached 50% signal loss ($T_{50, \text{signal}}$). Each point represents the average T_{50} value

for encapsulated or non-encapsulated groups, including standard deviations. A connecting line was added to visualize the global trend, and its slope provides a comparative indicator of the relative decay rates.

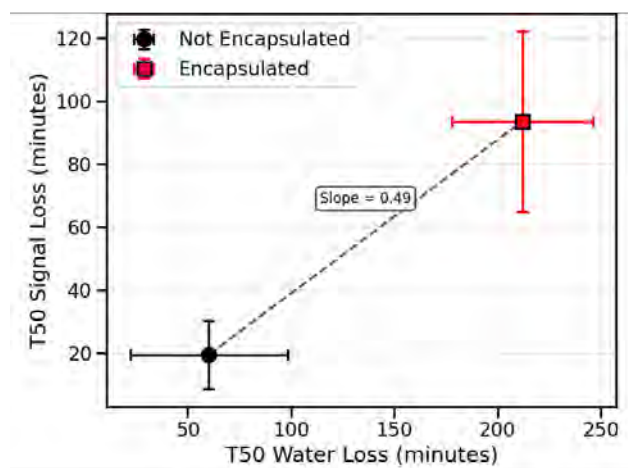


Figure 6.25 Scatter plot comparing the average time to 50% water loss ($T_{50,\text{water}}$) and 50% signal loss ($T_{50,\text{signal}}$) for encapsulated (red) and non-encapsulated (black) electrodes. Points represent group means with standard deviation error bars. A connecting line illustrates the overall trend, with a calculated slope of 0.49, indicating that delays in water loss translate to smaller delays in signal retention.

In figure 6.25, encapsulated electrodes reached both water and signal loss thresholds substantially later than their non-encapsulated counterparts. The connecting line between group means yielded a slope of approximately 0.49, indicating that for every minute of water loss delay, signal loss was delayed by 49%. This relationship implies that the presence of hydrated structure contributes to the electrochemical performance, the signal is more susceptible to drying than water loss alone would suggest. In other words, a relatively small decrease in water content may lead to a much larger decrease in response, and the global water content of the system is a poor indicator of electrochemical fidelity. The fidelity of the signal is determined by other local effects such as electrode wetting, stability of the electrode/analyte interface and the charge carrier mobility. These can deteriorate well before significant evaporation is observed macroscopically, and the system could therefore fail more quickly than an overall loss of water content would suggest.

FC-40 encapsulation markedly prolongs signal retention: encapsulated electrodes maintain most of their initial I_{pred} and I_{pox} after over 60 min, and show a noticeably slower decline over the full 120 min. We attribute this stabilization to the FC-40 layer acting as a hydrophobic “lid,” which traps a thin film of analyte solution in the PDMS chamber and minimizes losses

due to evaporation.

Interestingly, encapsulated electrodes exhibited a resurgence of peak signals in both reduction and oxidation currents at 60 min, before the eventual decline resumes. However, upon statistical analysis, both paired t-tests returned high p-values ($p = 0.52$ for reduction, $p = 0.54$ for oxidation), meaning there is no statistically significant difference between the 45 min and 60 min relative signal intensities for encapsulated electrodes. A slighter rebound is also present at the 75 min time point of non-encapsulated electrodes.

We hypothesize that this rebound arises from one or more of the following phenomena: Electrochemical resurgence and resilience may act synergistically with physical changes occurring within the encapsulated system. For example, mechanical relaxation or subtle swelling of the PDMS chamber walls could disrupt surface fouling layers, temporarily exposing additional active carbonized area and enhancing signal recovery [91]. Furthermore, as the aqueous phase within the PDMS-defined chamber gradually evaporates, it does not retreat uniformly. Instead, it often breaks into discrete droplets or micro-volumes that re-concentrate solutes as they shrink. This process may partially explain the resurgence in relative peak current observed even in non-encapsulated electrodes, where droplet redistribution or analyte accumulation near the electrode could briefly restore electrochemical activity before complete signal loss.

Under encapsulated conditions, microdroplet formation may become even more pronounced. Brewer *et al.* demonstrated that FC-40 can form robust immiscible barriers that remain stable under pressure differentials up to approximately 3.4 kPa [5]. Owing to FC-40's hydrophobic nature and immiscibility, the fluorocarbon layer surrounding the microdroplets can dynamically reflow to minimize interfacial energy, thereby encapsulating the shrinking sample volumes and exerting gentle pressure on them.

Other studies have also demonstrated that proper channel geometry and surface treatments can stabilize aqueous droplets within oil phases, preventing coalescence and surface dehydration, thereby preserving the microenvironment for extended periods [92]. Conchouso *et al.* also noted enhanced analyte detection when droplets were gently compressed within confined geometries, mirroring how FC-40 may increase analyte-electrode proximity and signal intensity in our system [90].

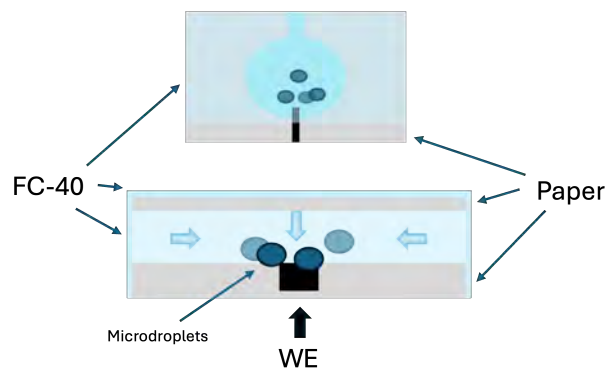


Figure 6.26 Schematic representation of microdroplet formation within the PDMS-defined electrode chamber. As the aqueous sample dries, it separates into discrete microdroplets that are confined by the surrounding FC-40 fluorocarbon layer (light blue). This immiscible oil phase applies gentle pressure to the droplets (depicted by light blue arrows), maintaining contact with the WE surface and supporting extended electrochemical activity.

This dynamic redistribution may drive the remaining sample volume toward the WE surface, thereby enhancing local analyte concentration and maintaining electrochemical contact even as total water content decreases. Such a mechanism could possibly partially account for the superior signal retention observed in encapsulated electrodes at later time points, by preserving the functional interface between analyte and electrode under drying conditions.

FC-40 encapsulation possibly enhances electrochemical stability as both reduction and oxidation peak currents decay more slowly over time demonstrating that encapsulation effectively protects the electrode/electrolyte interface against degradation during repeated CV measurements.

6.4.4 Electrochemical Peak Separation Stability via Peak-to-Peak Separation Potential Analysis

To further assess the stability of the redox response over time, we monitored the evolution of the peak-to-peak separation potential ($\Delta E = E_{\text{ox}} - E_{\text{red}}$) for both FC-40-encapsulated and non-encapsulated electrodes during repeated CV measurements. As shown in Figure 6.27, encapsulated electrodes exhibited a gradual and consistent decrease in ΔE , from approximately 0.12 V at $t = 0$ to around 0.04 V after 135 minutes. These final values are comparable to those typically observed on polished GCE (0.03 V), where surface smoothness and minimal kinetic barriers support near-ideal reversibility [86]. The progressive narrowing of ΔE suggests an enhancement in electrochemical reversibility over time. Notably, values of ΔE below 50 mV are characteristic of thin-layer diffusion regimes, where redox species are con-

finned to a small volume near the electrode, enabling rapid equilibration between oxidized and reduced forms [75].

Non-encapsulated electrodes displayed highly variable and less defined ΔE values, dropping below 0.05 V as early as 60 minutes and exhibiting substantially larger standard deviations. The rapid decrease and variability of ΔE in the absence of encapsulation are consistent with the onset of thin-layer diffusion effects. As evaporation progresses, the sensing chamber transitions from semi-infinite to thin-layer diffusion, where redox species are confined between the electrode surface and the diminishing liquid layer. In this regime, both oxidation and reduction peaks begin to overlap due to rapid equilibration within a restricted volume, thereby reducing ΔE .

According to Bard and Faulkner, thin-layer behavior emerges when the diffusion layer thickness becomes comparable to the dimensions of the electrochemical cell [75]. This leads to deviations from classical diffusion-controlled kinetics: instead of the expected $\sqrt{SR}$ dependence, the current becomes linearly dependent on SR. Peak separation narrows, and voltammograms increasingly resemble those of surface-confined species.

By contrast, encapsulated electrodes likely maintain semi-infinite diffusion conditions for a longer duration due to sustained hydration and physical confinement by the FC-40 layer. In semi-infinite diffusion, redox species are assumed to diffuse into an effectively infinite bulk volume, maintaining a stable concentration gradient near the electrode [75]. In this regime, ΔE approaches 59 mV for a reversible one-electron system, such as RuHex [75]. When drying or confinement occurs (as in unencapsulated devices) this assumption no longer applies, and the system enters a thin-layer regime in which the entire analyte volume is sampled rapidly, resulting in altered peak shapes and kinetics.

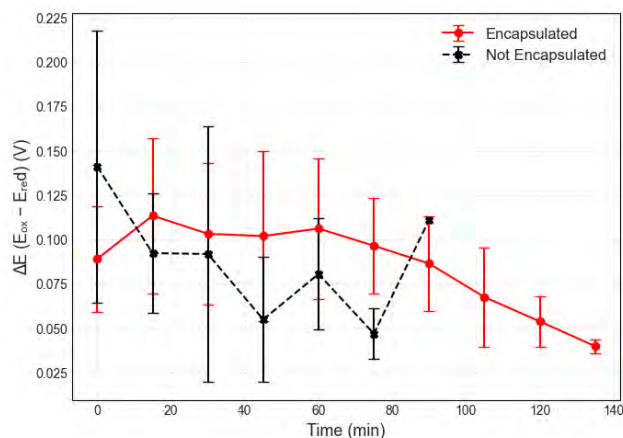


Figure 6.27 Evolution of peak-to-peak separation potential ($\Delta E = E_{\text{ox}} - E_{\text{red}}$) measured at 100mV/s over time for encapsulated (red, $N = 10$) and non-encapsulated (black, $N = 17$) paper-based electrodes in 1mM RuHex and 1M KCl. Error bars represent standard deviation. Note that not all electrodes survived the full duration of the measurement; the number of devices contributing to each time point may vary accordingly.

These findings indicate that FC-40 encapsulation contributes not only to improved signal retention, but also to the preservation of electrochemical reversibility over time. The pronounced variability and progressive collapse of ΔE in the absence of encapsulation likely reflect the effects of evaporation, including capacitive interference, peak broadening, and eventual signal loss due to electrode degradation.

6.4.5 Electrode Survival

As shown in Figure 6.28a, the number of electrodes capable of generating valid redox signals decreased more rapidly in the non-encapsulated group compared to their encapsulated counterparts. Encapsulated electrodes demonstrated improved stability, with a majority retaining functionality beyond 90 minutes. The dropout of devices over time further emphasizes the role of FC-40 encapsulation in preserving electrode integrity and signal fidelity in extended electrochemical measurements.

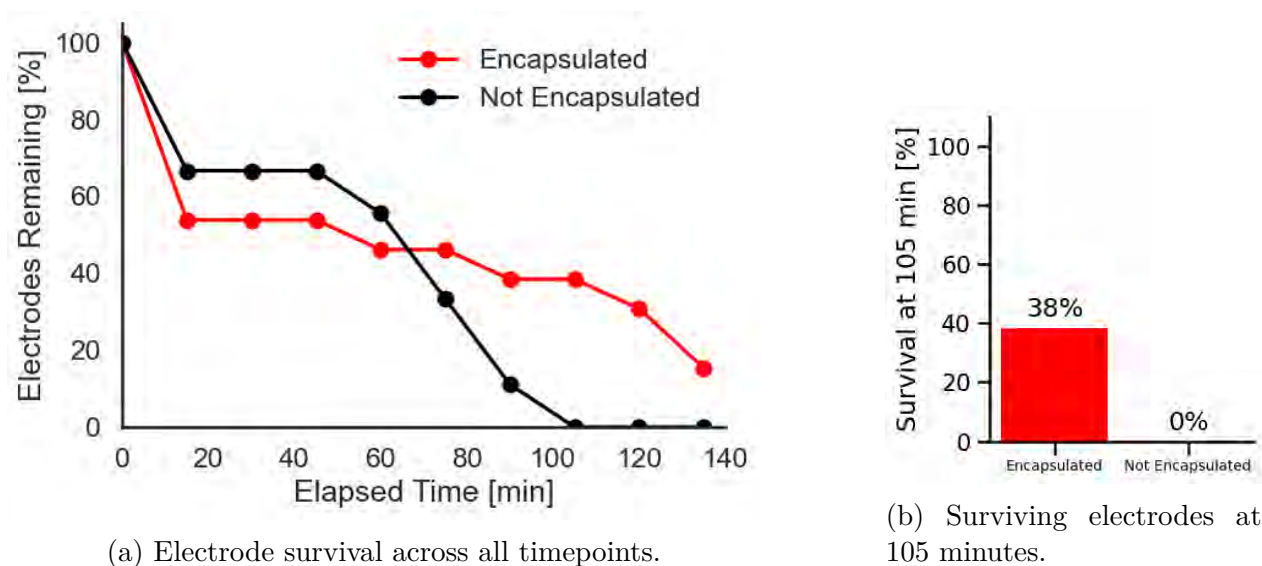


Figure 6.28 (a) Number of electrodes contributing valid electrochemical data at each timepoint for encapsulated (red) and non-encapsulated (black) conditions. (b) Proportion of electrodes still contributing signal at the 105-minute timepoint.

It is worth noting that a pronounced drop in electrode viability occurred at the initial 15-minute time point, particularly in the encapsulated group. This early loss may be attributable to electrical disconnection rather than chemical degradation. Specifically, the presence of FC-40 around the electrode surface may have interfered with the electrical interface between the copper tape and the crocodile clips, possibly displacing or insulating the contact. While the majority of encapsulated electrodes remained functional beyond 90 minutes, this observation suggests that care must be taken to ensure stable mechanical and electrical connections in oil-encapsulated systems, particularly during the initial stages of operation.

In addition, a significant proportion of failures throughout this study stemmed from mechanical fragility at the point of connection. The act of attaching three crocodile clips required to interface with the potentiostat, places considerable mechanical stress on the carbonized paper, which is already structurally weakened from the laser exposure. Tearing or delamination of the carbonized strip frequently occurred during initial clipping or readjustments, also contributing to the early dropout of otherwise functional electrodes.

6.5 Limit of Detection Enhancement via PEDOT:PSS Coating Assessed by Voltammetry

6.5.1 Cyclic Voltammetry for Pyocyanin Detection at Different Concentrations Using the Simple Electrode Design

Initial CVs were recorded in PBS to establish a baseline response and confirm the absence of any redox-active features. Both unmodified control electrodes and electrodes modified with PEDOT:PSS 1:30 (N=3 respectively) were measured under identical conditions.

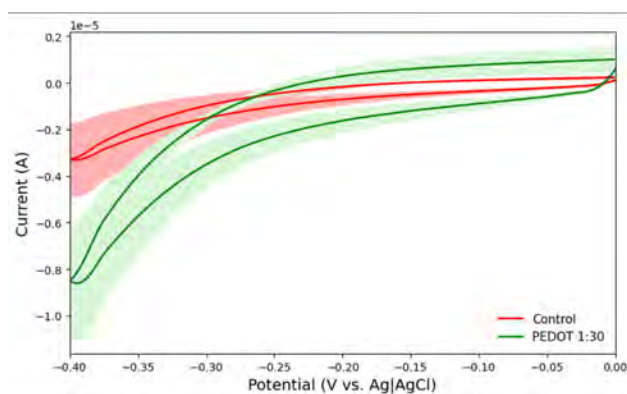


Figure 6.29 Baseline cyclic voltammograms in PBS for unmodified control electrodes (red, N=3) and PEDOT:PSS (1:30) modified electrodes (green, N=3). No discernible redox peaks are observed, confirming the featureless background current in the absence of redox-active analyte.

The voltammograms in PBS are essentially featureless, there are no well-defined oxidation or reduction peaks in either curve, which confirms that neither the bare laser-exposed control nor the PEDOT:PSS-modified laser-exposed electrodes exhibit faradaic activity in this blank electrolyte. Instead, both traces display the typical capacitive charging currents.

Quantitatively, the PEDOT:PSS (1:30)-coated electrodes (green) show a systematically larger magnitude of charging current (more negative cathodic current at negative potentials and more positive anodic current at positive potentials) compared to the unmodified control (red), which is less broad. This is expected from a conductive polymer film increasing the effective surface area and hence the double-layer capacitance [93].

The shaded bands ($\pm 1\sigma$) around each mean indicate good reproducibility: the control electrodes are very consistent (narrow band), while the PEDOT:PSS replicates show slightly greater spread likely due to small variations in film thickness or morphology. Overall, this baseline characterization demonstrates the absence of redox signals in blank PBS and an

increased capacitive background upon PEDOT:PSS modification, establishing a stable reference for all subsequent analyte-detection experiments.

Next, CV measurements were performed in increasing concentrations of PYO to compare their respective electrochemical responses. As shown in Figure 6.30, PEDOT:PSS modification enhances signal definition and amplifies peak current intensity, particularly at lower analyte concentrations.

CVs obtained at varying PYO concentrations exhibited characteristic redox behavior consistent with previous literature. Elkhawaga *et al.* [42], for example, reported quasi-reversible peaks centered around -0.25 V vs. Ag|AgCl using PANI-AuNPs/ITO electrodes, with peak currents increasing proportionally with concentration. The observed peak separation of 50–70 mV and the symmetry of the redox couple are indicative of diffusion-controlled electron transfer and closely resemble our results in phosphate buffer systems.

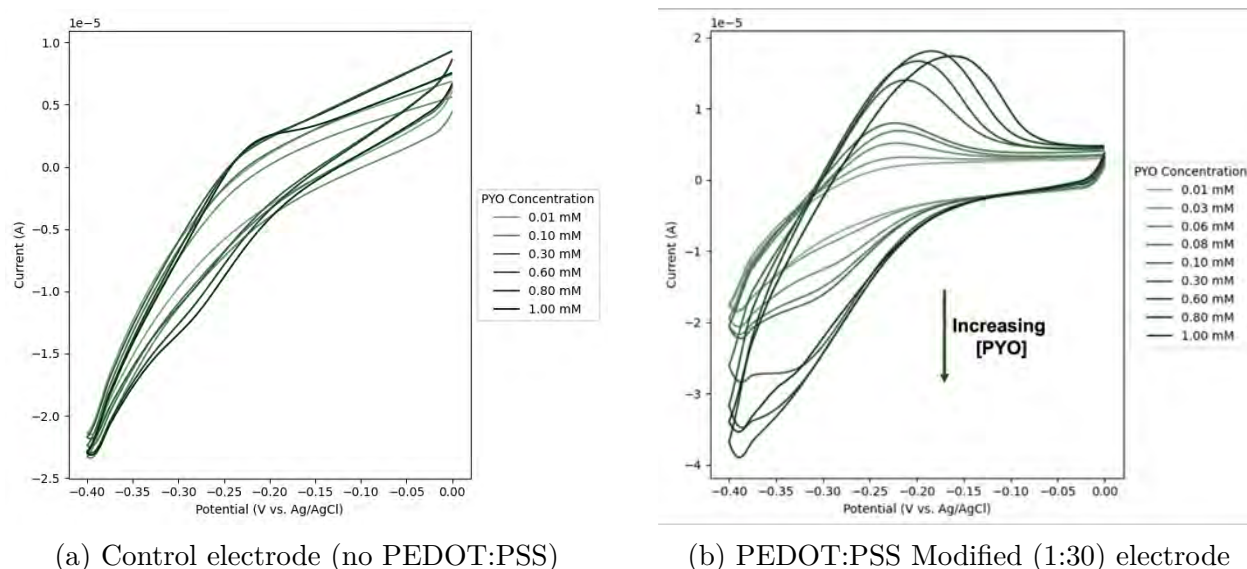


Figure 6.30 Cyclic voltammograms showing response to increasing PYO concentrations on (a) control and (b) PEDOT:PSS-modified (1:30) electrodes. Modification with PEDOT:PSS improves peak definition and signal amplitude across the tested range.

CV measurements using a commercial GCE electrode were performed across a range of PYO concentrations to establish a baseline electrochemical response. As illustrated in Figure 6.31, peak currents magnitudes remain negligible between 0.001 mM and 0.1 mM, indicating poor sensitivity at low concentrations. However, a steep increase in peak intensity is observed at 1.0 mM, suggesting the electrode operates effectively only above a certain threshold.

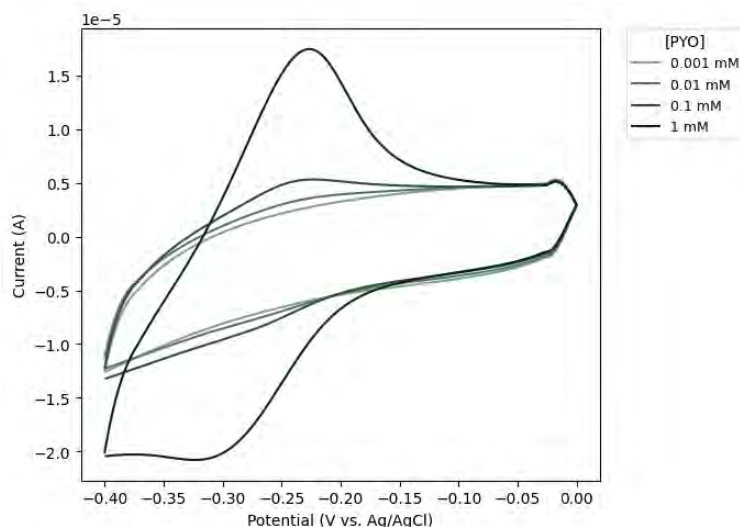


Figure 6.31 CV of a commercial GCE in 1 mM PYO, 1 M PBS, at 100 mV/s.

6.5.2 Square Wave Voltammetry Detection of Varying Pyocyanin Concentrations

SWV was performed using a reverse scan, from 0 V to -0.6 V, which allowed us to selectively analyze the cathodic (reduction) peak of PYO. This peak consistently appeared between -0.2 V and -0.3 V vs Ag|AgCl and was characterized by a negative current response. Although most published methods for PYO detection emphasize anodic detection through forward scans [19, 20, 40], cathodic detection of PYO using SWV has also been successfully reported [69, 94]. In particular, and Jarosova *et al.* demonstrated a prominent reduction peak at approximately -0.31 V using boron-doped diamond electrodes [69].

In our preliminary CV trials, the reduction peak was more pronounced and reproducible than the oxidation counterpart across all electrode types. This guided our choice to emphasize the reductive mode in SWV, providing a more reliable analytical window for PYO quantification.

SWV was used to characterize the electrochemical response of non-encapsulated carbonized paper electrodes toward a range of PYO concentrations, from $0.1 \mu\text{M}$ to 1 mM. SWV analysis can help confirm the responsiveness of the electrodes as the resulting peak-shaped voltammogram should be proportional to the analyte concentration [95]. Electrodes with and without PEDOT:PSS (1:30) modification were tested to assess the influence of surface conductivity and redox mediation on detection sensitivity. Ranges of concentrations plotted are adapted to the signal measured. In unmodified electrodes, a differential current was only measurable around 0.1 mM PYO, whereas in modified electrodes, currents could be identified as of

0.01 μ M. For this reason, the concentrations were adapted to include 0.01, 0.03, 0.06, 0.08 mM to clearly observe the effects of concentration.

As depicted in Figure 6.33, control electrodes exhibited a weaker differential current. In contrast, PEDOT:PSS-modified electrodes produced a well-defined, concentration-dependent reductive peak centered near -0.26 V as expected in SWV. Refer to Table 6.10 for mean and standard deviation of differential peak intensities (I_{peak} (A)) and their respective potentials (E_{peak}).

At higher concentrations of PYO (> 0.3 mM), a second anodic feature emerged around -0.41 V. This behavior can be attributed to three complementary phenomena. First, multilayer adsorption may occur beyond monolayer surface coverage, where only the first layer of PYO molecules is in direct electronic contact with the electrode. Subsequent layers interact indirectly, leading to a secondary redox feature, possibly involving multielectron transfer with shifted potential [95]. Second, PYO may undergo additional redox transitions at high surface concentrations, involving distinct protonation or resonance-stabilized intermediates, which become electrochemically visible under saturating conditions. Third, surface saturation effects and changes in adsorption kinetics may result in the appearance of spatially or energetically distinct populations of PYO, contributing to multiple overlapping redox peaks. These interpretations are consistent with prior electrochemical studies of PYO on modified surfaces [1,32]. They support the hypothesis that the secondary peak reflects concentration- and surface-dependent dynamics rather than the formation of new chemical species.

pH-Dependent Redox Behavior as a Contributing Mechanism

A fourth complementary explanation for the appearance of the second anodic peak at higher PYO concentrations is the pH-dependence of its redox behavior (Figure 3.2). As redox events accumulate near the electrode surface, particularly in low-buffer-capacity environments such as paper-based sensors, localized changes in pH may occur, especially if the pH is not previously optimized. These microenvironmental shifts can alter the protonation state of PYO or its redox intermediates, thereby modifying the redox potential.

Indeed, PYO is known to undergo proton-coupled electron transfer (PCET), and prior studies have demonstrated that its redox behavior is highly sensitive to pH. A study by Buzid *et al.* found that the CV oxidation peak potential of PYO shifted toward more negative values as the buffer pH increased from 4.0 to 7.0, with peaks appearing in the range of approximately -0.8 V to -0.25 V. Concurrently, the oxidation peak current decreased with increasing pH, indicating reduced redox activity in more alkaline conditions. Based on peak resolution and sensitivity, this study identified 50 mM acetate buffer at pH 5.0 as the optimal medium [96].

A second study examining the pH range 5–9 observed a similar trend: as pH increased, SWV peaks shifted linearly toward more negative potentials, with a slope of -0.054 V/pH on a porous CNT/graphene electrode [97]. These findings support the idea that changes in local proton activity can shift redox equilibria, potentially activating alternative oxidation pathways or stabilizing distinct PYO species. The same study also reported that deviations from the optimal pH led to significant errors in quantification: PYO concentrations were overestimated by 11.6% at pH 6.5 and underestimated by 30.0% at pH 8.5 [97]. This emphasizes the critical importance of pH control in voltammetric measurements involving PYO. In our study, all voltammetric analyses were conducted in phosphate-buffered saline (PBS) at pH 7.4, which falls within the physiologically relevant range but may not correspond to the optimal redox sensitivity window for PYO. This may partially explain the appearance of broadened or overlapping peaks at higher concentrations, as well as the possible emergence of additional redox features.

Effect of pH on PEDOT:PSS Performance

In addition to influencing PYO redox behavior, pH can also affect the electrochemical stability and conductivity of PEDOT:PSS films. Rathore and Schiffman investigated PEDOT:PSS fiber mats processed at various precursor pH levels and found that although fabrication was tolerant to a wide pH range (up to pH 12), subsequent exposure of cross-linked mats to aqueous environments between pH 5 and 11 caused conductivity losses of up to 60–70 % due to dedoping and morphological changes [98]. Complementary findings by Gao *et al.* showed that under basic conditions, deprotonation of PSS sulfonate groups induces phase separation and swelling, while hydroxide ions can neutralize PEDOT chains, further reducing conductivity and increasing resistance [99]. These results demonstrate the vulnerability of PEDOT:PSS to local pH variations, particularly in weakly buffered or confined systems like ours, and suggest that apparent changes in signal shape or intensity could partly stem from pH-driven alterations in the film's conductive properties.

Square-Wave Voltammetry to Analyze Simple Carbonized Electrodes, PEDOT:130 Modified Electrodes and GCE Response to PYO

To assess the electrochemical baseline in the absence of redox-active analytes, both control and PEDOT:PSS-modified electrodes ($N=3$ for each) were tested in 1 M PBS solution without PYO. As shown in Figure 6.32, neither electrode type produced distinct redox peaks, confirming the absence of interfering faradaic processes in the buffer alone. However, PEDOT:PSS-modified electrodes exhibited substantially higher capacitive current across the

potential window, attributable to the increased surface conductivity and double-layer capacitance introduced by the polymer film.

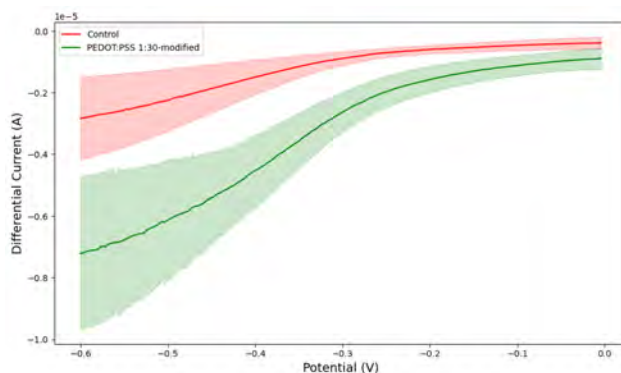
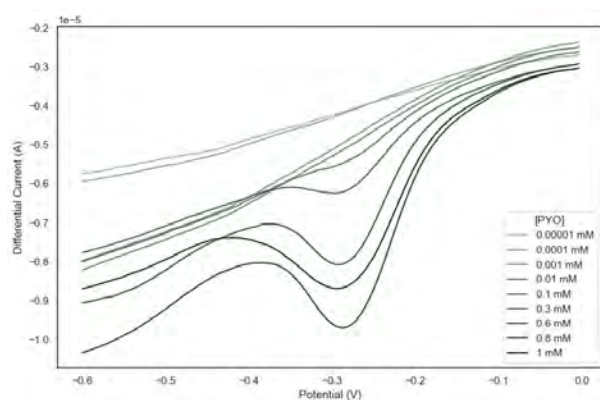
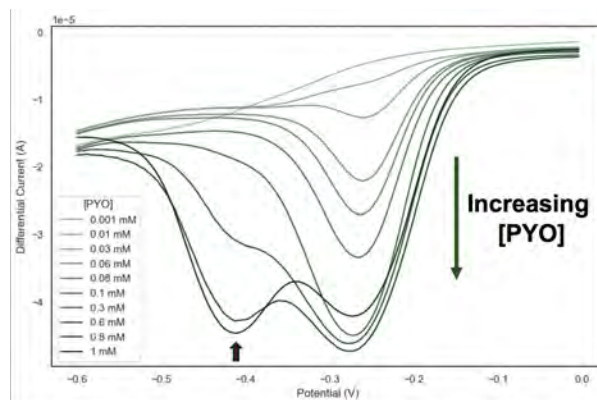


Figure 6.32 SWV of control (N=3) and PEDOT:PSS-modified (1:30) electrodes (N=3) in 1 M PBS (0 mM PYO). No faradaic peaks are observed in either condition, confirming electrochemical silence of the buffer.



(a) Unmodified electrode (control)



(b) PEDOT:PSS (1:30) modified electrode

Figure 6.33 SWV of PYO at increasing concentrations using (a) control and (b) PEDOT:PSS-modified paper electrodes. Only the modified electrode exhibits a characteristic redox signal for PYO at low concentration. Red arrow to point-out emerging second current peak in PEDOT:PSS-modified electrode.

When compared against a GCE (Table 6.11), PEDOT:PSS electrodes provided comparable peak currents and redox potentials, positioning them as a cost-effective, flexible, and scalable alternative to traditional materials.

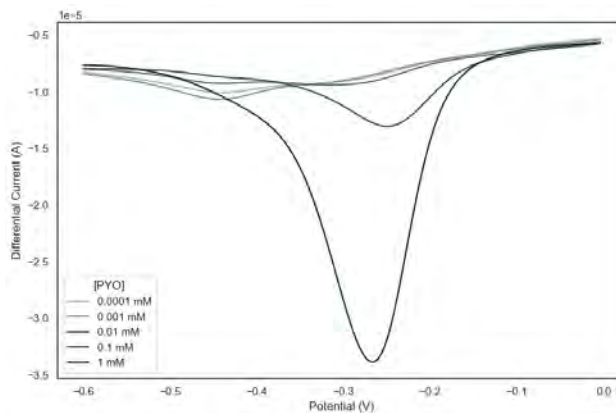


Figure 6.34 SWV response of a commercial GCE in the presence of increasing concentrations of PYO (0.0001 to 1 mM) in 1 M PBS. A clear increase in peak height is observed above 0.1 mM.

Table 6.10 Comparison of SWV peak current density (J_{peak}) and potential (E_{peak}) for control and PEDOT:PSS-modified electrodes at varying PYO concentrations (2×2 mm WEs).

[PYO]	Control			PEDOT:PSS		
	J_{peak} (A mm^{-2})	E_{peak} (V)	n	J_{peak} (A mm^{-2})	E_{peak} (V)	n
0.01 mM	-4.17×10^{-8}	—	1	$(-5.00 \pm 0.50) \times 10^{-7}$	-0.254 ± 0.006	5
0.03 mM	—	—	0	$(-1.96 \pm 0.99) \times 10^{-6}$	-0.259 ± 0.006	4
0.06 mM	—	—	0	$(-3.05 \pm 1.33) \times 10^{-6}$	-0.260 ± 0.006	4
0.08 mM	—	—	0	$(-3.98 \pm 0.73) \times 10^{-6}$	-0.262 ± 0.007	4
0.10 mM	$(-2.05 \pm 0.12) \times 10^{-7}$	-0.253 ± 0.017	6	$(-4.78 \pm 0.95) \times 10^{-6}$	-0.264 ± 0.007	5
0.30 mM	$(-5.05 \pm 1.58) \times 10^{-7}$	-0.264 ± 0.017	7	$(-6.92 \pm 1.83) \times 10^{-6}$	-0.271 ± 0.008	5
0.60 mM	$(-9.82 \pm 3.20) \times 10^{-7}$	-0.264 ± 0.014	7	$(-7.23 \pm 1.65) \times 10^{-6}$	-0.275 ± 0.007	5
0.80 mM	$(-1.39 \pm 0.29) \times 10^{-6}$	-0.259 ± 0.014	6	$(-7.98 \pm 1.95) \times 10^{-6}$	-0.271 ± 0.006	5
1.00 mM	$(-1.46 \pm 0.35) \times 10^{-6}$	-0.257 ± 0.018	5	$(-6.82 \pm 2.10) \times 10^{-6}$	-0.271 ± 0.009	5

Table 6.11 SWV results for GCE at varying PYO concentrations, normalized to current density (3 mm $\varnothing$ WE).

[PYO] (mM)	J_{peak} (A mm^{-2})	E_{peak} (V)
0.0001	-1.12×10^{-7}	-0.432
0.001	-9.68×10^{-8}	-0.308
0.01	-1.91×10^{-7}	-0.308
0.1	-7.80×10^{-7}	-0.248
1.00	-3.69×10^{-6}	-0.264

6.5.3 Electrode Sensitivity

The sensitivity of the electrodes was evaluated by plotting the SWV peak current as a function of PYO concentration. Sensitivity is defined here as the slope of the linear fit between current and PYO concentration (A/mM). As shown in Figure 6.35, control electrodes exhibited a strong linear response, with coefficients of determination (R^2) consistently exceeding 0.95 (Table 6.12). In contrast, PEDOT:PSS-modified electrodes displayed a nonlinear, bimodal response: at low concentrations (up to approximately 0.1mM), they exhibited steep slopes indicative of high sensitivity, but this trend began to plateau at higher concentrations, suggesting a saturation effect. This nonlinearity could possibly reflect the finite number of available redox-active sites within the PEDOT:PSS matrix or diffusion limitations through the polymer film at higher PYO concentrations. For this reason, when evaluating the sensitivity of PEDOT:PSS-modified (1:30) electrodes, only the range of peak currents corresponding to 0–0.1 mM PYO was included for slope computations. This range aligns with previously reported linear quantification intervals for PYO, which have been established between 1 and 100 μ M in clinically relevant matrices [40].

Referring to Table 6.12, the calculated sensitivities for modified electrodes are beyond 10-fold greater than those of control electrodes, meaning that modified electrode is able to detect smaller changes in PYO concentration more efficiently.

Statistical analysis was performed to compare the sensitivity of control and modified electrodes. As summarized in Table 6.12, modified electrodes exhibited a higher mean sensitivity (-1.97×10^{-4} A/mM) compared to unmodified controls (-7.18×10^{-6} A/mM). The difference in sensitivities was statistically significant ($p = 0.004$), indicating that PEDOT:PSS (1:30) modification significantly improves detection sensitivity in this system.

The progressive increase in peak current with concentration, followed by nonlinear saturation behavior at higher levels, observed in PEDOT:PSS (1:30)-modified electrodes, is characteristic of surface-confined electron transfer processes. Despite this nonlinearity at elevated concentrations, all electrodes except for one that exhibited prominent dual-peak behavior showed strong linearity in the low-concentration range, with R^2 values approaching or exceeding 0.90. This trend is consistent with the Langmuir adsorption model, which was subsequently applied to fit the full response curve and extract key analytical performance parameters.

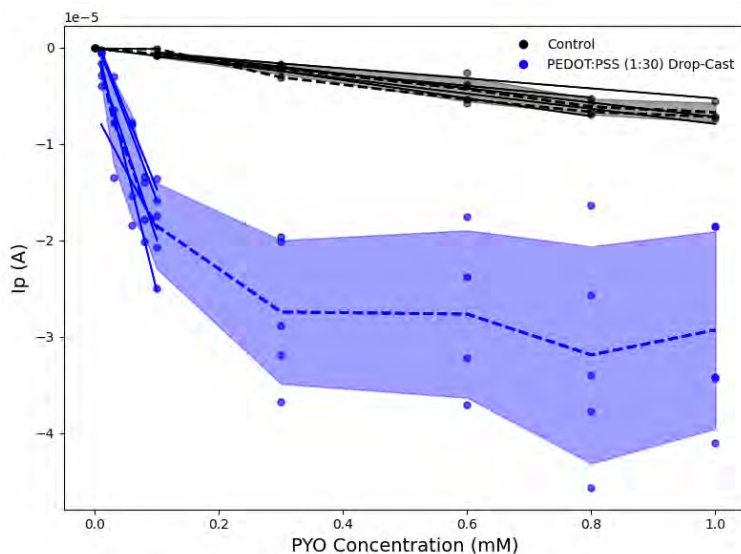


Figure 6.35 SWV peak current intensity (I_p) as a function of PYO concentration for control (black) and PEDOT:PSS-modified (blue) paper electrodes. Shaded regions represent the standard deviation across replicates. Control electrodes display a linear response, while modified electrodes exhibit a biphasic trend with an initial step increase followed by a plateau at higher concentrations, suggesting saturation effects.

A previously reported paper sensor achieved a sensitivity of $4.30 \mu\text{A}/\mu\text{M}$ [32], equivalent to $4.30 \times 10^{-3} \text{ A}/\text{mM}$. In comparison, our unmodified control electrodes showed a mean sensitivity of $7.18 \times 10^{-6} \text{ A}/\text{mM}$, which is approximately 0.17% of the reported value, demonstrating that without conductive enhancement, the carbonized paper platform is substantially less responsive. PEDOT:PSS (1:30)-modified electrodes improved this sensitivity significantly, reaching $1.97 \times 10^{-4} \text{ A}/\text{mM}$, remaining about 21.8 times less sensitive than the benchmark. These differences may be attributed to variations in electrode composition, surface area and redox coupling efficiency.

Table 6.12 Linear regression results for individual control and modified electrodes using SWV measurements. Sensitivity is reported as the slope of the Ip vs. concentration curve (A/mM).

Sensitivity (A/mM)	R^2
<i>Control Electrodes</i>	
-7.84×10^{-6}	0.9746
-5.19×10^{-6}	0.9653
-7.45×10^{-6}	0.9939
-6.04×10^{-6}	0.9896
-9.39×10^{-6}	0.9720
Mean $\pm$ SD	$(-7.18 \pm 1.53) \times 10^{-6}$
<i>Modified Electrodes</i>	
-1.69×10^{-4}	1.00
-2.64×10^{-4}	0.998
-9.56×10^{-5}	0.29 [†]
-1.60×10^{-4}	0.96
-1.95×10^{-4}	0.89
Mean $\pm$ SD	$(-1.97 \pm 0.47) \times 10^{-4}$

[†] This electrode was excluded from the calculation of the mean and standard deviation due to its poor R^2 value caused by its prominent dual-peak behavior as shown in Figure 6.33(b).

6.6 Langmuir for LOD Calculation

Electrodes included in the analysis required at least four valid data points across the concentration range. Within the PEDOT:PSS-modified group, five devices met the criterion, whereas three control electrodes were retained. The limit of detection (LOD) for each electrode was calculated from the slope of the Langmuir fit at low concentrations ($I_{\max} \cdot K$), using baseline noise (σ) obtained from control and pedot-modified electrodes in PBS solution according to the 3σ method:

$$\text{LOD} = \frac{3\sigma}{I_{\max} \cdot K}$$

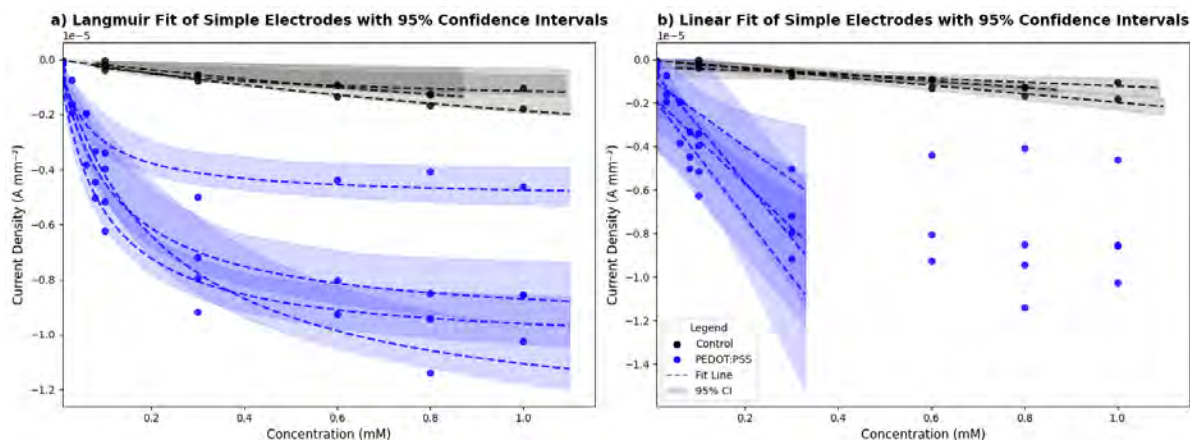


Figure 6.36 SWV calibration curves for PYO detection using simple PEDOT:PSS 1:30-modified (blue) and uncoated control (black) LIG paper electrodes. Langmuir adsorption isotherms (a) and linear fits (b) are shown for each individual electrode, fitted across the concentration range. Linear fits for PEDOT-modified electrodes were truncated to correspond the linear range, before the start of saturation effects. Confidence intervals (CI) of 95% are represented as shaded bands.

Calibration Performance

The limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for both the Langmuir and ordinary least-squares (linear) models (Table 6.13). For the Langmuir model the PEDOT:PSS electrodes achieved an average LOD of $0.2 \pm 0.1 \mu\text{M}$, representing a ~ 16 -fold enhancement relative to the unmodified controls ($3.3 \pm 1.0 \mu\text{M}$; Welch's t -test, $p = 0.0310$). This statistically significant improvement is attributed to the higher electroactive surface area provided by the PEDOT:PSS coating. The improvement is accompanied by a high goodness-of-fit ($R^2 = 0.935 \pm 0.041$) and a correspondingly low LOQ of $0.7 \pm 0.2 \mu\text{M}$.

Although the linear model yielded slightly higher LODs overall, the same trend was observed: PEDOT:PSS LOD $0.8 \pm 0.2 \mu\text{M}$ versus control $6.8 \pm 2.8 \mu\text{M}$ (difference not statistically significant, $p = 0.0676$). The slight decrease in R^2 for the linear model (0.840 ± 0.084) reflects the reduced concentration range used in the regression but remains acceptable for quantitative work.

Table 6.13 Summary of calibration statistics for control and PEDOT:PSS electrodes. Values are mean $\pm$ standard deviation; n denotes the number of electrodes.

Fit model	Group (n)	R^2	LOD (μM)	LOQ (μM)
Langmuir	Control (3)	0.949 ± 0.066	3.3 ± 1.0	10.8 ± 3.2
	PEDOT:PSS (4)	0.935 ± 0.041	0.2 ± 0.1	0.7 ± 0.2
Linear	Control (3)	0.933 ± 0.074	6.8 ± 2.8	22.5 ± 9.5
	PEDOT:PSS (4)	0.840 ± 0.084	0.8 ± 0.2	2.7 ± 0.8

To support our estimated limit of detection (LOD), representative SWV data are shown below for 4 PEDOT:PSS-modified electrodes (2x2mm) exposed to a $0.1 \mu\text{M}$ PYO solution.

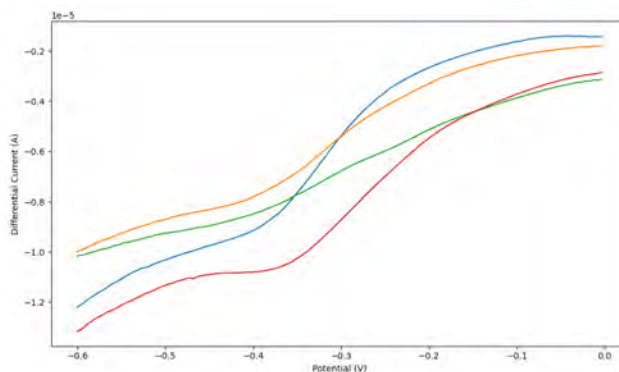


Figure 6.37 Square wave voltammogram demonstrating the detection of $0.1 \mu\text{M}$ PYO using a PEDOT:PSS-modified simple paper electrode.

Table 6.14 Measured SWV differential currents (I_p) for $0.1 \mu\text{M}$ PYO using simple PEDOT:PSS treated 2x2mm sensors.

Measurement	I_p (A)
1	-3.67×10^{-6}
2	-3.17×10^{-6}
3	-1.21×10^{-6}
4	-6.99×10^{-6}
Mean	-3.76×10^{-6}
Standard Deviation	2.40×10^{-6}

The simple-form sensor is capable of producing a measurable signal at concentrations approaching the submicromolar range.

6.6.1 Evaluation of Encapsulated Electrode Designs

To further assess the detection performance under more realistic sensing conditions, electrodes incorporating an encapsulated design were evaluated. Both PEDOT:PSS-modified and control encapsulated electrodes were analyzed using the same SWV protocol and Langmuir fitting approach.

As shown in Figure 6.38, PEDOT:PSS-modified encapsulated electrodes continued to show submicromolar LODs. Control encapsulated electrodes, in contrast, demonstrated broader variability and generally poorer detection performance.

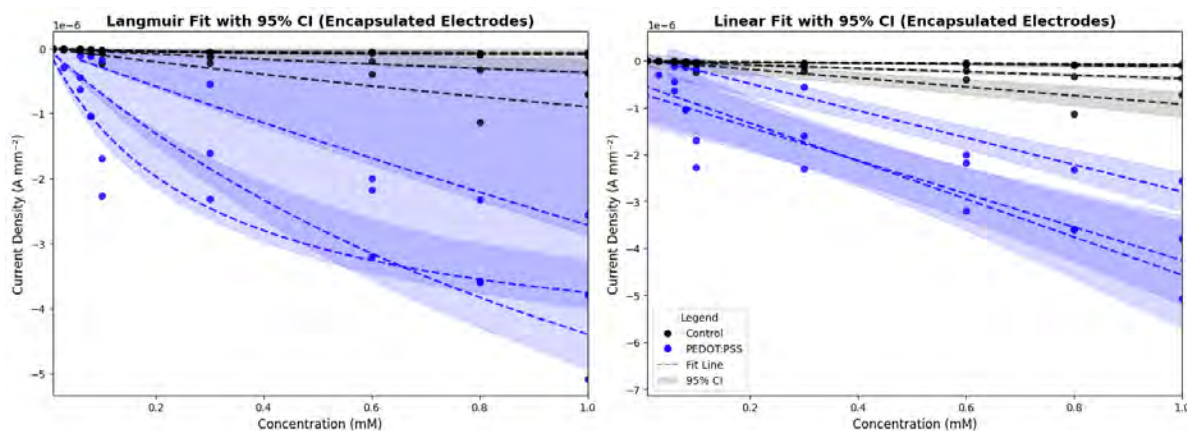


Figure 6.38 Langmuir adsorption isotherm and linear fits of PYO peak current densities as a function of concentration for encapsulated electrodes. PEDOT:PSS-modified electrodes are shown in blue, and unmodified controls in black.

Calibration Performance of Encapsulated Electrodes

The encapsulation strategy markedly improved the analytical figures of merit when the electrodes were further coated with PEDOT:PSS (Table 6.15). For the Langmuir model the PEDOT:PSS encapsulated electrodes achieved an average limit of detection of $3.1 \pm 2.5 \mu\text{M}$, an ≈ 6 -fold reduction relative to the encapsulated controls ($19.4 \pm 8.2 \mu\text{M}$; Welch's t -test, $p = 0.0084$). A corresponding decrease in the limit of quantitation ($10.2 \pm 8.2 \mu\text{M}$ vs. $64.7 \pm 27.2 \mu\text{M}$) and a comparable goodness-of-fit ($R^2 = 0.913 \pm 0.097$) confirm that the PEDOT:PSS layer enhances sensitivity.

A similar, statistically significant trend is observed for the ordinary least-squares fit: PEDOT:PSS electrodes reached an average LOD of $5.0 \pm 0.9 \mu\text{M}$ compared with $66.3 \pm 48.2 \mu\text{M}$ for the controls ($p = 0.0469$).

Although the linear regression covers a narrower concentration window and exhibits slightly

lower R^2 values overall (0.900 ± 0.059 for PEDOT:PSS; 0.861 ± 0.080 for control), the fit quality remains adequate for quantitation in the clinically relevant range.

The slightly wider confidence intervals observed for the PEDOT:PSS devices indicate that while the conductive polymer network mitigates some diffusion resistance, heterogeneities in film thickness, morphology, and drying dynamics on the porous paper still contribute to electrode-to-electrode variability.

Taken together, these data show that encapsulation by itself yields reliable electrodes, with PEDOT:PSS functionalization enabling sensitivity gains offered by the confined geometry: the film provides additional catalytic sites while the encapsulation limits convective dilution, resulting in a one-order-of-magnitude decrease in LOD compared with encapsulated controls.

In summary The results in Table 6.16 demonstrate that PEDOT:PSS-modified electrodes consistently outperformed unmodified controls in both simple and encapsulated formats. These results highlight the importance of conductive polymer integration and validate that our encapsulation design, when paired with PEDOT:PSS, preserves high electroanalytical sensitivity with minimal performance loss due to encapsulation.

Table 6.15 Calibration statistics for encapsulated electrodes (mean $\pm$ SD; n = number of electrodes).

Fit model	Group (n)	R^2	LOD (μM)	LOQ (μM)
Langmuir	Control (5)	0.912 ± 0.068	19.4 ± 8.2	64.7 ± 27.2
	PEDOT:PSS (3)	0.913 ± 0.097	3.1 ± 2.5	10.2 ± 8.2
Linear	Control (5)	0.861 ± 0.080	66.3 ± 48.2	220.9 ± 160.8
	PEDOT:PSS (3)	0.900 ± 0.059	5.0 ± 0.9	16.8 ± 2.9

Table 6.16 Langmuir fit parameters and calculated limits of detection (LOD) for both simple and encapsulated paper-based electrodes with and without PEDOT:PSS modification. Peak currents $I_{\max}$ have been normalized to current density because the simple (2×2 mm) and encapsulated ($\emptyset$ 4 mm) formats differ in area, $J_{\max} = I_{\max}/A$. Values are individual-electrode results followed by means $\pm$ standard deviations.

Format	$J_{\max}$ (A mm ⁻²)	K (1/mM)	R^2	LOD (μ M)	Group
Simple	-5.66×10^{-6}	0.493	0.984	3.1	Control
Simple	-1.67×10^{-6}	2.223	0.873	2.4	Control
Simple	-5.54×10^{-6}	0.372	0.989	4.3	Control
	$-4.29e-6 \pm 2.06e-6$	1.03 ± 0.93	0.949 ± 0.066	3.3 ± 1.0	<i>Control</i>
Simple	-1.35×10^{-5}	4.495	0.981	0.3	PEDOT:PSS
Simple	-1.05×10^{-5}	11.171	0.953	0.1	PEDOT:PSS
Simple	-5.08×10^{-6}	14.442	0.887	0.2	PEDOT:PSS
Simple	-9.73×10^{-6}	8.525	0.918	0.2	PEDOT:PSS
	$-9.00e-6 \pm 3.03e-6$	9.66 ± 4.29	0.935 ± 0.041	0.2 ± 0.1	<i>PEDOT:PSS</i>
Encapsulated	-1.89×10^{-6}	0.245	0.976	24.5	Control
Encapsulated	-1.18×10^{-7}	3.913	0.945	19.0	Control
Encapsulated	-8.21×10^{-8}	5.436	0.957	19.7	Control
Encapsulated	-1.32×10^{-7}	2.142	0.866	31.2	Control
Encapsulated	-5.38×10^{-6}	0.201	0.817	8.1	Control
	$-2.57e-6 \pm 3.29e-6$	2.19 ± 2.12	0.912 ± 0.068	19.4 ± 8.2	<i>Control</i>
Encapsulated	-4.86×10^{-6}	3.405	0.974	1.0	PEDOT:PSS
Encapsulated	-1.07×10^{-5}	0.692	0.802	2.3	PEDOT:PSS
Encapsulated	-3.26×10^{-5}	0.091	0.964	5.8	PEDOT:PSS
	$-1.61e-5 \pm 1.40e-5$	1.40 ± 1.67	0.913 ± 0.097	3.1 ± 2.5	<i>PEDOT:PSS</i>

Table 6.17 Ordinary least-squares (linear) calibration parameters for simple and encapsulated electrodes. The slope has been normalized to current density ($J' = \frac{dJ}{dC}$); LODs were calculated as $3\sigma_{\text{blank}}/|\text{slope}|$. Values are individual electrodes followed by group means $\pm$ SD.

Format	J' (A mm ⁻² mM ⁻¹)	R^2	LOD (μ M)	Group
Simple	-1.943×10^{-6}	0.961	4.5	Control
Simple	-8.826×10^{-7}	0.850	9.9	Control
Simple	-1.511×10^{-6}	0.990	5.8	Control
	$-1.45e-6 \pm 5.3e-7$	0.933 ± 0.074	6.8 ± 2.8	Control
Simple	-2.576×10^{-5}	0.960	0.7	PEDOT:PSS
Simple	-2.768×10^{-5}	0.830	0.6	PEDOT:PSS
Simple	-1.536×10^{-5}	0.769	1.1	PEDOT:PSS
Simple	-2.014×10^{-5}	0.800	0.9	PEDOT:PSS
	$-2.22e-5 \pm 5.6e-6$	0.840 ± 0.084	0.8 ± 0.2	PEDOT:PSS
Encapsulated	-3.582×10^{-7}	0.983	24.5	Control
Encapsulated	-9.681×10^{-8}	0.898	90.7	Control
Encapsulated	-6.992×10^{-8}	0.780	125.6	Control
Encapsulated	-1.082×10^{-7}	0.830	81.1	Control
Encapsulated	-9.306×10^{-7}	0.814	9.4	Control
	$-3.13e-7 \pm 3.6e-7$	0.861 ± 0.080	66.3 ± 48.2	Control
Encapsulated	-3.546×10^{-6}	0.880	4.9	PEDOT:PSS
Encapsulated	-4.051×10^{-6}	0.854	4.3	PEDOT:PSS
Encapsulated	-2.879×10^{-6}	0.967	6.0	PEDOT:PSS
	$-3.49e-6 \pm 5.9e-7$	0.900 ± 0.059	5.0 ± 0.9	PEDOT:PSS

Table 6.18 Reported limits of detection (LOD) for PYO. All LODs are expressed in μ M for direct comparison.

Method / platform	LOD (μ M)	Notes (matrix, range)	Ref.
Electrochemical methods			
Carbon-fibre microelectrode (SWV)	0.030	30 nM detection limit	[40]
B-doped diamond FIA / SWV	0.09	Flow-injection, square-wave voltammetry	[69]
Printed paper electrochemical sensor (SWV)	0.095	95 nM; bacterial cultures	[31]
AuNPs/rGO glassy-carbon (SWV)	0.27	PBS (best case); 1.34 μ M saliva; 2.3 μ M urine	[45]
PANI/AuNP-ITO (DPV)	0.50	500 nM detection limit	[42]
3-D printed CNT electrodes (SWV)	0.5	0.5 μ M (GC); 2.5 μ M (MWCNT)	[100]
Gold screen-printed electrode (SWV)	2.0	LOD rises to 6 μ M in complex matrices	[31]
Spectroscopic / biosensing / immunochemical			
SERS hand-held probe	23.1	Linear 15–100 μ M; <i>P. aeruginosa</i> culture	[25]
BrIR whole-cell GFP biosensor	1.3	LB medium; $S/N = 3$	[28]
ELISA microplate	7×10^{-5}	0.07 nM antibody-HRP assay	[7]
Paper competitive immunoassay	4.7×10^{-3}	Dynamic range 0.47–47.6 μ M	[30]
Chromatographic			
HPLC-UV quantification *	3.1	LOQ reported (13 ng)	[21]

* Converted with $m = 13$ ng and $M_w = 210$ g mol⁻¹ in a 20 μ L HPLC loop: [PYO] $\approx$ 3.1 μ M.

For comparison, Alatraktchi *et al.* [101] reported a detection limit of 95 nM using screen-printed paper sensors, with detectable currents observed down to 10 nM. However, their working-electrode (WE) diameter was 4 mm, whereas in this study WE were 2×2 mm for the simple electrodes and 4 mm in diameter for the encapsulated design. Although our lowest limit of detection (LOD) of 200 nM (Table 6.16) remains slightly higher, this result demonstrates that carbonized paper electrodes, when properly modified with PEDOT:PSS, can approach the analytical performance of commercial printed platforms while offering a simpler, low-cost fabrication method without requiring advanced materials or surface treatments.

Among electrochemical methods from Table 6.18, state-of-the-art microelectrodes and engineered surfaces report sub- μ M LODs (e.g., carbon-fibre microelectrode 0.030 μ M [40], boron-doped diamond 0.09 μ M [69], and printed paper sensors 0.095 μ M [31]). Our simple PEDOT:PSS-modified paper electrodes therefore fall within similar orders of magnitude as these electrochemical platforms, and outperform more conventional gold SPEs measured under clean conditions (2.0 μ M [31]). Showing promise that conductive-polymer modification of a low-cost carbon paper substrate can achieve literature-level performance without precious metals or nanofabrication.

For encapsulated electrodes, PEDOT:PSS modification reduced the LOD from $19.4 \pm 8.2 \mu$ M (control) to $3.1 \pm 2.5 \mu$ M (Langmuir model; $p = 0.0084$), and from $66.3 \pm 48.2 \mu$ M to $5.0 \pm 0.9 \mu$ M (linear model; $p = 0.0469$), with comparable R^2 across groups. These encapsulated LODs are higher than the best microelectrode and engineered-surface benchmarks (0.03–0.50 μ M) but are competitive with gold SPEs tested in complex mixtures (2–6 μ M [31]) and markedly lower than label-free SERS (23.1 μ M [25]). They are also similar to the HPLC-UV value converted from mass to molarity (LOQ $\sim 3.1 \mu$ M [21]).

Wider confidence intervals (CIs) were also observed among PEDOT:PSS-modified encapsulated electrodes relative to controls. This suggests that while the conductive polymer network mitigates some diffusion resistance, heterogeneities in film thickness, morphology, and drying dynamics on the porous substrate still contribute to electrode-to-electrode variability. Increased double-layer capacitance and residual wetting gradients under the PDMS-coated layer may further broaden the noise distribution, especially around the onset of surface coverage and the approach to saturation.

Literature reports achieving the lowest LODs often rely on either extremely short diffusion paths, as in carbon-fibre microelectrodes, or on highly engineered inorganic interfaces such as boron-doped diamond or AuNP/rGO composites. These architectures minimize variability in mass transport and electron transfer, offering a level of uniformity that polymer films

deposited on porous paper substrates cannot yet match. Variability becomes especially impactful when aiming to detect analyte levels at the low micromolar concentrations found in patient samples.

The concentration range used in this study, particularly around 1–100 μM , is clinically relevant. PYO concentrations within this range have been reported in the sputum and biofilms of patients with chronic PA infections, including those with cystic fibrosis and ventilator-associated pneumonia reported in the range of 1–130 μM , with a median value of 8.1 μM [100], and ear infections have been reported in millimolar concentrations [102].

Non-electrochemical approaches span a very broad performance envelope; from SERS at 23.1 μM [25] to immunochemical assays in the pM–nM regime (7×10^{-5} μM [7]; 4.7×10^{-3} μM [30]). These methods typically require labels, multiple reagents, or optical instrumentation, whereas our electrochemical format remains single-use, reagent-free, and readily integrable into POC hardware.

Several factors limited the fidelity of Langmuir-based LOD fitting across all electrodes. First, the 4 mm diameter encapsulated electrodes did not consistently reach saturation within the tested concentration range (0–1 mM), resulting in poorly defined plateaus and suboptimal curve fits. Future experiments should extend the calibration range beyond 1 mM PYO (*e.g.*, to 2–3 mM) to more accurately capture the full adsorption profile.

Second, the appearance of a secondary redox peak around -0.1 V vs. Ag|AgCl in several electrodes, particularly under higher concentrations, likely contributed to peak distortion, splitting, and broadening. This altered the apparent height of the main redox peak, and reduced the reliability of peak height-based quantification. Because these distortions prevented robust automated peak picking, peak maxima were selected manually, which introduces subjectivity and limits throughput. As mitigation, future work could (i) narrow or shift the potential window to avoid partial overlap, (ii) tune SWV parameters (step, amplitude, frequency) to maximize peak separation, and/or (iii) use charge (peak area) with baseline subtraction as a more robust response metric when broadening is significant.

Despite these limitations, the marked improvement in LOD for PEDOT:PSS electrodes, even under encapsulated conditions, highlights their potential for low-cost, deployable sensing.

The encapsulated PEDOT:PSS LODs (Langmuir: 3.1 ± 2.5 μM ; linear: 5.0 ± 0.9 μM) are higher than the other electrochemical benchmarks that use microelectrodes or engineered surfaces, but they remain within or near clinically relevant PYO levels and are comparable to gold screen-printed electrodes tested in complex matrices (Table 6.18).

By contrast, the simple (non-encapsulated) PEDOT:PSS paper electrodes reach sub- μM per-

formance and cluster with several electrochemical platforms found in literature, suggesting that further control over encapsulation-induced transport (e.g., side-flap geometry, PDMS-coated layer permeability) and PEDOT:PSS film uniformity could close the remaining sensitivity gap while retaining the advantages of simplicity, cost, and disposability.

6.7 Summary of Suggestions to Increase Sensitivity and Improve LOD

To obtain lower detection limits and stronger signals for PYO with PEDOT:PSS modified carbonized paper electrodes, the strategies focus on enhancing charge transfer, improving mass transport, and suppressing background currents.

Electrode Architecture and Materials

- **Nanotexturing of the carbonized surface.** Light overburn or controlled laser rescans increase the electroactive area and density of edge-plane sites, which enhances electron transfer and elevates the current peak [103].
- **PEDOT:PSS refinement.** Use ultrathin PEDOT:PSS films or graded coatings to preserve conductivity while limiting capacitive loading. High-boiling cosolvents can promote phase separation that favors percolation pathways and reduces background capacitance.
- **Hybrid conductive modifiers.** Small fractions of graphene nanoplatelets or carbon nanotubes blended with PEDOT:PSS improve electron transfer while maintaining thin coverage, as reported in previous work [4, 104]. Similar PEDOT:PSS–graphene oxide composites have shown marked sensitivity gains for small-molecule detection [105].
- **Metal nanoparticle seeding.** Sparse decoration with AuNPs provides catalytic sites for phenazine redox and enhances adsorption [106].

Surface Chemistry for Preconcentration and Selectivity

- **Permselective overcoats.** Nanometric Nafion or chitosan films can enrich PYO at the interface while excluding interferents, leading to stronger peak currents [107].
- **Open-circuit accumulation.** Holding the electrode in solution at open circuit for 30–120 s allows PYO adsorption on the carbonized surface, increasing initial coverage and amplifying the first SWV response [108].

- **pH and ionic strength tuning.** Adjusting buffer conditions to favor PYO redox while suppressing competing adsorption can sharpen SWV peaks. Higher ionic strength reduces double-layer thickness and enhances current response.

Mass Transport and Confinement

- **Thin-layer operation.** Encapsulation with FC-40 and PDMS confines microdroplets and shortens diffusion paths. By optimizing sample confinement and quasi thin-layer conditions, the current peak depends less on $\sqrt{SR}$, peaks sharpen, and ΔE shifts toward reversible values.
- **Microscale geometry.** Narrowing the working zone or adding microchannels refreshes analyte near the electrode, with capillary flow enhancing flux without external pumps.

Electrochemical Protocol Optimization

- **SWV parameter sweep.** Along with frequency, amplitude, and step potential should be tuned systematically.
- **Background suppression.** Acquiring a blank in the same encapsulated configuration allows differential SWV or harmonic analysis to reduce capacitive currents from PEDOT:PSS, improving the faradaic-to-background ratio.
- **Oxygen management.** Removing dissolved oxygen or maintaining a controlled level minimizes overlapping reduction features and stabilizes the baseline.

Antifouling and Matrix Management

- **Low-fouling interlayers.** Brief adsorption of BSA or casein can block nonspecific binding and preserve redox features in future experiments using complex media [109].
- **Surfactant-assisted release.** Trace surfactant below the critical micelle concentration can increase PYO mobility within paper pores, improving signal replenishment between SWV pulses [110,111].

Signal Processing and Calibration

- **Replicate averaging with drift correction.** Applying baseline correction across short replicates and averaging reduces noise without biasing the current peak.

- **Standard addition.** Performing standard additions directly in the matrix corrects for adsorption or viscosity effects, yielding more reliable slopes.

CHAPTER 7 CONCLUSION AND FUTURE PROSPECTS

7.1 Summary of Works

This research focused on the development of low-cost, paper-based electrochemical sensors for the detection of PYO, a redox-active biomarker secreted by *Pseudomonas aeruginosa*. The core objective was to fabricate functional, conductive paper electrodes via CO₂ laser-induced carbonization under ambient conditions and to improve their analytical sensitivity through conductive polymer coatings and encapsulation strategies.

Inspired by the LIG approach, cellulose filter paper was carbonized using optimized laser parameters to yield conductive, porous electrodes. Their electrochemical behavior was first validated using a standard redox probe (RuHex), and benchmarked against commercial glassy carbon electrodes. Subsequent CV in PYO confirmed the successful redox activity of the carbonized paper surface.

To enhance signal strength and stability, electrodes were modified with PEDOT:PSS ink, exploring both 1:3 and 1:30 dilution formulations. While the 1:3 ink generated higher peak currents, it also introduced greater capacitance and signal broadening, limiting its utility for quantification. The 1:30 formulation provided a more balanced tradeoff, improving peak definition and linearity, which proved advantageous for sensitive detection.

Two deposition methods, dipping and drop-casting, were evaluated. Although dipped electrodes produced stronger faradaic responses, drop-casting offered more reproducible and predictable electrochemical performance.

SWV was then employed to quantify the limit of detection for PYO across control and PEDOT:PSS-coated devices. Modified electrodes demonstrated submicromolar LODs (as low as 0.2 μ M), representing an approximate 16-fold improvement over uncoated controls. Encapsulated designs featuring a PDMS-defined sensing zone and an FC-40 barrier were developed to minimize evaporation and prolong signal stability. These devices maintained comparable LODs and exhibited markedly improved electrochemical retention over time, with enhanced peak current preservation and reduced degradation due to drying.

Finally, gravimetric water-loss assays and contact angle measurements quantitatively confirmed that FC-40 encapsulation effectively slowed evaporation.

This work demonstrated that encapsulation with FC-40 effectively extends the functional lifetime of paper-based electrochemical sensors, doubling the available assay time from 60 to 120

minutes under ambient drying conditions. Encapsulated electrodes maintained peak-to-peak separation and electrochemical reversibility far longer than unprotected counterparts, suggesting preserved semi-infinite diffusion behavior. In parallel, modification with PEDOT:PSS significantly enhanced analytical performance: the mean LOD improved from $5.69 \pm 1.66 \mu\text{M}$ in uncoated controls to $0.17 \pm 0.06 \mu\text{M}$ in modified electrodes, approaching the sensitivity of advanced printed systems reported in literature [32]. Sensitivity was also increased by a factor of 27. These results validate the combined use of conductive polymer coatings and passive FC-40 encapsulation as a promising strategy for improving both sensitivity and signal stability in carbonized paper-based sensing platforms.

7.2 Limitations

While the carbonized electrodes and PEDOT:PSS coatings demonstrated high sensitivity and versatility, several limitations must be acknowledged. First, the reproducibility of the PEDOT:PSS ink layer remains sensitive to deposition technique and environmental factors such as humidity and drying rate. Variability in ink spreading or film thickness can impact peak shape and capacitance, particularly with dipped electrodes.

Second, although encapsulation with FC-40 significantly improved signal longevity, the PDMS stamping process used to define the encapsulation zone was not fully standardized and its process remains under optimization. Inconsistencies in PDMS patterning can alter the size and shape of the encapsulation chamber, which directly impacts the extent of sample volume retained and absorbed by the paper substrate. These variations may influence diffusion dynamics and electrochemical response, introducing additional variability across encapsulated sensors.

Third, the number of replicates available for some experimental conditions (*e.g.*, encapsulated PEDOT:PSS group) was limited, which may reduce the statistical power of certain comparisons. While trends were consistent, additional replication would strengthen confidence in observed differences.

Fourth, a notable limitation in the electrochemical detection of PYO lies in the presence of multiple oxidation peaks, which may overlap with endogenous redox-active species in biological fluids. PYO is known to exhibit characteristic oxidation peaks at approximately -0.560 V , -0.311 V , and $+0.699 \text{ V}$ versus $\text{Ag}|\text{AgCl}$ reference electrodes. While the peaks at -0.560 V and $+0.699 \text{ V}$ lie outside the redox windows of most interfering compounds, the central peak at -0.311 V often overlaps with the electrochemical signatures of biological matrices such as artificial sputum medium and human saliva [31]. This overlap can compromise specificity in

complex sample environments, possibly limiting the utility of this peak for quantification. Consequently, the higher-potential oxidation window between 0.58–0.82 V, which is uniquely associated with PYO, may offer a more selective and reliable basis for detection in biosensing applications. However, reliance on a single high-potential window may also limit sensitivity in low-concentration regimes, particularly in systems with high background noise or capacitive interference.

Finally, the electrodes were tested using model analytes (RuHex and PYO) in controlled aqueous solutions. Their performance in complex biological samples, such as sputum or serum, remains to be validated.

7.3 Future Research

Future work should focus on further standardizing the PEDOT:PSS deposition process to minimize variability. Integration of automated or controlled drop-casting techniques, such as microfluidic or inkjet printing, could yield more consistent coatings and support scalable manufacturing.

Improvements in the PDMS stamping process are also essential. Custom molds or high-resolution 3D-printed stamps may improve reproducibility of the encapsulated chamber and reduce variability in sample volume and diffusion behavior. Exploring alternative superhydrophobic encapsulation materials, such as wax and titanium dioxide, or microfluidic structures may further enhance performance [112].

Beyond fabrication, expanding the electrode characterization to real biological matrices will be a key step toward clinical application. This includes evaluating fouling resistance, cross-reactivity, and response in complex fluids.

A key direction will be to test the biological compatibility of the encapsulated sensor. We aim to assess whether viable PA cells can be cultured directly within the encapsulated microenvironment. The ability to detect redox-active metabolites directly from living cells could enhance the specificity and functional relevance of our platform.

Additionally, this approach may be extended to other microorganisms that secrete redox-active compounds. Exploring different bacterial species or co-culture models could broaden the utility of our device and enable multiplexed detection of microbial activity. Collectively, these efforts will support the translation of carbonized paper sensors from proof-of-concept devices to biologically integrated diagnostic platforms.

Lastly, integration with portable electronics (e.g., smartphone-based readers) and development of multiplexed or multi-layer sensor formats could enable translation of these paper-

based platforms into reliable user-friendly diagnostic tools for POC detection of infectious disease biomarkers.

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