5-2015

Immobilization of electrochemical mediators to derivatives of a conducting polymer: toward the development of biofuel cells

Pilar Bare
University of Arkansas, Fayetteville

Immobilization of Electrochemical Mediators to Derivatives of a Conducting Polymer: Toward the Development of Biofuel Cells

An Honors Thesis submitted in partial fulfillment of the requirements of Honors Studies in Chemistry and Biochemistry

Pilar Bare

May 2015

Department of Chemistry and Biochemistry

J. William Fulbright College of Arts and Sciences

The University of Arkansas

Table of Contents

1. Abstract

The goal of this project is to develop a more efficient biofuel cell with the use of mediators and modification of electrode surfaces. This project focuses on a mediator, ferroceneaceticc acid (FcAA), which is expected to assist the transfer of electrons between the electrode surface and enzyme, resulting in a more efficient cell. This project is aimed toward the electropolymerization of monomer, coupling of mediator to monomer, and electropolymerization of mediator-monomer product. The monomer, hydroxymethyl 3,4-ethylenedioxythiophene (HMEDOT), was successfully polymerized onto the surface of a gold electrode using a solution of 0.01 M HMEDOT, 0.001 M β-CDSS, and ultrapure 18.2 MΩ*cm water from 0.0 V to 1.2 V at a scan rate of 5 mV/s. Coupling of the mediator, ferroceneacetic acid (FcAA), to the monomer, HMEDOT, using an esterification procedure was attempted. Future work is needed in order to optimize the coupling of FcAA to HMEDOT and to verify the product of the esterification. The product can then be electropolymerized onto a gold electrode using the parameters established above for HMEDOT.

2. Introduction

The long-term purpose of this project deals with biofuel cells, which have the ability to convert chemical energy to electrical energy. Specifically, the goal is to develop a more efficient biofuel cell with the use of mediators and modification of electrode surfaces. This project focuses on the characterization of a mediator in relation to electron shuttling within the galvanic cell. The mediator is expected to assist the transfer of electrons between the electrode surface and the enzyme, resulting in a more efficient cell.

Electropolymerization is a chemical reaction influenced by a potential or an electric current to produce polymers from monomers on an electrode surface. The electrodes used throughout this experiment are gold because of their chemical stability and biocompatibility.[1] Individually addressable gold microelectrodes photolithographically patterned on silicon chips were available in the laboratory for electropolymerization studies. These particular chips are advantageous because each electrode can be electropolymerized separately, under the same or different conditions. Multiple electrodes on one chip allow for replicates to be taken with relative ease. An example of the chip used is shown in Figure 1. The monomer used is HMEDOT (hydroxymethyl-3,4-ethylenedioxythiophene), rather than the widely experimentally used EDOT (3,4-ethylenedioxythiophene), because its polarity causes increased solubility under aqueous conditions.[1] The structures of EDOT and HMEDOT shown in Figure 2. This increase in solubility introduces a polymer that could potentially be more

applicable to biomolecules. Additionally, the hydroxymethyl functional group can be conjugated to an enzyme or coupled to a mediator.

Enzymes in a biofuel cell catalytically oxidize fuels and reduce oxidizing agents via redox reactions. Most enzymes cannot exchange electrons directly with a solid electrode, so a mediator is required.[2] Mediators assist in the shuttling of electrons from enzymes to the surface of the electrode at the anode, while helping to shuttle electrons from the electrode surface to the enzyme at the cathode. A mediator can diffuse to the catalytic site of the enzyme, due to its small character, and assist in the transfer of electrons. If enzymes are attached to the surfaces of an electrode, they can be concentrated there. If the mediator is also confined there, rather than freely diffusing throughout the cell, then the efficiency of the collection of electrons at the electrode from the enzyme-catalyzed reaction can be much greater. The conducting polymer HMEDOT serves as the immobilizing agent (via covalent coupling, esterification, for example) for the mediator, a means to direct the immobilization to a specific location (via electropolymerization), and an extensive matrix of conductive pathways to transfer electrons with the electrode. A schematic of the electrode, mediator, conducting polymer, and enzyme is shown in Figure 3.

A good mediator should be a small molecule that can be relatively close to the active site of the enzyme, and has an oxidation-reduction potential that is similar to the redox potential of the enzyme so that electron transfer will proceed spontaneously. The redox potential of the mediator needs to be 50 mV more positive of that of the enzyme active site at the anode, and more negative of that

for an enzyme at the cathode.[5] If the redox potential of the mediator is too similar to that of the enzyme, then the electron transfer will not be able to occur. Furthermore, if the potential of the mediator is too far from that of the enzyme active site (more than 100 mV), then the voltage of the cell will be impaired.[2] Ferrocene is the model mediator for this project, specifically ferroceneacetic acid (FcAA).[3] Ferrocene is convenient for multiple reasons: several derivatives are commercially available, it is electrochemically reversible, and it can be synthetically modified to tune its redox potential.[4] Ferroceneacetic acid (shown in Figure 4) was selected because the functional group can be used in a coupling reaction with HMEDOT. In addition, the carboxylic acid group is at least one carbon unit away, as to not interfere with the redox potential of ferrocene.

This project is aimed to obtain a more efficient enzymatic biofuel cell from the modification of the electrode surface and use of electrochemical mediators. *Specific Aim 1:* The optimization of electropolymerization conditions to obtain polymerized HMEDOT on the surface of a gold electrode. *Specific Aim 2:* The immobilization of the mediator by coupling ferroceneacetic acid (FcAA) to the monomer HMEDOT. *Specific Aim 3:* The immobilization of the mediator-monomer product to a specific location on a gold electrode via electropolymerization, using the conditions optimized in Specific Aim 1.

2.1 Figures

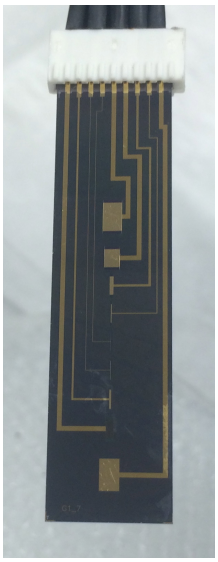

Figure 1. A representative chip used for the electropolymerization studies.

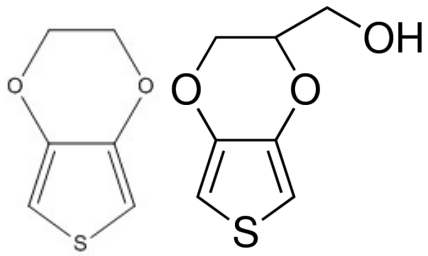

Figure 2. Schematic for EDOT (left) and HMEDOT (right).

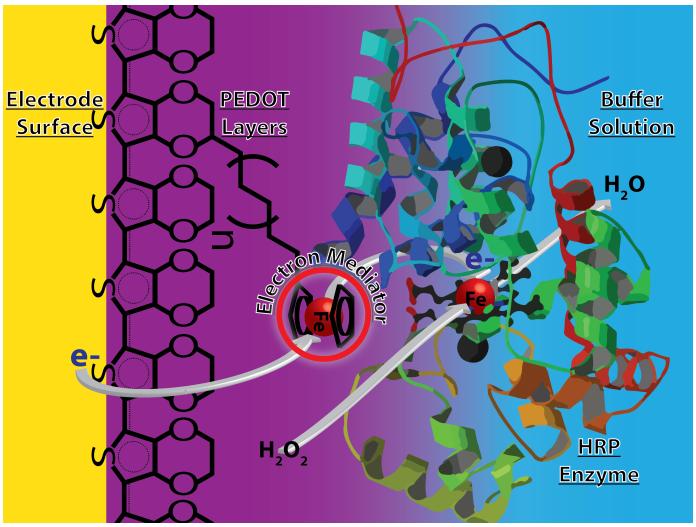

Figure 3. Interaction of an electrode, an electron mediator (a ferrocene derivative), a conducting polymer (poly 3,4-ethylenedioxythiophene, PEDOT), and an enzyme (horse radish peroxidase (HRP)). *Figure courtesy of Benjamin J. Jones.*

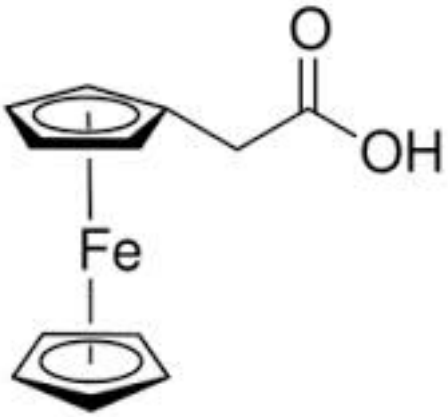

Figure 4. Schematic for ferroceneacetic acid (the mediator used in these studies).

2.2 References

1. Xiao, Yinghong, Xinyan Cui, Jessica M. Hancock, Mohamed Bouguettaya, John R. Reynolds, David C. Martin. “Electrochemical polymerization of poly(hydroxymethylated-3,4-ethylenedioxythiophene) (PEDOT-MeOH) on multichannel neural probes.” *Sensors and Actuators.* B. 99 (2004): 437-443.

2. Atanassov, Plamen; Chris Apblett; Scott Banta; Susan Brozik; Scott Calabrese Barton; Michael Cooney; Bor Yann Liaw; Sanjeev Mukerjee; Shelley D. Minteer. “Enzymatic Biofuel Cells.” *The Electrochemical Society Interface.* Summer 2007: 28-31.

3. Shim, Joonmok, Gha-Young Kim, and Seung-Hyeon Moon. "Covalent Co-immobilization of Glucose Oxidase and Ferrocenedicarboxylic Acid for an Enzymatic Biofuel Cell." *Journal of Electroanalytical Chemistry* 653.1-2 (2011): 14-20.

4. Okawa, Y., M. Nagano, S. Hirota, H. Kobayashi, T. Ohno, and M. Watanabe. "Tethered Mediator Biosensor. Mediated Electron Transfer between Redox Enzyme and Electrode via Ferrocene Anchored to Electrode Surface with Long Poly(oxyethylene) Chain." *Biosensors and Bioelectronics* 14.2 (1999): 229-35.

5. Atanassov, Plamen; Chris Apblett; Scott Banta; Susan Brozik; Scott Calabrese Barton; Michael Cooney; Bor Yann Liaw; Sanjeev Mukerjee; Shelley D. Minteer. “Enzymatic Biofuel Cells.” *The Electrochemical Society Interface.* Summer 2007: 28-31.

3. Electropolymerization and Characterization of Conducting Polymer

3.1 Introduction

Electropolymerization is a chemical reaction that turns monomers into polymers using electric current or potential (for this project, potential was applied to polymerize a monomer on the surface of an electrode). More specifically, the electrochemical oxidation of an aromatic heterocyclic molecule (for example, a thiophene group) typically leads to the formation of a conducting polymer on an electrode surface.[1] EDOT (3,4-ethylene dioxythiophene) is a widely used monomer, but the hydroxymethyl derivative of EDOT (HMEDOT) is potentially more applicable to biomolecules due to its increased solubility under aqueous conditions.[2] HMEDOT not only has improved ability to electropolymerize in water, but also has an increased level of electroactivity after polymerization in aqueous environments.[3] In addition to its polarity, the hydroxymethyl functional group of HMEDOT can also be coupled to a mediator (for example, via esterification). While PEDOT (the polymer of EDOT) has been used in several experiments, its more polar derivative HMEDOT has been selected as the polymer due to the applications of the hydroxymethyl group.

Cyclic voltammetry (CV) is performed throughout these experiments to measure the current, as the voltage is swept through a potential range. The instrument used to sweep the potential, set in the parameters, is the potentiostat. During CV, the voltage of the working electrode is cycled (biased by the reference electrode), and the current measured. CV works through a potentiostat and a three-electrode electrochemical cell.[4] The working electrode, typically

made of inert material (usually Au, Pt, or glassy carbon), is the electrode where the electrochemical reaction of interest occurs; the counter electrode, placed in a solution having ionic conductivity with the working electrode, is also characteristically made of inert material (usually Pt or graphite) and completes the electrochemical circuit (passes current to and from the working electrode).[5] The reference electrode simply defines the reference potential, without actually passing current. The counter electrode used throughout these experiments is a platinum flag, and the reference electrode is Ag/AgCl (in saturated KCl). The electrochemical solution contains the necessary ions for the redox reaction to proceed. The shape of a cyclic voltammogram helps identify specific electrochemical properties of the redox processes in an electrochemical cell.[5]

3.2 Experiment

3.2a Materials

Hydroxymethyl-3,4-ethylenedioxythiophene (HMEDOT), and β-cyclodextrin sulfated sodium salt (β-CDSS), obtained from Sigma US, were used in these experiments. Potentiostat models 650A and 760B from CH Instruments in Austin, TX were used to perform the electrochemical studies. For the platinum electrode experiments, a commercially available platinum macrodisk electrode from CHI was used as the working electrode. For the gold electrode experiments, the chips had gold working electrodes (0.02 cm^2, 0.04 cm^2, and 0.06 cm^2) insulated with benzocylcobutene (BCB). These gold-electrode chips were plasma cleaned and stored in ultrapure 18.2 MΩ*cm water within 72 h before use. A

platinum flag counter electrode was used along with a Ag/AgCl (in saturated KCl) reference electrode.

3.2b Electropolymerization of EDOT and HMEDOT on a Platinum Electrode

Electropolymerization was first tested on a platinum macrodisk electrode, with a platinum flag counter electrode and a Ag/AgCl (in saturated KCl) reference electrode. The Pt electrode was first polished before use. To polish the electrode, a standard polishing technique was used with 1 μm diamond and 0.5 μm alumina polishing solutions, sonicating in between solutions with ethanol and water. After polishing, electropolymerization of the monomer was completed on the platinum electrode. The polymerization procedure was done for both the EDOT monomer and the HMEDOT monomer.

The electropolymerization of EDOT was first tested to ensure that the polymerization procedure and conditions were possible and repeatable. A solution of 0.01 M PBS, 0.001 M β-CDSS, 0.01 M EDOT was used for the polymerization of EDOT. The solution was sonicated for 1 hour. The Pt electrode, Pt flag counter electrode, and Ag/AgCl (in saturated KCl) reference electrode were placed in the solution and connected to the potentiostat via alligator clips. The parameters were set at a scan rate of 0.005 V/s from -0.455 V to 1.25 V. The electrode was then characterized in 0.1 M KCl from 0.0 V to 0.4 V at a scan rate of 0.05 V/s.

For the electropolymerization of HMEDOT, a solution of 0.1 M HMEDOT, 0.001 M β-CDSS, and 0.01 M PBS (phosphate buffered saline) was used. The

HMEDOT solution was sonicated for one hour. The working, counter, and reference electrode were then placed in the solution and connected to the potentiostat. Parameters were set at a scan rate of 0.005 V/s from -0.455 V to 1.25 V. After polymerization, the electrode was characterized in 0.1 M KCl from 0.0 V 0.4 V at a scan rate of 0.05 V/s.

Because of the relative inefficiency, the one platinum macrodisk electrode was replaced with a multiple-electrode gold chip for further experimentation.

3.2c Electropolymeization of HMEDOT on a Gold Electrode

Gold electrodes are used due to their chemical stability and biocompatibility.[2] Specifically, individually addressable gold microelectrodes patterned on a chip photolithography patterned on a Si wafer with a top insulating SiO_2 layer, which are available in the laboratory, are used because each electrode can be electropolymerized separately. This permits a more efficient, repeatable process by allowing electropolymerization to take place under the same or different conditions on one chip, rather than multiple chips.

A multi-electrode gold chip was used to electrodeposit HMEDOT on the surface of each electrode. Before the chip could be used for experiments, it had to be plasma cleaned to remove any organic impurities on its surface. Such organic impurities on the electrode surface could affect the ability of the polymer to stick. During the plasma cleaning process, the pressure is kept very low (around 0.001 atm), and oxygen is slowly entered into the system – oxygen plasma forms through the ionization of the low-pressure oxygen. A chemical

reaction occurs to clean off the surface of the electrode, which involves breaking organic bonds, and the subsequent reaction of oxygen species in the plasma to form compounds that are evaporated from the chamber during the cleaning process, resulting in a clean electrode.

Optimization of Polymerization Conditions for HMEDOT

For optimization of polymerization conditions on a gold electrode, four different solutions were used to electropolymerize the monomer on separate electrodes on the same chip. Solution 1 contained 0.01 M PBS, 0.01 M HMEDOT, and 0.001 M β-CDSS; solution 2 contained 0.01 M PBS, 0.01 M HMEDOT, and 0.01 M β-CDSS; solution 3 contained 0.01 M HMEDOT, 0.001 M β-CDSS and ultrapure 18.2 MΩ*cm water; solution 4 contained 0.01 M HMEDOT, 0.01 M β-CDSS, and ultrapure 18.2 MΩ*cm water. These solutions are summarized in Table 1. After sonication of the polymerization solution for one hour, electropolymerization took place under the following parameters: start potential at 0.0 V, end potential at 1.12 V, scan rate at 0.005 V/s, sensitivity at 1.0×10^{-5} A/V.

Using the two solutions that produced dark films (a visual check of polymerization), each electrode on another separate chip was then polymerized to evaluate reproducibility.

The scan rate was then optimized for polymerization. To test these scan rates, the scan rate parameter was simply changed for each subsequent experiment, with the other parameters (start potential, end potential, sensitivity)

held constant throughout. Scan rates of 0.005 V/s, 0.05 V/s, and 0.1 V/s were tested. Different start and end potentials were also tested, with the start varying from -0.5 to 0, and the end varying from 1.0 to 1.2.

The electrode, following polymerization, was characterized in 0.1 M KCl at a scan rate of 0.1 V/s, start potential of 0.0 V, end potential of 1.2 V, and a sensitivity of 1×10^{-5} A/V. The charging current for each electrode before and after polymerization were then overlaid to get a quantitative indication of how much the capacitance has increased as a result of the deposited film.

3.3 Results & Discussion

3.3a Electropolymerization on Platinum Electrode

The electropolymerization of EDOT was first tested to ensure that the polymerization procedure and conditions were possible and repeatable. EDOT was polymerized on the Pt electrode, forming a dark blue film covering the electrode surface (Figure 3). The charging current noticeably increased, further showing that PEDOT (polymer of EDOT) was actually formed on the surface of the electrode (Figure 2). The oxidation of the thiophene ring occurred around 1.1 V. A representative example of the polymerization of EDOT on Pt can be seen in Figure 1.

The polymerization procedure used for EDOT was then applied to HMEDOT. The polymerization of HMEDOT on a platinum electrode to produce HMPEDOT (polymer of HMEDOT) resulted in a dark film of polymer and a slight

increase in charging current, but was relatively inefficient and inconsistent. A representative polymerization of HMEDOT on Pt is shown in Figure 4, and the characterization of the Pt electrode before and after polymerization is shown in Figure 5. A better option would be a chip containing multiple electrodes so replicates can be taken with ease. Luckily, chips (each with multiple gold electrodes) were readily available in the laboratory; as a result, further experimentation occurred using the more efficient multi-electrode gold chip.

3.3b Electropolymerization on Gold Electrode

The polymerization conditions were performed in replicates (multiple electrodes on six separate chips), each yielding similar visual results, with a dark blue film appearing on the surface of the electrode (Figures 9 and 10). Different scan rates were tested for optimization of the scan rate parameters, and it was found that the slowest scan rate yielded the most consistent visual results, with a dark blue film on the surface of the electrode. The overlay of the CVs before and after polymerization shows a clear increase in charging current, which is expected for a polymer deposited on the surface of the electrode because of the increase in surface area. The capacitance also increased approximately 100 times after polymerization (shown in Table 2), expected with an electrodeposited conducting polymer on the surface. Both the current and capacitance should theoretically increase when adding a conducting polymer to the electrode surface, and this was found to be experimentally true for the polymer of HMEDOT. The smaller electrodes (0.02 cm^2) were deemed to be unfit for

consistent polymerizations due to the large standard deviation of capacitance values (shown in Table 2); as a result, such electrodes were avoided in subsequent experiments.

In the electropolymerization solution, HMEDOT was used as the monomer, and β-CDSS was used as the solubilizer, while also serving as the electrolyte (from the sulfated sodium) in the absence of PBS. It was found that solutions 3 and 4 produced consistently dark films, while solutions 1 and 2 did not. Thus, the solutions with water rather than PBS were used for further experimentation. Those two solutions were polymerized on individual electrodes in replicate on separate chips. A dark film covered the surface on both, and both had an oxidation of the thiophene ring at about 0.9 V. The polymerization of solution 3 (0.01 M HMEDOT, 0.001 M β-CDSS and ultrapure 18.2 MΩ water) is shown in Figure 6, and the polymerization of solution 4 (0.01 M HMEDOT, 0.01 M β-CDSS and ultrapure 18.2 MΩ water) is shown in Figure 7. The polymerization results obtained from these two solutions were quite similar, both having dark films and oxidation around 1 V; as a result, the solution using 0.001 M β-CDSS rather than 0.01 M β-CDSS is ideal. Figure 8 shows a representative example of the overlay of before and after electropolymerization of this solution, showing that the deposition of polymer on the surface of the electrode causes an increase in charging current. The electrode before and after polymerization can be seen in Figure 9, with the dark polymer noticeably deposited on the surface of the electrode. Different scan rates were tested to see if it was actually necessary to have the solution polymerize at 0.005 V/s or if it could be done faster. It was

found that the faster scan rates (0.1 V/s and 0.05 V/s) were not effective at producing polymer, so a scan rate of 5 mV per second is the scan rate that produces a repeatable, dark film of polymer on the electrode surface. Additionally, the optimal voltage was found to go from 0.0 V to 1.2 V, noting that the end voltage of 1.12 V produced similar results as the end voltage of 1.2 V. Thus, the solution composed of 0.01 M HMEDOT, 0.001 M β-CDSS and ultrapure 18.2 MΩ*cm water is optimally polymerized from 0.0 V to 1.2 V at a scan rate of 0.005 V/s to obtain a consistent, even film of polymer.

3.4 Figures

3.4a Electropolymerization on Platinum Electrode

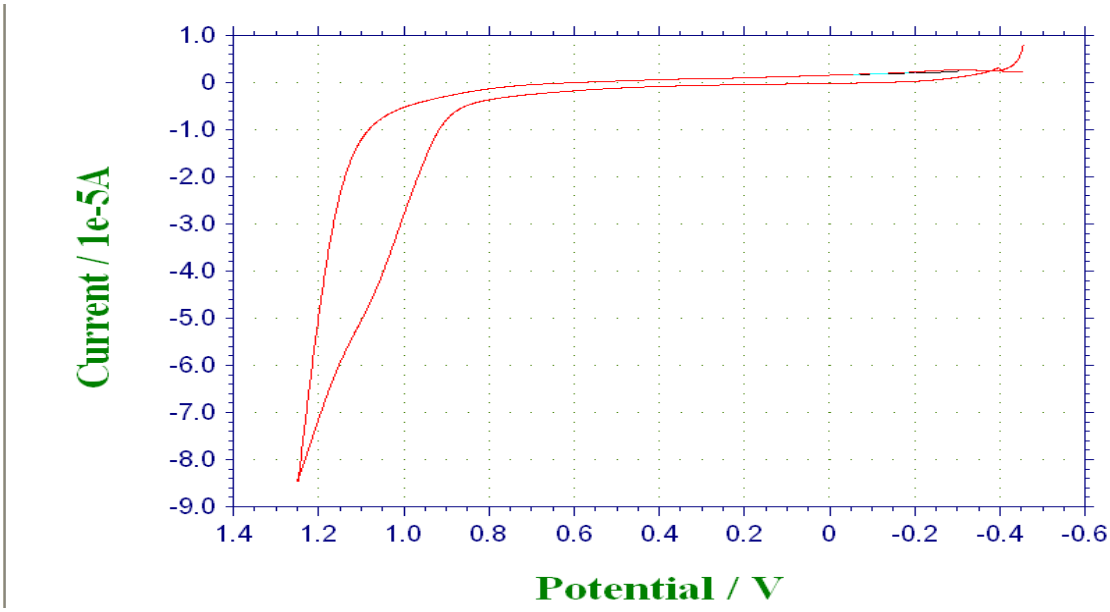

Figure 1. The polymerization of an EDOT solution (0.01 M PBS, 0.001 M β-CDSS, and 0.01 M EDOT) from -0.455 V to 1.25 V at a scan rate of 0.005 V/s on a platinum electrode.

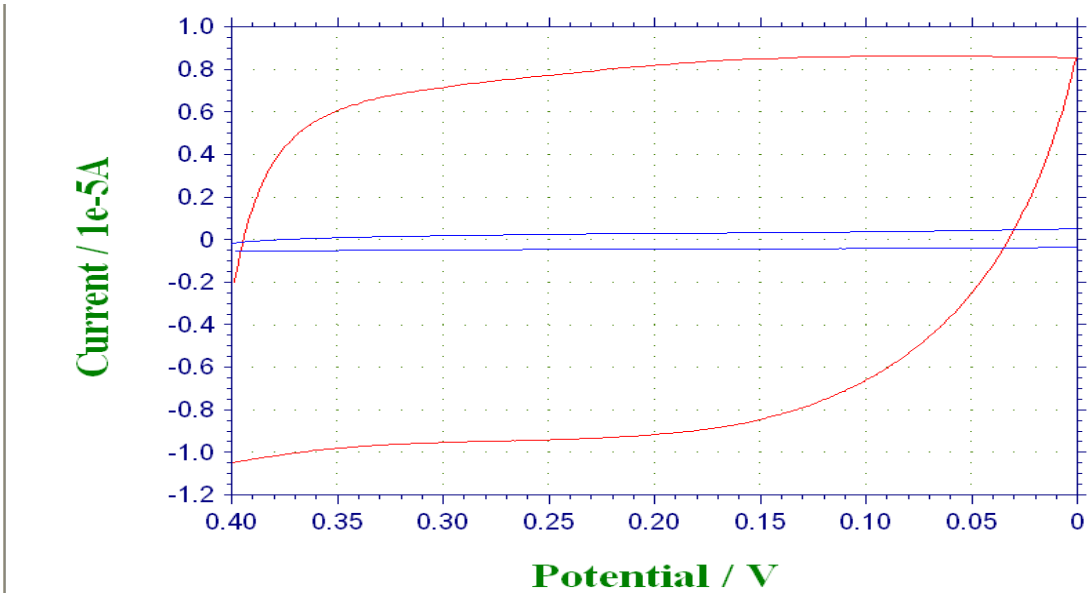

Figure 2. The characterization of a platinum electrode in 0.1 M KCl before (blue) and after (red) polymerization of an EDOT solution (.01 M PBS, 0.001 M β-CDSS, and 0.01 M EDOT) from 0.0 to 0.4 V at a scan rate of 0.05 V/s.

Figure 3. The platinum electrode before and after polymerization of EDOT. The red arrow points to the electrode surface.

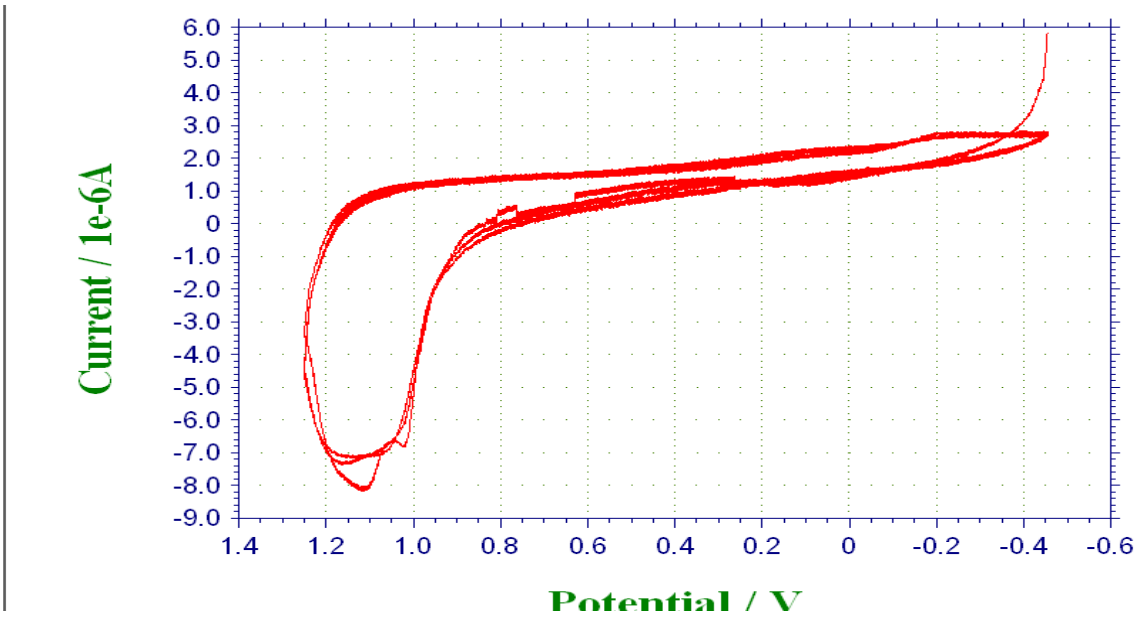

Figure 4. The polymerization of an HMEDOT solution (0.1 M HMEDOT, 0.001 M β-CDSS, and 0.01 M PBS) on a Pt electrode from -0.455 V to 1.25 V at a scan rate of 0.005 V/s.

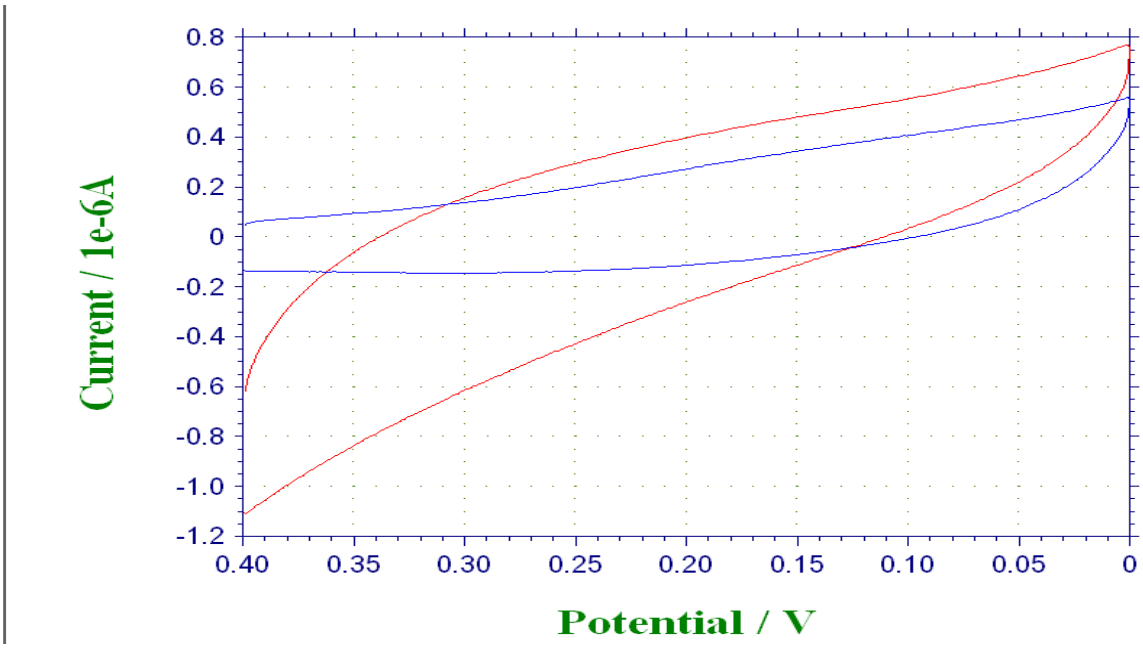

Figure 5: Characterization of Pt electrode in 0.1M KCl before (blue) and after (red) polymerization of HMEDOT solution (0.1 M HMEDOT, 0.001 M β-CDSS, and 0.01 M PBS) from 0.0 – 0.4 V at a scan rate of 0.05 V/s.

3.4b Electropolymerization on Gold Electrode

Table 1. Electropolymerization solution compositions. The β-CDSS acts as the electrolyte in addition to the solubilizer in the solution made up of ultrapure 18.2 MΩ*cm H_2O.

Solution	**Monomer**	**Solubilizer**	**Electrolyte**
1	0.01 M HMEDOT	0.001 M β-CDSS	0.01 M PBS
2	0.01 M HMEDOT	0.01 M β-CDSS	0.01 M PBS
3	0.01 M HMEDOT	0.001 M β-CDSS	No added electrolyte
4	0.01 M HMEDOT	0.01 M β-CDSS	No added electrolyte

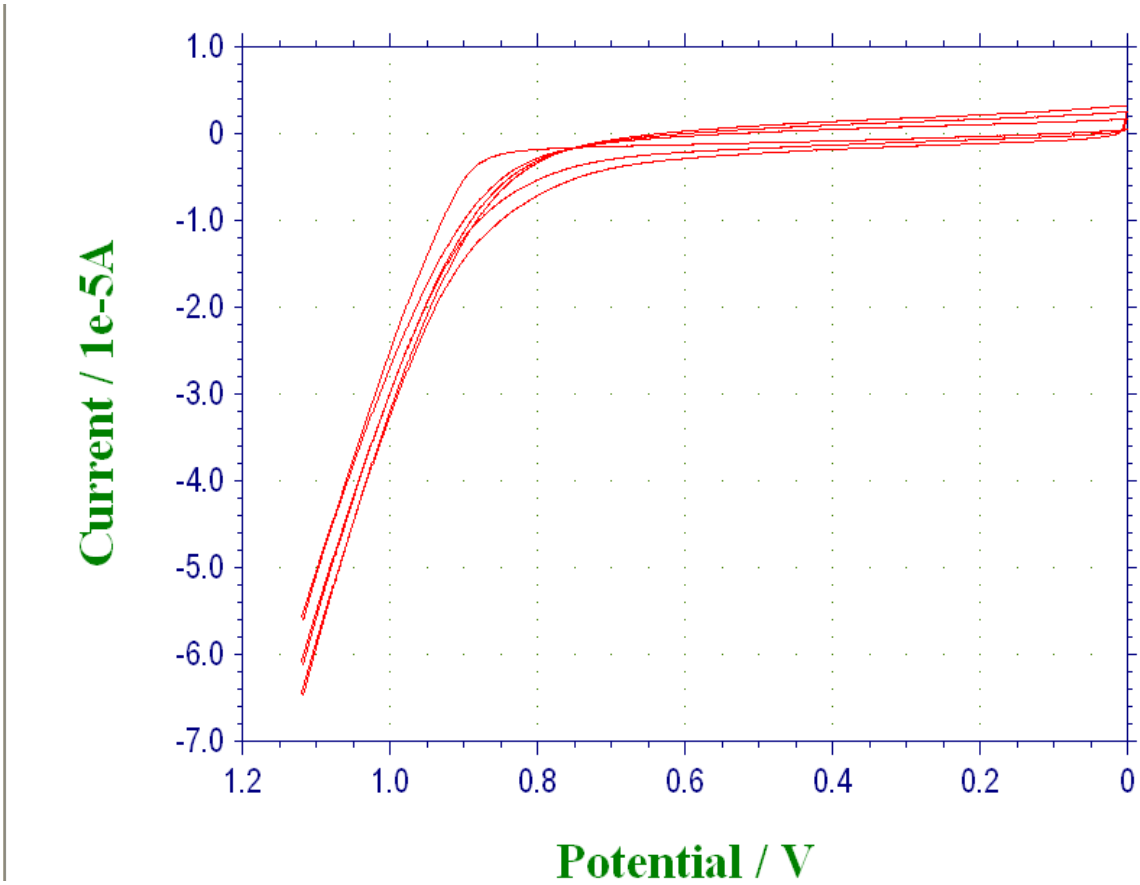

Figure 6. An example CV response of electropolymerization from an HMEDOT solution (containing 0.01 M HMEDOT, 0.001 M β-CDSS, and ultrapure 18.2 MΩ water) from 0.0 V to 1.12 V at a scan rate of 0.005 V/s on a 0.04 cm^2 gold electrode.

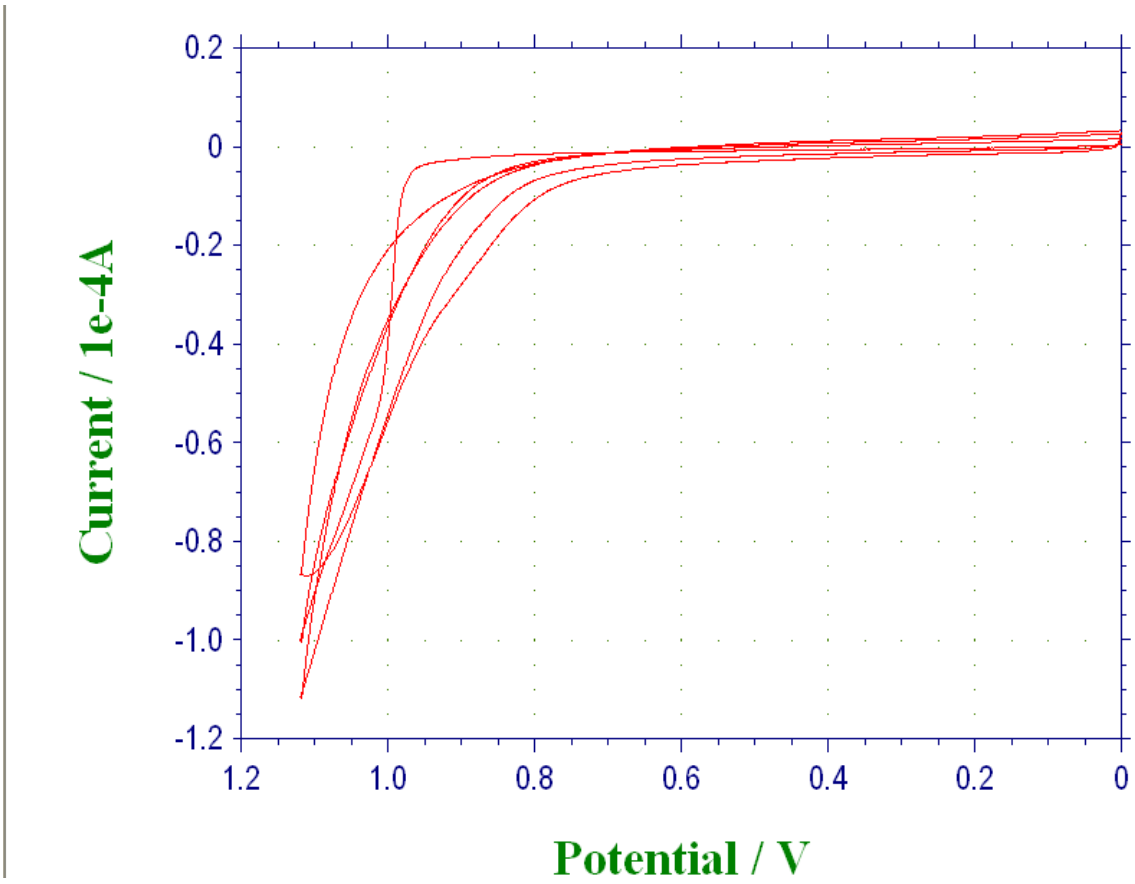

Figure 7. An example CV response electropolymerization from an HMEDOT solution (containing 0.01 M HMEDOT, 0.01 M β-CDSS, and ultrapure 18.2 MΩ water) from 0.0 V to 1.12 V at a scan rate of 0.005 V/s on a 0.06 cm^2 gold electrode.

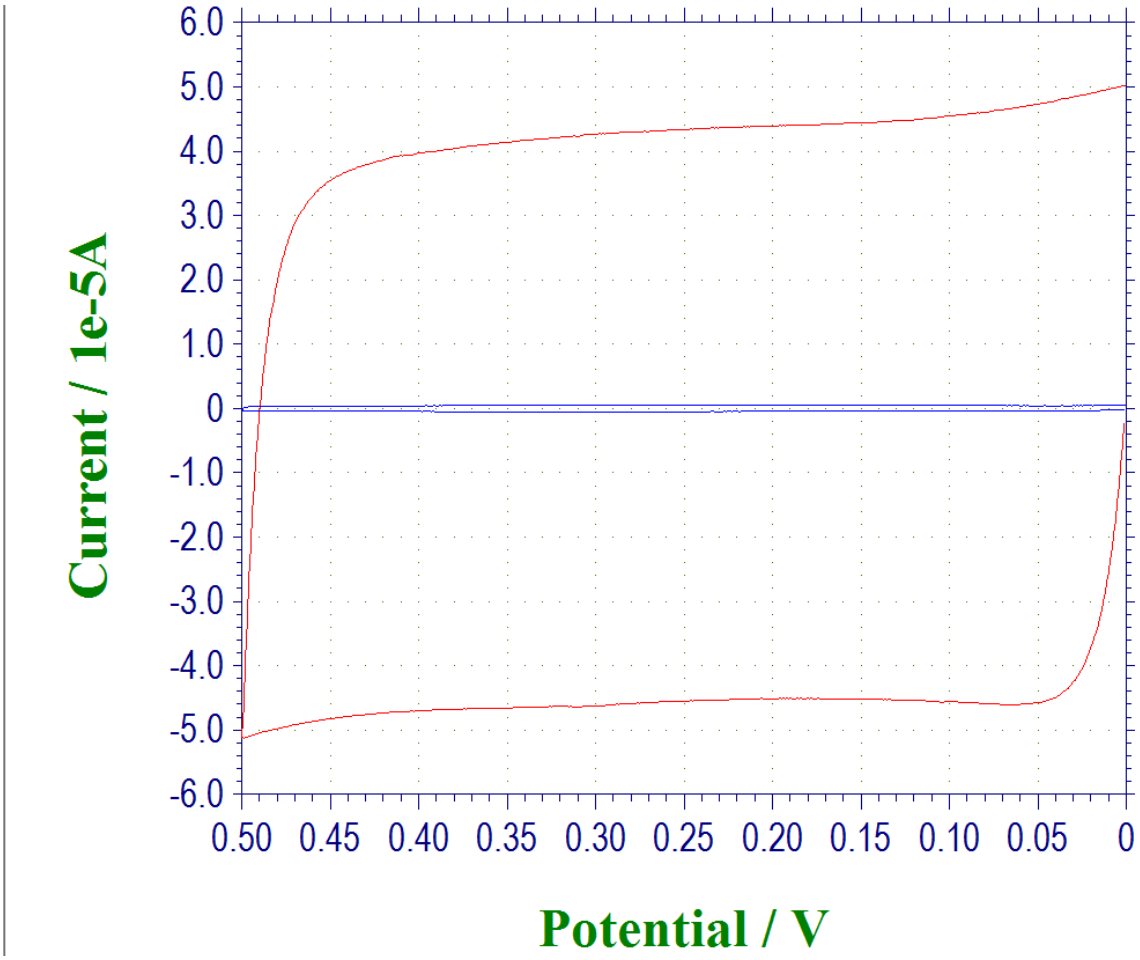

Figure 8. A representative CV response for characterization of a gold electrode in 0.1 M KCl before and after polymerization of a solution of HMEDOT (0.01 M HMEDOT, 0.01 M β-CDSS, and ultrapure 18.2 MΩ water) from 0.0 V to 0.5 V at a scan rate of 0.1 V/s.

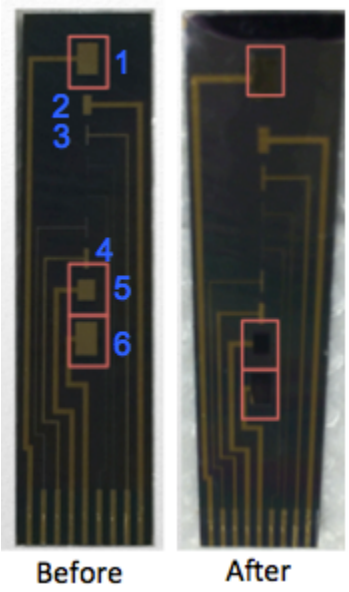

Figure 9. The gold electrodes (on chip type 1) before and after electropolymerization of HMEDOT. The red boxes highlight the electrodes that were polymerized (electrodes 1, 5, and 6).

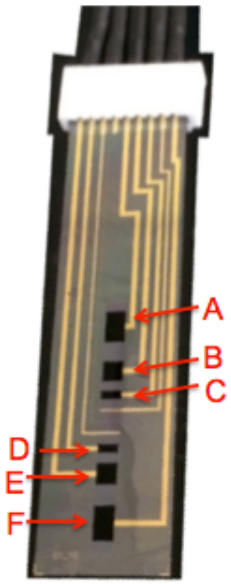

Figure 10: The gold electrodes (on chip type 2) after electropolymerization of HMEDOT. The letters A – F correspond to the electrodes that were polymerized.

Table 2: Characterization of Au electrode before and after polymerization of HMEDOT in 0.1 M KCl. See Figure 10 for the specific electrodes (on chip type 2) corresponding to each letter. The ± refers to the standard deviation of each electrode, where the number of replicates N = 5.

Electrode	**Area (cm^2)**	**Current Density ($\mu A/cm^2$) Before**	**Current Density ($\mu A/cm^2$) After**	**Capacitance ($\mu F/cm^2$) Before**	**Capacitance ($\mu F/cm^2$) After**
A	0.06	11.0 ± 1.60	1100 ± 26	110.3 ± 16	10960 ± 260
B	0.04	9.89 ± 2.3	1080 ± 78	98.9 ± 23	10790 ± 780
C	0.02	7.65 ± 2.2	747 ± 220	76.5 ± 22	7469 ± 2200
D	0.02	7.91 ± 2.4	555 ± 260	79.1 ± 24	5552 ± 2600
E	0.04	9.85 ± 3.3	1120 ± 25	98.5 ± 33	10940 ± 250
F	0.06	11.1 ± 4.3	1090 ± 63	111.0 ± 43	10880 ± 630

3.5 References

1. Waltman, R.J; J. Bargon. “Electrically conducting polymers: a review of the electropolymerization reation, of the effects of chemical structure on polymer film properties, and of applications towards technology.” *Can. J. Chem.* 64.76 (1984): 76-95.

2. Xiao, Yinghong, Xinyan Cui, Jessica M. Hancock, Mohamed Bouguettaya, John R. Reynolds, David C. Martin. “Electrochemical polymerization of poly(hydroxymethylated-3,4-ethylenedioxythiophene) (PEDOT-MeOH) on multichannel neural probes.” *Sensors and Actuators.* B. 99 (2004): 437-443.

3. Akoudad, Said, and Jean Roncali, “Modification of the electrochemical and electronic properties of electrogenerated poly**(**3,4-ethylenedioxythiophene**)** by hydroxymethyl and oligo**(**oxyethylene**)** substituents.” *Electrochemistry Communications.* 2 (2000): 72-76.

4. Kissinger, Peter T., and William R. Heineman. “Cyclic Voltammetry.” *Journal of Chemical Education*. 60.9 (1983): 702-06.

5. Skoog, D.; Holler, F.; Crouch, S. Principles of Instrumental Analysis. 2007.

6. Heinze, J. Cyclic Voltammetry—“Electrochemical Spectroscopy”. *Angewandte Chemie International Edition in English.* 23.11 (1984): 831-847.

4. Characterization and Immobilization of Electrochemical Mediator

4.1 Introduction

Electropolymerization, a chemical reaction influenced by electric current or potential to produce polymers from monomers, can be used to immobilize a small molecule (a mediator) to an electrode surface. In this project, the potential is controlled to induce the polymerization. With the mediator immobilized to the electrode surface (for instance, via coupling to a monomer and subsequent electropolymerization), it can then that can assist in transferring electrons to or from enzymes. A representation of the role the mediator may play in a biofuel cell is shown in Figure 4. This strategy is one approach toward making an electrode for a more efficient biofuel cell.

Enzymes in a biofuel cell work through redox reactions to catalytically oxidize fuels and reduce oxidants. Most enzymes cannot exchange electrons directly with a solid electrode; thus, a small molecule (a mediator) is required.[1] The mediator helps to shuttle electrons from the redox site within the enzyme to the surface of the electrode at the anode, while it helps to shuttle electrons from the electrode surface to the enzyme at the cathode. The mediator's small character allows diffusion to the catalytic site of the enzyme and assistance in the transfer of electrons. The ease of diffusion by mediators could pose a potential problem concerning its ability to escape from the electrode without exchanging electrons. If the enzymes are attached to the surfaces of the electrodes, they can be concentrated there. If the mediator is also confined to that area, then the mediator can more efficiently transfer electrons; as compared to a mediator freely

diffusing throughout the cell, since the loss of electrons to other reactions could occur. The conducting polymer (HMPEDOT) will serve as an immobilizing agent (via covalent coupling) for the mediator, a means to direct the immobilization to a specific location (via electropolymerization), and a source of conductive pathways to transfer electrons with the electrode.

A good mediator should be a small molecule that can be relatively close to the active site of the enzyme and has an oxidation-reduction potential similar to the redox potential of the enzyme so that the electron transfer process can be completed spontaneously. Specifically, the mediator should have a redox potential about 50 mV more negative of that of the enzyme.[1] The mediator must be able to quickly cycle between the oxidized and reduced states, and be stable enough in those states to effectively continue mediation.[2] The model mediator used in this project is a ferrocene derivative.[3] Ferrocene is convenient for a few reasons: several derivatives are commercially available, it's electrochemically reversible, and it can be synthetically modified to tune its redox potential.[4] Ferroceneacetic acid (Figure 3) has been chosen because of the carboxylic acid functional group, which allows for conjugation (for example, via esterification). Additionally, this particular derivative was chosen since the carboxylic acid group needs to be at least one carbon-unit away so that it does not interfere with the redox potential of ferrocene.

4.2 Experiment

4.2a Materials

Hydroxymethyl-3,4-ethylenedioxythiophene (HMEDOT), ferroceneacetic acid (FcAA), dichloromethane, tetrabutylammonium hexafluorophosphate, magnesium sulfate ($MgSO_4$), para-toluenesulfonic acid monohydrate (APTS) and β-cyclodextrin sulfated sodium (β-CDSS) were obtained from Sigma US. For the gold electrode experiments, the chips had gold electrodes insulated with benzocylcobutene (BCB). These fabricated gold-electrode chips were plasma cleaned and stored in ultrapure 18.2 MΩ*cm water within 72 h before use.

4.2b Electrochemistry with FcAA

Electrochemistry was performed, using potentiostat models 650A and 760B from CH Instruments in Austin, TX, on ferroceneacetic acid (FcAA) alone, FcAA on HMEDOT film, and FcAA on PEDOT film. Cyclic voltammetry (CV) was performed using a three-electrode system with a Ag/AgCl (in sat'd KCl) reference electrode, platinum flag counter electrode, and gold working electrode. 1 mM FcAA (in PBS) was first characterized from 0.0 – 0.4 V at a scan rate of 0.1 V/s on a clean gold electrode. HMEDOT was polymerized on a clean gold electrode, as outlined previously in section 3.2c.1 mM FcAA was then characterized from 0.0 V – 0.6 V at a scan rate of 0.1 V/s on a gold electrode newly polymerized with HMEDOT. Subsequently, 1 mM FcAA was characterized from -0.1 – 0.4 V at a scan rate of 0.1 V/s on a gold electrode newly polymerized with EDOT.

4.2c Synthesis of FcA-MEDOT

The reaction for the synthesis of FcA-MEDOT (ferroceneacetic-methylEDOT) is shown in Figure 5. Approximately 43.9 mg of FcAA (0.18 mmol) was placed in a 100 mL three-neck round bottom flask (RBF) along with approximately 31 mg HMEDOT and 3.8 mg APTS. The RBF was attached to a N_2 line and flushed with N_2. A drying column was made by filling a 50 mL buret with approximately 6 cm of Al_2O_3. Approximately 20 mL of toluene was poured into the drying column, and 10 mL collected in the RBF. The RBF was attached to the N_2 line, plugged, and allowed to stir for 8 h at room temperature.

5 mL of DI H_2O was added to the RBF, swirled and the contents transferred to a 125-250 mL separatory funnel. About 2.5 mL diethyl ether was added to the separatory funnel. The separatory funnel was stoppered and then inverted, The stopcock was then opened to release pressure. The stopcock was closed, and the separatory funnel inverted 4-5 times. The stopcock was again opened to release pressure. The inversion and release process was repeated 4-5 times to ensure complete mixing. The separatory funnel was set onto ring and the phases allowed to separate completely. The aqueous phase, on bottom, was drawn out through the stopcock into a 20 mL vial. The organic phase was then washed with once with 5 mL water using the same inversion procedure. The aqueous phase was removed through the stopcock; the organic phase was poured out of the top of the separatory funnel into a clean 20 mL vial. Approximately 20 mg $MgSO_4$ was slowly added to the vial, swirling until the clumping ceases, and let sit for 30-60 seconds. The $MgSO_4$ was removed from

the vial using a pipet with glass wool. The solvent was slowly poured into the filter, leaving the majority of the $MgSO_4$ in the vial. $MgSO_4$ (approximately 10 mg) was again added to the filtered solvent, and let sit for 10 minutes. The solvent was filtered through glass wool, and collected.

The solvent was evaporated from the RBF using a rotovap (rotary evaporator). The product was subsequently purified by column chromatography. The column was prepared with a Pasteur pipet, glass wool, and silica gel. The column was then flushed with dichloromethane: solvent flowed down the silica gel, never letting the column dry out. The sample was then loaded onto the silica gel column, and the column eluted by flash chromatography. Fractions were taken approximately every milliliter until the color of the column ceases to change (after 28 fractions).

4.2d Determination of Product

Each fraction was then analyzed using thin-layer chromatography (TLC): 2 µL of each fraction was placed on a pencil-marked line (at about 0.5 cm from the bottom) of the TLC plate, the plate was placed in a jar of dichloromethane (solvent below the line), the plate was taken out when the solvent level reached the top of the plate (at about 0.5 cm from the top), and the solvent line marked with pencil. Each plate was placed under UV light, and the spots circled with pencil. The distance the solvent and compound traveled, and the Rf values calculated (Rf = distance traveled by sample/distance traveled by solvent). The

fraction with the largest Rf value (most hydrophobic fraction) was then tested with electrochemistry for further analysis.

Electrochemistry was performed on the product using a Ag/AgCl (in saturated KCl) reference electrode, a platinum flag counter electrode, and a gold working electrode. The product was dissolved in a solution of 0.1 M tetrabutylammonium hexafluorophosphate in dichloromethane. The electrodes were placed in the solution, and cyclic voltammograms taken from 0.0 – 0.4 V at a scan rate of 0.1 V/s on a clean gold electrode. The results were then compared to the CV of the 1 mM FcAA control performed using the same reference and counter electrodes at the same parameters, with the same type of 0.06 cm^2 gold working electrode.

4.3 Results and Discussion

4.3a Electrochemistry with FcAA

The electrochemistry performed with FcAA provided results that could be used as a comparison point for further experimentation. The CV response of 1 mM FcAA on a gold electrode (shown in Figure 1) gave an $E_{1/2}$ (half reduction potential) of 0.155 V, and a ΔE_p (change in peak potential) of 0.061 V. Likewise, the CV response of 1 mM FcAA on a gold electrode polymerized with an HMPEDOT film (shown in Figure 2) gave an $E_{1/2}$ of 0.154 V, and a ΔE_p of 0.067 V in addition to the typical rectangular shape, consistent with that of the polymer. The $E_{1/2}$ values as well as the ΔE_p values for FcAA on gold and FcAA on HMPEDOT film on gold are consistent. This shows that the redox properties of

FcAA are consistent whether on the electrode surface or on the conducting polymer (on an electrode surface). Thus, FcAA should be able to transfer electrons through a conducting polymer with similar properties to that through an electrode surface. Additionally, the change in peak potential for that of FcAA and that of FcAA on an HMPEDOT-modified electrode are similar, specifically 6 mV apart. Thus, the reversibility of FcAA's redox process is similar with and without a conducting polymer.

4.3b Synthesis of FcA-MEDOT

A schematic of the reaction of FcAA with HMEDOT to form FcA-MEDOT (ferroceneacetic-methylEDOT) is shown in Figure 5. After evaporation of solvent off the product, a golden substance was left in the RBF. During the column chromatography, the initial color was very dark brown (almost black), but proceeded to separate into three main colors: brown/black, yellow, and orange. The orange band progressively moved down the column, eventually leaving only brown/black and yellow bands on the column.

4.3c Determination of Product

TLC was performed on each of the fractions obtained from column chromatography, and the Rf values subsequently calculated. Dichloromethane (a nonpolar substance) was used as the solvent for the mobile phase. The product expected (FcA-MEDOT) is a relatively nonpolar substance. Thus, the product

should have a higher affinity for the solvent than for the plate, meaning that the product will move down the plate and have a higher Rf value. The fraction with the highest Rf value most likely contains the desired product. The Rf values obtained from TLC are shown in Table 1 and Table 2.

The fraction with the largest Rf value (shown in Table 2) would be the most hydrophobic molecule, which is consistent with the desired product. The FcAA and HMEDOT would both have a greater affinity for the column than the FcA-MEDOT. The fraction with the highest Rf value was tested with electrochemistry to determine if the FcAA could be detected: there should be a shift in the redox potential of the FcAA upon coupling. FcAA and its electrochemical signal would not be present in the film on the electrode if it did not couple to HMEDOT. There was no oxidation or reduction peak detected for the fraction tested. It seems as though the coupling of FcAA to HMEDOT was not entirely successful even though TLC showed a more hydrophobic molecule (compared to FcAA or HMEDOT alone) that is consistent with FcA-MEDOT. It is also possible that side reactions of the product occurred. The electrochemical analysis of the product did not take place immediately after the synthesis process. Additionally, the fraction was dissolved in several different solutions (evaporating one before dissolving the next) to test the solubility of the product in different solvents. The prolonged exposure to air could have an effect on the FcAA moiety of the product, possibly resulting in a loss of redox activity. The prolonged exposure to light could have had similar effects.

4.4 Figures

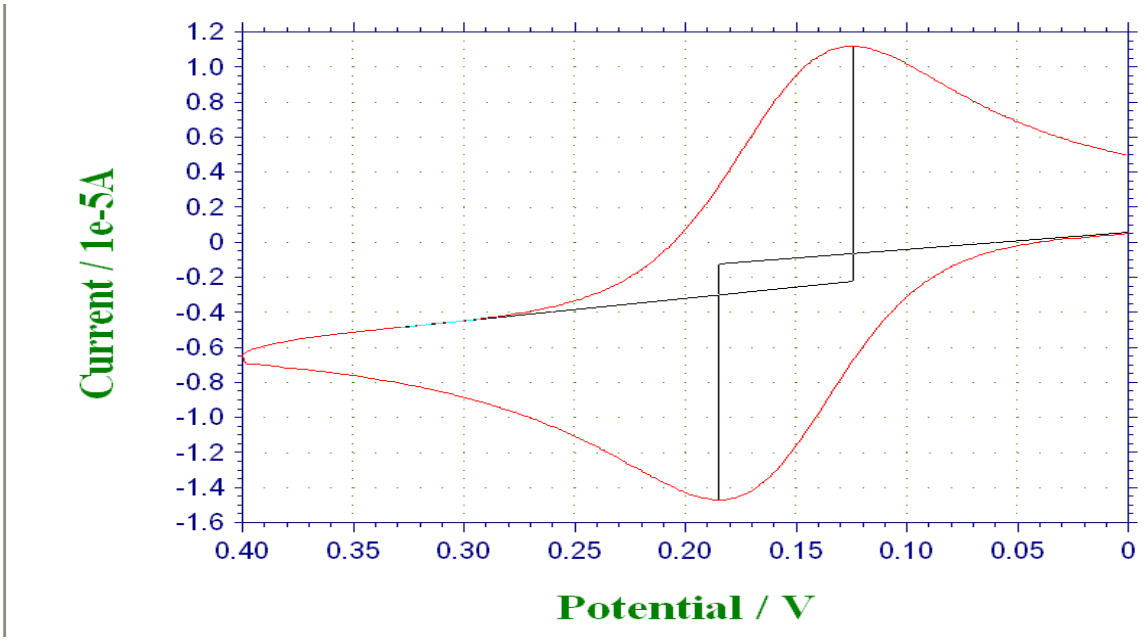

Figure 1. 1 mM FcAA (in 0.1 M PBS) from 0.0 – 0.4 V at a scan rate of 0.1 V/s.

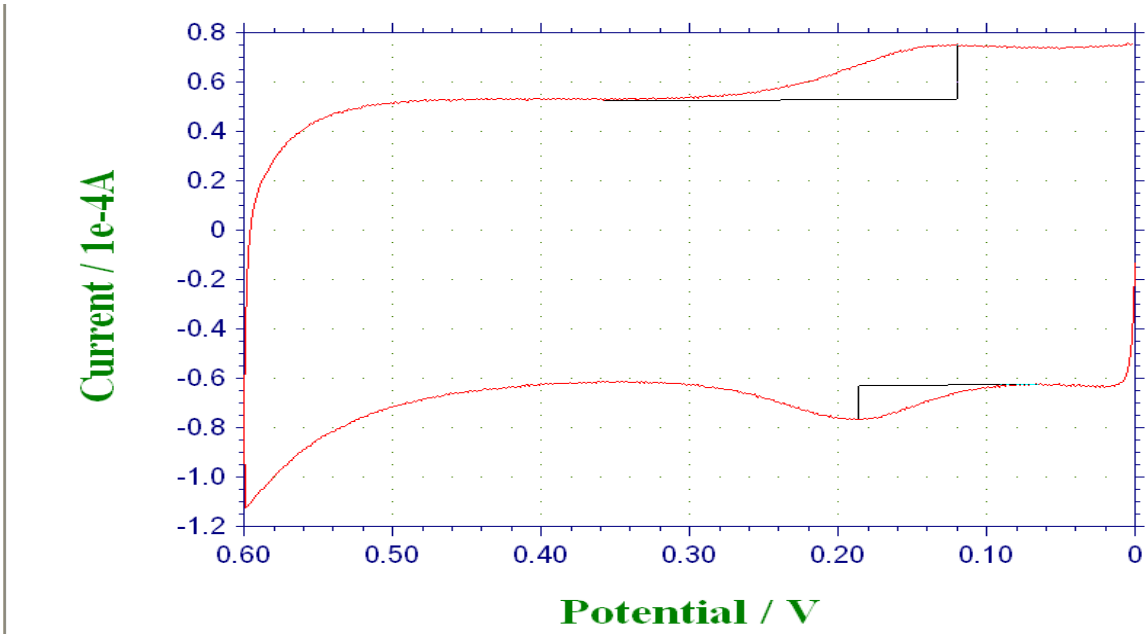

Figure 2. CV response of an HMPEDOT-modified electrode in a solution containing 1 mM FcAA (in 0.1 M PBS) from 0.0 – 0.6 V at a scan rate of 0.1 V/s.

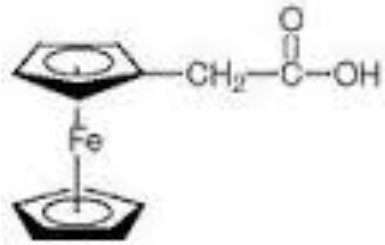

Figure 3. Schematic for ferroceneacetic acid (the mediator used in these studies).

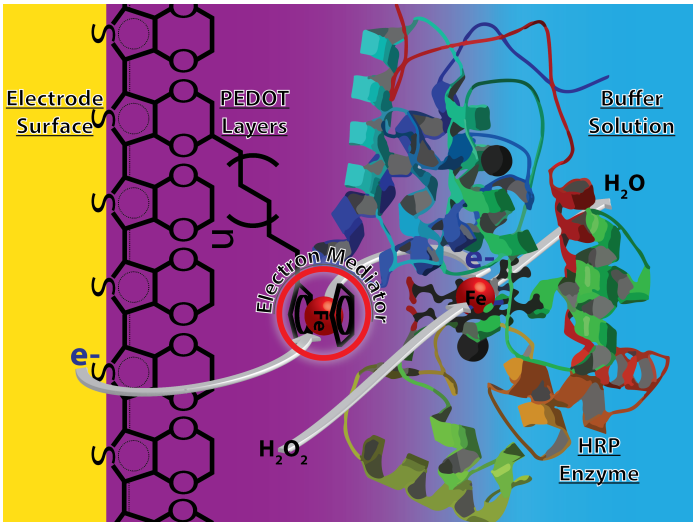

Figure 4. Interaction of an electrode, an electron mediator (a ferrocene derivative), a conducting polymer (poly 3,4-ethylenedioxythiophene, PEDOT), and an enzyme (horse radish peroxidase (HRP)). This interaction shows the role that FcAA might play in a biofuel cell. *Figure courtesy of Benjamin J. Jones.*

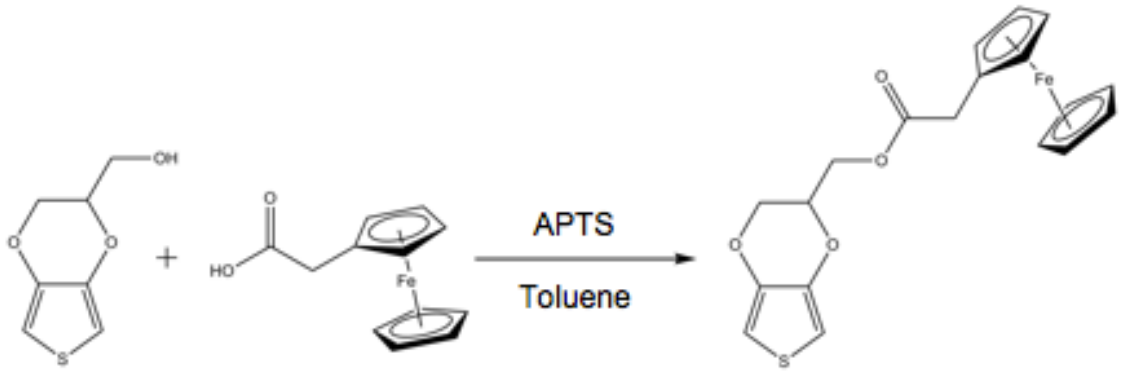

Figure 5. The esterification reaction of FcAA with HMEDOT to form FcA-MEDOT.

Table 1. Rf values from thin-layer chromatography (TLC) for the first five fractions and the starting materials (FcAA and HMEDOT).

Starting Materials	FcAA	HMEDOT	**Fraction**	1	2	3	4	5
Rf Values	-	-	**Rf Values**	-	-	0.847	0.102	0.197
	0.0667	-		-	-	-	0.0847	-
	0.0370	0.0250		-	-	-	0.0508	0.0492

Table 2: Rf values from TLC for fractions 6-12 and the starting materials (FcAA and HMEDOT).

Starting Materials	FcAA	HMEDOT	**Fraction**	6	7	8	9	10	11	12
Rf Values	-	-	**Rf Values**	-	-	-	-	-	-	-
	0.0667	-		-	-	-	-	-	0.0667	0.0667
	0.0370	0.0250		-	-	-	0.0583	-	0.0333	0.0333

4.5 References

1. Atanassov, Plamen; Chris Apblett; Scott Banta; Susan Brozik; Scott Calabrese Barton; Michael Cooney; Bor Yann Liaw; Sanjeev Mukerjee; Shelley D. Minteer. “Enzymatic Biofuel Cells.” *The Electrochemical Society Interface.* Summer 2007: 28-31.

2. Harper, Alice; Mark R. Anderson. “Electrochemical Glucose Sensors – Developments Using Electrostatic Assembly and Carbon Nanotubes for Biosensor Construction.” *Sensors*. 10 (2010): 8248-8274.

3. Shim, Joonmok, Gha-Young Kim, and Seung-Hyeon Moon. "Covalent Co-immobilization of Glucose Oxidase and Ferrocenedicarboxylic Acid for an Enzymatic Biofuel Cell." *Journal of Electroanalytical Chemistry* 653.1-2 (2011): 14-20.

4. Okawa, Y.; M. Nagano; S. Hirota; H. Kobayashi; T. Ohno; M. Watanabe. “Tethered mediator biosensor. Mediated electron transfer between redox enzyme and electrode via ferrocene anchored to electrode surface with long poly(oxyethylene) chain.” *Biosensors & Bioelectronics.* 14 (1999): 229-235.

5. Conclusion

The ultimate purpose of this project is to create a more efficient biofuel cell with the use of mediators and modified electrodes. The mediator transfers electrons between the electrode surface and the enzyme, producing a more efficient complex. With a mediator confined to a specific area on the surface of the electrode, the electron transfer process is much more effective than it would be if the mediator were freely diffusing throughout the cell. HMPEDOT, the conducting polymer, serves as the immobilizing agent for the mediator (via covalent coupling), as a means to direct the immobilization to a specific location (via electropolymerization), and as the matrix of conductive pathways in which electrons are transferred with the electrode.

In this project, the electropolymerization of HMEDOT was optimized, and attempts made to covalently couple FcAA to HMEDOT. The optimal solution composition and polymerization conditions for HMEDOT was found to be 0.01 M HMEDOT, 0.001 M β-CDSS, and ultrapure 18.2 MΩ*cm water at a scan rate of 0.005 V/s from 0.0 V to 1.2 V to consistently obtain a film of polymer. The chosen mediator, ferroceneacetic acid (FcAA), was coupled to HMEDOT via esterification. The product was analyzed using TLC and electrochemistry. Future work needs to be done to optimize the coupling procedure and verify the synthesis product. It is possible that the percent yield is so low that the product cannot be detected, especially considering that the synthesis on such a small scale. Increasing the scale of synthesis could help alleviate this issue. Once

synthesized and purified in large enough quantities, FcA-MEDOT can then be electropolymerized using the conditions optimized for HMEDOT. Once the FcA-MEDOT electropolymerization conditions and parameter have been set, the immobilized mediator can be tested with an immobilized enzyme. This could take a variety of forms. For example, the FcA-MEDOT could possibly be co-polymerized with an enzyme-HMEDOT product. Another strategy would be to layer thin films of FcA-MEDOT, enzyme-HMEDOT, FcA-MEDOT, enzyme-HMEDOT, etc. on the electrode.

6. Acknowledgements

Ingrid Fritsch, Benjamin J. Jones, Fritsch Group, University of Arkansas Honors College, National Science Foundation (CBET-1336853), Arkansas Biosciences Institute.