WPI

A Study of the Effectiveness of Electron Transfer for a CNT/CNF Based Anode in an Enzyme Fuel Cell

A Major Qualifying Project Report

Submitted to the faculty of
WORCESTER POLYTECHNIC INSTITUTE
in partial fulfillment of the requirement for the
Degree of Bachelor of Science

April 25th, 2013

Written by:
Nancy Bezies, Connor Ellison, Justin Hollinger, Xavier Miller

Advised by:

Professor Ravindra Datta and Professor Jianyu Liang

Abstract

With the dwindling supply of fossil fuels, the scientific community is looking at the fundamentals by which biological systems obtain energy so efficiently, in order to develop novel sustainable energy sources. To date, enzymatic biofuel cells (EFCs) have attracted attention due to their potential to power medical devices implanted into human bodies; however, if EFCs are able to overcome obstacles such as poor stability and low power output they have the potential to be used in many other areas. This research project investigated the potential advantages and effectiveness of using carbon nanofibers (CNFs) versus multi-walled carbon nanotubules (MWCNT) as the medium for electron transfer. The ultimate goal was to increase the current generated by effectively increasing the total surface area available for enzyme immobilization as well as stability.

In our study, fuel cell electrodes were fabricated by depositing a PEI/CNT/GOx based mixture onto a glassy carbon electrode and the electrochemical performance of the electrodes was characterized through cyclic voltammetry. The morphology of the electrodes was studied through SEM analyses. On average, the current density of MWCNTs to CNFs was nearly fourfold greater, and the MWCNT electrode better supported enzyme immobilization as evidenced by a higher longevity. It is also notable that the current densities obtained for MWCNTs were slightly higher than that in published literature, implying a more effective electrode nanostructure. In summary, our results convincingly show that MWCNTs remain a good choice for further EFC research.

Acknowledgements

We would like to thank our advisors Professor Ravindra Datta and Professor Jianyu Liang for their guidance throughout the year and prompt feedback on various preliminary drafts. We were immensely grateful for the help provided by Yinjie Cen both in maintaining the laboratory and for the support he provided when we encountered a problem. Lastly, we would like to recognize Miaoyue Shi for the assistance that she provided with our laboratory testing.

The behind the scenes job of coordinating MQP shipments goes largely unrecognized. That said, we would like to thank Felicia Vidito, Tiffany Royal and students who staff the Chemistry Stockroom for their service. Lastly, this project could not have been possible without funding from WPI's Chemical Engineering Department.

Executive Summary

With Earth's fossil fuels are expected to be depleted or in perilously low levels just one century from now. Consequently, the scientific community is looking at the fundamentals by which biological systems obtain energy, in order to develop novel sustainable energy sources. To date, enzymatic biofuel cells (EFCs) have attracted attention due to their potential to power medical devices implanted into human bodies; however, if EFCs are able to overcome obstacles such as poor stability and low power output they have the potential to be used in many other areas.

Single-wall carbon nanotubules (SWCNTs) have been well explored in various different applications of biofuel cells, however the consensus is that they do not demonstrate the same amount of promise as multi-walled carbon nanotubules (MWCNTs). It should be noted the exact mechanics regarding the electrons pathways to the electrode are far from clear to most researchers. Most recent publications have focused solely on the optimization of MWCNT based anodes for a variety of different oxireductases, however no consideration has been given to a carbon nanofiber (CNF) based support matrix. This research project, however, looked into investigating the potential advantages and effectiveness of using CNFs versus MWCNTs as the medium for electron transfer. With the ultimate goal being to increase the current generated by effectively increasing the total surface area available for enzyme immobilization.

In our study, fuel cell electrodes were fabricated by depositing a PEI/CNT/GOx based mixture onto a glassy carbon electrode and the electrochemical performance of the electrodes was characterized through cyclic voltammetry. On average, the current density of MWCNTs to CNFs was nearly four times greater and the MWCNT electrode better supported enzyme immobilization as evidenced by a higher longevity. After less than twenty minutes the

normalized peak current for the CNF based electrode was twenty eight percent of its original value, nearly half of the standard decline observed for similar conditions of MWCNT anodes in the literature. Much of this decline in current was likely the result of the electrode diffusing into solution – a phenomenon that was not seen with the MWCNT based electrode. It is also notable that the current densities obtained for MWCNTs were slightly higher than that in published literature, implying a more effective electrode nanostructure. Lastly, the morphology of the MWCNT and CNF electrodes was studied through SEM analyses. From this significant difference in packing density and nanomaterial orientation were observed that lead us to believe our particular CNF formulation is not fully optimized. In summary, our results convincingly show that MWCNTs remain a good choice to pursue for further EFC research.

Table of Contents

Table of Figures

1. Background

1.1 Why Biofuel Cells?

The ever decreasing supply of fossil fuels demands the production of a clean energy supply. Fuel cells could be the answer to this problem, providing their clean energy at relatively efficient means. Fuel cells can range from large molten or phosphoric fuel cells to compact designs that can fit into cars, such as the Proton Exchange Membrane (PEM) fuel cells. One design that could provide the best solution to the problem of clean energy is the biological fuel cell or biofuel cell. Whereas other fuel cells currently provide only a partially clean energy source or are extremely expensive, biofuel cells are: a completely clean source of energy, far smaller than comparable fuel cells, and can be produced at very low costs (Osman, 2011).

Biofuel cells are not without their problems. They suffer from two main flaws that prevent them from becoming a practical power option; lack of power and longevity. These flaws arise due to the distance between the enzyme activation site and the electron and proton pathways. This distance causes internal resistance within the fuel cell resulting in a decreased power density.

If these problems can be overcome, biofuel cells would be a practical and cheap alternative to conventional fuel cells, such as the PEM fuel cell. With an increase in power output and longevity biofuel cells could be used as a safe energy source for bioimplants, such as pace makers, and portable power for the numerous devices that modern society uses. Ideally, this would be able to be done without polluting the environment at any stage of energy production.

1.2 Design

In an enzymatic fuel cell you have two fuel compartments directly in contact with the surface of the anode and the cathode on opposed sides of the fuel cell assembly. In between these two electrodes there is typically a selectively permeable proton membrane which allows

positively charged particles to pass through, while forcing electrons to travel through an external circuit thereby producing current. An example of a Glucose Oxidase and Laccase based biofuel cell is depicted below in Figure 1.

An electrode in its most basic form is a carrier of charge, in an enzymatic fuel cell the electrode uses enzymes in order to transfer the charge. This is done by using applying a thin layer of immobilized enzymes on the anode and cathode. The enzyme's selectivity toward certain molecules will elicit a reaction on the surface of the electrode thus producing electrons. As a result, the catalytic materials chosen for the anode and cathode largely define the efficiency of the electrode. There are three major components to an enzymatic electrode, the electrode material, the enzyme, an optional mediator. The purpose of the anode and cathode material is to provide conductivity and support; materials commonly selected are gold, platinum, and carbon. Since the cost of common electrode materials such as gold and platinum has been on a constant increase attention has shifted towards new materials. The most popular electrode materials that have been recently studied are: conducting polymers, functionalized polymers, composite materials, sol-gel materials and nanomaterials. The enzymes used are chosen for their selectivity and depending on which of the many type of fuel they wish to use. Finally ionic polymers, such as Nafion, are not required in a fuel cell but have become increasingly popular because they increase the electron transfer efficiency of the enzyme. (Sarma, 2007)

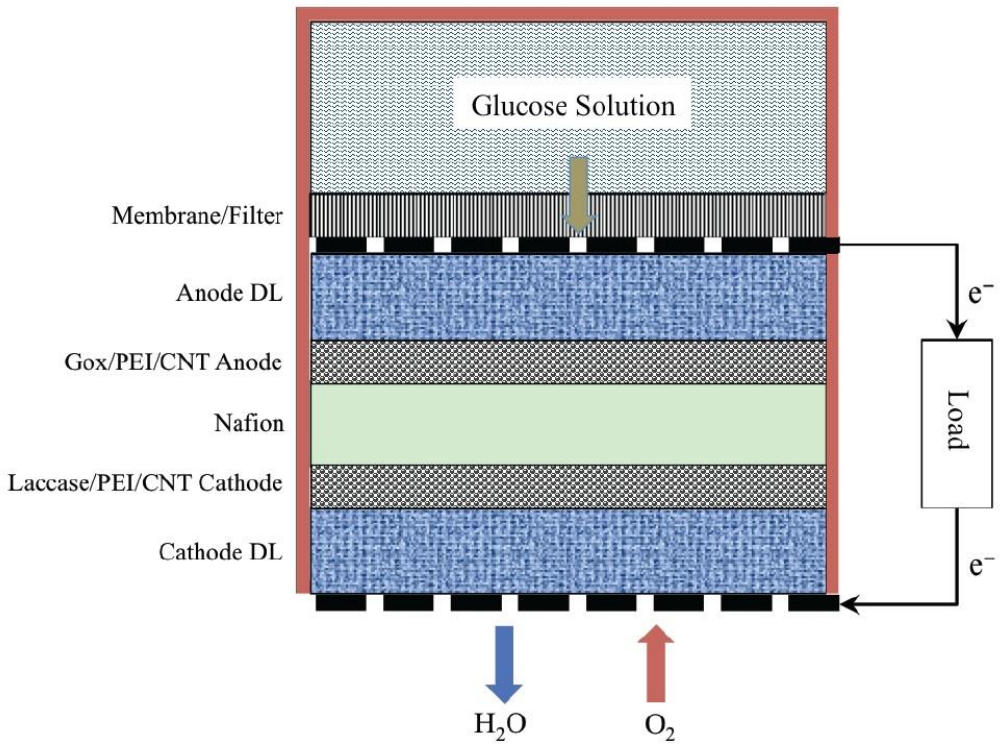


Figure 1 - Schematic of Proposed EFC

1.2.1 Fuel Sources

There are an assortment of organic compounds that can be used as a fuel source depending upon the enzyme used and the power output desired. No different than enzymes in biological systems, immobilized enzymes in fuel cells require a specific substrate that will properly accommodate the enzyme's active site. This becomes critical because in order to achieve full oxidation of the fuel, several different enzymes are needed to oxidize the by-products into further reduced states. For example, the human body makes use of nine distinct enzymatic reactions to reduce a single molecule of glucose, a monosaccharide containing six carbons, into two molecules of pyruvate, a further oxidized compound containing three carbons. This complexity is extremely difficult to duplicate in an environment outside a biological

organism where there is a lack of natural dynamics that keeps all the elements operating optimally (Sarma, 2007).

Due to the difficulty in fully oxidizing the substrate's by-products, the most frequently used fuels for enzymatic fuel cells are basic carbohydrates such as monosaccharides and disaccharides. The most well-known and often used is glucose due to its low cost and high abundance. In most glucose-powered EFCs the majority of the fuel's potential energy is wasted, as they only use a single oxidoreductase which produces just two electrons per glucose molecule used. These monosaccarides and disaccharides are easy to process but are low in energy content, however they form the building blocks for more complex high energy molecules such as starch, cellulose and many other complex sugars known as polysaccharides. Other than sugars there are other fuel sources with significantly higher energy content, such as alcohols or alpha-hydroxy acids, however their use is limited by abundance, cost and safety concerns (Zhu, 2012).

Extensive research is currently being done to improve the extent of fuel oxidation by constructing biofuel fuel cells that have multiple oxidoreductases that can achieve total or deep oxidation of a variety of organic fuels. In the case of glucose, this would result in more than two electrons being generated per glucose molecule fed into the system. Many of these attempts have been successful but it typically involves a trade-off, lowering the maximum power density while achieving a more complete oxidation of the fuel (Zhu, 2012). Ultimately the choice of fuel type is highly dependent upon which enzymes are going to be used and what the desired power density.

1.3 How to Immobilize Enzymes

In their natural state, enzymes are free flowing. Yet in order to utilize enzymes as catalysts in a bioelectrode it must be set on to the electrode, this can be achieved through enzyme immobilization. (Atanassov et al., 2007) Further immobilization increases the stability of the enzyme, increasing its active lifetime. One of the main problems with EFC's is their short lifespan; this has to do with the typically short lifetime of enzymes, only eight hours to two days. Yet with the correct form of immobilization the active lifetimes of the enzymes can be typically extended from between seven and twenty days, in fact the active lifetimes have been extended for up to a year. (Minteer, 2007) This is because the right method of immobilization will increase the stability of the enzyme and in turn prevent denaturing. (Sheldon, 2007) Because immobilization has a high impact the functionality of the EFC the selection for an immobilization method and materials is crucial.

There are two ways to immobilize enzymes, chemically and physically, only physical immobilization has been studied for use in bio-electrodes. While there are over one hundred methods for physical immobilization, there are five methods that are widely used; covalent attachment (A), adsorption (B), entrapment (C), cross-linkage (D), and encapsulation (E) depicted in Figure 2 below. (Klibanov, 1983)

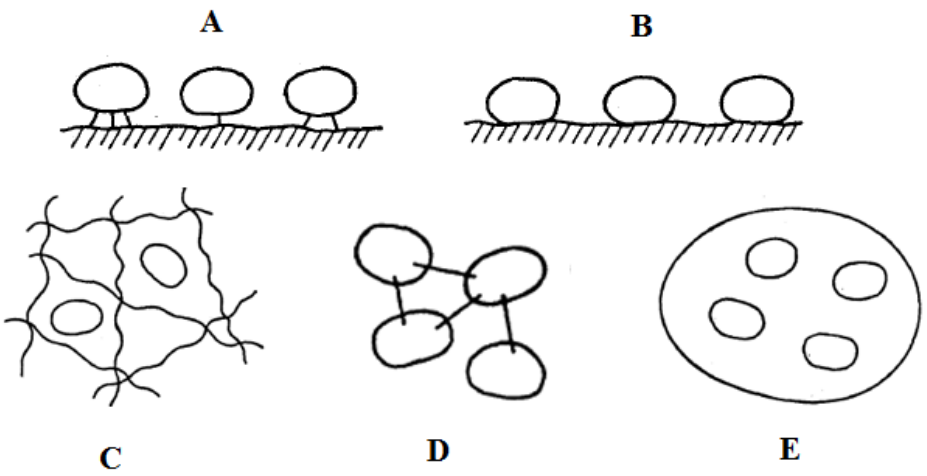


Figure 2 - Immobilization Techniques

Covalent attachment of enzymes to solid supports is achieved by using the enzymes amino or carboxyl groups, which are activated in order to attach the enzyme on to a support. Because these fictionalized groups do not affect catalytic activity the process does not deteriorate the efficiency of the EFC. Cross-linkage, like covalent attachment, uses functional groups. Yet cross-linkage may be inter or intra molecular cross-linkage. Entrapment is achieved by adding enzymes to a monomer solution, as the monomer solution becomes a gel the enzymes become trapped. Adsorption of enzymes is very simply done by mixing an enzyme solution with a solid support. Because the stability of the enzyme is paramount, methods for immobilization that provide stronger bonds are preferable in the design of EFC. In this aspect cross-linkage and covalent attachment are the best choice, yet the process requires more work and can become expensive. On the other hand adsorption and entrapment methods are simple but these methods produce weak bonds. Finally, encapsulation of enzymes is still a relatively new method for encapsulation which encases enzymes with a membrane which is impermeable by enzymes but permeable by low molecular weight products. (Minteer, 2007)

1.4 Proton Exchange Membranes

Proton exchange membranes operate just as its name would lead you to believe. Being sandwiched between the oxidizing terminal and the reducing terminal this thin electrolytic polymer film's primary purpose is to conduct protons from the anode to the cathode. However, it is crucial that electrons are prevented from traversing the membrane en route to the cathode, instead forcing the electrons to travel along an exterior circuit thereby generating current. If the membrane did not exhibit this selectivity the electrons would take the least resistive path which would effectively short circuit the fuel cell.

1.4.1 Nafion

There are a number of other polymer electrolyte membranes, however Nafion is often used as the benchmark material to which others are compared due to its superior performance. Nafion ionomers were first developed by Dr. Walther Grot while working for E. I Dupont Company in the late 1960's after nearly 15 years of research. This was created by altering Dupont's heavyweight commercial product, Teflon, which is extremely hydrophobic and lacks the ability to conduct electric current. Nafion was a novel development for its time because it created a class of polymers that had unique ionic and hydrophobic properties, made possible by the strategic placement of the perfluorovinyl ether with a sulfonate ending group onto Teflon's tetrafluoroethylene repeating mer units (Mauritz, 2004). The general chemical structure of a Nafion mer is shown in Figure 3below.

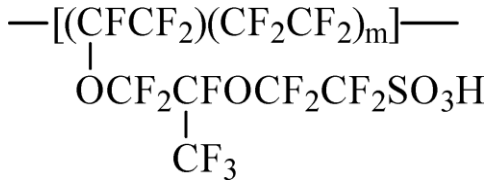


Figure 3 - A Nafion Mer Unit

Nafion has some very unique properties that make it ideal for use in fuel cells. The first is its ability to withstand relatively high temperatures. Nafion maintains its overall integrity and is very resilient to exposure from chemicals for temperatures up to at least 100 degrees Celsius. It does, however, become susceptible to degradation when exposed to alkali metals in solution under normal operating temperatures and pressures, thus preventing its use with lithium, sodium, potassium and other like Group 1A elements. Most notable are its water transport properties that arise due to the extremely high water of hydration levels for the sulfonic groups. This high permeability to water paired with the sulfonic groups tacked onto the Teflon backbone make Nafion very effective in shuttling cations through the membrane. Due to the mechanics of how the membrane is hydrated, membrane dehydration is a concern at temperatures above 80 degrees Celsius. Nafion must be stored in a controlled environment, preferably away from sunlight and in a well-sealed container, to prevent discoloration or buildup of organic material on the membrane surface which will obstruct active sites on the surface and decrease the membranes efficiency (Adigoppula, 2008).

The convention for naming Nafion is to use the letter N followed by a three to four digit number. The equivalent weight (EW), with respect to 100, is given by the first two numbers and the last digit or two represents the thickness of the membrane in mills (where 1 mil = 0.0254 mm). For example, the two most commonly used Nafion membranes are NR211 and NR212. This means that their EW are both 21 with their thicknesses being 1 and 2 mils respectively (Adigoppula, 2008).

1.5 Carbon Nanotubes

Carbon nanotubes (CNTs) have played a vital role in the development and function of fuel cells. In modern fuel cells, the usage of a Platinum-Rubidium (PtRu) catalyst has become a

financial obstacle in the fuel cell industry; the price of platinum and the amount required for an effective and useful surface area presents a significant barrier financially. To still be able to utilize known effective catalysts, nano-scale solutions have been investigated. By dispersing catalytic particles across a nano-scale support matrix composed of CNTs, the ratio of required amount of catalyst to the effective reactive surface area is lowered significantly. CNTs are an ideal and attractive candidate for this purpose not only due to their large surface area, but also because of their electric conductivity and relative stability (Geng, 2012). By increasing surface area and decreasing the amount of platinum required, CNTs allow fuel cells to become increasingly economically feasible. Additionally, the CNT matrix provides a conductive framework that surmounts the problem of connecting the enzymes to the electrodes of fuel cells.

1.5.1 Types of CNTs

Carbon nanomaterials can be categorized in many different ways depending on their structure. These materials can be broken into two primary categories: carbon nanotubes and carbon nanofibers. Within the family of nanotubes, there are multiwalled carbon nanotubes (MWCNTs) and singlewalled carbon nanotubes (SWCNTs). MWCNTs are concentric strands of CNTs. Single walled CNTs are single graphene sheets rolled into a tube. Depending on their intended function, each type of CNT serves its purpose.

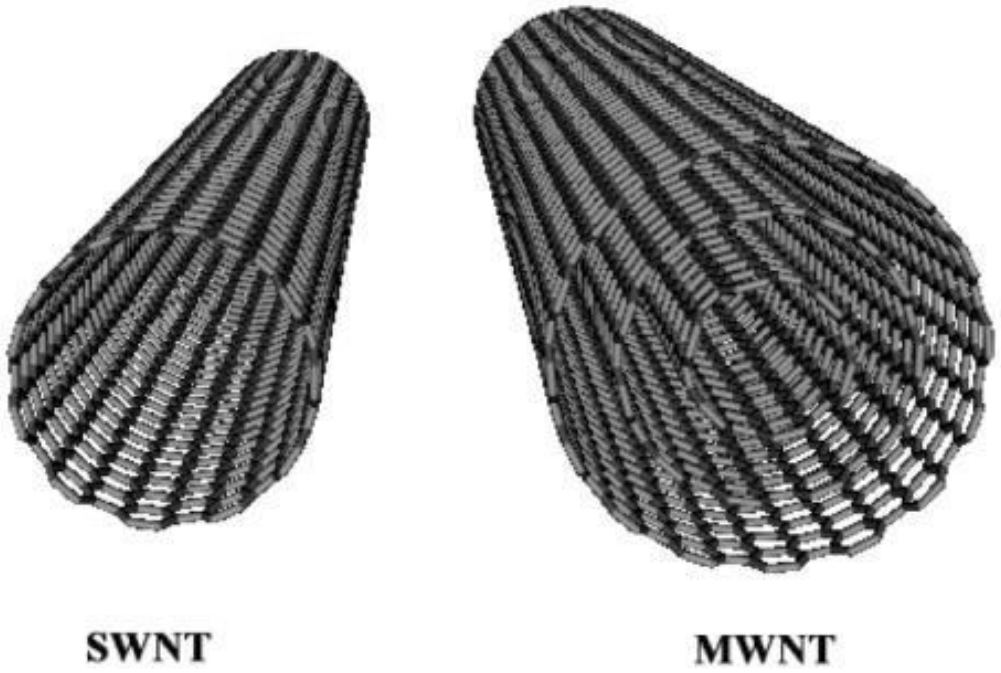


Figure 4 - Comparison of SWCNT's and MWCNT's

Both of these nanotubes can be modified in various ways to alter their properties. One such modification is known as graphenation. This process dramatically increases the surface area of the nanotubes while also increasing the charge density of the nanotubes (Stoner, 2011). Other modifications include attaching nanobuds to the external wall of the nanotube. Each specific type of modification accentuates part of the nanotube's physical behavior.

1.5.2 Which Types of CNTs Should Be Used?

Each type of CNT product presents benefits, disadvantages, challenges, and unique characteristics. For our purposes, maximizing the effective electrochemically active surface area is our primary concern. This must be accomplished while retaining an effective conductive pathway to the electrodes. MWCNTs appear to provide the best solution to this; they have the conductive characteristics of carbon nanotubes, but afford a larger surface area due to their increased diameter that is a function of their multi-walled structure. Ongoing experimentation continues to be done to determine the true electrochemically active surface of SWCNTs and MWCNTs to determine whether the increased surface area is beneficial (Zebda, 2011). From experiments conducted by Li et al., the choice to pursue research with MWCNTs is further validated; results indicate that the direct electron transfer (DET) of MWCNTs is adequate and more desirable than that of amorphous SWCNT structures. The treatment of the CNTs is indicated as a point of interest related to the ultimate effectiveness of the electrode; when produced, they are coated with amorphous carbon, which interferes with electron transfer between the catalyst and the electrode (Li, 2002). Heat treatment in air is a proposed method of removing these impurities, and the effects of this preparation method will be investigated in this experiment.

1.6 Literature Review of GOx Based Enzymatic Fuel Cells

In order to achieve a homogeneous dispersion of carbon nanomaterials in a particular solvent, one must wrap the CNTs in a polymeric chain such as polyetherimide (PEI) or polystyrene sulfonate (PSS). This polymer interferes with the hydrophobic interaction that would normally be exhibited between the water molecules and the smooth nanotube. When sonicated with CNTs the polymeric mixture is remarkably stable maintaining its original CNT suspension

appearance prolonged periods of time as a result of the polymeric wrapping adding mechanical stability to the CNTs.

Investigation of direct electron transfer was conducted by Cai and Chen (2004) using a Nafion-CNT/GC electrode and a Nafion-GOx-CNT/GC electrode in a 0.1 M PBS solution at a scan rate of 40 mV/s. As one would expect, Cai and Chen observe that there are not any notable voltammetric peaks obtained in the electrode without GOx. Conversely, reduction and oxidation peaks are observed around -0.5 V for the Nafion-GOx-CNT/GC electrode (Figure 5). This electrode was the subjected to exposure to a 3M guanidine hydrochloride (GndCl) solution overnight, which is capable of stripping FAD active centers from the GOx complex. The result, shown in Figure 6, was a disappearance of redox peaks in the overnight incubated case, suggesting the FAD built into the enzyme was responsible for this and had no impact on the FAD groups that were absorbed onto the electrode surface.

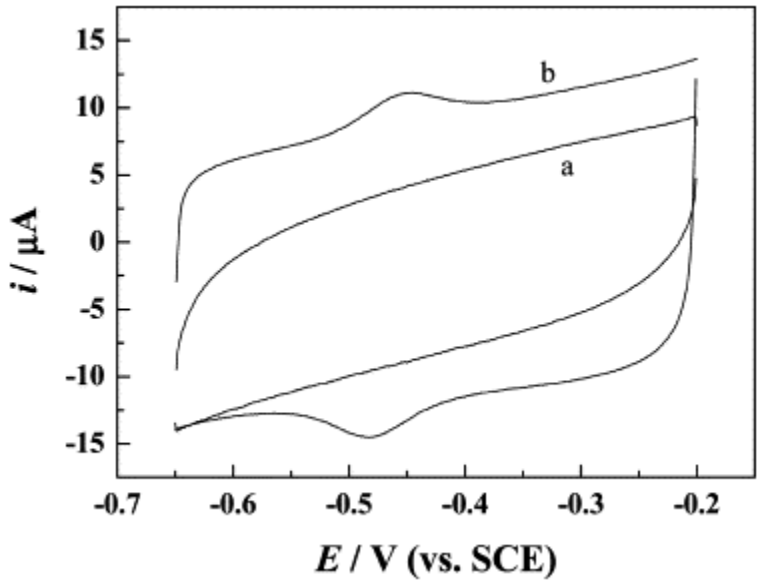


Figure 5 - Cyclic voltammogram of (a.) Nafion/CNT and (b.) Nafion/CNT/GOx

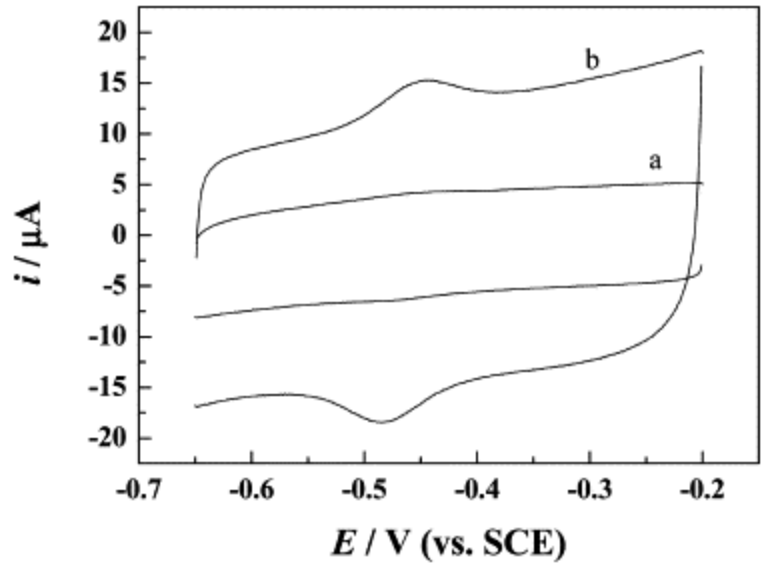


Figure 6 - Cyclic voltammogram of Nafion/GOx/CNT (a.) with GndCl and (b.) without GndCl

To determine whether the surfactant or nanomaterial was responsible for the direct electron transfer an electrode was prepared sans CNTs. The result was an absence of an electrochemical response – meaning the mechanism for DET is almost entirely provided by GOx molecules immobilized on the CNTs. The exact reasons for how CNTs exhibit this property are unknown, but there are postulations DET can be attributed to CNTs small dimensions and the presence of carboxylic acid and alcohol groups on GOx's surface. For a GC electrode using an GOx/Nafion/CNT electrode in 0.1 M PBS, the half-wave potential was found to conform to the equation $E_{1/2}$=−0.466±0.001 V for scan rates ranging from 20-140 mV producing an electron transfer rate constant (k_s) of 1.53 ± 0.45 s^{-1}.

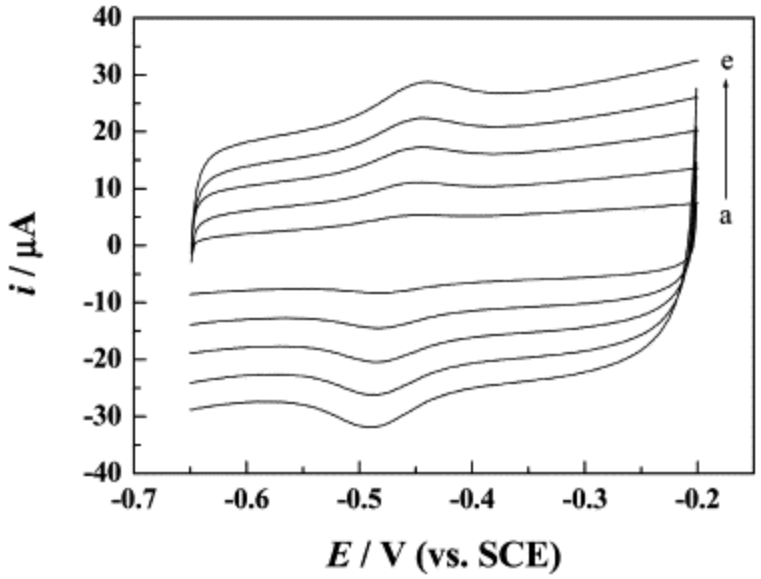


Figure 7 - Cyclic voltammograms of Nafion/GOx/CNT for scan rates of 20-100 mV/s

Through the modification of the electrolyte pH, Cai and Chen demonstrate that the shifting of the cathodic and anodic peak potentials produces a linear relationship between $E_{1/2}$ and pH that has a slope of -53 mV/pH close to the theoretical value of -58.6 mV/pH for a reversible, two-proton/two-electron reduction oxidation reaction. This confirms FAD deeply imbedded in GOx is indeed responsible for the redox currents observed.

1.7 Fundamentals of Cyclic Voltammetry

According to Bard and Faulkner (2000), cyclic voltammetry is one of the first techniques used to evaluate the effectiveness of the system's kinetics and thermodynamics. More specifically, when applied to fuel cells, these tests evaluate the reactivity of analyte at a given electrode. The peaks that are seen in the output from cyclic voltammetry runs help provide information about the kinetics of the reaction. When anodic current is generated, oxidation of products appears as anodic peaks on the upper portion of the CV graphical output.

The device used to perform cyclic voltammetry is called a potentiostat. It is created by using three electrodes, a working electrode, a reference electrode and a counter electrode, that are submerged in a supporting electrolyte that does not interfere with the cell's redox reactions. Potential, or voltage, is applied to the working electrode and swept over time using either a square wave pattern or a triangle wave pattern, depending on what the future use of the fuel cell. The triangle wave, shown above, is most frequently used. The reference electrode controls the potential applied while the counter electrode must conduct the current as it is being created at the working electrode. Maintaining the properties of the working electrode at a constant level is very important to ensure surface properties are not improperly skewed. The data collection process is all automated by an electroanalyzer program, and depending on the system you wish to study various CV inputs are specified such as the sampling technique (e.g. sweep, linear, etc.), scan rate, and minimum and maximum energy voltage values.

1.7.1 The Concept of Electrochemical Reversibility

Electrochemical reversibility is said to be when the redox system is able to sustain equilibrium for the entire scanning duration and is not hindered by electron transfer or rates of reactions. A cyclic voltammogram for a one electron process is said to be reversible if the peak potential separation ΔEp should is about 58 mV, the peak current ratio is around one, and the the peak current is independent of scan rates chosen. Inefficient electron transfer, or low k_s values, at the surface of the electrode can cause the peak potential separation to increase resulting in irreversibility of the system. This irreversibility is often termed quasi-reversibility, as the overall electrochemistry depends upon the scan rate chosen. For example, a low testing frequency can oftentimes help push the system in a reversible direction since k_{sv} dominates (Andrienko, 2008).

1.7.2 Experimental Variables and Uncertainties

The selection of correct variables can be critical to the success of determining the underlying reaction mechanisms. The most important choice one is confronted with when running voltammetry testing is how the voltage will be applied and over what range. Voltage, when applied to an electrochemical cell, is often referred to as potential as it represents the driving force available for oxidation or reduction reactions. The voltage can either undergo a single instantaneous step from V_1 to V_2 or a gradual increase over time from V_1 to V_2, where the voltage is said to be ramped until the ending voltage is achieved. There are benefits to each of these different voltage ramping techniques seen below.

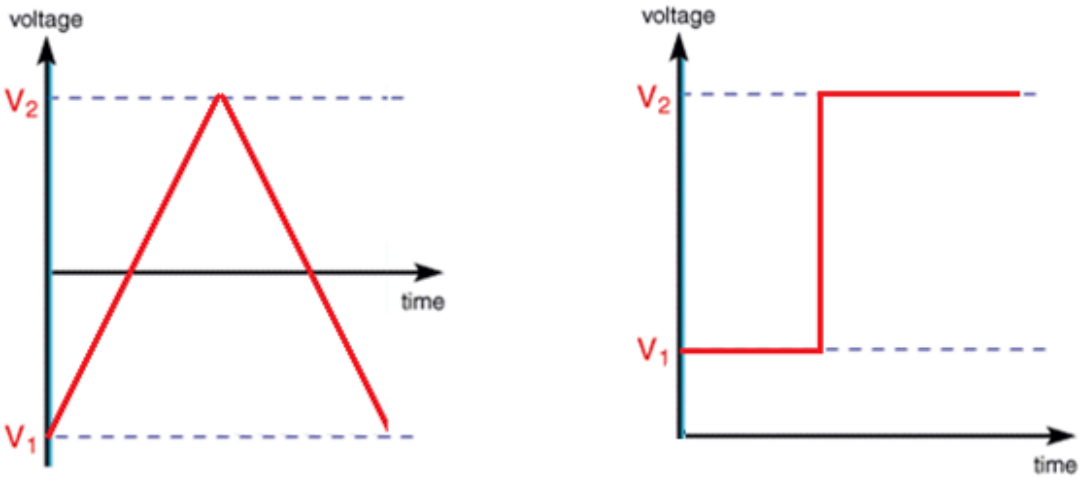


Figure 8 - Linear Potential Ramp vs. Potential Step

1.7.3 Maintenance of the equipment

Responsiveness of the electrode can gradually diminish over time do to the development of a coating (from reaction products) on the electrode surface. To ensure accurate results this surface must be adequately cleaned and polished. Complete and thorough cleaning procedures allow one to compare results even if they are collected several days apart. Included in Appendix

D is a summary of the protocol BASi recommends to ensure the electrodes are adequately cleaned.

2. Methods

Testing was completed in several cycles, with each cycle effectively achieving one of the following objectives until the final comparison of MWCNT and CNF is reached:

1. To optimize the CNF/GOx anode based upon MWCNT formulation in literature.
2. To test the difference in performance between MWCNT and CNF in a GOx based anode.
3. To assess which type of electrode is financially advantageous based upon longevity and potential current output.

The final electrode materials were characterized by scanning electron microscopy (SEM) in an attempt to understand better the results observed.

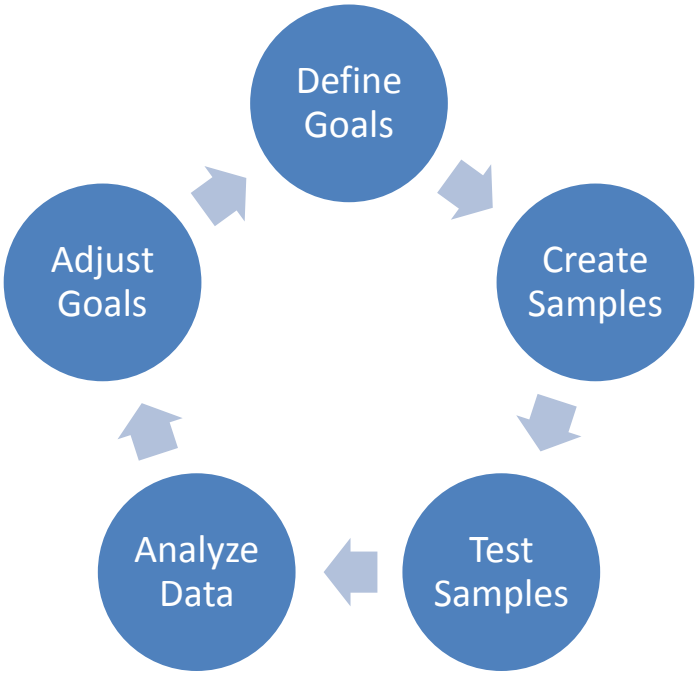


Figure 9 - Methods/Optimization Cycle

The ability to preserve samples for future use was an important aspect of our preparation procedure. Two separate intermediate samples, one for the enzyme dissolved in PBS and another for CNT/PEI, were prepared and then preserved until needed later. This was done to increase

shelf life of our testing sample, as previous test had shown the potential for a significant decrease in performance when frozen together.

2.1 Creation of Intermediate Enzyme Sample

To prepare the glucose oxidase enzymatic solution, phosphate buffer solution (PBS) of pH 7 was combined in a mini centrifuge tube (MCT) with a pre-specified amount of GOx (Sigma Aldrich) and sonicated for ten minutes in a Branson 2510 Ultrasonic bath so the contents would be evenly dispersed. It was important that the temperature of this bath be closely monitored to not exceed 100 degrees Fahrenheit. To ensure consistent amounts of GOx are used, forty milligrams was weighed on a scale with a sensitivity of 0.1 mg - variances of ± 0.2 mg were deemed acceptable. This intermediate GOx mixture was then frozen for later combination with the CNT/PEI solution that will be discussed in the following section. This GOx solution mixture is critical in that it allows one to withdraw a small but consistent amount of GOx while ensuring the enzyme is kept in a neutral environment to minimize the amount of enzymatic deactivation that might occur.

Figure 10 - 2510 Branson Sonicator

2.2 Creation of Intermediate Carbon Nanomaterial Sample

In the same manner as the enzyme testing sample, the carbon nanomaterial, either CNFs or MWCNT (Sigma Aldrich), was weighed in masses ranging from six to fifteen milligrams and combined with five weight percent PEI. The only difference between the nanomaterial testing solution and the enzymatic solution, was the acceptable variance in the weights that were used. In the case of the carbon material a stricter tolerance was used and only ±0.1 mg was allowed. In most cases a PEI concentration of five weight percent was used; however, in several runs we made use of ten and twenty weight percent solutions to study the effects of higher concentrations of PEI. Similar to before the prepared mixture was sonicated for ten minutes in a Branson 2510 Ultrasonic bath. These testing samples were stored in dark environments when not in use to mitigate the possibility of light damage.

2.3 Final Sample Preparation

To test the final anode mixture both of the intermediate enzyme and carbon material testing samples were combined. To ensure a representative sample was withdrawn both intermediate samples were once again sonicated for ten minutes after which approximately 100 μL of each was transferred into a microtubule. This GOx/CNT/PEI mixture must then be sonicated one more time before the it is ready for applied to the glassy carbon electrode for cyclic volammetry testing.

2.4 CV Testing

The fuel cell testing used the BASi Cell Stand (Figure 11) and the BAS100B Electrochemical Analyzer (Figure 12), and BAS100W Electrochemical Analysis Software. Once the BAS100W program was opened the parameters for the cyclic voltammetry runs had to be input. There were six different parameters that had to be accounted for: Initial E, High E, Low E,

Scan Rate, Sensitivity, and Segment Length. The Initial E and High E were kept the same constant throughout all CV runs at 800 mV and 800 mV respectively. Low E was set at -800 mV for the majority of tests, however Low E was changed to 0 mV for a few runs to better determine what the performance of the anode would be in a fuel cell. Scan rates were varied from 20 mV/s to 400 mV/s. Testing was started at higher scan rates (200-400 mV/s), but it was later determined that the cell was unable to reach steady state conditions at these levels. Once the scan rate parameter was reduced to 50 mV/s the cell was nearly able to reach steady state conditions at each data point. The sensitivity parameter was changed based on the performance of the cell to yield the optimal resolution.

Finally the segment length was chosen depending on the variables that were being tested. For samples that needed peak current measurements or location of redox peaks, four segments or just two cycles were sufficient, however when the stability of the electrode was of interest the tests were extended to thirty to fifty cycles allowing the performance of the electrode to be measured over an extended period of time.

Figure 11 - BASi Cell Stand

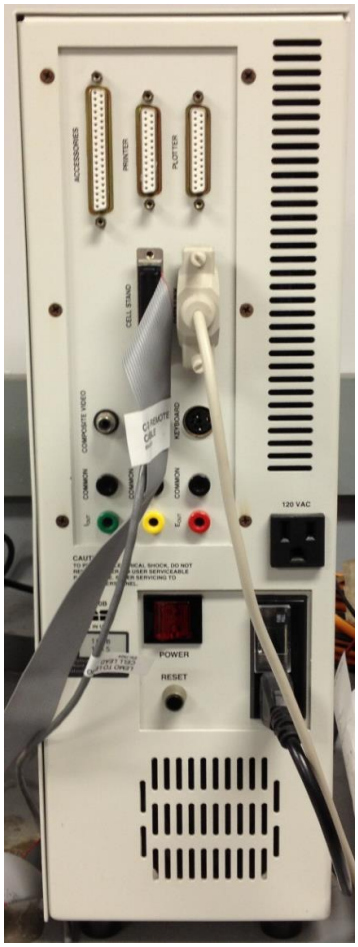


Figure 12 - BAS100B Electrochemical Analyzer

Once the parameters were established, the glassy carbon electrode, shown in the center of Figure 13 received a casting layer of the electrode nanomaterial and a Nafion layer. Specifically, this coating was applied by placing 6 μL of the electrode nanomaterial solution right in the center of the glassy carbon (GC) or working electrode. The application of the nanomaterial was critical in that it had to be placed just above the glassy carbon active portion of the electrode, the black central area in Figure 14. All accompanying liquids were then evaporated in standard ambient conditions; however, this was accelerated by the use of a fan blowing air at approximately 90 degrees Fahrenheit. Once fully dried, 20 μL of Nafion is then cast over the entire surface of the

working electrode in effect providing a layer of protection for the underlying electrode. Once dried, the working electrode is fully ready to undergo cyclic voltammetry testing.

Figure 13 - Reference, Working and Counter Electrodes

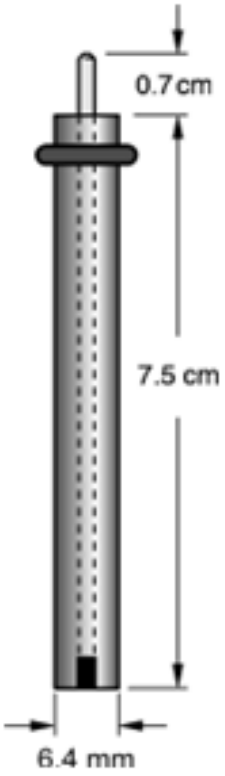


Figure 14 - Diagram of the Working Electrode.

After the preparation of the glassy electrode was complete, the CV testing glass was filled with 10 mL of CV electrolyte solution of PBS containing 20 μM glucose. This volume was just enough to sufficiently submerge all of the electrodes. The final result, shown below in Figure 15 is the CV testing stand ready to undergo electrochemical testing. All data was then exported from the BAS100W analysis software to Microsoft Excel for further analysis.

Figure 15 - The assembled CV testing stand

2.5 SEM Testing

To conduct SEM testing on the prepared electrode nanomaterial mixtures they must first be sonicated to ensure even distribution and full emulsification. Next the sample was cast onto copper foil to simulate the glassy carbon electrode, and dried in a furnace at 70 degrees Celsius for twenty four hours to ensure no water remains in the sample. After the sample is fully dried a small piece is then taped onto the small circular sample holder by carbon tape. This is done to ensure it remains stationary during testing and to enhance the electroconductivity. Steps for loading, focusing the image and unloading the sample from the SEM were followed from the JSM – 7000F SEM manual. Images were taken at several different magnifications and a scale was added to facilitate the taking of any rough measurements.

3. Results and Analysis

A series of electrochemical tests provide information about the feasibility and long term potential of using CNFs as a substitute for CNTs. This was done by first establishing a standard method by which to cast the electrode, then a multitude of parameters were adjusted and the effects on cyclic voltammetry current output were observed to determine ideal electrode compositions. Many of the tests utilize cyclic voltammetry tests, the individual run result obtained from this electrochemical testing can be seen in more detail in the Appendix.

3.1 Standardization of CNT/PEI/GOx Casting Layer

The standardization of the electrode casting layer presented the largest problem area in our initial tests. As a result, for cyclic voltammetry testing it was critical to develop an accurate method of metering in order to ensure all cyclic voltammetry runs would be easily repeatable and comparable. Many literature results provide the power density, but fail to normalize this with respect to the enzyme loading. The result of this is significantly different power densities depending on the amount of electrode nanomaterial cast onto the glassy carbon electrode. Figure 16 below shows the wide range of current densities that can be obtained by varying the drop size from small to large represented by 6μL to 20 μL, respectively. Lastly, given the minute volumes used it was important to ensure the mixture was fully emulsified and in full colloidal suspension.

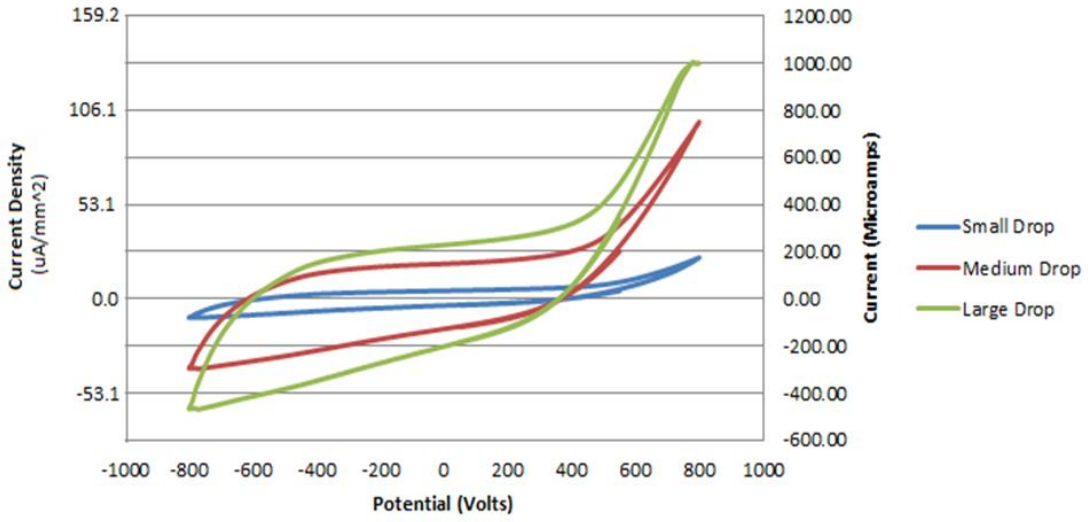


Figure 16 - Impact of Nanomaterial Droplet Size

This appreciable difference in power density for increased volumes is simply the result of a directly proportional increase in the amount of enzyme available to oxidize the glucose which thereby generates additional electrons achieving an increase in current. Past literature has shown the difficulty for an enzyme carbon nanomaterial matrix to exhibit direct electron transfer, especially in when using GOx due to its highly inaccessible active site. As a result, it was necessary to ensure that the majority of the electrode casting was applied directly on top of the glassy carbon electrode. When the largest drop was applied there was a significant amount of the electrode that was directly contact with the electrode's plastic casing. The current generated by enzymes above this outer ring is effectively unused as a majority of the electrons would be unable to travel the several millimeters required to reach the glassy carbon material. This problem was remedied, and the electrode's surface was fully covered with the application of a smaller casting volume of just six microliters. It should be noted all future current densities that are based upon an electrode application of six microliters. A representative illustration of three

different size electrode casting layers is shown below in Figure 17**Error! Reference source not found.**.

Figure 17 - Representative Illustration of Electrode Casting Layer vs. Volume

As briefly mentioned in the methodology section our experiments made use of a fan that blew air at approximately 90 degrees Fahrenheit, just below the glucose oxidase's optimal operating temperature of 104 degrees Fahrenheit. This air supply greatly increased the rate at which the liquid would evaporate and increased consistency of the electrode casting layers as it eliminated the presence of any bubbles that might otherwise form leaving an undesirable void on the electrode surface. Ultimately, the use of this slightly warm air generated no changes in currents observed and exhibited no observable negative impact on the stability of the electrode, so in response this accelerated drying procedure was used for all future tests.

3.2 Optimization of CNF Based Electrode

To assess the viability of a CNF/GOx anode it was necessary to determine the ideal ratio of CNF to PEI and increase the synthesized electrode's stability. First, peak current was obtained to be 0.25 µA/mm2 per mg at a CNF mass loading of 12 milligrams. CNF loading levels of greater than 12 mg produced similarly high peak currents, however loading levels below 12 mg exhibited a steep decline in power output.

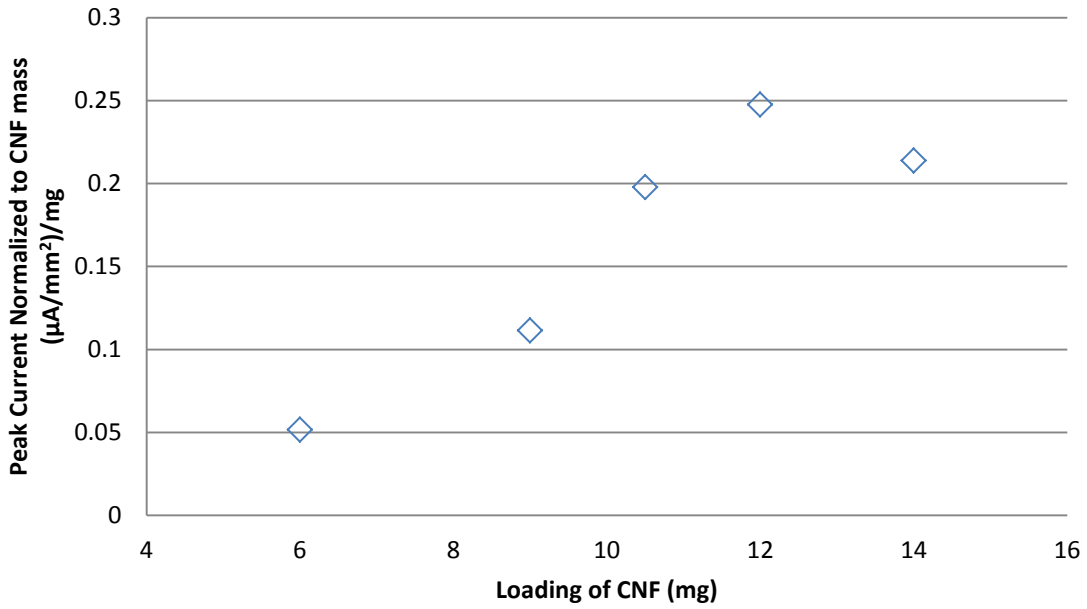


Figure 18 - Optimization of CNF Electrode (basis of 1 mL PEI)

After completing several CV runs with this newly optimized CNF electrode, it became clear the electrode suffered from a rapid decline in current within the first two to three cycles. This was confirmed by visual inspection, which showed the applied electrode diffusing into the analyte despite the presence of a coat of Nafion. With the degradation of the electrode being purely due to a physical phenomenon our goal was to increase the overall stability of the electrode.

Based upon literature sources, one possible way to prevent the failure of the Nafion casting layer was to ensure it is the evenly distributed and fully dried for around two hours at room temperature. This procedure, however, is to be balanced with the risk of enzyme deactivation. When allowing the Nafion additional time to dry we observed a significantly lower power output and a negligible increase in electrode stability. The second method used to increase the electrode's longevity was the application of a second casting layer of Nafion. This additional casting layer created a barrier to diffusion when applied in two separate applications reducing the

peak current from around 80 µA to just less than 10 µA in the two coat case. Figure 19 below shows the importance of a single casting layer of the 5 weight percent Nafion in retarding the leeching of unimmobilized enzymes into the electrolyte.

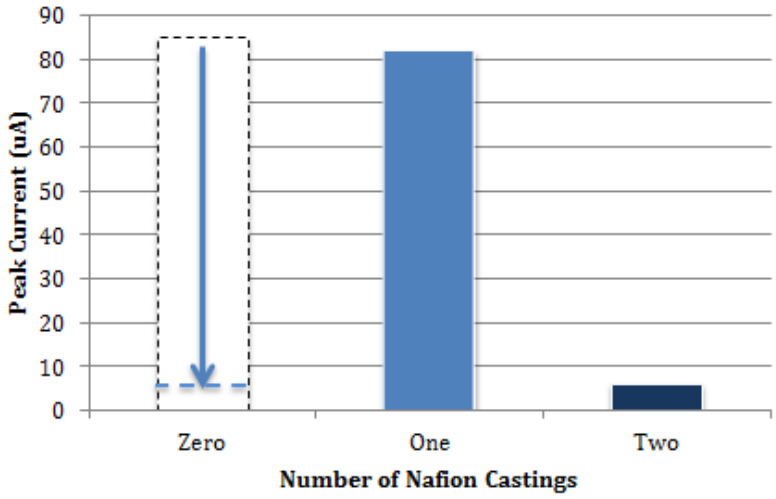


Figure 19 - Nafion Casting Impact

3.3 Comparison of CNT and CNF Anodes

Ultimately we wish to compare the performance of our optimized CNF based electrode with the CNT based electrode. Since mass of carbon nanomaterials differed for each electrode type it was necessary to normalize the peak current to the CNT/CNF mass. The results, shown graphically below in Figure 19, reveal the superior performance for CNTs. It can be easily seen that the normalized peak current for the CNF based electrode declines to twenty eight percent of its original value within fifteen minutes of testing whereas the CNT electrode does not drop below forty percent of its initial peak current reading in the twenty two minute testing duration. This tells us that the CNT based electrode is both superior at facilitating electron transfer and being much more stable as measured by the decline in peak current output.

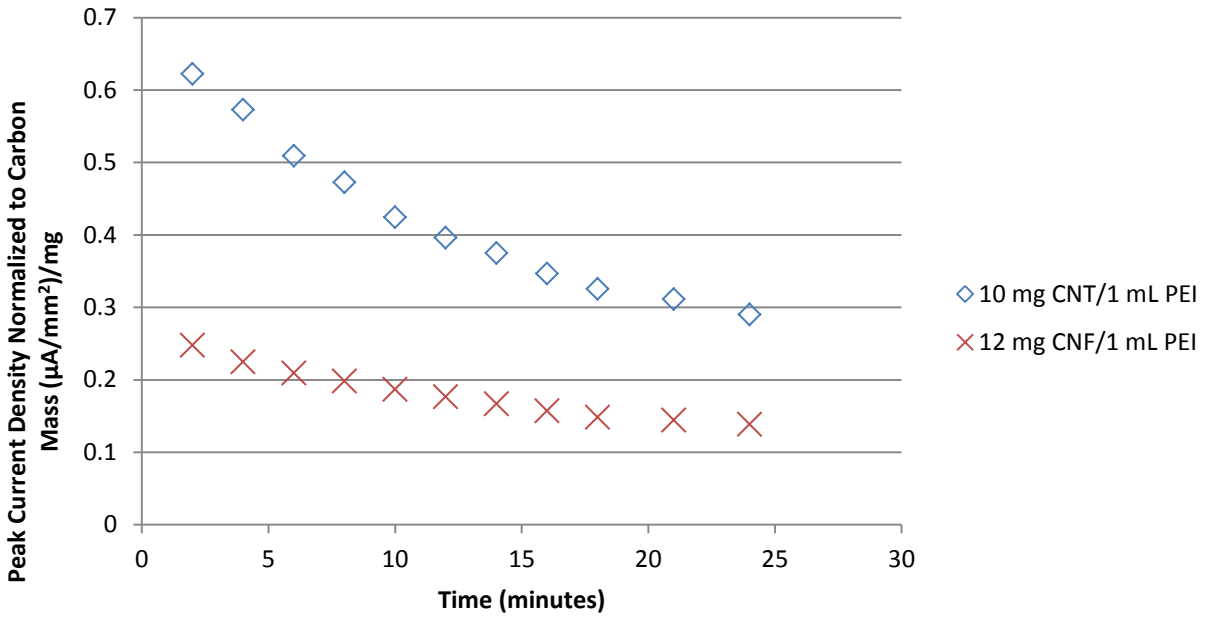


Figure 20 - CNF vs. CNT Performance and Longevity

The theory that the CNT based electrode exhibited better electron transfer was further strengthened by the appearance of redox peaks in the several cyclic voltammetry runs as can be seen in Appendix B. While many times these reduction and oxidation peaks were small, especially in comparison to the peak output they demonstrated that the CNT formulation made direct electron transfer from the FAD deeply embedded in the enzyme active site to the GC electrode achievable. As expected based upon literature sources, these FAD redox peaks were visible within their characteristic potential range of -500 V to -400 V. It must be noted, however, that the appearance of DET was observed on less than ten percent of CNT CV tests suggesting there is a difficulty in consistently achieving DET. This is not unusual as the electrode does not make use of a substrate such as Toray Paper which can facilitate better transfer of electrons from carbon nanomaterial to the electrode itself.

3.4 Study of Surface Morphology

The morphology of the two electrodes was studied via SEM, in an attempt to elucidate why the CNF based electrode performs significantly better. The SEM imagery, shown on the following page in **Error! Reference source not found.**, provides a clear visual of the differences between the CNF and CNT based electrode. Image D shows the uniform appearing CNF electrode at 2,000 times magnification. Here the electrode surface is smooth and individual tubules are only visible in the region where there is appears to be a defect from the process of synthesizing the electrode. In contrast, Image B which provides the CNF electrode at a similar magnification shows the nanofibers to be highly interwoven and the electrode surface full of nanofibers projecting in various directions suggesting that the PEI perhaps is not as effective at wrapping up nanofibers. Using the higher magnification images it is clear that the nanomaterial packing of the CNF based electrode is hindered by the diameter of the nanofibers that are in some cases as large as 0.25 micrometers. This in turn produces a loosely packed support matrix that is not conducive to facilitating direct electron transfer. Again the CNT based electrode displays tight packing and where a defect is observed the tubules appear to be naturally aligned which can provide a high amount of contact between adjacent nanotubes.

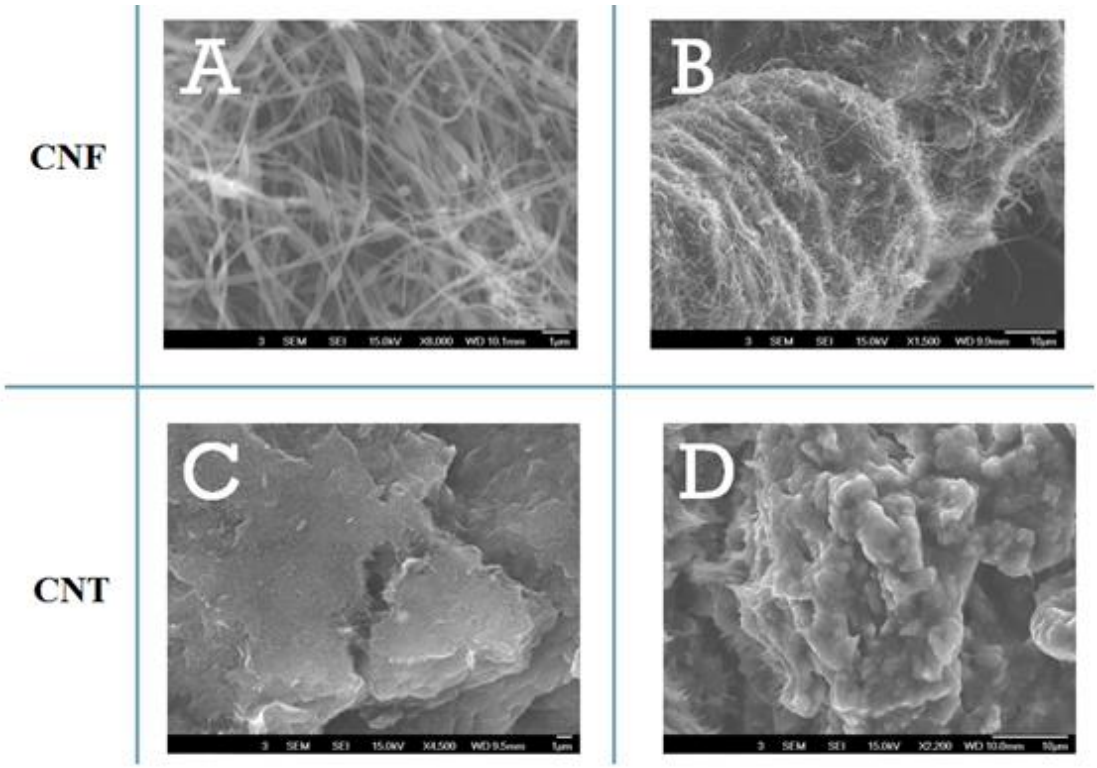


Figure 21 - SEM of CNT/CNF Electrodes (higher magnification on left)

4. Conclusions and Recommendations

CNFs were found to be an inferior support material for a glucose oxidase based anode in comparison to MWCNTs. The MWCNTs demonstrated consistently higher currents and in some cases the appearance of redox peaks were even visible, none of which were observed when using the CNF electrode. This demonstrated that based upon our electrode formulation, MWCNT were able to provide a better electron conduction pathway from the enzyme active site to the electrode. Even with CNFs being at a lower price point the cost per ampere was never favorable. Despite these unfavorable results, a CNF based electrode was developed and optimized. Attempts at increasing the stability of this electrode were made; however all such attempts were unsuccessful.

While the MWCNT results appear promising, it could be worthwhile to investigate the performance of another enzyme in the event glucose oxidase has poor compatibility with CNFs. Attempts to better align the nanofibers to create a tighter packing density could also potentially yield a fruitful increase in the current density, as the loose highly random packing evident in SEM images would be a hindrance to transfer of electrons to adjacent carbon fibers. As discussed in the *SEM Imagery* section, the use of another polymeric suspension could potential produce a better CNF packing arrangement.

References

Adigoppula, Vinay Kumar. "A Study on Nafion Nanocomposite Membranes for Proton Exchange Membrane Fuel Cells." Master of Science Wichita State University, 2011. Print.

Akers, Nick L., Christine M. Moore, and Shelley D. Minteer. "Development of alcohol/O2 Biofuel Cells using Salt-Extracted Tetrabutylammonium bromide/Nafion Membranes to Immobilize Dehydrogenase Enzymes." *Science Direct* (2005). Print.

Atanassov, Pamen, Chris Apblett, Scott Banta, Susan Brozik, Scott Barton, Michael Cooney, Sanjeev Mukerjee, and Shelley Minteer. "Enzymatic Biofuel Cells." *The Electrochemical Society Interface* (2007): 28-31. Web. 1 Oct. 2012.

Cai, Chenxin, and Jing Chen. "Direct Electron Transfer of Glucose Oxidase Promoted by Carbon Nanotubes." *Analytical Biochemistry* 332.1 (2004): 75-83. Print.

Geng, Xi, et al. "In-Situ Synthesis and Characterization of Polyethyleneimine-Modified Carbon Nanotubes Supported PtRu Electrocatalyst for Methanol Oxidation." Print.

Geng, Xi. "Synthesis and Characterization of Nanostructured Carbon Supported Pt-Based Electrocatalysts." Master of Science Worcester Polytechnic Institute, 2012. Print.

Guiseppi-Elie, Anthony, Chenghong Lei, and Ray H. Baughman. "Direct Electron Transfer of Glucose Oxidase on Carbon Nanotubes." *Nanotechnology* (2002). Print.

Hu, Chengguo, and Shengshui Hu. "Carbon Nanotube-Based Electrochemical Sensors: Principles and Applications in Biomedical Systems." *Journal of Sensors* (2009). Print.

Ivanov, Ivan, Tanja Vidaković-Koch, and Kai Sundmacher. "Recent Advances in Enzymatic Fuel Cells: Experiments and Modeling." *Energies* 3 (2010): 803. Print.

Ivnitski, Dmitri, et al. "Glucose Oxidase Anode for Biofuel Cell Based on Direct Electron Transfer." *Electrochemistry Communications* 8 (2006): 1204. Print.

Kim, Jinseong, et al. "Development of a Biofuel Cell using Glucose-Oxidaseand Bilirubin-Oxidase-Based Electrodes." *Solid State Electrochem* (2009). Print.

Klibanov, Alexander M. "Immobilized Enzymes and Cells as Practical Catalysts." *Science* 219.4585 (1983): 722. Print.

Li, Jun, et al. "Novel Three-Dimensional Electrodes: Electrochemical Properties of Carbon Nanotube Ensembles." *Physical Chemistry* 106 (2002): 9299. Print.

Li, Xianchan, et al. "A Miniature glucose/O2 Biofuel Cell with Single-Walled Carbon Nanotubes-Modified Carbon Fiber Microelectrodes as the Substrate." *Electrochemistry Communications* 10 (2008): 851. Print.

Mauritz, Kenneth A., and Robert B. Moore. "State of Understanding of Nafion." *Chemistry Review* 104 (2004): 4535. Print.

Minteer, Shelley D., Bor Yann Liaw, and Michael J. Cooney. "Enzyme-Based Biofuel Cells." *Current opinion in biotechnology* 18.3 (2007): 228-34. Web.

Osman, M. H., A. A. Shah, and F. C. Walsh. "Recent Progress and Continuing Challenges in Bio-Fuel Cells. Part I: Enzymatic Cells." *Biosensors and Bioelectronics* 26 (2011): 3087. Print.

Sarma, Anil Kumar, et al. "Recent Advances in Material Science for Developing Enzyme Electrodes." *Biosensors and Bioelectronics* 24 (2009): 2313. Print.

Sheldon, Roger A. "Enzyme Immobilization: The Quest for Optimum Performance."*Advanced Synthesis & Catalysis* 349.8-9 (2007): 1289-307. Print.

Stoner, Brian R., Akshay S. Raut, Billyde Brown, Charles B. Parker, and Jeffrey T. Glass. "Graphenated Carbon Nanotubes for Enhanced Electrochemical Double Layer Capacitor Performance." *Graphenated Carbon Nanotubes for Enhanced Electrochemical Double Layer Capacitor Performance* 99.18 (2011): 3 pgs. Print.

Thampan, Tony, et al. "PEM Fuel Cell as a Membrane Reactor." *Catalysis Today* 67 (2001): 15. Print.

"The Transition Electron Microscope." Print.

Wang, Shiunchin C., et al. "Membrane-Less and Mediator-Free Enzymatic Biofuel Cell using Carbon nanotube/porous Silicon Electrodes." *Electrochemistry Communications* 11 (2009): 34. Print.

Willner, I., et al. "Integrated Enzyme-Based Biofuel Cells." *Fuel Cells* (2008)Print.

Zebda, Abdelkader, et al. "Mediatorless High-Power Glucose Biofuel Cells Based on Compressed Carbon Nanotube-Enzyme Electrodes." *Nature Communications* (2011). Print.

Zhou, Chunmei, et al. "MnO_2/CNT Supported Pt and PtRu Nanocatalyst for Direct Methanol Fuel Cells." *American Chemical Society* (2009)Print.

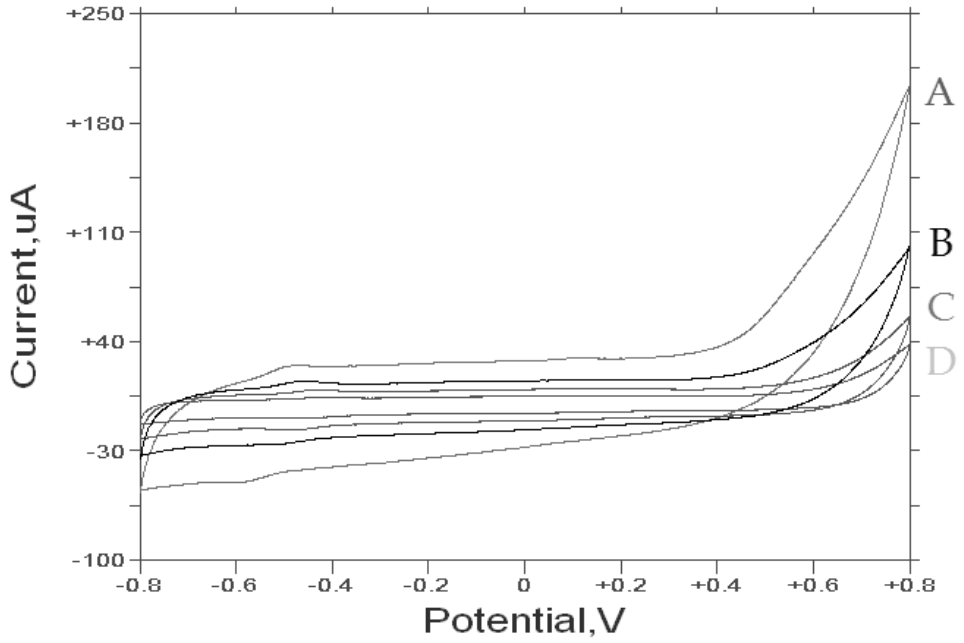
Zhu, Zhiguang, et al. "Deep Oxidation of Glucose in Enzymatic Fuel Cells through a Synthetic Enzymatic Pathway Containing a Cascade of Two Thermostable Dehydrogenases." *Biosensors and Bioelectronics* (2012)Print.

Appendix A: Detailed CV Testing Procedures

1. Using testing solution place one drop ONLY on glassy carbon portion of the electrode and let dry.
    a. Creation of continuous air flow either by blowing lightly or fanning the electrode will speed up drying without compromising the test.
2. Place one drop of 5 wt% Nafion solution on top of testing solution completely covering the first application layer and let dry.
    a. Same drying procedure as testing solution can be applied here
3. CV testing glass should be filled with approximately 10 ml of phosphate buffer (25 mM glucose and .1 M NaCl).
    a. 10 ml should be enough to completely cover electrodes. If this is not the case, increase the volume of solution used.
4. CV testing glass should then be capped and a parafilm seal should be applied to remove any possible contamination from air.
5. Three electrodes then need to be inserted into the top of the CV testing glass; glassy carbon electrode (test sample), working electrode, and counter electrode.
6. CV testing glass placed into CV testing station and all electrodes connected to appropriate wires
7. EAS100W program used to collect data. Parameters must be chosen to obtain most accurate data.
    a. Parameters used were; High E = 800 mV, Low E = -800 mV, Scan Rate = 100 mV/s, and Sensitivity = mA/V. Number of segments were varied based on information needed from test.

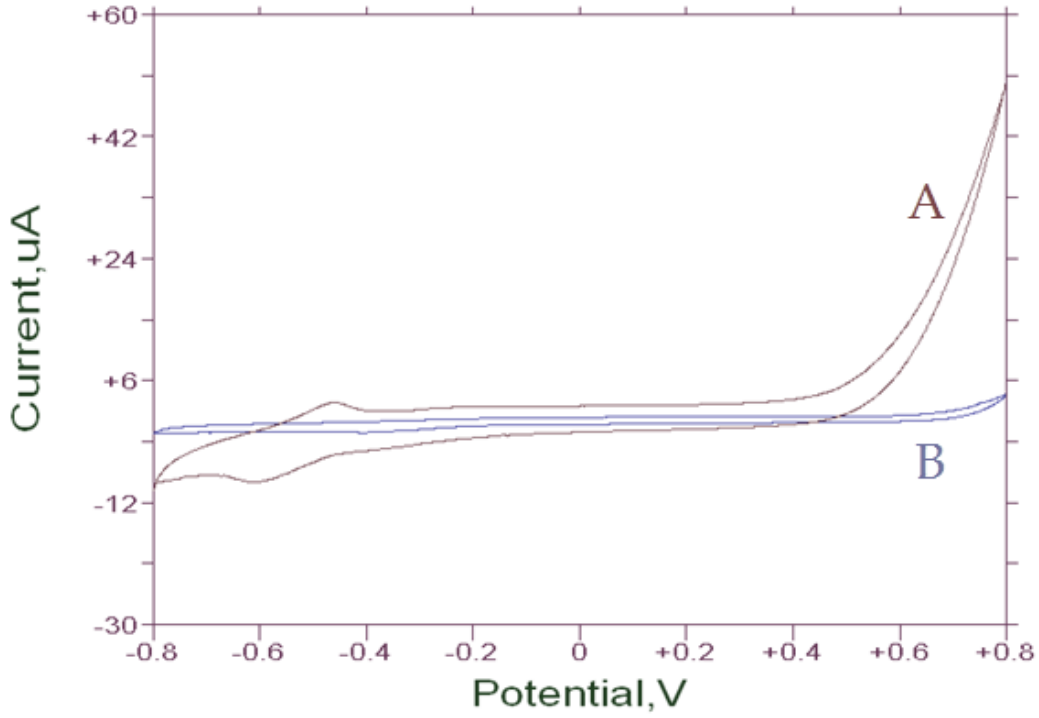
Appendix B: Cyclic Voltammetry Data

CNT/PEI/GOx-GC Electrode: Scan rate varied from (a.) 100 (b.) 50 (c.) 30 (d.) 15 mV/s



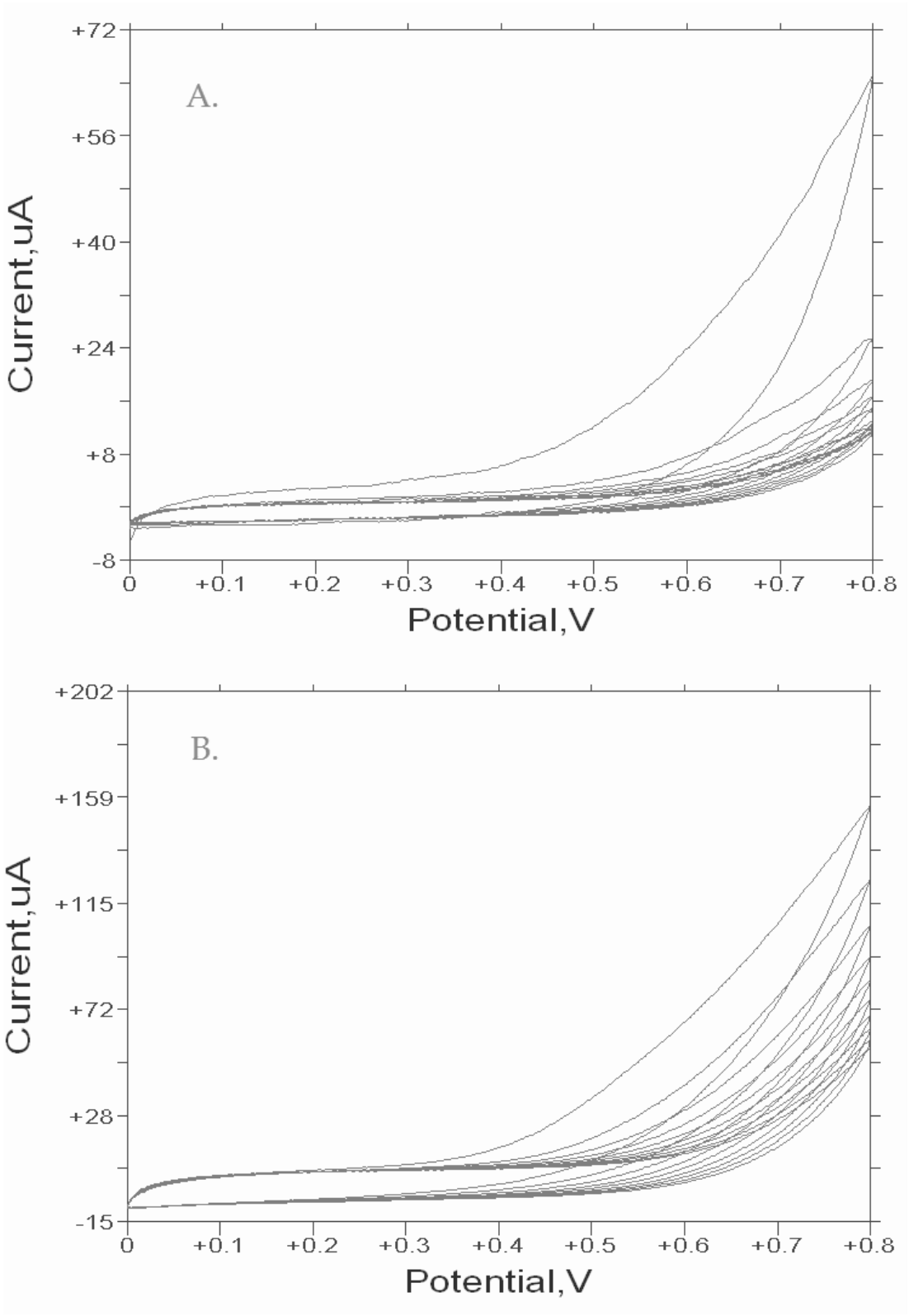
CNF/PEI/GOx - GC Electrode: Increase stability using (a.) one Nafion coat vs. (b.) two coats

(40 segments, Scan Rate – 50 mV/s)



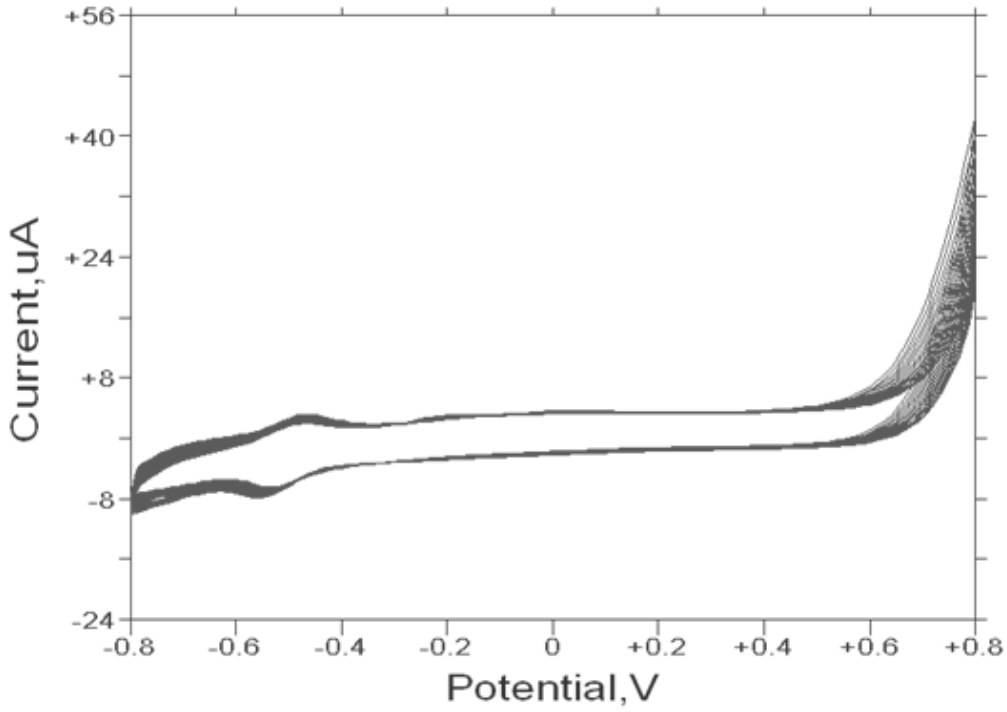
Positive Potential Testing – (a.) CNF/PEI/GOx (b.) MWCNT/PEI/GOx

(20 segments, Scan Rate – 50 mV/s)



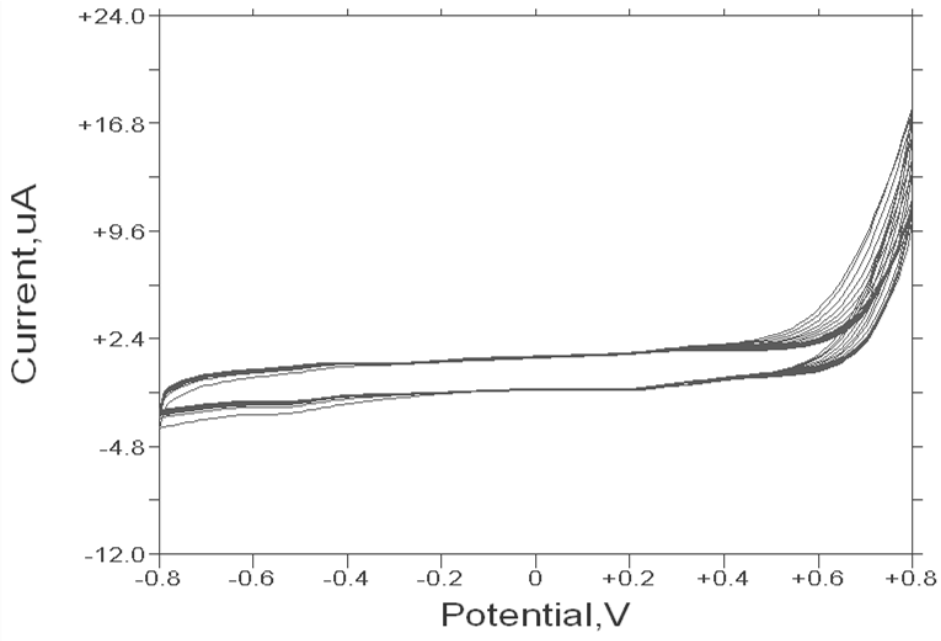
CNT/PEI/GOx-GC electrode

(40 segments, Scan Rate – 50 mV/s)



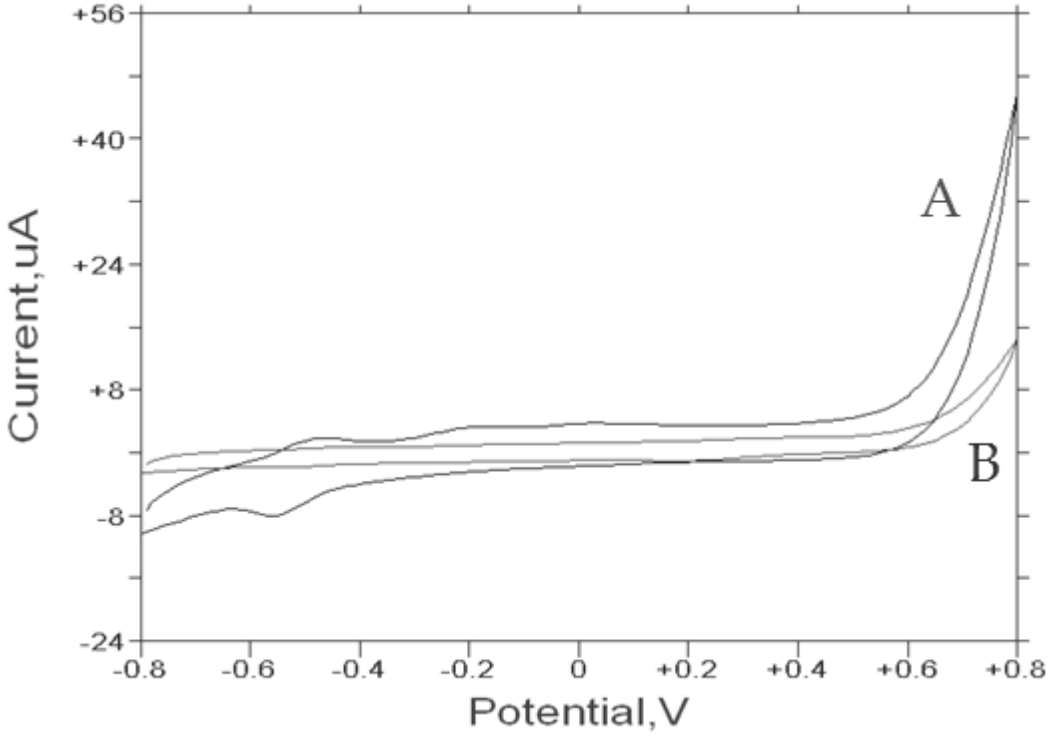
CNF/PEI/GOx-GC electrode

(40 segments, Scan Rate – 50 mV/s)



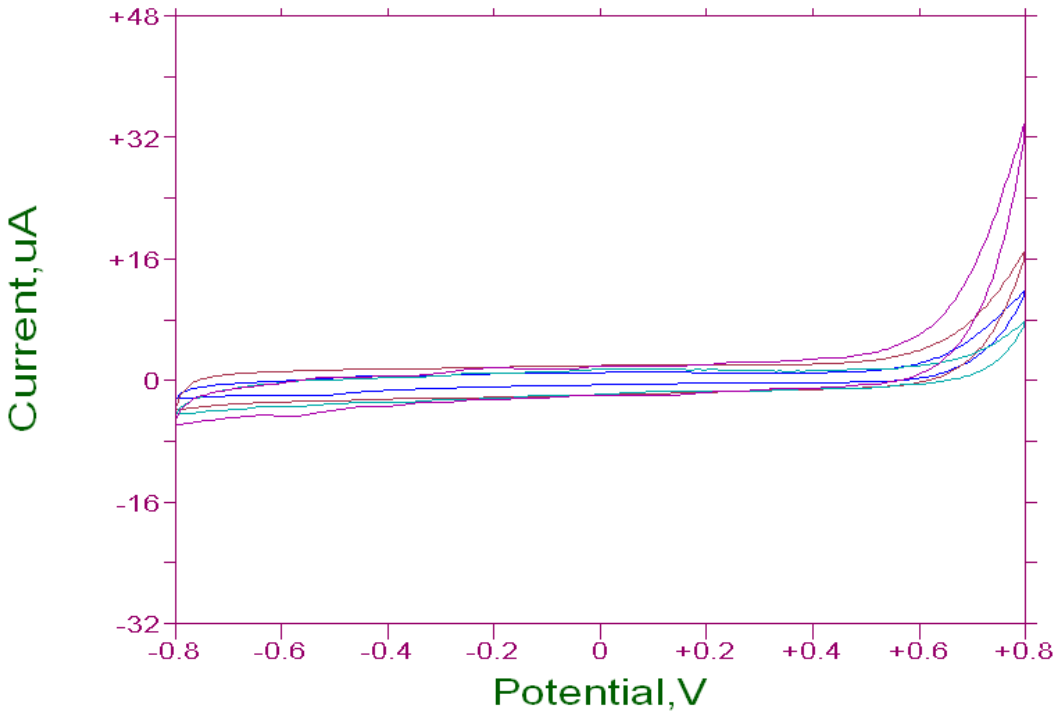
Single Cycle Comparison of (a.) MWCNT and (b.) CNF

(Segments 2 and 3, Scan Rate – 50 mV/s)



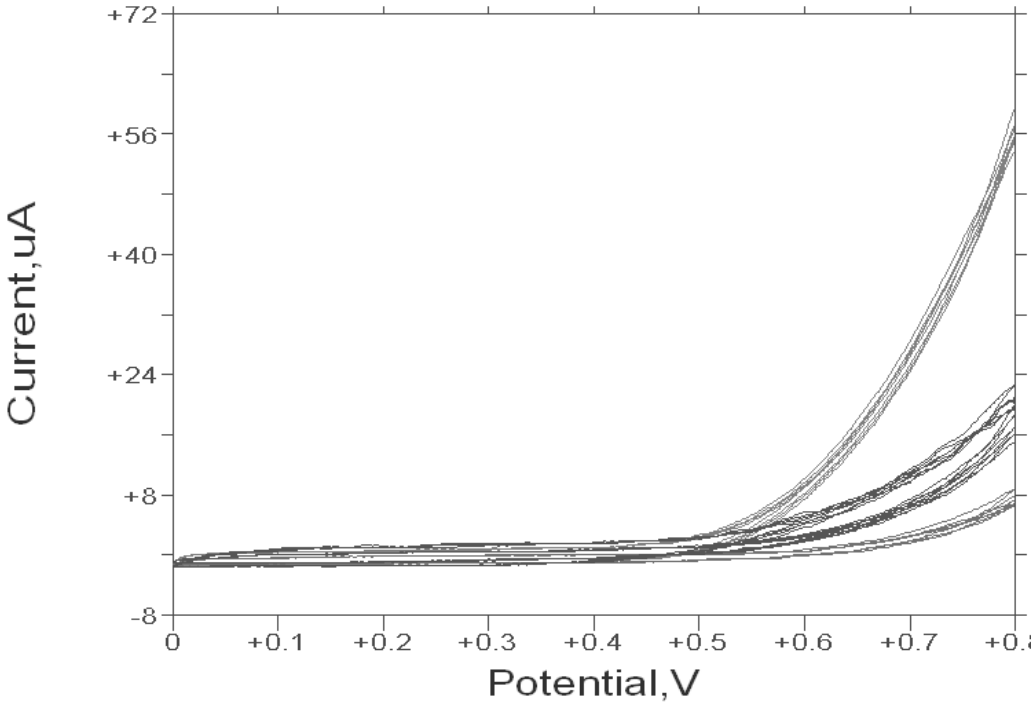
Single Cycle Comparison of CNF Anode using (a.) 8 (b.) 10 (c.) 14 and (d.) 12 mg CNF

(Segments 2 and 3)



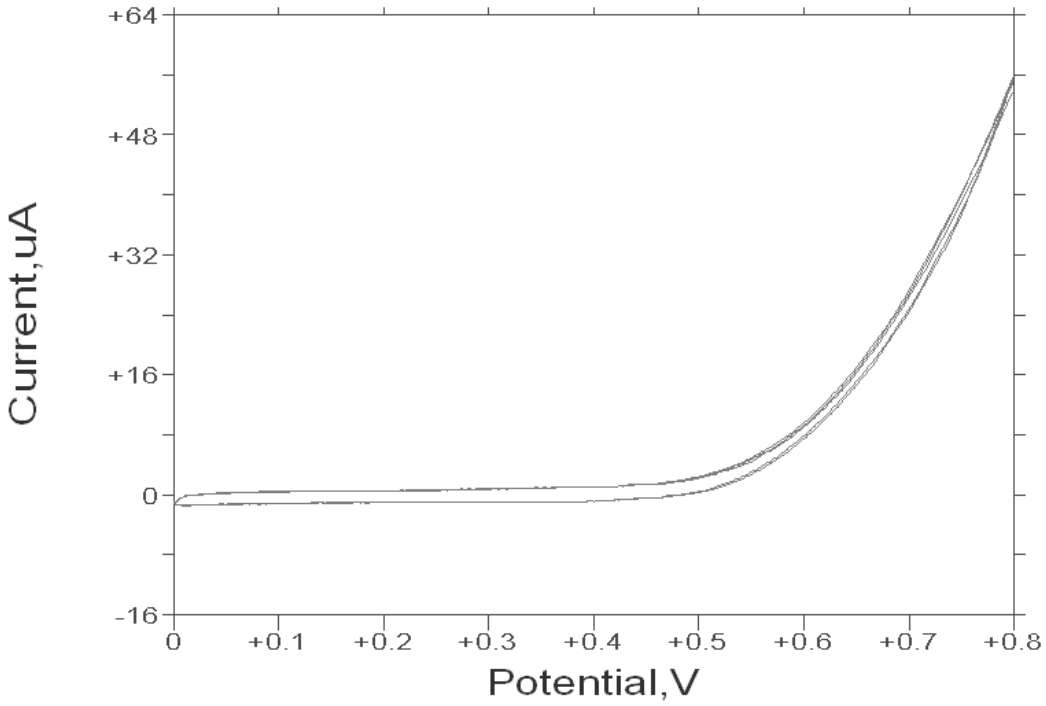
Comparison of CNF Anode using (a.) 6 (b.) 8 (c.) 10 and (d.) 12 mg CNF

(6 segments, Scan Rate – 20 mV/s)



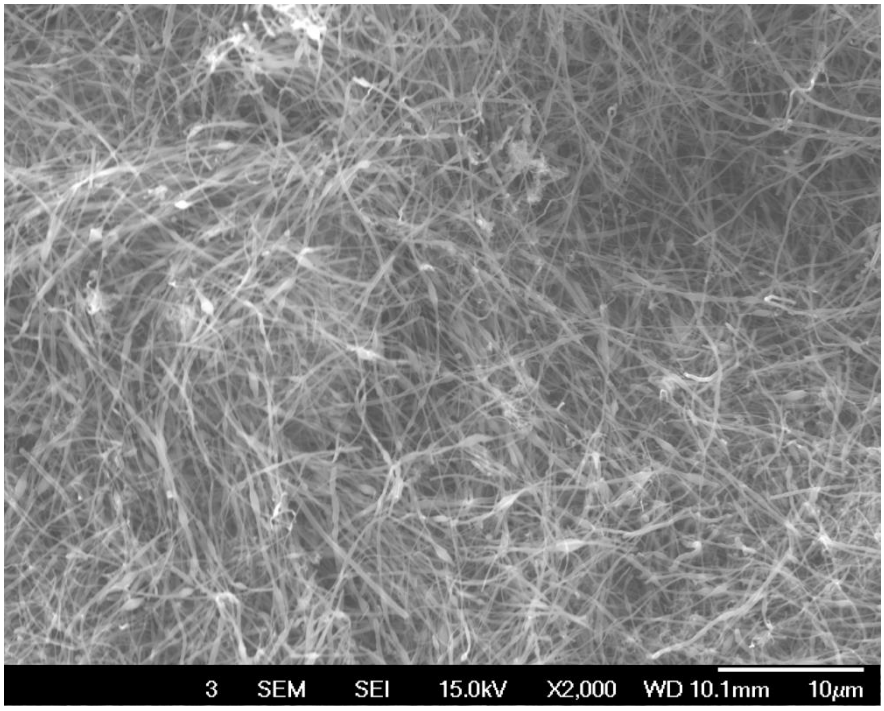
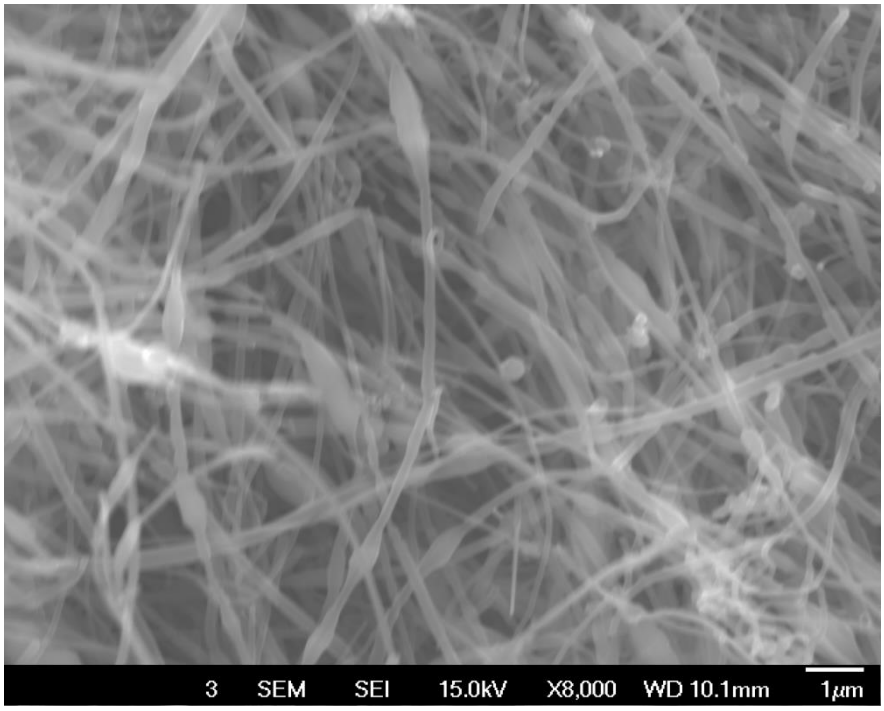
CNF/PEI/GOx Anode 12 mg CNF

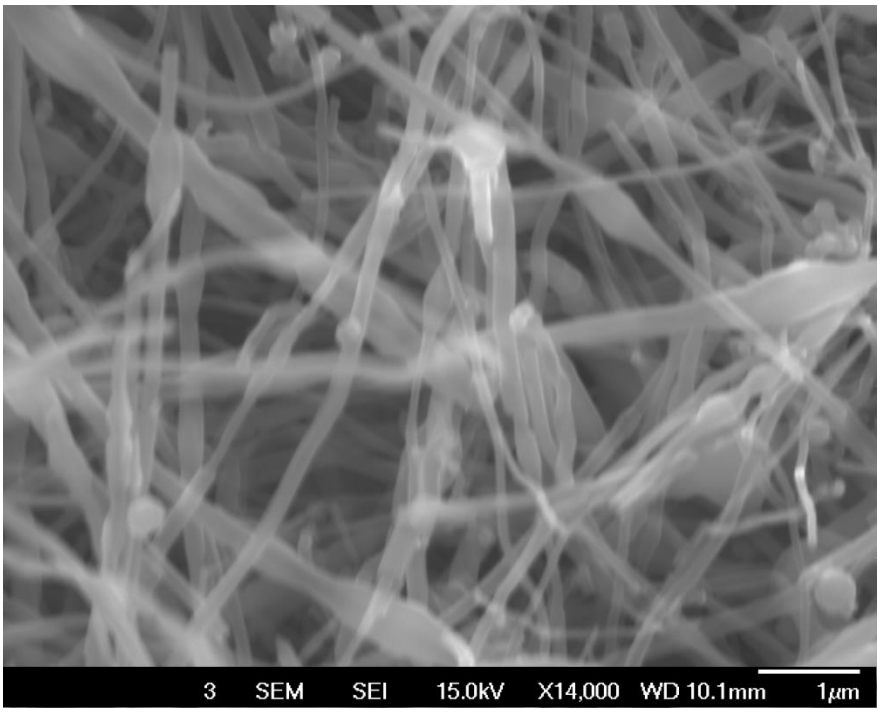
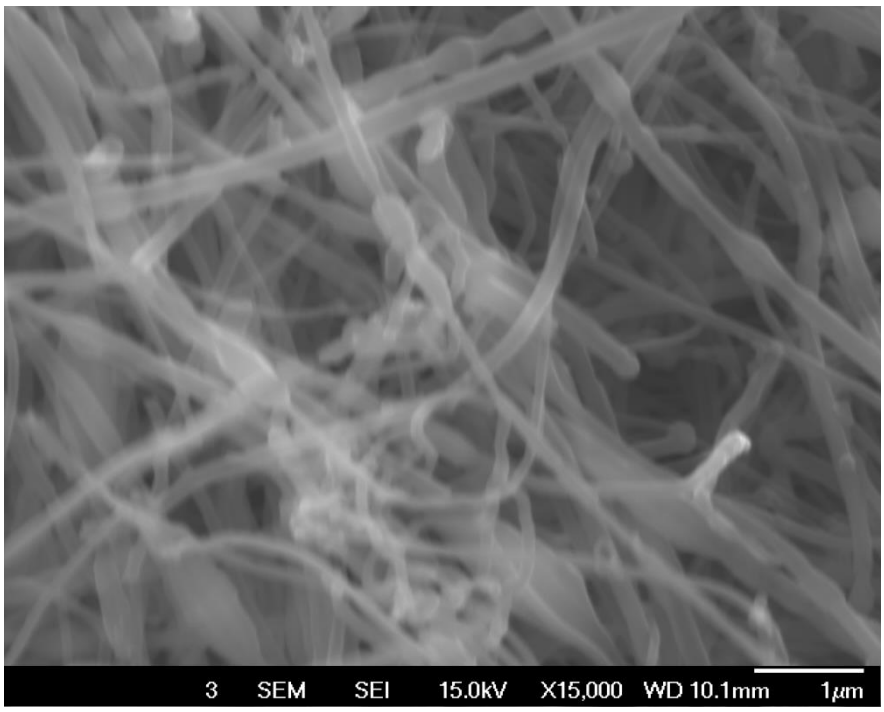
(3 segments, Scan Rate – 20 mV/s)


+64
+48
+32
+16
0
-16
Current, uA
0
+0.1
+0.2
+0.3
+0.4
+0.5
+0.6
+0.7
+0.8
Potential, V

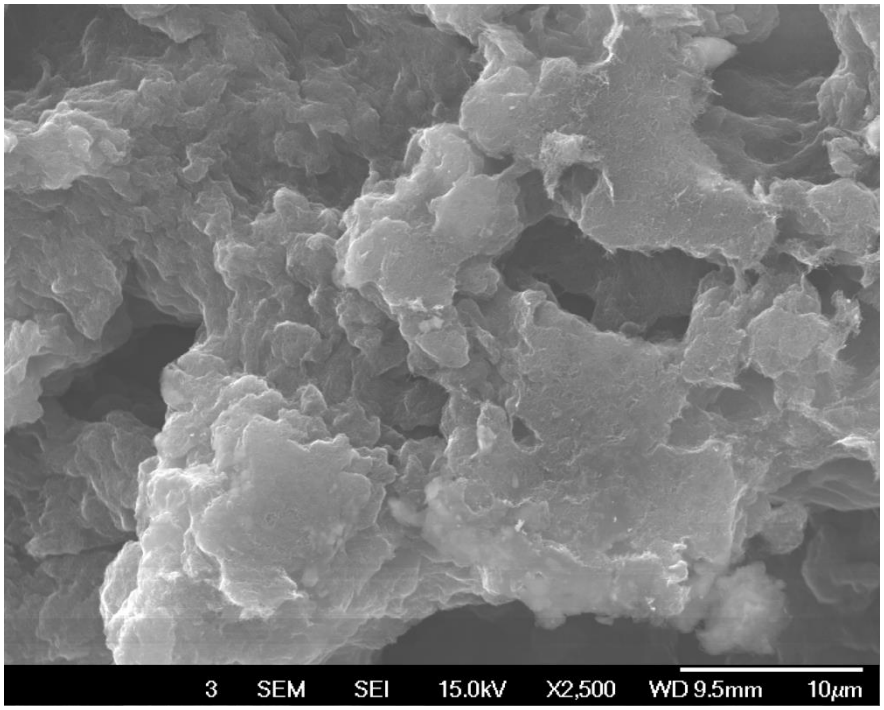
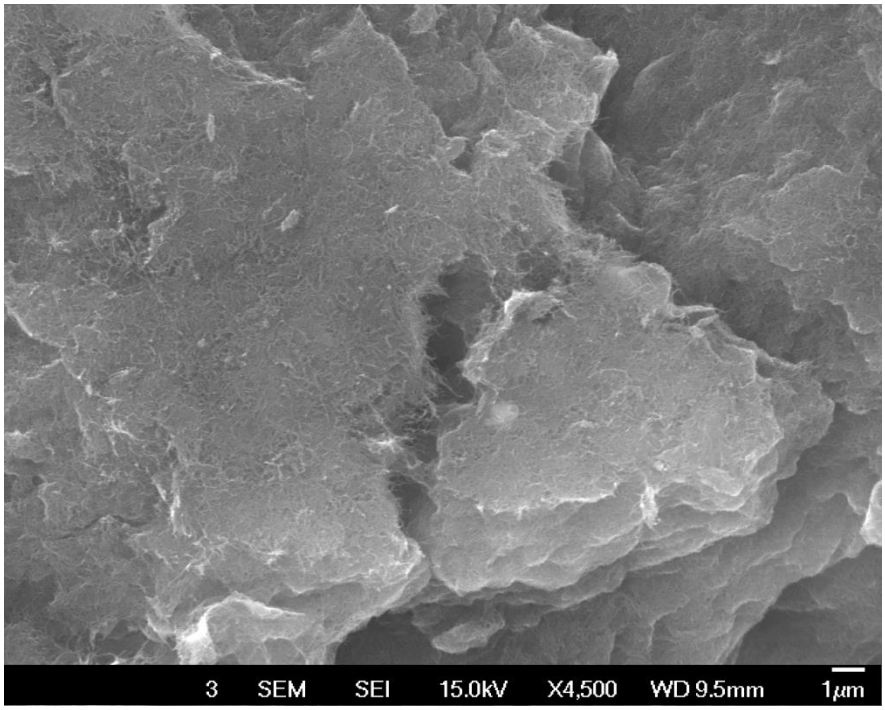
Appendix C: SEM Imagery

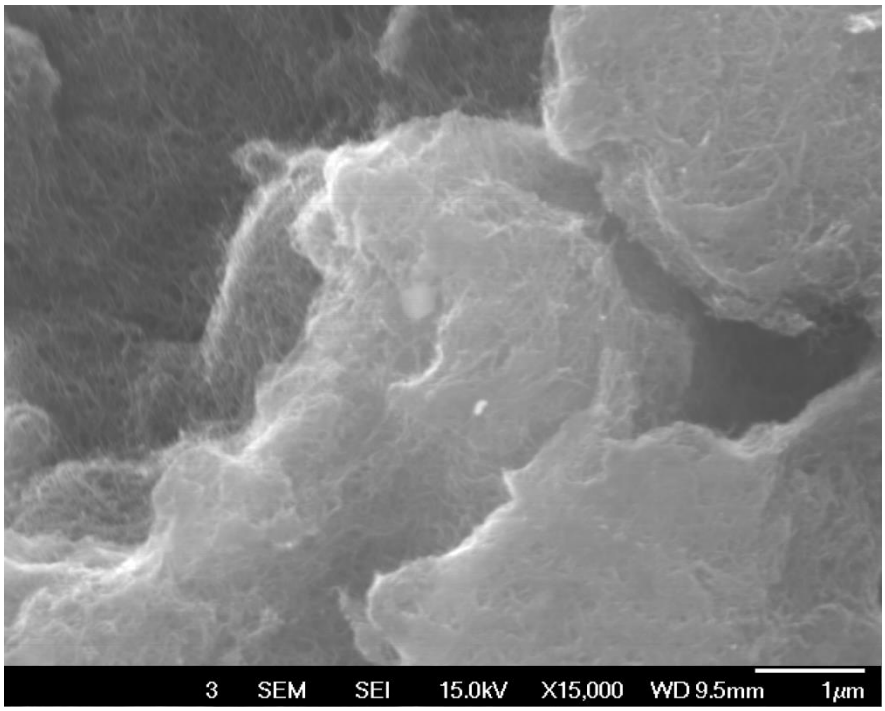
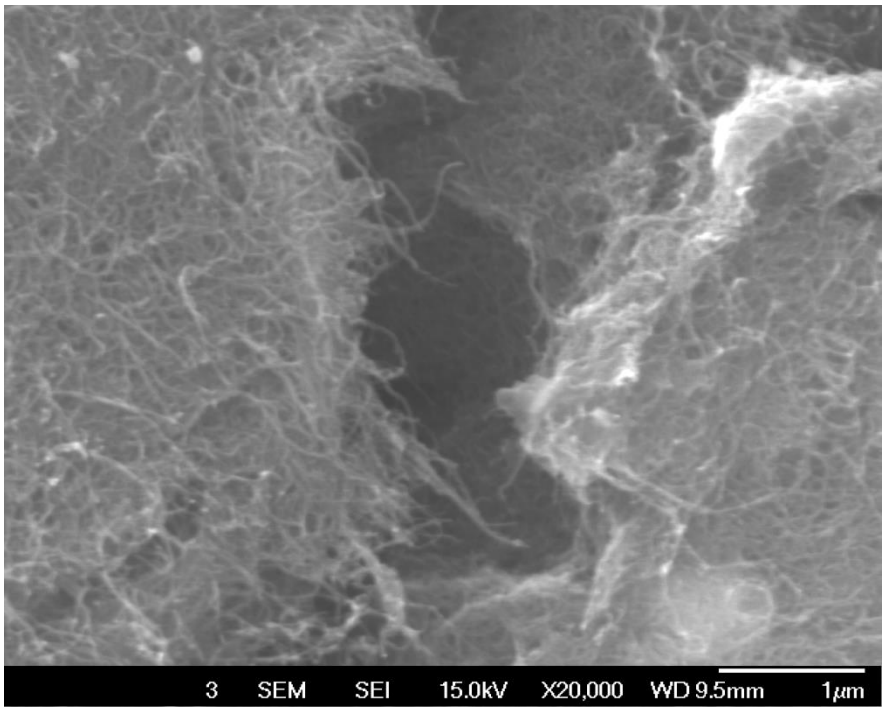
12mg CNF with 1mL of 5 wt% PEI solution:

Magnification	Resulting SEM Image
X2,000	
X8,000	

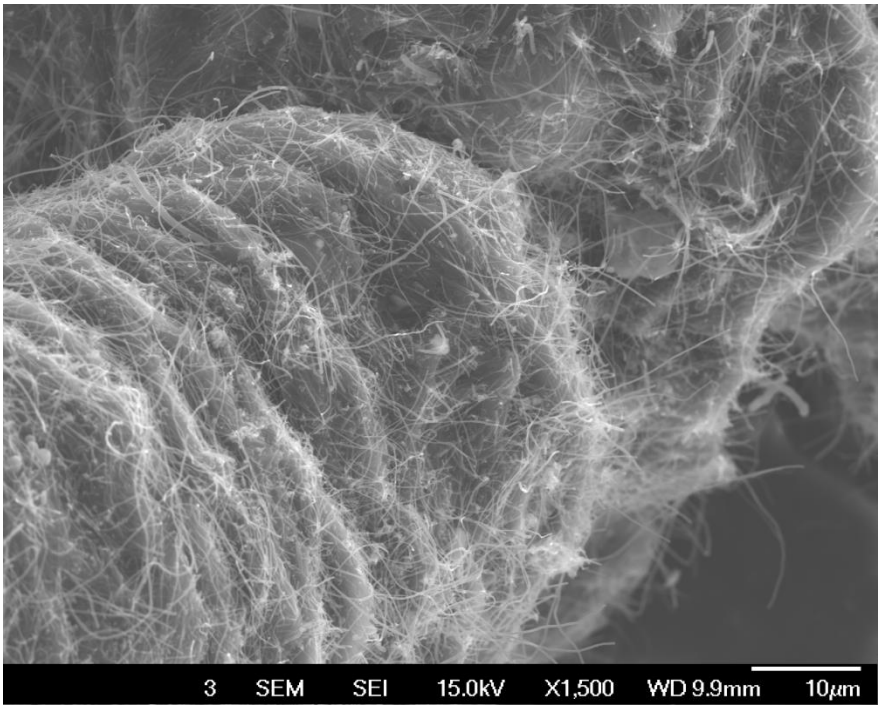
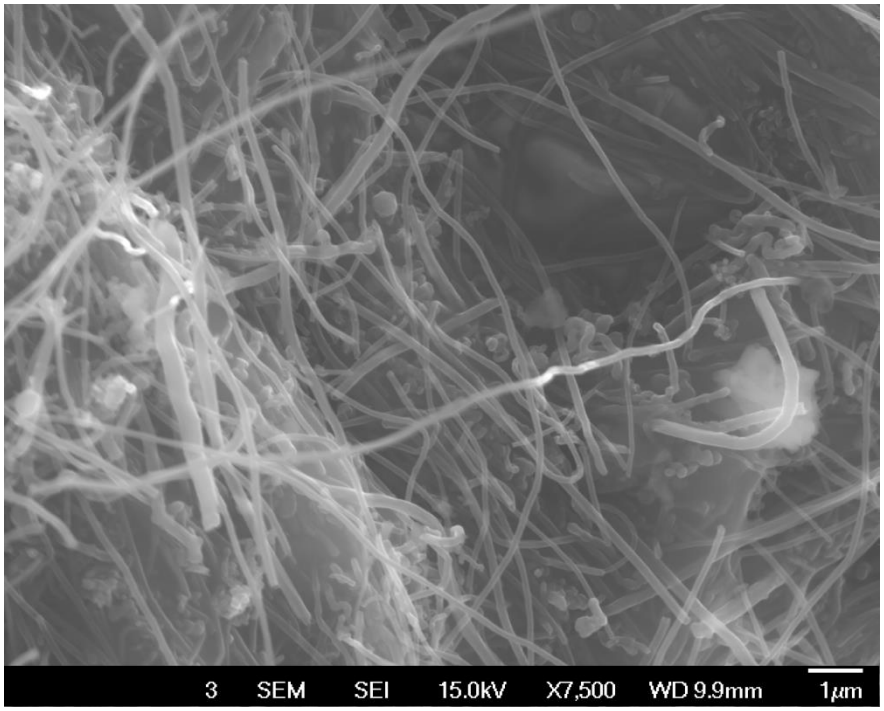
X14,000	
X15,000	

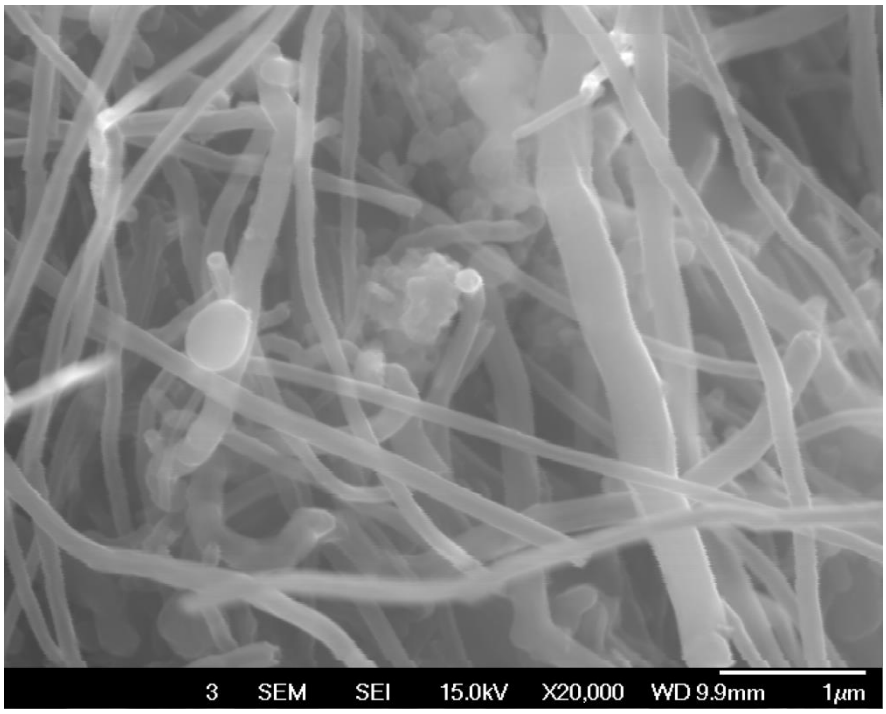
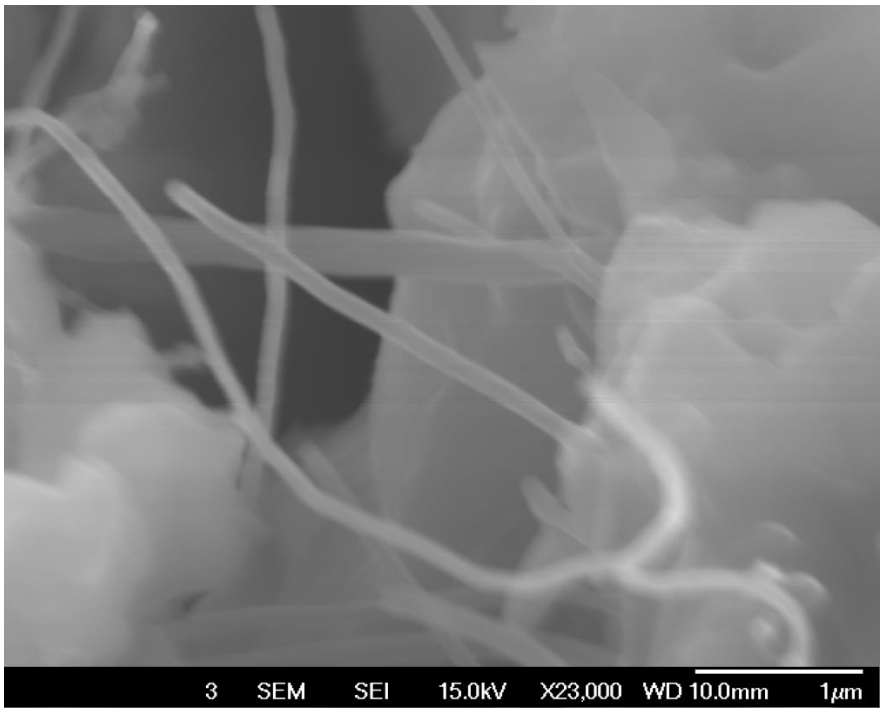
10mg CNT with 1mL of 5 wt% PEI solution:

Magnification	Resulting SEM Image
X2,500	
X4,500	

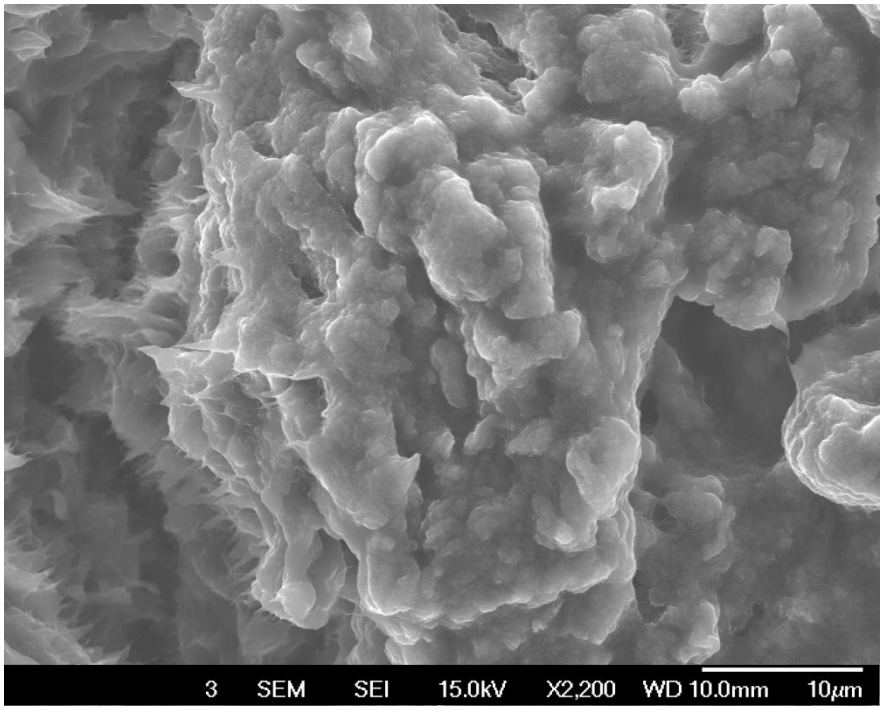
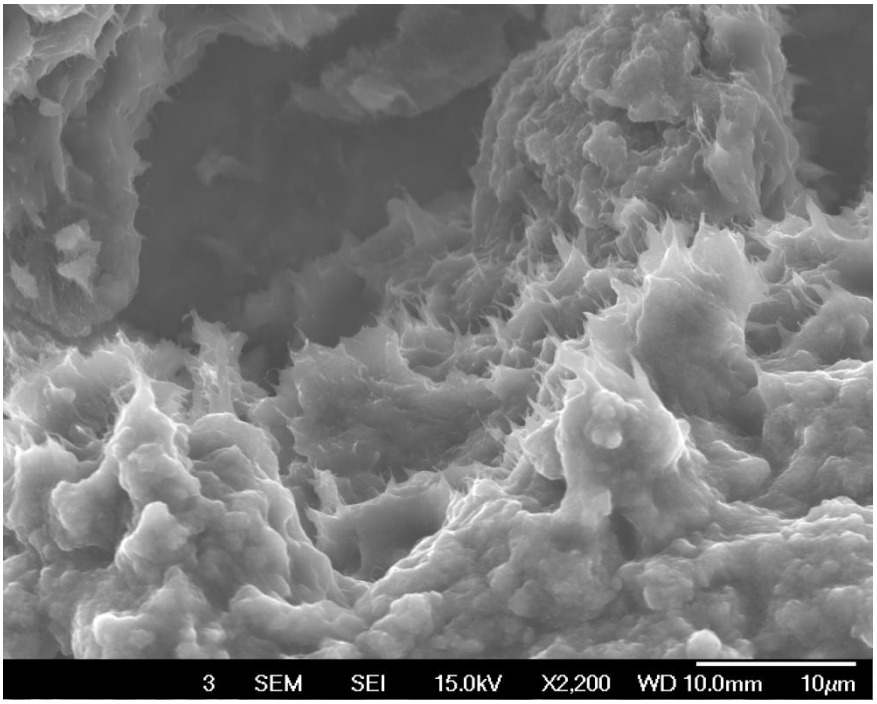
X15,000	
X20,000	

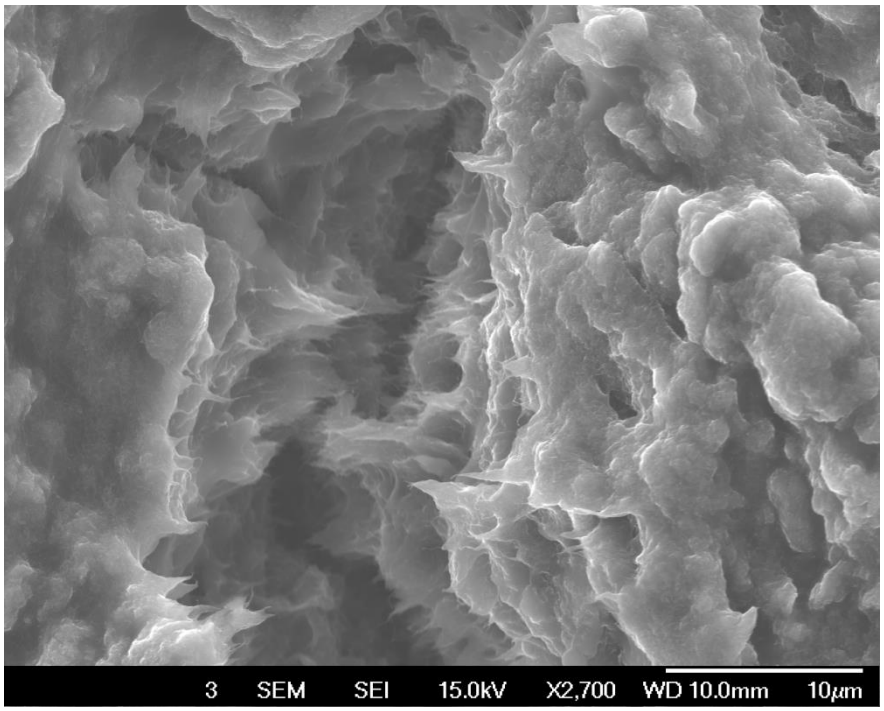
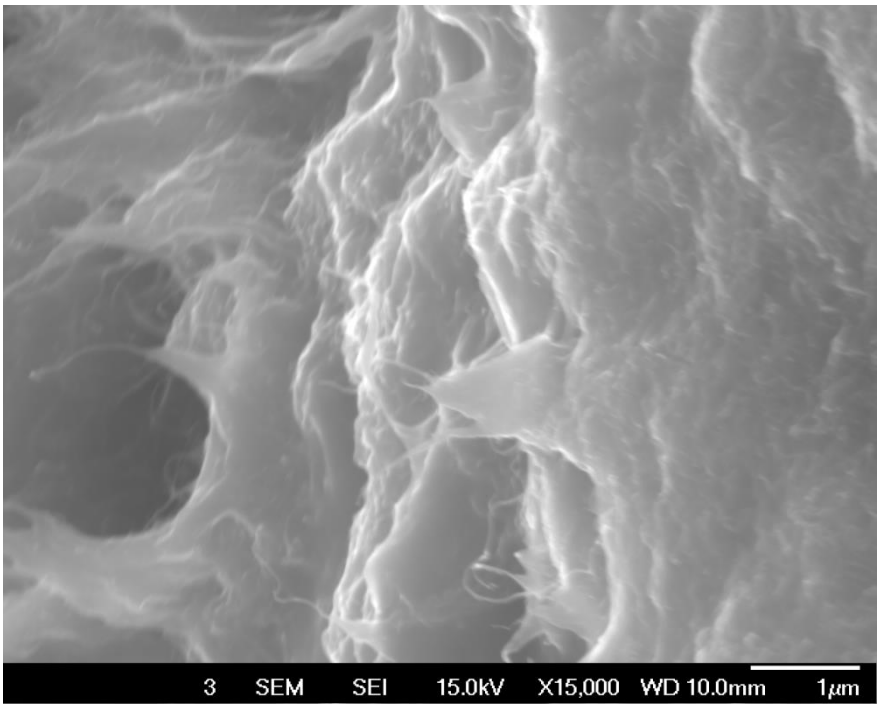
12mg CNF (w/ 1mL 5 wt% PEI) and 40mg GOx (w/ 1mL PBS pH 7)

Magnification	Resulting SEM Image
X1,500	
X7,500	

X20,000	
X23,000	

10mg CNT (w/ 1mL 5 wt% PEI) and 40mg GOx (w/ 1mL PBS pH 7)

Magnification	Resulting SEM Image
X2,200	
X2,200	

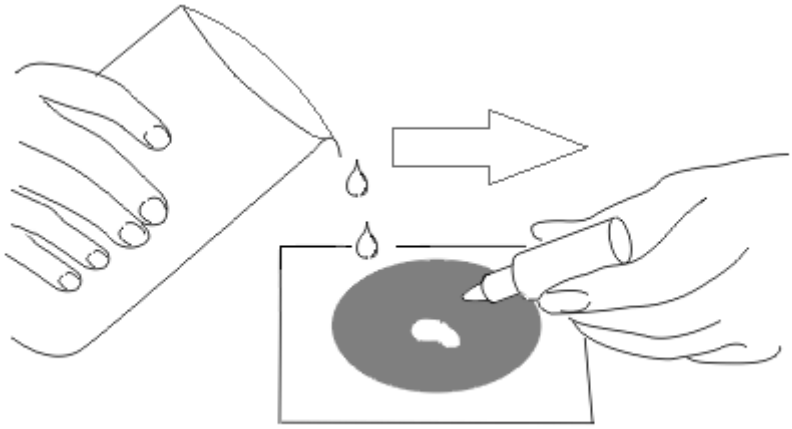
X2,200	
X15,000	

Appendix D: Electrode Handling and Cleaning

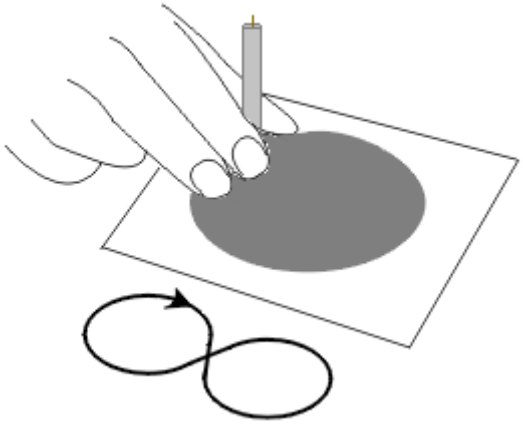
It is important to never touch the electrode or scratch the electrode's active area, as this has the potential to render the electrode. Additionally, the electrode shouldn't be heated or cooled to temperatures significantly different than ambient conditions, as the glassy carbon and out plastic have different rates of thermal expansion. If subjected to these extreme conditions, irreversible damage in the form of a cracked glassy electrode is frequently observed.

The following of the procedure below (Adapted from the BASi Handbook) is critical to avoid damaging the electrode. These should be replicated before and/or after every run to ensure the electrodes can continue to function optimally.

1. Remove any electrode material on the surface of the GC electrode by rinsing with distilled water followed by a rinse or light buffing of the surface with a methanol soaked lab wipe.
2. Prepare the polishing pad by rinsing with distilled water if any large aggregated particles remain. Then applying an alumina powder to various locations on the nylon polishing pad.



3. Polish the electrode by holding it parallel to the glass plate and moving the electrode in figure eight motions for at least ten complete cycles in each direction. This ensures the electrode surface and plastic casing will experience a uniform wear.



4. To remove any alumina particles that may still be on the electrode submerse the GC electrode into a distilled water bath and sonicate for up to 5 minutes.
5. Once the sonication is finished rinse the electrode with additional water followed by methanol. Blot dry with a clean lab wipe.
6. Electrodes should be fully cleaned and dried following testing, to avoid damage to the active area place the cleaned electrode into its plastic case provided.