3D Printed Glucose Monitoring Sensor

by

Anngela Adams

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2017 by the
Graduate Supervisory Committee:

Jeffrey LaBelle, Chair
James Abbas
Vincent Pizziconi

ARIZONA STATE UNIVERSITY

May 2017

ABSTRACT

The American Diabetes Association reports that diabetes costs $322 billion annually and affects 29.1 million Americans. The high out-of-pocket cost of managing diabetes can lead to noncompliance causing serious and expensive complications. There is a large market potential for a more cost-effective alternative to the current market standard of screen-printed self-monitoring blood glucose (SMBG) strips. Additive manufacturing, specifically 3D printing, is a developing field that is growing in popularity and functionality. 3D printers are now being used in a variety of applications from consumer goods to medical devices. Healthcare delivery will change as the availability of 3D printers expands into patient homes, which will create alternative and more cost-effective methods of monitoring and managing diseases, such as diabetes. 3D printing technology could transform this expensive industry. A 3D printed sensor was designed to have similar dimensions and features to the SMBG strips to comply with current manufacturing standards. To make the sensor electrically active, various conductive filaments were tested and the conductive graphene filament was determined to be the best material for the sensor. Experiments were conducted to determine the optimal print settings for printing this filament onto a mylar substrate, the industry standard. The reagents used include a mixture of a ferricyanide redox mediator and flavin adenine dinucleotide dependent glucose dehydrogenase. With these materials, each sensor only costs $0.40 to print and use. Before testing the 3D printed sensor, a suitable design, voltage range, and redox probe concentration were determined. Experiments demonstrated that this novel 3D printed sensor can accurately correlate current output to glucose concentration. It was verified that the sensor can accurately detect glucose levels

from 25 mg/dL to 400 mg/dL, with an R^2 correlation value as high as 0.97, which was critical as it covered hypoglycemic to hyperglycemic levels. This demonstrated that a 3D-printed sensor was created that had characteristics that are suitable for clinical use. This will allow diabetics to print their own test strips at home at a much lower cost compared to SMBG strips, which will reduce noncompliance due to the high cost of testing. In the future, this technology could be applied to additional biomarkers to measure and monitor other diseases.

DEDICATION

Dedicated to my mother and father who always loved, encouraged, and supported me. I appreciate every opportunity that they afforded me and the many sacrifices that they made along the way. Thank you for always encouraging me to serve God, value education, to pursue my dreams, and to persevere in life.

ACKNOWLEDGMENTS

I would like to express my deep gratitude to the outstanding faculty who supported me in completing my master's degree and thesis. Dr. Jeffrey LaBelle, my thesis committee chairperson, has mentored me in the La Belle Lab during the past six years. He is a tremendous and inspirational leader; and I appreciate his insightful mentorship along with the many opportunities that working in the LaBelle Lab has afforded me. Dr. LaBelle is a strong role model, dedicated researcher, and innovative problem-solver. His example has instilled an inquisitive spirit balanced with a good measure of hope, creativity, and persistence in so many students that future biomedical accomplishments will reflect his dedication. His generous support and careful attention to details facilitates more than knowledge and skills in his students; it provides his students with the deep appreciation of excellence and a desire to contribute to biomedical research. I am proud to have been a part of the LaBelle Lab, as we strived to better the lives of people all over the world. I would also like to acknowledge Dr. LaBelle's doctoral students Chi Lin, Mackenzie Honikel, Steven Lathers, and other members of the LaBelle Lab, Garrett Repp, and Aldin Malkoc for their collaboration. I truly appreciate having worked on the LaBelle Research Team and am proud to have worked under the guidance of Dr. Jeffrey LaBelle.

I am also most grateful to Dr. James Abbas and Dr. Vincent Pizziconi, who graciously contributed their time in serving on my thesis committee. Dr. Pizziconi was the first Arizona State University biomedical engineering professor that I met when I was a high school student visiting Arizona State University. His friendly welcome and impressive articulation of research in the field of biomedical engineering solidified my decision to study biomedical engineering at Arizona State University! One of my best decisions ever!

Dr. Pizziconi has been an invaluable confidant and supporter on my journey to medical school.

My time at Arizona State University was challenging and enjoyable; and, it was always enriched by the outstanding Ira A. Fulton College of Engineering biomedical engineering faculty. I am appreciative of their leadership; and, in the future I will pass that supportive leadership along to those that I mentor. I am deeply indebted and most appreciative for all who have supported me along my educational path, especially those who have supported me in completing my thesis. Thank you!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Market Need

Current devices on the market for monitoring and managing diabetes are invasive and expensive. Many times, the pain and price of managing diabetes leads to noncompliance among diabetics. There is a need for a device that is not only non-invasive, but also inexpensive. New technologies, such as 3D printing, could be utilized to provide new options for diabetics to monitor their blood glucose levels. Use of 3D printed technology in healthcare is growing in popularity as 3D printing is becoming an inexpensive technology and the availability of 3D printers is expanding. The focus of this research was to utilize concepts of electrochemistry to develop a viable and affordable 3D printed sensor for diabetics to monitor their blood glucose levels.

Overview of Diabetes

According to the World Health Organization, there are 422 million diabetics worldwide [10]. The American Diabetes Association reports that there are 29.1 million diabetics in the United States [23]. Globally, there are 1.5 million diabetes related deaths each year [10]. The prevalence of diabetes is expected to grow and it is projected to be the seventh leading cause of death by 2030 [10].

There are two types of diabetes - type 1 and type 2. Individuals with type 1 diabetes usually develop a predisposition for the disease early in life and affects 5% of all diabetics [25]. Type 1 diabetes is a chronic autoimmune disorder where the body's immune system attacks beta cells [5]. Beta cells are important as they produce insulin,

which is a hormone produced by the pancreas that metabolizes glucose. Insulin is responsible for keeping blood glucose levels within a healthy range and avoiding glycemic spikes. Once the beta-cells have been destroyed by the immune system, production of insulin is no longer possible [6]. Individuals with type 1 diabetes are therefore unable to regulate their own blood glucose levels.

Type 2 diabetes is more common than type 1 and occurs when individuals can no longer metabolize sugar [19]. Individuals with type 2 diabetes are either resistant to insulin or unable to produce enough insulin which leads to above normal blood glucose levels [19]. Risk factors for type 2 diabetes include: poor diet, lack of exercise, family history, overweight, and high blood pressure [21].

The glycemic spikes in both types of diabetes can lead to very serious complications. These complications include: cardiovascular disease, neuropathy, nephropathy, retinopathy, and ketoacidosis [9]. If left untreated, these complications can lead to premature death in diabetics. To avoid these complications, diabetics must maintain a healthy diet, exercise, take all prescribed medication, and comply with doctors' blood glucose testing schedules [22]. These steps help to control glycemic levels, which can slow or prevent diabetes related complications [22]. Although it is very important to comply with prescribed testing schedules, patients often do not adhere to these schedules as testing with current monitoring devices is often painful and expensive.

Current Blood Glucose Monitoring Technology

Self-monitoring blood glucose (SMBG) devices are the most widely used monitoring technology for diabetics. SMBG devices require the user to obtain a blood sample using a

lancet. This blood sample is then placed onto a test strip, which is then inserted into a monitor device. This monitor provides the user with their current blood glucose levels. If the blood glucose levels are outside of the normal range, the patient must correct them accordingly. Hyperglycemic levels require the diabetic to inject insulin, while hypoglycemic levels require the diabetic to ingest food with glucose or simple carbohydrates [14]. Although SMBG devices are relatively easy to use, provide fast results, and allow the user to test anywhere, noncompliance remains an issue with this device. This is because collecting the blood sample is often painful and the test strips are expensive. The average price for a single SMBG test strip is $0.98 [27]. These test strips should not be reused, raising the overall cost of monitoring this disease when testing multiple times a day. The number of tests required per day depends on the severity and management of the disease. Physicians help create a testing schedule for diabetics, which is critical to help patients maintain healthy glycemic levels. Therefore, it is essential these SMBG monitoring devices provide accurate readings but unfortunately, many are prone to various errors. The reported blood glucose levels can easily produce errors if the user does not have clean skin in the area where the blood sample is collected [3]. Errors can also occur if there is a delay in getting the blood sample testing strip into the monitor [3]. Continuous glucose monitoring (CGM) devices are a growing technology in managing diabetes. In CGM devices, a glucose sensor is implanted subcutaneously in the abdomen [30]. This sensor continuously measures glucose levels and sends the blood glucose levels to an external monitor [30]. The monitor then alerts the user if their blood glucose levels are out of the healthy range [30]. SMBG devices only provide a single blood glucose reading for each test; however, CGM devices continually measure blood glucose

levels [30]. Continuously measuring blood glucose levels allows patients and physicians to detect trends. These trends can be used to determine the optimal time and amount of insulin to administer [3]. Although this device allows the user not to worry about adhering to a strict testing schedule, there are several downsides to this device. The patient must have the glucose sensor internally implanted and live with an implanted device. CGM devices also require calibration every 12 hours, but it is recommended that the device be calibrated three to four times a day [30]. This calibration requires the user to use a SMBG device to collect and test a blood sample, so the patient is still subjected to the pain of a fingerpick [30]. Although this technology is very expensive, most insurance companies will either cover or reimburse CGM devices, so cost is usually not an issue with this type of device.

The most accurate method of testing blood glucose levels is a blood test at a physician's office. Bloodwork is not an option for managing diabetes, as the results are not available quickly enough for diabetics to correct blood glucose levels. This is also very expensive, requiring the diabetic to pay to be seen by a physician and to have bloodwork performed. In addition, having blood drawn is painful and invasive.

Currently, there is a lot of research in alternative diabetes monitoring devices. The TearTOUCH device is a blood glucose monitoring device that utilizes tear fluid instead of blood [11]. This device consists of polydimethylsiloxane (PMDS) microfluidic attached to a screen-printed electrode [4]. A current is produced when the tear fluidic reacts with the liquid reagents in the device. These reagents include a mixture of glucose dehydrogenase flavin adenine dinucleotide (GDH-FAD), phosphate buffered saline, and potassium ferricyanide [4]. The research on this device determined the correlation

between tear fluid glucose levels and blood glucose levels, proving the tear fluid could be a viable alternative to blood [3]. The current produced from the reaction is proportional to the glucose concentration of the sample. This device provides an alternative to the painful fingerpicks associate with SMBG and CGM devices [3]. The TearTOUCH device is not yet ready for the market, as there are still multiple factors that must be addressed before the device is ready for consumers. The liquid reagents must be dried down so that the devices can be used outside of the lab and has a shelf life comparable to SMBG test strips. A drying technique has been developed but still requires animal and human testing. The TearTOUCH device also needs to be redesigned so that the required tear sample size can be smaller and more realistic for the tear volume contained in the average human eye. Although the TearTOUCH device has been tested in rabbits, this technology has not yet been tested on humans.

A tattoo-based glucose monitoring device is also being developed [2]. This flexible, peel-and-stick tattoo for the arm uses the capillary fluid to determine blood glucose levels [2]. A secondary device is used to report the blood glucose levels measured from the device using reverse iontophoretic extraction of glucose in the interstitial fluid [2]. This proof-of-concept device was tested in several human subjects. The blood glucose levels from the biosensing tattoo were compared to results from a commercially available SMBG device, ultimately supporting the data that this technology is possible. This device could be a pain free alternative to SMBG and CGM devices but requires a lot of research and development before its introduction on the market.

There are also advances in the development of an ophthalmic lens to measure blood glucose levels. This device contains a loop antenna, a communication chip, and a

polymer substrate glucose sensor [15]. The glucose levels in the tear fluid are correlated to blood glucose levels and is reported to a device that the user can see [15]. Although this provides an alternative to current blood glucose monitoring devices, this device has not been tested in animal or human subjects. All testing of this device was conducted on a polydimethylsiloxane eye model under optimal conditions, so it is still unclear how this device would perform in non-ideal conditions, such as in a patient with dry eyes.

Electrochemistry Overview

Electrochemistry is the study of chemical reactions that result in electron movement. A common example in which this is found is the concept of oxidation-reduction reactions, also known as redox reactions. These reactions are based upon a coupled event where there is a loss of an electron on a molecule by oxidation, and a gain of an electron on a molecule by reduction. Many of these processes occur naturally, such as the green plant photosynthesis and the biochemistry involved with converting food into an energy source for the body. Each involves the transfer of electrons and have played a critical role in nature for millions of years. A simple redox reaction can be seen in the production of NaCl, commonly known as table salt, by utilizing the following stoichiometricly balanced equation:

$$\mathbf{2\ Na^0 + Cl_2 \rightarrow 2\ NaCl}$$

The positively charged sodium atom promotes the acceptance of electrons whereas the negatively charged chlorine atom promotes the loss of an electron. In order to show the electron movement, the equation reaction above can be broken down further by the respective atoms:

Oxidation: $Na^0 \rightarrow Na^+ + e^-$

Reduction: $Cl_2 + 2\ e^- \rightarrow 2\ Cl^-$

This relationship is based upon creating equilibrium through bonds to achieve the greatest amount of stability between the two atoms. The transfer of electrons caters to the ability of creating equilibrium states for molecules through the inherently coupled events of oxidation-reduction reactions [24]. By taking the concept of electron movement for redox reactions and applying it to the field of physics, chemistry can be used to look at a similar reaction performed through a conductive electrical wire that creates an electrochemical change [24]. This is the fundamental concept that is used in the study of electrochemistry and can be seen throughout various commonly used items. Batteries utilize the changes in electron flow from redox reactions to create electrical energy and provide the power for almost all portable electronics. Electrical laboratory measuring equipment allows for monitoring of chemical reactions [24]. Industry utilizes electrochemistry to produce important chemicals, such as those in liquid bleach [24]. The possibilities of applications for electrochemistry are boundless, with various techniques and branches that permit new advances in technology and healthcare [24]. Such techniques will be explored throughout this section in order to provide reasoning for utilizing such methodology and illustrate its importance to scientific study.

Electrochemistry can be broken up into two fundamental fields – potentiometry and amperometry. Potentiometry is the measurement of the voltage between two electrodes, while amperometry is the measurement of current between two electrodes [8]. An application that can be utilized to test both of these measurements within electrochemistry is through the creation of an electrochemical cell. The electrochemical

cell is an electrical circuit composed of two half-cells, one cell a cathode and the other an anode, immersed in respective ion solutions, with a salt bridge connecting the two electrodes. The salt bridge, commonly KCl, allows the electrons to flow freely across ion solutions, completing the circuit. The following Figure showcases a typical electrochemical cell along with the redox reaction equilibrium equation for the respective electrodes:

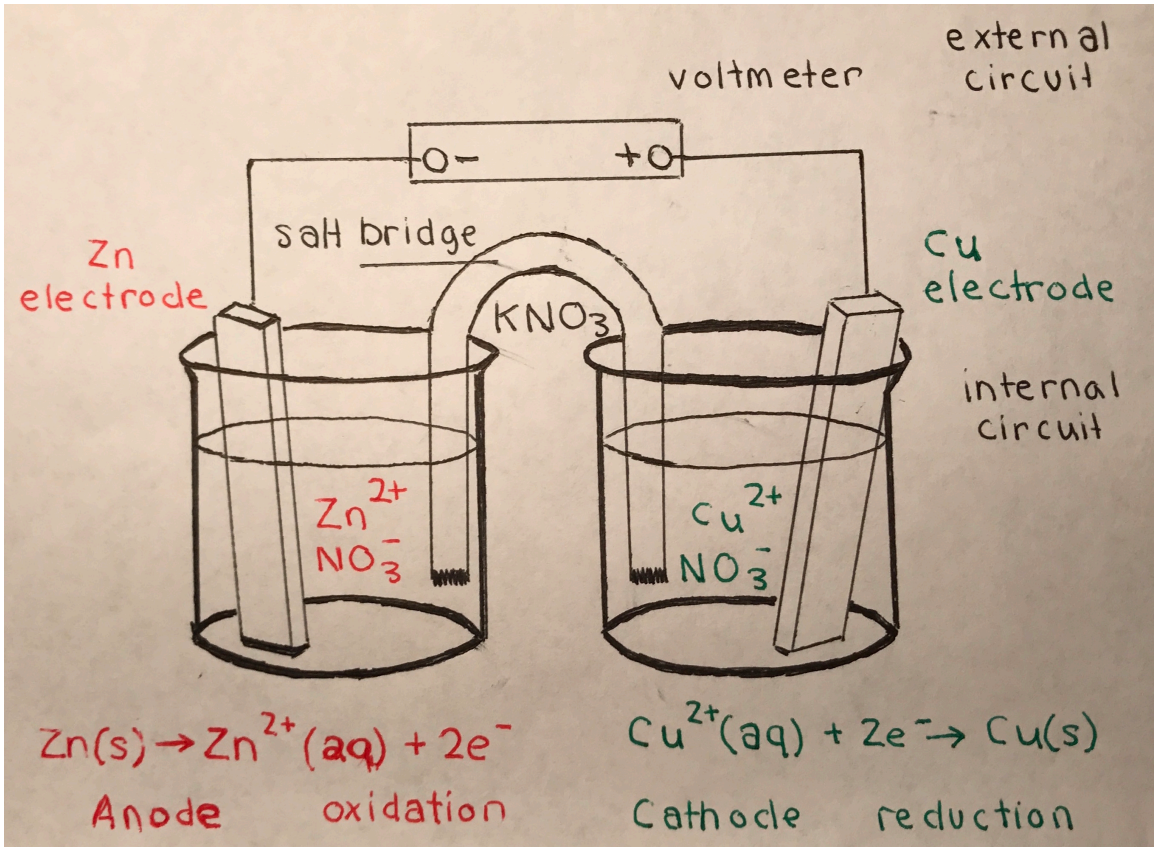

Figure 1: A commonly used electrochemical cell and the redox reaction performed.

The cathode and anode electrodes play important roles that follow the conventions of redox reactions. The cathode is reduced where the anode is oxidized, a permissible event by the use of the salt bridge [17]. Each reaction of oxidation or reduction has an

associated change in energy produced by the electron flow that is dependent on the material used. This change in energy is known as the electrode potential and can be used to determine the total amount of energy potential of an electrochemical cell at standard-state (performed under standard conditions of 1 mole per liter (1 M) and pressure of 1 atmosphere (1 atm) at 25^{o}C) by utilizing the Nernst Equation [17]. This equation is defined as:

$$\boldsymbol{E_{cell} = E_c - E_a}$$

Where E_c and E_a are the reduction potentials for the electrode redox reactions which both follow an equation to achieve determination of respective reduction potentials under standard-state:

$$\boldsymbol{E = E^0 - \frac{RT}{nF} \ln (Q)}$$

$$\boldsymbol{E = E^0 - \frac{0.05916}{n} \log (Q)}$$

Where E is the reduction potential of the respective electrode, E^o is the standard-state reduction potential, n is the number of electrons in the redox reaction and Q is the reaction quotient of the electrode. The reduction potential of the entire system can be calculated based upon such standard-state values but can also be found utilizing methods of potentiometry. This involves using a potentiometer connecting the two electrodes together to measure the change in the reduction potentials caused by electron flow in the redox reductions [17]. This method of connection between the two electrodes is also useful in amperometry to find the rate of electron flow known as current. The current between the two electrodes can be determined using an ammeter [8]. The applications of potentiometry and amperometry are critical to understand so that measurements within

systems can be monitored and used to provide accurate scientific results. As shown with an electrochemical cell, natural systems with qualitative electron flow characteristics can now be quantified and measured objectively to provide insight on how the system functions. The fundamental principles of both fields can be used to proceed with further in-depth analysis of various systems, not just limited to electrochemical cells, by way of modified techniques. Cyclic voltammetry (CV) is a type of potentiometry electrochemical measurement that utilizes oscillating low to high voltages on an electrode to obtain analytical, thermodynamic, kinetic, and mechanistic information about chemical systems involving redox reactions [8]. Such measurements include concentration, redox potentials, equilibrium constants, or rate constants for reactions involving electrogenerated species [8]. Understanding of how cyclic voltammetry works can be described within the electrochemical cell system and the governing electrical reduction potential equations [20]. Cyclic voltammetry utilizes low and high voltage sweeps that are predicted by the Nernst Equation for cathode and anode reduction potentials. The constant voltage sweep is applied to a working electrode that is connected to a reference (grounded) electrode to produce a resulting current [16]. This current is measured as a function of potential which is known as a voltammetry experiment, commonly performed in electroanalytical chemistry [20]. In terms of usefulness, this relationship can help in comparison between the effectiveness of various materials and their possible applications based on resulting voltage vs. current measurements. In addition, by knowing the variables within the Nernst Equation, concentration, reduction potentials, and the number of electrons per molecule of analyte oxidized or reduced can be found which may provide useful information for experimental significance [20].

Similar in methodology to cyclic voltammetry, an amperometric i-t test is a type of amperometry electrochemical measurement that uses the resulting current from the cyclic voltammetry test and is measured against time. This generates a current vs. time plot that can assist in determining various information about the materials and mediums used within the chemical system [18]. A direct form of application is through Fick's first law of diffusion to determine diffusion rates based upon concentration differences over time [18]. This ability to determine diffusion rates by a simple experiment aids in the effectiveness of using such an experiment and its potential to quantize characteristics of the experimental system [18].

Electrochemistry is a fundamental branch of chemistry that incorporates various concepts of physics to quantitatively characterize and determine properties of a given chemical system under analysis [18]. The core chemistry concept of oxidation-reduction reactions allows for two branches, voltammetry and amperometry, to be created that create a methodology for studying a system through measurements of its electrical potentials and electron flow [18]. Cyclic voltammetry, used in conjunction with an electrochemical cell, provides information regarding the current (flow of electrons) through the system during application of oscillating voltages [18]. Amperometric i-t tests can be created from the resulting cyclic voltammetry current when measured against time. Independently, this data provides only basic information regarding the physics behind the experimental system [18]. When conjoined and applied to the Nernst Equation, Fick's Law of diffusion, and Faraday's Law, a whole host of system characteristics can be quantitatively described to give insight on how the component composition of the chemical system can be applied to other areas. Electroanalytical chemistry techniques are used to obtain

analytical, thermodynamic, kinetic, and mechanistic information [18]. From one single voltammetry experiment, quantitative data regarding concentration, redox potentials, equilibrium constants, rate constants for reactions involving electrogenerated species, diffusion rates, diffusion coefficients, and number of electrons per molecule of analyte oxidized or reduced can be calculated. This information is essential towards advancements in science and technology to facilitate the creation of new material coatings, implantable devices, more efficient batteries, and more efficient, effective, and affordable diabetes glucose sensor strips. Glucose sensors strips utilize the concept of electrochemistry with every measurement obtained. Therefore, by utilizing techniques of voltammetry and amperometry, research into the possibilities of finding new methods or materials for creating glucose strips through experimentation can ultimately lead to providing a more affordable and comfortable way of life for diabetics globally.

Glucose Biosensors

There are three main parts of a biosensor. The first part is the biological recognition element [13]. In a glucose monitoring biosensor, this is the enzyme that reacts with glucose [13]. A common enzyme is glucose dehydrogenase flavin adenine dinucleotide (GDH-FAD). The second part of a biosensor is the transducer [13]. The transducer converts the transfer of electrons into current output [13]. The third part of a biosensor is a processing system [13]. The processing system transforms the current output to the glucose output reading. This processing is important as it allows the user to receive results that are easily interpretable.

There are three generations of glucose monitoring biosensors [12]. The first generation use oxygen as the final electron acceptor [12]. These devices usually contain glucose oxidase enzyme to catalyze the reaction. This enzyme is highly specific to glucose and can withstand a wide range of pH readings and temperatures; however, glucose oxidase can react with oxygen [7]. These devices can easily produce inaccurate results, as oxygen from the surroundings can affect the glucose output levels of these devices [12]. Many SMBG devices are considered a first-generation device. Second-generation glucose biosensors have an artificial electron acceptor that serves as the final electron acceptor [12]. This artificial acceptor is referred to as a redox mediator or a redox probe. Potassium ferricyanide is a typical redox mediator. GDH-FAD is a common enzyme in second generation devices and does not react with oxygen but can react with sugars in the body other than glucose. Although these devices eliminate possible errors from oxygen, the redox mediators are typically toxic [12]. In third-generation glucose biosensors, the electrode surface serves as the final electron acceptor [12]. These devices eliminate the toxic redox mediator and possible errors from oxygen [12].

Below is the reaction that is occurring in the proposed glucose monitoring biosensor. In reaction (1), the glucose reacts with the GDH-FAD enzyme. In this reaction, the glucose is oxidized and the GDH-FAD enzyme is reduced. In reaction (2), the reduced GDH-FAD enzyme from reaction (1) then reacts with the redox mediator, which is potassium ferricyanide ($[Fe(CN)_6]^{3-}$). The GDH-FAD enzyme is oxidized back to its original form, while the redox mediator is reduced. In reaction (3), the redox mediator reacts with the surface of the electrode. The reduced redox mediator is oxidized to its original form while the sensor is reduced.

(1) Glucose + GDH-FAD → Glucono-1,5-lactone + GDH-$FADH_2$

(2) GDH-$FADH_2$ + $[Fe(CN)_6]^{3-}$ → GDH-FAD + $[Fe(CN)_6]^{4-}$

(3) $[Fe(CN)_6]^{4-}$ + Sensor → $[Fe(CN)_6]^{3-}$ + Sensor (reduced)

The three reactions show the movement of electrons. The electron is passed from the glucose molecule, to the enzyme, to the redox mediator, and then finally to the sensor. The electron is then allowed to move through the electrode where the current is measured. The current is proportional to the concentration of glucose molecules which can be utilized for monitoring glucose levels.

3D-Printing Overview

Additive manufacturing is the technique of building objects by layering material [1]. 3D printing is one of the most popular forms of additive manufacturing. In 3D printing, a computer aided design (CAD) file of the object is created on a software program [28]. There are software programs that allow for very technical designs, such as SolidWorks; however, there are more intuitive design programs, such as Tinkercad, that sacrifice technical details in exchange for simplicity while still maintaining overall functionality. The CAD file is then transferred to the 3D printer software, where the print can begin. The designed object is created by adding layers of heated substrate material on top of each other [29]. The additive substrate material, including various types of plastics, metals, waxes, carbons, and even biomaterials, creates density for which the object of interest is specified [29].

Although 3D printing has been around since the early 1980s, it has grown recently in popularity due to the development of low-cost 3D printers [26]. More user-friendly

software has allowed people to come up with unique applications for 3D printers. This ultimately transformed the industry from an expensive method to develop trinkets, into a more inexpensive means to develop healthcare and industrial goods. The options for substrate materials has expanded, allowing for more application of 3D printed objects. 3D printing also has many distinct benefits when compared to traditional manufacturing [29]. Objects that are 3D printed can be customized much more easily [29]. The amount of time to build an object from a conceptual design is much shorter when compared to traditional manufacturing processes [29]. This proves beneficial not only to prototyping but also to companies trying to get products on the market more quickly. In addition, 3D printing not only simplifies the manufacturing process, but it also reduces wasted materials, contributing to its overall cost effectiveness [29].

Currently, the four main market areas utilizing 3D printing include: aerospace, industrial, healthcare, and automotive industries [29]. The current applications of 3D printed parts include: automotive engine components, consumer sporting goods, orthodontic implants, and surgical guides [29]. The 3D printing industry is drastically growing and is projected to be over a $17 billion market by 2020 [29]. It is also projected that stents and prosthetics will soon be added to the list of 3D printed healthcare items and marketed to patients [29]. Research is currently being conducted to bio-print organs and body parts using living cells as the substrate material [28]. The use of 3D printing in the medical field will allow for more personalized care that will improve outcomes for patients [26].

CHAPTER 2

METHODOLOGY

Creating the CAD Model of the 3D Printed Sensor

The first step in the development of the 3D printed glucose sensor was designing the sensor in SolidWorks. This sensor was designed to have similar dimensions and features to Zensor TE100 SPEs (Zensor, Taiwan). The final SolidWorks model of the 3D printed sensor is seen below in Figure 2.

Figure 2: SolidWorks model of sensor.

3D Printing the Sensor

The SolidWorks file was opened and saved as a STL/object file. A conductive filament was required to create this sensor. The conductive graphene PLA filament, purchased through BlackMagic3D, was determined to be the most conductive filament available. The MakerBot printer was selected to create this sensor as it is specifically designed to print PLA filament. This sensor file was then imported into the MakerBot Print software. The sensor model was then rotated 90 degrees along the x-axis, so that the sensor was arranged properly on the build plate. The sensors on the build plate can be seen below in Figure 3.

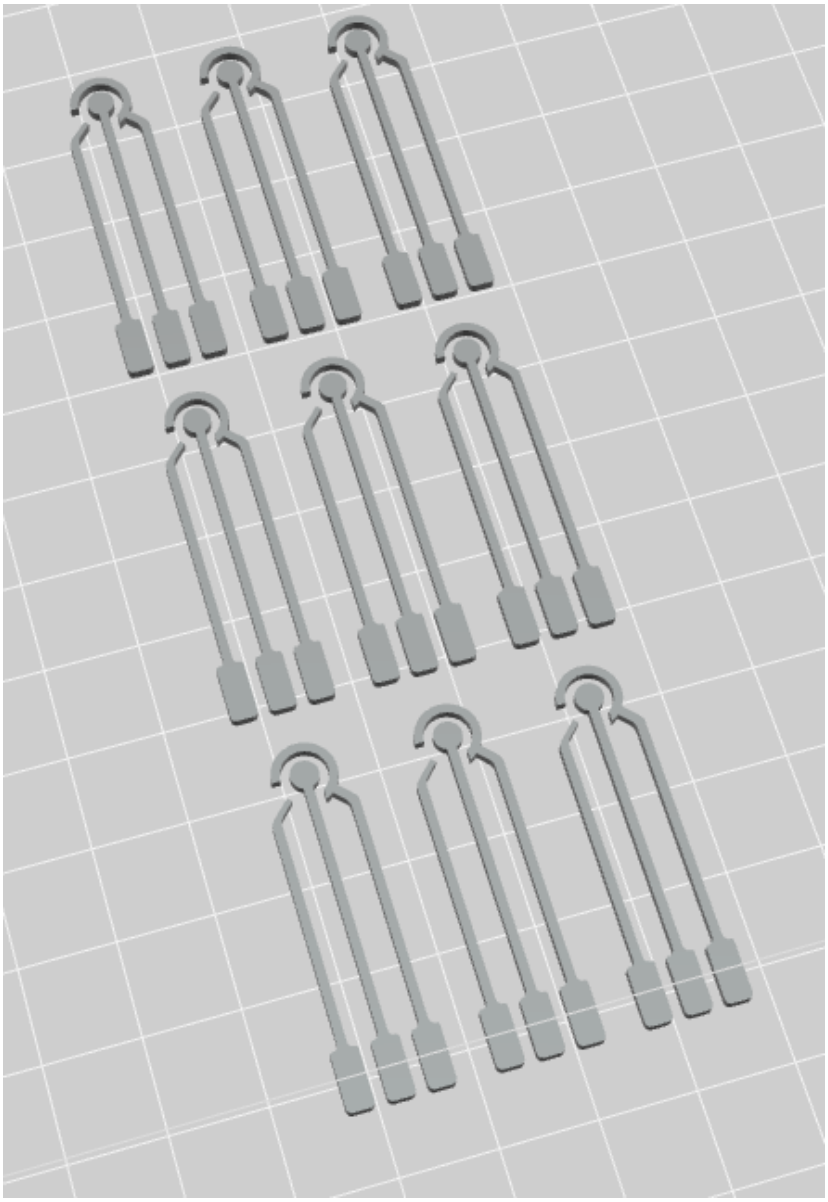

Figure 3: Sensor models on MakerBot build plate.

To adjust the print settings, the custom settings button was selected. The temperature settings for the extruder were changed to 220 C, as this was determined to be the melting point for the conductive graphene filament. The infill density was selected to be 100% to create a solid print and maximize the conductivity of the sensor. The travel speed was

changed to 30 mm/s, as this was determined to produce a print with the highest resolution and prevent slipping. The retraction distance was also adjusted from 0.5mm to 1.5mm. This change was important to prevent the filament from oozing out of the extruder between layers. The final printer settings can be seen below in Figure 4.

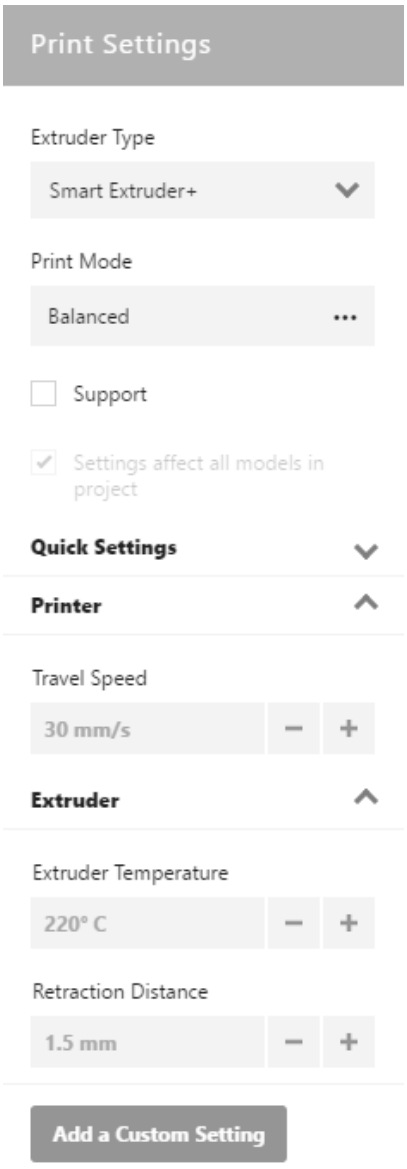

Figure 4: Final settings for using conductive graphene filament on MakerBot printer.

Once these settings were selected and the sensors were arranged on the build plate, the print file was exported to the 3D printer. The filament was then loaded into the printer and the mylar substrate was taped onto the platform. Taping the mylar substrate was determined to be critical to ensure that no warping occurred during the print. The file was then selected on the printer and the print was started.

Chemicals

All the chemicals used in experiments were purchased through Sigma-Aldrich unless stated otherwise. The enzyme used in these experiments, glucose dehydrogenase flavin adenine dinucleotide (GDH-FAD), was donated from Amano Inc. (Japan). The GDH-FAD used had an activity of 209 units/mg. Phosphate-buffered saline (PBS), with a pH of 7.4, was used to prepare all glucose solutions, the redox probe solution, and enzyme solution.

Making Redox Probe Solution

To test the electrochemical properties of the sensor, the reagent mixture was first created. This mixture consists of enzyme and a redox probe. To create the redox probe, potassium ferricyanide and phosphate buffered saline were mixed together to form a solution with a concentration of 100mM. The mixture was stored in an amber vial as the mixture is light sensitive. This redox probe solution was mixed using a vortexer for 30 seconds and then allowed to sit for ten minutes. After ten minutes, the redox probe mixture was tested to ensure that it was made correctly.

To test the redox probe, the CHI 1230A (CH Instruments, Austin, TX) was checked to ensure that the hardware was working correctly. Results of a successful hardware test can be seen below in Figure 5.

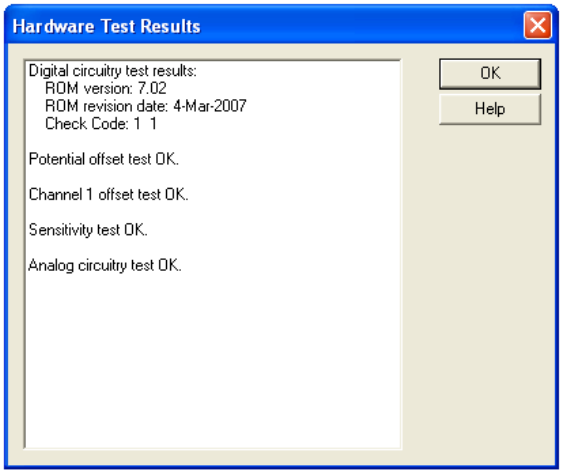

Figure 5: Results of successful hardware test on CHI 1230A.

A Zensor was then hooked up to the CHI 1230A using alligator clips. The red alligator clip was attached to the counter lead of the electrode. The green alligator clip was attached to the working lead of the electrode. The reference lead was then attached to the white alligator clip. The technique on the CHI 1230A machine was selected to be cyclic voltammetry. The parameters were set to have a voltage between -1V and 1V. The rest of the parameters are seen below in Figure 6.

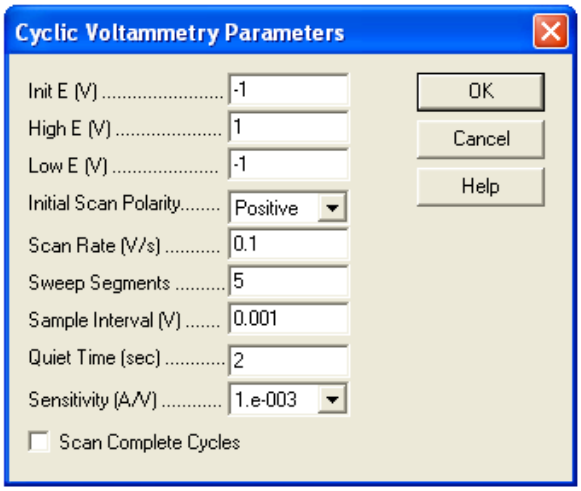

Figure 6: Parameters for running cyclic voltammetry to test redox probe.

Once the parameters were set, 90uL of the redox probe mixture was pipetted onto the working circle of the Zensor and the run button was selected. Once the CHI 1230A machine was done running, a current verse potential graph was produced. A successful redox probe mixture could be confirmed if the graph resembled the shape and magnitude of that in Figure 7. If the graph did not resemble Figure 7, the redox probe mixture was disposed of and remade.

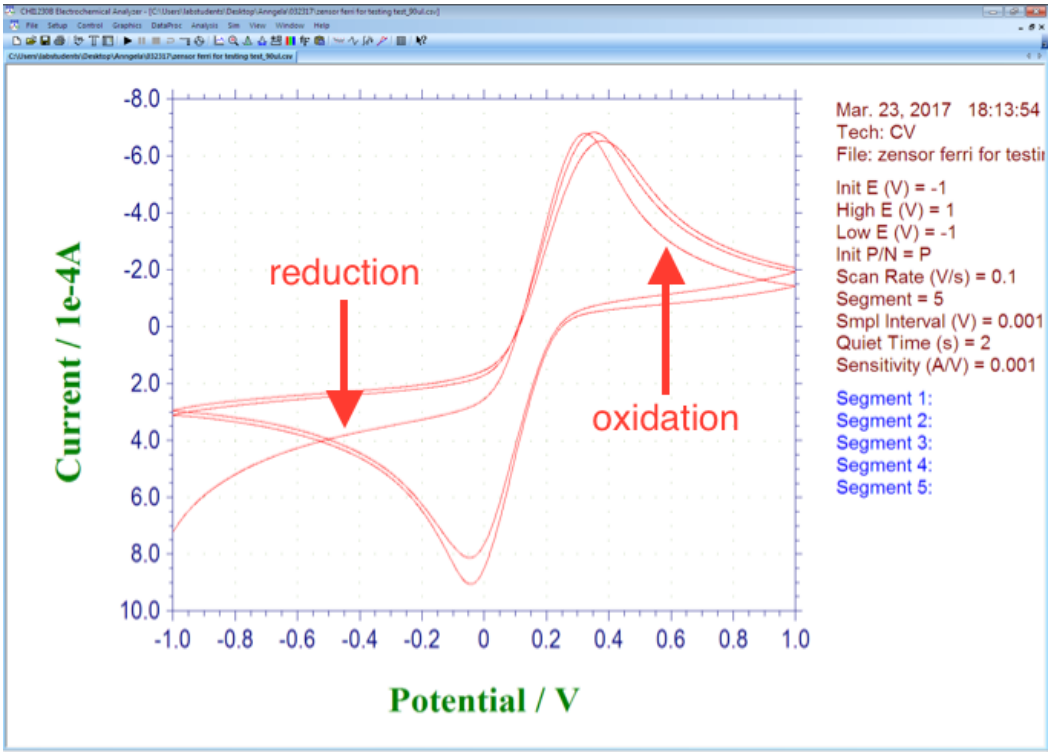

Figure 7: Successful cyclic voltammetry test to verify redox probe solution.

Making Enzyme Solution

To create the enzyme solution, GHD-FAD and the 100mM redox probe solution was mixed together in a one-to-one ratio, 1 mg of the GHD-FAD enzyme was mixed with 1 mL of the redox probe solution. This solution was created and stored in a light sensitive amber vial. It was lightly mixed, but not mixed using the vortexer. This is because the enzyme could be denatured by vortexing. Before using the enzyme solution, it was allowed to fully dissolve for ten minutes.

Making Glucose Solutions

Glucose solutions of various concentrations were prepared using PBS. The concentrations of glucose solutions that were prepared included: 0, 25, 50, 100, 200, and 400 mg/dL. A vortexer was used to mix the glucose and PBS. The solutions were prepared 24 hours prior to their use. This allowed the glucose to fully dissolve.

Determining Optimal Settings to Test 3D Printed Sensor

To determine the proper parameters for testing, cyclic voltammetry tests were conducted with control leads. The reference electrode lead was replaced by an Ag/AgCO wire. The working electrode lead remained on the 3D printed sensor. The counter electrode lead was replaced by a platinum wire. These leads were placed into a small beaker and alligator clipped to the CHI 1230A machine. Redox probe solutions at various concentrations were added to the beaker and data was collected. The voltage range was also changed to determine the optimal voltages. The set-up of this experiment is seen below in Figure 8.

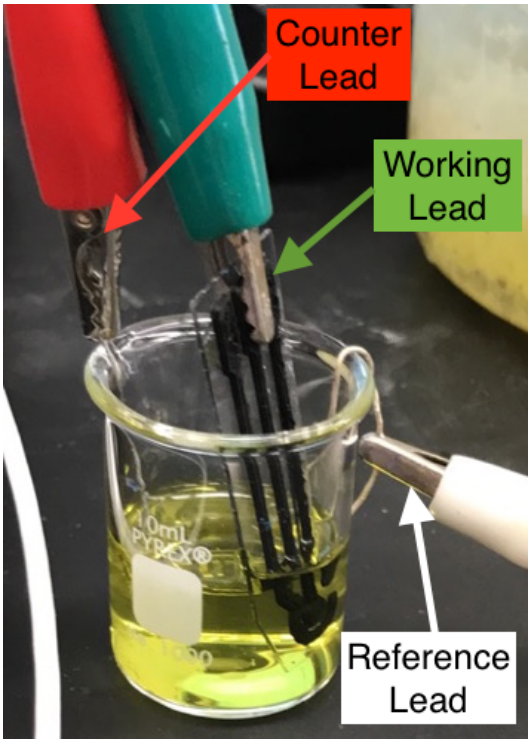

Figure 8: Set up of experiment to determine test parameters using control leads.

Testing 3D Printed Sensor

The 3D printed sensors were separated and cut apart. The sensors were cut so that minimal mylar was surrounding the leads. The leads were attached to the CHI 1230A machine, so that the red alligator clip was attached to the counter lead, the green alligator clip was attached to the working lead, and the white alligator clip was attached to the reference lead. The technique was selected to be amperometric i-t. The parameters were selected to that the initial voltage was 0.35V. The run time was selected to be 90 seconds for all the tests to ensure that enough data was collected to capture all trends. The rest of the parameters are seen below in Figure 9.

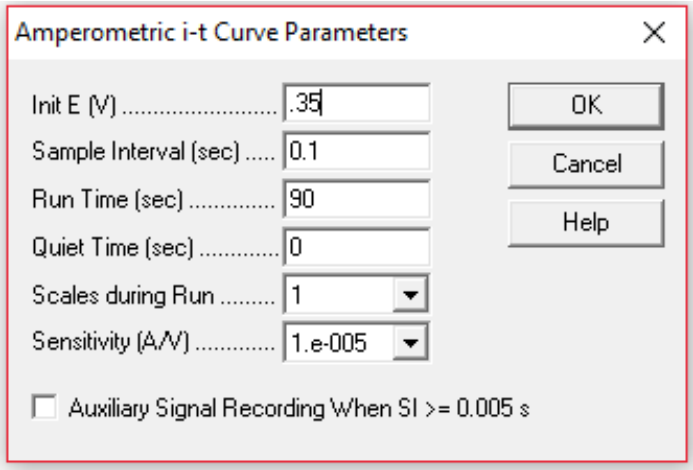

Figure 9: Parameters for running amperometric i-t test to test 3D-printed sensor.

Once the CHI 1230A machine was set up, 810uL of the enzyme solution was pipetted to into a testing vial. Then, 90uL of the glucose solution was pipetted into the vial. This mixture was stirred 10 times counterclockwise. The 3D printer attached to the CHI 1230A machine was then dipped into a testing vial and the test was immediately started. The set-up of this test is seen below in Figure 10.

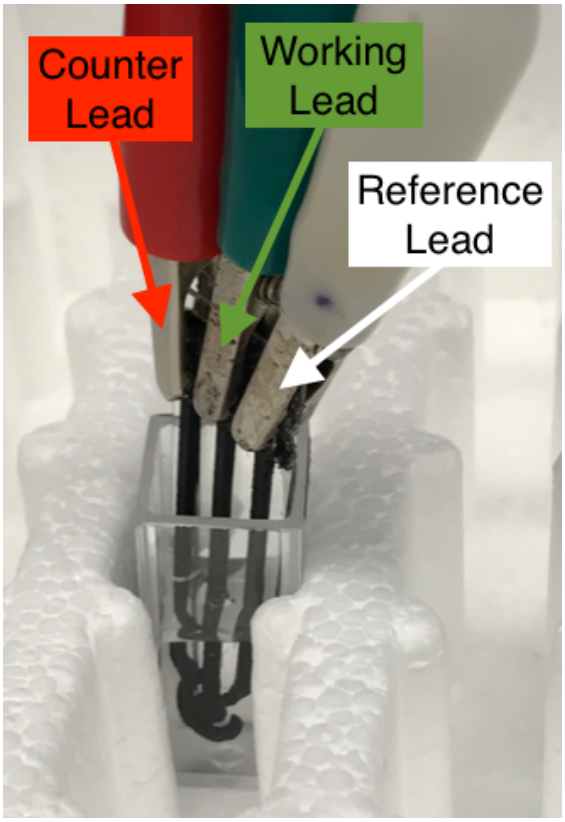

Figure 10: 3D printed sensor in testing vial.

A current verses time graph was produced and all the data was saved in both a text file and an Excel file. The data was then analyzed in Excel.

Overview of Sensor

Figure 11A shows the CAD model of the 3D-printed sensor, while Figure11B shows the sensor printed onto the mylar substrate. The current output from the sensor is a reaction between GDH-FAD and glucose solutions of various concentrations. This can be seen below in Figure 11C.

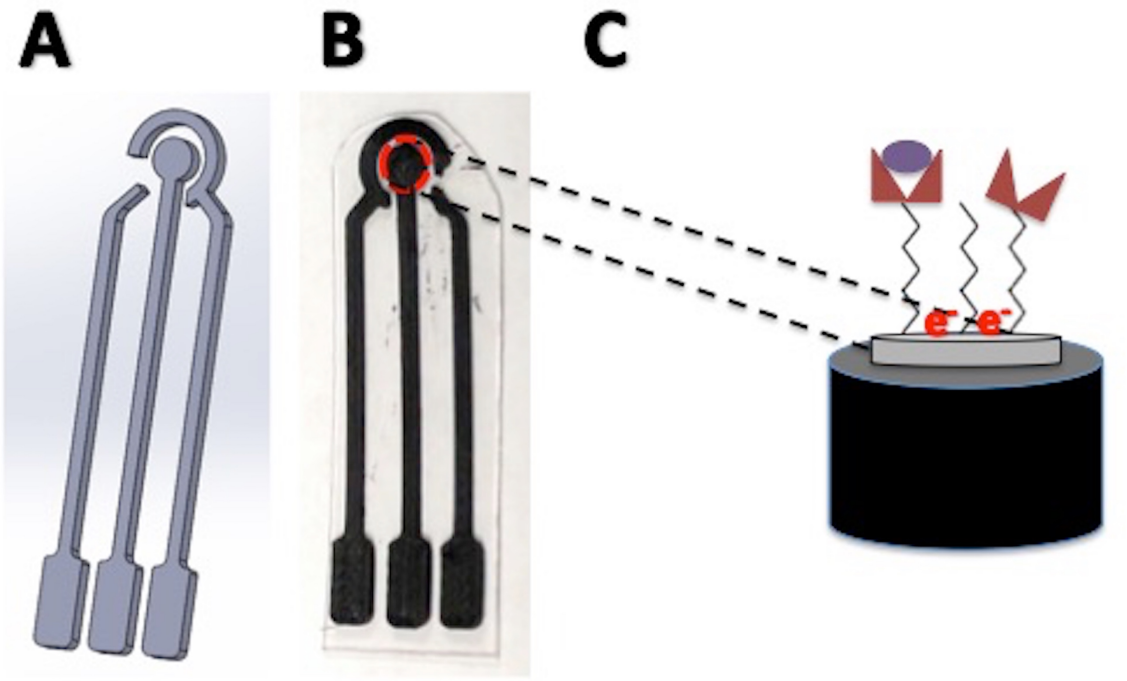

Figure 11: A) A CAD model of the 3D printed sensor with dimensions 11.07x37.72x0.95 mm. B) Sensor printed with conductive graphene filament onto a mylar substrate. C) The reaction of the glucose and glucose dehydrogenase reacting generating electrons that are converted to a current output.

CHAPTER 3

RESULTS AND DISCUSSION

Development of the CAD Model of the 3D-Printed Sensor

A three-lead electrode was designed in SolidWorks, as seen in Figure 12 and Figure 13. The three-lead design was used because it is an industry standard and allows for a counter, working, and reference lead. This CAD model was designed to have the leads and substrate printed together.

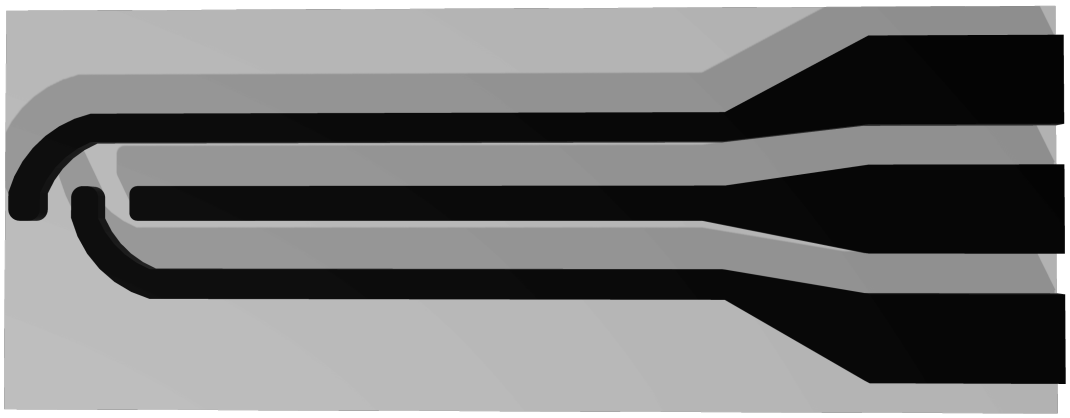

Figure 12: Original SolidWorks design of 3D-printed sensor.

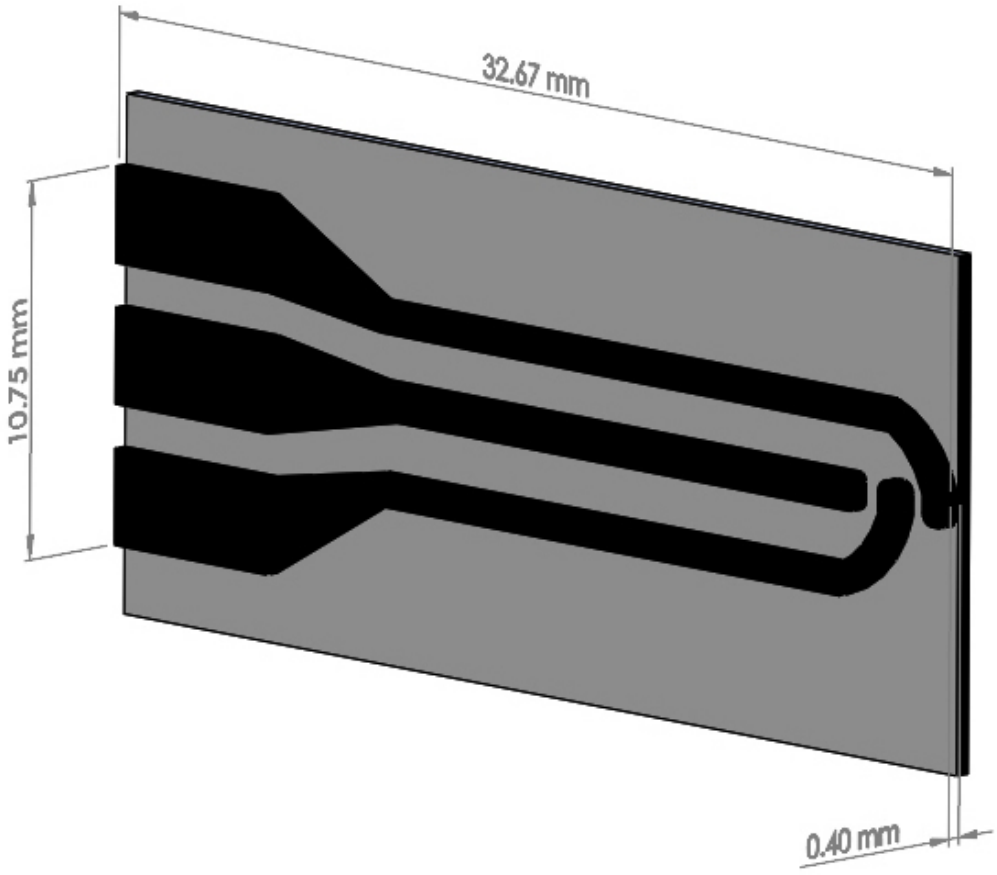

Figure 13: Original SolidWorks design of 3D-printed sensor with dimensions.

This design did not prove acceptable for several reasons. It required two extruders for printing, which resulted in many wasted sensors from calibration issues. In addition, all the amperometric i-t tests performed on these sensors were highly variable and produced very low current outputs. The physical sensor can be seen below in Figure 14.

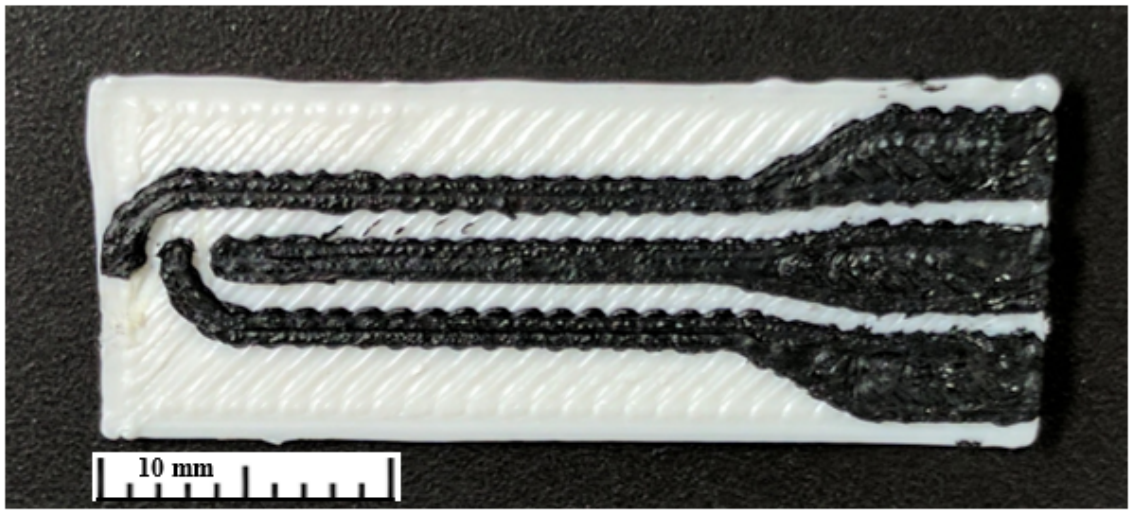

Figure 14: Sensor with 3D-printed leads and substrate.

Development of the Updated CAD Model of the 3D-Printed Sensor

An updated SolidWorks model, seen below in Figure 15 and Figure 16, was created to more closely resemble the structure, dimensions, and design of the industry standard, Zensor. Both the 3D printed sensor and Zensor have a three-lead design with a 3mm working diameter. This was important to allow data output between the two sensors to be more easily comparable. In this design, the substrate was not printed. Instead, the sensor was designed to be printed onto a mylar substrate that was secured to the printing platform of the printer.

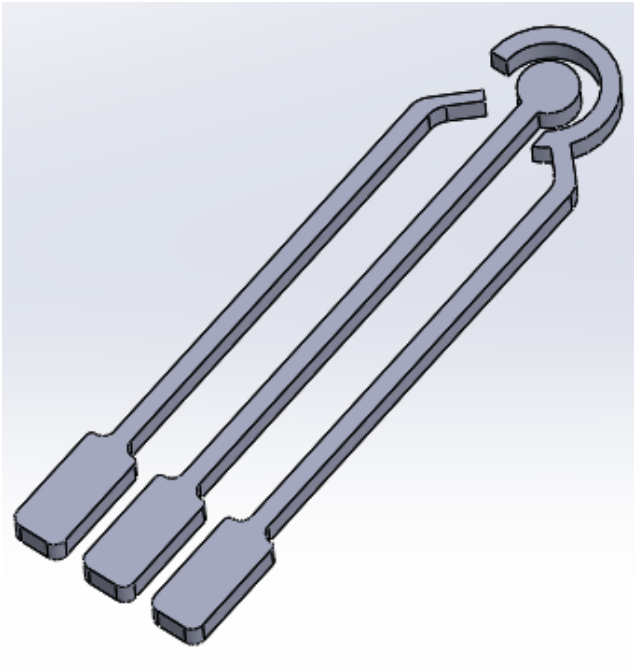

Figure 15: Updated CAD model of sensor.

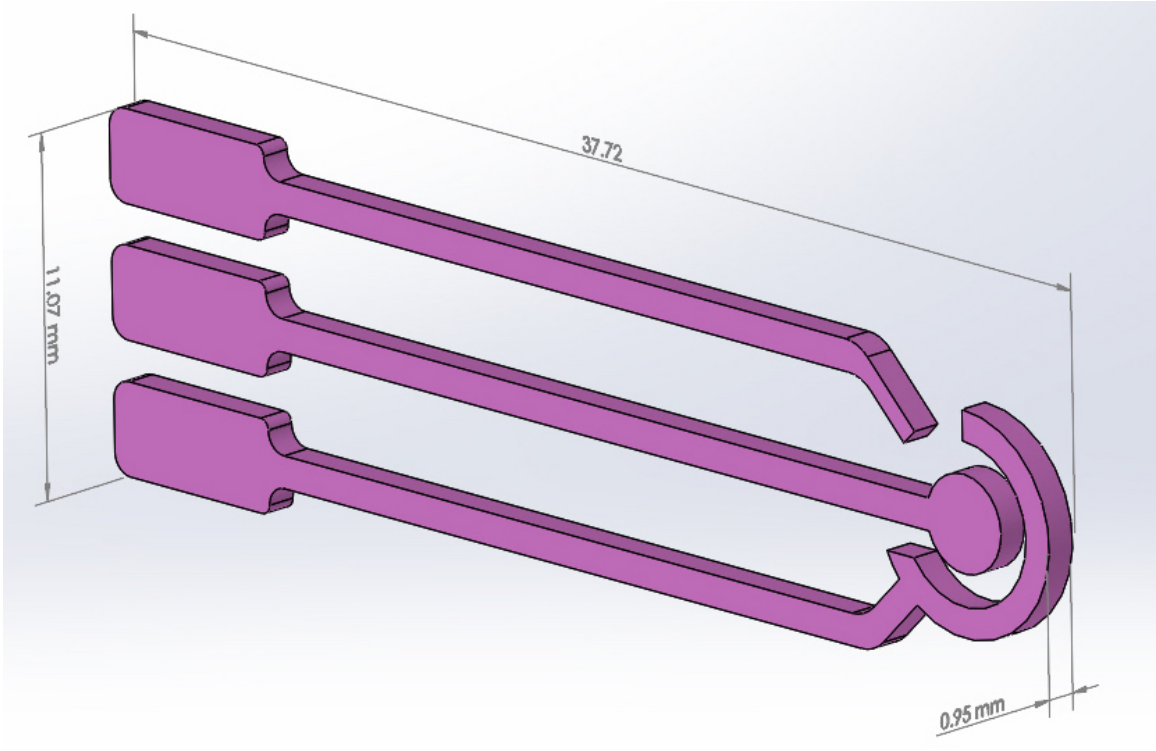

Figure 16: Updated CAD model of sensor with dimensions.

Test Print of CAD Model of 3D-Printed Sensor

With the updated CAD model, a test print was conducted to see if the printer resolution was small enough for the details of the sensor and if the sensor could be printed onto the mylar substrate. For this print, a blue ABS filament (Adafruit, USA) was used in the MakerGear Printer. This filament was chosen because it is a common printing filament that was readily available for this test print. As seen below in Figure 17, the printer successfully printed the sensor onto the substrate with high resolution; however, the printed sensor was not conductive. The resistance of the working electrode was determined to be on the order of hundreds of mega ohms for three centimeters of filament, meaning that the filament was nonconductive. Filament with such a high resistance would not allow electrons to move freely through the sensor, so it was determined that a more conductive filament was necessary.

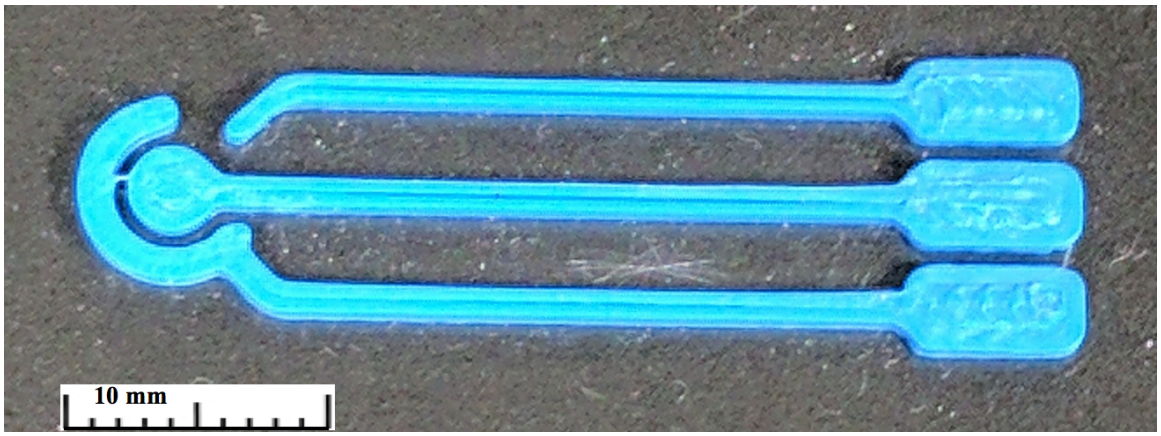

Figure 17: Test print with blue ABS filament to verify that the sensor can be printed.

Development of Alpha Prototype of 3D-Printed Sensor

The Octave ABS filament (Octave, USA) was determined to be more conductive with a preprinted resistance of about 37 MΩ for three centimeters. The updated CAD file was

printed using the MakerGear printer. The printed sensor can be seen below in Figure 18. The print quality was still reasonably good and provided a printed resistance of around 30 MΩ for the length of the working electrode. Although this resistance was lower than the previously 3D printed sensors, the resistance was still too high for an electrochemical sensor. The resistance of the Zensor's working lead is about 100 Ω, so the 3D-printed electrode had to have a filament that was similar.

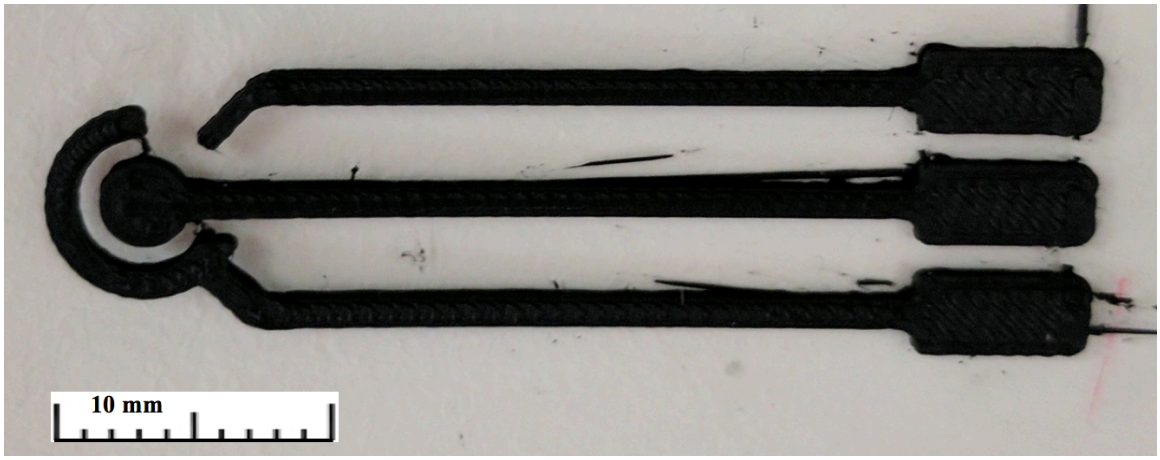

Figure 18: Alpha prototype of 3D-printed sensor made from Octave ABS filament.

Development of Beta Prototype of 3D-Printed Sensor

To increase the electrochemical properties of the sensor, an ABS conductive black filament (Black Magic 3D, USA) was purchased. This filament was advertised to be conductive with a preprinted resistance on the order of kilo ohms; however, it was determined that the preprinted resistance was approximately 20 MΩ per three centimeters. The difference in resistivity could be due to manufacturing impurities or errors, as this filament is no longer sold by Black Magic. The updated CAD model was printed using this filament on the MakerGear printer. Although optimal printer settings

had to be determined once again, printing the ABS conductive black filament with carbon still proved to be challenging. The retraction settings of the printer were increased to the maximum level to reduce the bleeding of the leads. The extruder head temperature was increased to 220°C - which was critical for the filament not to clog the extruder head - and the platform temperature was increased to 55°C. These changes prevented the printer from etching the sensor into the mylar substrate and not extruding out filament. The resolution of the 3D-printed sensor with this filament was not ideal, so after each print, a razor was used to clean up the areas of the leads that had bled together. The beta prototype of the sensor printed with the ABS conductive black filament can be seen below in Figure 19.

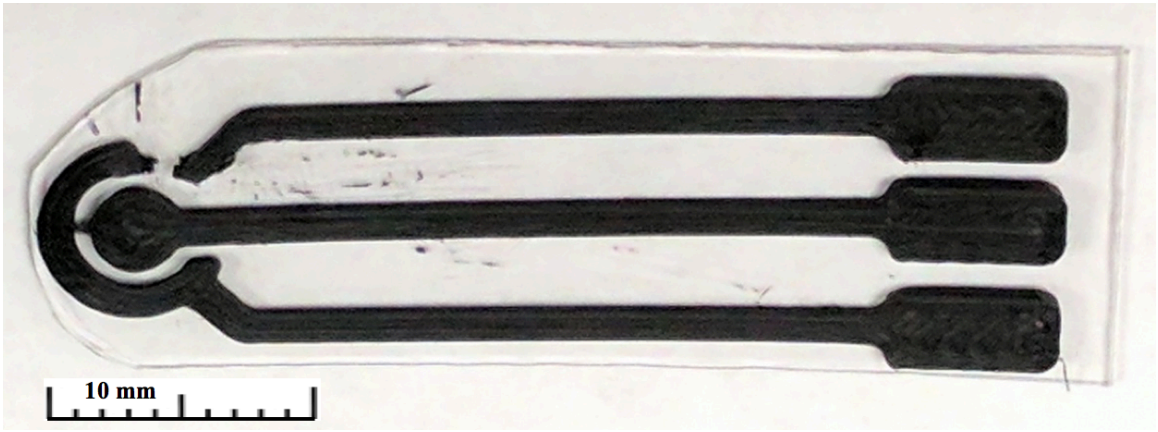

Figure 19: Beta prototype of 3D-printed sensor printed using ABS conductive black filament.

The printed resistance of the working lead of this electrode was determined to be approximately 22 MΩ. Due to a somewhat smaller resistance compared to the previous 3D printed sensors, the electrochemical properties of this sensor were tested. The current

output at various glucose concentrations was measured for the 3D printed sensor and compared to the Zensor. The current outputs for the 3D-printed sensor and the Zensor were compared because it was determined to be a comparable device on the market. The 3D printed sensor produced current output values that were two to three orders of magnitude less than the Zensor current output values. This demonstrated that the resistance was still too high for the electrical sensor. The R-squared value for this data was determined to be very low at 0.01, as seen in Figure 18. The inconsistencies of the physical characteristics of the printed sensors were determined to be the cause for the extremely low R-squared value. The inconsistencies included differences in porosity, mass, and density of the filament between the 3D printed sensors. The data from Figure 20 showed that a more conductive filament and a printer with a higher resolution was required.

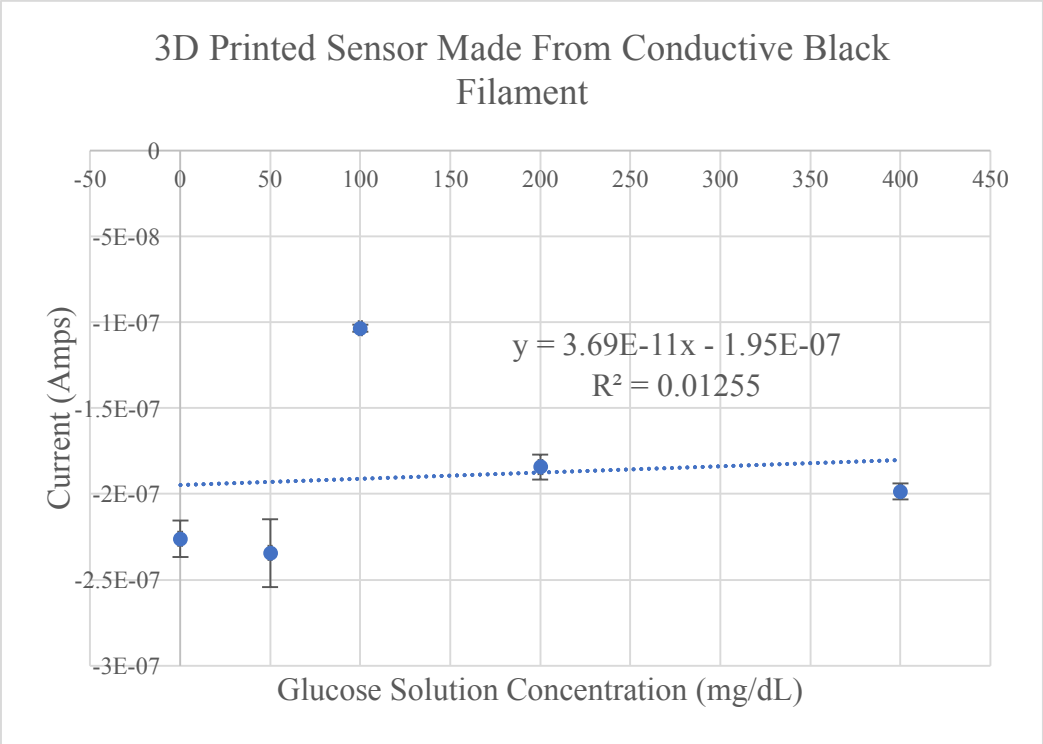

Figure 20: Current verses glucose solutions of various glucose concentrations for sensor printed with conductive black filament.

Development of Gamma Prototype of 3D-Printed Sensor

A conductive graphene filament was ordered (Black Magic 3D, USA), with a preprint resistivity of 1.8 Ω per three centimeters. Graphene was determined to be a good material as it is strong, flexible, and conductive. This filament is a PLA based filament, so a printer specifically designed for PLA filaments was used to increase the resolution and quality of the print. The print settings for this can be found in the methods section. Determining these settings was critical as the graphene in the filament proved to be challenging to print with. Even with the optimal setting for the print, the sensors still had some inconsistencies that could be visually observed. To control for these

inconsistencies, a razor was used to thoroughly and precisely clean filament that had bled between the three leads. After the sensor was printed, the working lead of the electrode was determined to have a resistivity of approximately 700 Ω. This was much more comparable to the Zensor's resistivity of approximately 100 Ω. The sensor printed using the conductive graphene filament can be seen below in Figure 21.

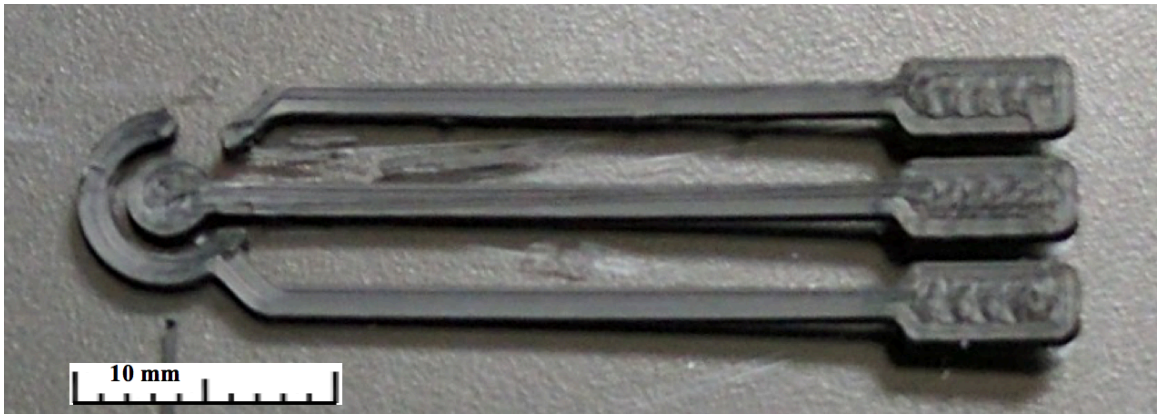

Figure 21: Gamma prototype of 3D-printed sensor made from conductive graphene filament onto a mylar substrate.

Calibration of Gamma Prototype of 3D-Printed Electrode

Before testing could be conducted on the conductive graphene 3D-printed sensors, the specific testing parameters had to be determined. The electrochemical analyzer CV was performed using various concentrations of redox probe at various voltages to determine the suitable parameters for amperometric i-t testing. The parameters were selected to have the best signal-to-noise ratio (SNR). A bias potential of 0.35V was selected, as seen in Figure 22.

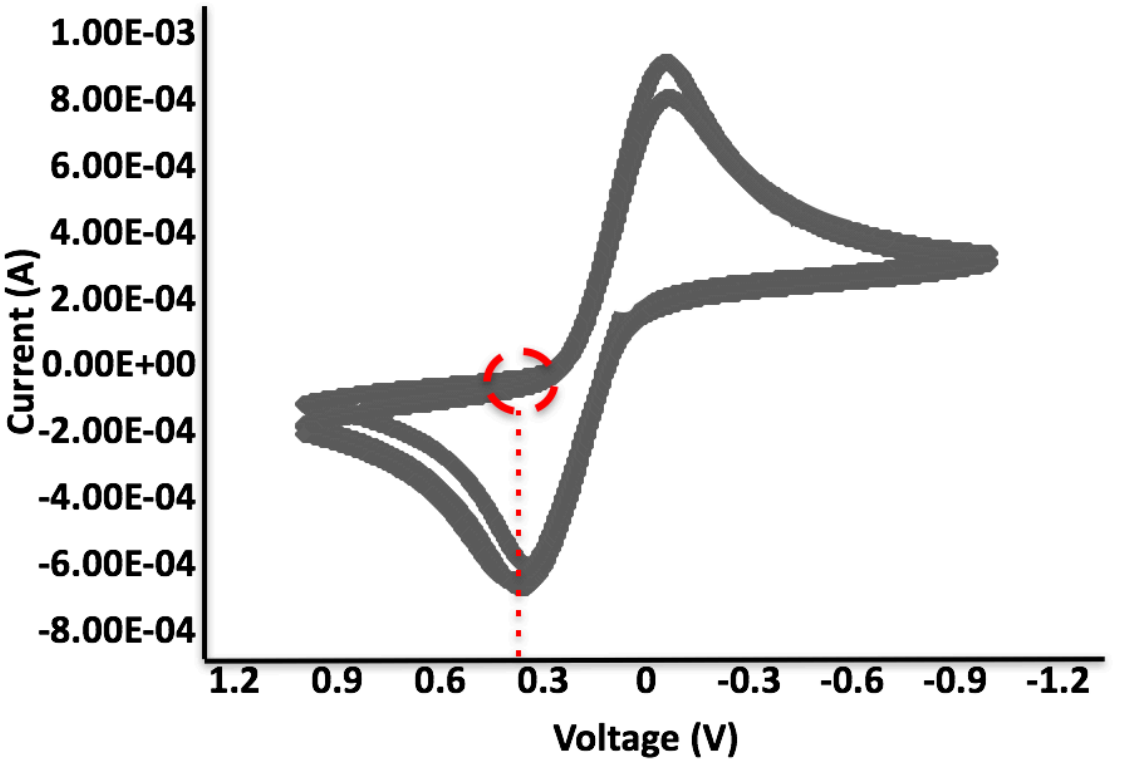

Figure 22: Shows a CV using a Zensor with redox probe. The red circle shows the most optimal current responses observed in amperometric i-t is at 0.35V.

Using this voltage, the various glucose solutions were tested using amperometric i-t. An analysis determined that the electrical current at 42.3 seconds was the best representation of the signal produced by the sample, as shown in Figure 23A. At this time, the electrical current output had the highest correlation and slope. Current values at 42.3 seconds were used in the construction of calibration curves.

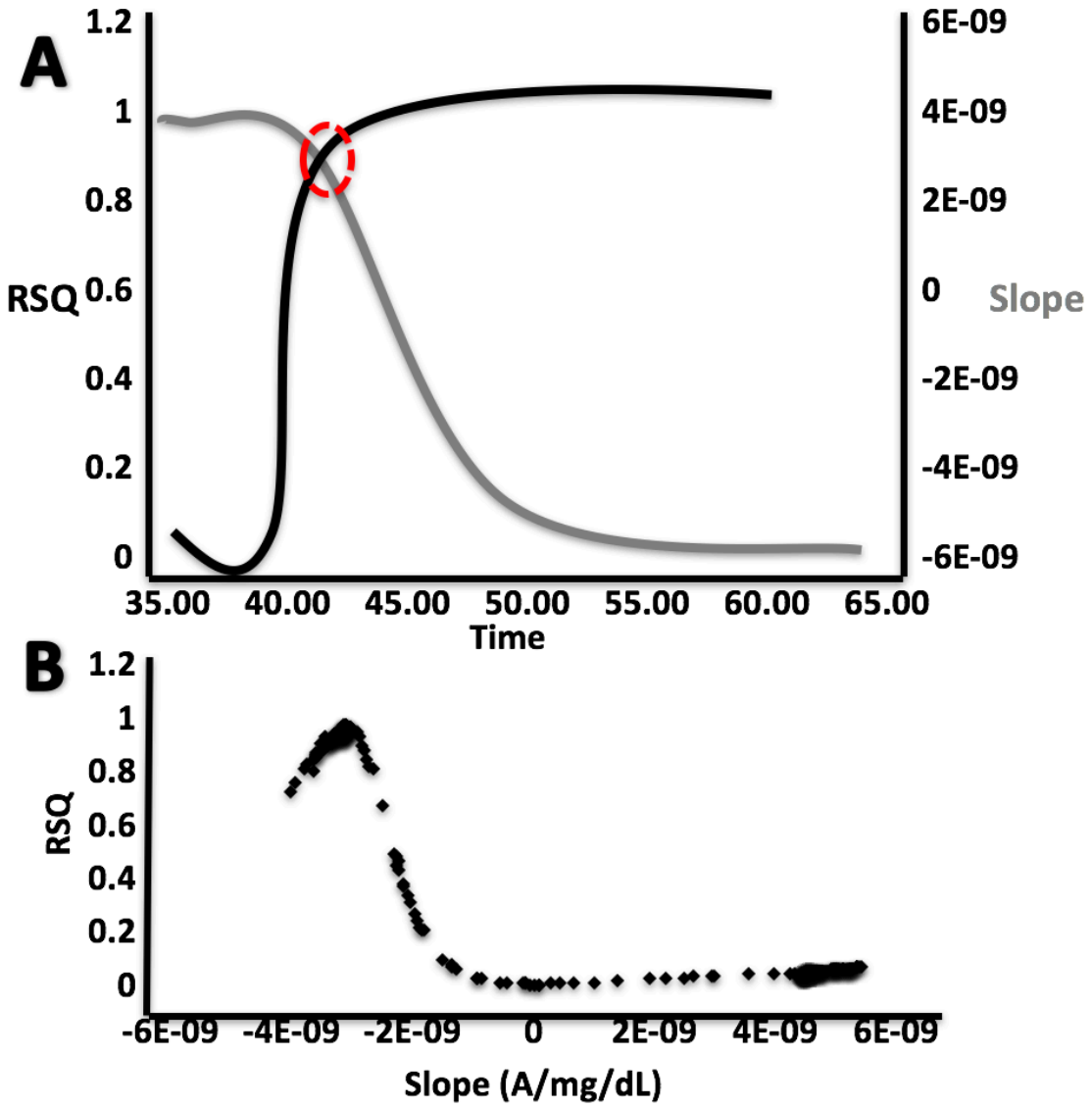

Figure 23: A) A representation of the highest slope and R^2 at the most optimal time point. B) The peal representation of the R^2 versus the slope.

Initial Verification of Gamma Prototype of 3D-Printed Sensor

With a 3D printed sensor with a resistivity comparable to the Zensor and the ideal testing parameters, an amperometric i-t test was performed. The current output was measured for glucose solutions of various concentrations. The results from this data showed that there

was a positive relationship between the measured current output and the glucose concentration; however, the current differences between the various glucose concentrations were very low. This can be seen below in Figures 24 through 26. This data was further analyzed for the current output at 42.3 seconds. The results can be seen in Figures 27 through 30. Sensor 1 was determined to have an R-squared correlation value of 0.75 and a slope of 4.70E-13x with representing the concentration of glucose and the y-intercept being -2.50E-05 Amps. Sensor 2 was determined to have an R-squared correlation value of 0.86 and a slope of 8.89E-13x with representing the concentration of glucose and the y-intercept being -2.50E-05 Amps. Sensor 3 was determined to have an R-squared correlation value of 0.79 and a slope of 1.51E-08x with representing the concentration of glucose and the y-intercept being -2.29E-05 Amps. The average of sensors 1, 2, and 3 was determined to have an R-squared correlation value of 0.79 and a slope of 5.02E-09x with representing the concentration of glucose and the y-intercept being -2.43E-05 Amps. This supported that the 3D printed graphene sensors were accurately detecting the various glucose solutions.

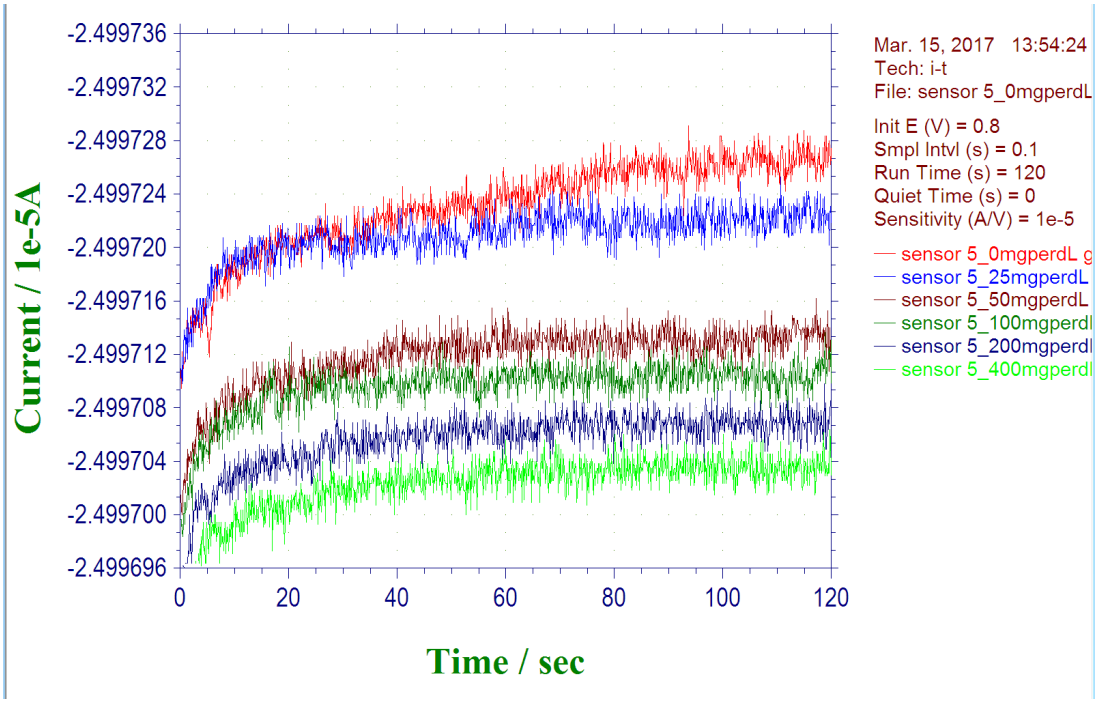

Figure 24: Current versus time for sensor 1 showing the expected trend for various glucose solution concentrations.

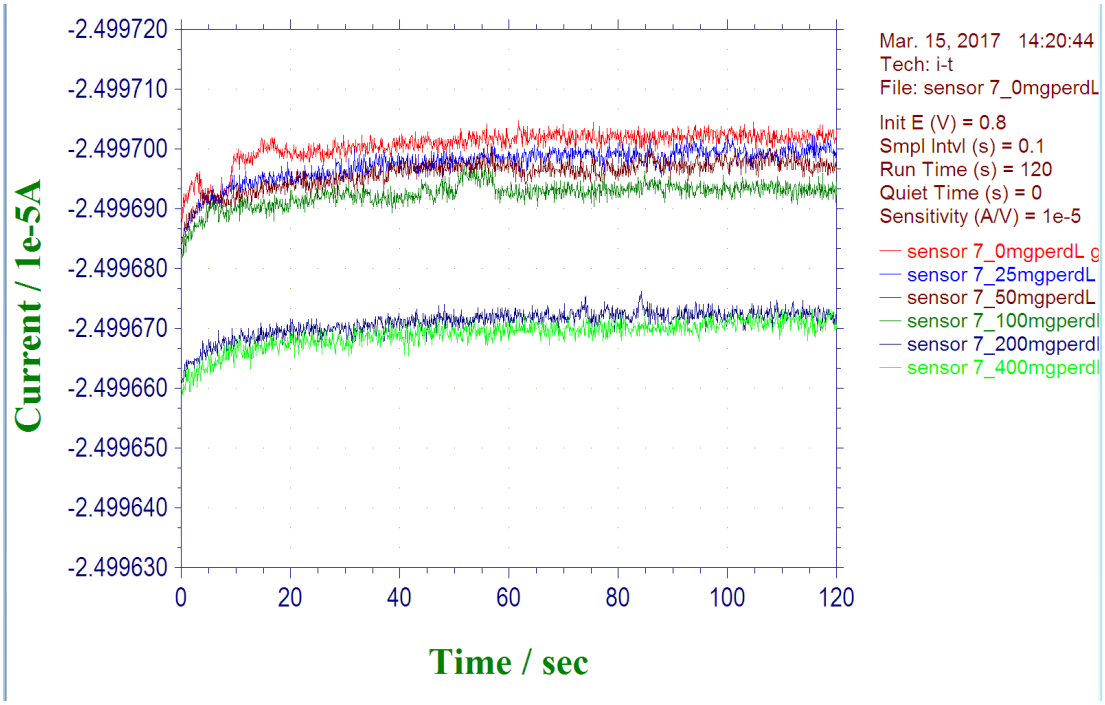

Figure 25: Current versus time for sensor 2 showing the expected trend for various glucose solution concentrations.

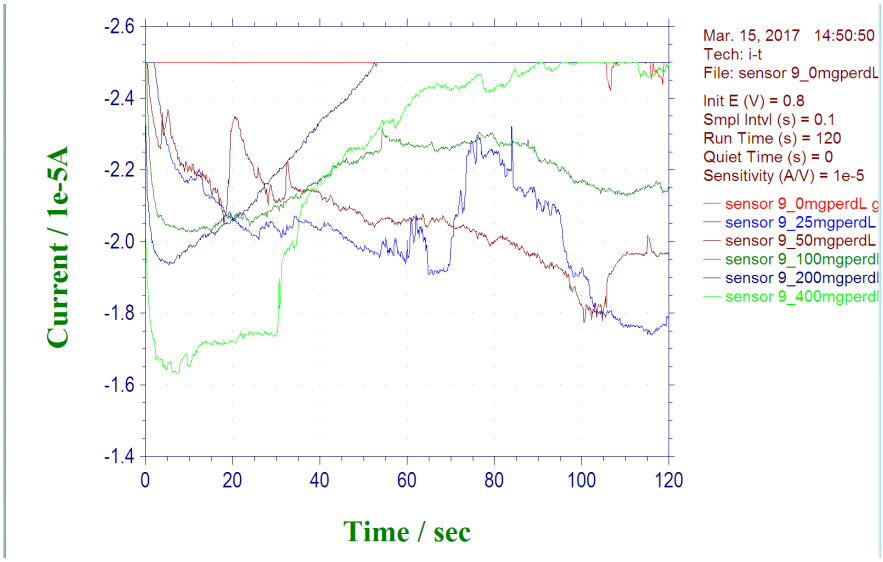

Figure 26: Current versus time for sensor 3 showing somewhat of the expected trend for various glucose solution concentrations. This trend was not as consistent as the other sensors.

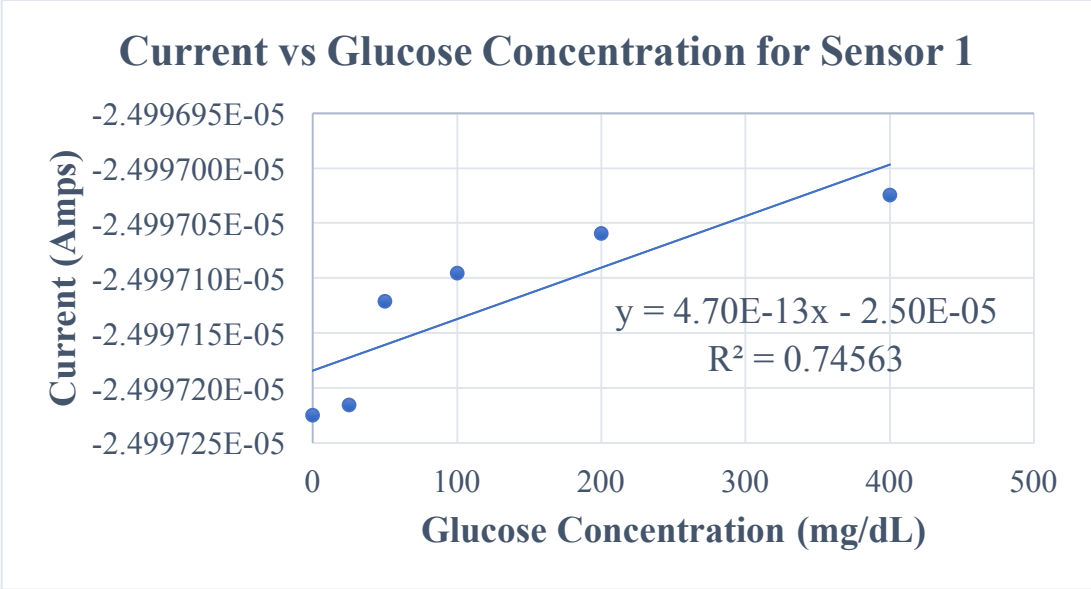

Figure 27: Current verses glucose solutions of various concentrations for sensor #1, which was printed with conductive graphene filament.

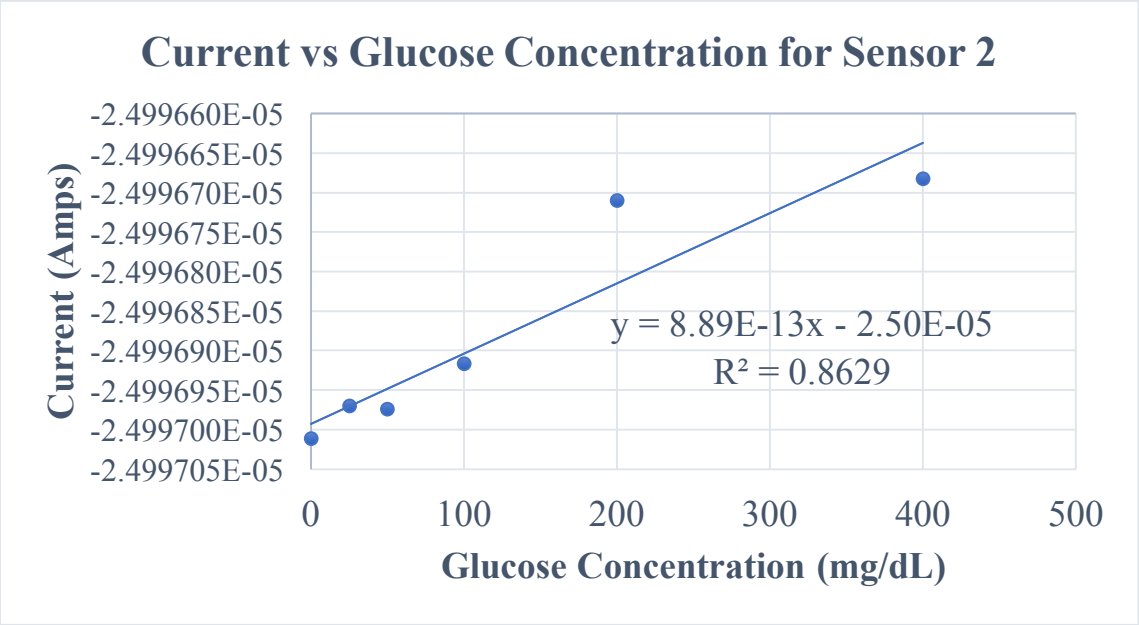

Figure 28: Current verses glucose solutions of various concentrations for sensor #2, which was printed with conductive graphene filament.

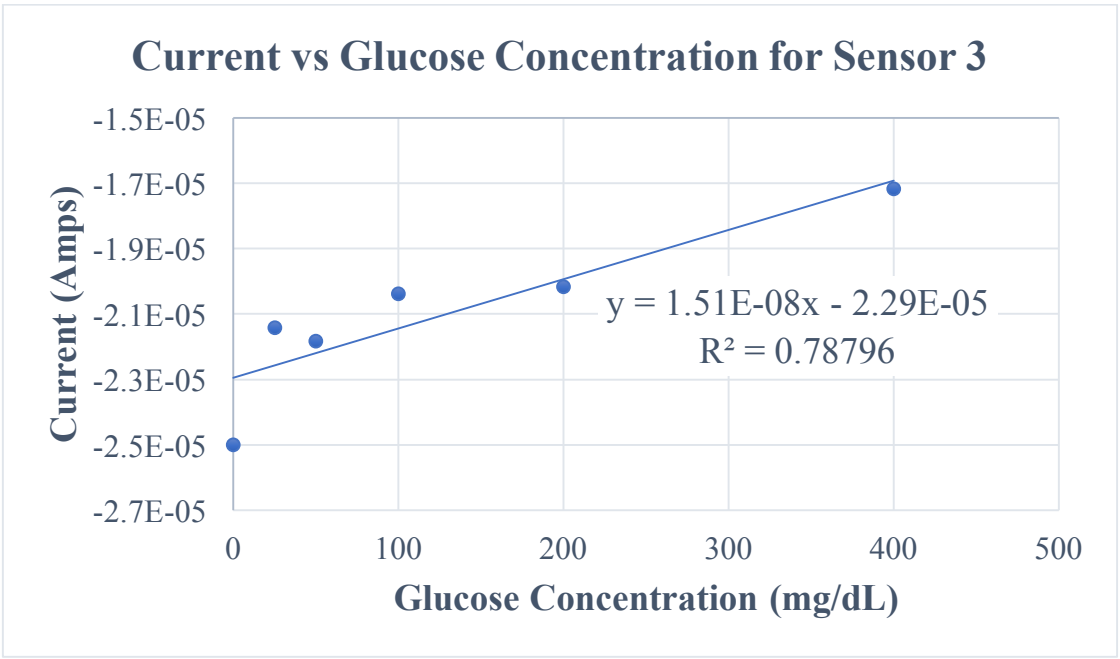

Figure 29: Current verses glucose solutions of various concentrations for sensor #3, which was printed with conductive graphene filament.

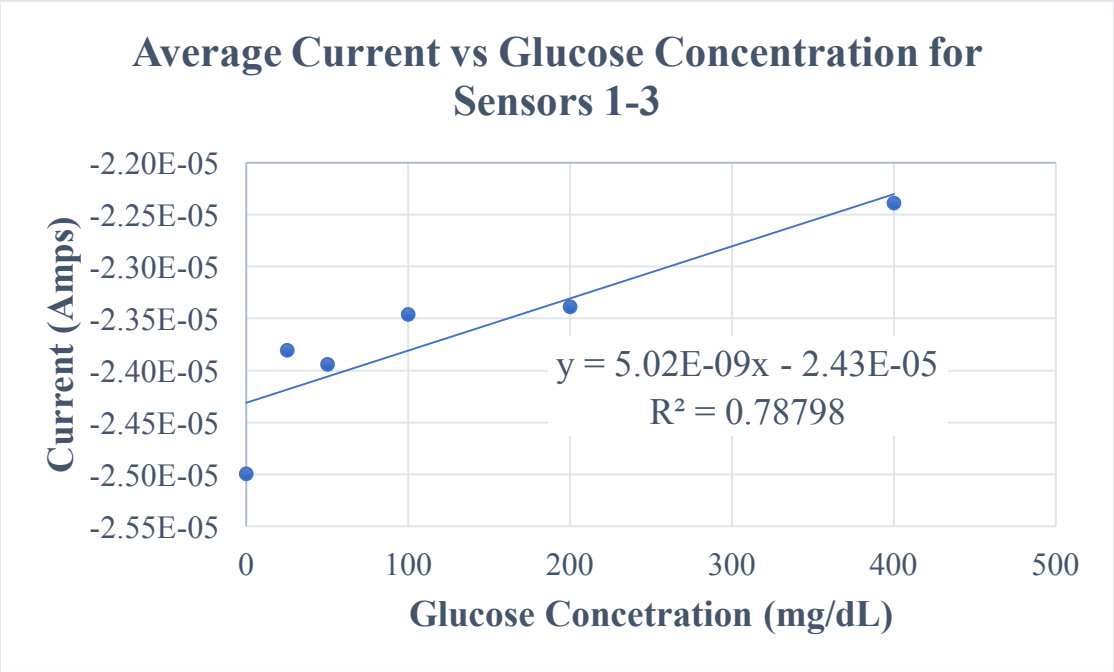

Figure 30: Average of the current verses glucose solutions of various concentrations for sensors 1, 2, and 3, which were printed with conductive graphene filament.

Post Print Modification of 3D-Printed Sensor

It was hypothesized that the low signal difference between glucose concentrations was because the redox probe mixture was not reacting properly with the graphene filament due to the inconsistency of the print quality. Although the printed sensors appeared to be similar, there were differences in the density and porosity of the filament. To correct for this, the sensors were soaked in enzyme solution containing potassium ferricyanide for 420 minutes while in the refrigerator, as seen below in Figure 31.

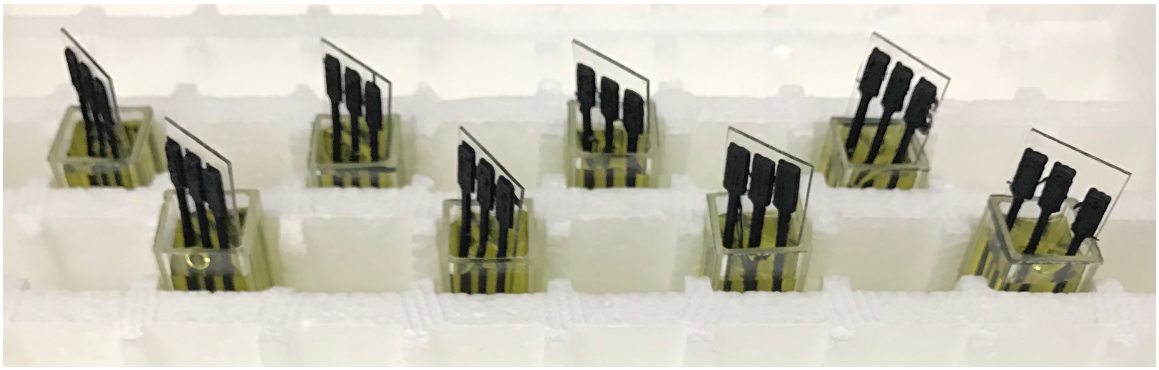

Figure 31: This illustration shows the soaking procedure of the 3D printed graphene sensors. The procedure required using 100 mM concentration of ferri and 209 units/mg concentration of enzyme.

To determine how long the 3D-printed sensors needed to soak in the enzyme solution, sensors were soaked for various lengths of time. The times that were tested included: 0 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 420 minutes. While the sensors were soaking in the enzyme solution, they were stored at 277.15K due to the temperature sensitivity of the GDH-FAD enzyme. The results from this can be seen in Figure 32. A soak time of 60 minutes was determined not to be enough time, as the R^2 value was determined only to be 0.11. Of the soak times tested, 420 minutes produced the best results, as the R^2 value was determined to be 0.92. The R^2 value for the 420-minute soak time was comparable to the R^2 value of the Zensor, which was determined to be 0.99. Soaking the electrodes allowed for the enzyme solution to fill in the pores of the printed sensor, which allowed for easier movement of electrons through the working sensor, resulting in an increase in the current output.

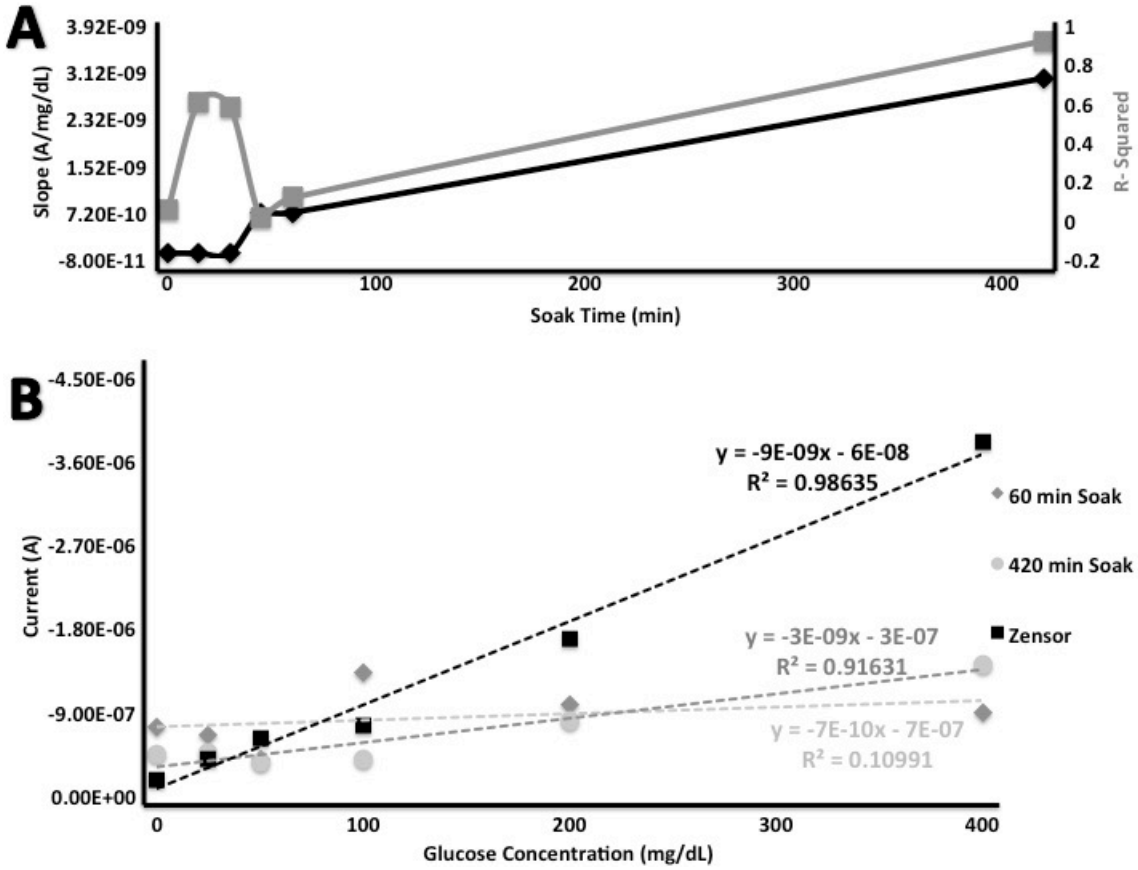

Figure 32: A) Shows the effects of a soak time of 0, 15, 30, 45, 60, 420 minutes on the slope and linear correlation values. B) This shows an overlay plot of the current with soak times of 60 and 420 minutes. The current output for glucose solutions of various concentrations for each individual soak time were overlaid with a Zensor to show that 420 minutes gave the best comparable result when compared with a Zensor. Soak time below 60 minutes produced a flat line when graphed alongside the scale. Additionally, the magnitude of slope increased with increasing soak time.

Quality Control of 3D-Printed Sensor

The electrodes were printed and prepared in batches of nine. The baseline current output readings were compared and the sensors were placed into batches. These batches contained sensors that produced similar baseline current output readings. Placing sensors into batches according to baseline readings is a standard procedure in the SMBG device industry.

Verification of Soaked Gamma Prototype of 3D-Printed Sensor

The current output for the soaked 3D-printed sensors were compared and calibration curves were created. Although 42.3 seconds was determined to be the optimal current output time, calibration curves were also created for 3 seconds and 13 seconds. These times were selected to generate a more reasonable range comparable for SMBG devices when compared to the optimal experimental current output time. As seen in Figure 33C, the calibration curve for 42.3 seconds was determined to have an R-squared correlation value of 0.97, a slope of -3.14E-09x (with x representing the concentration of the glucose solution), and a y-intercept of -3.45E-07 Amps. As seen in Figure 33B, the calibration curve for 13 seconds was determined to have an R-squared correlation value of 0.94, a slope of -3.20E-09x (with x representing the concentration of the glucose solution), and a y-intercept of -4.16E-07 Amps. As seen in Figure 33A, the calibration curve for 3 seconds was determined to have an R-squared correlation value of 0.80, a slope of -3.50E-09x (with x representing the concentration of the glucose solution), and a y-intercept of -6.44E-07 Amps.

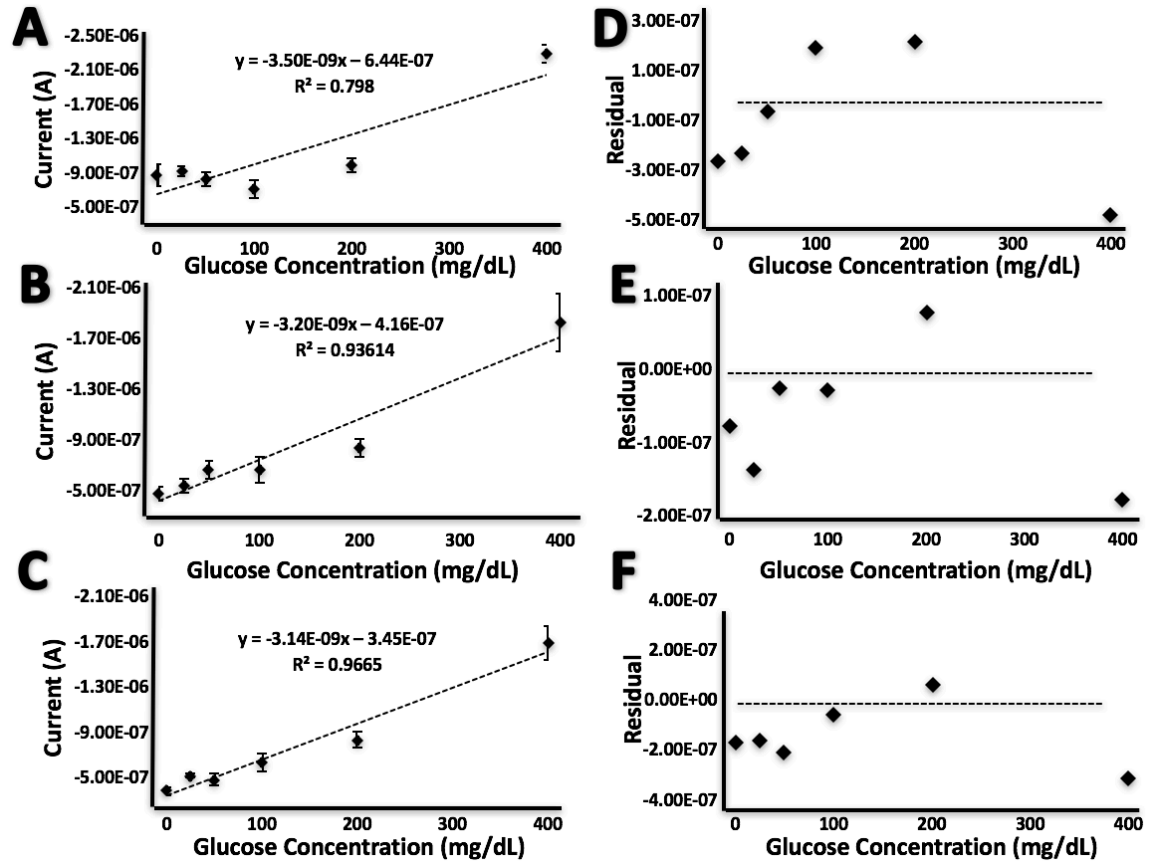

Figure 33: A) This is a representation of the 3D printed strip sensor calibration curve with glucose solutions based off current readings at 3 seconds (N = 4). B) This is a representation of the 3D printed strip sensor calibration curve with glucose solutions based off current readings at 13 seconds (N = 4). C) This is a representation of the 3D printed strip sensor calibration curve with glucose solutions based off current readings at the optimal time of 42.3 seconds (N = 4). Error bars shown for all figures are standard error. D) Normal probability plot of residuals versus the glucose concentrations at time of 3 seconds. E) Normal probability plot of residuals versus the glucose concentrations at time of 13 seconds. F) Normal probability plot of residuals versus the glucose concentrations at time of 42.3 seconds. We can see in the residual plots that after 13

seconds, there is no obvious pattern when plotting the residual vs the run order, which would be evident of a good model.

The calibration curve at a time of 13 seconds is significantly better than the calibration curve at a time of 3 seconds. Although the calibration curve at 42.3 seconds is slightly better than the calibration curve at 13 seconds, the improvements are negligible. This means that the current outputs after 13 seconds are just as reliable as the current outputs at the optimal time of 42.3 seconds.

To determine if the calibration curves fit the data well, the residuals were plotted for the three current output times. These results can be seen above in Figure 33. As shown in Figure 33D, it was determined that the calibration curve at a time of 3 seconds did not fit the data well, as the residuals were not random and a pattern could be observed. As shown in Figure 33E, it was determined that the calibration curve at a time of 13 seconds did fit the data well, as the residuals were random and had a relatively constant spread, allowing a linear regression model to be used. The calibration curve at a time of 42.3 seconds was also determined to be a good fit to the data, as seen in Figure 33F. Figure 33F shows that the residuals do not have a visible pattern and were not spread unevenly. This also supported that a linear regression model fit the data. The calibration curves for the three times were compared to the Zensor calibration curve as seen below in Figure 34 and Figure 35. The calibration curve for the current output time of 42.3 seconds is the most comparable to the calibration curve for the Zensor. This data supported that the 3D-printed sensor could accurately detect glucose solution concentrations between 0mg/dL and 400mg/dL.

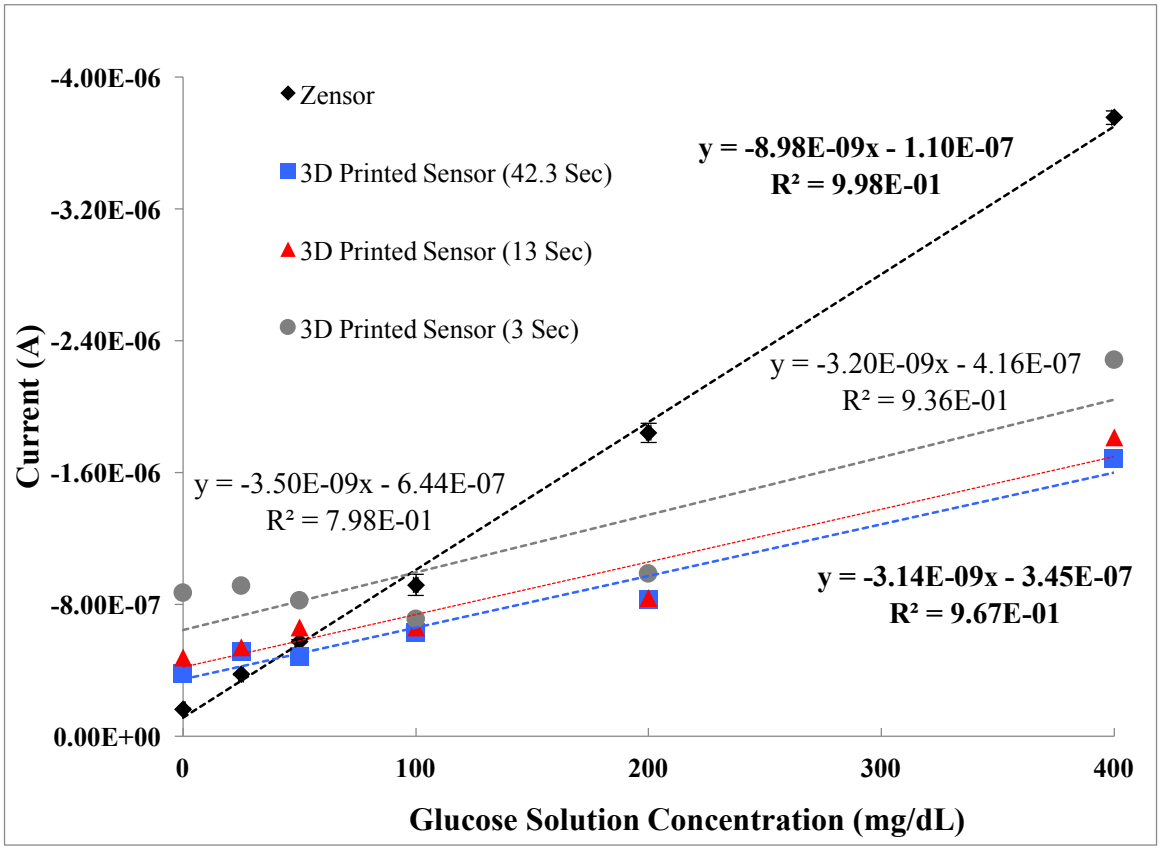

Figure 34: Current verses glucose solution concentration for 3D-printed sensor based off readings at 3 seconds, 13 seconds, and 42.3 seconds, with Zensor comparison.

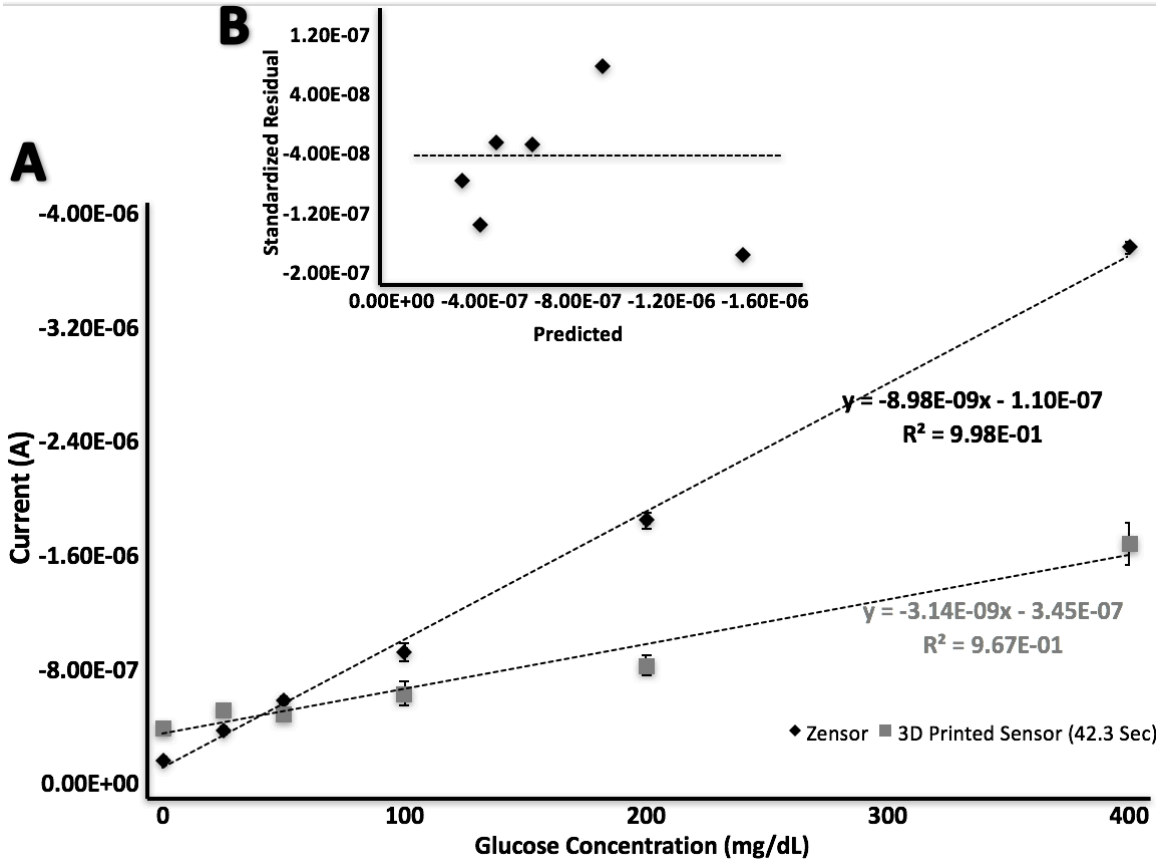

Figure 35: A) This is a representation of the 3D printed strip sensor calibration curve with the glucose solutions based off current readings at 42.3 seconds (N = 4) alongside the Zensor calibration curve. B) Normal probability plot of residuals versus the predicted current value for glucose concentrations at time of 42.3 seconds; there is no obvious pattern, which is evident of a good model.

CHAPTER 4

CONCLUSION

3D-Printed Sensor Verified Through Amperometric i-t Testing

This research represents the first known work in developing a 3D-printed glucose monitoring sensor. It was determined that it is possible to 3D-print a glucose monitoring sensor. The sensor was determined to be sensitive to glucose concentrations between 0 mg/dL to 400 mg/dL, which is a large enough range to properly manage diabetes. The results showed that there were minimal differences between the current output at various glucose solution concentrations between the industry standard Zensor and the 3D-printed graphene electrode. This showed that the 3D-printed sensor was responsive and sensitive. It was also determined that this device could be printed at a much lower cost than currently available devices. As seen below in Table 1, the cost of manufacturing the sensor was determined to be $0.40; however, this low cost for each sensor does not include the high initial cost of a 3D-printer. 3D-printers are continuing to become more affordable, so it is reasonable that in the future they will be a common household appliance. This would allow diabetics to print their own test strips at home for much less than SMBG test strips. This would provide diabetics with a cost effective, accurate device that could reduce noncompliance issues due to the high cost of the test strips, ultimately improving their health outcomes.

Material	Cost Per Test Strip
Conductive Graphene PLA Filament	$0.05
Mylar Substrate	$0.06
GDH Enzyme	$0.18
PBS	$0.05
Ferricyanide	$0.06
Total:	**$0.40**

Table 1: Manufacturing cost for each 3D-printed test strip.

This device could potentially prevent the serious complications caused by diabetes for millions of individuals around the world. Providing diabetics with this device not only will improve their health outcomes, but also will drive down the cost of other devices on the market. This technology also could be utilized in developing countries, as a local clinic could be supplied with a 3D-printer and filament and trained on how to use them. This would provide the clinics with the necessary testing supplies for managing and monitoring diabetes.

This technology could be applied to other biomarkers. Biomarkers for pregnancy, cancer, and various diseases could be monitored using a 3D-printed sensor. This could allow hospitals to print the sensors as needed, which could reduce wasted resources. The SMBG industry could also transition from screen-printed electrodes to 3D-printed electrodes, which could lower the cost of SMBG test strips.

Future Work

There is a lot of work that still needs to be done on this device before it can be marketed to diabetics. A 3D-printer with a much higher resolution should be used to reduce the variance between prints. This would make all the sensors more uniform and reduce the

porosity of the leads. Work must also be completed to reduce the volume of the liquid reagents needed in order to test the device. This can be done by creating a capture area to hold the reagents onto the leads. The technology from the TearTOUCH device could also be translated to this device, making the 3D-printed sensor not only affordable but also non-invasive. This could reduce noncompliance due to high cost of test strips and the pain that is associated with testing.

It would also be useful to create a 3D-printer filament that contained the chemical reagents. This would eliminate the need for reagents to be applied after printing. Creating this filament could potentially be challenging as an enzyme could not be included in the filament. This is because of the high extrude temperature used in the printer would denature most enzymes. An enzyme with a high heat tolerance or a different chemical such as prussian blue could be used in this filament.

REFERENCES

[1] "AM Basics." *Additive Manufacturing (AM)*. N.p., n.d. Web. 04 Apr. 2017.

[2] Bandodkar, Amay J., Wenzhao Jia, Ceren YardÄ±mcÄ±, Xuan Wang, Julian Ramirez, and Joseph Wang. "Tattoo-Based Noninvasive Glucose Monitoring: A Proof-of-Concept Study." *Analytical Chemistry* 87.1 (2015): 394-98. Web.

[3] Belle, Jeffrey T. La, Anngela Adams, Chi En Lin, Erica Engelschall, Breanna Pratt, and Curtiss B. Cook. "Self-monitoring of Tear Glucose: The Development of a Tear Based Glucose Sensor as an Alternative to Self-monitoring of Blood Glucose." *Chemical Communications*. Royal Society of Chemistry, 16 Aug. 2016. Web. 04 Apr. 2017.

[4] Belle, Jeffrey T. La, Daniel K. Bishop, Stephen R. Vossler, Dharmendra R. Patel, and Curtiss B. Cook. "A Disposable Tear Glucose Biosensor—Part 2: System Integration and Model Validation." *Journal of Diabetes Science and Technology*. Diabetes Technology Society, Mar. 2010. Web. 04 Apr. 2017.

[5] Belle, Tom L. Van, Ken T. Coppieters, and Matthias G. Von Herrath. "Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies." *Physiological Reviews*. American Physiological Society, 01 Jan. 2011. Web. 04 Apr. 2017.

[6] "Beta Cell Dysfunction." *Diabetes and the Environment*. N.p., n.d. Web. 04 Apr. 2017.

[7] Chen, Chao, Qingji Xie, Dawei Yang, Hualing Xiao, Yingchun Fu, Yueming Tan, and Shouzhuo Yao. "Recent advances in electrochemical glucose biosensors: a review." RSC Advances 3.14 (2013): 4473-491. RSC Publishing. Web. 5 Apr. 2017.

[8] "Cyclic Voltammetry." *Indiana University*. N.p., n.d. Web.

[9] "Diabetes Complications." *Mayo Clinic*. N.p., n.d. Web. 04 Apr. 2017.

[10] "Diabetes Programme." *World Health Organization*. World Health Organization, n.d. Web. 04 Apr. 2017.

[11] DK, Bishop, La Belle JT, Vossler SR, Patel DR, and Cook CB. "A Disposable Tear Glucose Biosensor-part 1: Design and Concept Testing." *Journal of Diabetes Science and Technology*. N.p., n.d. Web. 04 Apr. 2017.

[12] Ferri, Stefano, Ph.D., Katsuhiro Kojima, Ph.D., and Koji Sode, Ph.D. "Review of Glucose Oxidases and Glucose Dehydrogenases: A Bird's Eye View of Glucose

Sensing Enzymes." Journal of Diabetes Science and Technology 5.5 (September 2011): 1068-076. JournalofDST. Web. 4 Apr. 2017.

[13] Grieshaber, Dorothee, Robert Mackenzie, Janos Vörös, and Erik Reimhult. "Electrochemical Biosensors - Sensor Principles and Architectures." Sensors 8.3 (2008): 1400-458. NCBI. Web. 7 Apr. 2017.

[14] "Hypoglycemia (Low Blood Glucose)." *American Diabetes Association*. N.p., n.d. Web. 04 Apr. 2017.

[15] Liao, Yu-Te, Huanfen Yao, Andrew Lingley, Babak Parviz, and Brian P. Otis. "A 3-$muhbox{W}$ CMOS Glucose Sensor for Wireless Contact-Lens Tear Glucose Monitoring." *IEEE Journal of Solid-State Circuits* 47.1 (2012): 335-44. Web.

[16] Libretexts. "Cyclic Voltammetry." *Chemistry LibreTexts*. Libretexts, 16 Feb. 2017. Web. 04 Apr. 2017.

[17] Libretexts. "11.2: Potentiometric Methods." *Chemistry LibreTexts*. Libretexts, 21 July 2016. Web. 04 Apr. 2017.

[18] Madkour, Loutfy. "Comparison of Various Electrochemical Methods." *Academia.edu*. N.p., n.d. Web. 04 Apr. 2017.

[19] Mayo Clinic Staff Print. "Overview." *Mayo Clinic*. N.p., 13 Jan. 2016. Web. 04 Apr. 2017.

[20] "Model 600C Series Electrochemical Analyzer/Workstation User's Manual." *CH Instruments*. N.p., n.d. Web.

[21] "Risk Factors." *International Diabetes Federation*. N.p., n.d. Web. 04 Apr. 2017.

[22] Shrivastava, Saurabh, Prateek Shrivastava, and Jegadeesh Ramasamy. "Role of Self-care in Management of Diabetes Mellitus." *Journal of Diabetes & Metabolic Disorders* 12.1 (2013): 14. Web.

[23] "Statistics About Diabetes." *American Diabetes Association*. N.p., n.d. Web. 04 Apr. 2017.

[24] Stretton, Tom. "SCH4U Electrochemistry: Introduction." *SCH4U Electrochemistry: Introduction*. N.p., n.d. Web. 04 Apr. 2017.

[25] "Type 1 Diabetes." *American Diabetes Association*. N.p., n.d. Web. 04 Apr. 2017.

[26] Ventola, C. Lee. “Medical Applications for 3D Printing: Current and Projected Uses.” *Pharmacy and Therapeutics* 39.10 (2014): 704–711. Print.

[27] Yeaw, J., W. C. Lee, M. Aagren, and T. Christensen. "Cost of Self-monitoring of Blood Glucose in the United States among Patients on an Insulin Regimen for Diabetes."*Journal of Managed Care Pharmacy : JMCP*. U.S. National Library of Medicine, n.d. Web. 04 Apr. 2017.

[28] 2602, Issn 2320 -. *Jabbar Qasim Al-Maliki Et Al., International Journal of Advances in Computer Science and Technology, 4(10), October 2015, 161 - 165* (n.d.): n. pag. Web.

[29] "3D Printing: A Manufacturing Revolution." *A.T. Kearney*. N.p., n.d. Web.

[30] "4 Ways to Get Started." *Continuous Glucose Monitoring | CGM | Medtronic Diabetes*. N.p., n.d. Web. 04 Apr. 2017.