DESIGN AND CHARACTERIZATION OF CONTINUOUS GLUCOSE MONITORING SENSOR

A Thesis

by

Chedia Ben Ali Hassine

Submitted to the
Graduate School of Sciences and Engineering
In Partial Fulfillment of the Requirements for
the Degree of

Master of Science

in the
Department of Electrical and Electronics Engineering

Özyeğin University
August 2022

DESIGN AND CHARACTERIZATION OF CONTINUOUS GLUCOSE MONITORING SENSOR

Approved by:

Assistant Professor Ahmed AKGIRAY,
Advisor
Department of Electrical and Electronics Engineering
Özyeğin University

Assistant Professor Ahmet TEKIN
Department of Electrical and Electronics Engineering
İstanbul Technical University

Assistant Professor Ramazan Ünal
Department of Mechnical Engineering
Özyeğin University

Date Approved: 12 August 2022

To my grandparents, my parents, sister and loved ones.

ABSTRACT

The main objective of this thesis is the development and the elaboration of a low-cost continuous glucose monitor. This thesis describes the design of miniaturized continuous glucose monitoring sensor based on two needles. The sensor is based on enzymatic detection of glucose. The used enzyme is glucose oxidase entrapped inside a protecting film of chitosan. Chapter I provides a general introduction to diabetes and the different types of biosensors used for continuous glucose monitoring. "Continuous" monitoring approaches involve subcutaneous implantation of a micro-needle allowing real-time glucose level measurements in the interstitial fluid. The working principals of continuous glucose monitors (CGM)s are introduced. An overview of electrochemical sensors is presented, and the different methods of common electrode modification are also discussed in this chapter. The electrical circuits that are used for glucose sensors are reviewed. The second chapter contains the methods and materials used in the performed experiments. It describes the steps of sensor elaboration from the electrical circuit assembly on the PCB board to the electrode modification process. The measurement setup is also presented in this chapter. Chapter 3 discusses the results obtained for the sensor tests. Chapter 4 is the conclusion, it stipulates that after the tests carried on the elaborated biosensor both in the buffer solution and in the real sample, the sensor can be improved and it has potential to be used in tests on humans.

ÖZETÇE

Bu tezin temel amacı, düşük maliyetli bir sürekli glikoz monitörünün geliştirilmesi ve detaylandırılmasıdır. Bu tez, iki iğneye dayalı minyatür sürekli glikoz izleme sensörünün tasarımını açıklamaktadır. Sensör, glikozun enzimatik tespitine dayanmaktadır. Kullanılan enzim, koruyucu bir kitosan filmi içinde tutulan glikoz oksidazdır. Bölüm I, diyabete ve sürekli glikoz izleme için kullanılan farklı biyosensör türlerine genel bir giriş sağlar. "Sürekli" izleme yaklaşımları, interstisyel sıvıdaki glikoz konsantrasyonlarının gerçek zamanlı ölçümüne izin veren bir mikroiğnenin subkutan implantasyonunu içerir. Sürekli glikoz monitörlerinin (CGM) çalışma prensipleri tanıtılmaktadır. Elektrokimyasal sensörlere genel bir bakış sunulur ve bu bölümde yaygın elektrot modifikasyonunun farklı yöntemleri de tartışılır. Glikoz sensörleri için kullanılan elektrik devreleri gözden geçirilir. İkinci bölüm, gerçekleştirilen deneylerde kullanılan yöntem ve malzemeleri içermektedir. PCB kartındaki elektrik devresi montajından elektrot modifikasyon işlemine kadar sensör detaylandırma adımlarını açıklar. Ölçüm kurulumu da bu bölümde sunulmaktadır. Bölüm 3, sensör testleri için elde edilen sonuçları tartışır. Bölüm 4 sonuçtur, ayrıntılı biyosensör üzerinde hem tampon çözeltide hem de gerçek numunede yapılan testlerden sonra sensörün geliştirilebileceğini ve insanlar üzerinde testlerde kullanılma potansiyeline sahip olduğunu şart koşar.

ACKNOWLEDGEMENTS

This thesis work was developed within the Analog Laboratory at Özyeğin University, Istanbul under the co-supervision of Prof. Ahmet Tekin and Dr. Ahmed Akgiray. I would like to thank them for welcoming me into their laboratory team, for the interest they took in my work and for all the knowledge they shared with me during my thesis. Members of the jury, you do me the honor of accepting to examine my work. Please find here, the testimony of my gratitude and my deep respect. I would like to express my gratitude to all the members of the jury for the time they devoted to evaluating my work. Their comments and questions allowed me to clarify my writing and gave me new avenues for reflection. I will not forget to also thank my friends at OZU as well as the stuff working at OZU who accompanied me in my thesis. Finally, I wish good luck to all ÖzÜ students who would be defending their thesis in the near future.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 Diabetes

Diabetes is a severe and chronic disease having a considerable influence on the well-being and lives of individuals. It was estimated that diabetes caused four million deaths worldwide in the year 2017 [6]. Patients with diabetes can have fluctuations of their blood glucose concentrations during the day resulting in hyperglycemia or hypoglycemia. Hypoglycemia can lead to loss of counter regulatory mechanisms. Hypoglycaemia is an abnormally low blood sugar (glycaemia) concentration, that is to say, less than 0.80g/l [7]. Inadequacy of the treatment can cause hypoglycemia which is associated with increased risk of hypoglycemia, decreased quality of life, increased healthcare costs, and increased risk of subsequent mortality and cardiovascular disease. Hyperglycemia is an abnormally high blood sugar (glycemia) concentration. For a human being, this corresponds to a blood sugar level above 1.20 g/L on an empty stomach, and 2.00 g/L the rest of the time. Since 2000, the International Diabetes Federation has reported on the incidence of diabetes at the regional, national and global level [6]. In 2021, 537 million adults have diabetes, they estimate that this number will increase in 2030 to 643 million and in 2045 this number will increase to 783 million [8]. Blood sugar should be frequently monitored in such a way that this information will be used in an appropriate way for the treatment of diabetes in order to maintain the glucose concentrations in the recommended range. Continuously measuring blood glucose can help these patients manage their diabetes and have a better quality of life [1].

1.2 Glucose

Glucose is stored as starch in plants and as glycogen in animals, which can be hydrolyzed at any time to give back glucose molecules ready to be broken down to

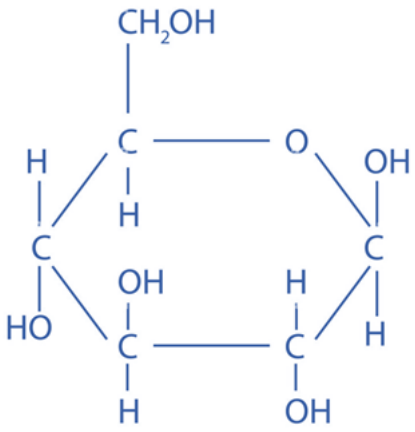

Figure 1: Glucose molecule structure.

provide energy as soon as the cell needs it. It also plays a structural role as cellulose in plants. Glucose circulates through the body in the blood. The concentration of glucose in blood plasma is called blood sugar. As shown in Figure 1, glucose is a simple sugar, with the chemical formula C6H12O6. The suffix -ose is a chemical classifier indicating that it is a carbohydrate; it is also the body's main source of energy. Other simple sugars with the same composition, but different structure are fructose and galactose [9, 10, 11].

1.3 Continuous glucose monitoring sensors

Solutions must be found to reduce the hardship and pain caused by measuring blood sugar. One solution consists of implanting a biosensor so that the blood sugar level is measured in vivo. This has the advantage of preventing diabetics from having to repeat the procedure several times a day to measure their blood sugar and acquiring almost continuous blood sugar measurements and not just occasionally during the day. Moreover, such a sensor can be coupled to an insulin pump to operate in a closed circuit. The biosensor can measure blood sugar, and based on blood sugar, the pump regulates the amount of insulin that needs to be injected into the blood. This device with an insulin pump thus plays the role of an artificial pancreas and has the additional advantage of delaying the development of chronic complications linked to diabetes [12]. In the last few years, significant

advances have been made in continuous glucose monitors (CGMs) but only some of them have been manufactured. Many recent developments have focused on CGM technologies. Several studies have shown that using continuous glucose sensors provides many glycemic benefits for patients with type-1 and type-2 diabetes [13, 14, 15, 16, 17, 18]. Improvements in the accuracy and lifetime of continuous glucose monitors have led to increase in the use of these kind of devices. CGM sensors have been shown to ameliorate the glycemic control significantly for diabetic patients [19]. In order to reduce the complications related to diabetes strict monitoring of glucose level is recommended for diabetes. Glucose monitors can help in the suitable management of diabetes; evaluate managed treatment and prevent or delay the development of diabetes complications [20]. CGMs provide detailed, real-time glucose level information through intermittent blood sampling and alert the patient to impending hypoglycemia and hyperglycemia. These sensors provide maximum needed information about blood sugar fluctuations during the day, including the duration, frequency, and magnitude of these fluctuations. This information can help diabetics improve the quality of their treatment [21]. Accordingly, the use of continuous glucose monitoring in parallel with insulin pumps made the management of diabetes easier. CGMs provide a better alternative for patients, eliminating the need for frequent blood sugar sampling as it is inconvenient for them [1]. Mostly, these invasive sensors rely on amperometry measurements that measure glucose concentrations in the extracellular fluid and can procure alerts against fluctuations in the case of high glucose levels throughout the day [22, 23, 24]. Since 2010, several research focused on continuous monitoring sensors. As shown in Figure 2 taken from the dimensions database showing the evolution of the annual number of scientific publications dealing with Continuous Glucose Monitoring sensors; there is a growing increase in the publications number. Indeed, in the last 10 years it increased from 20,041 in 2010 to 53,731 in 2021. The increasing number of publications per year confirms the importance of such devices. Efforts to realize this type of technology have led to several successful

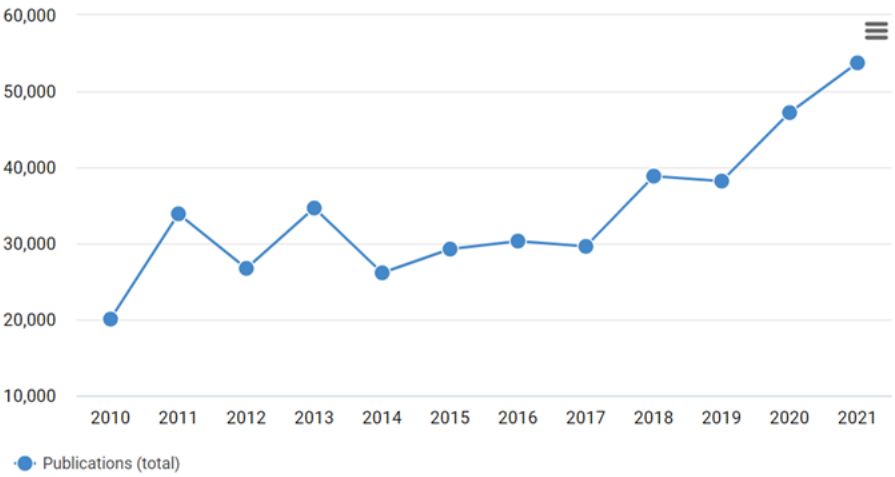

Figure 2: Number of publications in each year for the keywords 'Continuous Glucose Monitoring' [3]

commercial glucose sensors [21]. Table 1 and 2 show some of the market available sensors and their analytical performances. Several CGMs are available and usually consist of a body-worn sensor that can automatically take frequent glucose readings. Data is sent via Bluetooth integrated system to the receiver to display the results [25].

The first next-generation of sensors was the Medtronic Enlite continuous glucose system. This device extends the lifetime to 6 days. Additionally, the comfort of the sensor was improved by reducing its weight and size, it is designed to be waterproof, and allows BG storage for up to 10 hours in the event that the receiver's connection to the transmitter is interrupted for some reason. After that, Abbott released the Freestyle Navigator II, a new product that provides minute-by-minute blood sugar readings. In 2012, Dexcom designed and produced the G4 Platinum, which features a smaller detector that lasts 7 days due to new algorithms built directly into the device. In 2015, Dexcom launched the G5 Mobile with a consumption time up to 7 days, which allows blood glucose data to be transmitted to a cell phone and therefor there is no need to use another receiver. Abbott released the Freestyle Libre in 2016. This CGM sensor is the first that does not require a finger prick test during wear. In accordance with this technology trend, in 2017, Dexcom launched the G6, a CGM which can be used and doesn't need in vivo

calibration, for 10 days and ensure the same accuracy as the previous model G5. In 2017 also, Medtronic has introduced as new product the Guardian Sensor 3 with an accuracy of 10.6 percent . This glucose sensor is smaller by 80 percent than the previous Enlite one and provides 7 days lifetime and a shorter start-up time. Manufactured glucose sensors are very expensive for diabetics who really need them. A study was conducted that assessed the harms and benefits in users and non-users of glucose sensors. The authors found that most non-users and some users said currently available glucose sensors were too expensive to wear on a regular basis. Therefore, if glucose sensors are offered at a lower price in the market, more patients will use glucose sensors to monitor their blood sugar daily [26].Our aim according to this thesis is to provide an accurate and low-cost sensor. A simple assembly and coating methods will be used to achieve our objective. The final product will be lower in its price and therefore can be used by all diabetic patients all around the world.

Table 1: Examples of commercialized sensors [1].

Device	Lifetime	M.C.R/day
Medtronic Paradigm Minimed Veo(530 G)	6 days	2/day
Medtronic 630G with Smart Guard	6 days	2/day
Medtronic 670G	3, 7 days	2/day
IPro 2	6 days days	4/day
Dexcom G4 /G5/G6	7 days	2/day No cal G6
Abbott Freestyle Libre Flash	14 days	No cal

M.C.R is Minimum calibration requirement and cal is calibration

Table 2: Comparison of commercial continuous glucose monitors[2]

Product name	Accuracy	Calibration frequency per day	lifetime
Medtronic	10.55%	Min 2, (3–4 Recommended)	7 days
Dexcom	9%	2 (every 12 h)	7 days
Abbott	9.7%	0	10 days

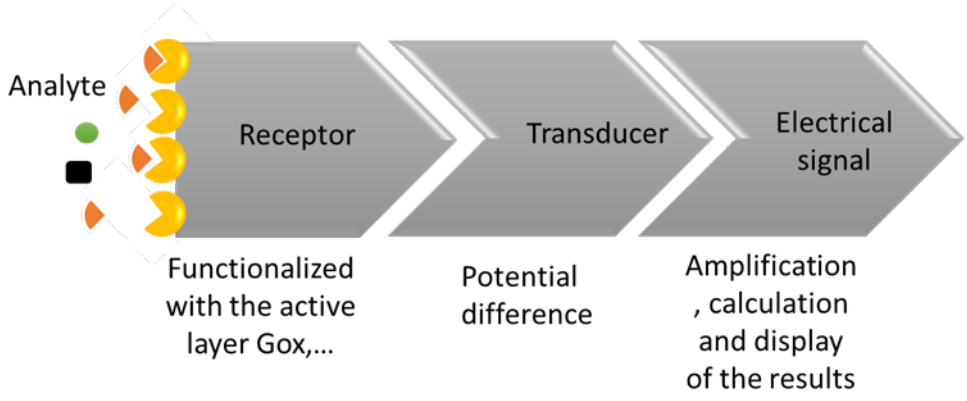

Figure 3: The principle of operation of the sensor.

1.4 Electrochemical sensors

An electrochemical sensor provides information about its physical, chemical and biological environment. A sensor generally consists of two major components connected in series. These components are a recognition system or a receiver and a transducer (Figure 3). The transducer transfers the signal from the output domain of the recognition system directly to the electrical domain. The transducer is as well named a detector, electrode or sensor however the term transducer is mostly used to avoid any confusion [27, 28]. A biosensor is mainly composed of a biological entity (enzymes, antibodies, bacteria, tissues) as a recognition agent, in the other hand a chemical sensor does not contain biological agents and mostly is containing chelating molecules. Sensor devices are manufactured from conventional solid electrolytes, semiconductors, insulators, catalytic materials and metals. Sensor modeling consists of preparing the sensor surface in such a way that it is sensitive for only one type of analyte. All sensors in electro-analytical detection contains two functional units. A receiving unit, that transforms the chemical information into energy and a transducer that transforms the energy, keeping the chemical information, to an easy-to-process signal [29, 30].

Electrochemical sensors have the ability to generate immediately an electrical signal. It is their principal advantages. Electrochemical sensors can be classified according to their operating principles as shown in the following Table 3 [27].

Table 3: Principles of transducers and measurement techniques for electrochemical sensors.

Type of transducer	Transducer	Measured entity
Potentiometric	Convective energy	Tension
Amperometric and coulometric	Current limit	Current
Conductimetric and impedimetric	Resistive	Resistance or conductance

1.5 Electrodes

During an electrochemical reaction, two half-reactions occur at two different electrodes. The oxidation reaction occurs at the anode and the reduction reaction occurs at the cathode. In electroanalytical chemistry, an electrode often alternates being the anode and then the cathode. Thus, the terms "anode" and "cathode" are uncommonly used designations. Indeed, there is an electrode where the chemical species of interest (analytes) react, which is called the working electrode. In addition, due to the fact that it is not possible to quantify the electric potential of a single electrode, another electrode is used to measure the voltage (ddp: difference in potentials) between the working electrode and this electrode. This second electrode is called the reference electrode. This electrode is mainly a half cell of a system whose potential must be known and constant during the experiments [27].

1.6 Electrode surface modification

There are several methods allowing the surface modification among these methods we will quote the most frequently used:

1.6.1 Ionic liquids

Modification of a solid surface after immersion in a liquid has been around since the 1970s and this method was not used until 1990. Markin and his colleagues observed that deposition of oil droplets on the surface of the electrode forms a stable deposition. In most cases, electrodes modified with ionic liquids are prepared by direct precipitation of the ionic liquid on the surface of the electrode

or its solution diluted in a volatile solvent. These two processes can give deposits of different geometric shapes [31].

1.6.2 Spin coating

The spin coating method is widely used to prepare polymer films on the surface because it results in homogeneous films, which are repeatable over a large surface. This method consists of liquid deposition on the substrate, followed by rotating the substrate at the chosen speed. The liquid can be applied while the substrate is in motion. However, these thin films may have surface defects. For example, radial streaks were often detected both at the center level and at the periphery of the substrate. Researchers dedicate much effort to study the surface stability and morphology of spin-coated thin films in order to understand the reason behind these defects in the structures of the formed film at the surface through the spin-coating procedure [32]. Most research currently focuses on elucidating film surface shapes with analytical tools like optical microscopy and atomic force microscopy (AFM) [33].

1.6.3 Dip-coating

When the substrate is dipped in a polymerizing chemical solution, some polymer is deposited on its surface. This process takes place on different substrates such as metals, carbon, plastic..., and the thickness of the film is controlled by modifying the dipping speed. A similar process requires alternating immersion of the substrate in monomer and oxidizing solutions. The monomer adsorbed on the surface of the substrate will be polymerized [34]. Dip-coating is an easy and low-cost method used in many industrial applications. The process can be defined as the deposition of an aqueous liquid phase coating solutions on the surface of a chosen substrate. In general, the materials are dissolved in solutions and coated directly on the surface of the substrate, and then the solvent is evaporated in order to have a dry film. This method requires immersing the substrate in the coating dissolved material, to make sure that the substrate is completely drained and after that the

substrate is removed from the solution. It has to be noted that the apparently simple film-forming technique by dip coating method involves multivariate chemical and physical parameters. While immersion and coating, the morphology and thickness of the deposited thin films is determined by several parameters like immersion time, shrinkage rate, dip coating cycles, viscosity and density, substrate surface, surface tension, and evaporation parameters of the coating solutions [35].

1.6.4 Electrochemical modification

Electrochemical deposition is usually performed in a single or double cell, using a three-electrodes system in a bath containing a supporting electrolyte and a monomer dissolved in the suitable solvent. A set of three electrodes is used and consists of a working electrode, a reference electrode and a counter electrode. The working electrode is the surface where the sensing film is deposited. Metals like titanium, gold, nickel, platinum, carbon and palladium are used. Electrodes made of glass coated with indium tin oxide or tin oxide can be used. A metal plate of nickel, gold or platinum is in general used as the counter electrode. For the reference electrode, in aquatic systems, silver or mercury sulfate electrodes are often used [27, 36].

1.6.5 Enzyme immobilization techniques

It is necessary to immobilize biological elements on the surface of the transducer to design the biosensor's biorecognition. Choosing the appropriate immobilization technique is key to prepare a sensor since after immobilization the biorecognition element or molecule could get inactivated or detached. The biomolecules used have to conserve their biological activity, function and structure when the biosensor is being used. There are two most familiar important immobilization methods: Physical which is reversible and chemical which is irreversible. the physical-chemical environment, the transducer, the biorecognition element selected, and the analyte's characteristics must all be considered when choosing the appropriate technique [37, 38, 39, 40, 41].

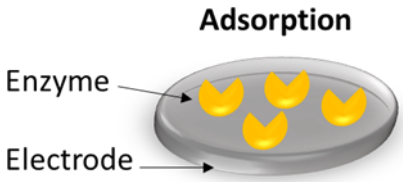

Figure 4: Enzyme adsorption.

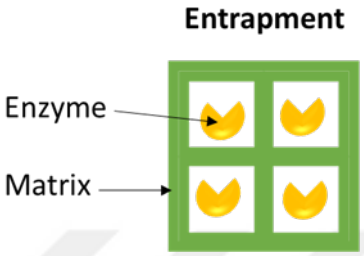

Figure 5: Enzyme adsorption.

-Physical adsorption Concerning the adsorption coating method, weak attractive forces, for example Van Der Waals force, ionic bonding, the electrostatic force, or hydrogen bonding are used to immobilize the biological recognition element on the superficial surface of the inert solid material [37, 38, 39, 41]. Figure 4 shows the physical adsorption of an enzyme at an electrode surface.

-Physical entrapment In this process, non-covalent or covalent bonds physically entrap the biorecognition elements within the 3D matrices. this can be done it two ways. The enzyme is either mixed with a monomer solution, then monomer solution polymerization is performed with a chemical reaction, or it is immobilized by varying the experimental parameters. The enzymes are trapped inside the 3-dimensional network of inorganic or organic materials [39, 41]. The Figure 5 shows the physical entrapment of an enzyme inside a polymeric matrix.

-Electropolymerization This simple process involves the application of a potential or current to an aqueous solution or electrolytic solution that contains monomer molecules or biomolecules. On the electrode's surface, the monomer is either reduced or oxidized. This produces reactive radical species which attach to

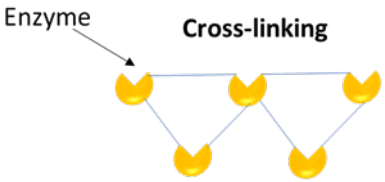

Figure 6: Cross-linking process.

each other to form a polymer. This polymer traps the enzymes near the electrode in the electrolytic solution [39, 41].

-Cross-linking Cross-linking happens when biorecognition elements (enzymes) create intermolecular covalent cross-linkages between them or by the use of functionally inert proteins like bovine serum albumin. The Figure 6 shows the cross linking mechanism. Multi-functional reagents are used for his since they can connect enzyme molecules in 3 dimensions cross-linked attaches to the surface of the transducer [39, 41].

1.7 Materials used for Glucose sensors

1.7.1 Glucose oxidase

Glucose oxidase or GOx was discovered by Müller in 1928. He demonstrated that GOx can oxidize glucose molecules to gluconic acid in the presence of oxygen [4, 42, 43, 44]. GOx extracted from A. niger are homodimeric glycoproteins. Their molecular mass is between 147 and 180 kDa. The Figure 7 shows the structure of GOx. Monomers in GOx contain flavin adenine dinucleotide (FAD). Two consecutive monomers are connected with salt bridges and hydrogen bonds. A large deep structure 10 Å × 10 allows access to the active site it becomes adequately large to entrap the substrate. The dimension of GOx is about 60 Å×52 Å×77 Å [45, 46, 47] [48]

Glucose enzymatic electrodes were firstly introduced by Clark and Lyons in 1962. The initial enzymatic electrode was based on a thin layer of glucose oxidase trapped above an oxygen electrode through a semi-permeable membrane. The measurements relied on monitoring the consumed oxygen by the enzyme. The

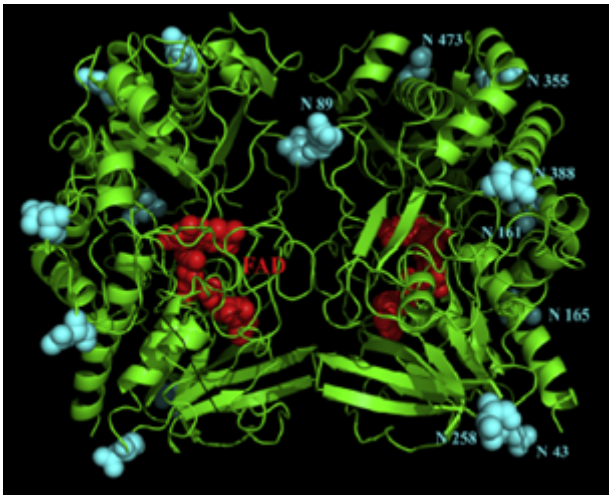

Figure 7: Images of GOX. Red color: FAD; Blue color: glycosylation.[4]

$$\text{glucose} + O_2 \xrightarrow{\text{glusoce oxidase}} \text{gluconic acid} + H_2O_2$$

reaction is showed in the following equation.

The origin of the field of biosensors is coming mostly from the glucose enzymatic electrode developed by Clark. Clark's discovery was transferred to a company named Yellow Spring Instrument that launched the first glucose analyzer in 1975 for measurement of glucose concentrations in blood samples [48, 49] Glucose Oxidase optimal working concentration have been determined by previous researchers work and is estimated to be between 27–37 °C for A. niger [50]. The human body temperature is arround 37°C for a healthy person so a small variation of the temperature will not affect the Glucose oxidase activity.

1.7.2 Ferrocene

Artificial mediators have been used in glucose sensors to transport electrons between the flavin adenine dinucleotide center and the electrode surface. The following reactions describes the reactions happening at the electrode surface.

where M is the mediator. At the electrode's surface, the reduced molecule is oxidized, generating a current signal which is proportional to the glucose concentration and giving the oxidized mediator. The mediation cycle is recapitulated in Figure 8. Most of the commercial blood glucose test meters uses ferricyanide or

$$\text{glucose} + \text{GOx}_{(ox)} \rightarrow \text{gluconic acid} + \text{GOx}_{(red)}$$

$$\text{GOx}_{(red)} + 2\text{M}_{(ox)} \rightarrow \text{GOx}_{(ox)} + 2\text{M}_{(red)} + 2\text{H}^{+}$$

$$2\text{M}_{(red)} \rightarrow 2\text{M}_{(ox)} + 2\text{e}^{-}$$

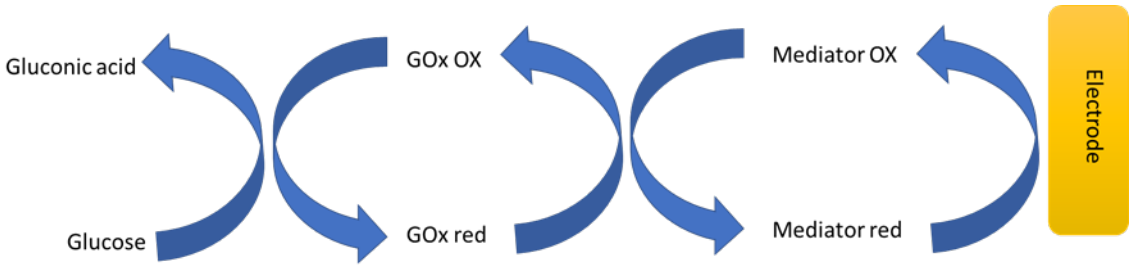

Figure 8: Glucose biosensor mediated sensor

ferrocene mediators [51, 52, 53].

The table 4 recapitulates the analytical performances of some emzymatic sensors [54].

1.7.3 Chitosan

Chitosan is a cationic, semi-crystalline polysaccharide that is rare in marine sources, it comes mainly from the deacetylation of chitin. It has a linear chain of repeating units of 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy D-glucopyranose, linked by type osidic bonds (1→4), whose degree of deacetylation is greater than 50%. Chitosan is a natural material of high molecular weight with a fluffy, white and odorless solid appearance. It is non-toxic, renewable, biodegradable and biocompatible. It also has significant chemical and biological reactivity. It also has various properties such as chelation, film-forming power and adsorption capacity. The Figure 9 shows the chitosan monomer structure [55, 56].

1.8 Overview on the electrical circuits used in biosensors

The electronic circuit that measures the output signal and biases the biosensor is in general called a potentiostat. The potentiostat involve a voltage follower, a constant voltage source, and a current-to-voltage converter. Figure 10 presents

Table 4: Overview of the enzymatic electrochemical glucose sensors

Sensor material	Sensitivity[A.mM^{-}1.cm^{-}2]	selectivity
GOx-Au-ZnO-GCE	1.409 A.mM^{-}1	AA,UA,DA
GOx-Cu-hemin MOFs	22.77	AA, UA, Fru, Gal, Man
PAA-rGO-GOx	15.31	AA, UA
PtNWA-AuNP-GOx	184	AA, CA, UA
GOx-3D $MoS_2 - graphene$	3.36 36 A.mM^{-}1	AA, DA, UA
GOx-Pt-MWCNTs-CSF	288.86	AA, UA, AP
GOx-CNT-Mucin-Pt	15 mA.M^{-}1.cm^{-}2	AA
GOx-GO–SH–Au-SPE	3.17	AA, UA,L-Cys, H2O2
PTTzFr-GOx on graphite	65.44	CA,U
CdS-ITO-GOx	1.345A.cm^{-}2	AA, DA, UA
GOx-Pt-rGO-P3ABA-SPE	22.01	AA, UA, Cho, Suc
GOx-Au-Ni-AuNW	123.3mV/decade	Ca^2+, Cl^-, K^+, Na^+
GOx-SPE-3D-PMED	35.7	AA, UA,Lactate, NaCl

Abbreviations: 3D: Three dimensional, AA: Ascorbic acid, AP: Acetaminophen, CA: Citric acid, Cho: Cholesterol, CNT: Carbon nanotubes, CSF: Carbonized silk fabric, CV: Cayclic voltammetry, Cys: Cysteine, DA: Dopamine, Fru: Fructose, G: Graphene, Gal: Galactose, GO: Graphene oxide, GOx: Glucose oxidase, ITO: Indium tin oxide, LuPc2: Lutetium phthalocyanine, Man: Mannose, MOF: Metal-organic framework, MFH: Multi functional, MWCNT: Multi-walled carbon nanotube, NP: Nanoparticle, NW: Nanowire, NWA: Nanowire array, P3ABA: Poly(3-aminobenzoic acid), PAA: Polyacrylic acid, PMED: Paper-based microfluidic electrochemical integrated device, rGO: Reduced graphene oxide, SPE: Screen printed electrode, Suc: Sucrose, U: Urea, UA: Uric acid.

the schema of the glucose sensor biasing circuit [5].

A group of researchers have developed an implantable device for continuous glucose monitoring based on a three electrodes system. The potentiostat used in their work controls the potential difference between reference electrode and the working electrode of an electrochemical cell by creating a current from or into the electrochemical cell through the electrode. They also designed a converter that converts the current into frequency. The Figure 11 shows the designed circuit for this particular glucose monitor [57].

Measuring with microelectrodes is easier, since there is no need for electronic control. The current used with these electrodes can vary from nanoamperes to picoamperes. The voltage drop at the homogeneous solution resistance can be

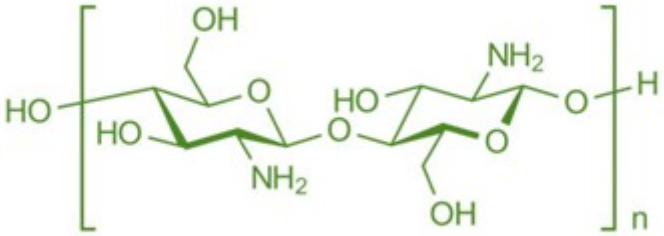

Figure 9: Chitosan structure

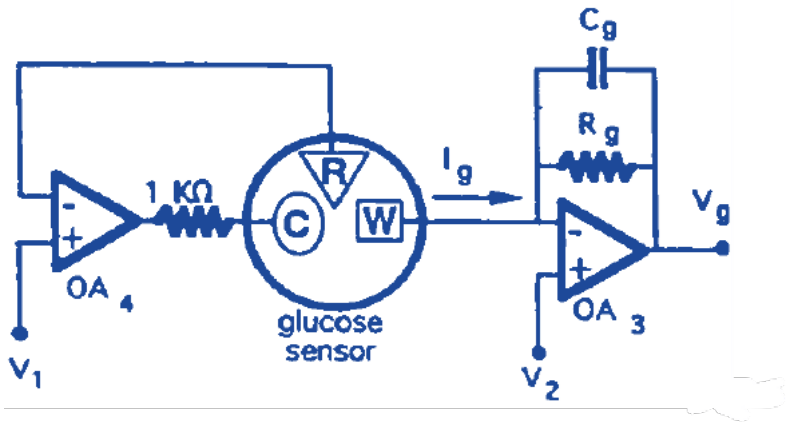

Figure 10: Typical circuit used for biosensors [5]

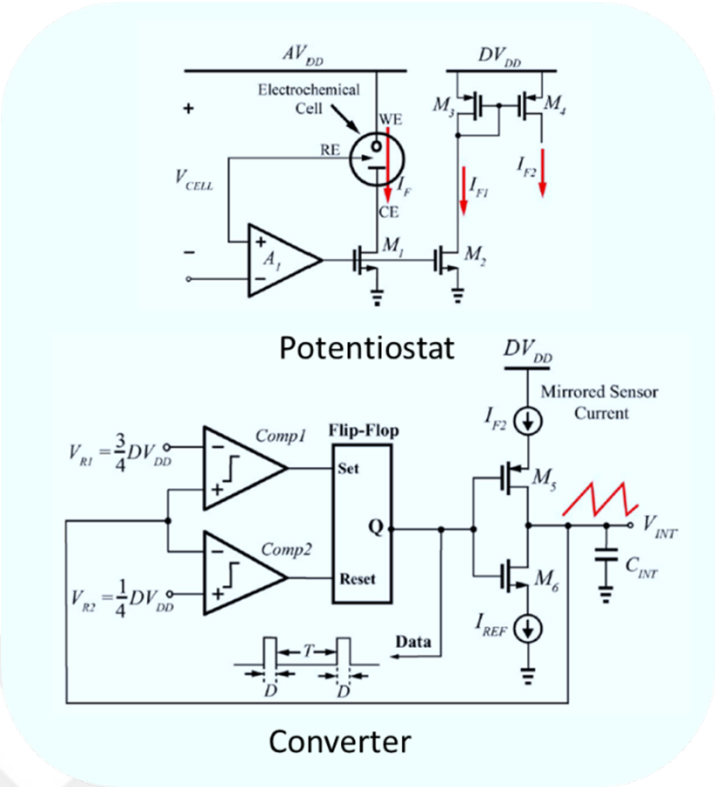

Figure 11: Electrical circuits used.

neglected. the counter electrode has a low current load that can be performed by commonly used reference electrodes. instead of using a three-electrode circuit, the variation of voltage is just imposed between the working and the reference electrodes. However, measuring the current response is not easy if we consider that the current produced by external effects is low. The invention of low-noise operational amplifiers made it easier to do research on microelectrodes. These amplifiers are commonly integrated to the potentiostat as a current measuring system between the ground and the working electrode terminal. the working electrode's potential is kept equal to zero (ground potential) by the current measuring system, and it also generates an output voltage in proportion to the actual electrolysis current. When using low-current circuits, it is necessary to use damping and filtering units to adjust them carefully (Figure 12) [27].

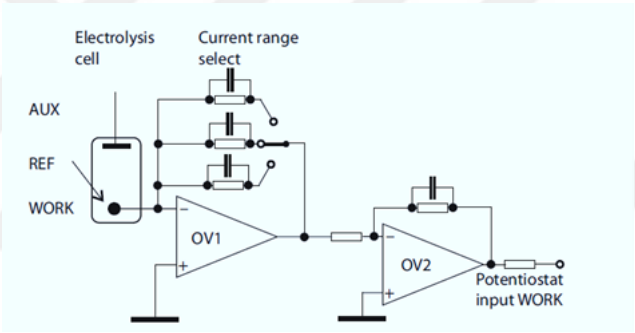

Figure 12: Electrical circuit used for measurements with microelectrode.

CHAPTER II

MATERIALS, METHODS AND RESULTS

2.1 Materials and methods

The electrochemical measurements were conducted by a voltmeter (MESTEK) and a GPC3060D Triple-Output DC Power Supply. The working electrode was a gold micro-needle electrode and the reference electrode was a silver micro-needle electrode. The two needles were supported by a Printed Circuit Board. Deionized water and phosphate buffer pH, 7.4 were used to prepare glucose solutions. Wires soldered to PCB were incorporated to the sensor to connect it to the power supply and the voltmeter for the measurements as shown in Figure 13.

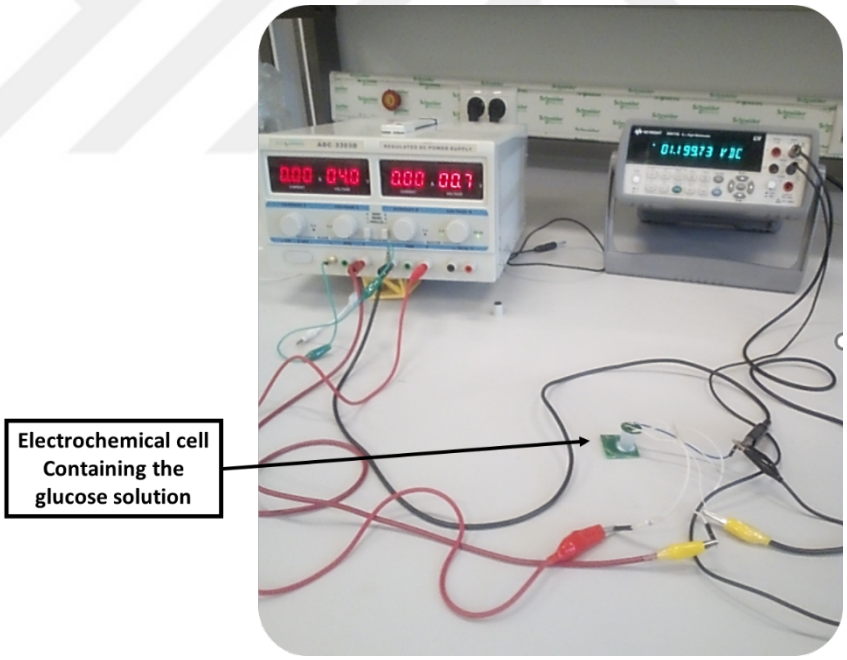

Figure 13: Experimental setup.

2.1.1 Sensor assembly

The glucose sensor was designed as following: - The electronic part soldered directly on the PCB containing the electronics and the electrical circuit as shown in

the Figure 14. - A sensing part containing two needles inserted and soldered into the printed circuit board. A gold electrode served as a working electrode and a silver electrode as a reference.

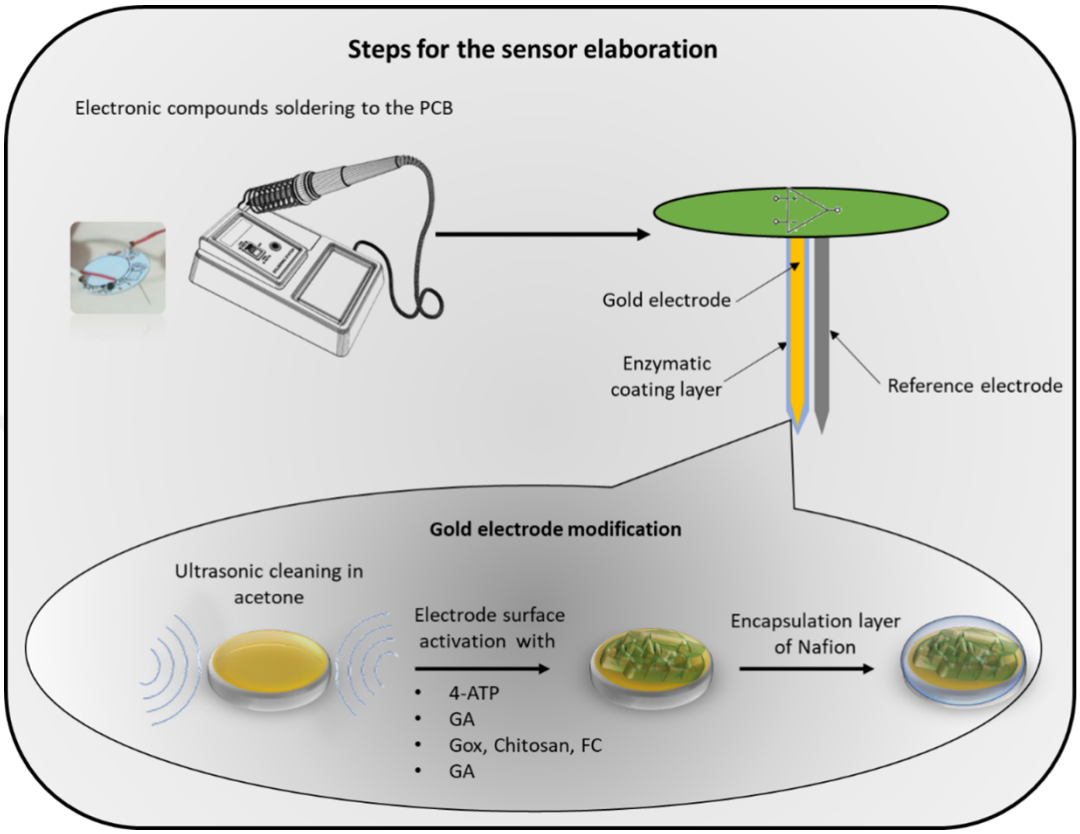

Figure 14: The steps for the glucose sensor assembly and elaboration.

The glucose level measurement is based on the potential difference between the electrodes. The gold needle is modified dip-coating method. Figure 15 shows the elaborated continuous glucose sensor.

2.1.2 Gold and silver electrodes modification

First, silver and gold electrodes electroplating was performed. Titanium electrode was used as an anode electrode, and gold and silver electrodes were used separately as cathode electrode for electroplating. Prior to electroplating, the silver and gold electrodes were cleaned using a cleaning bath. The potentials used for electroplating were optimized and the potentials chosen for silver electroplating were 1.5 V and for gold 2 V. After electroplating, the gold and silver electrodes

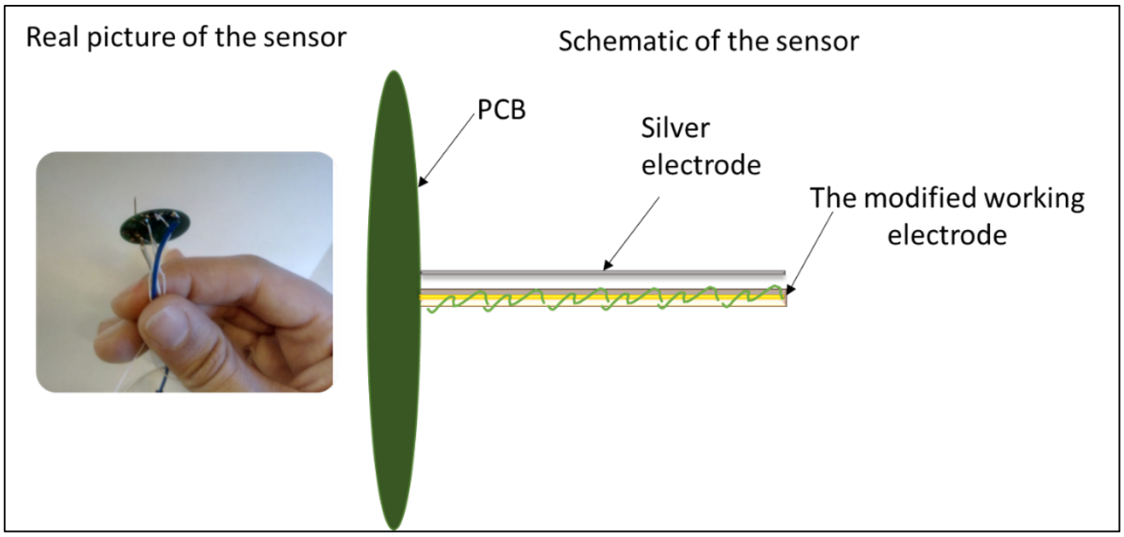

Figure 15: Real picture and schematic of the continuous glucose sensor.

were cleaned in hot pure water to remove residual salts. Then the gold electrode was modified by the sensitive layer.

2.1.3 Gold electrode modification steps

1. Apply 4-Aminothiophenol with a concentration of 10mM dissolved in ethanol. 2. Dip in Glutaraldehyde. Ga is liquid initially. 3. Apply the mixture of Ferrocene, Gox, and chitosan. A solution of ferrocene 10-2M in ethanol was prepared. 3mg of the Gox powder was added to it and 1ml of PBS. after mixing 1ml of Chitosan and 1ml of Ferrocene are added and then mixed together. the final concentration of GOx is 1mg/ml. 4. Out under condensed steam of Glutaraldehyde. 5. Dip in Nafion. 6. Apply Nafion a second time after the insertion of the silver electrode as a final coating protective layer.

2.1.4 Electrical circuit description

The electronic sensing part of the glucose sensor is based on a trans-impedance stage that supplies the current to the gold electrode acting as an anode as shown in the Figure 16. The electrode current, which is represented by Is, is multiplied by the feedback gain resistor Rg providing a voltage output for the subsequent ADC.

The sensor bias voltage is generated through the central BLE microcontroller current source which is dynamically adjusted during the sensor's active lifetime in order to extend the useful lifetime of the device. The same microchip converts the voltage output of the sensor front-end into digital enabling digital transmission of the measurement results to a mobile device, after an initial averaging operation that is performed locally. In addition to sensed signal level, the ambient temperature and other user specific information are transferred to the mobile device for further processing.

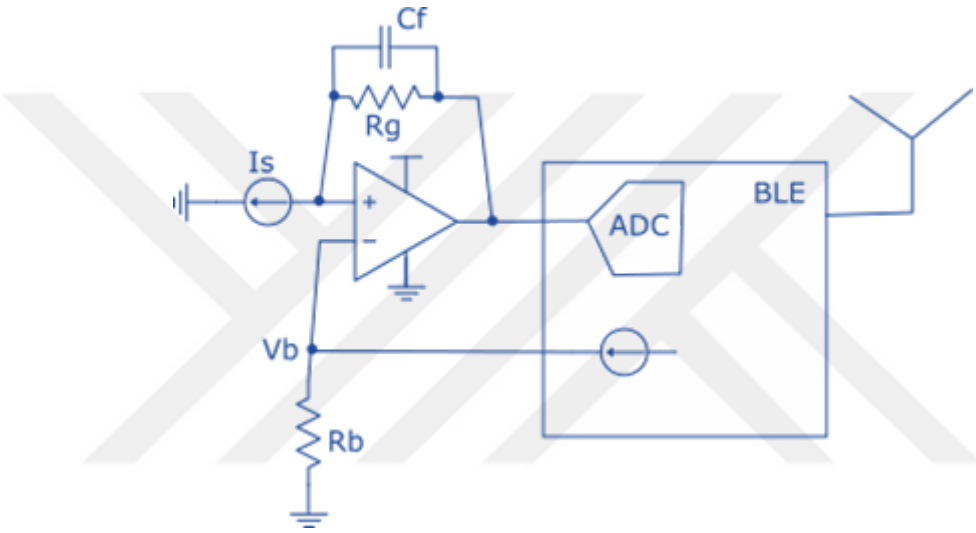

Figure 16: A small footprint integrated glucose sensor electronics.

2.2 Results

2.2.1 Reference electrode elaboration

Reference electrode elaboration is a crucial step during the manufacturing of the sensor. An optimization step was performed, and different methods were tested for stable coating. Figure 17 shows the silver electrode before and after electroplating. A layer of silver is formed, and it could be observed clearly under the microscope. The concentration, voltage and timing of electrodeposition of AgCl were optimized carefully. The concentration of KCl was also optimized during the experiments from 0.1M to 0.8M in order to obtain a uniform and repeatable amount of AgCl formation. The electrolysis time was varied from 1min to 5min

during the experiments. The final electrical parameters were 4V, 0.8M for 5min as we obtained the best coating with these parameters during the experiments. A dense and condensed layer of salt is clearly visible as shown in Figure 18.

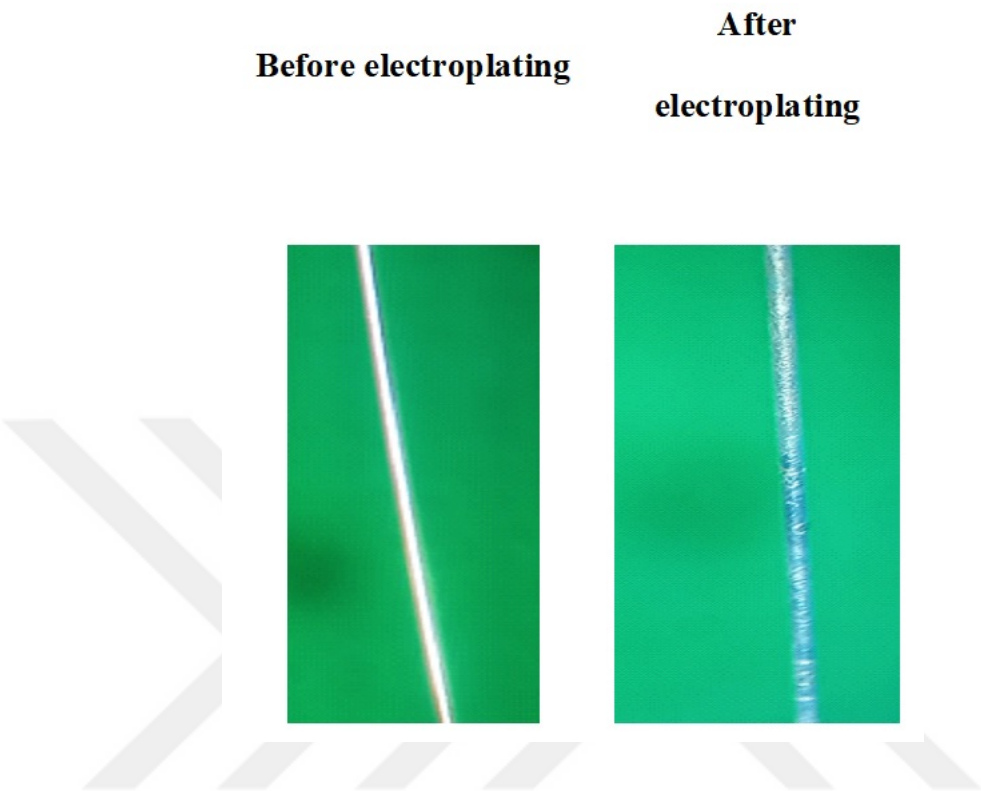

Figure 17: Microscope graph of the silver needle.

the plating effect of silver electrode on the sensor response toward glucose molecules in PBS buffer was evaluated and the results are presented in the Figure 19. These results show that the plated silver electrode gave better liner curve in terms of sensitivity. Somehow the linearity was not good for both. That is why further optimization of the experimental parameters will be done in the following parts.

2.2.2 Applied potential optimization

The electrochemical characterization of the elaborated sensor was performed. The variation of the bias voltage was carried out as shown in Figure 20. The Figure 20 below shows the bare output voltage reading of the sensor with the applied bias voltage sweep for a glucose concentration of 89 mg/dL. A linear shape is observed with the increasing of the bias voltage.

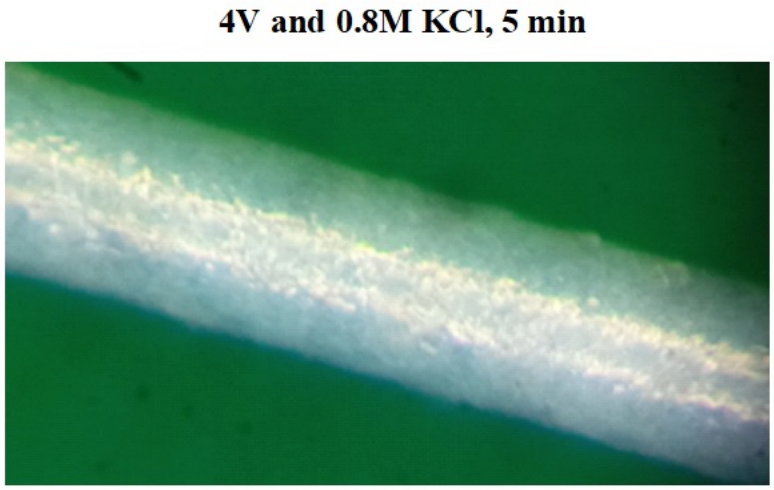

Figure 18: Microscope picture of the optimized AgCl layer on silver micro-needle.

Manual cyclic voltammetry was performed in order to have an idea of the electrochemical behavior of the sensor while the voltage varies. As shown in Figure 21, in the PBS buffer there was a slight increase in the current and in presence of glucose the current from -0.45µA to 11µA. This increase indicates the sensitivity of the elaborated sensor towards glucose.

Redox potential is a key parameter that needs to be optimized for a given sensing mechanism. Three potentials were chosen, and the obtained curves are presented in Figure 22. There is a significant improvement in the current and the linear behavior at a value of 700mV of applied potential for the proposed sensor sub-assembly.

2.2.3 Sensing layer optimization

As an effort to optimize the performance of the gold electrode for glucose detection, a set of parameters were optimized such as the final coating, use of ferrocene as a catalyzer, the enzyme concentration, and the applied redox potential. For the functionalization of the gold electrode, the aim was to obtain the highest current in a linear shape response to corresponding glucose concentrations.

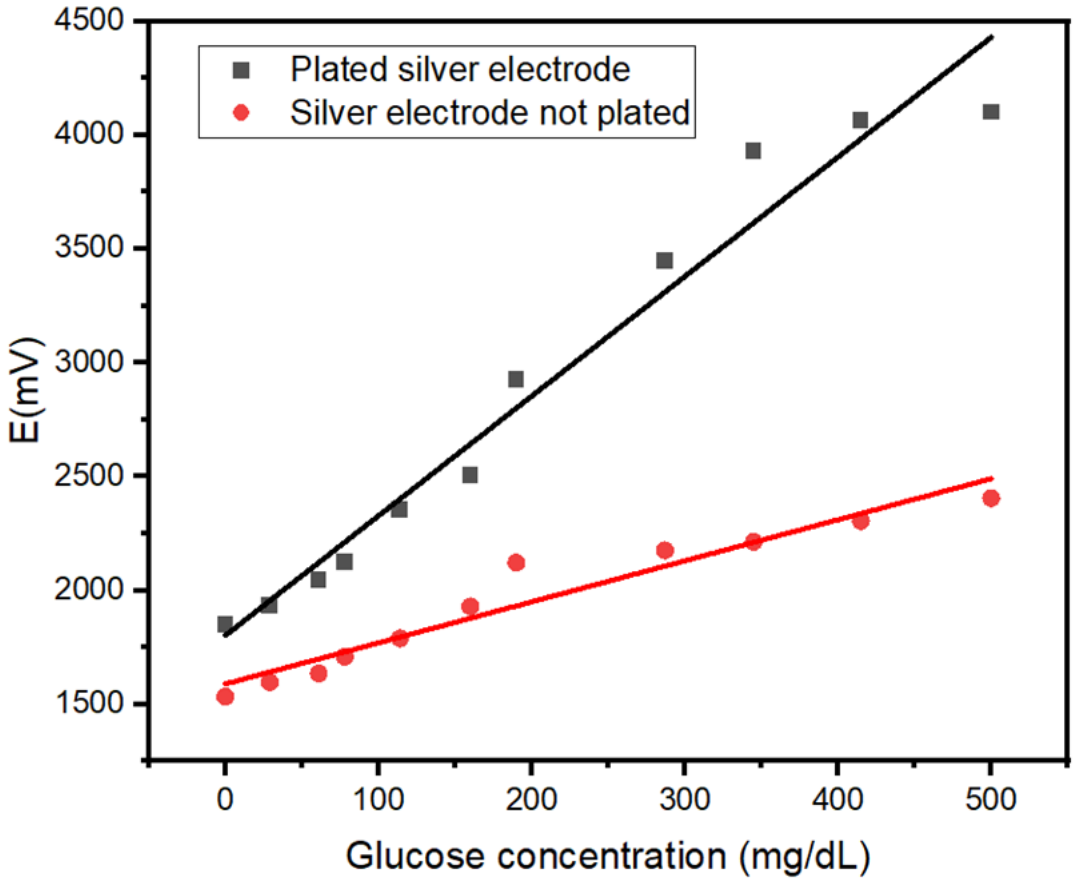

Figure 19: Calibration curve for glucose detection.

-The use of ferrocene Ferrocene is frequently used in the development and elaboration of glucose biosensors and has been used as a mediator that carries electrons between the active center of the enzyme and the electrode surface. The incorporation of ferrocene mediator on metallic electrodes has been studied in recent years to improve the rate of electron transfer [53]. Thus, electrodes modified by ferrocene are increasingly used in many electrochemical applications. Therefore, we have carefully studied the effect of ferrocene on the response of our glucose sensor.

After processing, the obtained results for the elaborated sensors, we plotted the sensitivity curve giving the variation of the voltage as a function of the glucose concentration. Figure 23 represents the calibration curves obtained for the detection of the tested glucose solutions. A linear dependence (R2 = 0.94623) in

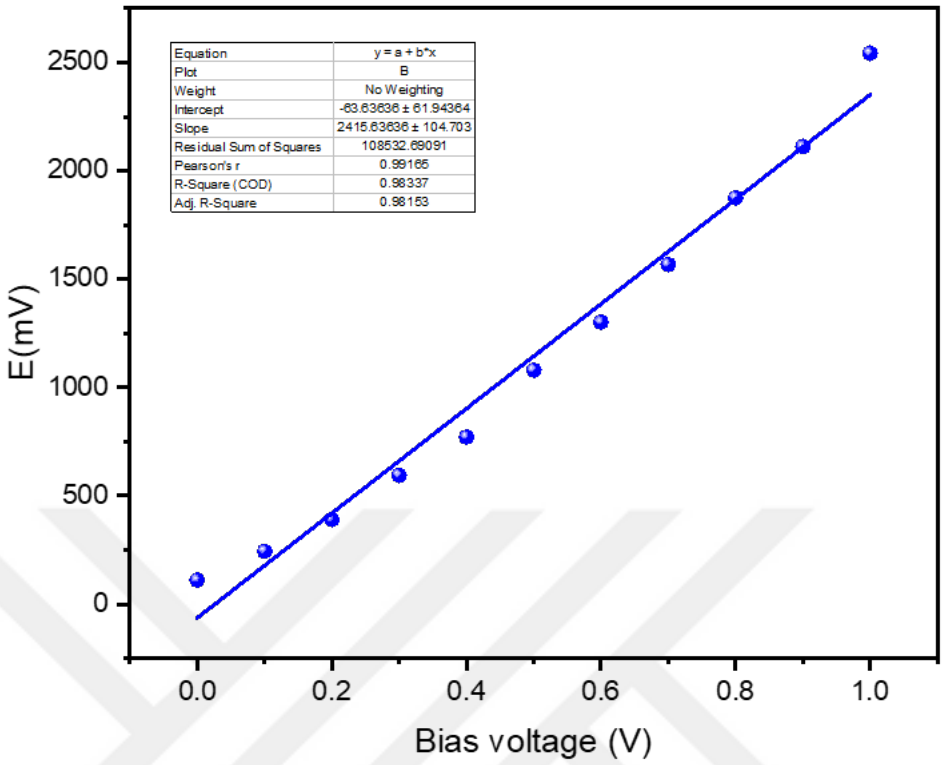

Figure 20: Sweep of the electrode bias potential in glucose solution with a concentration of 89mg/dL.

a concentration range of 0 to 500 mg/dL is obtained for the sensor with ferrocene with a sensitivity of 1.404 (mV.dL)/mg. A linear dependence (R2 = 0.96012) in the concentration range of 0 to 500 mg/dL is obtained for the sensor without FC with a sensitivity of 0.982 (mV.dL)/mg. It can be observed that the gold electrode modified with FC presents a greater sensitivity compared to the other electrode.

-Glucose oxidase concentration optimization The glucose oxidase concentration was optimized since excess doping of GOx was also degrading the measurement accuracy. Five concentrations of glucose oxidase were tested during the experiments to reach the best needle-based coating density: 0.25 mg/mL, 0.5mg/mL,1 mg/mL, 2 mg/mL and 4 mg/mL. The results are presented in Figure

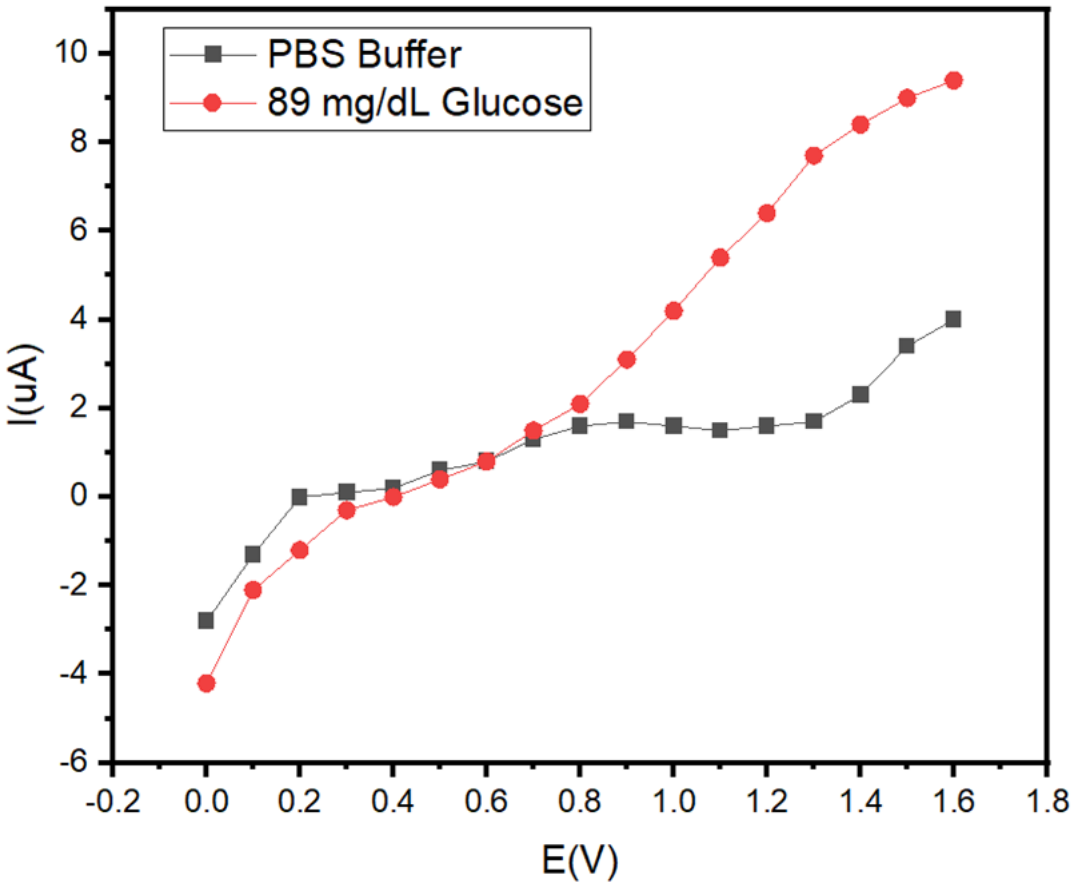

Figure 21: Manual Cyclic voltammetry.

24. The voltage increased with the increase of glucose concentration on the electrode surface. The relationship of the voltage as a function of glucose concentration is plotted in the Figure 24.The Table 3 recapitulates the obtained results.For a concentration of Gox equal to 0.25mg/mL we observed a small variation in the current with the increasing glucose concentration. For 0.5mg/mL of Gox concentration, we obtained the highest sensitivity and a less linear range. The sensor shows a good linearity from 0 to 400mg/dL for a concentration of glucose oxidase equal to 1 mg/mL as shown in Table 5. Thus, this concentration will be used for the future experiments.

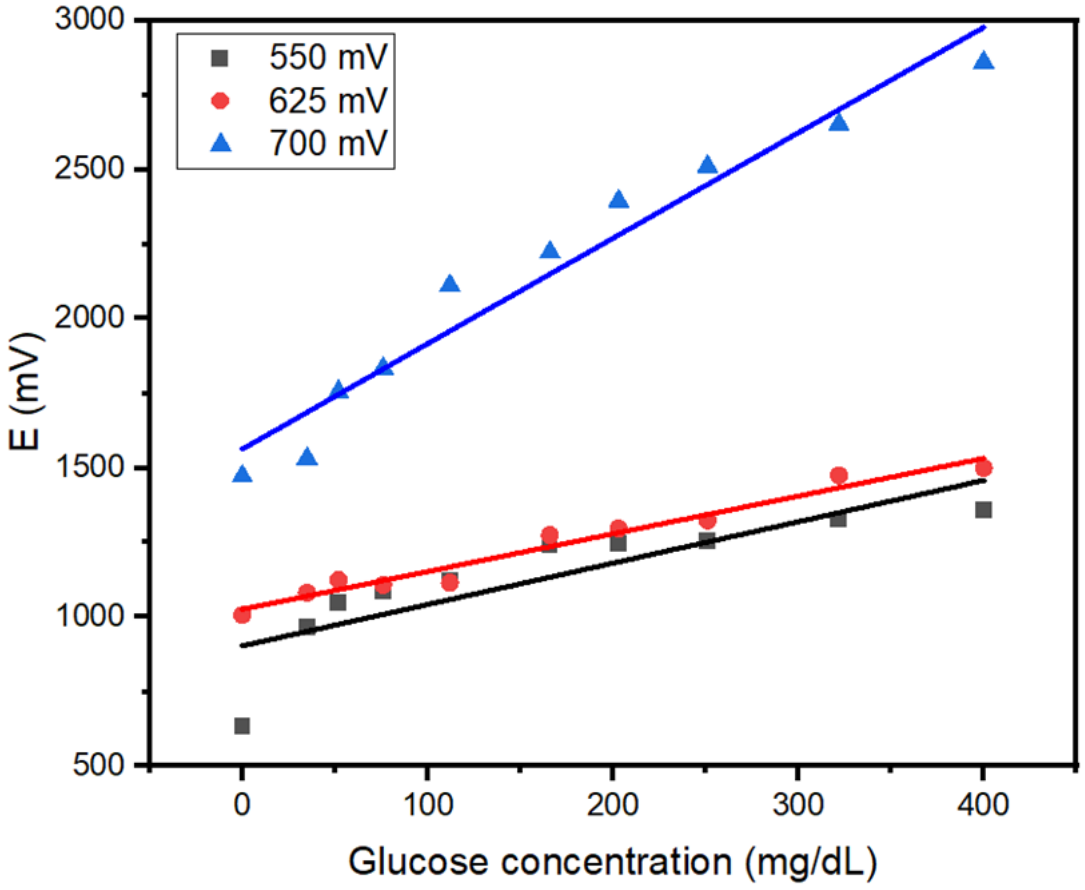

Figure 22: Tested sensor at 550mV (y = 1.2647x + 1026.2 R^2 = 0.9638) , 625mV (y = 1.3866x + 903.68 R^2 = 0.7252) and 700mV (y = 3.5341x + 1562.7 R^2 = 0.9543). .

2.2.4 Applied potential re-optimization

Figure 25 shows the obtained results. From the linear relation between the voltage and the glucose concentration, it can be deduced that 700mV is giving the best analytical performance. Table 6 recapitulates the analytical performance of the sensors in terms of sensitivity and linear range, and we could observe the highest sensitivity for 700mV bias voltage. the parasitic offset current was also higher in this case.

2.2.5 Encapsulating layer

The final coating is an important layer as it protects and encapsulates the active enzymatic layer. In this study we have tested the sensor with and without Nafion

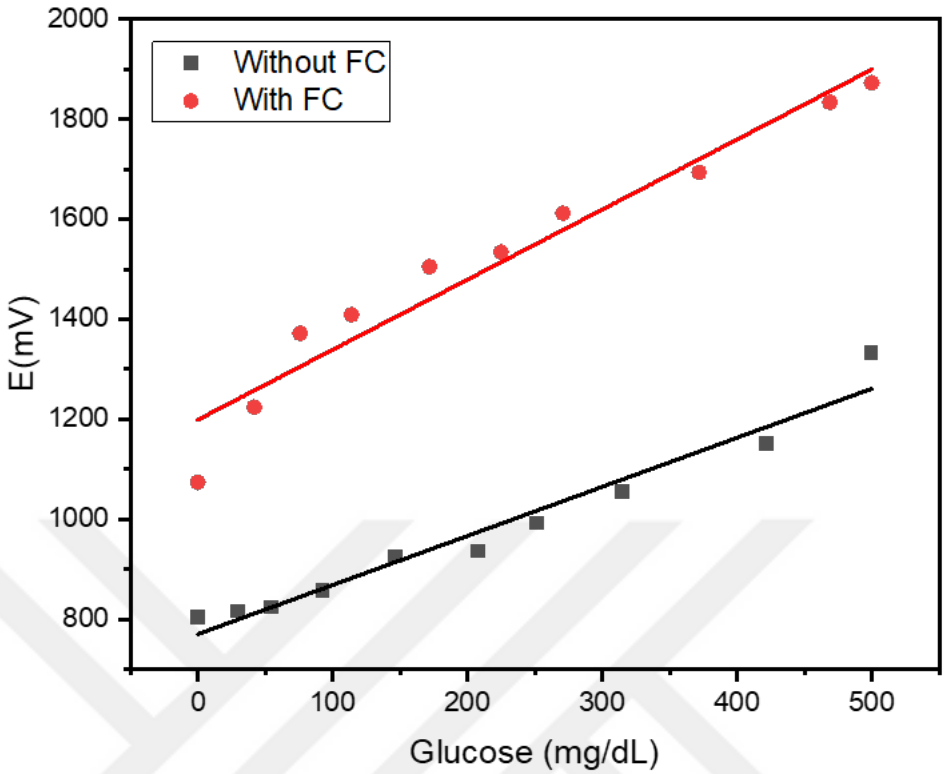

Figure 23: Tested glucose sensors with and without ferrocene in the deposited layer.

membrane, in order to check whether it affects the sensor response towards glucose or not. Figure 26 shows the obtained results. We can observe a significant increase in the current and the linear shape for the sensor with Nafion membrane application as a final coating.

2.2.6 Real sample test

The blood glucose monitoring sensors are in general considered as minimally invasive sensors and are based on a reliable relationship between skin interstitial fluid and blood glucose [19–22] [58, 59, 24]. The Figure 27 shows a simplified schema of CGMs. For this reason, we inserted the elaborated sensor into meat and measured the glucose levels.

Meat experiments. As a first step and in order to check the sensor performances

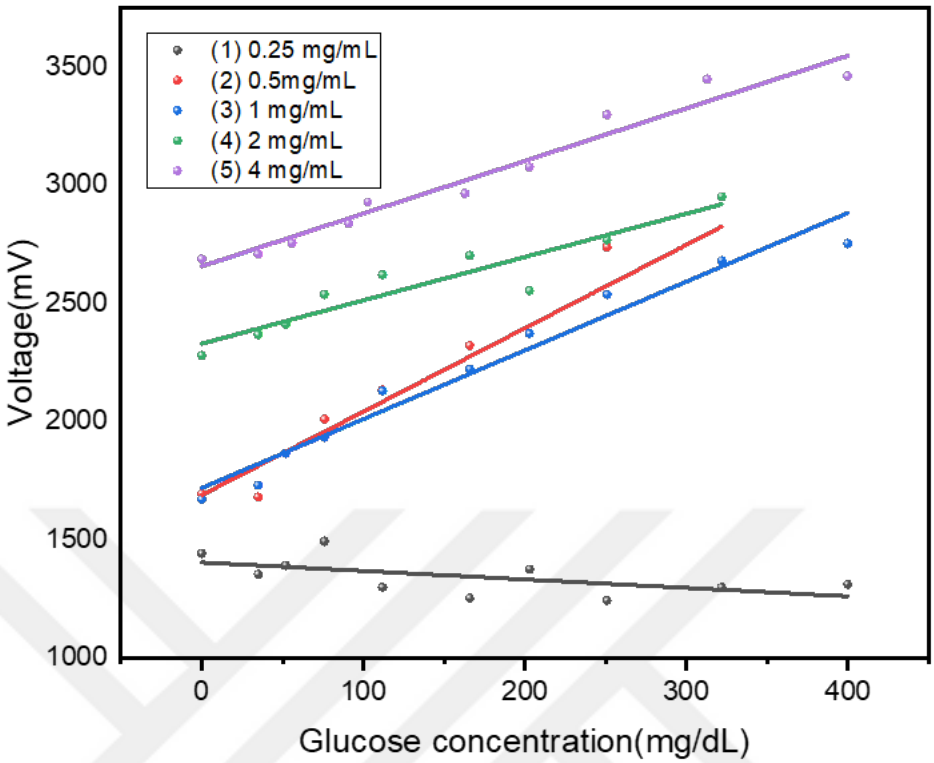

Figure 24: The linear relationship between voltage and the concentrations of glucose. 0.25 mg/mL, (2) 0.5mg/mL, (3) 1 mg/mL, (4) 2 mg/mL and (5)4 mg/mL .

in real environments, the elaborated sensor was inserted in beef meat and several glucose concentrations were injected using a syringe (Figure 28). The results are depicted in the Figure 29.

The calibration curve obtained for the sensor inserted in the meat has a linear shape and a good analytical performance. Indeed, it presented a high sensitivity of 2.8649 and a linear range from 0 to 500 mg/dL. These results are promising and suggest that the elaborated sensor can be used for human blood sugar tests.

Table 5: Performance of the sensor.

Concentration	Sensitivity	Linear range	R^2
0.25 mg/dL	0.345	0-400 mg/dL	0.9435
0.5mg/dL	3.529	0-300 mg/dL	0.9318
1 mg/dL	3.059	0-400 mg/dL	0.9819
2 mg/dL	1.833	0-300 mg/dL	0.8600
4 mg/dL	2.228	0-400 mg/dL	0.9545

Table 6: Performance of the sensor.

Voltage	Sensitivity	Linear range	R^2
700mV	3.059	0-400 mg/dL	0.9819
625mV	2.477	0-400 mg/dL	0.92841
550mV	1,467	0-400 mg/dL	0.9213

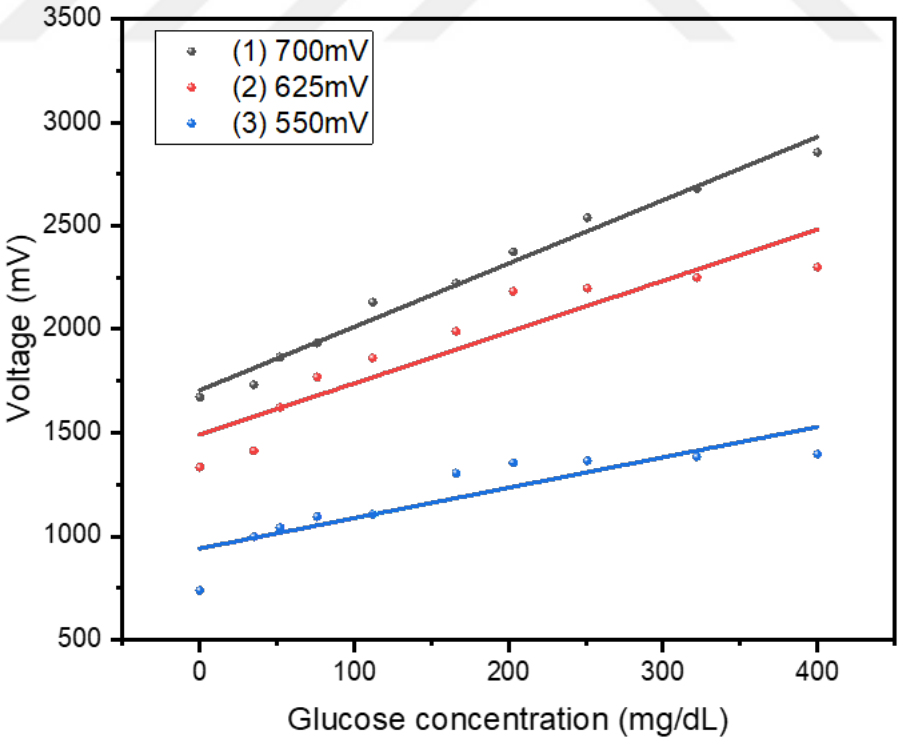

Figure 25: Voltage optimization (1) 700mV,(2) 625mV, (3) 550mV.

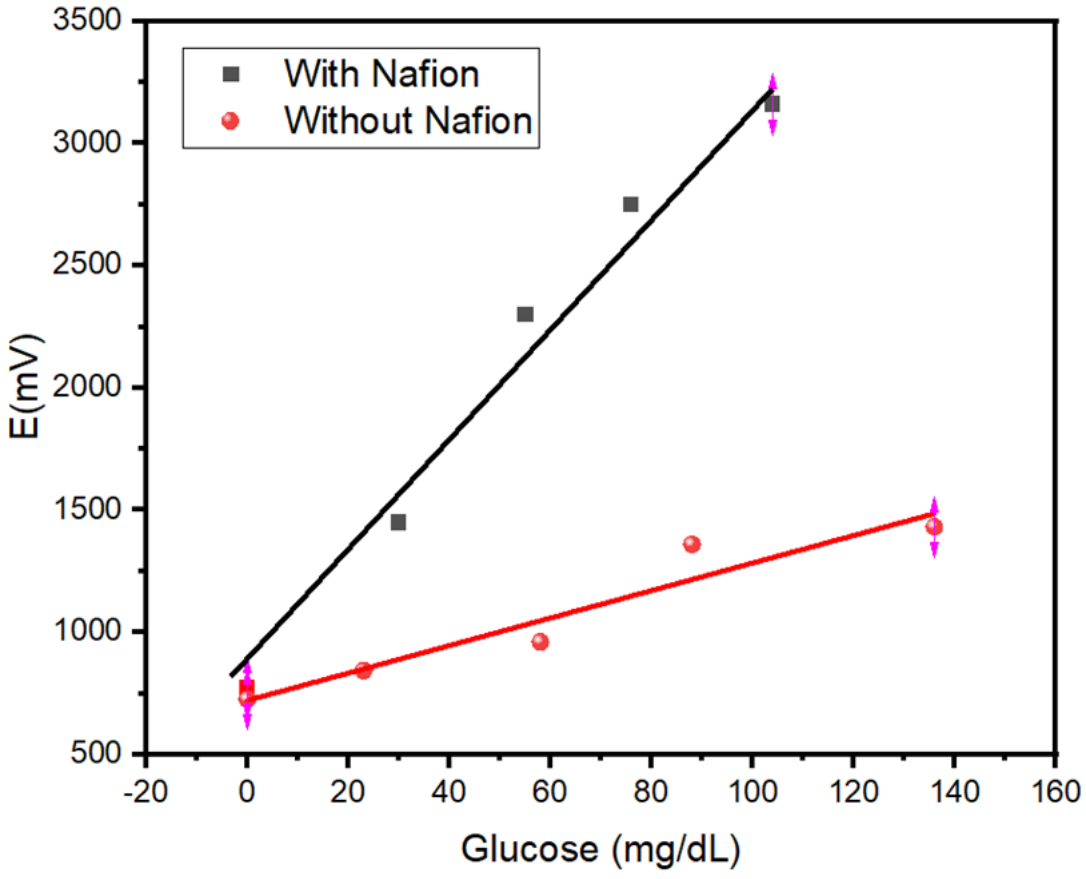

Figure 26: Linear curves for the tested sensors. With Nafion (y = 17.42x + 1074.1 R^2 = 0.8796) and Without Nafion (y = 7.4651x + 668.35 R^2 = 0.9103)

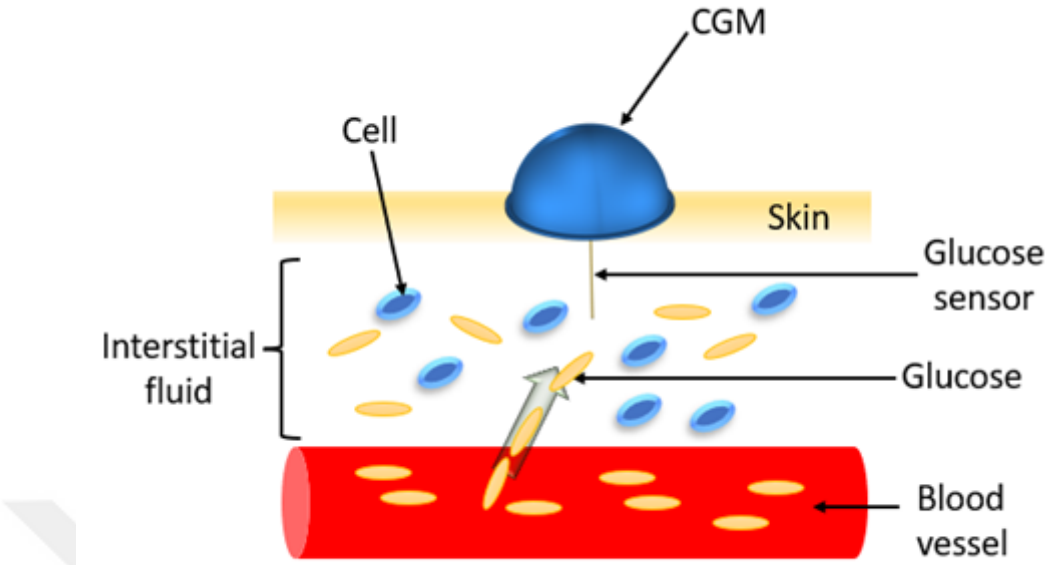

Figure 27: Schematic showing a glucose monitoring sensor inserted into the skin.

Figure 28: Meat experiment picture.

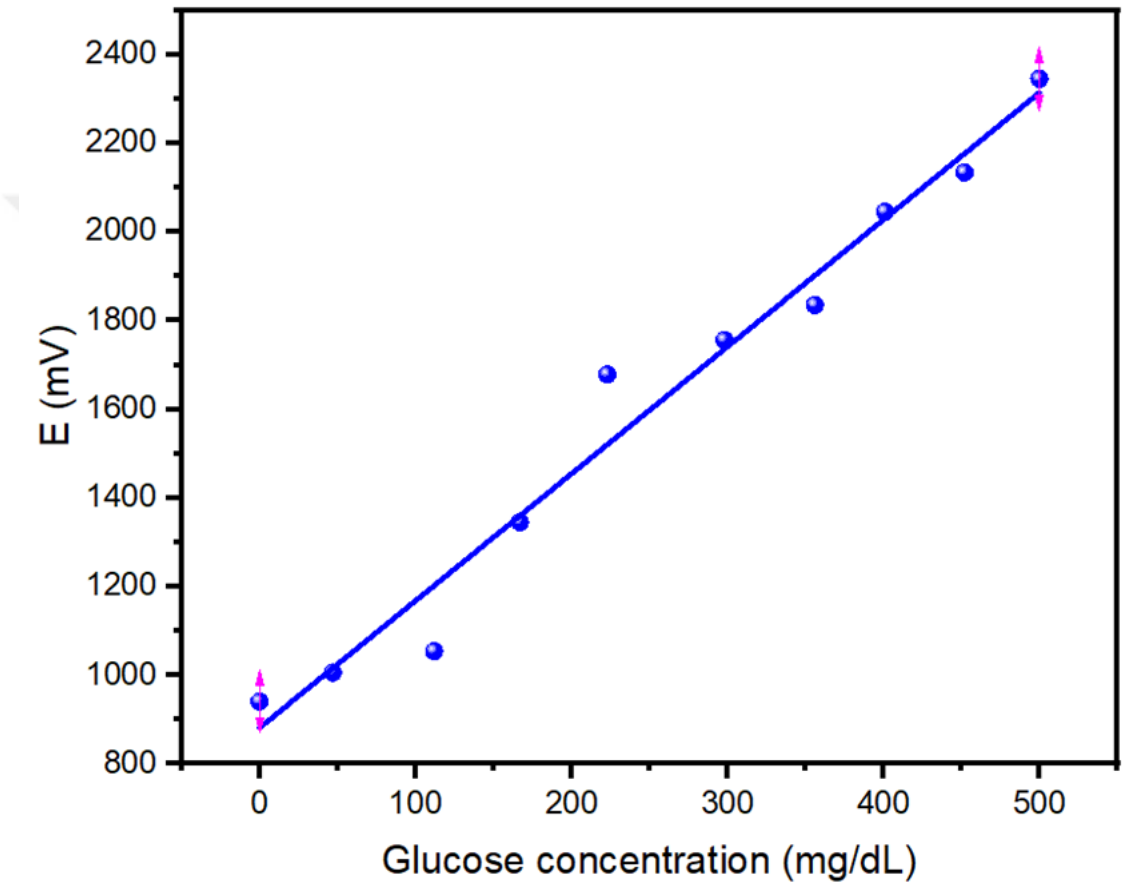

Figure 29: Calibration curve. Measurement carried out inside meat (y = 2.8649x + 881.63 R^2 = 0.9742).

CHAPTER III

CONCLUSION

The objective of this thesis was the design and the elaboration of a continuous glucose sensor that would be used by diabetes patients all around the world. The work of this thesis went form the sensor design, assembly and finally testing. This type of sensors are the subject of several research groups. Although there are some sensors on the market, these sensors remain expensive and are not accessible to all patients. For a diabetic patient, the use of continuous monitors would ease their lives and facilitate proper the blood sugar management during the day. These sensors are less painful then the classical finger-stick glucose sensors requiring poking the finger for each measurement.

As a conclusion, in this work a low-cost and simple glucose sensor was elaborated based on a two needles system inserted into an electronic PCB. The electrical components were soldered carefully on the PCB using a solder machine and magnifying glasses. Several sensors were elaborated, tested, the chemical and electrical parameters were optimized. The sensors were tested firstly in phosphate buffer to determine their analytical performances. Then they were tested in a real sample which is meat as a first test. Beef meat was chosen due to its similar texture to human skin. The preliminary results were satisfactory and promising to continue the work and be able to test the sensor in humans. There is still a need to further validate and improve the performance of the designed CGM. Before validation, optimization of various parameters should continue to be improved.

As perspectives to this work, we are aiming to:

- Enhance the linearity and the sensitivity of the sensor
- Use of non-enzymatic layer to detect the glucose in order to erase the limitation due to the denaturation and lifetime of the enzyme. The criteria that

need to be considered should be the linear range and the sensitivity.

- Study of interference species
- Study of lifetime
- The Bluetooth integration into the system and perform the tests without any cabling.
- Test the sensor in humans

Bibliography

[1] Z. Mian, K. L. Hermayer, and A. Jenkins, "Continuous Glucose Monitoring: Review of an Innovation in Diabetes Management," tech. rep., 2019.

[2] Y. J. Heo and S. H. Kim, "Toward long-term implantable glucose biosensors for clinical use," *Applied Sciences (Switzerland)*, vol. 9, no. 10, 2019.

[3] Dimensions, ""continuous glucose monitoring." https://app.dimensions.ai/discover/publication (assessed july 05,2021)."

[4] N. Mano, "Engineering glucose oxidase for bioelectrochemical applications," *Bioelectrochemistry*, vol. 128, pp. 218–240, aug 2019.

[5] "A Telemetry-Instrumentation System for Long-Term Implantable Glucose and Oxygen Sensors," *Analytical Letters*, vol. 29, pp. 2289–2308, oct 1996.

[6] P. Saeedi and et al, "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition," *Diabetes Research and Clinical Practice*, vol. 157, p. 107843, 2019.

[7] M. Buysschaert, J.-L. Medina, B. Buysschaert, and M. Bergman, "Send Orders for Reprints to reprints@benthamscience.ae Definitions (and Current Controversies) of Diabetes and Prediabetes," tech. rep., 2016.

[8] H. Sun and et al, "Idf diabetes atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045," *Diabetes Research and Clinical Practice*, vol. 183, p. 109119, 1 2022.

[9] T. R. R. McDonald and C. A. Beevers, "The crystal and molecular structure of α-glucose," *Acta Crystallographica*, vol. 5, no. 5, pp. 654–659, 1952.

[10] G. K. Brown, "Glucose transporters: Structure, function and consequences of deficiency," in *Journal of Inherited Metabolic Disease*, vol. 23, pp. 237–246, 2000.

[11] A. L. Olson and J. E. Pessin, "Structure, function, and regulation of the mammalian facilitative glucose transporter gene family," tech. rep., 1996.

[12] M. Gougis, A. Tabet-Aoul, D. Ma, and M. Mohamedi, "Laser synthesis and tailor-design of nanosized gold onto carbon nanotubes for non-enzymatic electrochemical glucose sensor," *Sensors and Actuators, B: Chemical*, vol. 193, pp. 363–369, 2014.

[13] B.-g. Tir, "A Randomized Clinical Trial Assessing Continuous Glucose Monitoring (CGM) Use With Standardized Education With or Without a Family Behavioral Intervention Compared With Fingerstick Blood Glucose Monitoring in Very Young Children With Type 1 Diabetes," *Diabetes care*, vol. 44, no. 2, pp. 464–472, 2021.

[14] G. P. Forlenza, N. B. Argento, and L. M. Laffel, "Practical considerations on the use of continuous glucose monitoring in pediatrics and older adults and nonadjunctive use," *Diabetes Technology and Therapeutics*, vol. 19, pp. S13–S20, jun 2017.

[15] T. Danne and et al, "International consensus on use of continuous glucose monitoring," *Diabetes Care*, vol. 40, pp. 1631–1640, dec 2017.

[16] A. Wallia and et al, "Consensus Statement on Inpatient Use of Continuous Glucose Monitoring," *Journal of Diabetes Science and Technology*, vol. 11, pp. 1036–1044, sep 2017.

[17] A. L. Carlson, D. M. Mullen, and R. M. Bergenstal, "Clinical use of continuous glucose monitoring in adults with type 2 diabetes," *Diabetes Technology and Therapeutics*, vol. 19, no. S2, pp. S4–S11, 2017.

[18] M. Phillip and et al, "Use of continuous glucose monitoring in children and adolescents," *Pediatric Diabetes*, vol. 13, pp. 215–228, may 2012.

[19] R. J. Galindo and G. Aleppo, "Continuous glucose monitoring: The achievement of 100 years of innovation in diabetes technology," dec 2020.

[20] E. L. Johnson and et al, "Standards of medical care in diabetes—2019 abridged for primary care providers," *Clinical Diabetes*, vol. 37, pp. 11–34, jan 2019.

[21] J. Kim, A. S. Campbell, and J. Wang, "Wearable non-invasive epidermal glucose sensors: A review," *Talanta*, vol. 177, pp. 163–170, jan 2018.

[22] G. McGarraugh, "The chemistry of commercial continuous glucose monitors," *Diabetes Technology & Therapeutics*, vol. 11, no. S1, pp. S–17–S–24, 2009. PMID: 19469674.

[23] S. N. Thennadil, J. L. Rennert, B. J. Wenzel, K. H. Hazen, T. L. Ruchti, and M. B. Block, "Comparison of glucose concentration in interstitial fluid, and capillary and venous blood during rapid changes in blood glucose levels," *Diabetes Technology and Therapeutics*, vol. 3, no. 3, pp. 357–365, 2001.

[24] T. M. Gross, B. W. Bode, D. Einhorn, D. M. Kayne, J. H. Reed, N. H. White, and J. J. Mastrototaro, "Performance Evaluation of the MiniMed ® Continuous Glucose Monitoring System During Patient Home Use," Tech. Rep. 1, 2000.

[25] D. C. Klonoff, D. Ahn, and A. Drincic, "Continuous glucose monitoring: A review of the technology and clinical use," *Diabetes Research and Clinical Practice*, vol. 133, pp. 178–192, 2017.

[26] V. Divan, M. Greenfield, C. P. Morley, and R. S. Weinstock, "Perceived Burdens and Benefits Associated With Continuous Glucose Monitor Use in Type 1 Diabetes Across the Lifespan," *Journal of Diabetes Science and Technology*, vol. 16, pp. 88–96, jan 2022.

[27] P. Gründler, *Chemical sensors: An introduction for scientists and engineers*. Springer Berlin Heidelberg, 2007.

[28] D. R. Thévenot, K. Toth, R. A. Durst, G. S. Wilson, and D. R. Thévenot, "Electrochemical biosensors: recommended definitions and classification," tech. rep., 2001.

[29] B. Adhikari and S. Majumdar, "Polymers in sensor applications," 2004.

[30] M. A. Rahman, P. Kumar, D.-S. Park, and Y.-B. Shim, "Electrochemical Sensors Based on Organic Conjugated Polymers," *Sensors*, vol. 8, pp. 118–141, 2008.

[31] M. Opallo and A. Lesniewski, "A review on electrodes modified with ionic liquids," jun 2011.

[32] F. C. Krebs, "Fabrication and processing of polymer solar cells: A review of printing and coating techniques," 2009.

[33] X. Ren, L. T. Weng, C. M. Chan, and K. M. Ng, "Hollow interior structure of spin-coated polymer thin films revealed by ToF-SIMS three-dimensional imaging," *Analytical Chemistry*, vol. 84, pp. 8497–8504, oct 2012.

[34] H. Bai and G. Shi, "Gas Sensors Based on Conducting Polymers," *Sensors*, vol. 7, pp. 267–307, 2007.

[35] X. Tang and X. Yan, "Dip-coating for fibrous materials: mechanism, methods and applications," feb 2017.

[36] T. Ahuja, I. A. Mir, D. Kumar, and Rajesh, "Biomolecular immobilization on conducting polymers for biosensing applications," feb 2007.

[37] V. Naresh and N. Lee, "A review on biosensors and recent development of nanostructured materials-enabled biosensors," feb 2021.

[38] A. Arya, A. Gangwar, and A. Kumar, "Biosensors in animal biotechnology," in *Nanotechnology in Modern Animal Biotechnology: Concepts and Applications*, pp. 75–95, Elsevier, jan 2019.

[39] A. Sassolas, L. J. Blum, and B. D. Leca-Bouvier, "Immobilization strategies to develop enzymatic biosensors," may 2012.

[40] P. Martinkova, A. Kostelnik, T. Valek, and M. Pohanka, "Main streams in the construction of biosensors and their applications," aug 2017.

[41] H. H. Nguyen and M. Kim, "An Overview of Techniques in Enzyme Immobilization," *Applied Science and Convergence Technology*, vol. 26, pp. 157–163, nov 2017.

[42] P. Wu, Q. Shao, Y. Hu, J. Jin, Y. Yin, H. Zhang, and C. Cai, "Direct electrochemistry of glucose oxidase assembled on graphene and application to glucose detection," *Electrochimica Acta*, vol. 55, pp. 8606–8614, dec 2010.

[43] D. R. Cavener, "GMC Oxidoreductases A Newly Defined Family of Homologous Proteins with Diverse Catalytic Activities," tech. rep., 1992.

[44] W. P. Dijkman, G. De Gonzalo, A. Mattevi, and M. W. Fraaije, "Flavoprotein oxidases: Classification and applications," jun 2013.

[45] S. Liu, S. Oeljeklaus, B. Gerhardt, and B. Tudzynski, "Purification and characterization of glucose oxidase ofBotrytis cinerea," *Physiological and Molecular Plant Pathology*, vol. 53, pp. 123–132, aug 1998.

[46] K.-S. Shin', H.-D. Youn', Y.-H. Han', S.-O. Kang, Y. Chi1 Hah', and S. Korea, "Purification and characterisation of D-glucose oxidase from white-rot fungus Pleurotus ostreatus," tech. rep., 1993.

[47] S. Nakamura and S. Fujiki, "Comparative Studies on the Glucose Oxidases of Aspergillus niger and Penicillium amagasakiense," Tech. Rep. I, 1968.

[48] L. C. Clark, "Membrane polarographic electrode system and method with 2 sheets-sheet," tech. rep., 1965.

[49] V. Mani, B. Devadas, and S.-M. Chen, "Direct electrochemistry of glucose oxidase at electrochemically reduced graphene oxide-multiwalled carbon nanotubes hybrid material modified electrode for glucose biosensor," *Biosensors and Bioelectronics*, vol. 41, pp. 309–315, mar 2013.

[50] S. B. Bankar, M. V. Bule, R. S. Singhal, and L. Ananthanarayan, "Glucose oxidase — an overview," *Biotechnology Advances*, vol. 27, no. 4, pp. 489–501, 2009.

[51] J. E. Brady, P. W. Carr, M. Mascini, G. G. Guilbauk, H. Lineweaver, D. Bruke, I. Deng, and C. Enke, "Ferrocene-Mediated Enzyme Electrode for Amperometric Determination of Glucose," Tech. Rep. 2, 1984.

[52] J. E. Frew and H. A. Hill, "Electrochemical Biosensors," vol. 59, no. 15, pp. 933–944, 1987.

[53] D. J. Claremont, I. E. Sambrook, C. Penton, and J. C. Pickup, "Subcutaneous implantation of a ferrocene-mediated glucose sensor in pigs," tech. rep.

[54] E. Sehit and Z. Altintas, "Significance of nanomaterials in electrochemical glucose sensors: An updated review (2016-2020)," *Biosensors and Bioelectronics*, vol. 159, no. January, p. 112165, 2020.

[55] R. B. Northrop, D. J. Hawkes, R. B. Northrop, R. B. Northrop, N. Methods, and W. E. Finn, *Edited by.* CRC Press, 2011.

[56] M. Rinaudo, "Chitin and chitosan: Properties and applications," *Progress in Polymer Science*, vol. 31, pp. 603–632, jul 2006.

[57] M. M. Ahmadi and G. A. Jullien, "A wireless-implantable microsystem for continuous blood glucose monitoring," *IEEE Transactions on Biomedical Circuits and Systems*, vol. 3, pp. 169–180, jun 2009.

[58] P. H. Scott, D. J. Andrews, and A. Taylor, "Glucose monitors," jul 1980.

[59] "A Tale of Two Compartments: Interstitial Versus Blood Glucose Monitoring," *Diabetes Technology Therapeutics*, vol. 11, pp. S–11–S–16, jun 2009.