ELECTROCHEMICAL SENSING OF GLUCOSE USING CONJUGATED POLYMER/CHITOSAN/MWCNT ARCHITECTURE

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ABSTRACT

ELECTROCHEMICAL SENSING OF GLUCOSE USING CONJUGATED POLYMER/CHITOSAN/MWCNT ARCHITECTURE

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In this thesis an amperometric biosensor consisting of a conjugated polymer, chitosan and multi-walled carbon nanotubes constructed for the detection of glucose. Conjugated polymers have opened a new era for the development of biosensing platforms with their unique electronic properties, high stabilities and processabilities. They serve both as immobilization matrices for biorecognition elements and as transducers in biosensing devices. As an additional modification material, chitosan was participated in the construction of the proposed biosensor due to its promising properties such as excellent film formability, biocompatibility, biodegradability, and nontoxicity. Chitosan served as a great immobilization matrix together with the conjugated polymer and improved the stability and sensitivity of the biosensor. Multi-walled carbon nanotubes have also extensively used in electrochemical sensing devices since they improve the response performances of biosensors due to their superior electrochemical properties. Furthermore, they are also promising as supporting matrix materials while preserving the catalytic activity and stability of enzymes. From this point of view, by modifying a graphite electrode with a monomer via electropolymerization followed by casting with CHIT/MWCNTs solution, the proposed biosensor was fabricated having a good linear response for glucose between

0.01-0.75 mM with a detection limit of 0.032 mM and the sensitivity value of 63.76 $\mu AmM^{-1}cm^{-2}$. Moreover, the biosensor presented promising kinetic parameters with the K_M^{app} value of 0.05 mM. For investigating the surface modifications, cyclic voltammetry and SEM techniques were utilized. Besides, in order to prove the applicability of the proposed biosensor, the system was tested with a commercial beverage sample.

Keywords: Electrochemical biosensors, Conjugated polymers, Chitosan, Carbon nanotubes, Glucose oxidase

ÖZ

KONJUGE POLİMER/KİTOSAN/ÇOK DUVARLI KARBON NANOTÜP YAPISI KULLANILARAK GLİKOZUN ELEKTROKİMYASAL OLARAK ALGILANMASI

Özel, Hande
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Bu tezde glikoz tespiti için bir konjuge polimer, kitosan ve çok duvarlı karbon nanotüplerden oluşan bir amperometrik biyosensör imal edilmiştir. Konjuge polimerler benzersiz elektronik özellikleri, yüksek stabiliteleri ve işlenebilirlikleri ile biyoalgılama platformları geliştirme alanında yeni bir çağ açtılar. Konjuge polimerler hem biyoalgılama elemanları için immobilizasyon matrisi olarak ve hem de biyoalgılama cihazlarında transdüser olarak görev yaparlar. Ek bir modifikasyon materyali olarak kitosan, mükemmel film biçimlendirilebilirliği, biyouyumluluk, biyobozunurluk ve toksik olmayanlık gibi gelecek vaadeden özellikleri nedeniyle, önerilen biyosensörün yapısına katıldı. Kitosan, konjuge polimer ile birlikte mükemmel bir immobilizasyon matrisi olarak görev yaptı ve biyosensörün stabilitesini ve duyarlılığını arttırdı. Çok duvarlı karbon nanotüpler, elektrokimyasal algılama cihazlarında da yaygın olarak kullanılmaktadırlar. Sebebi üstün elektrokimyasal özellikleri sayesinde biyosensörlerin tepki performanslarını arttırmalarıdır. Ayrıca, enzimlerin katalitik aktiviteleri ve stabilitelerini koruyarak destekleyici matris malzemeleri olarak da gelecek vaat etmektedirler. Bu açıdan, elektropolimerizasyon yoluyla fonksiyonel bir monomer ile bir grafit elektrotun modifiye edilmesi ve ardından kitosan/çok duvarlı karbon nanotüp çözeltisi ile döküm

yapılmasıyla; 0.032 mM algılama sınırına, 63.76 $uAmM^{-1}cm^{-2}$ duyarlılık değerine ve 0.01-0.75 mM glikoz için iyi bir lineer tepki aralığına sahip bir biyosensör imal edilmiştir. Ayrıca biyosensör 0.05 mM gibi bir K_M^{app} değeri ile umut vadeden kinetik parametreler ortaya koymaktadır. Yüzey değişikliklerinin araştırılması için, dönüşümlü voltametri ve SEM teknikleri kullanılmıştır. Ayrıca, önerilen biyosensörün uygulanabilirliğini kanıtlamak için sistem ticari bir içecek numunesi ile test edilmiştir.

Anahtar Kelimeler: Elektrokimyasal biyosensör, Konjuge polimer, Kitosan, Karbon nanotüp, Glikoz oksidaz

To my dear family…

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LIST OF ABBREVIATIONS

Ag	Silver
CE	Counter Electrode
CNTs	Carbon Nanotubes
CHIT	Chitosan
CP	Conjugated Polymer
CV	Cyclic Voltammetry
Eg	Band Gap
GA	Glutaraldehyde
GOx	Glucose Oxidase
LOD	Limit of Detection
MWCNTs	Multi-walled Carbon Nanotubes
PBS	Phosphate-buffered Saline
Pt	Platinum
RE	Reference Electrode
RSD	Relative Standard Deviation
SEM	Scanning Electron Microscope
SD	Standard Deviation
WE	Working Electrode

CHAPTER 1

INTRODUCTION

1.1. Biosensors

Many definitions can be made for the term ‘’biosensor’’ according to the application area (Mohanty & Kougianos, 2007). But if a common definition is made; a biosensor is an analytical device consisting of a biorecognition element and a transducer which converts the recognition incident into an analytically useful signal (Thevenot et al., 2001).

In 1955-56, the history of biosensing began with the scientist Leland C. Clark's invention of the oxygen electrode. On this development, in 1962, the invention of first glucose biosensor and improvement of electrochemical sensors were reported (Gottschalk, Breulmann, Fetter, Kretschmer, & Bastian, 2006). In 1975, the first commercial biosensor was produced for assaying glucose in blood samples (Pohanka, 2008). Since that time, biosensors continued to develop and gain ground as an interdisciplinary field of study.

The biosensor is the integration of two main parts: a bioelement and a detecting element (Mohanty & Kougianos, 2007). Bioelement part provides the biosensor a high selectivity specific to the measured analyte. The detecting element, transducer, transfers the signal comes from the recognition part to the electronic circuit (Thevenot et al., 2001) (Figure 1.1).

Biosensors are used and gain more importance in many areas such as medicine, food industry, environmental monitoring, and many more. In environmental monitoring, they play a major role such as for estimation of pesticides, phenolic compounds, heavy metals and other toxic environmental chemicals. In medicine, biosensors are used for detecting and quantifying glucose, cholesterol, urea and lactate in blood. In food

industry, they are used for microbial contamination, freshness of animal products and so on (D'Souza, 2001). Biosensing techniques are strongly growing with combining various scientific fields such as chemistry, biology, biochemistry, physics, electronics and computer science (Choi, 2004).

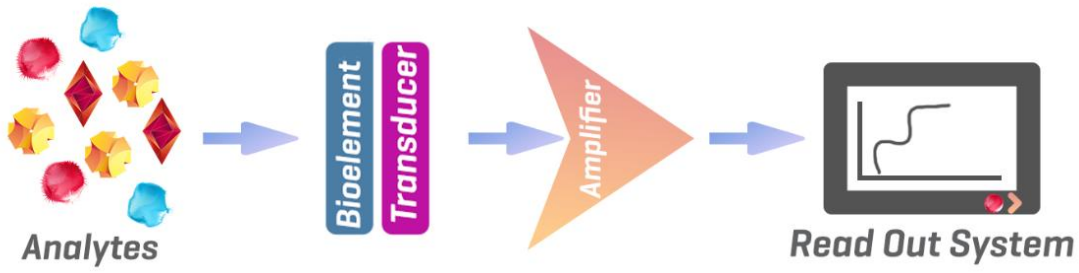

Figure 1.1. General configuration of a biosensor

1.1.1. Biosensor Types

Biosensors can be categorized depending on the types of transducers and bioelements. Bioelement part can be enzymes, antibodies, whole cells, membrane receptors, plants, animal tissues etc. depending on the particular analyte (Thevenot et al., 2001) (Figure 1.2).

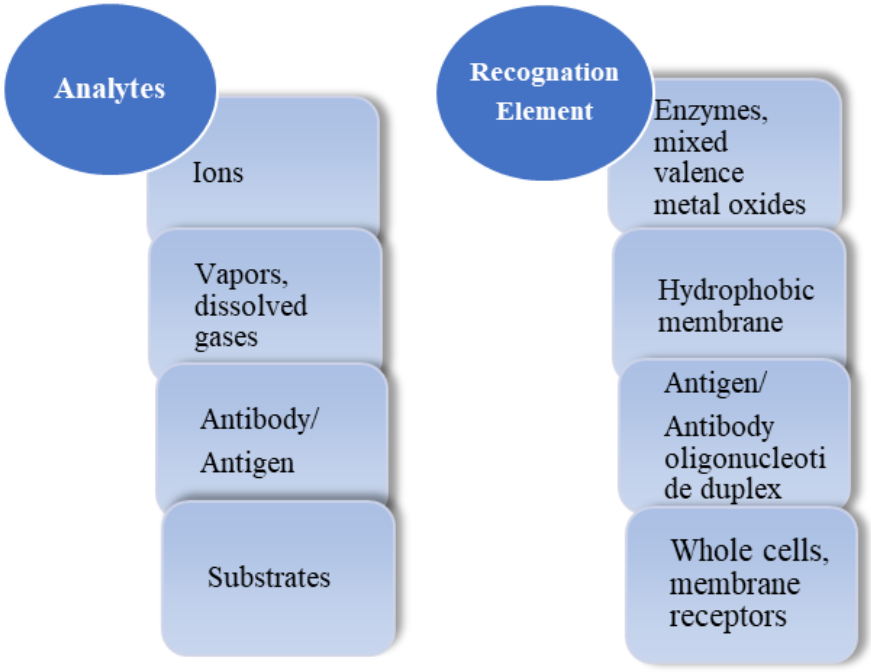

Figure 1.2. Types of analytes and recognition elements

And also biosensors can be divided into electrochemical, thermal, optical, and piezoelectric depending on the transducer types or converted signals (Bhardwaj, 2015) (Table 1.1).

Table 1.1. Types of biosensors depending on the converted signals

Type of Biosensor	Converted Signals
Thermal	Temperature signal into electrical signal
Optical	Optical signal into electrical signal
Piezoelectric	Change in mass, density or viscosity into electrical signal
Electrochemical	Chemical signal into electrical signal

Thermal biosensors consists of an enzyme molecule and a temperature sensor (Mohanty & Kougianos, 2007). This type of biosensors use the main properties of biological reactions as follows absorption or production of heat that can be seen as a

change in temperature in the reaction medium (Ramanathan & Danielsson, 2001). The temperature measurement is performed via thermistors. They are highly sensitive to thermal changes and their sensitivity makes them the most suitable choices in such cases. In general, thermal biosensors are used for detecting pathogenic bacteria and pesticides (Mohanty & Kougianos, 2007).

Optical biosensors consist of a biorecognition element and an optical transducer. The main purpose of these biosensors is to produce a signal in proportion to the concentration of the analyte. As biorecognition elements; enzymes, receptors, antigens, whole cells, nucleic acids, and tissues can be used. The optical biosensing is basically done in two ways: label-free and label-based. In label-free mode, the optical signal is achieved directly by the interactive relation between the analyte and the transducer. In label-based mode, the detected signal is generated by a fluorescent, colorimetric or luminescent method. Application areas of optical biosensors are generally microelectronics, micro/ nano systems, biotechnology and microelectromechanical systems (MEMs) (Ligler & Taitt, 2008).

Piezoelectric biosensors detect the alteration in the medium viscosity, density or mass of analytes. Piezoelectricity is related to the mechanically stressed material's ability of producing voltage or vice versa. The working principle of piezoelectric biosensors is basically affinity interaction recording (Pohanka, 2018).

In between all types of biosensors, electrochemical biosensors are known to be outstanding comparing with many costly, complicated and difficult techniques. These biosensors are simple to operate, sensitive, selective and cheaper comparing with optical, thermal and piezoelectric biosensors. Also electrochemical biosensors have need less amount of sample for examination (Bhardwaj, 2015).

1.1.1.1. Electrochemical Biosensors

Due to the fact that the electrochemical biosensors have many advantages like low cost, simplicity, sensitivity and high selectivity; numerous fields interest particularly

with electrochemical biosensors. Some of these fields are food, clinical, environmental and pharmaceutical.

The most basic parts of electrochemical biosensors are biorecognition layer procuring electroactive species and a transducer providing a signal in measurable format (Pohanka, 2008).This type of biosensors convert the chemical change resulting from the interaction between biomolecule and analyte into an electrical signal (Bhardwaj, 2015).

Electrochemical detection devices can be categorized into two main classes depending upon biorecognition process:

i. Biocatalytic devices
ii. Affinity sensors

Biocatalytic devices contain enzymes, tissues or whole cells that selectively recognize the target analyte and produce a detectable signal. These devices are easy to handle, cost efficient, adaptable to clinical or industrial analysis. Electrochemical detection techniques mostly use enzymes since enzymes have high specificity and biocatalytic activity. Also, enzymes can increase the reaction rate and can detect their individual substrate molecules in complex mixtures. In the meantime, due to lack of adequate enzymes for many biochemical analytes, affinity sensors are regarded as an alternative method (Barsan, Emilia Ghica, & Brett, 2013).

Affinity sensors contain selective and strong binding biological components such as antibodies, receptors or nucleic acids that selectively interacts with analyte to produce an electrical signal. These biomolecules high specifities and affinities for their analytes make affinity sensors highly selective (Barsan et al., 2013).

Based on the mode of transduction technique, electrochemical biosensors can be described as potentiometric, conductometric and amperometric (Gottschalk et al., 2006).

Potentiometric biosensors determines the potential difference between a reference electrode and an indicator which is proportional to the gas concentration or ion activity when there is zero or near zero current flowing between them (Thevenot et al., 2001). Potentiometric transducers can be ion-selective electrodes (ISE) or ion-sensitive field effect transistors (ISFET). The output signal is based on ions accumulated at interface of ion-selective membrane (Pohanka, 2008). pH electrodes, gas (NH_3, CO_2) and ion (Na^+, K^+, I^-, CN^-) selective electrodes are the most general potentiometric devices (Thevenot et al., 2001).

Conductometric biosensors detects the alteration in the electrical conductivity of the sample medium resulting from a biochemical reaction. Most conductometric devices include enzymes; as a result of an enzymatic reaction, the concentration of charged species and thus the conductivity change between two electrodes. Conductometric detection is generally used for clinical and environmental analysis (Barsan et al., 2013).

Amperometric biosensors measure the produced current which is directly related to the electroactive substances in the reaction medium resulting from an electrochemical oxidation or reduction. Amperometry is mostly performed at a constant potential at a working electrode such as Au, Pt or C based electrodes containing biorecognition elements with respect to a reference electrode (Thevenot et al., 2001). Amperometric biosensors are very sensitive devices by comparison with other methods this is because, for detecting oxidation and reduction potential values are specific for examined sample.

Electrochemical biosensors use either two or three electrode configurations. Two electrode system consists of a working electrode containing bioelement for recognition and a reference electrode. In this configuration, at high current values, it is difficult to control the potential on the working electrode. This leads in shortening in the linear range of the biosensor. This problem is solved by a third electrode. In this case, applied voltage is still between the working and Ag/AgCl reference electrodes

and current flows between the working and the counter electrodes (Pohanka, 2008) (Figure 1.3).

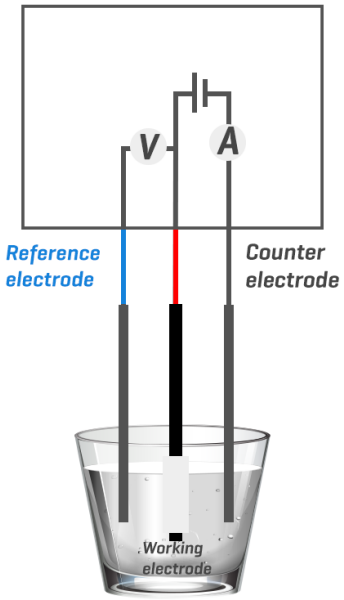

Figure 1.3. Representative three electrode configuration

Amperometric detection is mainly used with affinity sensors and biocatalytic devices due to their easiness and low limit of detection values. Also, hydrodynamic amperometric methods increase the mass transfer to the electrode surface (Barsan et al., 2013).

1.1.2. Immobilization of Bioelements for Biosensor Applications

One of the crucial features of a biosensor is the construction of the biorecognition element or biorecognition site for interacting with the target substrate. Many factors affects the choice of biorecognition element such as; physicochemical properties of the analyte to be detected, the type of transducer used, working conditions, environmental stability etc. (Jeanmonod, Rebecca, & Suzuki, 2018). These biologic sensing elements can be enzymes, receptors, antigens or antibodies, microorganisms, DNA, and other low molecular weight molecules that interacts with certain biomaterials.

Enzyme based biosensors represent the most comprehensive studied technique in the biosensing area. In fact, the first biosensor in the scientific literature is known as enzyme electrode presented by Clark in 1956 and by Clark and Lyons in 1962. In this biosensor, the GOx (glucose oxidase) enzyme was coupled with an oxygen electrode.

Enzymes as biorecognition elements are excellent catalysts under very mild conditions with their unique functional groups. Furthermore, there is a very significant factor affecting the success of enzyme-based biosensors, that is the fixation (immobilization) of enzymes on solid platforms. Immobilization of enzymes affect the selectivity, sensitivity, analytical parameters, and life time of biosensors. Also by means of immobilization of enzymes, immobilized enzyme can be reused over a long time therewith cost savings and simple biosensor operation (Choi, 2004).

Several methods have been used for an effective immobilization for various systems. Generally, there are four basic methods for enzyme immobilization; adsorption, covalent binding, entrapment and cross-linking (Jeanmonod et al., 2018).

1.1.2.1. Physical Adsorption

Physical adsorption is the simplest and quickest method for immobilization of enzymes. This method consists of weak physical bonds such as electrostatic attractions, hydrophobic interactions and van der Waal's forces. By this method, enzymes do not lose their activity, however, the immobilized enzymes prepared by physical adsorption are loosely bound and tend to resolve from the surface of the solid materials after reuse. Therefore, the biosensor may lose its operational stability (Choi, 2004)

1.1.2.2. Covalent Binding

In covalent binding method, formation of covalent bonds occurs between a functional group of the bioelement and solid supporting matrix. Covalent bonds between enzyme and the solid support is achieved by activating the surface of the immobilization matrix and coupling the enzyme to the activated surface. The unreacted bioelements can be

removed with buffer solutions. Covalent binding technique leads to a stable and efficient binding between the enzyme and supporting matrix. However, during the coupling reaction some of enzymes may lose their bioactivity and this leads in the construction of an instable biosensor (Choi, 2004).

1.1.2.3. Entrapment

Entrapment is the caging of biomolecules by covalent or non-covalent bonds within gels. The gels can be starch gels, conducting polymers, silica gels etc. In this method, biomolecule, supporting material and other additives are positioned onto the sensing surface at once. The main disadvantage of entrapment method is diffusion of substrate can be hindered and this leads to the reaction delays and long response times (Jeanmonod et al., 2018).

1.1.2.4. Crosslinking

In crosslinking method, enzyme is chemically bonded to supporting material's surface via multifunctional reagents. Multifunctional reagents provide linking biomolecules with each other and to the immobilization matrix.

For crosslinking method, using adequate crosslinking agent in the optimum amount is very crucial for maintaining the activities of enzymes. Excess crosslinking may interfere with the enzyme activity. Glutaraldehyde is the most commonly used bifunctional crosslinker because of its solubility in aqueous solvents and ability to form stable covalent bonds (Datta, Christena, & Rajaram, 2013) (Figure 1.4).

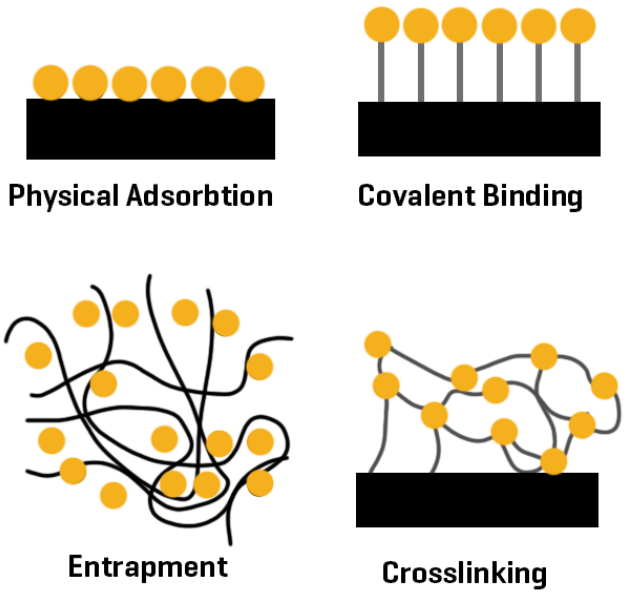

Figure 1.4. Enzyme immobilization methods

1.1.3. Glucose Biosensors

Diabetes mellitus is a metabolic disorder of carbohydrate metabolism results from insulin deficiency. This endocrine disorder is indicated by different concentrations of blood glucose than the normal range. This health problem leads to the highest mortality and disease rate worldwide. The prevalence of diabetes worldwide was reported to be 285 million people (adults) in 2010. What is more, it is estimated that 439 million people (adults) will have this disorder by 2030 (Yoo & Lee, 2010).

Frequent and correct testing of blood glucose level is very crucial for management and treatment of diabetes mellitus. In addition, glucose is the most widespread monitored analyte and glucose biosensors involve approximately 85% of whole biosensor market based on 2004 data. This large market size and enormous economic expectations for controlling diabetes have led to remarkable research and development strategies, not only in medicine but also in food industry (J. Wang, 2008).

Numerous methods have been comprehensively developed for creating sensitive, selective, cost efficient, reliable and fast glucose biosensors such as electrochemical methods, optical methods, colorimetry, fluorescent spectroscopy etc. Among all these methods, electrochemical glucose biosensors are pointed as more sensitive, selective, stable, cheap, practically applicable and easily operable ones. The electrochemical glucose biosensors can be mainly categorized into three groups depending upon the measurement principles; potentiometric, amperometric or conductometric biosensors (M. M. Rahman, Ahammad, Jin, Ahn, & Lee, 2010).

Amperometric methods have been commonly used in glucose sensing. Amperometric glucose sensing can also be categorized into two; Amperometric nonenzymatic glucose biosensors and amperometric enzymatic glucose biosensors. Amperometric nonenzymatic glucose biosensors are being founded on the direct electrochemical oxidation of glucose. The biggest advantage of this method is that the problem of not being able to maintain the enzyme stability for a long time is radically eliminated which is the most extensive and significant problem for enzymatic glucose biosensors. Noble metals like Au and Pt and their composites have been utilized as electrodes in nonenzymatic glucose biosensors due to their high electrocatalytic properties and high selectivity to electrochemical oxidation of glucose. But yet, the adsorption of active species and oxidation intermediates in the medium hinder the electrode activity which is a quite important problem for this system. Moreover, in nonenzymatic glucose sensing method, electrocatalytic materials used cannot be as characteristic as enzymes for catalyzing the oxidation of glucose. This ensures that enzymatic glucose biosensing systems have higher selectivity than nonenzymatic glucose biosensors (Chen et al., 2013).

Amperometric enzymatic glucose biosensors have been commercially utilized and commonly studied in recent years. These biosensors monitor the current which is linearly dependent on the glucose concentration at constant potential (M. M. Rahman et al., 2010). Glucose is oxidized at the working electrode which consists of the enzyme such as glucose oxidase (GOx) or glucose dehydrogenase (GDH) (Yoo & Lee,

2010). The main reason for being different from each other is their ability to react with electron acceptors; GOxs can use oxygen as the electron acceptor unlike GDHs. In addition to this, they also differ from each other in selectivity for glucose, redox potentials, ionic strength, pH and temperature tolerance etc. (Ferri, Kojima, & Sode, 2011).

1.1.3.1. Glucose Oxidase (GOx)

Glucose oxidase is a flavoprotein which is derived from the Fungus Aspergillus Niger and widely used in glucose detection and monitoring systems. GOx can also be produced from a few insects, however GOx obtained from Aspergillus Niger have higher specifity for glucose. Glucose oxidases are commercially available in the market and they are very important in glucose sensing systems due to their high specifity for glucose, cost efficiency and usability in different medium conditions (Ferri et al., 2011).

Glucose oxidase catalyzes oxidation of β-D-Glucose by using oxygen as the electron acceptor. In electrochemical sensing of glucose, generally amount of oxygen consumption or amount of hydrogen peroxide production is measured (Yoo & Lee, 2010). In 1973, first H_2O_2 production monitoring and GOx based glucose detection was described. The GOx based glucose detection reaction includes the reduction of the redox cofactor, flavin adenine dinucleotide (FAD) group in the enzyme after reacting with analyte glucose to obtain GOx - $FADH_2$, reduced form of the enzyme.

$$\text{Glucose} + \text{GOx} - \text{FAD} \longrightarrow \text{Gluconic acid} + \text{GOx} - \text{FADH}_2$$

This is followed by regeneration of GOx – FAD by oxidation of GOx – $FADH_2$ by the Med_{ox} (electron acceptor). Regarding to the nature of the electron acceptor, amperometric glucose biosensors can be categorized into three generations; first, second and third generation of amperometric glucose biosensors (Chen et al., 2013).

1.1.3.2. Generations of Enzyme Based Amperometric Glucose Biosensors

In the first generation enzyme based amperometric glucose biosensors, O_2 is used as the Med_{ox} (physiological electron acceptor) for regeneration of GOx – FAD. This generation of biosensing measures the O_2 consumption or H_2O_2 formation. The biosensor response is directly related to the O_2 concentration in the reaction medium. In these systems major drawback is the oxygen deficit and to this respect, glucose sensitivity is limited by the O_2 concentration in the medium. To overcome this drawback, first generation sensing system was upgraded to second generation by using artificial electron acceptors.

Second generation enzyme based amperometric glucose biosensors utilize artificial electron acceptors with low oxidation potentials. The steps of catalytic process of this system are; transferring electrons from substrate to $FADH_2$, then transforming Med_{ox} to Med_{red} by transferring the electrons from $FADH_2$, and transporting the electrons to the electrode from the artificial mediators.

The third generation enzyme based amperometric glucose biosensing system utilizes the principle of directly coupling of the enzyme to the electrode in the absence of mediators. This system is efficient in electrodes which electrically wired with redox enzymes (Chen et al., 2013) (Figure 1.5).

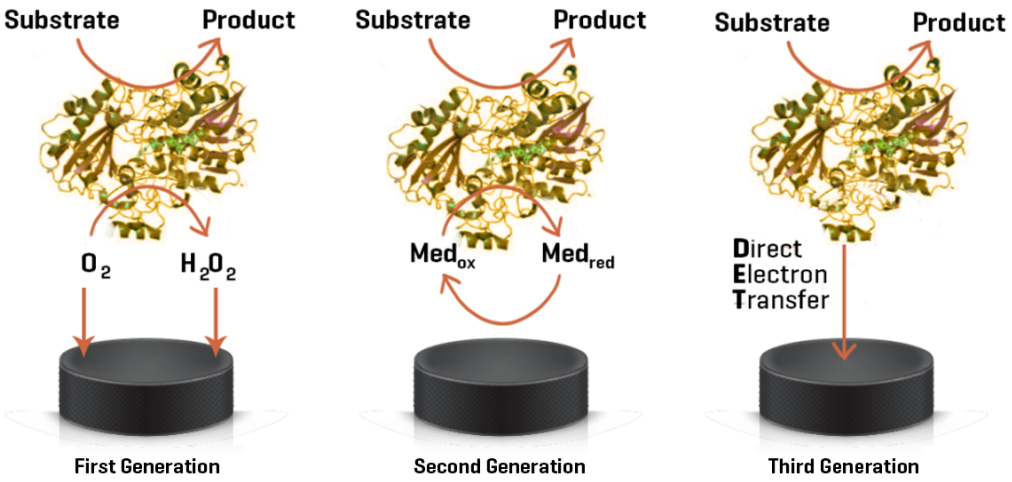

Figure 1.5. Working principles of 1st, 2nd and 3rd generations of biosensors

1.2. Conjugated Polymers (CPs)

In 1976 a new field of research including chemistry and physics was opened with the discovery of conducting polymers by Alan J. Heeger, Alan MacDiarmid, Hideki Shirakawa. In fact, this innovative discovery even brought them a Nobel Prize in Chemistry in 2000. The invention of conducting polymers, as well as being a significant milestone, has paved the way for many studies and developments such as the development of polymeric materials that can be processable, but which can also have the characteristics of optical and electrical properties of metals or semiconductors (Heeger, 2001).

Charge mobility along the backbone of the polymer chain is provided by electronic delocalization. Chemical bonding in conjugated polymers involves an unpaired electron per carbon atom. This π bonding causes the delocalization of electrons along the polymer chain (Heeger, 2001). As a consequence, such polymers are defined mainly as organic macromolecules that contain an extended π-orbital system and have electronic properties like conductivity (M. A. Rahman, Kumar, Park, & Shim, 2008).

Alternation in bond lengths causes a band gap (E_g). Generally, wider band gaps result from larger differences in C-C lengths. Band gap determines the electron motion and a semiconductor has narrow band gap which is limited for electron transfer. Conducting polymers are generally insulators inherently or semiconductors with wide band gap. Band gap of a semi conductive organic polymer can be lowered by some methods such as by designing structures with donor-acceptor concept or these polymers can be doped for being conductive by electrochemical methods. Doping can be defined as injecting charge to conjugated polymer chains, more clearly oxidizing (p-doping) or reducing (n-doping) of a neutral polymer (Swager, 2017). Doping process puts in local charge carriers which can exist in the form of polarons, bipolarons into the chain. And the movement of electrons along or between the polymer chains (called as electron hopping) results in charge mobility and electrical conductivity. Besides, electrical conductivity range of these polymers can be controlled by changing the dopant concentration (Ravichandran, Sundarrajan, Venugopal, & Mukherjee, 2010).

Conjugated polymers draw attention as new functional materials in numerous application and research fields due to their metal-like conductivity, processability, biocompatibility, light-weight, cost efficiency and many more attractive properties. Some of the areas CPs find applications are sensor technology, electrochromic devices, polymer light emitting diodes, electrocatalyst, drug delivery (Ates, Karazehir, & Sarac, 2012).

1.2.1. CPs for Biosensor Applications

Conjugated polymer-based biosensors by means of their peerless electronic characteristics and very low detection limits are considered as the new generation of sensing systems for bio assaying. Conducting polymers have been utilized in many analytical application areas from the day they were discovered. Furthermore, in the recent years, CPs have come into play as one of the operable materials for the electrochemical biosensors with their tunable electronic properties. Beforehand, inert

polymers were used as supporting materials for enhancing the mechanical strength of these systems but nowadays CPs are used both as structuring materials which provide mechanical support, and as immobilization matrix for biorecognition elements and transducers which generate analytical signals in biosensing devices (Pan, Gonuguntla, Li, & Trau, 2017).

CPs are biocompatible with biological molecules and further they can bind the biomolecules to the sensing system and preserve their catalytic activities over a long period of time. Another advantage of the conducting polymers in biosensor applications is that they can be directly deposited over the determined areas of electrodes which provides the orientational control of the film thickness and the immobilization of different recognition elements (M. A. Rahman et al., 2008).

In a few words, conjugated polymers have opened a new era by enhancing the selectivity, sensitivity, response times and many more properties of the biosensing platforms with their unique characteristic properties.

1.3. Chitosan for Biosensor Applications

Chitosan is a cellulose-based biopolymer obtained from partial deacetylation of natural chitin (Dervisevic, Dervisevic, Çevik, & Şenel, 2017). Chitosan (CHIT) has attracted much attention recently in many application fields especially for biological applications due to its marvelous biocompatibility, biodegradability, nontoxicity and high stability. Moreover, CHIT has a grand potential for electrochemical biosensor applications (Congur, Eksin, & Erdem, 2018).

Chitosan can be utilized as a modification agent by means of its amino, hydroxyl and acetyl functional groups providing sites for chemical bonding. Also, CHIT has excellent film formability. These features lead to chitosan being a suitable matrix for biomolecules such as enzymes. Moreover, CHIT preserves enzyme stability and activity against organic solvents and other damaging environments (Warner & Andreescu, 2016).

In addition to these advantages of CHIT, chitosan-based electrochemical biosensors encounter a problem such as failure to transmit the electric signal to the transducer due to its relative low conductivity. In order to overcome this problem; a method, which also will be mentioned in the continuation of this study, that is combining CHIT with materials such as carbon nanotubes and some nanoparticles are used in the fabrication of the biosensor (Dervisevic et al., 2017).

1.4. Carbon Nanotubes (CNTs) for Biosensor Applications

In recent years searching for advanced materials has become very important in the many innovative technologic areas. From this point, CNTs exhibit unique electrical, thermal and mechanical properties which made them quite advantageous in several applications. In 1985, Buckminster fullerene (C_{60}) was discovered by Kroto et al. and in 1991, another new form of carbon, multi-walled carbon nanotubes (MWCNTs) was reported by Sumio Ijima. Approximately two years after, Ijima discovered single-walled carbon nanotubes (SWCNTs) and at the same times Dresselhaus et al. synthesized (SWCNTs). CNTs can be defined as tubular fullerenes, rolled up graphene sheets of sp^2 carbon atoms. They can be produced with methods like chemical vapor deposition, and laser ablation and the arc-discharge method (Saeed, 2017) (Figure 1.6-1.9).

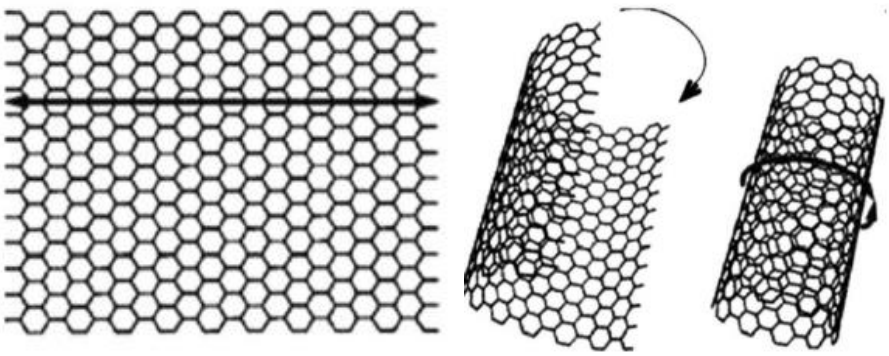

Figure 1.6. Graphene sheets rolled into carbon nanotubes

CNTs can be classified as single-walled CNTs (SWCNTs), double-walled CNTs and multi-walled CNTs (MWCNTs). (SWCNTs) is rolled of a single graphene sheet.

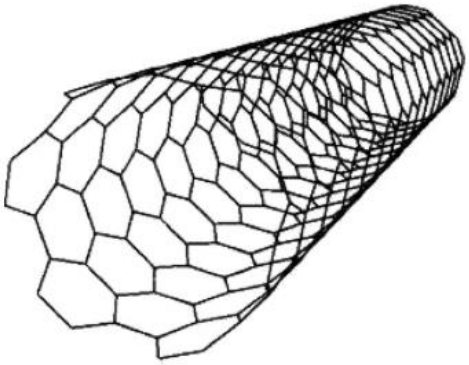

Figure 1.7. Single-walled carbon nanotubes (SWCNTs)

Double-walled CNTs consist of two concentric carbon nanotubes enclosing each other.

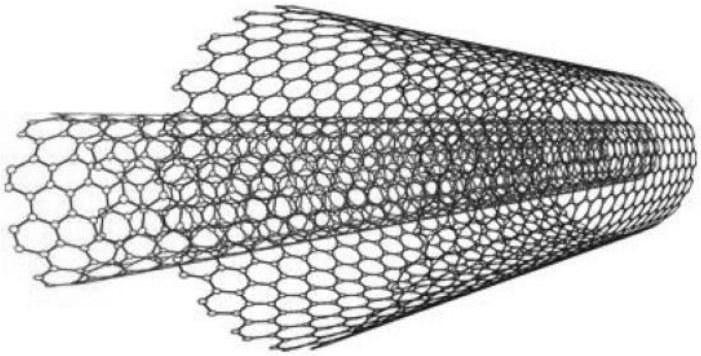

Figure 1.8. Double-walled carbon nanotubes

MWCNTs are rolled up graphene sheets stack .

Figure 1.9. Multi-walled carbon nanotubes (MWCNTs)

CNTs have incredible mechanical properties within sp^2 carbon-carbon bonds and in fact the weakest types of CNTs have tensile strengths in GPas. Also, the electronic properties of CNTs are also tremendous, even compared to the copper (Saifuddin, Raziah, & Junizah, 2013). Due to these features, CNTs and their modified materials are utilized in sensing systems, micro/nano electronics, photovoltaic devices, optics, biological fields etc. (Saeed, 2017).

In the fabrication of biosensors, CNTs are optimal nanomaterials due to their high electrical conductivity, high sensitivity, high stability, and biocompatibility. They are great transducers with superior electrochemical properties in sensing systems. Also, they are promising supporting matrix materials since most of chemical species can be attached to CNTs such as enzymes with preserving their catalytic activities and stabilities (Yang, Chen, Ren, Zhang, & Yang, 2015).

1.5. Aim of the Thesis

In this thesis study a conjugated polymer based amperometric biosensor consisting of chitosan and multi-walled carbon nanotubes was designed for an improved glucose detection system. For this purpose, a graphite electrode was modified with CP, CHIT and MWCNTs and GOx was immobilized onto the modified electrode surface using

glutaraldehyde as the crosslinking agent. In the construction of the biosensor; CP coated surface served as a stable immobilization matrix due to its electroactive nature, CHIT utilized as an additional modification material and provided sites for chemical bonding for the enzyme molecule by means of its functional groups, and MWCNTs increased the charge transfer and enhanced the electrical conductivity. The response of the fabricated biosensor was measured by amperometric detection technique monitoring the oxygen consumption results from the enzymatic reaction between GOx and the substrate glucose at -0.7 V. In order to obtain the best combination for the proposed biosensor, optimization studies were performed. With the optimum surface design, characterization and sample application studies were carried out. To investigate the surface morphology of the final design SEM technique were applied.

CHAPTER 2

CP/CHIT/MWCNT/GOX BIOSENSOR

2.1. Experimental Studies

2.1.1. Materials

Glucose oxidase (GOx, β-D-glucose: oxygen 1-oxidoreductase, EC1.1.3.4, 17,300 units/g solid) from Aspergillus Niger, D-glucose, $NaClO_4$, $LiClO_4$, dichloromethane (≥99.8%), acetic acid (≥99%), glutaraldehyde (GA), chitosan (low viscosity) and multi-walled carbon nanotubes (MWCNTs) were purchased from Sigma–Aldrich Co., LCC. (St. Louis, USA). For the immobilization of the enzyme GOx, a 50 mM pH 7.0 phosphate buffer solution (PBS) consisting of 0.025 M Na_2HPO_4 (Fisher Scientific Company) and 0.025 M NaH_2PO_4 (Fisher Scientific Company) was used. For the substrate (0.1 M glucose solution) preparation, 0.18 g of glucose was dissolved in 10 mL pH 7.0 PBS solution. All chemicals were of analytical reagent grade.

2.1.2. Instrumentation

All the amperometric measurements and cyclic voltammetry studies were performed by using PalmSens potentiostat (PalmSens, Houten, The Netherlands). Three electrode system consisting of a graphite rod as the working electrode (Ringsdorff Werke GmbH, Bonn, Germany, typeRW001, 3.05 mm diameter and 13% porosity), Pt wire as the counter electrode (Metrohm, Switzerland) and Ag wire as the reference electrode was used for both electropolymerization and amperometric measurements. For the surface investigation of the fabricated biosensor, scanning electron microscope (SEM) (JEOL JSM-6400 model) was used. All measurements were performed at ambient conditions.

2.1.3. The Monomer Used in the Construction of the Biosensor

The monomer, 4,7-bis(3,4-dihydro-2H-thieno[3,4-b][1,4]oxathiepin-8 yl)benzo[c][1,2,5]thiadiazole, was provided from Günbaş group, Department of Chemistry - Middle East Technical University. The synthesis of the monomer was conducted by PhD students Figen Varlıoğlu and Aliekber Karabağ (Figure 2.1).

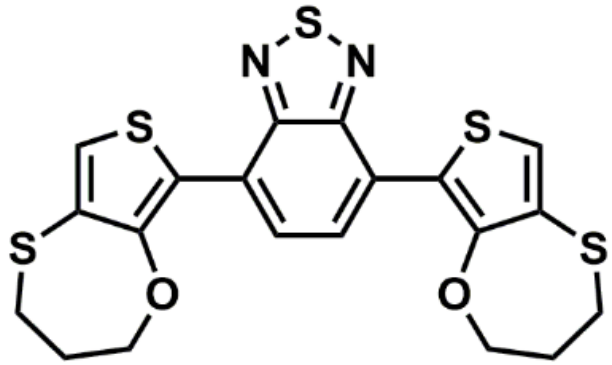

Figure 2.1. Structure of the 4,7-bis(3,4-dihydro-2H-thieno[3,4-b][1,4]oxathiepin-8 yl)benzo[c][1,2,5]thiadiazole

2.1.4. Biosensor Preparation

Prior to electropolymerization of the monomer 4,7-bis(3,4-dihydro-2H-thieno[3,4-b][1,4]oxathiepin-8 yl)benzo[c][1,2,5]thiadiazole, surface of the graphite electrodes was prepared by polishing them with emery paper and then washing them with distilled water. After the washed electrodes have dried, electropolymerization of the 1.0 mg of monomer was performed via cyclic voltammetry on a clean graphite electrode in 95:5 ACN:DCM solution containing 0.1 M $LiClO_4$/$NaClO_4$ electrolyte between 0.0 and 1.3 V potentials with a scan rate of 100 mV s^{-1} for 30 cycles. After the electropolymerization, the polymer coated electrode was rinsed off with distilled water for removing the impurities. Then, a chitosan solution was prepared as: 0.25 g chitosan in 50 mL of 2.0 M acetic acid with stirring efficiently for 1 hour (Hassanein, Salahuddin, Matsuda, Kawamura, & Elfiky, 2017). After preparation of 0.5% of viscous chitosan solution, 0.50 mg MWCNTs in 5 mL of 0.5% of chitosan solution dispersed by 15 min ultrasonication to obtain a black suspension. After the CP

modified electrode was dried at ambient conditions, 10 µL aliquots of the prepared CHIT/MWCNTs solution were cast on the electrode surface and the electrode was left to dry for 1 h at room temperature. After 1 hour, 10 µL of GOx solution (0.25 mg GOx in 10 µL of 50 mM pH 7.0 PBS buffer solution) was immobilized on the dry electrode surface and followed by the immobilization of 5.0 µL of GA solution (1% in 50 mM pH 7.0 PBS buffer solution) to the electrode surface. Then, the electrode was left to dry for 2 h at ambient conditions. The prepared biosensor was washed with distilled water for sending away the impurities and unbound molecules. Figure 2.2 represents the procedure of the construction of the proposed biosensor schematically.

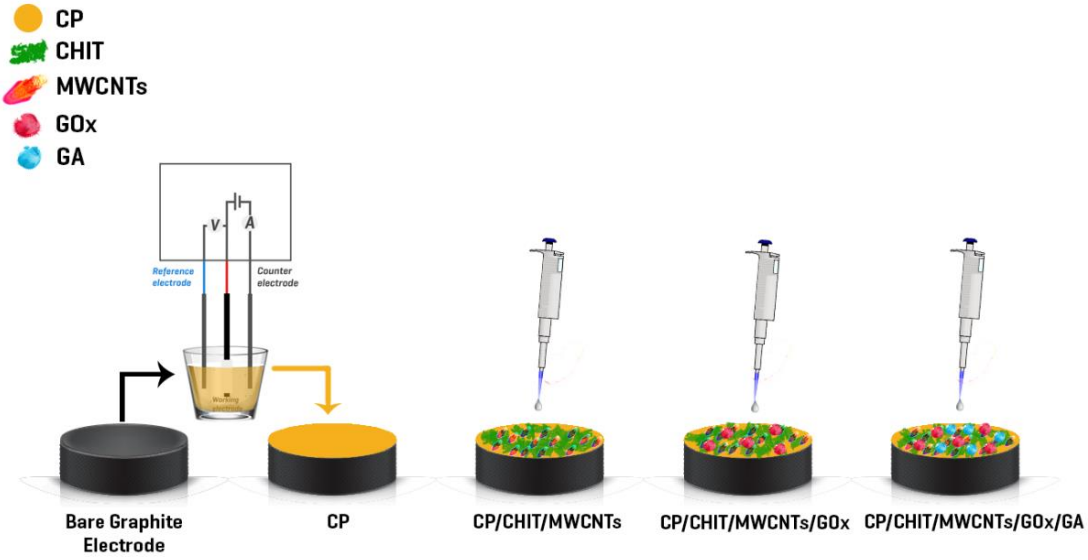

Figure 2.2. Schematic representation of CP/CHIT/MWCNTs/GOx biosensor

2.1.5. Amperometric Measurements

For amperometric measurements, a reaction cell filled with 10 mL pH 7.0 PBS solution and all the electrodes (working, reference, counter electrodes) were inserted into the cell. Measurements were performed by applying constant potential under mild stirring. After each measurement, the buffer solution was refreshed in the reaction cell and the surfaces of the electrodes rinsed off with distilled water. All the amperometric studies were conducted at ambient conditions.

In consequence of the enzymatic reaction between the enzyme (GOx) and the substrate (glucose solution), oxygen consumption which is associated with the concentration of the substrate was monitored at a specific potential of -0.7 V. This specific potential was applied for all the amperometric measurements since electrochemical oxidation of the H_2O_2 produced occurs nearly at this potential vs. Ag/AgCl for pH 7.0 and the biosensor response for this enzymatic reaction is most sensitive at this potential (Gorton, 1995).

As conducting the measurements, a certain amount of substrate was added into the reaction cell when the baseline current equilibrated. The current changed in a balanced way as a result of the enzymatic reaction between GOx and added glucose solution. Then, the current reached to a new equilibrium after the change. The biosensor response was considered as the difference between these two constant current values (µA). Each amperometric measurement was repeated at least three times. Results of the measurements were given as the average of these measurements and standard deviations were recorded as ±SD (Figure 2.3).

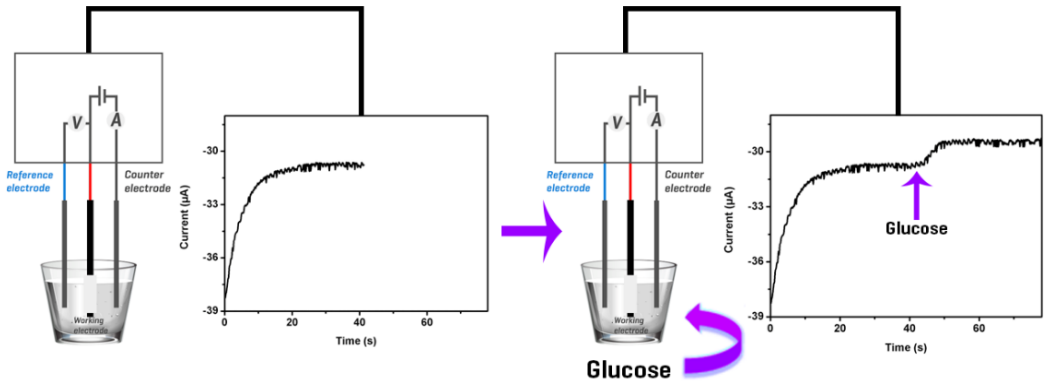

Figure 2.3. Amperometric measurement process

2.1.6. Optimization Studies

For the development of a stable, sensitive, reproducible and long-lived biosensor, all the parameters affecting the construction of the biosensor were optimized. For this

reason, the effects of different amounts of CP, CHIT and MWCNTs as well as GOx concentration and pH of the buffer solution on the biosensor response were examined.

The amount of the parameter to be optimized was changed and all the other parameters were kept constant. By applying this method, different electrodes were prepared, and the current signal values were measured. The most stable and highest response of the biosensor was chosen as the optimum value. After all the parameters were optimized, best combination of the biosensor was achieved.

2.1.7. Characterizations

2.1.7.1. Analytical and Kinetic Characterizations

After achieving the optimum construction of the biosensor, analytical parameters of the proposed biosensor were calculated. A calibration curve for the substrate glucose was plotted and limit of detection (LOD) and sensitivity values were determined by fixing the intercept of the linear range of the curve to zero using criterion of S/N (signal-to-noise ratio) is equal to three. For investigating the repeatability of the fabricated biosensor, at least 10 consecutive measurements were taken for 0.5 mM glucose solution. Using the obtained results, the standard deviation (SD) and the relative standard deviation (RSD) values of the constructed system were calculated.

Michaelis-Menten enzyme kinetics model was utilized for the kinetic characterizations. This model provides an equation which defines the relation between the rate of the enzymatic reaction and the concentration of the substrate. This equation is: $v = \frac{V_{max}[s]}{K_M + [S]}$

V_{max} is the maximum reaction rate and K_M is the enzyme affinity to its substrate and these are the parameters for characterizing the biochemical reaction kinetics (Cornish-Bowden, 1976).

For obtaining I_{max} and K_M^{app} values, a Lineweaver- Burk plot (1/I vs 1/[S]) is used which is the linear form of the Michaelis-Menten plot.

Moreover, for proving the reproducibility of the fabricated biosensor, three optimum electrodes were prepared and amperometric measurements were performed by measuring at least three current values.

2.1.7.2. Surface Characterizations

In order to characterize the effective electroactive surface area of the modifications, cyclic voltammetry (CV) technique was used. Within this method, performed experiments were conducted in a solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$, 0.1 M KCl and 50.0 mM pH 7.0 PBS buffer solution at the potential between 0 and 1.0 V with a scan rate of 100 mV s^{-1}. By using Randles-Sevcik equation, the electroactive surface areas of each surface modification were calculated. Randles-Sevcik equation is as follows $I_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} v^{1/2} C$

In this equation, A is the area of the electrode in cm^2, D is the diffusion coefficient of the molecule in solution in cm^2/s, n is the number of electrons involved in the reaction, V is the scan rate in V/s and C is the concentration of the probe molecule in the bulk solution in mol/cm^3.

Also, for investigating the surface morphology of the different surface modifications, SEM technique was used. Images of CP modified, CP/CHIT/MWCNT modified, and CP/CHIT/MWCNT/GOx modified electrode surfaces were analyzed.

2.1.8. Investigation of Interferents

Several variables can affect the accuracy of the glucose detection tests. Urea and ascorbic acid are the electrochemically interfering molecules found in blood and these species can cause false reading of the obtained response from the glucose biosensor (Yoo & Lee, 2010). For this reason, the selectivity of the proposed biosensor only to glucose was also verified by performing amperometric measurements with urea, ascorbic acid and glucose solutions having concentrations of 0.5 mM. Measurements were performed by adding urea, ascorbic acid and glucose solutions into the reaction

medium at constant -0.7 V potential under mild stirring and response of the biosensor was recorded.

2.1.9. Sample Application

The fabricated biosensor was tested for detecting and analyzing the glucose concentration on commercially available beverage samples. A beverage sample having the glucose concentration in the linear range of the biosensing system (adjusted by changing added volume to 10 μL) was added to the reaction medium and amperometric measurements were taken. The biosensor response to this sample was compared with the glucose quantity indicated on the product label.

2.2. Results And Discussion

2.2.1. Biosensor Preparation

Many experiments were performed to achieve the best response in the biosensor preparation process. One of these trials was as follows; after electropolymerization of the monomer onto the graphite electrode surface, MWCNTs-DMF (dimethylformamide) dispersion was cast on the CP modified electrode surface without using chitosan in the construction of the sensing system. The other one was the immersing the CP modified electrode into the CHIT/MWCNTs solution before immobilization of the enzyme GOx. Moreover, it was also tried to cast CHIT (in acetic acid) and MWCNTs (in DMF) separately on the CP modified electrode surface as the immobilizing matrix for GOx. Many more methods have been tried to obtain the best architecture for sensing glucose, however the most stable and highest response was taken from the proposed method which was preparing an immobilization matrix for the enzyme by casting CHIT/MWCNTs solution on the electrode surface after electropolymerization of the monomer (Figure 2.4).

Moreover, the cyclic voltammogram was performed in a monomer free solution (0.1 M $LiClO_4/NaClO_4$) with a scan rate of 100 mV/s for 2 cycles on an ITO electrode in order to investigate the doping properties of the polymer (Figure 2.5).

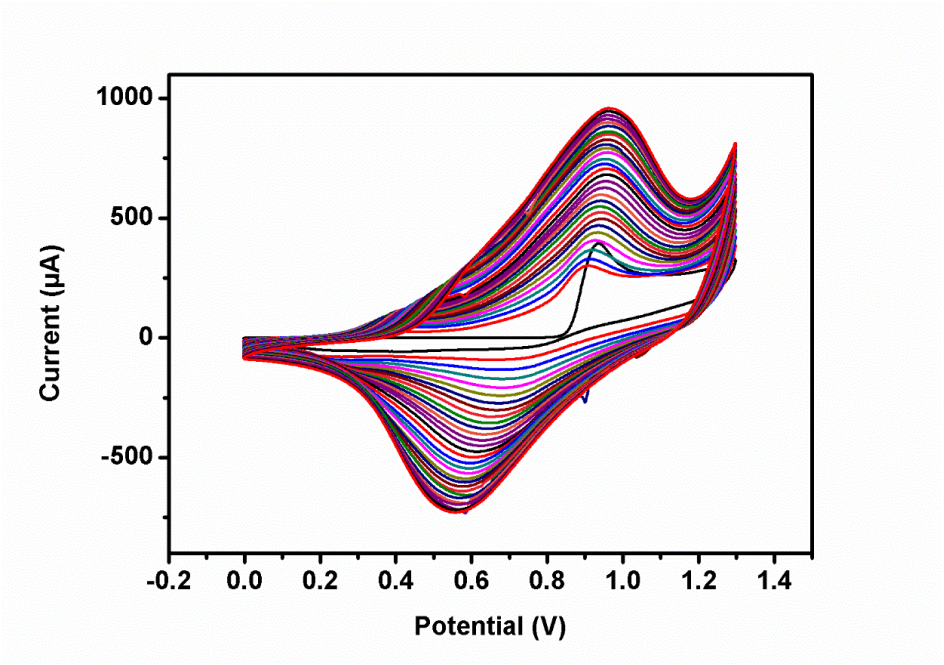

Figure 2.4. Repeated potential scan polymerization of monomer 4,7-bis(3,4-dihydro-2H-thieno[3,4-b][1,4]oxathiepin-8 yl)benzo[c][1,2,5]thiadiazole in 95:5 ACN:DCM solution containing 0.1 M $LiClO_4/NaClO_4$ electrolyte between 0.0 and 1.3 V potentials with a scan rate of 100 mV/s for 30 cycles on an ITO electrode

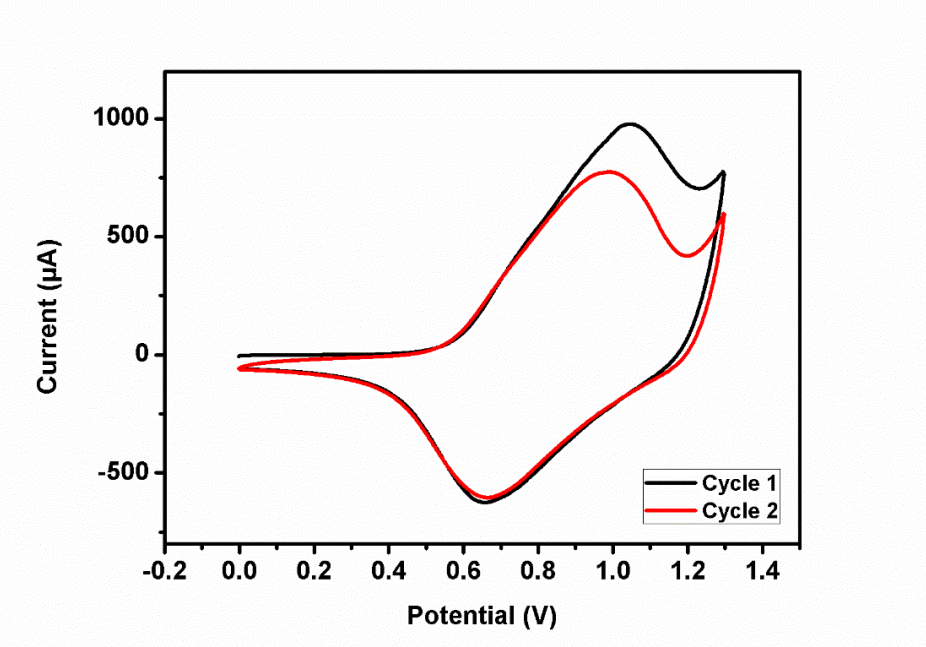

Figure 2.5. Cyclic voltammogram of the polymer in a monomer free 0.1 M $LiClO_4/NaClO_4$ solution on an ITO electrode

2.2.2. Optimization Studies

2.2.2.1. Optimization of the Biosensor Parameters

Optimum polymer film thickness was determined by adjusting the scan number in the electropolymerization. Polymer film thickness affects the orientation and binding effectiveness of the enzyme in the electrode surface, correspondingly it also affects the electron transfer between the enzyme and the electrode (Buber, Soylemez, Udum, & Toppare, 2018). Therefore, the monomer was coated on the electrode surface by electropolymerization with different cycle numbers: 10, 20, 30, 40 and 50 cycles by keeping all the other parameters constant. With these electrodes having different polymer film thicknesses, amperometric measurements were taken and the most balanced and highest biosensor response was obtained with 30 scans (Figure 2.6).

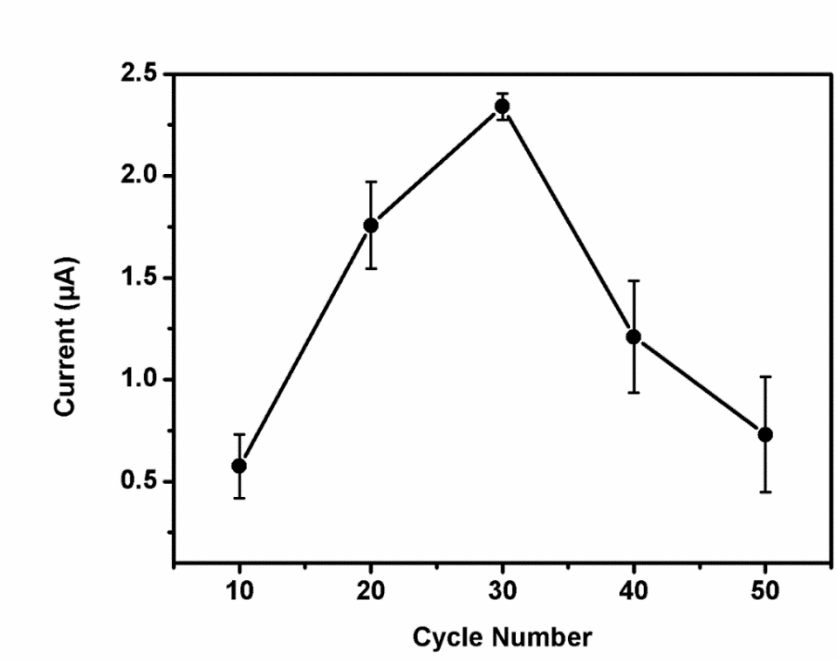

Figure 2.6. The effect of cycle number on biosensor response (in 50.0 mM PBS, pH 7.0, 25◦C). Error bars show the standard deviation (SD) of three measurements

After the determination of the optimum polymer film thickness, the amount of the CHIT used in the proposed biosensor was optimized by adjusting the concentration of the CHIT solution by keeping all the other parameters constant and setting the cycle

number as 30. Following 0.25%, 0.40%, 0.50%, 0.65%, 0.80% CHIT solutions were prepared and 0.50 mg MWCNTs were dispersed in 5.0 mL of these solutions by 15 min ultrasonication and 10 µL aliquots of these solutions were cast on the CP coated electrode surfaces. After amperometric measurements, obtained signals of the electrodes were compared and biosensor with 0.50% CHIT solution gave the most balanced and highest response (Figure 2.7).

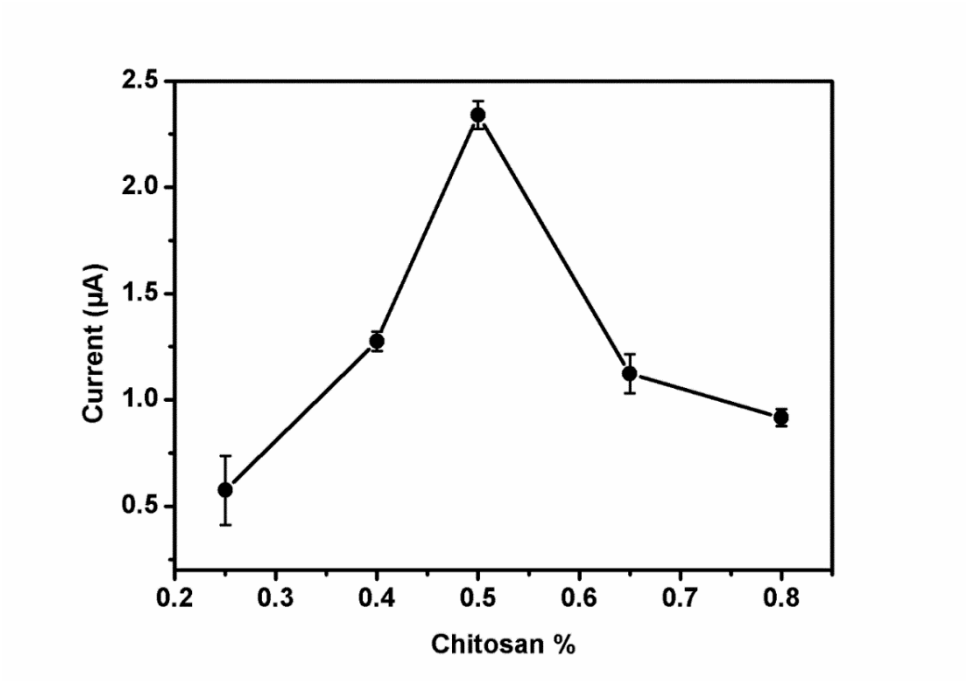

Figure 2.7. The effect of chitosan % on biosensor response (in 50.0 mM PBS, pH 7.0, 25◦C). Error bars show the standard deviation (SD) of three measurements

The amount of MWCNTs was also optimized since excessive use may result in limitation of the diffusion and this lead lowering of biosensor response. On the other hand, smaller amounts of use may leads to enzyme fixation problem (Buber, Yuzer, et al., 2017). Therefore, the amount of MWCNTs was optimized by dispersing 0.05, 0.1, 0.25, 0.40, 0.50, 0.65 mg of MWCNTs in 5.0 mL of 0.50% CHIT solution by 15 min ultra-sonification and 10 µL aliquots of these solutions were cast on the CP coated electrode surfaces. After amperometric measurements, obtained signals were compared and biosensor with 0.50 mg of MWCNTs gave the most balanced and highest response (Figure 2.8).

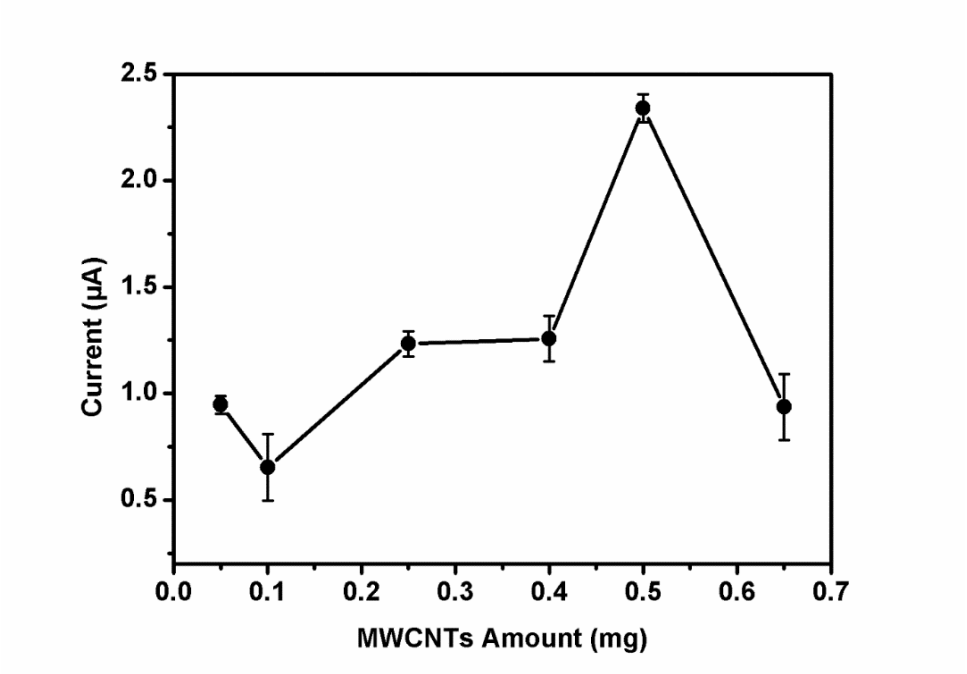

Figure 2.8. The effect of amount of MWCNTs on biosensor response (in 50.0 mM PBS, pH 7.0, 25◦C). Error bars show the standard deviation (SD) of three measurements

Enzyme (GOx) amount was also optimized since a large amount of the enzyme used may not stand on the electrode surface due to the enzyme loading capacity of the immobilization matrix. Besides, use of smaller amounts of enzyme may affect the biosensor response negatively (Buber, Kesik, Soylemez, & Toppare, 2017). Therefore, GOx amount was optimized by preparing the electrodes having different enzyme amounts: 0.1, 0.25, 0.50, 1.0, 1.25, 1.50 mg of GOx. After amperometric measurements, obtained signals of the electrodes were compared and biosensor with 0.25 mg of GOx gave the most balanced and highest response (Figure 2.9).

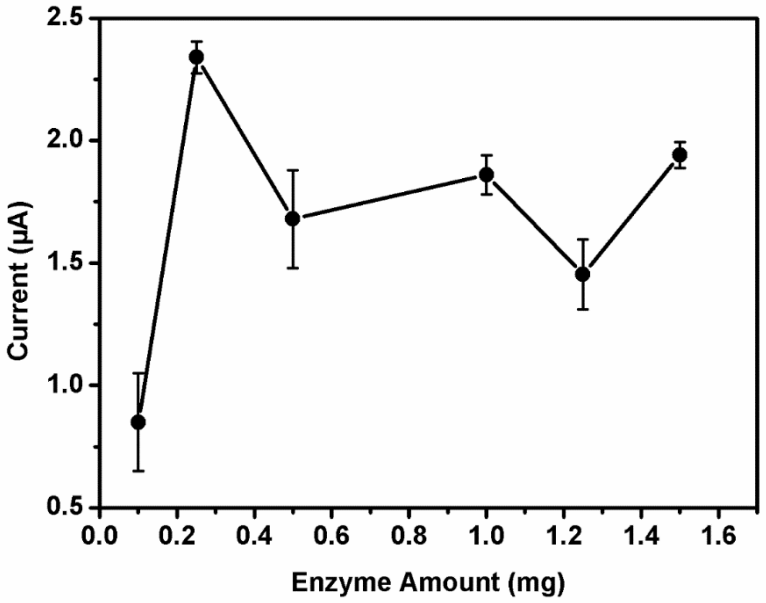

Figure 2.9. The effect of enzyme (GOx) amount on biosensor response (in 50.0 mM PBS, pH 7.0, 25◦C). Error bars show the standard deviation (SD) of three measurements

Terminally, the pH value for the sensing system was optimized due to high sensitivity of the enzyme molecules to the pH alterations (Buber, Yuzer, et al., 2017). For this reason, 50 mM buffer solutions in a pH range of 5.0-8.0 (sodium acetate buffer at pH 4.0-5.5, sodium phosphate buffer at pH 6.0-7.5, tris buffer at pH 8.0-9.0, 25◦C) were prepared and amperometric measurements were conducted with these buffer solutions at pH 5.0, 5.5, 6.5, 7.0, 7.5, 8.0 without changing other parameters. After the measurements, the most balanced and highest response of the biosensor was obtained with pH 7.0 (Figure 2.10).

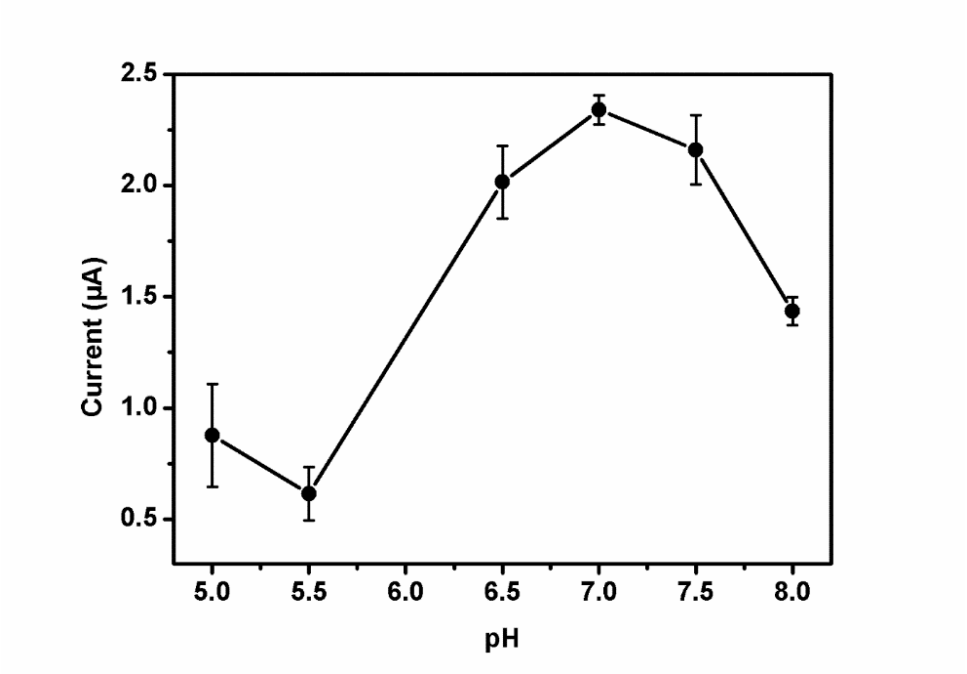

Figure 2.10. The effect of pH on biosensor response (in 50 mM sodium acetate buffer at pH 5.0; 5.5, 50 mM PBS at pH 6.5;7.0;7.5 and 50 mM Tris buffer at pH 8.0, 25◦C). Error bars show the standard deviation (SD) of three measurements

2.2.2.2. Determination of the Best Combination

For achieving the most stable and precise biosensing system, different combinations of CP/GOx, MWCNT/GOx and CP/CHIT/MWCNT/GOx were prepared as glucose sensing surfaces using the optimum parameters. Then, amperometric measurements were taken with alternating concentrations of glucose solution and obtained signals were compared (Figure 2.11).

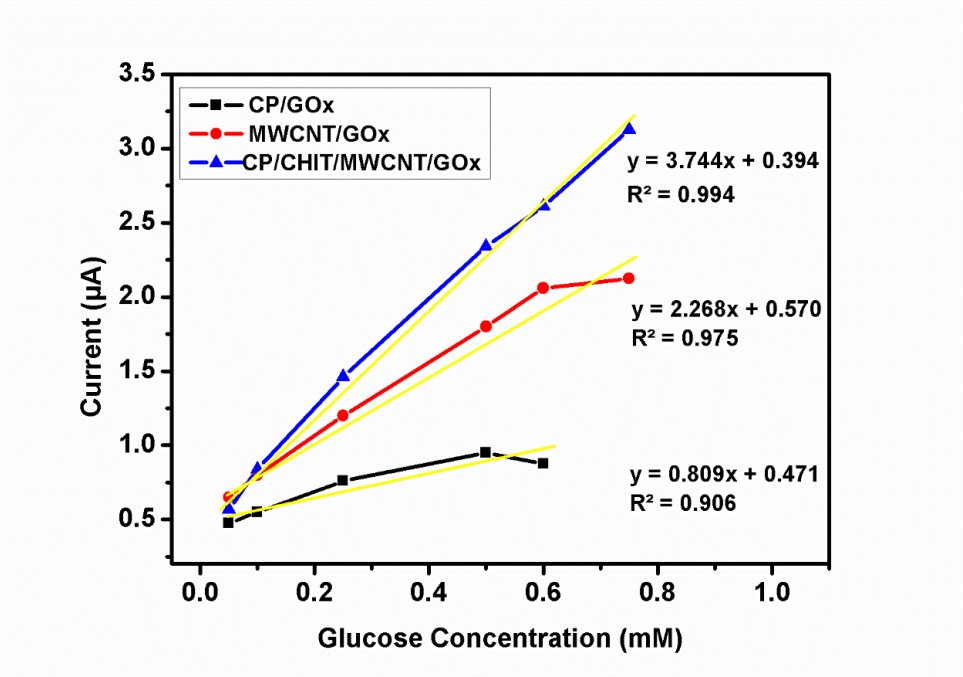

Figure 2.11. The effect of different surface modifications on performance of the biosensor (in 50.0 mM PBS, pH 7.0, 25◦C)

According to the calibration curves of these combinations, LOD and sensitivity values were calculated (Table 2.1).

Table 2.1. Comparison of analytical performances of different combinations

Combination	**LOD (mM)**	**Sensitivity ($\mu AmM^{-1}cm^{-2}$)**
CP/GOx modified electrode	0.058	26.2
MWCNT/GOx modified electrode	0.136	47.1
CP/CHIT/MWCNT/GOx modified electrode	0.032	63.76

With the evaluation of the data, the following comments can be made; CP and CHIT combination enhanced the stability of the biosensor, on the other hand MWCNTs increased the charge transfer ability of the electroactive surface and promoted the biosensor response. However, CP/CHIT/MWCNT/GOx combination resulted in more stable, sensitive and improved sensing performance when compared to the individual use of the species.

2.2.3. Characterizations

2.2.3.1. Analytical and Kinetic Characterizations

A calibration curve for glucose was plotted after reaching the optimum biosensing system (Figure 2.12).

A linear response range was obtained as 0.01-0.75 mM glucose in 50 mM PBS pH 7.0 with the equation $y = 3.744x + 0.394$ with $R^2 = 0.994$. Substrate saturation was observed at higher glucose concentrations than 0.75 mM. The limit of detection (LOD) and sensitivity values were calculated as 0.032 mM and 63.76 $\mu AmM^{-1}cm^{-2}$, respectively.

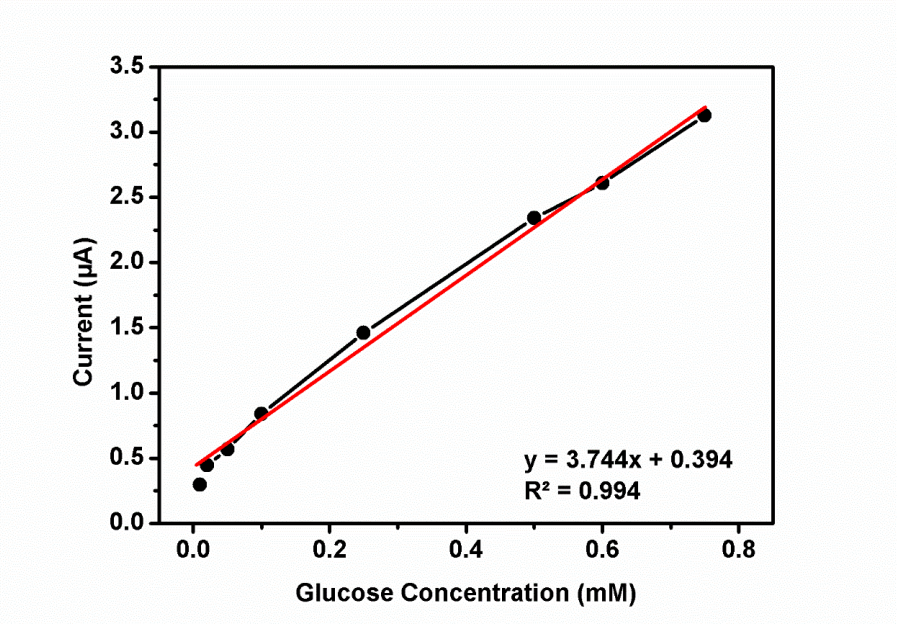

Figure 2.12. Calibration curve for glucose (in 50 mM PBS, pH 7.0, 25◦C)

For investigating the repeatability of the fabricated biosensor, at least 10 consecutive measurements were taken for 0.5 mM glucose solution. Using the obtained results, the standard deviation (SD) and the relative standard deviation (RSD) values were calculated as ±0.12 and 5.13%, respectively.

The kinetic parameters, K_M^{app} and I_{max} values of the fabricated biosensor were calculated as 0.05 mM and 1.69 μA, respectively by using a Lineweaver-Burk plot. When these results are compared with other glucose sensing systems in the literature, it was seen that this biosensor is superior with outstanding K_M^{app}, low LOD and high sensitivity values to other systems (Table 2.2).

Table 2.2. Comparison of analytical performances of glucose biosensors in the literature

Structure of Biosensor	LOD	Sensitivity	K_M^{app}	Reference
MWCNTs/Chi-BSA-Fc/GOD	10 μM	7.8 $\mu AmM^{-1}cm^{-2}$	1.5 mM	(Fatoni et al., 2013)
MWNT-Fc	3.4 μM	10 $\mu AmM^{-1}cm^{-2}$	6.3 mM	(Qiu, Deng, Liang, & Xiong, 2008)
GOx-CH/PPy-Au NPs/GCE	68 μM	0.58 $\mu AmM^{-1}cm^{-2}$	1.83 mM	(Şenel, 2015)
Chi-PB	0.397 μM	2.57 μAmM^{-1}	3.73 mM	(X. Wang, Gu, Yin, & Tu, 2009)
GOx/Graphene-chitosan nanocomposite	20 μM	37.93 $\mu A\ mM^{-1}cm^{-2}$	4.4 mM	(Kang et al., 2009)
GOD/CNTs-chitosan matrix	Not reported	0.52 μAmM^{-1}	8.2 mM	(Liu, Wang, Zhao, Xu, & Dong, 2005)
CP/CHIT/MWCNT/GOx	**32 μM**	**63.76 $\mu AmM^{-1}cm^{-2}$**	**0.05 mM**	**This work**

Moreover, for proving the reproducibility of the fabricated biosensor, three optimum electrodes were prepared and amperometric measurements were performed by measuring at least three current values close to each other (Figure 2.13). The mean current values taken from these three devices for 0.5 mM glucose solution are very close to each other and their standard deviations are quite low. On this basis the following comments can be made; CP/CHIT/MWCNT matrix provides suitable

environment for the immobilization of the enzyme and preserves the enzymatic activity of GOx.

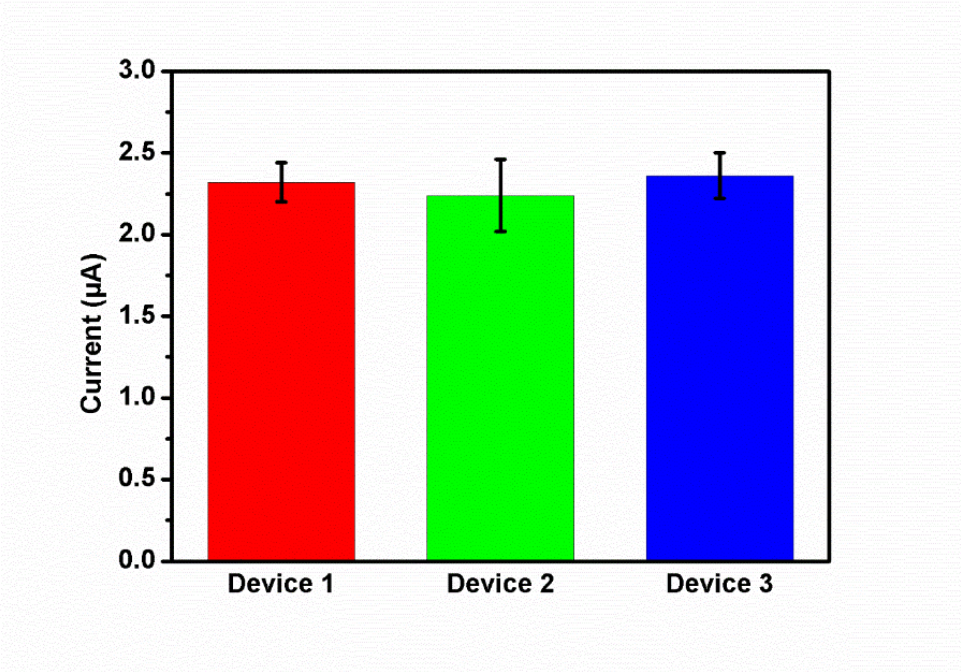

Figure 2.13. Response measurements of three prototypes of the proposed biosensor (in 50.0 mM PBS, pH 7.0, 25◦C for 0.5 mM glucose solution)

2.2.3.2. Surface Characterizations

From the peak currents of the corresponding cyclic voltammograms (Figure 2.14), the electroactive surface areas of CP/CHIT/MWCNT and CP/CHIT/MWCNT/GOx were calculated as 0.138 cm^2 and 0.092 cm^2 respectively. Immobilization of the enzyme resulted in the decrease in the peak current and effective surface area due to the insulating nature of biomolecules.

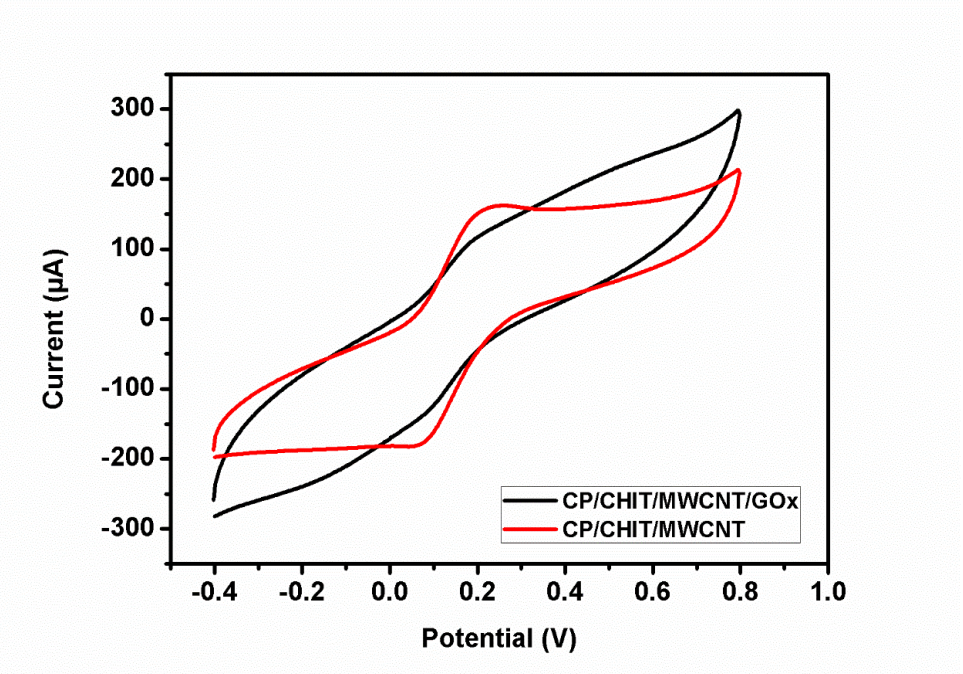

Figure 2.14. Cyclic voltammograms resulting from CP/CHIT/MWCNT and CP/CHIT/MWCNT/GOx in 5.0 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl

Moreover, scanning electron microscopy (SEM) technique was utilized for investigating the surface morphology of the different surface modifications. Figure 2.15 demonstrates SEM images of CP, CP/CHIT/MWCNT and CP/CHIT/MWCNT/GOx modified electrode surfaces, respectively. In each surface modification, homogeneous coating of each layer and a very distinct morphology change were observed.

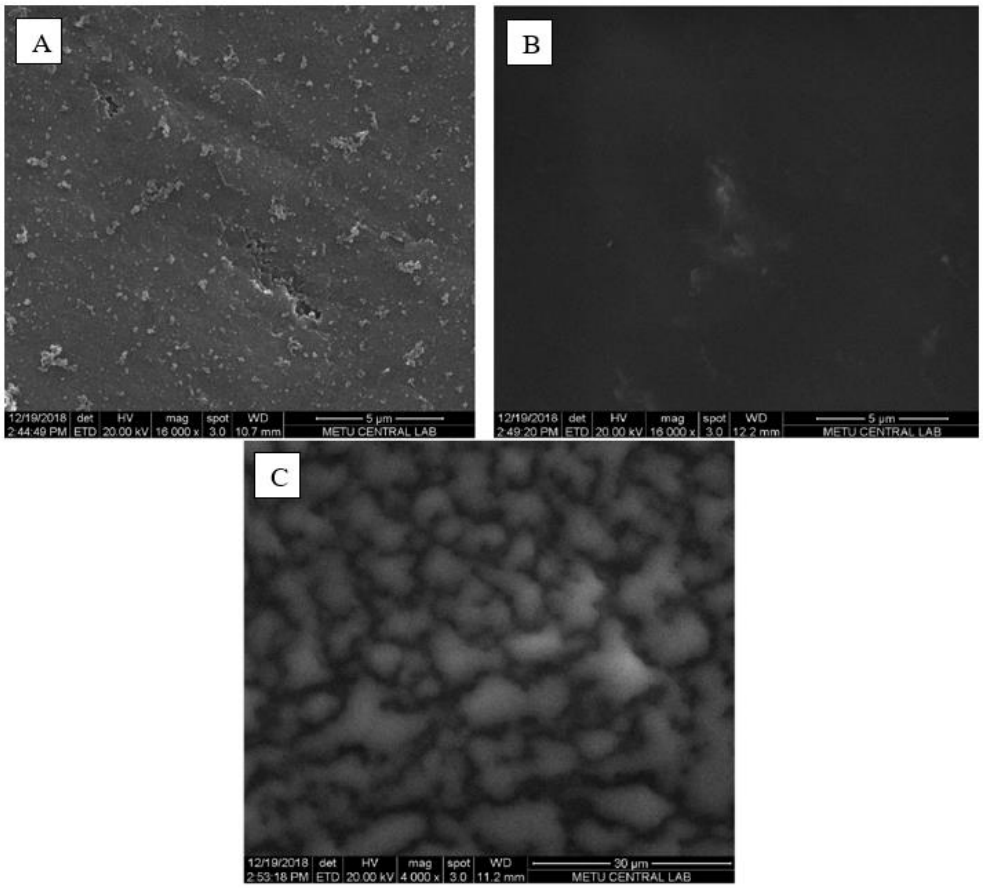

Figure 2.15. SEM images of (A) CP; (B) CP/CHIT/MWCNT; (C) CP/CHIT/MWCNT/GOx under optimum conditions

2.2.4. Investigation of Interferents

The main purpose of the fabricated glucose biosensor is the detection and quantification of glucose amounts in blood, in other words its target analyte is only glucose. Therefore, the fabricated biosensor was tested with other biological molecules to prove the selectivity of the biosensor. Urea, ascorbic acid and glucose solutions having concentrations of 0.5 mM were prepared and amperometric measurements were performed with these solutions by adding them into the reaction medium. Response of the biosensor was recorded and as shown in Figure 2.16 any significant response could not be obtained for these interfering substances.

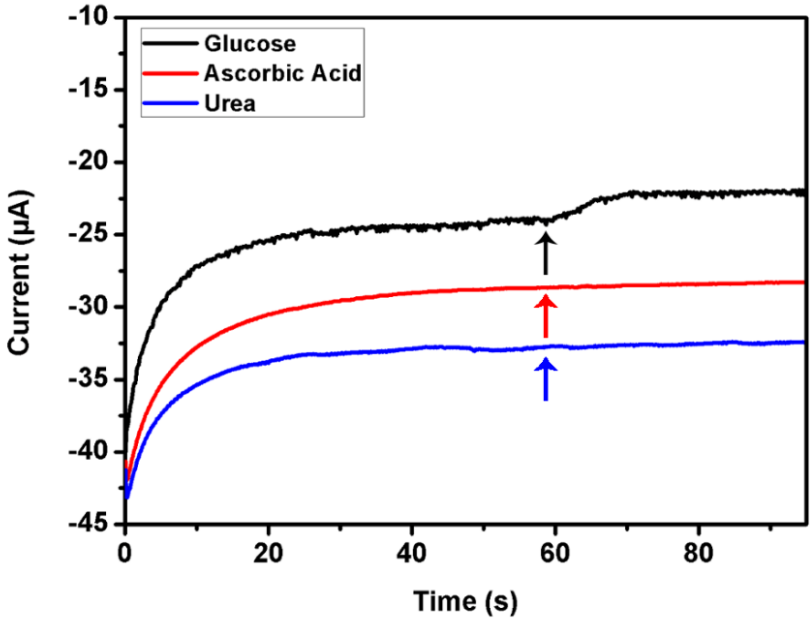

Figure 2.16. Responses of the proposed biosensor to glucose and interfering substances (in 50 mM PBS, pH 7.0, 25 °C)

2.2.5. Sample Application

In order to test the applicability of the proposed sensing system, amperometric measurements were performed by injecting 10 µl of beverage sample into the reaction medium instead of glucose solution. The glucose content of the product label was compared with the amount of glucose measured by the biosensor using the calibration curve. Table 2.3. shows the obtained results from the real sample analysis.

Table 2.3. Results of real sample analysis

Sample	**Glucose Content (mM)**	
	Product Label	CP/CHIT/MWCNT/GOx
L® Ice tea	0.37	0.32

CHAPTER 3

CONCLUSION

In this thesis a conjugated polymer based amperometric biosensor was constructed for detection of glucose. A monomer was electropolymerized onto the graphite electrode. After electropolymerization of the monomer, CHIT/MWCNTs solution was cast on the CP modified electrode surface in order to prepare an immobilization matrix for the enzyme GOx. Utilization of the CP enhanced the physical interactions and binding of the enzyme. CHIT was participated in this sensing system due to its promising properties such as biocompatibility, biodegradability, and nontoxicity. As well as performing all the duties in the construction, it also made great contributions to the stability of the biosensor. By having excellent film formability feature and preserving the stability and catalytic activity of the enzyme, CHIT served as a suitable immobilization matrix together with CP. MWCNTs as modification agents improved the response performance of the biosensor by increasing the electroactive surface area of the modified surface and the charge transfer rate by means of their superior electronic properties.

The biosensor response was investigated with amperometric measurements by monitoring the decrease in the oxygen level arising from the enzymatic reaction between the enzyme GOx and the substrate glucose solution at a specific potential -0.7 V. Also, optimization studies were performed in order to achieve the best surface design and the most sensitive biosensor architecture. After reaching the optimum combination of the components and conditions of the proposed biosensor, the parameters determining the performance of the sensing system were calculated with analytical and kinetic characterizations. The fabricated biosensor has a good linear response for glucose between 0.01-0.75 mM with a detection limit of 0.032 mM and the sensitivity value of 63.76 $\mu AmM^{-1}cm^{-2}$. Moreover, the biosensor presented promising kinetic parameters with the K_M^{app} value of 0.05 mM. Besides, cyclic

voltammetry and SEM techniques were utilized for investigating the surface modifications. Also, by testing the sensing system with interfering substances, specificity of the biosensor to glucose was proven. And finally, the proposed biosensor was tested with a commercial beverage sample for demonstrating the applicability.

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