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USAGE OF ORGANOBORON BASED POLYMERIC MATERIALS AT ENZYMATIC BIOSENSORS

M.Sc. Thesis by

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USAGE OF ORGANOBORON BASED POLYMERIC MATERIALS AT ENZYMATIC BIOSENSORS

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Zeycan Kalkan

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M.Sc. THESIS EXAMINATION RESULT FORM

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ABSTRACT

The application and characterization of organoboron polymers to electrochemical enzymatic biosensors, which have only been tested in a limited number, have been examined within this thesis's scope, and patent applications will be made since this is one of the pioneering studies. Therefore, contribution to the literature will be made. In this thesis, the use of biocompatible organoboron polymers as enzyme immobilization molecule is added value to the biosensor system to be prepared in many ways. Utilizing the direct electropolymerization (one-step) method, an electrochemical enzymatic biosensor system was developed with polyaniline film coated carbon screen printed electrodes, and poly 3-aminophenylboronic acid film coated gold screen printed electrodes/ glassy carbon electrode, and novel organoboron polymer film-coated platinum screen printed electrodes/glassy carbon electrode. Organoboron polymer-based enzymatic and electrochemical analysis that will be developed in this thesis will be used for the determination of catechol that is one of the mostly analyzed phenolic compounds in the chemistry and agriculture industry. Catechol analysis was developed in which the tyrosinase enzyme was used as the sample determination system. With the developed biosensor system, the phenolic components were tested in the linear range between 1 μM to 200 μM with different electrodes. After the biosensor performance conditions optimization, real sample analysis were also performed for controlled catechol added green tea samples with 3% to 10% range of standard deviation results. Finally, it should be noted that the developed biosensor system can be designed and commercialized as a portable end product that allows real-time detection. Phenolic compounds, which are determined to determine the antioxidant and antimicrobial activities of natural foods, are partly made within the scope of quality control analysis, and the developed organoboron polymer-based biosensor system will allow faster, cheaper, precise, and real-time tests.

Keywords: Boron, organoboron polymers, conducting polymers, polyaniline, enzymatic biosensors, electrochemistry, phenolic compounds, catechol analysis

ORGANOBOR TABANLI POLİMERİK MALZEMELERİN ENZİMATİK BİYOSENSÖRLERDE KULLANILMASI

ÖZ

Henüz çok sınırlı sayıda test edilmiş olan organobor polimerlerin elektrokimyasal enzimatik biyosensörlere uygulanması ve karakterizasyonu bu tez kapsamında incelenmiş olup öncü çalışmalardan biri olduğu için patent başvuruları yapılacaktır, dolayısıyla literatüre katkı yapılacaktır. Bu tezde,biyouyumlu organobor polimerlerin enzim immobilizasyon molekülü olarak kullanılması, hazırlanacak biyosensör sistemine birçok yönden değer katmıştır.Doğrudan elektropolimerizasyon (tek aşamalı) yönteminden yararlanılarak, polianilin film kaplı carbon yüzey baskılı elektrotlar ve poli 3-aminofenilboronik asit film kaplı altın yüzey baskılı elektrotlar / cam karbon elektrot ve yeni organobor polimer film kaplı platin yüzey baskılı elektrotlar / cam karbon elektrot ile elektrokimyasal bir enzimatik biyosensör sistemi geliştirilmiştir.Bu tezde geliştirilecek olan organoboron polimer esaslı enzimatik ve elektrokimyasal analizler, kimya ve tarım endüstrisinde en çok analiz edilen fenolik bileşiklerden biri olan katekolün belirlenmesinde kullanılacaktır. Numune belirleme sistemi olarak tirozinaz enziminin kullanıldığı katekol analizi geliştirilmiştir. Geliştirilen biyosensör sistemi ile fenolik bileşenler, farklı elektrotlarla 1 µM ile 200 µM arasındaki doğrusal aralıkta test edildi. Biyosensör performans koşulları optimizasyonundan sonra, yüzde 3 ila yüzde 10 standart sapma sonuçları aralığında kontrollü katekol eklenmiş yeşil çay numuneleri için gerçek numune analizi yapılmıştır.Son olarak belirtilmesi gerekir ki geliştirilmiş olan biyosensör sistemi taşınabilir ve gerçek zamanlı tayine imkân veren son ürün olarak tasarlanıp ticarileştirme potansiyeline de sahiptir. Doğal gıdaların antioksidan ve antimikrobiyel aktivitelerinin belirlenmesi amacıyla tayini yapılan fenolik bileşikler bir yönüyle kalite kontrol analizi kapsamında yapılmaktadır ve geliştirilen organobor polimer temelli biyosensör sistemi daha hızlı, ucuz, hassas ve gerçek zamanlı testlere imkân verecektir.

Anahtar Kelimeler: Bor, organobor polimerler, iletken polimerler, polianilin, enzimatik biyosensörler, elektrokimya, fenolik bileşikler, katekol analizi

CONTENTS

NOMENCLATURE

Abbreviations

mL	Milliliter
µL	Microliter
M	Molar
mM	Millimolar
µM	Micromolar
mg/mL	Milligram/ milliliter
ºC	Degrees Celcius (also termed Centigrade)
g	Grams
h	Hours
I	Current
min	Minutes
T	Temperature
Conc.	Concentration

Acronyms

ACN	Acetonitrile
Ag	Silver
Au	Gold
Au SPE	Gold screen-printed electrode
C	Carbon
C SPE	Carbon screen-printed electrode
Pt	Platinum
Pt SPE	Platinum screen-printed electrode
PBS	Phosphate-buffered saline
CV	Cyclic voltammogram
CE	Counter electrode
DCM	Dichloromethane
FeCN	Hexacyanoferrate (II + III) / Hexacyanoferrate(redox)
$[Fe(CN)_6]^{3-}$	Ferricyanide/hexacyanoferrate (III)
$K_3[Fe(CN)_6]$	Potassium ferricyanide/ Potassium hexacyanoferrate(III)
$[Fe(CN)_6]^{4-}$	Ferrocyanide

$K_4[Fe(CN)_6]$	Potassium ferrocyanide
KCl	Potassium chloride
GA	Glutaraldehyde
GCE	Glassy carbon electrode
H_2O	Water
$HClO_4$	Perchloric acid
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
ITO	Indium-tin oxide
$LiClO_4$	Lithium perchlorate
NaF	Sodium fluoride
novel monomer	2-phenyl-1,3,2-dioxaborolane
PANI	Polyaniline
3-APBA	3-aminophenylboronic acid
PABA	Poly 3-aminophenylboronic acid/ poly (aniline boronic acid)
RE	Reference electrode
SEM	Scanning Electron Microscope
SPE	Screen-printed electrode
SPEs	Sc reen-printed electrodes
WE	Working electrode
TBTU	2- (1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
TBAHFP	Tetrabutylammonium hexafluorophosphate
Tyr	Tyrosinase

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CHAPTER 1

INTRODUCTION

Conductive polymers possess a high conductivity/weight ratio, unique chemical properties [1], well electrical and optical features, and permit perfect control of the electrical stimulus [2]. A conductive polymer's main feature is that there are conjugated (sequentially ordered) double bonds along the polymer's backbone (main chain). In conjugation, the bonds among carbon atoms are arranged in alternating single and double bonds. Each bond includes a strong chemical bond, "sigma" (σ). Moreover, each double bond has a weaker (30%) and less localized "pi" π bond. However, conjugation is not adequate to do the polymer material conductive, and the conductivity can be increased by introducing dopant materials into them. The task of dopant materials is to raise electrons' count and "holes" in the material. The location where there is an electron deficiency is called a hole. When such a hole is get fulled with an electron jumping from a neighboring location, a new hole is created, and as a result of this situation, the charge migrates over a long distance [3]. By doping with different agents, the physical, chemical, and electrical properties of conducting polymers can be changed [4], and by adding antibodies, enzymes, and other biological moieties, these properties can be adapted to the unique needs of their application [2], specifically for biosensor design. The electrochemical polymerization technique is one of the most common methods used to prepare conductive polymers. [5]. By implement an electrical current by way of electrodes placed into a solution including the polymer monomer, the solvent, and the doping agent, electrochemical polymerization occurs. The electrical current brings about the monomer to coat and oxidizes on the positively loaded working electrode, occurring irresoluble polymer chains. Electrochemical polymerization merely permits a polymer's synthesis if the monomer may undergo oxidation in potential electrical availability [2]. The electrochemical process leads to its location, thickness, or morphology controlled by applying current or voltage [4].

One of the most known conductive polymer types is Polyaniline (PANI). Owing to

the finding of its high conductivity and low cost, PANI has recently captured the scientific community's attention. Research workers are actively finding out its applications, involving those in biosensors, due to a range of beneficial properties like direct and quick sensor electrode coating, control of thickness, redox conductivity, and polyelectrolyte properties, high surface area, chemical characteristics, long-term environmental stability, tuneable features [6] and fast synthesis, low price, good environmental stability, and the capability to switch between its conductive and resistive states electrically [2]. PANI can serve as a significant mediator for electron transfer in redox or enzymatic reactions as a material for sensor and biosensor interfaces. PANI is considered an attractive polymer because it shows two redox couples to enable the transfer of enzyme-polymer charge in the right potential range and thus serves as a mediator of self-contained electron transfer [6]. The various techniques can be used on the PANI surface for the immobilization of intended biomolecules. Control over PANI's shape and dimensions may end up with desired physical and electrochemical features for biosensing application through varying synthesis or processing conditions [6]. Excellent electroactivity can be preserved up to pH 12 in the PANI's significant application zone [7].

The phenolic compounds are widely used in the chemical industry and agriculture and are discharged into the environment. The organism can quickly adsorb these chemicals through skins and mucous membranes. They accumulate in the body due to the difficult removal of phenolic compounds during metabolic processes. Due to these properties, the number of phenolic compounds in natural foods should be determined within quality control. In determining phenolic compounds (HPLC-High-Performance Liquid Chromatography, GMS-Gas Chromatography-Mass Spectrometry, etc.), traditional analytical methods are also used enzymatic methods using free and immobilized polyphenol oxidase class enzymes [8, 9, 10]. Electrochemical techniques are among the numerous known methods; due to their real-time identification of clinical samples, they are desirable for polyphenol determination [11]. Building a biosensor for low phenolic compound detection limits, high sensitivity, quick response, efficacy, and simplicity in the tyrosinase enzyme-based amperometric biosensor is a significant challenge [12]. Tyrosinase

(Tyr) is also known as polyphenol oxidase or catechol oxidase and has two atoms of copper at its active center [13]. In the existence of molecular oxygen, this essential enzyme catalyzes oxidation reactions, containing monophenols' hydroxylation into o-dihydroxy phenols and subsequently oxidation-dihydroxy phenols into o-quinones [14].

Biosensors are called compact analytical tools that integrate or combine a biologically or biologically derived sensor element with a physicochemical converter [15, 16]. (Turner, 1987; Turner, 2000). A biosensor aims to generate a perpetual digital electrical signal proportional to the quantity of one or a group of analytes. The enzyme-based glucose sensor created by Clark and Lyons is the first biosensor. Since then, in different laboratories worldwide, hundreds of biosensors have been developed [17]. Biosensors contain three basic components: a bioactive constituent that selectively interacts with the substance to be examined, a transmitter system that transmits the signal originating from this interaction, and a measurement system. These are "biomolecule, bioagent" with selective recognition mechanism, transducer and electronic parts that can convert physico-chemical signals resulting from this biogen's interaction with the substance under study into electronic signals. The most important of these components are sensitive biomolecules that interact with the substance to be determined in a highly selective but reversible manner [18].

Due to various favorable features, poly conjugated conducting polymers possess excellent attention to biosensing applications mentioned above. Primarily, PANI film synthesized from aniline aqueous solution with various acidic media such as HCl, H_2SO_4, and perchloric acids is eco-friendly, acceptable, and the enzymes are not denatured [19]. In this thesis, therefore, PANI has been used to increase the analytical efficiency of electrochemical biosensors. An electrochemical biosensor has been generated by simultaneous deposition of PANI and enzyme trapping by a one-step process. This study describes an electrochemical biosensor's manufacture and applying the resulting enzyme electrode to voltammetric detect catechol in an aqueous medium. In this thesis, utilizing the direct electropolymerization (one-step) method, an electrochemical enzymatic biosensor system was developed with PANI film-coated C SPEs, and PABA film-coated Au SPEs/GCE, and novel boron-

containing monomer film-coated Pt SPEs/GCE. Electropolymerization of PANI, PABA, and novel boron monomer was carried out through various electropolymerizatıon conditions. The developed biosensor was utilized to quantify catechol in green tea samples after optimization of biosensor performance conditions. Utilizing the portable potentiostat and the electrodes, this developed enzymatic biosensor system has potential for on-site analysis of catechol detection in real samples. Therefore, the study's future perspective could be developing a portable, easy-to-use prototype product for real sample testing.

1.1 Boron Element

Boron is stated as an element with the symbol "B" in the periodic table, atomic number 5, atomic weight 10.81, density 2.84 gr/cm^3, melting point 2,200 °C, and a boiling point 2,250 °C, with a semiconductor feature between metal and nonmetal. More than 200 boron derivatives are used for different purposes in around 250 uses [20, 21, 22]. Boron products are classified into three groups as raw boron, refined boron, and end products. Their usage areas are specified. Glass and glass fiber, detergent, textile, agriculture, metallurgy, and chemical industry are the leading areas among boron products. The world's boron reserves are estimated to be 1.2 billion tonnes of reserves, and 63% are located in Turkey. If we consider the production of boron remains the same rate, Turkey could afford world demand 400 years; It can be said that reserves in the USA and Russia can only last for 70 years. Considering these reserves are emerging as crucial as what Turkey in terms of world boron consumption. It should be noted that boron element has some fundamental features such as the existence of a vacant p-orbital, the Lewis acidity of boron, and the geometric feature of boron.

It should be noted that there are some basic characteristics of the boron element, like the presence of a vacant p-orbital, the Lewis acidity of boron, and the geometric feature of boron. Owing to the boron compound is a Lewis acid so that it can bind an anionic kind, leading to the shift in the π- conjugation mode [21, 23, 24].

1.2 Biosensors

1.2.1 General Principle of Biosensors and Metrological Characteristics

Sensors register a physical, chemical, or biological change and convert that into a measurable signal. The sensor contains a recognition element that enables the selective response to a particular analyte or a group of analytes, thus minimizing interferences from other sample components. Another main component of a sensor is the transducer or the detector device that produces a signal. A signal processor collects, amplifies, and displays the signal.

Sensors record a physical, chemical, biological alteration and convert into a measurable signal. The sensor includes a recognition element which permits the selective response to a specific analyte or a group of analytes, in this way, minimizing interferences from other specimen components as shown on Figure. The transducer or the detector device that fabricates a signal and A signal processor gathers, amplifies, and displays the signal [25, 26].

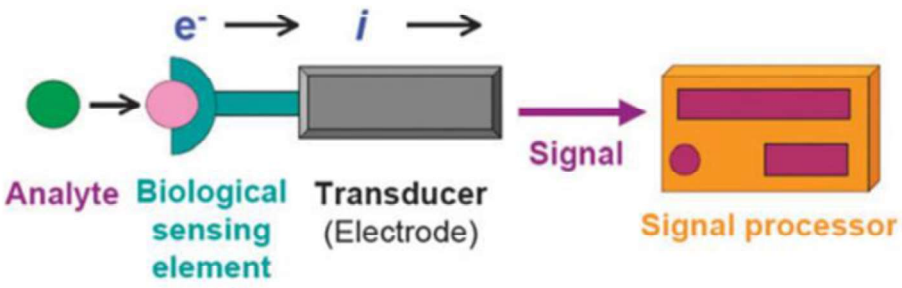

Figure 1.1 A schematic representation of a biosensor [26]

Especially, electrochemical biosensors, a subset of chemical sensors, integrate the sensitivity, as displayed by low detection limits, of electrochemical transducers with the elevated specificity of biological recognition processes. These tools include a biological recognition element (enzymes, proteins, antibodies, nucleic acids, cells, tissues or receptors) that selectively reacts with the target analyte and generates an electrical signal which is related to the concentration of the analyte. Electrochemical biosensors can be classified to two key categories based on the nature of the

biological recognition process (biocatalytic devices) and affinity sensors [27]. Biocatalytic devices include enzymes that identify the target analyte and produce electroactive species. Affinity sensors withstand a selective binding interaction between the analyte and a biological constituent like an antibody, nucleic acid, or a receptor [26].

1.2.2 Classification of Biosensors

In generally, a biosensor can be described as a tool consisting of a system of biological recognition, also named a bioreceptor, and a transducer.

For biosensors, there are several classifications that can be categorized according to their bioreceptor or type of transducer. A biological molecular species, such as an antibody, an enzyme, a protein, or a nucleic acid, is a bioreceptor. A bio-sensitive layer contains the sampling component of a biosensor that can either contain bioreceptors or be made of bioreceptors that are covalently linked to the transducer.The largest types of bioreceptors used in biosensing are focused on 1) antibody/antigen interactions, 2) nucleic acid interactions, 3) enzymatic interactions, 4) cellular interactions and 5) interactions using biomimetic materials in other words synthetic bioreceptors. Traditional techniques include, according to the category of transducers: 1) optical measurements 2) electrochemical and 3) mass-sensitive measurements [29].

Moreover, biosensors can be classified either by their biocatalytic or their bioaffinity categories. Enzymes, microorganisms, and tissue components that are involved in the catalytic activity of a particular biological reaction are biocatalytic sensors. Bioaffinity sensors rely on antibodies, receptors or binding proteins to molecularly recognize them [30].

1.2.2.1 Classification of Biosensors According to Transducer's Detection Type

Detection is basically divided into two as physical detection and chemical detection.

1.2.2.1.1 Physical Detection

In physical sensing, the signal formed by the bioreactor's reaction with the analyte is converted into electrical signals by the transducer due to the change of physical sizes such as potentiometry, amperometry, thermometry or photometry.

Potentiometric detection: Potentiometric measurement takes place by measuring the potential difference between two electrodes, one of which is a reference electrode and the other is a measurement electrode. The reference electrode has a constant voltage value independent of the environment in which is located. On the other hand, the measuring electrode has a voltage value that occurs due to oxidation and reduction reactions occurring at the solid and liquid interface. Potentiometric measurements can be carried out using different electrodes. Examples are glass pH electrodes used to measure hydrogen ion (H +) concentration. These electrodes can be coated with hydrophobic membranes and used to detect gases such as CO_2 or NH. Electrodes, which are sensitized to cations such as NH, Li ", Na, and K by being changed appropriately, can be used as biosensors by coating with enzymatic membranes [31].

Amperometric detection: Amperometric measurement is based on the principle of measuring the current density passing through the electrochemical cell under constant voltage. Here, two electrodes are used, the reference electrode and the measuring electrode. The oxidation or reduction reaction is carried out by the measuring electrode. During the electrolysis, the measuring electrode can treat as anode or cathode, depending on the structure of the analyte [31].

Thermometric detection: Optical, mechanical or electrical methods can be used to measure the temperature in thermometric sensing, where the enthalpy changes of the reactions between analyte and bioreceptor are measured. Since it is possible to obtain an electrical signal linearly with temperature change, the electrical method is accepted as the most useful method in the creation of thermal biosensors [31].

Piezoelectric detection: Piezoelectric sensing is based on measuring the change in the crystalline resonance frequency due to the mass increase caused by the analyte's

reaction with the bioreceptor on the piezoelectric crystal surface [31]. In this type of biosensors based on the principle of mass sensitive sensing, quartz (quartz) crystalline built-in beam (cantilever) is used [32, 33]. It is possible to detect even small changes in mass by using such devices. Piezoelectric based acoustic wave devices developed in this field are sensitive to changes in mass, density, viscosity, and acoustic force. Therefore, the resonance frequency series can be used as sensitive fit parameters [34].

Photometric detection: In the photometric detection method using optical fibers, bioreceptors are placed on optical fibers. It is based on the principle of measuring the change in luminous intensity due to absorption or irradiation resulting from the interaction between analyte and bioreceptor [34].

1.2.2.1.2 Chemical Detection

Some biosensors use conversion reactions or binding reactions to diagnose structures within the analyte. The enzyme used as a bioreceptor in transformation reactions turns the component to be analyzed into a product that can be detected by the transducer. An example of this is the conversion of the penicillinase enzyme into penicillin acid. In this structure, pH electrode is used for measurement later.

Association reactions are a particular detection method based on association reactions between antigens and antibodies. As a result of these reactions; the change in electrical charge and the change in mass or optical properties are diagnosed by the transducer types mentioned earlier [31].

1.2.2.2 Classification of Biosensors According to Bioreceptor Type

For biosensors to be created, it is necessary to choose a bioreceptor suitable for the structure and working environment to be analyzed. In a biosensor, the bioreceptor is designed to interact with a particular analyte of interest in order to produce an observable transducer impact. High analyte selectivity within a matrix of other chemical or biological components is a crucial requirement of the bioreceptor. Bioreceptors are grouped under five groups; 1. antibody/antigen, 2. enzymes, 3. nucleic acids / DNA, 4. Cellular structures / cells, 5. Biomimetic [29].

Antibody bioreceptors: Antibodies complex in protein structure, formed by connecting hundreds of amino acids in regular order. They are biomolecules. These

biological molecules show the ability to bind to special structures. The binding of an antigen to be diagnosed to an antibody used as a receptor can be perceived as a key lock mechanism. Just as a lock can be opened with a matching key, an antigen in the analyte can only be attached to an antibody that is suitable for its structure. It is possible to analyze special analytes with immunosensors (Figure 1.2) designed using these features. Nanometer-sized fiber optic sensors have been developed to make measurements in a single cell with the latest developments in biosensors designed with the use of antibody bioreceptors and nanotechnology [29].

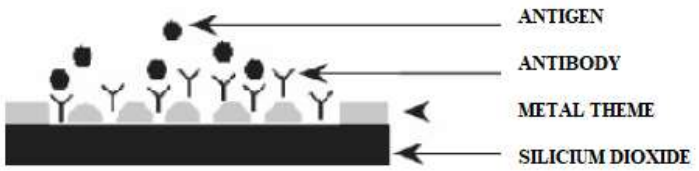

Figure 1.2 Immunosensor [35]

Enzyme bioreceptors: Enzymes are widely used as bioreceptors that stand out with their catalytic properties as well as their ability to bind to the sensor. In the biocatalytic diagnostic mechanism, detection occurs by the biocatalyst catalyzing the reaction. The catalytic effect provided by enzymes allows biosensors to measure between lower limits. The catalytic activities of enzymes depend on the formal integrity of natural proteins [29].

Nucleic acid bioreceptors: Nucleic acid sensors can analyze DNA, RNA, fragments, or molecules carrying them. DNA biosensors used in gene analysis are based on the complementary properties of bases (adenine = thymine, guanine = cytosine) in the structure of DNA [29] and reversible hybridization / dehybridization mechanism. They have been developed [36]. If the base sequence that makes up a certain part of the DNA molecule is identified, then it is possible to synthesize and mark the counter sequence that will accompany this sequence, called the probe, with an optically detectable compound. DNA double helix structure is separated by heat or chemicals and placed on the probe after labeling. Then, the probe placed in the analyte goes into hybridization reaction with the target molecules and forms the double helix

structure. The structure is then analyzed by an optical microscope or radiation [37].

Cellular bioreceptors: Cellular structures and cells have been highly effective components in the development of biosensors. It is quite easy for such receptors to bind to the electrode. These receptors, which are very effective in diagnosing a cell, microorganism, or a special cellular component, are divided into three main subgroups: cellular systems, enzymes, and non-enzymatic proteins. The use of such receptors narrows the measurement limits due to signal amplification. Since cell organelles are closed systems, they provide the advantage of being used for a long time. Tissues of all mammals, mammalian animal cells, and vegetable tissues obtained in vitro culture can be used as bioreceptors [29].

Biomimetic receptors: Artificially produced biomimetic receptors by the sampling of bioreceptors are obtained by genetically engineered molecules, production of artificial membranes, and molecular suppression methods [29]. Molecular suppression; is expressed as the formation of solid materials with chemical function by arranging functional monomers around a mold molecule by covalent or non-covalent interactions [38]. One of the most significant benefits of the molecular suppression technique is that the polymer structure is much more robust and durable compared to the biological receptor. Therefore, biomimetic receptors are preferred, especially in environments where bioreceptors are not suitable for use. However, due to the rigid structure of polymeric molecules, their flexibility and selectivity are weaker than biomolecules [29].

Many of the biological molecules used as bioreceptors are nondurable in the solution phase. Therefore, these components must be placed in a suitable matrix. To exemplify, the immobilization of enzymes against environmental conditions that decrease their activities prolongs their life. Therefore, the receptor component of the biosensors (enzyme, multiple enzyme systems, antibody, microorganisms, organelle, etc.) are immobilized to a membrane or gel [39, 40]. The activities of immobilized molecules depend on different parameters, such as surface area, porosity, hydrophilic character of the immobilization matrix, reaction conditions, and the method chosen for immobilization. The immobilization of the receptors on the transducer can be performed by physical (adsorption, arrest with polymer matrix, etc.) or chemical

(covalent binding, cross-linking with multi-functional reagents, etc.) methods [41]. Considering these reasons, it is necessary to use molecules in the immobilization step that ensure the most effective protection of enzyme activity in the preparation stages of the enzyme biosensor. Concordantly, the use of biocompatible molecules as immobilization molecules would add value to the biosensor system in many ways. Enzyme immobilization in polymeric molecules with high biocompatibility allows them to be immobilized by protecting their natural structure without being exposed to covalent bonding and cross-linking.

1.2.3 Electrochemical Biosensors

Electrochemical sensors are component of an electrochemical cell which occurs either three electrodes or two electrodes and a normal three electrode electrochemical cell occurs three parts which are a working electrode; a reference electrode, and a platinum wire auxiliary electrode [42]. After a recognition feature on the surface of the biosensor is contacted by the analyte, physical or chemical shifts create a reaction that is it has been translated into an electrochemical signal. To assess the concentration of the pathogen and the changes in the structure of the analyte, this knowledge can be further analyzed. A schematic illustration of the electrochemical biosensor as shown in Figure 1.3 [43]. Electrochemical biosensors are divided into four different classes which are amperometric biosensors, potentiometric biosensors, impedance biosensors and voltmetric biosensors [44].

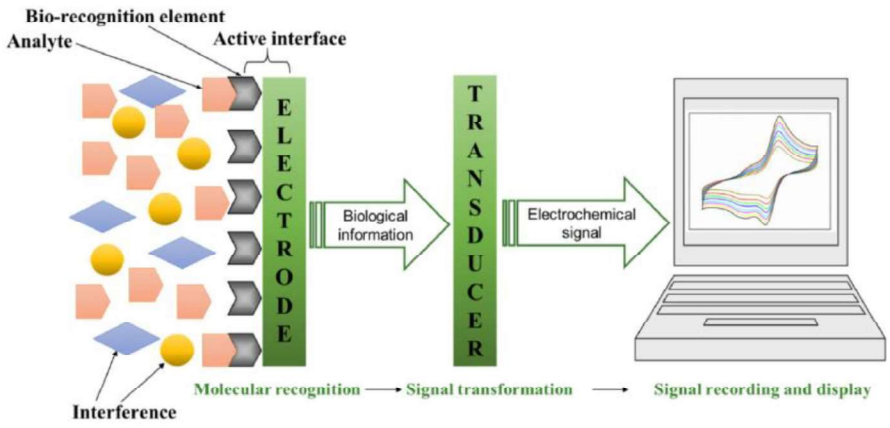

Figure 1.3 Electrochemical biosensor [43]

1.2.3.1 Amperometric Biosensors

For the electrochemical electrode coated with biologically active material, amperometric biosensors test the concentration-dependent current.

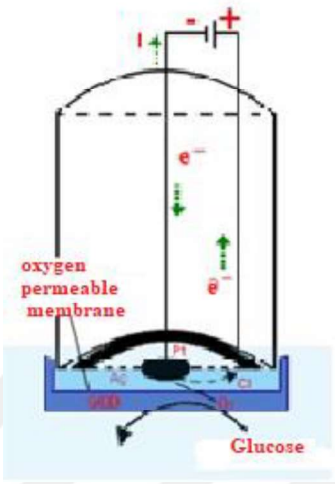

Figure 1.4 Representation for the amperometric biosensor system

This biosensor rule is based on the flow rate of the current generated by applying a voltage between the working electrode and the counter electrode. There is a wide range of uses, such as choosing analyte centers, high-throughput medical screening, quality control, problem finding and processing, and biological control and amperometric biosensor as shown in Figure1.4 [44].

1.2.3.2 Potentiometric Biosensors

Utilizing ion-selective electrodes, shifts in ionic concentrations are measured in such biosensors. Enzymes' sensitivity to ionic concentrations such as H^+ and NH^{4+} is the most significant drawback of potentiometric biosensors.

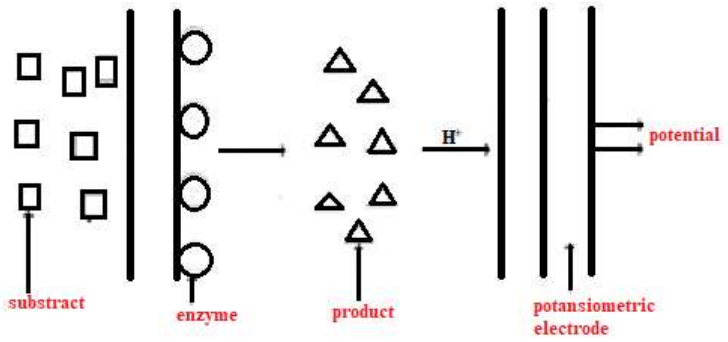

Figure 1.5 Schematic representation for the potentiometric biosensor system

The potential difference can be determined between the potentiometric electrode and the reference electrode, and this value is proportional to the substrate concentration and potentiometric biosensor as shown in Figure 1.5 [44].

1.2.3.3 Impedance Biosensors

Electrochemical impedance spectroscopy is a sensitive indicator of a wide range of physical and chemical properties. There is currently an increasing trend toward the use of impedance biosensors. Impedance techniques have been carried out to separate the invention of biosensors and to study the responses of enzymes, lactines, nucleic acids, receptors, and antibodies and impedance biosensor as shown in Figure 1.7 [44].

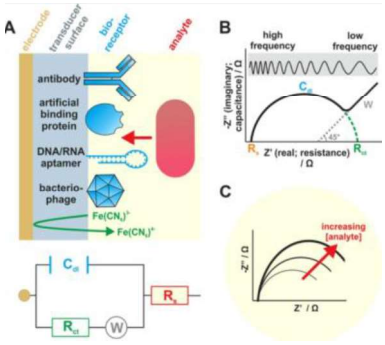

Figure 1.6 Representation for the impedance biosensor system. (A) Layer-by-layer sensor construction typically comprises an electrode surface (B) Nyquist plot showing the features of the Randles circuit. (C) Impedance changes resulting from analyte-surface interactions are proportional to analyte concentration

1.2.3.4 Voltammetric Biosensors

This biosensor is built with a carbon glue electrode adapted with Hb (hemoglobin). This type of electrode indicates the oxidation or reduction procedure that can be reversed. A voltammetric sensor can operate in other linear or cyclic voltammetric modes. As a result, the current and voltage will be different for each mode and this type as shown in Figure 1.7 [44].

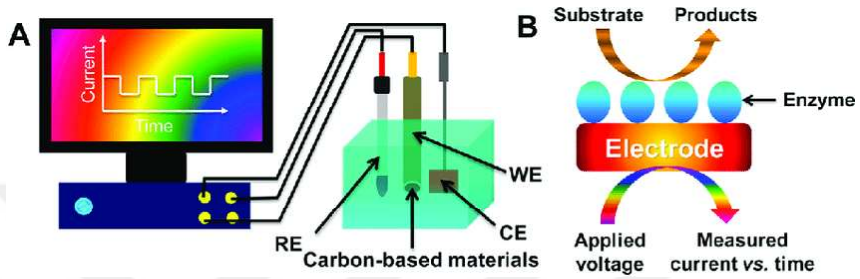

Figure 1.7 (A) Standard carbon nanomaterial voltammetric biosensor schematics consisting of the reference electrode (RE), carbon-based working electrode (WE), and counter electrode (CE). (B) The fundamental theory of biosensor voltammetry

1.3 Conductive Polymers

Conductive polymers are among the most frequently studied materials in recent years. Rechargeable batteries, optical devices, electrolytic capacitors, solar cells, sensors, and biosensor studies are the best known among the application areas of these materials. Because conductive polymers are easy to accumulate electrochemically, they allow a sensitive layer to form on the electrode surface. These properties make it preferable to use conductive polymers as an electron transmission mechanism (transducer) in biosensors. These molecularly electronic materials make it possible to control different parameters such as the thickness of the polymer layer, electrical properties and the ability to attach bioactive molecules to the structure. As well as conductive polymer-based biosensors meet the features needed for an ideal biosensor due to their biocompatibility, intracellular application, control of the release of drugs or their metabolites, and miniaturization. Organoboron based conductive polymers are highly suitable surface modification agents for the

proposed project thanks to the advantages of both organoboron synthesis and their compatibility with biosensors applications.

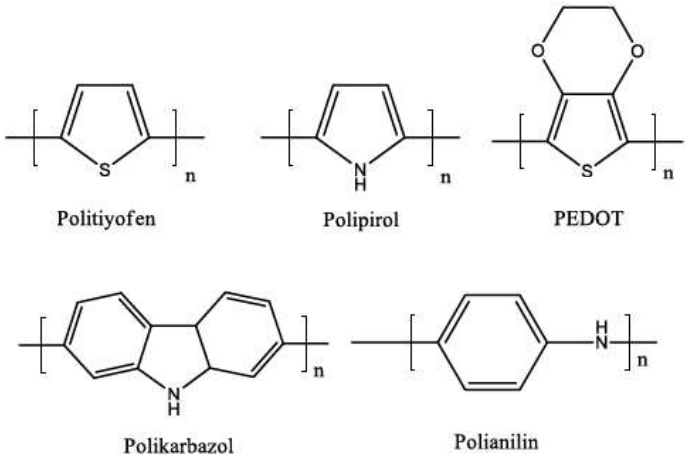

Figure 1.8 Structures of various conductive polymers in the literature [5]

The most known conductive polymer types are shown in Figure 1.8. Electrochemical polymerization technique is one of the most common methods used to prepare conductive polymers [5].

Electrochemical polymerization is performed in a three-electrode cell in a suitable solvent containing the monomer and support electrolyte. Electrochemical synthesis is performed in a cell consisting of a working, reference and counter electrode. As the working electrode, gold, platinum, titanium, nickel, indium doped-tin oxide coated glass (ITO), glassy carbon, carbon, etc. materials are used and electropolymerization is realized on the surface of these electrodes. Conductive polymers are used as a suitable immobilization medium for biological species. Due to the redox properties of these materials, their use has become very common in recent years [3].

The main feature of a conductive polymer is that there are conjugated (sequentially ordered) double bonds along the backbone (main chain) of the polymer.

One of the most known conductive polymer types is Polyaniline (PANI). As a material for sensor and biosensor interfaces, PANI can serve as an effective mediator

in redox or enzymatic reactions in order to pass electrons. PANI is considered a valuable polymer since it showcases two redox pairs in the right range of potential to facilitate enzyme-polymer charge transfer and thus acts as a mediator of self-contained electron transfer [6]. Different techniques for the immobilization of desirable biomolecules may be used on the PANI surface. Moreover, control over the form and dimensions of PANI is probable to lead to desired physical and electrochemical features via different synthesis or processing conditions for the application of biosensing [6]. The electropolymerization of PANI is illustrated in Figure 1.9 [45].

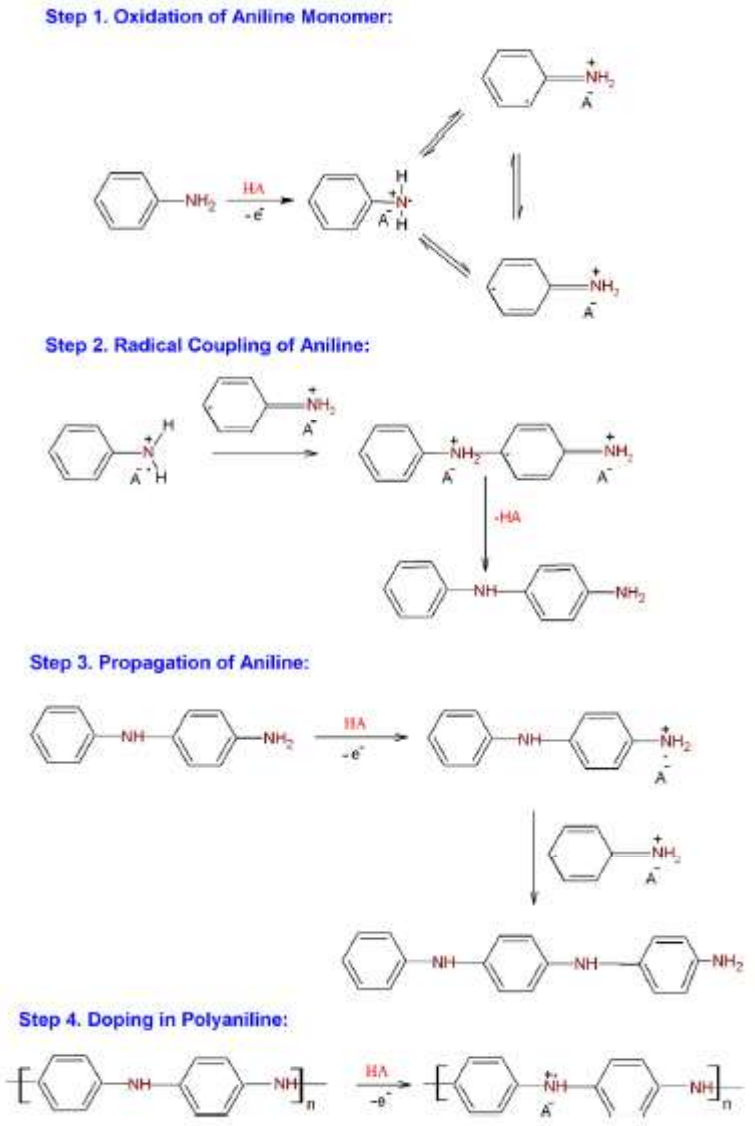

Figure 1.9 Electropolymerization mechanism of PANI [45]

1.4 Hybrid Conductive Polymers

In both conducting polymers (CPs) and organic / inorganic nanoparticles, hybrid conducting polymers (HCPs) possess unusual properties that have drawn a great deal of interest from scientists and researchers. HCP manufacturing, like weak processability, solubility, durability and low yield, has helped to overcome the drawbacks of CPs. By using one of two processes, chemical polymerization and electrochemical polymerization, HCPs can be generated. The various uses of hybrid conducting polymers are exhibited in Figure 1.10 [46].

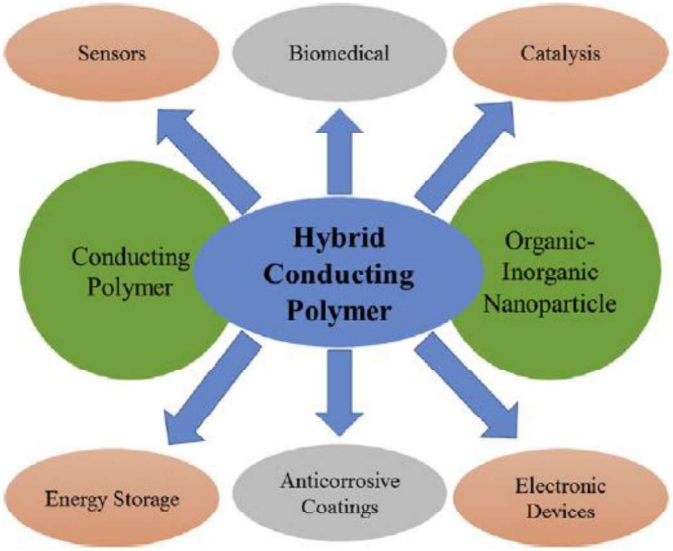

Figure 1.10 Applications of hybrid conducting polymers [46]

Literature research revealed that owing to their perfect electro-catalytic features, investigators are synthesizing HCPs in order to use them in biosensors. HCPs permit charge transfer and produce electrochemical signals between the electrode and the immobilized biomolecules [46].

1.5 Organoboron Based Polymers

Organoboron based polymers are among the highly biocompatible polymeric materials that are molecules that can serve the purpose of the proposed enzymatic biosensor system. Organoboron compounds and especially organoboranes such as

their stability to water and air, their ability to react with functional groups, the high stereoselectivity of their reactions and the environmentally friendly boric acid of the by-product [47, 48]. Organoboranes obtained by the addition of BH_3 to alkenes and alkynes by H.Brown are compounds containing C-B bonds and have been used mostly in organic synthesis to date [49]. Although its applications have not been tested in many other fields, there are promising studies. Organoboron polymers, which are the result of combining organoboron compounds with polymeric materials, are essential molecules that need to be studied due to the extra benefits they can add to the mentioned application areas. Organoboron polymers also have known optical and fluorescent properties, as well as lesser known properties such as conductivity and sensor signal amplification in electrochemical applications [48]. By combining these advantages that organoboron polymers provide to electrochemical systems and the biocompatibility features possible in the enzyme immobilization step, the necessary requirements for electrochemical enzyme biosensors will be completed.

As catalysts and cocatalysts, organoboron compounds play a significant role in organic conversion, involving polymerization reactions. The synthesis of organoboron polymers, however, has been a difficult task which has solely recently been successfully accomplished, leading to the discovery of new assisted reagents and immobilized catalysts. In synthesizing functionalized polymers with polar side groups, boron-containing polymers often act as intermediates and are used as preceramic and photoluminescent materials [50-54].

The incorporation into polymer structures of electron-deficient boron centers is especially interesting as it provides, for example, an oppurtinity to manipulate the polymers by donor acceptor bonding. For the design of new assisted reagents and immobilised catalysts and of highly selective sensor materials, the attachment of nucleophiles to organoboron polymers can be exploited [55].

Organoboron polymers are flexible and high-performance platforms for realizing functional materials with multifunctions. The majority of boron compounds have adequate stability to be handled under ordinary conditions and the complexes of organoborons have flexibility in their molecular structure [56].

The attitude of organoboranes as Lewis acids is a consequence of the empty p-orbital of tricoordinate boron. Either through π-overlap with an appropriate substituent X or through the constitution of Lewis acid-Lewis base complexes, Boron can achieve the necessary octet configuration as shown in Figure 1.11 and these interactions was utilized in a lot of the applications of organoboron compounds. Furthermore, in the area of sensor materials, the inclusion of Lewis acidic organoboron moieties into conjugated polymers was indicated to cause to sensor signal amplification influences such as improved stability and recoverability [47].

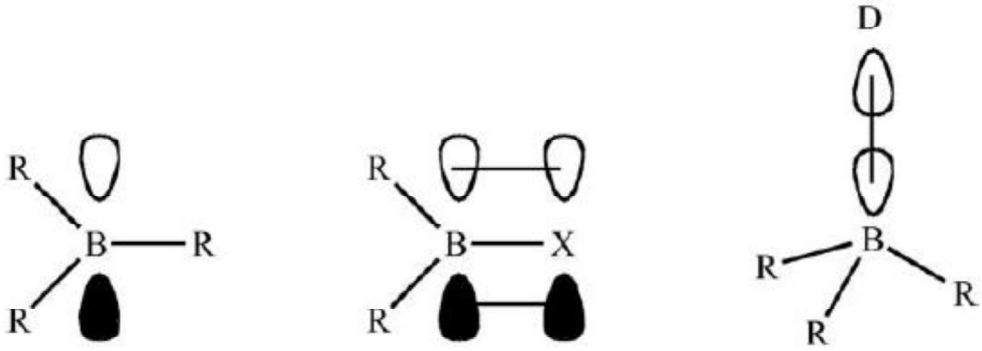

Figure 1.11 Stabilization of tri coordinate organoboranes through π -bonding or formation of Lewis acid–Lewis base complexes [47]

In generally, organoboron compounds are recognized as further versatile reagents or reaction intermediates for readying of an extensive diversity of functional compounds [57].

Boron-containing conjugated polymers, typical Lewis acid boron centers and very wealthy physical features like high specific surface and intramolecular charge transfer, have emerged as one of the most promising functional materials for selective sensors, catalysts, etc [58].

The production of organoboron sensor materials has been accelerated by the ability of boronic acid groups to combine with electron donor or withdrawal groups [59].

1.6 Review of Related Works

Nowdays, conductive polymers and organoboron based polymers by electropolymerization has recently captured the scientific community's attention and they are also studied by many researchers in recent years. Some of the studies in the literature are given below.

Firstly, an experimental study has been established by Wang et.al, and they investigated production, characterization and analytical performance for a phenol biosensor based on the covalent bonding of tyrosinase (TYR) onto a graphene oxide (GO)- modified glassy carbon electrode (GCE) via glutaraldehyde (GA) and a highly sensitive electrochemical biosensor was fabricated and this manufactured TYR/GA/GO/GCE biosensor has numerous benefits, involving a simple manufacturing protocol, perfect response rate efficiency, fast answer time, good stability, reproducibility, and sensitivity [60].

Sethuraman et al. examined the manufacture of effective biosensor polyaniline - polyphenol oxidase for catechol. The electroactivity of catechol on the formed biosensor is revealed by cyclic voltammetric studies. This research shows that to build the polyaniline, tyrosinase-based catechol biosensor, the optimum reaction condition is studied, and better response, sensitivity, and greater stability are observed with a relatively small enzyme have good performance and the developed biosensor was used to quantify catechol in green tea sample. The performance of biosensors and the integration of various polymer-based materials into cationic and anionic surfactants are currently in development [61].

In another study, various parameters such as solution pH, temperature, and electrode composition were amperometrically investigated in order to achieve optimal conditions for the enzyme electrode by Sdeghi et al. In the optimized state, the determination of the catechol content in tea samples was carried out using the established biosensor. The measurements were performed by interpolating the corresponding amperometric signals into catechol-based calibration plots. The benefits of the biosensor included excellent linearity, strong selectivity, and acknowledged long-term stability. The biosensor built for the determination of

catechol in tea samples satisfactorily [62].

Chen at al. reported that by immobilizing polyphenol oxidase (PPO) into polyaniline (PANI) film utilizing the direct electropolymerization (one-step) method in conjunction with cross-linking with glutaraldehyde, a highly stable and efficient catechol biosensor was created and cyclic voltammetry (CV) affirmed that the immobilization of PPO was successful and the biosensor showed good stability. The new catechol biosensor was simple to design, low price, stable and possess a good detection range of rapid response time, which could potentially be useful as a reliable technique for detecting catechol [63].

Based on tyrosinase (Tyr), single-wall carbon nanotubes (SWCNTs) and polyaniline, a sensitive biosensor was manufactured to determine phenolic compounds (PANI) by Wang at al. Results show that the biosensor has good sensitivity, repeatability and stability [64].

In another research, for aniline polymerization, various protonic acid was utilized as a dopant and supporting electrolyte by Hassan et al. the findings show that H_3PO_4 is a "weak" electrolyte supporting polymerization of aniline, while H_2SO_4 given the higher polymerization charge and higher current acid between the acids used on the CCG surface for electropolymerization of aniline. Whether in their polymerization charge or in the values of peak currents in monomer-free solutions, both HCl and HNO_3 produced very similar results. Also, by observing the impact of polymerization potential and polymerization time, optimization of the electropolymerization of PANI on the surface of CCG was accomplished. (Chemically converted graphene (CCG); Polyaniline (PANI)) [65].

In their study, Campanhã et al. investigated a biosensor based on gold nanoparticles, dihexadecylphosphate, and tyrosinase for the detection of catechol in natural water. As a result, the developed biosensor exhibited good repeatability and stability. There are benefits to the changed electrode with gold nanoparticles (AuNPs), containing biocompatibility with the Tyr enzyme and increasing the analytical signal for catechol. Utilizing the suggested biosensor, which can be applied in samples, amperometry was applied for catechol detection [66].

In their study, N. Nikitinaa, et. al. examined molecular imprinting of boronate functionalized polyaniline for enzyme-free selective detection of saccharides and hydroxy acids. When the results were examined, in the process of 3-aminophenylboronic acid (3-APBA) electropolymerization, imprinting with hydroxy acids results in an increase in conductive boronate functionalized polyaniline generating conductivity due to precise binding, allowing the specific signal to be discriminated against by non-specific interactions. They showed the enzyme-free selective detection of saccharides and hydroxy acids through conductivity improvement of the conductive polymer tuning binding features of boronate functionalized polyaniline [67].

In another study, Andreyev et al. have already stated that during the binding with polyols, boronate-substituted polyaniline can produce an increase in its conductivity [68].

Polyaniline production is reported to occur by electrophilic para-substitution in the comparatively amino group [69]. Polymerization, especially in the meta-position to-NH_2 [70], is favored by electron donor substituents in line with this. If the boronic acid residue, a low electron acceptor, produces a hydroxy acid complex in weakly acidic solutions, it is converted into an electron donor group because of the charge assigned to the boron atom [71, 72].

Badhulika et al. carried out a study on Poly (3-aminophenylboronic acid) functionalized carbon nanotubes based chemiresistive sensors for specification of sugars. Their studies included the electrochemical polymerization of 3-amino phenyl boronic acid (APBA) on the surface of single walled carbon nanotubes (SWNTs) in the presence of fluoride. A simple, cost-effective and enzyme-free approach has been improved to detect saccharides in the solution by utilizing a Polyaniline boronic acid (PABA) deposited SWNT sensor [73].

By reversible doping and undoping by means of chemical modification of the polymer backbone via its well-behaved electrochemistry, the capability to tune PANI's electrical features makes it an ideal sensing material. The synthesis of functionalized polyaniline has been reported in various literature papers [74, 75].

Shoji et.al stated that increasing sodium fluoride concentration results in important negative shifts in oxidation potential, therefore increasing polymerization ratios with the polymer film resulting in better stability and adhesion characteristics [76]. This high fluoride concentration led to sustained polymerization and substantial and persistent growth of the polymer. A self-doped polymer, as well as the formation of a tetrahedral anionic boronate species, was therefore formed on the surface of SWNT [77].

Complexation of saccharides (along with alkyl and aromatic diols) with aromatic boronic acids create a stable boronate anion and a proton in the 6-10 pH range, thus opening up probabilities for various electrochemical approaches to detect sugars based on interactions with saccharideboronic acid functionality [78, 79].

In another study, Andreyev et al. report on the novel concept of reagentless and label-free detection based on conductive polymers, taking into account sensors for polyols, especially saccharides and hydroxy acids. The mechanism for reducing resistance is polyaniline self-doping, that is the appearance of the negatively charged aromatic ring substituents in the polymer chain as an alternative to proton doping. In fact, the "freezing" negative charge at the boron atom is a consequence of complex formation with di- and polyols, particular binding. Following the addition of glucose, boronate-substituted polyaniline is similar to that caused by proton polymer doping [80].

It is probable to utilize the unprecented qualities of conducting polymers to build reagentless sensors. Indeed, through electropolymerization of 3-aminophenylboronic acid, boronate-substituted polyaniline was manufactured [81].

In their study, James et al reported that thanks to its high affinity, simplicity, and easiness of miniaturization, electrochemical sensing based on the bonding of boronic acid and cis-diol is a suitable choice. A traditional material for analytical analysis, poly (aniline boronic acid) (PABA), can be electrochemically polymerized from 3-amino phenyl boronic acid (APBA). It involves functional boronic acid groups that have been fine known by reversible boronic acid-diol esterification as a distinctive affinity ligand for diol-containing substances [82].

In another study, Li et al. stated that in order to create five or six-membered cyclic complexes, boronic acids can bind with 1,2- or 1,3- diols and can also interact with Lewis bases to produce boronate anions. Boronic acid, hence, has functionalized compounds. They addressed the recent development of boronic acid-based electrochemical sensors in the detection of biological analytes in both solution processes and surface processes [83].

Senel and coworkers studied on Polymer-Bound Boronic Acids. In this study, a polymer based on aniline boronic acid, which was created by electro-co-polymerization of aniline and aniline boronic acid, was developed in order to use in glucose detection. Electro-polymerization of aniline boronic acid carried out on the glassy carbon electrode [84].

1.7 Aim of the Study

The purpose of this thesis is to use organoboron-based polymers as immobilization material in enzymatic biosensors for the determination of phenolic components and to examine their effects on parameters affecting biosensor performance. In addition to the advantages of electrochemical test systems such as low-cost, portable use, and fast response, a more sensitive and selective biosensor system has been designed using organoboron polymers, known for their biocompatibility, in the enzyme immobilization step, allowing use with longer-term stability.

Organoboron components to be used as immobilization materials within the project's scope were explicitly synthesized by the BOREN (National Boron Research Institute) and given to be used in this study. First of all, the determined enzyme was immobilized on the electrode surface with organoboron polymers, and enzymatic based electrochemical phenolic compound determination was performed using this prepared organoboron polymer + enzyme surface. Some parameters affecting the biosensor performance (enzyme immobilization, pH, temperature, substrate concentration, interference effect of organic solvents and other similar compounds, etc.) were investigated, the stability of enzyme electrodes, and the usefulness of organoboron polymers to the biosensor system were examined. One or more organoboron components with different structures were tested, and the component

that could be used most effectively within the scope of the electrochemical-enzymatic biosensor system was determined.

By including some groups in conductive polymers with functional organic structures, especially in the molecule's design phase, the properties of the product obtained can be improved by addressing different purposes. More efficient polymers were synthesized and used in the biosensor application phase, thanks to organoboron polymer synthesis reactions, which have advantages such as high yield, lack of by-products, high tolerance to functional groups, and simple product isolation. With the advantageous synthesis properties of organoboron polymers and the superior properties of polymers related to their use in electrochemical systems, the new modified surfaces that will be obtained will be used in the fields of materials science and biotechnology.

Analysis of phenolic compounds (eg, catechol) for which tyrosinase enzyme was used as the sample detection system was developed. The phenolic component in natural foods was tested in the linear range of 10-80 μM to 5-60 μM with similar systems (Zoral and Turgay 2014) [85]. Considering these references, it is aimed to make determinations at similar intervals with the proposed biosensor system. Therefore, in the last step of the thesis, diluted and controlled catechol added food samples were analyzed with at least a margin of error. The developed biosensor system's responses with the catechol determination to be made with conventional analytical devices will be compared, and the accuracy of the organoboron-based system was determined. Finally, it should be noted that the developed biosensor system has the potential to be designed and commercialized as a portable end product that allows real-time detection.

1.8 Original Contribution

Novel organoboron monomer, which was synthesized by BOREN for the first time within the proposed project's scope, were used in enzymatic biosensor systems. Therefore, it will be a pioneering work and will contribute scientifically by making a patent application. The contribution of these specific organoboron polymers to the electrochemical enzymatic biosensor for the determination of phenolic components

was examined, and analyses were made with the highest sensitivity under optimum conditions. The biosensor parameters were developed from the important results obtained. The developed biosensor was used to quantify catechol in green tea samples after optimization of biosensor performance conditions. It should not be forgotten that using the portable potentiostat and these electrodes, this developed enzymatic biosensor system has the potential for on-site analysis of catechol detection in real samples. Thus, the study's future perspective could be developing a portable, easy-to-use prototype product for real sample testing.

CHAPTER 2

EXPERIMENTAL

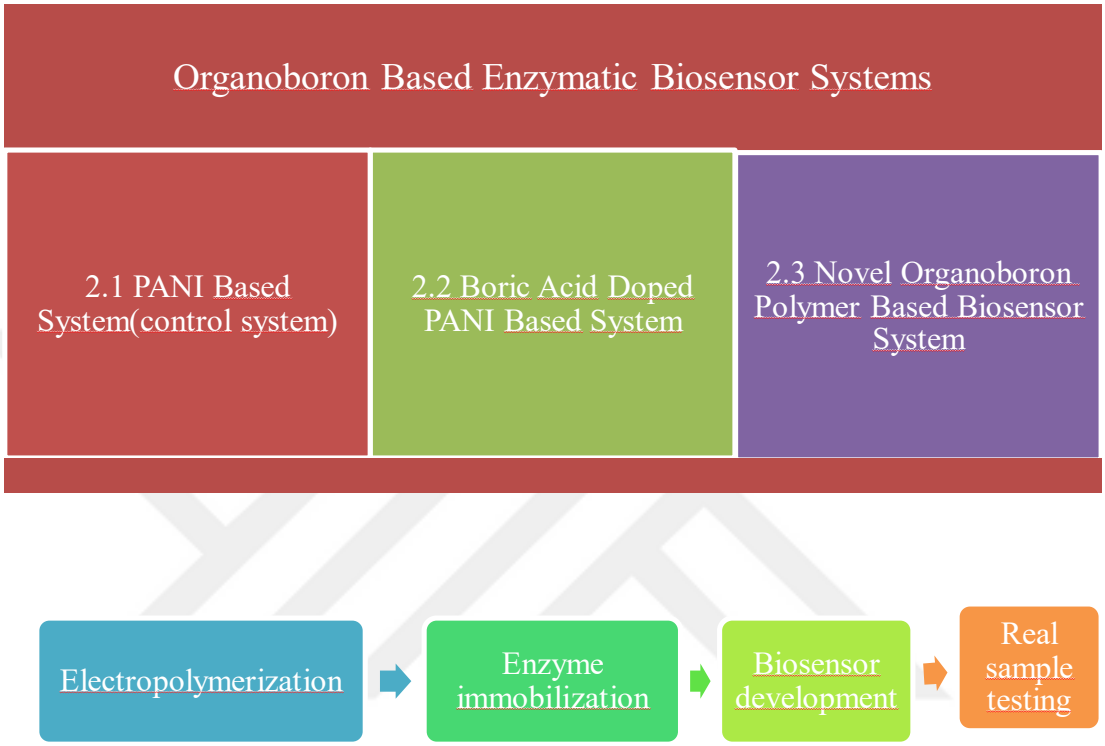

Figure 2.1 The flow chart for the experimental steps of the thesis

This study involved three different study which are PANI based system, boric acid doped PANI based system and Novel organoboron polymer based biosensor system. Each of them has some steps which are electropolymerization, enzyme immobilization, biosensor development, and real sample testing as shown in Figure 2.1.

2.1 PANI Based Electrochemical Enzymatic Biosensor System

2.1.1 Reagents

Tyrosinase from mushroom (Tyr, EC:1.14.18.1), catechol, aniline, sulfuric acid (H_2SO_4), hydrochloric acid (HCl), perchloric acid ($HClO_4$), and glutaraldehyde were purchased from Sigma Aldrich. Aniline was purified under vacuum before use. Phosphate buffer was prepared using di-potassium hydrogen phosphate and potassium dihydrogen phosphate. All the chemicals were used under the laboratory-grade. MilliQ TKA-Lab pure water used for the wet process.

2.1.2 Electropolymerization of PANI

The electrochemical measurements were recorded using screen printed electrodes that the SPE three-electrode system consisted of a carbon plate as the counter electrode(CE), carbon plate as the working electrode(WE), and Ag/AgCl as the reference electrode(RE). Electrochemical measurements were carried out using a potentiostat controlled by IviumSoft, software for control and data acquisition. Figure 2.2 represents the SPE and potentiostat setup used in this work.

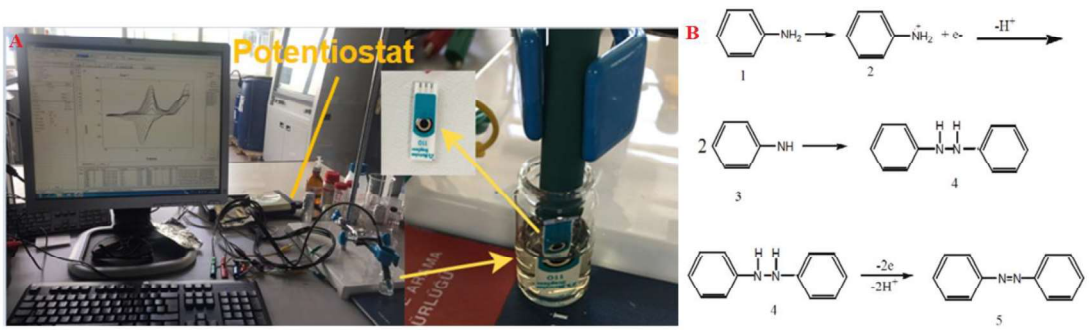

Figure 2.2 A) The illustration of the SPE and potentiostat setup that used in this work, B) Aniline electropolymerization mechanism

Electrochemical synthesis is an alternative way to obtain conductive polymers, making the synthetic procedure relatively straightforward. In this work, electropolymerization was carried out. The electrochemical method's process of obtaining conductive polymers is based on obtaining the Polyaniline (PANI) on the C SPE was immersed in an aqueous solution containing 0.1 M aniline monomer and

H_2SO_4, or $HClO_4$ or HCl. In the experiment, the screen printed electrodes (SPE) are scanned within the potential range of −0.5 V to 0.8 V in 0.5 M freshly prepared H_2SO_4 solution, 0.4 V to 0.8 V in 0.5 M $HClO_4$ solution, and -0.2 V to 1.0 V in 0.5 M HCl solution, respectively until stable curves of cyclic voltammograms are obtained. Cyclic voltammograms (CVs) were recorded in these different potential ranges in different acidic solutions at a scan rate of 100 mV s^{-1}. The current is passed through the solution, and the polymer begins to accumulate on the positively charged working electrode. During the oxidation process to form radical cations that react with other monomers, monomers on the working electrode surface form insoluble polymer chains on the electrode surface. After the aniline's electropolymerization, these PANI modified electrodes were washed with H_2SO_4 and distilled water, respectively. The surface activity and conductivity of the electrode are ascertained with $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ system and were carried out -0.2 V to 0.8 V in 5 mM $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ containing 0.1 M KCl solution.

2.1.3 Surface Characterization

The surface characterization of the polyaniline modified C-SPEs based on different acidic solutions was evaluated by scanning electron microscope.

2.1.4 Biosensor Preparation and Optimization of the Performance Conditions

After the polyaniline modification of the SPEs, enzyme immobilization was performed using glutaraldehyde crosslinking agents as support for Tyr immobilization. For enzyme immobilization, a mixture of 50 µl from 1 mg / mL Tyr in 50mM phosphate buffer solution (at pH 7.0) and 4 µl glutaraldehyde was prepared. Afterward, 8 µL of mixture dispersion was cast onto the electrodes' surface so that the solvent was allowed to evaporate at room temperature for approximately 1.5 hours.

A highly stable and effective catechol biosensor was prepared by immobilizing tyrosinase (Tyr) enzyme into PANI film in conjunction with cross-linking with glutaraldehyde so that an electrochemical enzymatic biosensor system was developed by using the direct electropolymerization (one-step) process. In Figure 2.3, a diagram

shows the tyrosinase enzyme's working mechanism and the electrochemical activity of the final product formed by the catechol component's enzymatic reaction. The ultimate product of catechol, o-quinone, is an electroactive molecule with electrochemical oxidation and reduction activity. In this mechanism, the enzyme played a key role. An immobilization method in which the enzyme activity was kept at maximum adds value to the biosensor system to be developed.

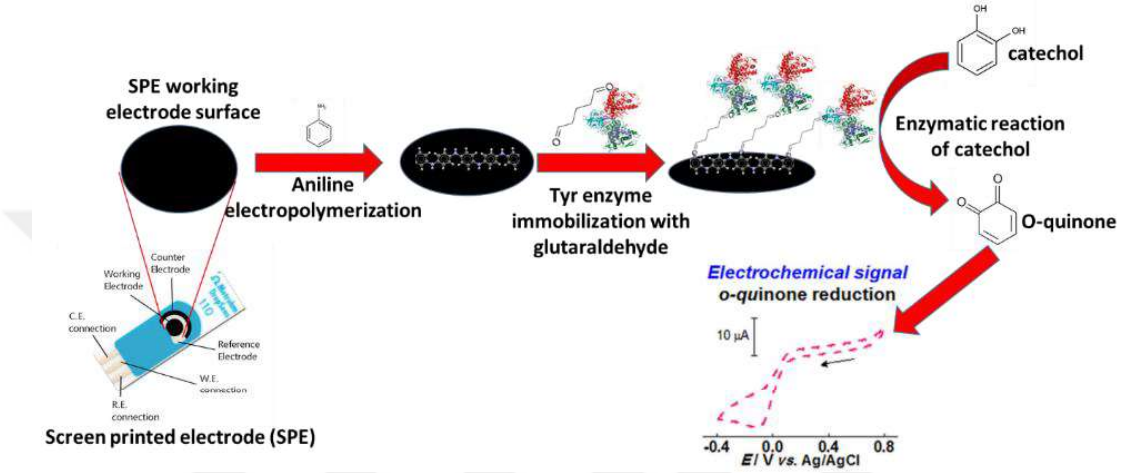

Figure 2.3 The diagram shows the tyrosinase enzyme's working mechanism and the electrochemical activity of the final product formed by the enzymatic reaction of the catechol

The biosensor was employed to determine catechol by voltammetric measurements at the applied potential of -0.9 V to 0.8 V in the steady-state condition in different amounts of catechol 10 µM, 25 µM, 50 µM, 100 µM, 200 µM, 300 µM, respectively in 50 mM pH PBS solution. To obtain optimum conditions of the enzymatic biosensor system was tested in different pH solutions of PBS (pH 6 to 8), including 200 µM of catechol. The developed enzymatic biosensor system's selectivity performance was tested with different phenolic compounds for biosensor selectivity observation in addition to these experiments. 200 µM of catechol, gallic acid, hydroquinone, n-nitrophenol were tested, and percentage signals were calculated depends on the biosensor signal with 200 µM of catechol, respectively. After optimizing the working conditions of the developed biosensor system, to evaluate the potential matrix effect of real samples on the biosensor performance, spiked green tea samples were analyzed that contained different catechol (50 µM, 100 µM, and 200 µM).

2.2 Boric Acid Doped PANI Based Electrochemical Enzymatic Biosensor System

2.2.1 Reagents

Tyrosinase from mushroom (Tyr, EC:1.14.18.1), catechol, aniline, sulfuric acid (H_2SO_4), 3-aminophenylboronic acid (3-APBA), and glutaraldehyde were purchased from Sigma Aldrich. Phosphate buffer was prepared using di-potassium hydrogen phosphate and potassium dihydrogen phosphate. All the chemicals were used under the laboratory-grade. All electrodes were washed by using MilliQ TKA-Lab pure water.

2.2.2 Electropolymerization of Boric Acid Doped PANI

GCE: The electrochemical measurements were recorded using a three electrode system that working electrode (WE) was a glassy carbon electrode (GCE), auxiliary electrode/counter electrode (CE) was platinum wire, and reference electrode was a Ag/AgCl electrode. Electrochemical measurements were performed through a potentiostat controlled by IviumSoft, software for control and data acquisition. Figure 2.4 represents the GCE and potentiostat setup used in this work.

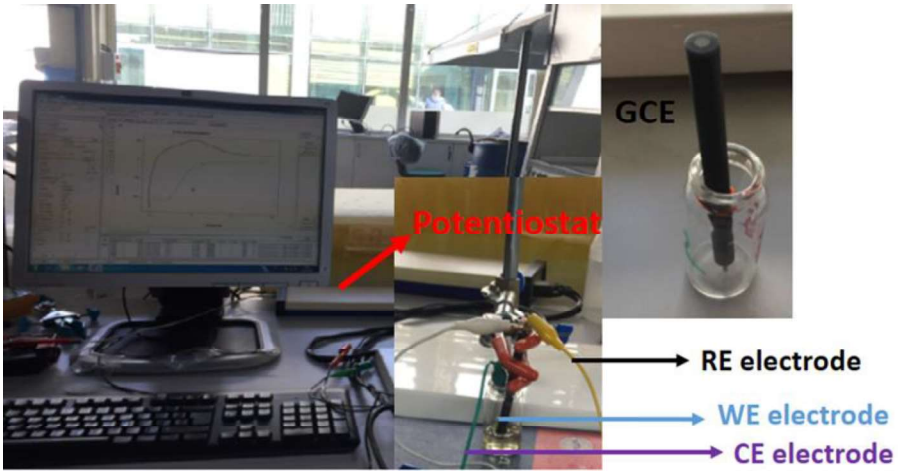

Figure 2.4 The illustration of the GCE electrode and potentiostat set up

Au SPE: The electrochemical measurements were recorded using screen printed electrodes (SPE) that the SPE three-electrode system consisted of an Au plate as the

counter electrode (CE), Au plate as the working electrode (WE), and Ag/AgCl as the reference electrode(RE). Electrochemical measurements were carried out using a potentiostat controlled by IviumSoft.

Poly 3-aminophenylboronic acid (PABA) was deposited electrochemically onto GCE and Au SPE surfaces. The solution containing 0.04 M 3-APBA monomer and 0.2 M NaF was prepared in the 4.5 mL distilled water and 0.5 mL H_2SO_4, respectively. In the electropolymerization experiment, the GCE electrode was scanned within the potential range of −0.4 V to 0.5 V, and the Au SPE electrode was scanned within the potential range of −0.5 V to 0.4 V, in this monomer solution, respectively, until stable curves of cyclic voltammograms were obtained. CVs were recorded at a scan rate of 40 mV s^{-1} and 100 mV s^{-1}. The current is passed through the solution, and the polymer begins to accumulate on the positively charged working electrode. During the oxidation process to form radical cations that react with other monomers, monomers on the working electrode surface form insoluble polymer chains on the electrode surface. Electrochemical polymerization of 3-aminophenylboronic acid was carried out on the surface of GCE, Au SPE. Afterward, these electrodes were washed with distilled water.

Another solution containing 0.04 M 3-APBA monomer and 0.3M NaF was prepared in the 0.1 M PBS solution at pH 5 (pH was adjusted with diluted HCl solution). After the poly 3-aminophenylboronic acid electropolymerization, these PABA modified electrodes were washed with deionized water.

After electropolymerization, the surface activity of the electrodes was ascertained with $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ system by the electrochemical studies that carried out in the presence of 5 mM $[Fe(CN)6]^{3-/4-}$ redox couple in 0.1 M of KCl. Cyclic voltammograms were between the applied potentials -0.2 V to 0.8 V. Afterwards, and electrodes were rinsed with deionized water.

2.2.3 Surface Characterization

HITACHI SU 5000 Scanning Electron Microscope (SEM) was used for investigation after electropolymerization processes by analyzing the surfaces of electrodes. The

surface characterization of the boron-doped PANI electrodes was evaluated and the best condition for the biosensor preparation was concluded.

2.2.4 Biosensor Preparation and Optimization of the Performance Conditions

After the PABA modification of the electrodes, enzyme immobilization was performed using glutaraldehyde crosslinking agents as a supporting material for Tyr enzyme. For enzyme immobilization, a mixture of 40 µl from 1 mg /mL Tyr in and 4 µl glutaraldehyde was prepared. Afterward, some of this mixture dispersion was cast onto these electrodes' surface so that the mixture was allowed to dry at room temperature.

A highly stable and effective catechol biosensor was prepared by immobilizing tyrosinase (Tyr) enzyme into PABA film in conjunction with cross-linking with glutaraldehyde so that an electrochemical enzymatic biosensor system was developed by using the direct electropolymerization process. The biosensor was employed to determine catechol by voltammetric measurements at the applied potential range of -0.9 V to 0.8 V in the steady-state condition with different amounts of catechol (1 µM, 1.5 µM, 2.5 µM, 5 µM, 10 µM, 25 µM, 50 µM, 100 µM, 200 µM, 300 µM, and 400 µM) respectively in the 10 mL PBS solution both for GCE (at pH 7.5) and Au SPE (at pH 6.5).

To obtain optimum conditions of the enzymatic biosensor system was tested in different pH solutions of PBS (pH 6 to 8), including 200 µM of catechol. The developed enzymatic biosensor system's selectivity performance was tested with different phenolic compounds for biosensor selectivity observation in addition to these experiments. Other phenolic compounds such as 4-hydroxybenzoic acid, gallic acid, hydroquinone, 4-nitrophenol, phenol were evaluated on the concentration level of 200 µM were tested.

After optimizing the working conditions of the developed biosensor system, to evaluate the potential matrix effect of real samples on the biosensor performance, spiked green tea samples were analyzed that contained different catechol concentrations (50 µM, 100 µM, and 200 µM).

2.3 Novel Organoboron Polymer Based Electrochemical Enzymatic Biosensor System

2.3.1 Reagents

Tyrosinase from mushroom (Tyr, EC:1.14.18.1), catechol, Acetonitrile (ACN), Sodium fluoride (NaF), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), Tetra butyl ammonium hexafluorophosphate (TBAHFP) and glutaraldehyde were purchased from Sigma Aldrich. Also, a novel organoboron monomer (2-phenyl-1,3,2-dioxaborolane) was synthesized by BOREN for the first time within the proposed project's scope, were used in enzymatic biosensor systems. All the chemicals were used under the laboratory-grade. MilliQ TKA-Lab pure water and Dichloromethane(DCM) were used for the wet process.

2.3.2 Electropolymerization of Novel Organoboron Monomer

GCE: The electrochemical measurements were recorded using conventional three-electrode system consisted of glassy carbon electrode as a working electrode (WE), platinum wire as an auxiliary electrode/counter electrode (CE), and Ag/AgCl as a reference electrode. With this three-electrode system, electrochemical measurements were performed through a potentiostat controlled by IviumSoft that is software for control and data acquisition.

Pt SPE: The electrochemical measurements were recorded using platinum screen printed electrodes (SPE) that the SPE three-electrode system consisted of a Pt coated plate as the counter electrode (CE), Pt coated plate as the working electrode (WE), and Ag/AgCl as the reference electrode(RE). Electrochemical measurements were carried out using a potentiostat controlled by IviumSoft.

GCE and Pt SPE electrodes were immersed in the solution containing different ionic solutions: NaF, $LiClO_4$, TBTU, and TBAHFP, respectively, and mM level of novel boron-containing monomer in the 10 mL ACN solution. Later, cyclic voltammetry scans were performed between -0.5 V to 1.2 V within the GCE, and Pt SPE electrodes' potential ranges until stable curves of cyclic voltammograms (CVs) are

obtained. Finally, the surface of GCE and SPEs were washed by using distilled water.

After electropolymerization, CVs were recorded at a different scan rate of 10 mV s^{-1}, 25 mV s^{-1}, 50 mV s^{-1},75 mV s^{-1}, 100 mV s^{-1}, 125 mV s^{-1}, and 150 mV s^{-1}, respectively that the surface activity and conductivity of the electrode are ascertained with $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ system which were carried out between -0.2 V to 0.8 V in 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$.

2.3.3 Surface Characterization

HITACHI SU 5000 Scanning Electron Microscope(SEM) was used for investigation after electropolymerization, and SEM analyzed the surfaces of electrodes. Surface characterization of Pt-SPE electrodes was evaluated for the novel boron-containing monomer's electropolymerization in the different salt solutions.

2.3.4 Biosensor Preparation and Optimization of the Performance Conditions

After the novel boron-containing monomer modification of the electrodes, enzyme immobilization was performed using glutaraldehyde crosslinking agents to support Tyr immobilization. For enzyme immobilization, a mixture of 40 µl from 1 mg / mL Tyr in and 4 µl glutaraldehyde was prepared. Afterward, some of this mixture dispersion was cast onto these electrodes' surface so that the solvent was allowed to evaporate at room temperature mixture was wait until it dries on the surface.

A highly stable and effective catechol biosensor was prepared by immobilizing Tyr enzyme into containing novel boron monomer film in conjunction with cross-linking glutaraldehyde so that an electrochemical enzymatic biosensor system was developed by using the direct electropolymerization process. The biosensor was employed to determine catechol by voltammetric measurements at the applied potential of -0.9 V to 0.8 V in the steady-state condition in different concentration of catechol 1 µM, 1.5 µM, 2.5 µM, 5 µM, 10 µM, 25 µM, 50 µM, 100 µM, 200 µM, 300 µM, and 400 µM, respectively both for GCE (in PBS solution at pH 8) and Pt SPEs (in PBS solution at pH 7.5).

To obtain optimum conditions of the enzymatic biosensor system was tested in different pH solutions of PBS (pH 6 to 8), including 200 μM of catechol and in PBS solution at pH 6, pH 6.5, pH 7, pH 7.5, and pH 8.

To determine the specificity of the developed enzymatic biosensor for detecting catechol, different phenolic compounds such as 4-hydroxybenzoic acid, gallic acid, hydroquinone, 4-nitrophenol, phenol were evaluated on the concentration level of 200 μM.

After optimizing the working conditions of the developed biosensor system, to evaluate the potential matrix effect of real samples on the biosensor performance, spiked green tea samples were analyzed that contained different catechol (50 μM, 100 μM, and 200 μM).

CHAPTER 3

RESULTS & DISCUSSION

3.1 PANI Based Electrochemical Enzymatic Biosensor System

3.1.1 Electropolymerization of Aniline in Different Acidic Solutions

SPEs were immersed in the solution of 0.1 M aniline and different acidic solutions of 0.5 M H_2SO_4, 0.5 M $HClO_4$, and 0.5 M HCl solution. SPEs were scanned within the potential range of −0.5 V to 0.8 for H_2SO_4 solution, 0.4 V to 0.8 V for $HClO_4$ solution, and -0.2 V to 1.0 V for HCl solution until stable curves of CVs are obtained as shown in Figure 3.1 (a) in HCl solution (b) H_2SO_4 solution (c) in $HClO_4$. Comparing the CVs of these in different solutions, it is found that both anodic and cathodic peak currents increased sharply in the case of aniline monomer in different acidic mediums, especially for $HClO_4$, which proves that it improves the electron transfer rate for PANI formed onto SPE.

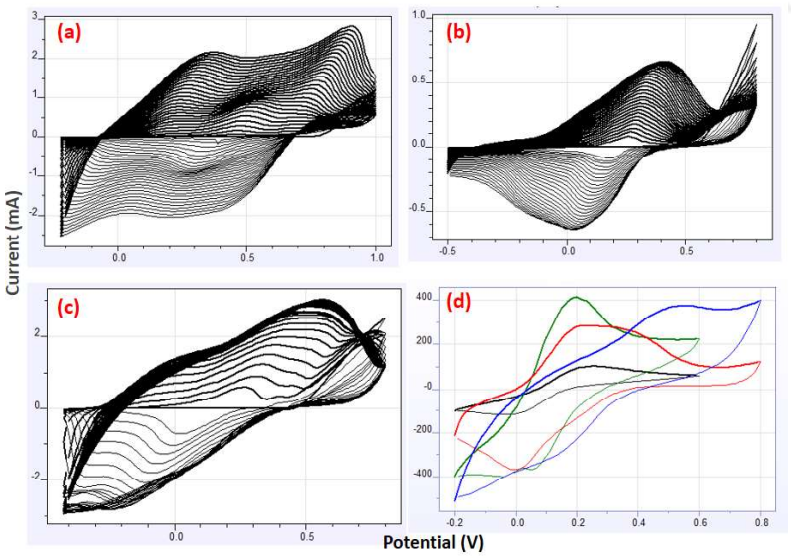

Figure 3.1 The polymerization bath consists of 0.1 M aniline monomer dissolved in 0.5 M different supporting electrolytes. Aniline electropolymerization (a) in HCl solution (b) H_2SO_4 solution (c)in $HClO_4$ solution (d) Electrode surface conductivity analysis with 5 mM $[Fe(CN)6]^{3-/4-}$ redox couple (Black: bare SPE, Blue: HCl electropolymerization, Red: H_2SO_4 electropolimerization, Green: $HClO_4$ electropolimerization)

After the electropolymerization, the electrode's surface activity and conductivity were ascertained by performing cyclic voltammograms between -0.2 V and 0.8 V in the solution of 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox molecule. As shown in Figure 3.1 (d), electrode surface conductivity analysis with $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox molecule results indicate that electrode surface conductivity after electropolymerization of aniline in $HClO_4$ solution has the highest voltammogram peak height, which is the evidence of high surface conductivity and this green curve coming from $HClO_4$ is the best. Blue curve coming from HCl shows that the oxidation peak was shifted more positive potential range, showing that electrode surface disrupted. So was quited testing HCl after this point. The more conductive the surface becomes, the better the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox molecule oxidation and reduction peaks.

The surface morphology of the bare and polyaniline modified SPEs based on different acidic solutions was characterized by SEM studies (Figure 3.2a–e). Depends on the SEM images for different acidic solutions, Figure3.2 (a) represented bare SPE as a homogeneous surface, Figure3.2 (b) not dense but homogeneous, Figure3.2 (c) the surface is very dense, nonhomogeneous, and quite dense, so it affects electrode surface conductivity, Figure3.2 (d) $HClO_4$ based modification represented the densest and most homogeneous surface and Figure3.2 (e) the results indicate that enzyme particles attached to the surface. Morphology permits the Tyr enzyme uniformly entrapped on the surface, and the porous structure disappeared utilizing the Tyr enzyme.

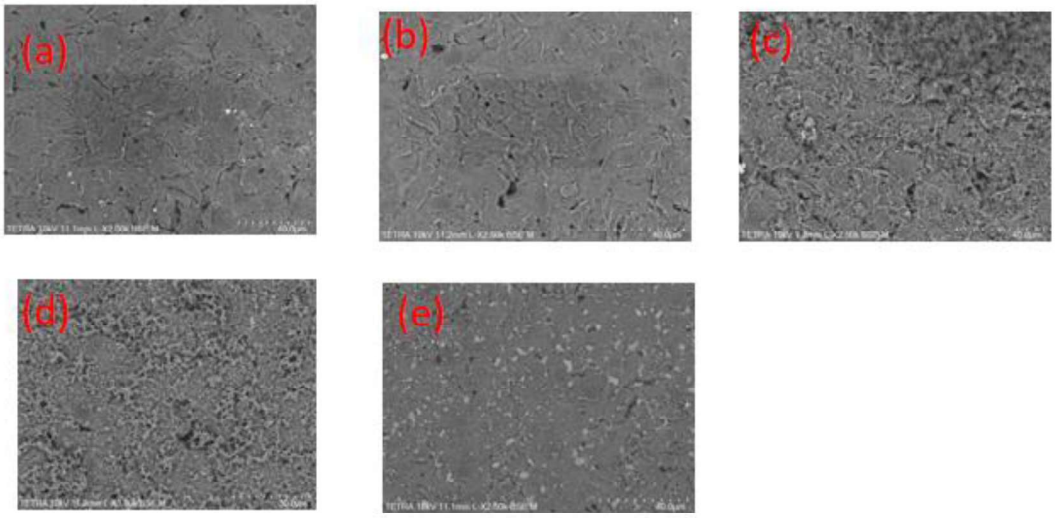

Figure 3.2 SEM images aniline electropolymerizatıon in acidic solutions (a) bare SPE (b) H_2SO_4 (c) HCl (d) $HClO_4$ (e) Enzyme immobilized SPE electrodes after aniline electropolymerization

3.1.2 Biosensor Performance Development

pH Optimization: 200 µM concentration of catechol was tested using different acidic solution based polyaniline modified and enzyme immobilized SPEs in different pH solutions of PBS including pH 6, pH 6.5, pH 7, pH 7.5, and pH 8. As represented in Figure 3.3, for both acidic solutions based on electropolymerized SPE, the optimum pH was observed at pH 6.5.

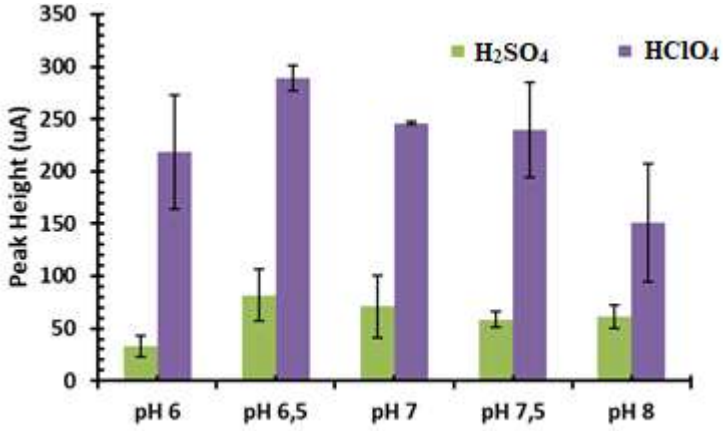

Figure 3.3 The effect of pH on the biosensor's performance was studied by incubating the electrode in 200 µM catechol solution buffered (10 mL PBS) at the pH range of 6–8

Dose-response curve: Different concentrations of catechol (10 µM, 25 µM, 50 µM, 100 µM, 200 µM, 300 µM) were added onto 50 mM pH 6.5 buffer solution, and the voltammetric measurements at the applied potential range of -0.9 V to 0.8 V were performed. The reduction peak for the o-quinone product of the enzymatic reaction of catechol was observed, and the peak heights were calculated for each concentration. The linear range was identified for both acidic solutions based on aniline modified SPEs between 10 µM and 300 µM where the $HClO_4$ based enzymatic biosensor system had higher signals and therefore sensitive response as shown in Figure 3.4.

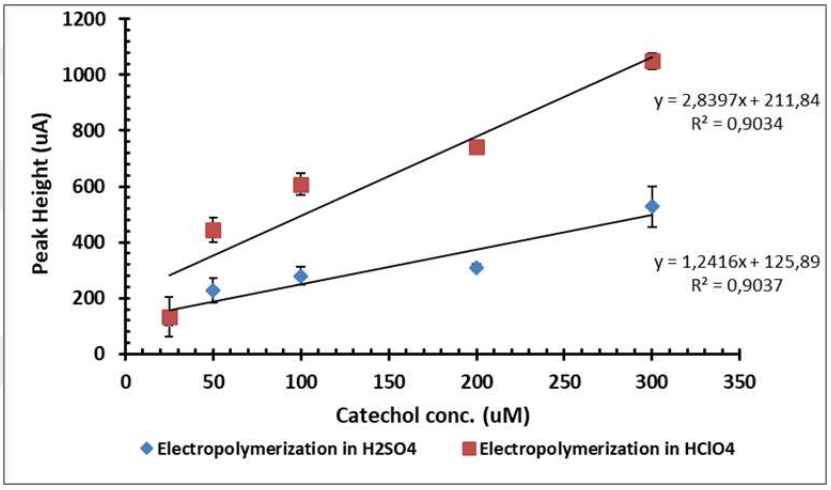

Figure 3.4 Catechol detection (25, 50, 100, 200, 300 µM) in H_2SO_4 and $HClO_4$ polymerization

Biosensor selectivity: To determine the specificity of the developed enzymatic biosensor for detecting catechol, different phenolic compounds such as gallic acid, hydroquinone, 4-nitrophenol were evaluated on the concentration level of 200 µM. The biosensor system's responses for these compounds were compared with the results of 200 µM of catechol detection. The results in Figure 3.5 clearly show that non-specific phenolic compounds do not generate any significant peak at the voltage that o-quinone's reduction peak located. Therefore, the developed biosensor system is quite specific for only catechol detection.

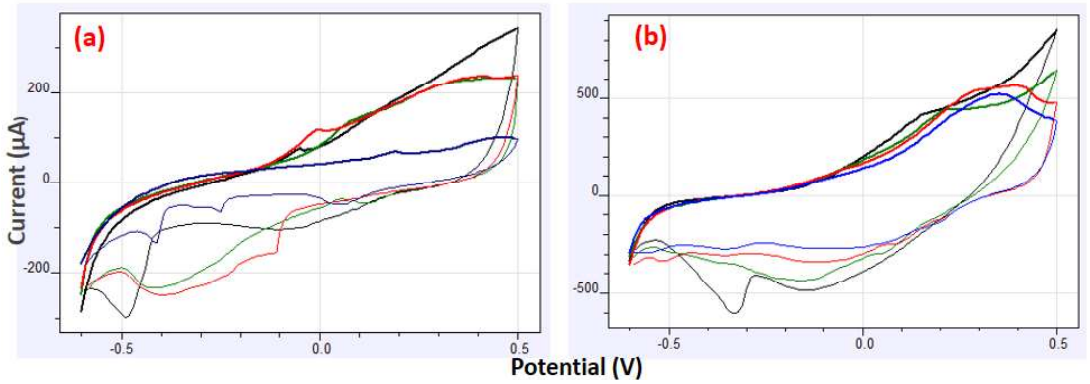

Figure 3.5 The biosensor system's response and selectivity for different phenol compounds (200 µM) (a) H_2SO_4 aniline electropolymerized SPE (b) $HClO_4$ aniline electropolymerized SPE. (Black: catechol, Blue: gallic acid, Green: 4-nitrophenol, Red:Hydroquinone)

3.1.3 Real Sample Testing

To show the biosensor's ability for the determination of catechol in tea samples under the optimized conditions. 50 µM,100 µM, and 200 µM spiked tea samples were tested with developed enzymatic biosensor systems in Figure 3.6. The results are summarized in Table 3.1. The recovery of all measured samples was between 93% and 110% for $HClO_4$, between 103% and 111% for H_2SO_4, and the parallel tests showed that sd% deviation between 2% and 9% for $HClO_4$, between 11% and 18% for H_2SO_4. These results indicated that the possible interference from the different background composition of real samples was within 16% and is considered acceptable for food quality monitoring tests.

Table 3.1 Green tea spiked samples result with developed polyaniline based enzymatic biosensor system

Aniline Electropolymerization in $HClO_4$				Aniline Electropolymerization in H_2SO_4			
Added	**Found**	**% Recovery**	**% sd**	**Added**	**Found**	**% Recovery**	**% sd**
50	55.28455	110.5691	5.942074	**50**	55.84989	111.6998	18.57475
100	93.73984	93.73984	2.192579	**100**	103.3113	103.3113	11.24118
200	205.6911	102.8455	9.198137	**200**	221.4128	110.7064	14.9494

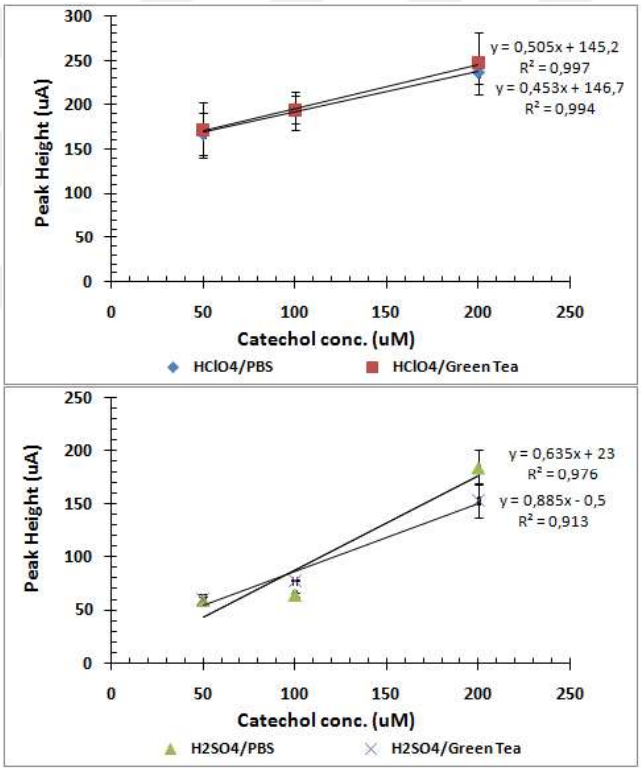

Figure 3.6 Catechol spiked green tea samples' testing results with developed polyaniline based enzymatic biosensor system

In this work, SPEs were modified with conductive PANI films using different acidic solution based electropolymerization of aniline. SPEs were scanned within the potential range of −0.5 V to 0.8 for H_2SO_4 solution, 0.4 V to 0.8 V for $HClO_4$

solution, and -0.2 V to 1.0 V for HCl solution until stable curves of CVs were obtained. Comparing the CVs of these in different solutions, it is found that the $HClO_4$ solution improves the electron transfer rate for PANI formed onto SPE. Electrode surface conductivity analysis with $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox molecule results also support this conclusion, which has the highest voltammogram peak height with $HClO_4$ based electropolymerization. On the SEM images of SPEs with PANI modification in different acidic solutions, we can see the significant influence of $HClO_4$ based modification. Hence the SPE surface with $HClO_4$ based modification lead to a more uniform and dense PANI film.

After the electropolymerization steps, a Tyr enzyme-based biosensor system was developed for catechol detection. Tyr enzyme was immobilized onto the PANI modified SPE surface, which was also observed by SEM images. The biosensor systems' linear ranges were identified for all acidic solutions based on electropolymerization between 10 μM and 300 μM, where the $HClO_4$ based enzymatic biosensor system had higher signals and, therefore, sensitive response. The biosensor system was also having quite a specificity for catechol detection compared to other phenolic compounds. Additionally, the developed enzymatic biosensor system was successfully tested with real tea samples with 93% to 110% recovery and 2% and 9% sd% deviation results. Thus, these results indicated that the possible interference from the different background composition of real samples was within 16% and is considered acceptable for food quality monitoring tests.

3.2 Boric Acid Doped PANI Based Electrochemical Enzymatic Biosensor System

3.2.1 Electropolymerization of Boric Acid Doped PANI on Different Conditions Using Different Electrodes

Different electrodes (SPE, GCE) were immersed in the solution containing 0.04 M 3-APBA monomer and 0.2 M NaF in the 4.5 mL distilled water and 0.5 mL H_2SO_4, respectively. Electrodes were scanned within the potential range of −0.4 V to 0.5 V for GCE and −0.5 V to 0.4 V for Au SPE until stable curves of CVs are obtained. As shown in Figure 3.7**,** comparing the CVs of GCE and Au SPEs, it is found that both

anodic and cathodic peak currents increased sharply in the case of 3-amino phenylboronic acid monomer. Therefore, it proves that electropolymerization of this boron-doped PANI (PABA) was performed successfully. Figure 3.7 (a) represented 3-APBA polymerization onto Au SPE in 0.3 M NaF and 0.1 M PBS solution at PH 5, (b) 3-APBA polymerization onto Au SPE in 0.1 M H_2SO_4 0.2 M NaF solution, (d) 3-APBA polymerization onto GCE in 0.3 M NaF and 0.1 M PBS solution at PH 5 (e) 3-APBA polymerization onto GCE in 0.1 M H2SO4 0.2 M NaF solution.

After electropolymerization,the surface activity and conductivity of the electrode are ascertained through -0.2 V to 0.8 V in 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox molecule containing 0.1 M KCl solution as shown in Figure 3.7 (c) and CVs were recorded. Figure 3.7 (c) represented electrode surface conductivity analysis with 5 mM Potassium FeCN redox molecule in KCl solution (Green: blank Au SPE, Red: PABA electropolymerization in pH 5, Black: PABA electropolymerization in H2SO4 electropolimerization), Figure 3.7 (g) FeCN CV before after electropolymerization (Black: blank GCE, Green: PABA electropolymerization in pH 5, Red: PABA electropolymerization in H_2SO_4).

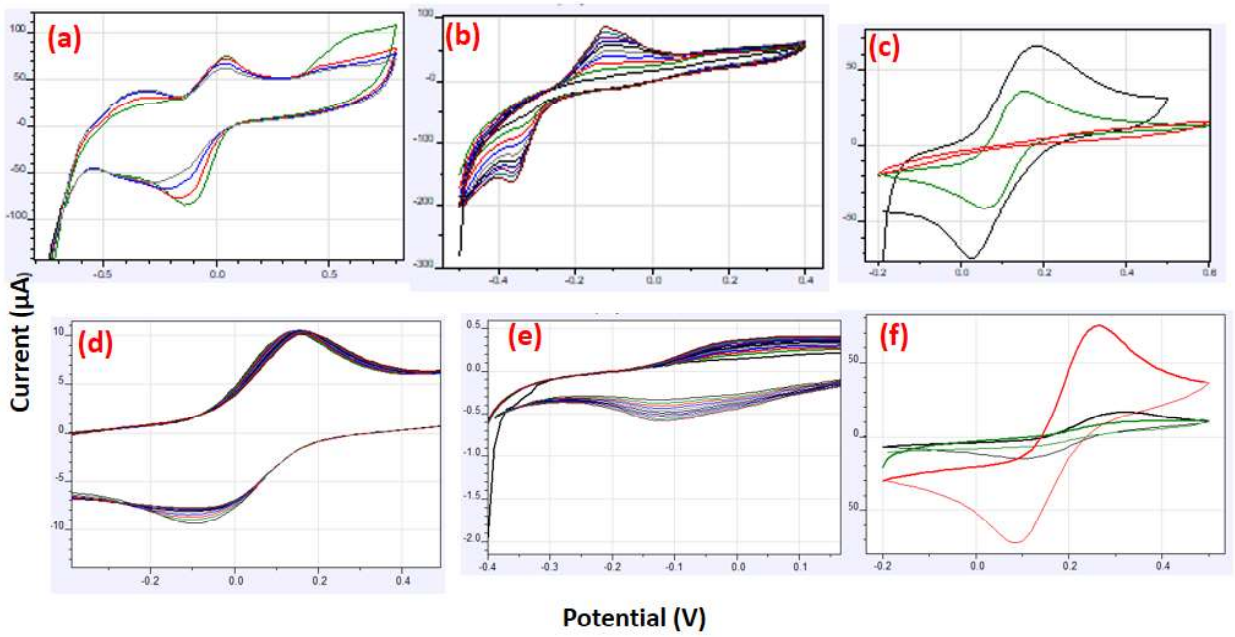

Figure 3.7 (a) 3-APBA polymerization onto Au SPE in 0.3 M NaF and 0.1 M PBS solution at PH 5, (b) 3-APBA polymerization onto Au SPE in 0.1 M H_2SO_4 0.2 M NaF solution, (c) Electrode surface conductivity analysis with 5 mM $[Fe(CN)_6]^{3-/4-}$ redox couple in KCl solution (Green: blank Au SPE, Red: PABA electropolymerization in pH 5, Black: PABA electropolymerization in H_2SO_4 electropolimerization), (d) 3-APBA polymerization onto GCE in 0.3 M NaF and 0.1 M PBS solution at PH 5 (e) 3-APBA polymerization onto GCE in 0.1 M H_2SO_4 0.2 M NaF solution (g) $[Fe(CN)6]^{3-/4-}$ redox couple CV before after electropolymerization (Black: blank GCE, Green: PABA electropolymerization in pH 5, Red: PABA electropolymerization in H_2SO_4)

The surface morphology of the bare and modified poly 3-aminophenylboronic acid (PABA) based on different electrodes were characterized by SEM studies Figure 3.8 (A–D). Depends on the SEM images both for Au SPE, the results indicate that Au SPE surface containing 0.04M 3-APBA monomer, 0.2 M NaF, 0.1 M H_2SO_4 and this surface was quite dense, as shown in Figure 3.8 (C).

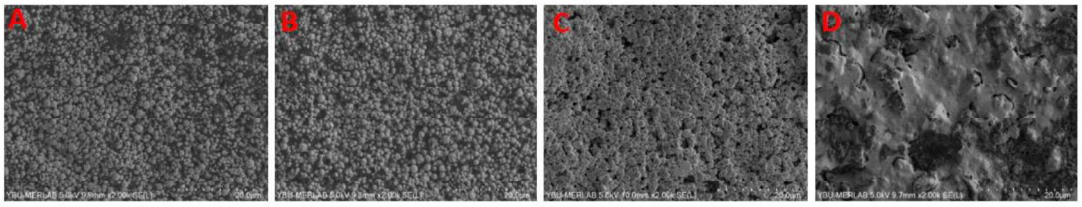

Figure 3.8 SEM images of PABA modified Au SPE electrodes by electropolymerization in acidic solutions; A) Blank, B) PH 5 buffer solution C) 0.1 M H_2SO_4 solution D) 0.1 M H_2SO_4 solution + immobilized enzyme

3.2.2 Biosensor Performance Development

pH Optimization: To obtain optimum conditions of the enzymatic biosensor system was tested in different pH solutions of PBS (pH 6 to 8), including 200 µM of catechol and pH 6, pH 6.5, pH 7, pH 7.5, and pH 8. As shown in Figure 3.9, the optimum pH value was observed at pH 7.5 for GCE. Similarly, the same procedure was applied for the Au SPE. As represented in Figure 3.10, the Au SPE was immersed in solutions containing different pH values, and pH 6.5 was found to be the optimum value.

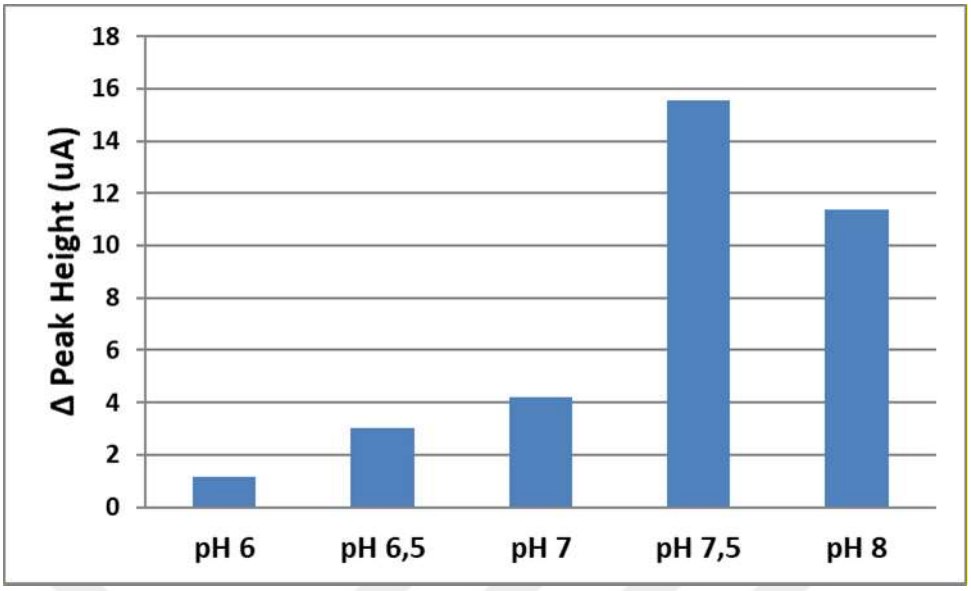

Figure 3.9 The effect of pH on the biosensor's performance by incubating the GCE containing in 200 µM catechol solution buffered (PBS) at the pH range of 6–8

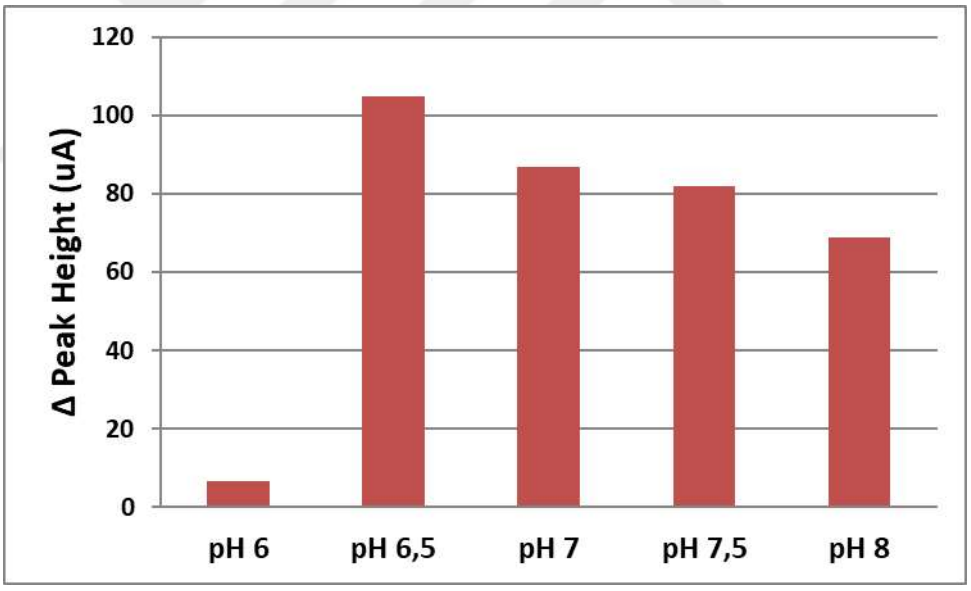

Figure 3.10 The effect of pH on the biosensor's performance by incubating the Au SPE (electropolymerization in H2SO4) in 200 µM catechol solution buffered (PBS) at the pH range of 6–8

Dose-response curve: Different concentrations of catechol (0 µM, 1 µM, 2.5 µM, 5 µM, 10 µM, 25 µM, 50 µM, 100 µM, 200 µM, 300 µM, 400 µM) were added in PBS solution, and the voltammetric measurements at the applied potential range of -0.9 V

to 0.8 V were performed both for GCE (at pH 7.5) and Au SPE (at pH 6.5). The reduction peak for the o-quinone product of the enzymatic reaction of catechol was observed, and the peak heights were calculated for each concentration. The linear range was identified both for GCE and Au SPE in different catechol concentration based on PABA modified electrodes between 1 µM and 200 µM for GCE and between 10 µM and 400 µM for Au SPE was seen in Figure 3.11 and Figure 3.12 where the GCE based enzymatic biosensor system had higher signals, and these results are clearly proved that even at a catechol value of 1 µM, they are detectable for GCE. Therefore, a sensitive response was seen in Figure 3.11 (B).

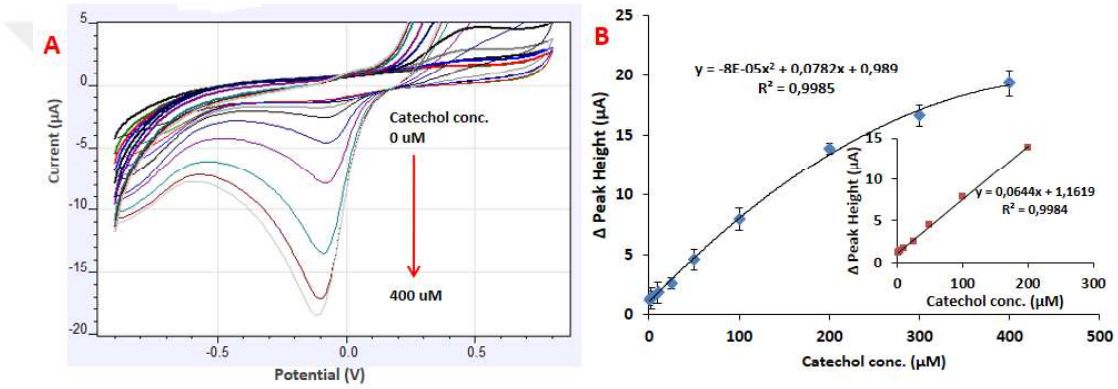

Figure 3.11 A) Different catechol concentrations for the GCE (APBA and H_2SO_4) in PBS at pH 7.5 B) Dose calibration curve, inner Figure; the linear range for catechol concentration between 1 µM to 200 µM

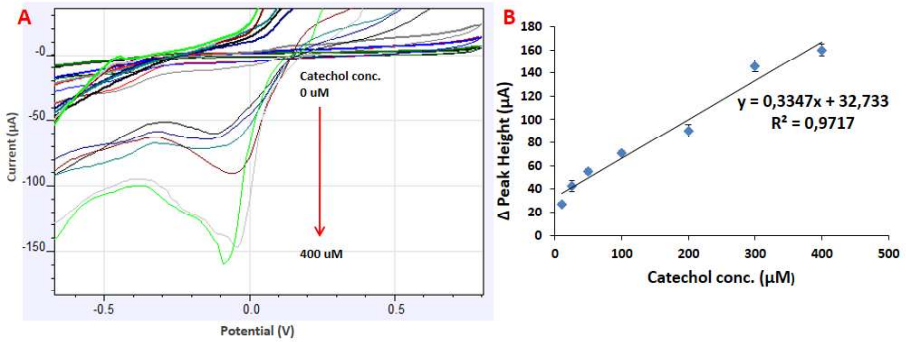

Figure 3.12 A) Different catechol concentrations for the Au SPE (H_2SO_4) in PBS at pH 6.5 B) The linear range for catechol concentration between 10 µM to 400 µM

Biosensor selectivity: To determine the specificity of the developed enzymatic biosensor for detecting catechol, other phenolic compounds such as 4-hydroxybenzoic acid, gallic acid, hydroquinone, 4-nitrophenol, phenol were evaluated on the concentration level of 200 µM. The biosensor system's responses for these compounds were compared with the results of 200 µM of catechol detection. The results in Figure 3.13 and Figure 3.14 clearly show that non-specific phenolic compounds do not generate any significant peak at the voltage that o-quinone's reduction peak located. Therefore, the developed biosensor system is quite specific for only catechol detection.

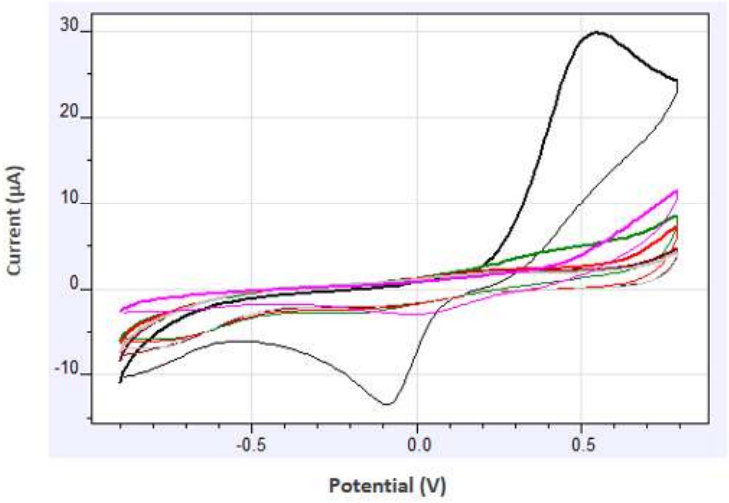

Figure 3.13 The biosensor system's response and its selectivity for different phenol compounds (200 µM) on GCE with PBS (APBA other phenolic compounds GCE)

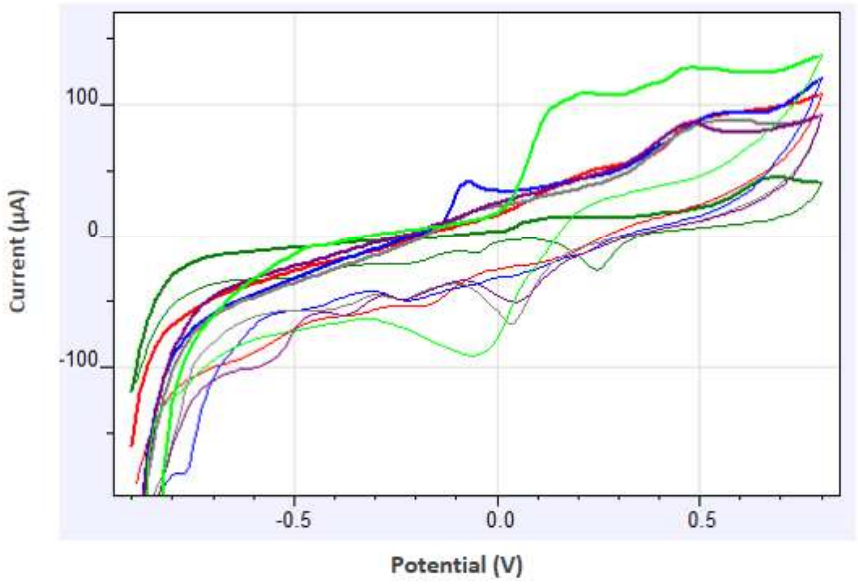

Figure 3.14 The biosensor system's response and its selectivity for different phenol compounds (200 µM) on Au SPE with PBS (APBA other phenolic compounds for Au SPE) (light green: catechol)

As seen here, when looking at the selectivity for these phenol compounds, it was seen that the Tyr enzyme worked only for catechol with a large and significant difference.

3.2.3 Real Sample Testing

To show the biosensor's ability for the determination of catechol in tea samples under the optimized conditions. 50 µM, 100 µM, and 200 µM spiked tea samples were tested with developed enzymatic biosensor systems, and the results are summarized for GCE and Au SPE in Table 3.2. The recovery of all measured samples was between 105.8% and 121.4% for Au SPE, between 89.1% and 102.78% for GCE and the parallel tests showed that sd% deviation between 4.2% and 1.7% for Au SPE and between 4.7% and 11.9% for GCE. These results indicated that the possible interference from the different background composition of real samples was within 16% and is considered acceptable for food quality monitoring tests.

Table 3.2 Real green tea testing with Gold SPE and GCE after electropolymerization in APBA in H_2SO_4+NaF

	Added catechol concentration	**Found catechol concentration**	**Recovery %**	**sd %**
Au SPE	50	60.7	121.4	1.7
	100	114.6	114.6	9.1
	200	211.6	105.8	4.2
GCE	50	51.39	102.78	4.7
	100	93.6	93.6	11.9
	200	178.2	89.1	10.9

3.3 Novel Organoboron Polymer Based Biosensor System

3.3.1 Electropolymerization of Novel Organoboron Monomer on Different Conditions by Using Different Electrodes

GCE and Pt SPEs were immersed in the solution containing different salts: NaF, $LiClO_4$, TBTU, and TBAHFP, respectively, and novel boron-containing monomer in the ACN solution. Since ACN is an organic solvent and it does not have an ionic content, the ionic solvent addition was needed for performing the electropolimerization process. Therefore, different salts were added to the solution each time so that it could form ions. During electropolymerization process, electrodes were scanned within the potential range of −0.5 V to 1.2 V for GCE and Pt SPEs until stable curves of CVs are obtained, as shown in Figure 3.15 and Figure 3.16. It is observed that both anodic and cathodic peak currents increased sharply on each scan till 10^{th} scan in the novel boron-containing monomer solution. Therefore, these results clearly proved that electropolymerization of this novel organoboron monomer was carried out successfully.

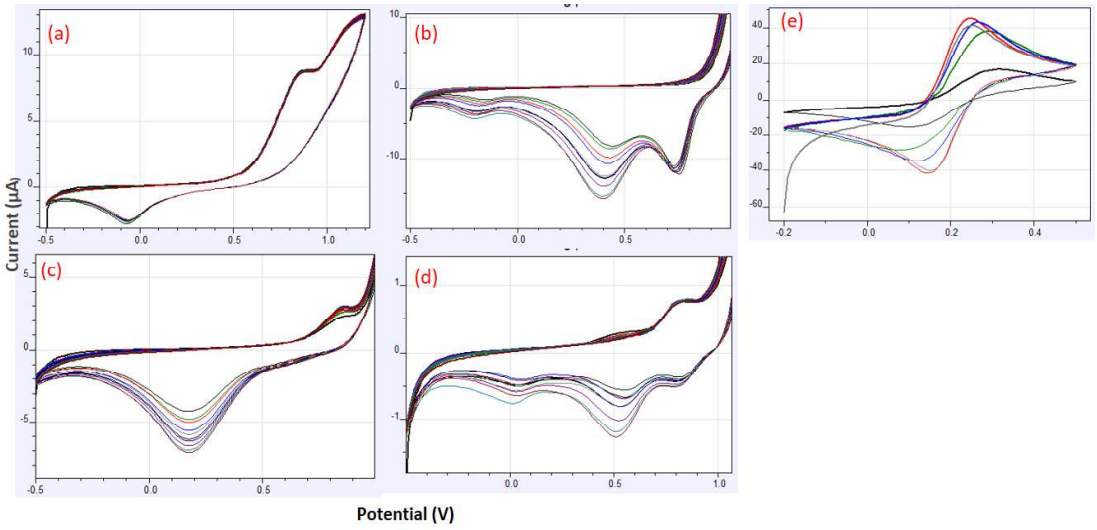

Figure 3.15 Electropolymerization of novel organoboron monomer on different conditions using GCE (a) in NaF (b) in LiClO4 (c) in TBTU (d)in TBAHFP (e) $[Fe(CN)_6]^{3-/4-}$ redox couple CV after electropolymerization(black: blank, green: in NaF, red: in LiClO4, blue: in TBTU, gray: in TBAHFP)

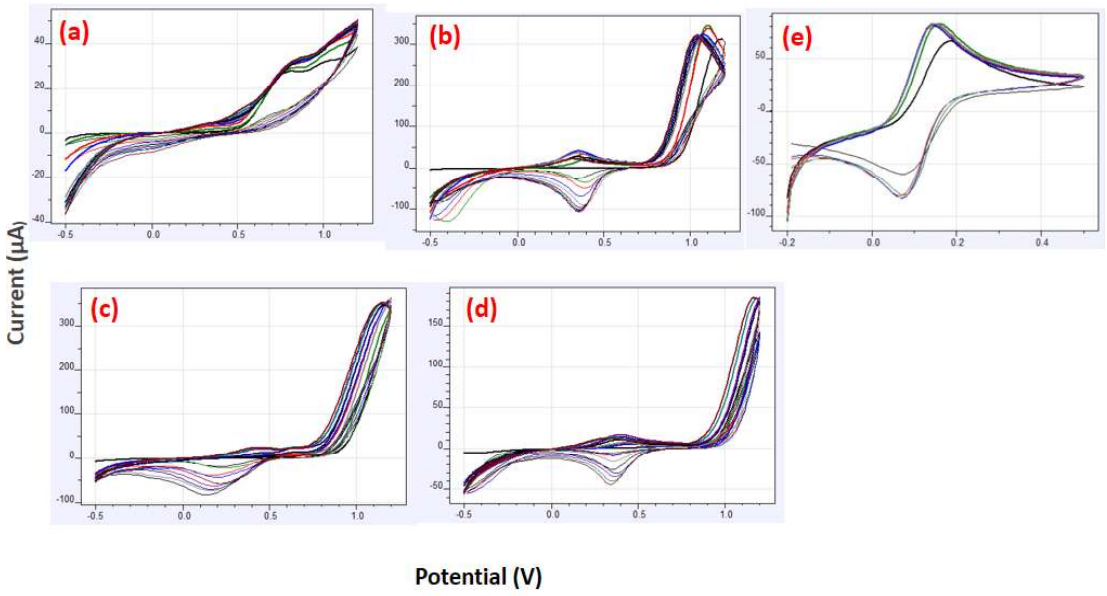

Figure 3.16 Electropolymerization of novel organoboron monomer on different conditions using Pt SPE (a) in NaF (b)in LiClO4 (c) in TBTU (d) in TBAHFP (e) $[Fe(CN)_6]^{3-/4-}$ redox couple CV after electropolymerization (black: blank, green: in NaF, red: in LiClO4, blue: in TBTU, gray: in TBAHFP)

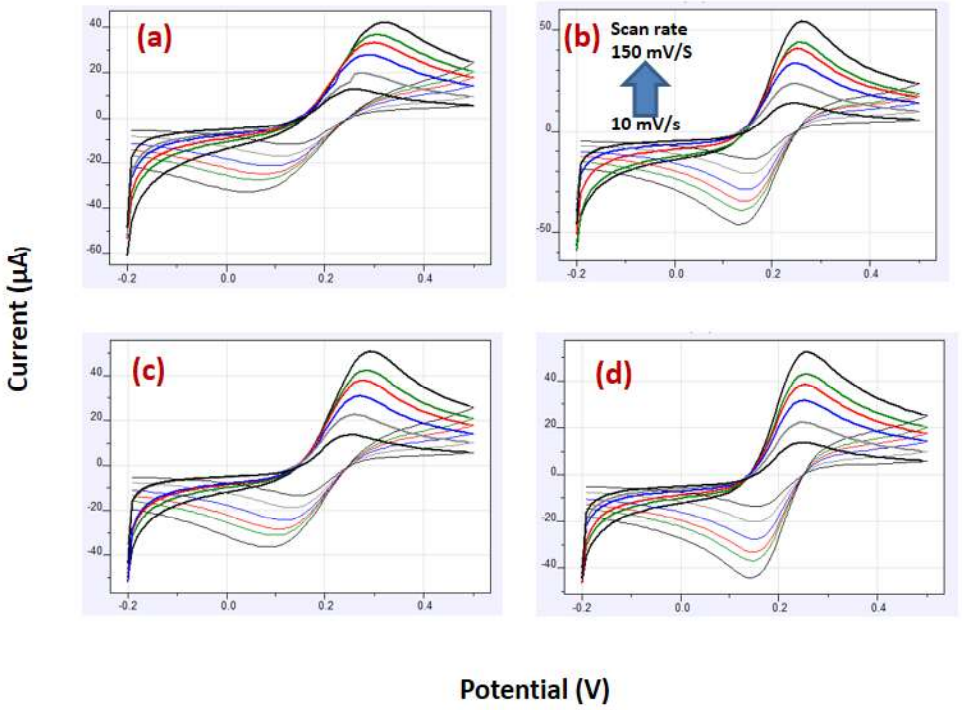

Figure 3.17 After electropolymerization of novel organoboron monomer, CVs were recorded at different scan rate of 10 mV s^{-1}, 25 mV s^{-1}, 50 mV s^{-1}, 75 mV s^{-1}, 100 mV s^{-1}, 125 mV s^{-1}, 150 mV s^{-1} as different peak height in 5 mM $[Fe(CN)_6]^{3-/4-}$ redox couple for GCE (a) NaF (b) LiClO4 (c) TBTU (d) TBAHFP

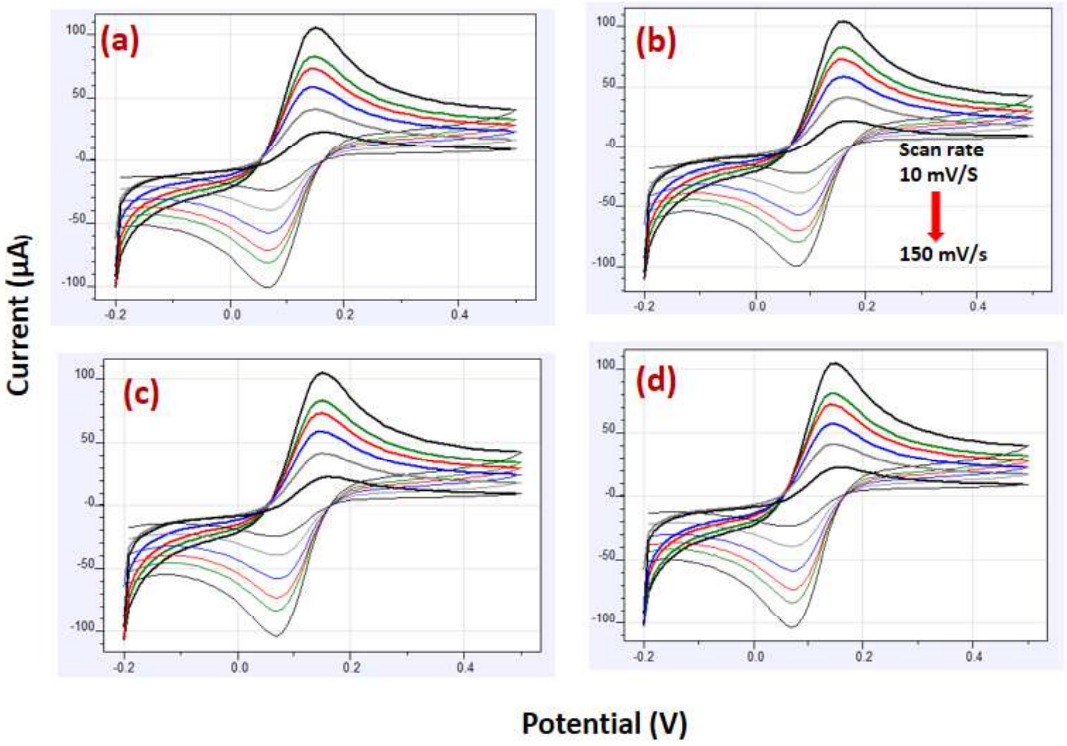

Figure 3.18 After electropolymerization of novel organoboron monomer, cvs were recorded at different scan rate of 10 mV s^{-1}, 25 mV s^{-1},50 mV s^{-1},75 mV s^{-1}, 100 mV s^{-1}, 125 mV s^{-1},150 mV s^{-1} as different peak height in5 mM $[Fe(CN)_6]^{3-/4-}$ redox couple for Pt SPE (a) NaF (b) $LiClO_4$ (c) TBTU (d) TBAHFP

After the electropolymerization, the electrode's surface activity and conductivity were ascertained by the potential range between -0.2 V and 0.5 V in the solution of KCl containing $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ redox molecule. As shown in Figure 3.17 and Figure 3.18, CVs were recorded at different scan rate of 10 mV s^{-1}, 25 mV s^{-1}, 50 mV s^{-1}, 75 mV s^{-1}, 100 mV s^{-1}, 125 mV s^{-1}, 150 mV s^{-1}, respectively in FeCN. It should be noted that according to the results, the cathodic peak current increased with the scan rates for cyclic voltammetry (CV) profiles. As seen in Figure 3.16 (e), after the electropolymerization in TBTU salt, the highest voltammogram peak height is evidence of high surface conductivity, and this TBTU curve is the best. These results clearly proved that increasing scan rate means there is something active on the surface that is the more conductive the surface becomes, the better the FeCN peaks, even at the empty electrode, and electropolymerization has occurred on the surface.

The surface morphology of the bare and novel boron-containing monomer modified SPEs based on different salt solutions was characterized by SEM studies (Figure 3.19 A–E). Depends on the SEM images for these electrodes, it should be noted that novel boron monomer containing $LiClO_4$ salt, the surface of Pt SPE is the most dense and uniform one, as seen in Figure 3.19 (C).

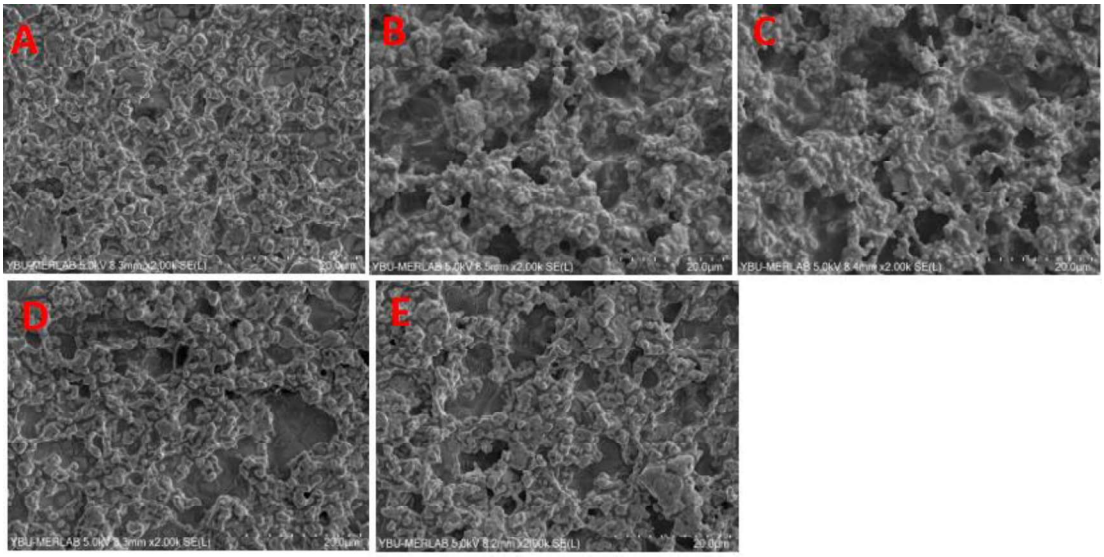

Figure 3.19 SEM images of novel organoboron monomer modified SPE platinum electrodes by electropolymerization in ACN and different salts; A) Blank, B) NaF C) $LiClO_4$ D) TBTU E) TBAHFP

3.3.2 Biosensor Performance Development

pH Optimization: To obtain optimum conditions of the enzymatic biosensor system was tested in PBS solution at different pH values (pH 6 to 8), including 200 µM of catechol and pH 6, pH 6.5, pH 7, pH 7.5, and pH 8. Pt SPEs were immersed in the solution containing different salts based on electropolymerized Pt SPE, and the optimum pHs were observed as pH 7.5, as shown in Figure 3.20. Similarly, the same procedure was applied to the GCE electrode. As shown in Figure 3.21, the GCE electrode containing the novel monomer and $LiClO_4$ salt was immersed in solutions containing different pH values, and pH 8 was found to be the optimum value.

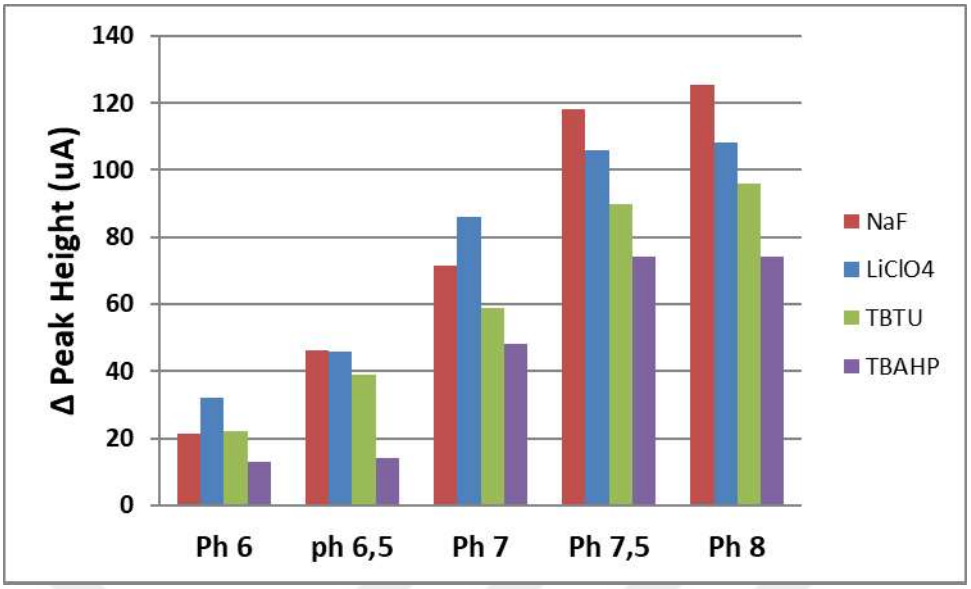

Figure 3.20 The effect of pH on the biosensor's performance by incubating the Pt SPE electrodes containing different salts in 200 µM catechol solution buffered (PBS) at the pH range of 6–8

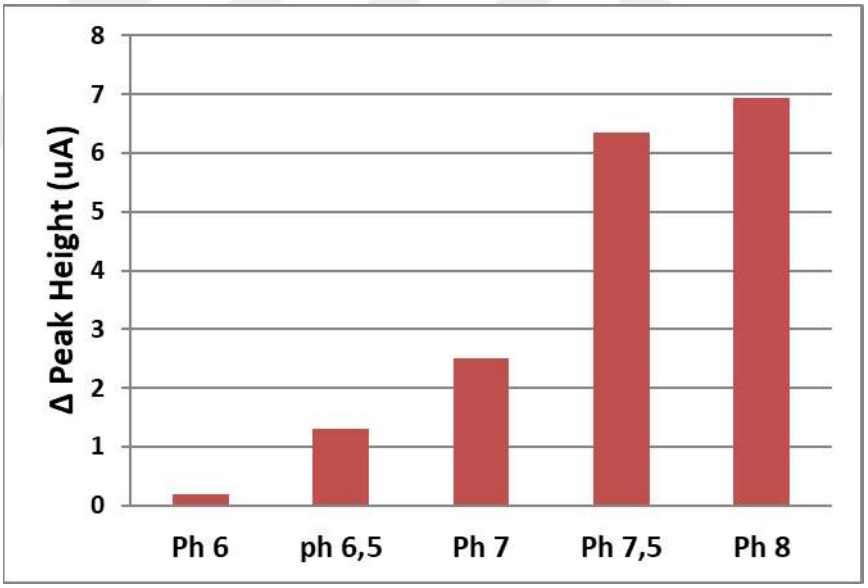

Figure 3.21 The effect of pH on the biosensor's performance by incubating the GCE electrodes containing novel organoboron monomer $LiClO_4$ in 200 µM catechol solution buffered (PBS) at the pH range of 6–8

Dose-response curve: Different concentrations of catechol (0 µM, 1 µM, 2.5 µM, 5 µM, 10 µM, 25 µM, 50 µM, 100 µM, 200 µM, 300 µM, 400 µM) were added onto 10 mL pH 8 buffer solution, and the voltammetric measurements at the applied

potential range of -0.9 V to 0.8 V were performed for both GCE and Pt SPEs. The reduction peak for the o-quinone product of the enzymatic reaction of catechol was observed, and the peak heights were calculated for each concentration. The linear range was identified for both GCE, and Pt SPEs in different salt solutions based on novel boron monomer modified electrodes between 5 µM and 300 µM for GCE (Figure 3.22) and between 5 µM and 100 µM (Figure 3.23(A)) for Pt SPEs and 5 µM and 200 µM (Figure 3.23(B)) for Pt SPEs where the novel boron monomer based enzymatic biosensor system had higher signals and therefore, these results are clearly proved that even at a catechol value of 5 µM, they are detectable for electrodes and so, the sensitive response was seen in Figure 3.22 and 3.23. Also, the reduction (cathodic) peak current of novel boron-containing monomer further increased with catechol concentration.

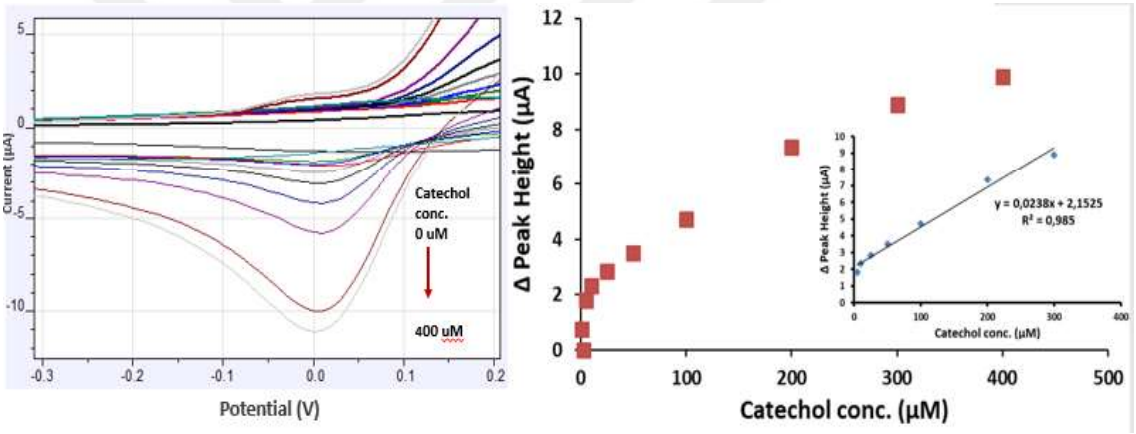

Figure 3.22 A) Catechol detection for the GCE containing novel organoboron monomer and $LiClO_4$ salts in PBS at pH 8 B) Dose calibration curve, inner Figure; the linear range for catechol concentration between 5 µM to 300 µM

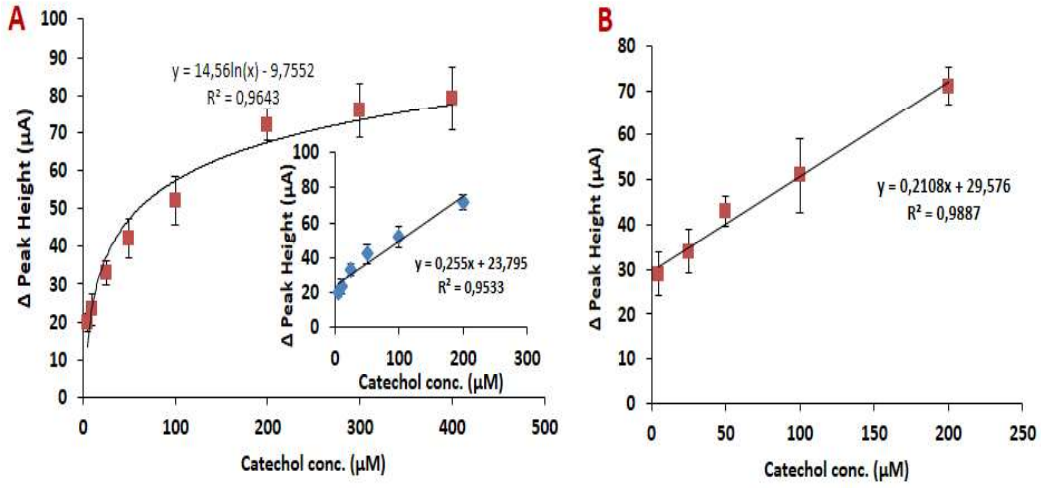

Figure 3.23 A) Catechol detection for Pt SPE containing novel organoboron monomer electropolimerization in NaF and dose calibration curve, inner Figure; the linear range for catechol concentration between 5 μM to 100 μM B) Catechol detection for Pt SPE containing novel organoboron monomer electropolimerization in $LiClO_4$, and dose calibration curve for catechol concentration between 5 μM to 200 μM

Biosensor specificity: To determine the specificity of the developed enzymatic biosensor for detecting catechol, different phenolic compounds such as 4-hydroxybenzoic acid, gallic acid, hydroquinone, 4-nitrophenol, phenol were evaluated on the concentration level of 200 μM. The biosensor system's responses for these compounds were compared with the results of 200 μM of catechol detection. The results in Figure 3.24, Figure 3.25, and Figure 3.26 clearly show that non-specific phenolic compounds do not generate any significant peak at the voltage that O-quinone's reduction peak located. Therefore, the developed biosensor system is quite specific for only catechol detection.

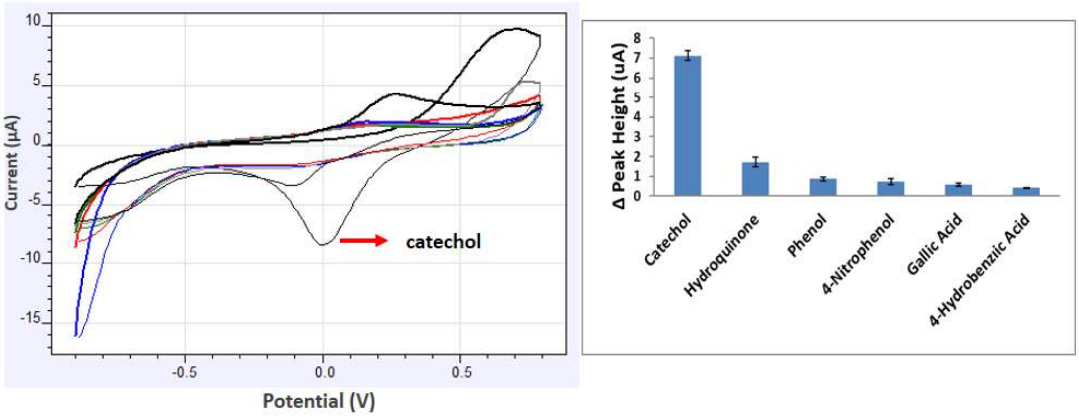

Figure 3.24 The biosensor system's response and selectivity for different phenol compounds (200 µM) on GCE with PBS at pH 8

As seen here, when looking at the selectivity for these phenol compounds, it was seen that the Tyrosinase enzyme worked only for catechol with a large and significant difference. Therefore, the Tyr-GCE/Pt SPE-based biosensor's sensitivity is the biggest for the determination of phenol derivatives.

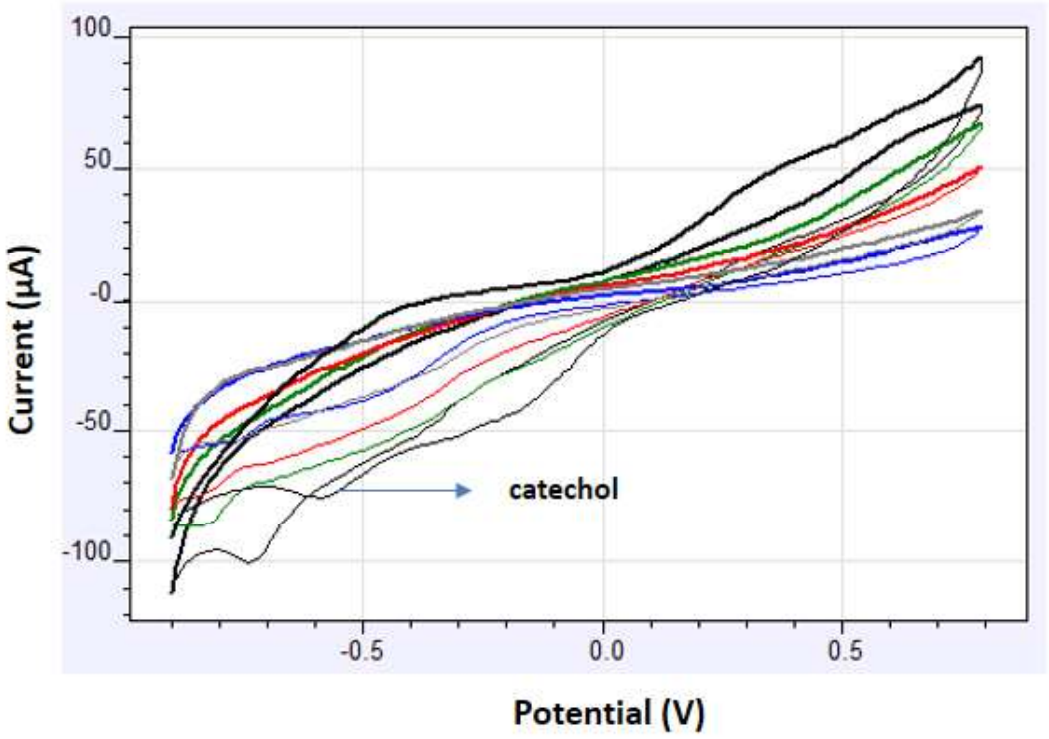

Figure 3.25 The biosensor system's response and selectivity for different phenol compounds (200 µM) on Pt SPE containing novel organoboron monomer electropolymerization in $LiClO_4$

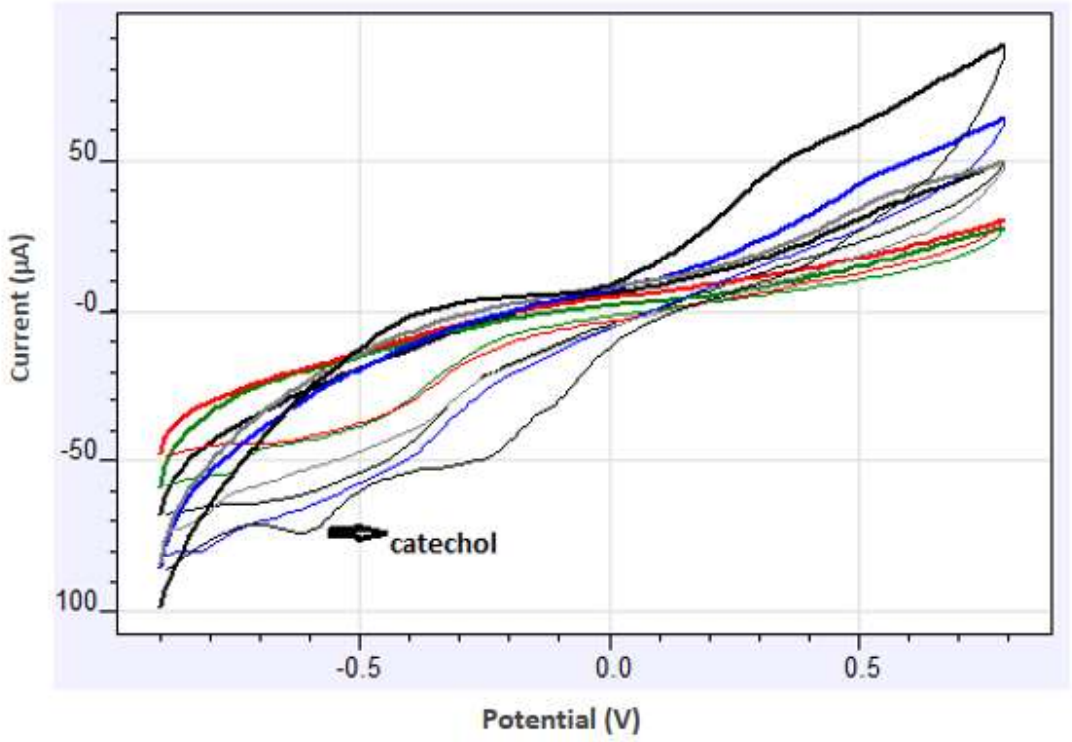

Figure 3.26 The biosensor system's response and selectivity for different phenol compounds (200 µM) on Pt SPE containing novel organoboron monomer electropolymerization in NaF

3.3.3 Real Sample Testing

To show the biosensor's ability for the determination of catechol in tea samples under the optimized conditions. 50 µM,100 µM, and 200 µM spiked tea samples were tested with developed enzymatic biosensor systems, and the results were summarized in Table3.3. The recovery of all measured samples was between 113.6% and 118.7% for $LiClO_4$, between 116% and 122.2% for NaF, and the parallel tests showed that sd % deviation between 1.7% and 9.1% for $LiClO_4$, and between 2.5% and 5.1% for NaF. These results indicated that the possible interference from the different background composition of real samples was within 5% and is considered acceptable for food quality monitoring tests.

Table 3.3 Real green tea testing with Pt SPE after electropolymerization in novel organoboron monomer and $LiClO_4$ and NaF, respectively

Added catechol conc.	**Found catechol conc.**	**Recovery %**	**sd %**
50	56.8	113.6	1.7
100	118.7	118.7	9.1
200	234.2	117.1	4.2
50	58	116	5.1
100	122.2	122.2	2.5
200	242	121	4.1

CHAPTER 4

CONCLUSION

In this master thesis, three different study which are PANI based system, boric acid doped PANI based system and novel organoboron polymer based biosensor system was developed. It should be noted that conductive polymers possess a high conductivity/weight ratio, unique chemical properties, well electrical and optical features, and permit perfect control of the electrical stimulus. A conductive polymer's main feature is that there are conjugated (sequentially ordered) double bonds along the polymer's backbone (main chain). Because of its excellent electrochemical properties, polyaniline (PANI) is one of the most favored and well-known conductive polymers for biosensor design. PANI is an efficient conducting platform for biosensor design because of its excessive redox behavior and its ability to mediate the electron transfer between the reaction site and the electrode surface.

In this thesis, using the direct electropolymerization (one-step) method, an electrochemical enzymatic biosensor system was developed with PANI film-coated C SPEs, and PABA film-coated Au SPEs/GCE, and novel organoboron polymer film-coated Pt SPEs/GCE. Electropolymerization of aniline, 3-aminophenylboronic acid and novel organoboron monomer was performed and developed using different electropolymerization conditions. It should be noted that, especially, novel boron-containing monomer, which was synthesized by BOREN for the first time within the proposed project's scope, were used in enzymatic biosensor systems. Therefore, it will be a pioneering work and will contribute scientifically by making a patent application.

The film surface morphologies were prepared with different processes, and the surface morphology was characterized by Scanning Electron Microscope (SEM). A highly stable and efficient catechol biosensor was prepared by immobilizing tyrosinase (Tyr) enzymes into the PANI film in conjunction with cross-linking with glutaraldehyde. In this thesis, with the developed biosensor system, the phenolic components were tested in the linear range between 1 μM to 200 μM with different

electrodes (in Table 4.1). After the biosensor performance conditions optimization, real sample analysis was also performed for controlled catechol added green tea samples with 3 % to 10 % range of standard deviation results. Furthermore, it should be noted that the developed biosensor system can be designed and commercialized as a portable end product that allows real-time detection. Phenolic compounds, which are determined to determine the antioxidant and antimicrobial activities of natural foods, are partly made within the scope of quality control analysis, and the developed organoboron polymer-based biosensor system will allow faster, cheaper, precise, and real-time tests. A combined comparison table for the current catechol detection based biosensor systems were represented in Table 4.1. Compared to other reported works for catechol detection, our results demonstrate more well sensitivity, linear detection range and stability efficiency than most of them. In addition, it should be noted that using the portable potentiostat and the screen printed electrodes, this developed enzymatic biosensor system can potentially analyze catechol detection in real samples. Thus, the study's future perspective could be developing a portable, easy-to-use prototype product for real sample testing.

Table 4.1 A combined comparison table for the current catechol detection based biosensor systems

Detection System	**Biosensor system**	**Detection range**	**Stability/ Reusability**	**Real sample testing**	**Ref.**
Amperometric Sensor	SWCNTs and PANI modified enzymatic system.	0.25 µM to 92 µM	24 days and over 10 times usage	Bear samples and river samples	[86]
Amperometric Sensor	PANI modified enzymatic system	5.0 µM to 165 µM	25 days and over 20 times usage	green tea samples	[87]
Amperometric Sensor	Fe3O4/ Polyaniline/ Laccase/Chitosan Biocomposite-Modified enzymatic biosensor system	0.5 µM to 80 µM	Not Specified	tea leaf samples	[88]
Electrochemical impedance spectroscopy (EIS)	PANI modified enzymatic system	1.0 µM to 100 µM	5 months	Wastewater samples	[89]

Amperometric Sensor	PANI copolymer-based enzymatic system	5.0 µM to 80 µM	over 120 times	Not Specified	[90]
Potentiometric Sensor	Polypyrrole film based enzymatic system	1 µM to 16 µM	1 month	Not Specified	[91]
Cyclic voltammetry Sensor	Carbon nanofibers used an enzymatic system	1 µM to 310 µM	30 days	Water samples	[92]
Differential Pulse voltammetry Sensor	Graphene oxide–metal oxide-PEDOT-enzyme modified System	0.4 µM to 62 µM	75 days	Green tea sample	[93]
Square-wave voltammetry Sensor	Gold nanoparticle on zein ultrafine fibers based enzymatic system	0.166 µM to 7 µM	30 days	Not Specified	[94]
Amperometric Sensor	Nitrogen-containing ordered mesoporous carbon (N-OMC) based enzymatic system	0.39 µM to 8.98 µM	30 days	wine samples	[95]
Voltammetric Sensor	PANI based enzymatic system	10 µM to 300 µM	Over 30 times in 2 weeks	Green tea sample	This work
Voltammetric Sensor	Boric acid doped PANI based enzymatic system	1 µM to 200 µM for GCE, 10 µM to 400 µM for Au SPE	Over 30 times in 2 weeks	Green tea sample	This work
Voltammetric Sensor	Novel organoboron polymer based enzymatic system	5 µM to 100 µM for Pt SPE, 5 µM to 300 µM for GCE	Over 30 times in 2 weeks	Green tea sample	This work

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