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Synthesis, electrochemical characterization and biosensor application of a novel organoboron monomer

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ABSTRACT

In this work, a novel organoboron-based monomer was synthesized and applied to enzymatic biosensor systems. Utilizing the direct electropolymerization (one-step) method, an enzymatic and electrochemical biosensor system was developed by a novel organoboron polymer film-coated platinum screen-printed electrode/glassy carbon electrode. Electropolymerization of novel organoboron monomer was carried out through several electropolymerization situations, and the most suitable conditions for biosensing systems were concluded. Organoboron polymer-based enzymatic and electrochemical analysis developed in work was used to determine catechol, which is one of the most analyzed phenolic compounds in the chemistry and agriculture industry. The established biosensing system tested the phenolic components in the linear range between 5 µM to 300 µM with different electrodes. After the biosensor performance conditions optimization, real sample analysis was also achieved for spiked green tea samples with 3% to 10% range of standard deviation results.

1. Introduction

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, 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 [1,2]. Organoboranes obtained by adding 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 [3]. 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 and lesser-known properties such as conductivity and sensor signal amplification in electrochemical applications [2]. By combining these advantages that organoboron polymers provide to electrochemical systems and the biocompatibility features possible in the enzyme immobilization step, the requirements for electrochemical enzyme biosensors will be completed.

Organoboron compounds play a significant role in organic conversion as catalysts and cocatalysts, involving polymerization reactions. However, the synthesis of organoboron polymers has been a difficult task that has solely recently been 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 [4-8].

The incorporation into polymer structures of electron-deficient boron centers is especially interesting as it provides, for example, an opportunity to manipulate the polymers by donor-acceptor bonding. For the design of new assisted reagents, immobilized catalysts, and highly selective sensor materials, the attachment of nucleophiles to organoboron polymers can be exploited [9].

Organoboron polymers are flexible and high-performance platforms for realizing functional materials with

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multifunctions. The majority of boron compounds have adequate stability to be handled under ordinary conditions, and the complexes of organoboron have flexibility in their molecular structure [2].

Furthermore, In the area of sensor materials, the inclusion of Lewis acidic organoboron moieties into conjugated polymers was indicated to cause sensor signal amplification influences such as improved stability and recoverability [1]. Nowadays, conductive polymers and organoboron-based polymers by electropolymerization have recently taken the scientific community's consideration, and they have also studied them in recent years.

The purpose of this study 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 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 TENMAK, Boron Research Institute and given to be used in this study. First, the determining 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 was 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 product's properties 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 materials science and biotechnology.

Analysis of phenolic compounds (e.g., catechol) for which tyrosinase enzyme was used as the sample detection system was developed. The phenolic component in natural foods was verified in the direct range of 10-80 μM to 5-60 μM with similar systems [10]. Considering these references, it is aimed to make determinations at similar intervals with the proposed biosensor system. Therefore, diluted and controlled catechol added food samples were analyzed with at least a margin of error [11]. 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 can be designed and commercialized as a portable end product that allows real-time detection.

Novel organoboron monomer, synthesized by TENMAK, Boron Research Institute for the first time within the proposed project's scope, was used in enzymatic biosensor systems. Therefore, it could be a pioneering work and 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 optimizing biosensor performance conditions. It had better not be forgotten that using the portable potentiostat and these electrodes, this established enzymatic biosensing system has the potential for on-site analysis of catechol analysis in real samples. Therefore, the future perspective of the study can be developing an easy-to-use and portable prototype invention for real sample detection.

2. Materials and Methods

2.1. Reagents

Catechol, acetonitrile (ACN), Tyrosinase enzyme from mushroom (Tyr, EC:1.14.18.1), Sodium fluoride (NaF), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), Tetra butyl ammonium hexafluorophosphate (TBAHFP) and chitosan 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 and was 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.

2.2. Synthesis of Novel Organoboron Monomer

Phenyl boronic acid (0.01 mol, 1.21 g) and catechol (0.01 mol, 1.10 g) were added into 100 mL of round flask bottom flask attached with dean-stark apparatus. Toluene was used as a solvent (115 mL). The reaction medium was set to 95°C after the dean-stark apparatus obtained the side product. The reaction was finished after 15 hours. The synthesis mechanism of the novel organoboron monomer is represented in Figure 1. The NMR analysis results for the synthesized novel monomer were observed as 1H NMR(CDCl3) 7.75-7.3 (m, 5H) 4.07 (t, 4H, J=15.12,15.13).

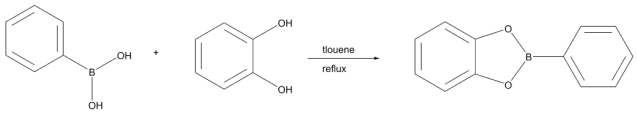

Figure 1. The synthesis mechanism of the novel organoboron monomer.

2.3. Electropolymerization of Novel Organoboron Monomer

GCE: The electrochemical measurements were recorded using a conventional three-electrode system consisting of a 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, 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 CE, Pt coated plate as 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 (CV) scans were performed between -0.5 V to 1.2 V within the GCE and Pt SPE electrodes' potential ranges until stable curves of CV profiles were 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 $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$.

2.4. Surface Characterization

For Scanning Electron Microscope (SEM), HITACHI SU 5000 was used to analyze the electrodes' surfaces after the electropolymerization. Surface characterization of Pt-SPE electrodes was evaluated for the novel organoboron monomer's electropolymerization in different salt solutions.

2.5. Preparation and Optimization of the Biosensor Performance Conditions

Afterward, the novel organoboron monomer modification of the electrodes enzyme immobilization was done using chitosan as an immobilization agent. A combination of 40 µL of 1 mg/mL Tyr and 2% chitosan in acetic acid was produced for enzyme immobilization. Following that, part of the mixture dispersion was cast onto the surface of these electrodes, allowing the solvent to evaporate at room temperature while the mixture was allowed to dry on the surface.

An electrochemical enzymatic biosensor system was developed using the direct electropolymerization procedure to immobilize Tyr enzyme into the organoboron polymer film in conjunction with chitosan, resulting in a very stable and effective catechol biosensor. Both for GCE (in PBS solution at pH 8) and Pt SPEs, the biosensor was used to determine catechol by voltammetric measurements in the steady-state condition at an applied potential of -0.9 V to 0.8 V in different concentrations of catechol (from 1 µM to 400 µM) respectively (in pH 7.5 PBS solution).

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

At a concentration level of 200 µM, several phenolic compounds such as 4-hydroxybenzoic acid, gallic acid, hydroquinone, 4-nitrophenol, and phenol were tested to assess the specificity of the proposed enzymatic biosensor for catechol detection.

To assess the possible matrix influence of actual samples on biosensor performance, spiked green tea samples with varying catechol (50 µM, 100 µM, and 200 µM) were evaluated after the working parameters of the designed biosensor system were optimized.

3. Results and Discussion

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 TBAH-

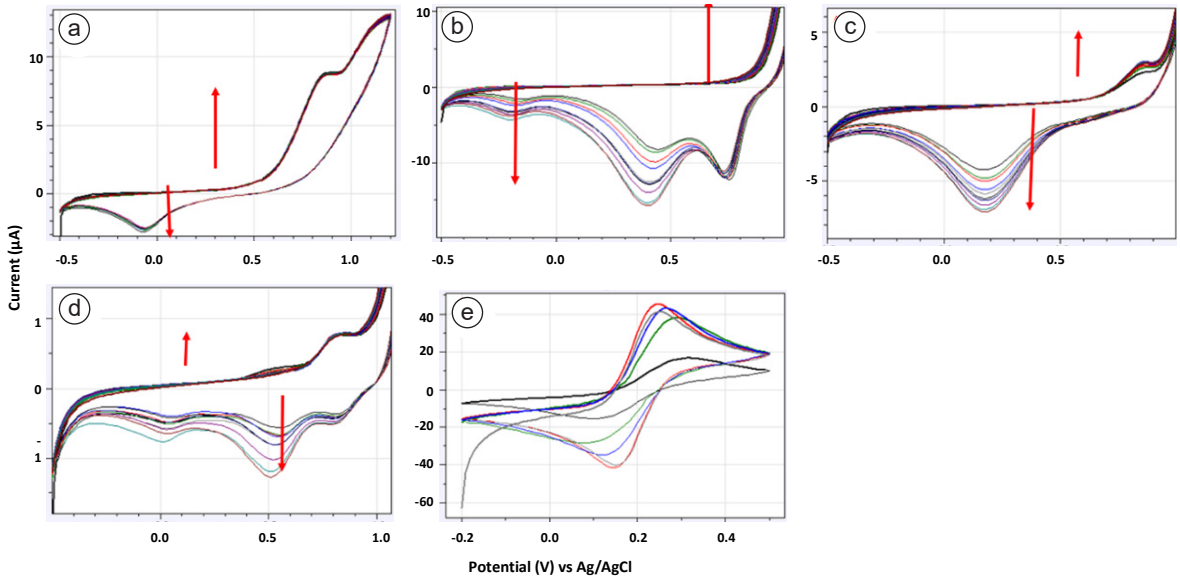

Figure 2. Electropolymerization of novel boron containing monomer on different conditions using GCE (a) in NaF (b) in $LiClO_4$ (c) in TBTU (d) in TBAHFP (e) $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox molecule CV after electropolymerization (black: blank, green: in NaF, red: in $LiClO_4$, blue: in TBTU, gray: in TBAHFP).

FP, respectively, and novel boron-containing monomer in the ACN solution. Since ACN is an organic solvent and does not have an ionic content, the ionic solvent addition was needed to perform the electropolymerization process. Therefore, different salts were added to the solution each time to form ions. Electrodes were scanned in the potential range of 0.5 V to 1.2 V for GCE and Pt SPEs during the electropolymerization process until steady CV curves were produced, as illustrated in Figures 2 and 3. The new boron-containing monomer solution's anodic and cathodic peak currents grew dramatically with each scan until the tenth scan. Therefore, these results proved that electropolymerization of this novel organoboron monomer was carried out successfully.

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 Figures 4 and 5, CVs were recorded at a different scan rate of 150 $mV.s^{-1}$, 125 $mV.s^{-1}$, 100 $mV.s^{-1}$,75 $mV.s^{-1}$,50 $mV.s^{-1}$, 25 $mV.s^{-1}$, and 10 $mV.s^{-1}$ respectively in FeCN. It should be noted that according to the results, the cathodic peak current increased with the scan rates for CV profiles. As seen in Figure 5c, af-

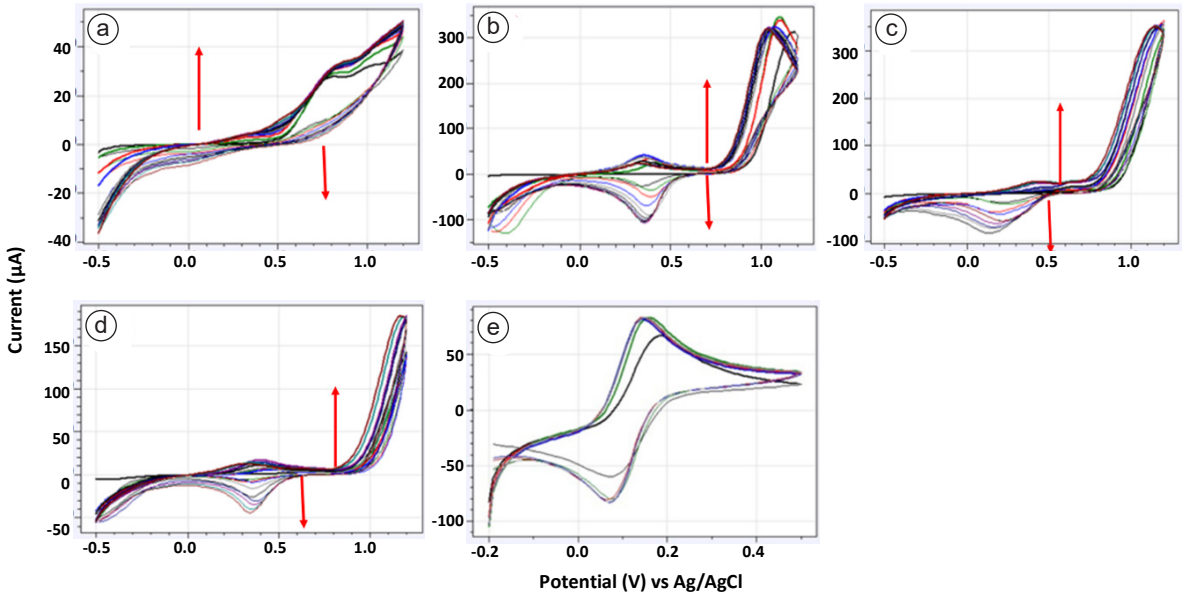

Figure 3. Electropolymerization of novel boron containing monomer on different conditions using Pt SPE (a) in NaF (b) in $LiClO_4$ (c) in TBTU (d) in TBAHFP (e) $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox molecule CV after electropolymerization (black: blank green: in NaF, red: in $LiClO_4$, blue: in TBTU, gray: in TBAHFP).

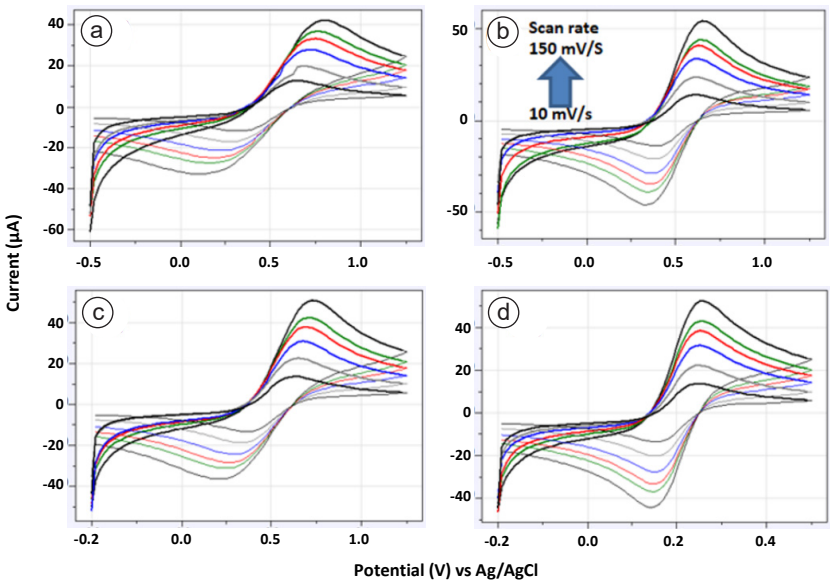

Figure 4. After electropolymerization of novel boron containing monomer, CVs were recorded at different scan rate of 10 mV s^{-1}, 25 mV s^{-1},50 mV s^{-1},75 mV s^{-1}, 100 mV s^{-1}, 125 mV s^{-1},150 mV s^{-1} as different peak height in FeCN for GCE (a) NaF (b) $LiClO_4$ (c) TBTU (d) TBAHFP.

ter 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 proved that increasing scan rate means there is something active on the surface; the more conductive the surface becomes, the better the FeCN peaks, even at the empty electrode, and electropolymerization has occurred on the surface.

SEM analyses were used to analyze the surface morphology of the bare and new organoboron monomer modified SPEs based on various salt solutions (Figure 6a-e). Depending 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 densest and uniform one, as seen in Figure 6c.

3.2. Biosensor Performance Development

pH Optimization: The enzymatic biosensor system was evaluated in PBS solution at multiple pH values (pH 6 to 8) to find the best circumstances, including 200 µM catechol. As shown in Figure 7a, Pt SPEs were submerged in a solution comprising various salts based on electropolymerized Pt SPE, and the best pH was found to be pH 7.5. Similarly, the GCE electrode was treated in the same way. The GCE electrode containing the new monomer and $LiClO_4$ salt was submerged in solutions with various pH values, as shown in Figure

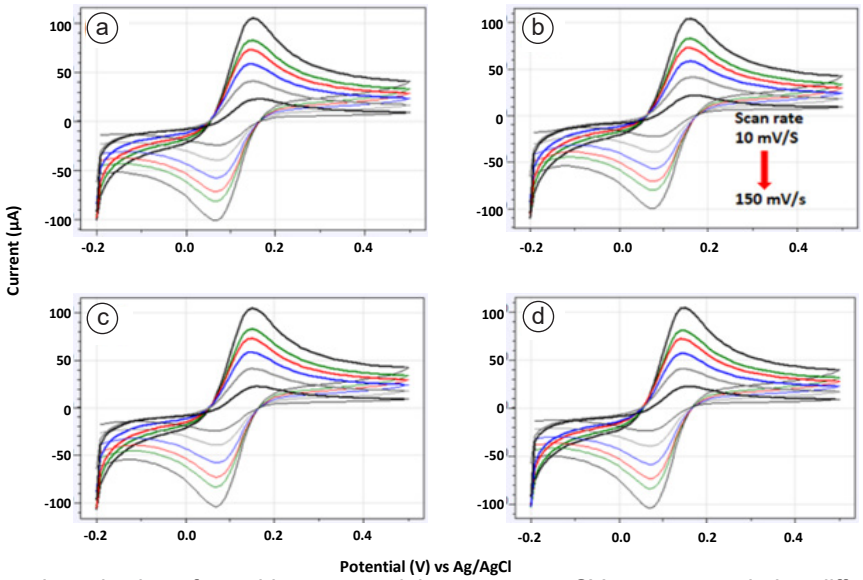

Figure 5. After electropolymerization of novel boron containing monomer, CVs were recorded at different scan rate of 150 $mV.s^{-1}$, 125 $mV.s^{-1}$, 100 $mV.s^{-1}$,75 $mV.s^{-1}$,50 $mV.s^{-1}$, 25 $mV.s^{-1}$, and 10 $mV.s^{-1}$ as different peak height in FeCN for Pt SPE (a) NaF (b) $LiClO_4$ (c) TBTU (d) TBAHFP.

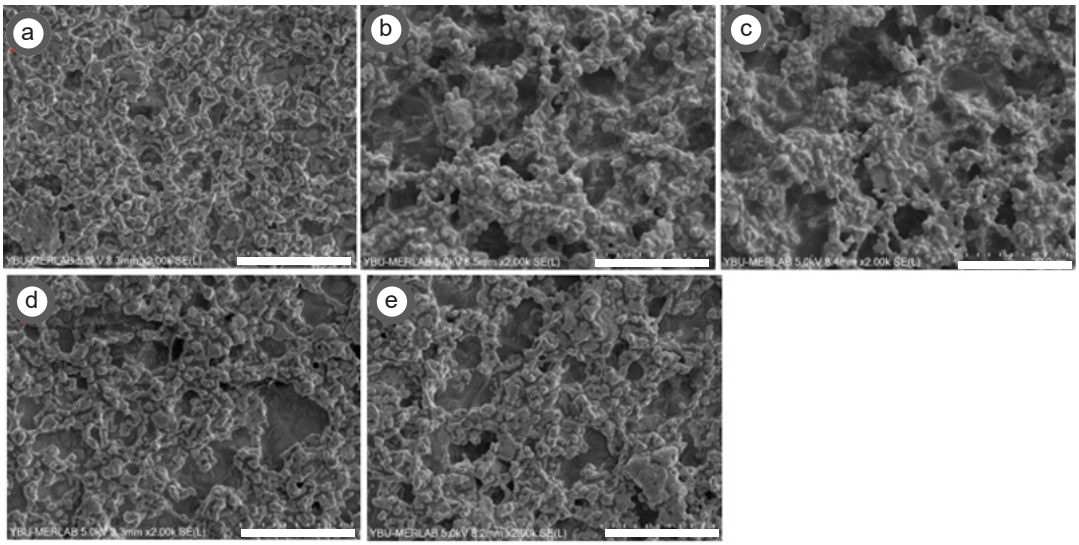

Figure 6. SEM images of boron monomer modified SPE Platinum electrodes electropolymerized in ACN and various salts: (a) Blank, (b) NaF, (c) $LiClO_4$, (d) TBTU, and (e) TBAHFP (Scale bar:20 µM) .

7b, and pH 8 was the best value.

Dose-response curve: Voltammetric measurements were performed for both GCE and Pt SPEs in the applied potential range of -0.9 V to 0.8 V using different concentrations of catechol (0 µM to 400 µM) in a 10 mL pH 8 buffer solution. The reduction peak for catechol's enzymatic reaction's o-quinone product was observed, and the peak heights for each concentration were calculated. Based on unique boron monomer modified electrodes, the linear range for both GCE and Pt SPEs in various salt solutions was determined between 5 µM and 300 µM for GCE (Figure 8) and between 5 µM and 100 µM (Figure 9a). In addition, when catechol concentration grew, the decreased (cathodic) peak current of the new boron-containing monomer increased. The limit of detections (LOD) for both electrode systems were calculated with the 3 times the standard deviation of the signals obtained from the blank standards rule [13], and the results were 2.25 µM and 1.8 µM, respectively for Pt SPE and CGE systems.

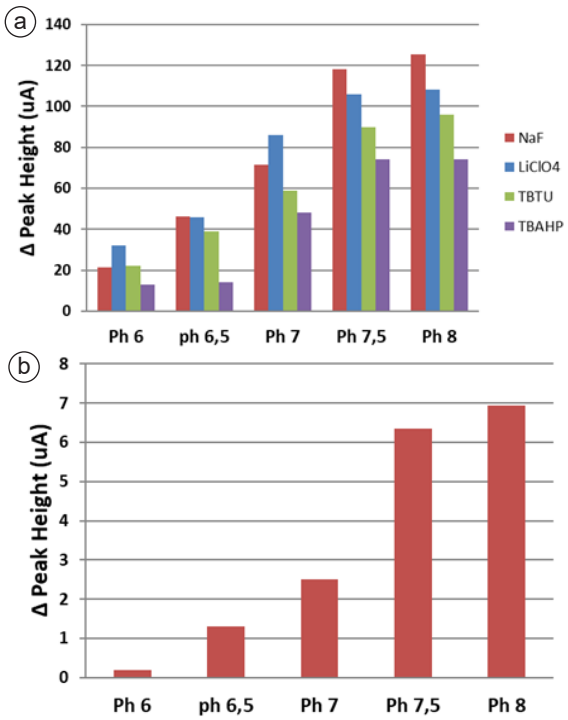

Figure 7. (a) The influence of pH on biosensor performance was investigated by incubating Pt SPE electrodes with various salts in 200 µM catechol solution buffered (10 ml PBS) at the pH range of 6-8. (b) By incubating the GCE electrodes containing the new boron monomer $LiClO_4$ in 200 µM catechol solution buffered (10 ml PBS) at pH 6-8, the influence of pH on the performance of the biosensor was investigated.

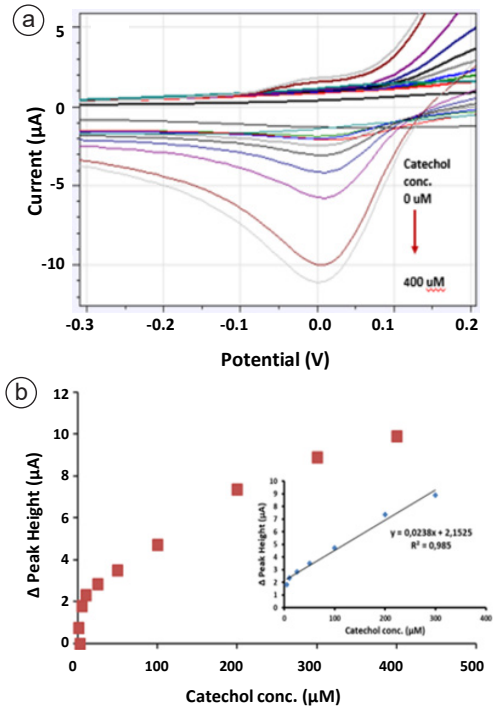

Figure 8. (a) Catechol detection for the GCE containing novel boron monomer and $LiClO_4$ salts in PBS at pH 8, (b) Dose-response curve for catechol concentration between 5 µM to 300 µM, and the inner figure is the linear range.

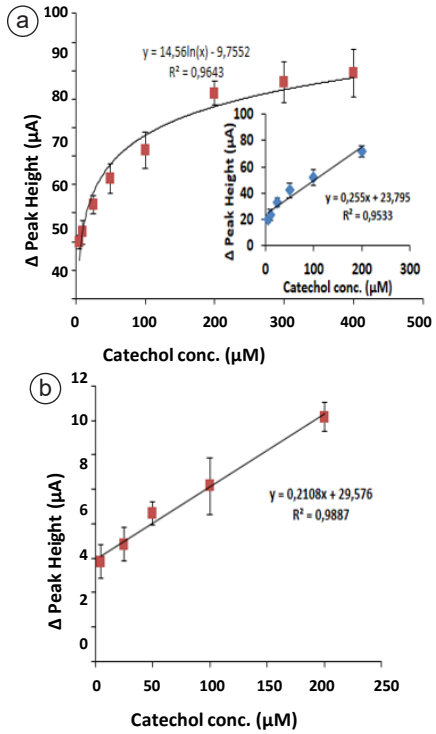

Figure 9. (a) Catechol detection for Pt SPE containing novel boron monomer in NaF and dose calibration curve for catechol concentrations between 5 M and 100 M, (b) Catechol detection for Pt SPE containing novel boron monomer in $LiClO_4$ and dose calibration curve for catechol concentrations between 5 μM and 200 μM.

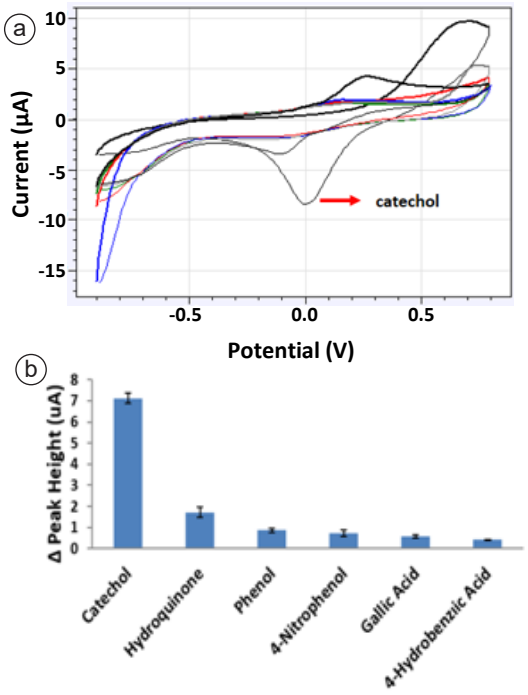

Figure 10. The sensitivity (a) and selectivity (b) of the biosensor device for various phenol compounds (200 μM) on GCE with PBS at pH 8 (black: catechol).

At a concentration level of 200 μM, several phenolic compounds such as 4-hydroxybenzoic acid, gallic acid, hydroquinone, 4-nitrophenol, and phenol were tested to assess the specificity of the proposed enzymatic biosensor for catechol detection. The biosensor system's reactions to these compounds were compared to catechol detection results at 200 μM. Figures 10 showed that non-specific phenolic compounds did not produce any significant peaks at the voltage where the O-reduction quinone's peak is located on the GCE system. However, on the Pt SPE system, we could observe that non-specific phenolic compounds still had some background signals, and they were potential for interference to the target molecule signal (Figures 11a and 11b). Thus we can conclude that the GCE system was more selective than the Pt SPE system for catechol detection.

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 determining phenol derivatives.

3.3. Real Sample Assessment

To show that the biosensor could detect catechol in tea samples under ideal conditions. Table 1 summarizes the results of using proven enzymatic biosensor equipment to evaluate 50 μM, 100 μM, and 200 μM spiked tea samples. In parallel testing, $LiClO_4$ recovery was between 113% and 118%, and NaF recovery was between 116% and 121%, with the percentage of standard deviation (sd) between 4.2% and 1.7% for $LiClO_4$ and between 2.5% and 5.1% for $LiClO_4$. According to these data, the potential influence from the

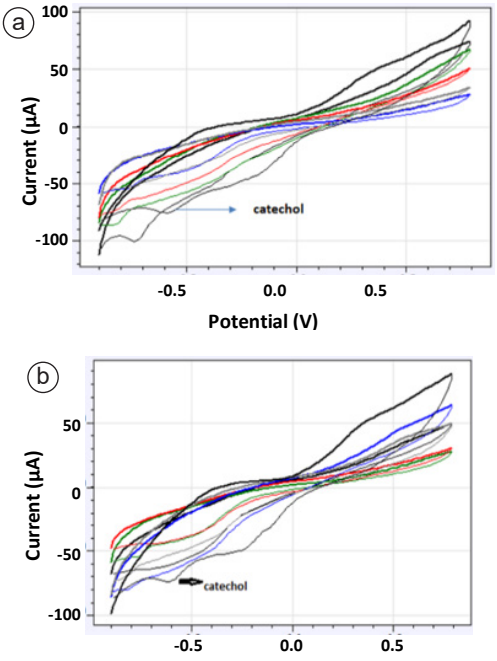

Figure 11. (a) Pt SPE containing novel boron monomer other phenolic compounds in $LiClO_4$. (b) Pt SPE containing novel boron monomer with other phenolic compounds in NaF.

varied background composition of authentic samples was less than 5%, which is acceptable for food quality monitoring tests.

Table 1. Real green tea assessment with novel boron monomer modified Pt SPE with $LiClO_4$ and NaF, as an electropolymerization salt, respectively.

Added Catechol Conc. (µM)	Found Catechol Conc. (µM)	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

4. Conclusions

In this study, using the direct electropolymerization (one-step) method, an electrochemical enzymatic biosensor system was developed with novel organoboron polymer film-coated Pt SPEs/GCE. Electropolymerization of novel organoboron monomer was performed and developed using different electropolymerization conditions. It should be noted that this novel boron-containing monomer, which Boron Research Institute synthesized for the first time within the proposed project's scope, was used in enzymatic biosensor systems. Therefore, it could be a pioneering work and 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). Immobilizing tyrosinase (Tyr) enzymes into the conducting polymer film, and chitosan resulted in a stable and effective catechol biosensor. The phenolic components were examined in the linear range between 1 µM and 200 µM with various electrode systems in this study using the proposed biosensor system. Following the tuning of biosensor performance settings, actual sample analysis was carried out on controlled catechol added green tea samples with standard deviations ranging from 3% to 10%. 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 natural foods' antioxidant and antimicrobial activities, are partly made within the scope of quality control analysis. The developed organoboron polymer-based biosensor system will allow faster, cheaper, precise, and real-time tests. Furthermore, this designed enzymatic biosensor system may theoretically test catechol detection in actual samples utilizing the portable potentiostat and screen-printed electrodes. As a result, the study's long-term goal might be to create a portable, easy-to-use prototype device for real-world testing.

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References

[1] Jäkle, F. (2006). Lewis acidic organoboron polymers. *Coordination Chemistry Reviews*, *250*(9-10), 1107-1121.

[2] Tanaka, K., & Chujo, Y. (2012). Advanced luminescent materials based on organoboron polymers. *Macromolecular Rapid Communications*, *33*(15), 1235-1255.

[3] Vedejs, E. (1980). The 1979 Nobel Prize for Chemistry. *Science*, *207*(4426), 42-44.

[4] Yamamoto, H. (1999). *Lewis acid reagents: A practical approach*. Oxford University Press, .

[5] Chung, T. C., & Janvikul, W. (1999). Borane-containing polyolefins: synthesis and applications. *Journal of Organometallic Chemistry*, *581*(1-2), 176-187.

[6] Boffa, L. S., & Novak, B. M. (2000). Copolymerization of polar monomers with olefins using transition-metal complexes. *Chemical Reviews*, *100*(4), 1479-1494.

[7] Kondo, Y., García-Cuadrado, D., Hartwig, J. F., Boaen, N. K., Wagner, N. L., & Hillmyer, M. A. (2002). Rhodium-catalyzed, regiospecific functionalization of polyolefins in the melt. *Journal of the American Chemical Society*, *124*(7), 1164-1165.

[8] Qin, Y., Cheng, G., Sundararaman, A., & Jäkle, F. (2002). Well-defined boron-containing polymeric lewis acids. *Journal of the American Chemical Society*, *124*(43), 12672-12673.

[9] Jäkle, F. (2006). Lewis acidic organoboron polymers. Coordination *Chemistry Reviews*, *250*(9-10), 1107-1121.

[10] Zoral, F., & Turgay, Ö. (2014). A Research on Total Phenolic Content, Antioxidant Activity and Antimicrobial Effects of Various Food Wastes. *Journal of Agriculture and Nature*, *17*(2), 24-33.

[11] Sadeghi, S., Fooladi, E., & Malekaneh, M. (2015). A new amperometric biosensor based on Fe3O4/polyaniline/laccase/chitosan biocomposite-modified carbon paste electrode for determination of catechol in tea leaves. Applied Biochemistry and Biotechnology, 175(3), 1603-1616..

[12] Kalkan, Z., Yence, M., Turk, F., Bektas, T. U., Ozturk, S., Surdem, S., & Yildirim-Tirgil, N. (2022). Boronic Acid Substituted Polyaniline Based Enzymatic Biosensor System for Catechol Detection. *Electroanalysis*, *34*(1), 33-42.

[13] Armbruster, D. A., & Pry, T. (2008). Limit of blank, limit of detection and limit of quantitation. *The Clinical Biochemist Reviews*, *29*(Suppl 1), S49.