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RESEARCH ARTICLE

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D-A-D type functional conducting polymer: Development of its electrochromic properties and laccase biosensor

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Abstract

Herein, a novel D-A-D type monomer that contains hexylthiophene as the donor and benzo[c][1,2,5]thiadiazole as the acceptor has been electropolymerized successfully to yield P(DTFBT) and its electrochromic properties and performance in amperometric biosensors have been investigated. The polymer shows great electrochemical features with low band gap and multichromic properties. Additionally, after polymer modification, laccase was immobilized on a modified electrode surfaces for catechol sensing. Biosensor optimization values, cycle number, enzyme amount, pH and also interfering effect of some possible compounds were investigated. With optimum values, analytical characterization of the sensor was determined. Kmapp, LOD and sensitivity values were estimated as 0.11 mM, 0.014 mM and 166.74 μA/(mM cm^2), respectively. The experimental results revealed that the sensor shows satisfactory accuracy and confirms the proposed sensor has a potential candidate for catechol quantification.

KEYWORDS

biosensor, catechol, conjugated polymer, electropolymerization, laccase

1 | INTRODUCTION

Highly toxic phenolic compounds are come from pesticides, dyes and pharmaceuticals and these types of compounds inflict both severe and long-lasting effects on both humans and animals.[1,2] One of the phenolic compounds family, catechol act as carcinogens and causes damage to the red blood cells and the liver.[3] Thus, their quantitative detection is significant to develop a facile and low-cost method. Up to now, different techniques have been used for catechol detection, including spectrophotometry and high-performance liquid chromatography.[4,5] But these techniques have not been considered as effective methods because of their high cost, time-consuming and requiring highly equipped person quality.[6]

Designing electrochemical biosensors are a very remarkable alternative to classical methods. Their quite simple manufacturing procedure are highly fast and sensitive properties makes them very useful for catechol detection.[7] One of these biosensor compounds is the laccase enzyme, (benzenediol: oxygen oxidoreductases, EC 1.10.3.2), known as a multi-copper oxidase and is commonly used in the construction of various biosensors.[8–11] To make enzyme molecules on the electrode surface more stable, efficient materials and immobilization strategies are necessary.

Conjugated polymers with their alternating double and single bonds are considered as one of the most suitable materials in various scientific fields of electrochromic device construction,[12,13] solar cell,[14] supercapacitor,[15] artificial muscle, light emitting diodes,[16] and biosensors.[17] In the last few decades, variable conjugated polymers with tunable electronic and optical properties have been synthesized via Suzuki or Stille cross-coupling polymerization reactions. Unfortunately, these chemical polymerization methods involve numerous synthetic steps. In this manner, a better alternative might be electrochemical polymerization with low cost and shorter reaction time advantages.[18] This polymerization method also gives the opportunity to the preparation of polymers for chemical sensors.[19–22] Among conjugated structures, benzothiadiazole is one of the most widely studied acceptor units and variable derivatives are developed for enhancing properties.[23] Fluorination is one of them and causes differences in electrochemical properties. Lowering HOMO and LUMO energy levels and increase in oxidation potential are observed in the literature.[24,25] Benzothiadiazole based polymers are applicable for the immobilization of glucose oxidase and alcohol oxidase in the fabrication of biosensors by our group.[26,27] In this study, fluorinated benzothiadiazole for biosensor is offered. Using this functional fluorine moiety on the polymer structure brings hydrogen bond formation between enzyme molecules and polymer. In addition to this binding, hydrophobic side chains of both polymer and enzyme molecules interact to contribute the efficient enzyme immobilization.

Herein, a novel monomer, 5-fluoro-4,7-bis (4-hexylthiophen-2 yl) benzo [c][1,2,5]thiadiazole (DTFBT), containing donor and acceptor functional groups in the same structure was electrochemically polymerized. Electrochemical and spectroelectrochemical properties of the synthesized electrochromic polymer have been investigated. It was determined that the conjugated polymer has a low band gap, low response time and showed multichromic properties. Then, a new amperometric biosensor is developed based on conductive polymer for the detection of toxic phenolic compounds. The polymer, poly(5-fluoro-4,7-bis (4-hexylthiophen-2 yl) benzo [c][1,2,5]thiadiazole (poly (DTFBT)), was deposited onto the graphite electrode surface by cyclic voltammetry technique. Laccase was immobilized on the polymer modified graphite electrode. The Optimum pH, film thickness and enzyme amount of the electrodes were optimized and analytical parameters of the biosensor were evaluated. The biosensor was characterized by scanning electron microscopy (SEM) and cyclic voltammetry (CV) techniques.

2 | MATERIALS AND METHODS

2.1 | Chemicals and instrumentation

All chemicals and solvents were purchased from Sigma Aldrich Chemical Co. Ltd. Triethylamine, and THF were distilled under nitrogen atmosphere before use. For purification step with column chromatography, Merck Silica Gel 60 was used. To verify structure of monomers, nuclear magnetic resonance (NMR) spectra were investigated on a Bruker Spectrospin Avance DPX-400 Spectrometer with internal reference as trimethylsilane (TMS) in deuterated chloroform ($CDCl_3$).

Laccase (oxygen oxidoreductase, EC 1.10.3.2) (21.8 U/mg) from *Trametes versicolor*, glutaraldehyde ((50%) wt in H_2O), $NaClO_4$, $LiClO_4$ were obtained from Sigma-Aldrich. Dichloromethane (DCM) and acetonitrile (ACN) were directly used without further purification. As a substrate, 0.05 M catechol solution (from Sigma-Aldrich) was prepared at room temperature.

Electrochemical polymerizations of the DTFBT monomer was performed by GAMRY Reference 600 Potentiostat/Galvanostat and Solarton 1285 using three electrode system containing platinum wire counter, ITO (indium tin oxide) working and silver wire pseudo-reference (+0.3 V vs. Fc/Fc^+) electrode. Amperometric measurements were performed using Palm Instrument (PalmSens, Houten, The Netherlands) with the same electrode configuration. Graphite electrodes (type RW001, 3.05 mm diameter and 13% porosity) were obtained from Ringsdorff Werke GmbH, Bonn, Germany and used as a working electrodes in biosensor studies. JEOL JSM-6400 models SEM was used to investigate the layers of the fabricated biosensor.

2.2 | Synthesis of DTFBT monomer

2.2.1 | Synthesis of 5-fluoro-2,1,3-benzothiadiazole

4-Fluoro-1,2-phenylamine (1.01 g, 7.93 mmol) was dissolved in chloroform ($CHCl_3$) (20 ml) and triethylamine

(4.4 ml, 31.71 mmol) mixture. After the mixture was cooled to 0°C, thionyl chloride (1.16 ml, 15.85 mmol) was added slowly. Then, the reaction was stirred at 70°C for 7 h. The mixture was allowed to cool at room temperature and extracted with DCM and water. Organic layer was dried over $MgSO_4$ and solvent was removed. The product was purified by column chromatography on silica gel (hexane/DCM: 1/3). Yield: 75% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (dd, J = 9.5, 5.2 Hz, 1H), 7.61 (dd, J = 8.8, 2.5 Hz, 1H), 7.43 (ddd, J = 9.5, 8.6, 2.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.78, 162.26, 154.79, 152.01, 122.59, 122.48, 121.49, 121.20, 104.92, 104.69.

2.2.2 | Synthesis of 4,7-dibromo-5-fluoro-2,1,3-benzothiadiazole

Br_2 (2.3 ml, 45.4 mmol) in 10 ml HBr was added slowly into 5-fluoro-2,1,3-benzothiadiazole (0.70 g, 4.54 mmol) and HBr (20 ml) mixture. Then, the reaction was heated at 120°C for 2 days. After the reaction was completed, saturated $NaHSO_3$ solution was added. The mixture was poured into water and solid was filtered. After recrystallization of it with ethanol, the product was obtained as a yellow solid. Yield: 52% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.94, 156.40, 150.35, 150.28, 147.87, 121.59, 121.27, 111.60, 111.49, 95.88, 95.64.

2.2.3 | Synthesis of 4,7-Bis(4-hexylthienyl)-5-fluoro-2,1,3-benzothiadiazole

In a 250 ml flame-dried 2-neck round-bottom flask with a condenser, 4,7-dibromo-5-fluoro-2,1,3-benzothiadiazole (0.35 g, 1.12 mmol), excess of tributyl(4-hexylthiophene-2-yl) stannane (1.17 g, 2.55 mmol) and 20 ml of dry THF were added. After the mixture was purged with nitrogen for 30 min, bis(triphenylphosphine)palladium(II) dichloride ($PdCl_2(PPh_3)_2$) (0.039 g, 0.056 mmol) was added and the reaction mixture was heated to reflux for 24 h. The reaction mixture was then cooled to room temperature and the solvent was evaporated. The product was purified by column chromatography with hexane/dichloromethane (1:1 v/v) as eluent. The solvent was evaporated and the final product was recrystallized from isopropyl alcohol as orange solid. Yield: 64% ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.99 (d, 1H), 7.74 (d, J = 13.0 Hz, 1H), 7.16 (s, 1H), 7.09 (s, 1H), 2.70 (dd, J = 15.7, 7.9 Hz, 4H), 1.78–1.63 (m, 4H), 1.45–1.29 (m, 12H), 0.91 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.46, 155.94, 151.88, 151.77, 148.10, 142.83, 141.80, 135.96, 135.93, 130.51, 130.46, 129.85, 129.77, 128.16, 124.26, 124.15, 121.27, 121.20, 121.01, 115.18, 114.85, 109.63, 109.47, 30.06, 30.04, 28.93, 28.88, 28.79, 27.40, 27.38, 20.99, 20.98, 12.44.

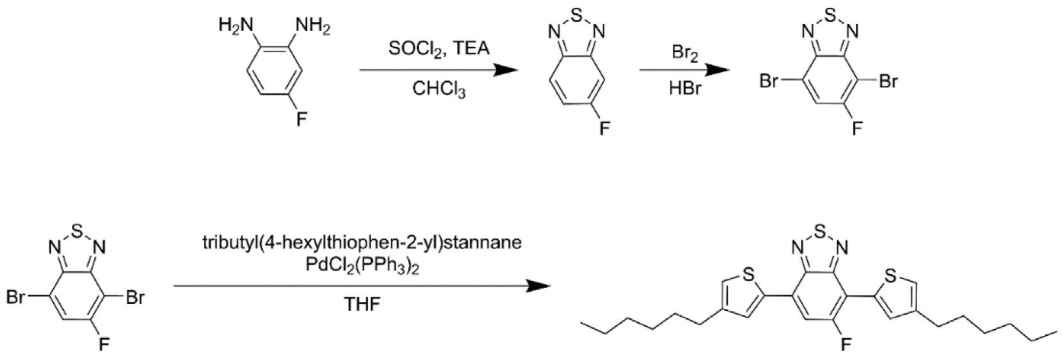
Synthesis pathway was given in Scheme 1.

2.3 | The preparation of P(DTFBT) polymer film by electropolymerization

The electrochemical polymerization of DTFBT was performed by cyclic voltammetry technique between 0.2 and 1.2 V with a scan-rate of 100 mV/s. Three electrode system was used during the electrochemical polymerization at room temperature. ITO electrode was used as the working electrode, Ag wire and Pt wire were used as reference electrode and counter-electrode respectively. To investigate the electrochemical properties of P(DTFBT), Gamry 600 potentiostat was used. Spectroelectrochemical studies of the P(DTFBT) film were performed with Varian Cary 5000 UV–vis Spectrophotometer.

2.4 | Fabrication of P(DTFBT)/Laccase biosensor and amperometric detection

Conducting polymer coated modified P(DTFBT)/Laccase biosensor was prepared as follows: Prior to use, graphite rod electrodes were carefully polished with emery paper,



SCHEME 1 Synthetic pathway of DTFBT monomer

were washed with distilled water, and was left to dry under natural conditions. Electrochemical polymerization of DTFBT was performed successfully in electrolyte solution of 0.1 M TBAP/DCM/ACN (5:95) by cycling the potential between 0.2 and 1.2 V at a scan rate of 0.1 V/s on a graphite electrode. The electrode was washed with distilled water to remove the organic impurities. After successful deposition Poly(DTFBT) by cyclic voltammetry, 10 μl freshly prepared laccase enzyme were immobilized onto the 40 cycle polymer coated graphite electrode surface with the help of the cross linking agent (gluteraldehyde (GA) 1%). Then, the modified electrode was allowed to dry for 2 h. The constructed laccase biosensor was stored at +4°C when not in use. Then, the surface was covered with 1% GA and was waited for 2 h at room temperature. The electrodes were cleaned with distilled water to get rid of both unbounded enzyme molecules and impurities.

Amperometric measurements were recorded at room temperature in a reaction cell filled with buffer under mild stirring by applying −0.3 V constant potential. P(DTFBT)/Laccase was conditioned with 50 mM acetate buffer pH 5.5 before the quantification step. Catechol was added at different concentrations on the electrochemical biosensor in drop mode and the results were recorded as current (μA). For consecutive measurements, the electrodes were washed several times with distilled water and the used buffer solution was regenerated. The responses of the biosensor were recorded and calibration plot was obtained by plotting current (μA) vs catechol concentration (mM).

3 | RESULTS AND DISCUSSION

3.1 | Electrochemical characterization of the polymer

Electropolymerization was carried out between 0.2 and 1.2 V at a scanning rate of 100 mV/s by cyclic voltammetry technique via three electrode system. During scanning in electrochemical polymerization, the polymer is coated on the electrode surface as an insoluble film in the electrolyte solution. Electrochemical polymerization of DTFBT was performed successfully in electrolyte solution of 0.1 M TBAP/DCM/ACN (5:95) as shown in Figure 1. While the synthesis of the polymer film on the ITO electrode surface, the cyclic voltammogram obtained after the first cycle belongs to the monomer. The oxidation potential of the monomer was determined as 1.1 V. During the cyclic voltammograms, increased current value responsible from the electropolymerization of DTFBT on the ITO electrode. Electroactive P(DTFBT) films were

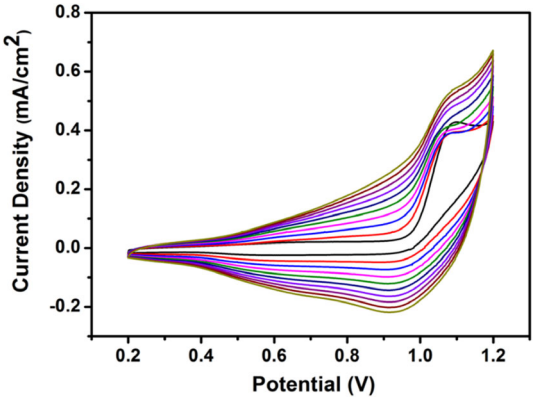


FIGURE 1 Cyclic voltammogram of electrochemical polymerization of DTFBT [Color figure can be viewed at wileyonlinelibrary.com]

obtained on the electrode surface after successive potential cycles. Electrochromic P(DTFBT) film on ITO electrode showed one anodic peak at about 1.06 V and one cathodic peak at about 0.98 V.

The scan rate dependence of anodic and cathodic peak currents of polymers were also investigated. Cyclic voltammetry measurements were carried out at scan rates between 50 and 300 V/s. Figure 2 shows the cyclic voltammograms at different scan rates for the P(DTFBT) modified electrode immerse in the blank solution. The linear dependence of current (Ip) obtained from maximum for anodic and cathodic branches vs. scan rate (V) could be evaluated as a characteristic of mass transfer in the electroactive film. Such observation indicates that, the electroactive polymers were well adhered and migration of the electroactive species were not diffusion controlled.

3.2 | Spectroelectrochemical and kinetic study

Spectroelectrochemical measurements were performed to reveal both electroactivity and electrochromic changes of the coated P(DTFBT) film. Spectroelectrochemistry is a combination of electrochemical and spectroscopic techniques performed simultaneously. For this purpose, UV–Vis–NIR spectroscopy is used to observe electronic transitions in conjugated polymers. The changes in the optical properties of the polymer film coated on the ITO were investigated by applying constant potentials in the range of 0–1.2 V as shown in Figure 3a. Thus, potential-dependent changes in the band gap (Eg), λ_{max}, polaron and bipolaron bands of the conjugated polymer were observed. The UV–vis spectrum is monitored as a function of the potential applied to the polymer films coated

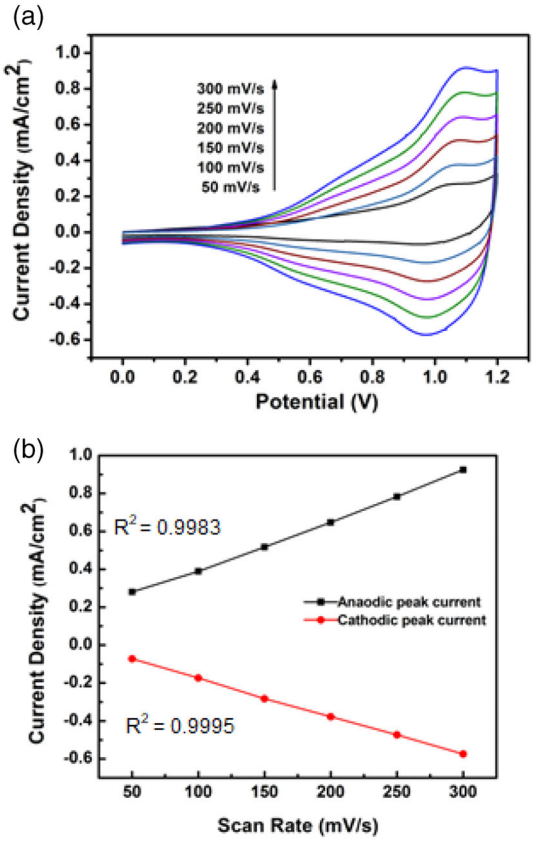


FIGURE 2 (a) Scan rate study of polymer and (b) linear relationship between maximum peak current density and scan rates [Color figure can be viewed at wileyonlinelibrary.com]

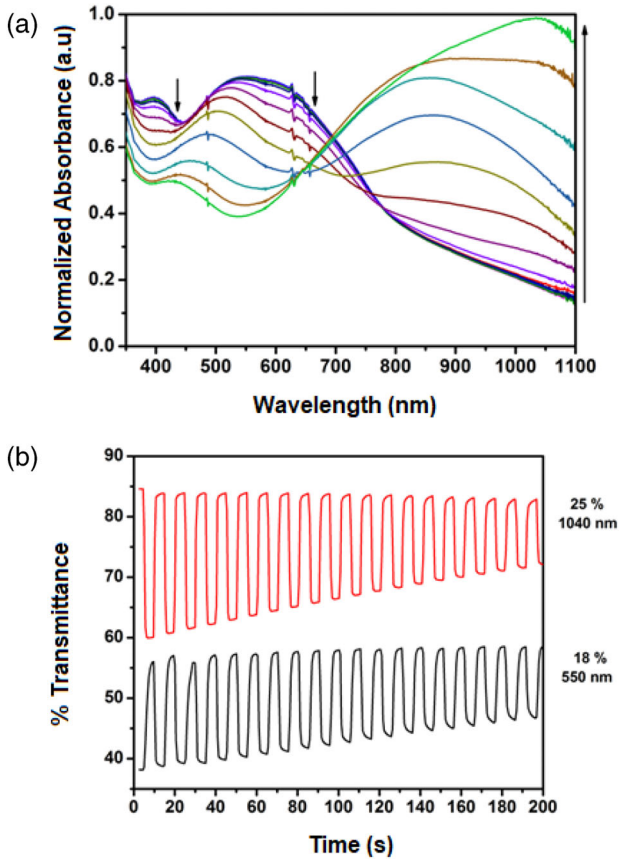
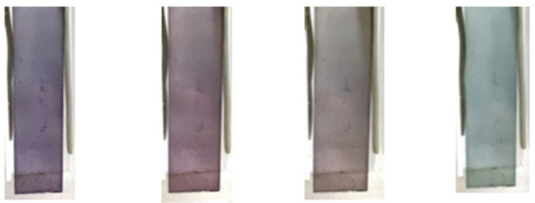


FIGURE 3 (a) Spectroelectrochemistry study and (b) kinetic study of the polymer film [Color figure can be viewed at wileyonlinelibrary.com]

onto ITO electrodes in the electrolyte solution. The reduced polymer shows a maximum of absorbance at 550 nm related to the interband π–π* transitions of the neutral P(DTFBT) film. When the oxidation starts via applied potentials, new polaronic and bipolaronic levels are generated. Upon electrochemical oxidation the band at 550 nm gradually lost its intensity with increasing of applied potential. At the same time the new absorption band at 850 nm and the broad band from 1040 nm grew up due to the formation of polarons an bipolarons. The electronic band gap of polymer, defined as the onset of the π–π* transitions, was determined as 1.3 eV.

One of the most important properties of electrochromic polymers is optical transmittance and sharp color change. Also, fast response time is very important for an electrochromic polymer. Optical contrast is the contrast difference between colors when oxidation and reduction potential is applied to an electrochromic material at a wavelength determined from the UV–vis spectrum and is expressed as $\Delta T\%$. The switching time of the polymer between the two colors varies depending on the ion conductivity of the electrode, the applied potential, the thickness of the film and its morphology. The switching

TABLE 1 *L*, *a*, and *b* values of polymer in neutral and oxidized states [Color table can be viewed at wileyonlinelibrary.com]

0.2 V → 1.2 V			
			
L: 40.67	**L:** 44.02	**L:** 52.39	**L:** 66.47
a: 6.46	**a:** 9.18	**a:** 6.93	**a:** -9.62
b: -12.74	**b:** -5.83	**b:** -1.62	**b:** -1.03

studies were carried out to show the contrast of the polymer as a function of time at given wavelengths. In these experiments, the transmittance ($T\%$) at λ_{max} is monitored as the films are oxidized and reduced state. This

experiment demonstrates that these polymers can undergo repeated switching while maintaining their optical contrast. Figure 3b shows the switching of P(DTFBT) between 0.0 and 1.2 V with a switching interval of 5 s in 0.1 M TBAP/ACN/DCM at two different wavelengths. The optical contrast for P(DTFBT) was 18% at 550 nm and 25% at 1040 nm. The polymer revealed a switching time of 1.5 s 550 nm and 1 s at 1040 nm while switched between 0.2 and 1.2 V.

In order to accurately express the colors of electrochromic materials with different colors and hues, a color system is used that characterizes the colors with numerical values. For this purpose, a colorimeter device (Konica Minolta CS-100a colorimeter) was used to define colors by giving coordinates in three-dimensional space. With this device, each color was positioned in the CIE Lab color space according to the measured values. The color changes were investigated by colorimetry studies and the color is quantified using the *L*, *a*, and *b* color space. In the colorimetric measurements, *L* represents the brightness of the color, a represents the color between red/magenta and green, b represents the color between yellow and blue. The P(DTFBT) film showed multichromic properties with prominent color changes at different applied potentials. The color coordinates, *L*, *a*, and *b* values, were summarized in Table 1. It was observed that the polymer film had a purple color at 0.2 V and a bluish-green at 1.2 V.

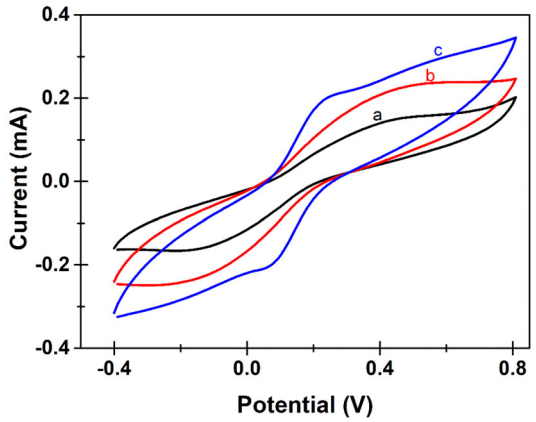


FIGURE 4 Cyclic voltammograms of (a) bare electrode, (b) P(DTFBT) and (c) P(DTFBT)/Laccase in 5.0 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Surface characterization of the biosensor

The cyclic voltammograms of bare graphite (a), P(DTFBT) (b) and P(DTFBT)/Laccase (c) electrodes were recorded in 5.0 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl to investigate the electroactive surface area of the corresponding surfaces (Figure 4). $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system is a convenient and valuable step to record the surface properties of the modified electrodes during each steps. According to the Figure 4, the average surface areas values were estimated as 0.124 cm^2 (bare graphite), 0.186 cm^2 (graphite/P(DTFBT)), and 0.2 cm^2 (P(DTFBT)/Laccase), respectively according to the Randles-Sevcik equation.[28] After polymer deposition on the bare graphite electrode, the electrode surface area was increased in 1.5 times compared to pristine one. After biomolecules deposition on the polymer modified surface, the decrease in the peak current associates with the insulator character of the biomolecules that is the common issue in biomolecule immobilization and proves the good localization of the biomolecules on the modified surfaces.

SEM images of before and after laccase immobilization on polymer coated surfaces were investigated and indicated in Figure 5. The porous surface of the deposited polymer layers looks a cauliflower like structure with high porosity (Figure 5 left). High porosity also means a larger surface area, which was stated in the electrochemical study in Figure 5. A difference was observed after deposition of laccase onto P(DTFBT) surface (Figure 5 right). It can be concluded that a smooth surface is a

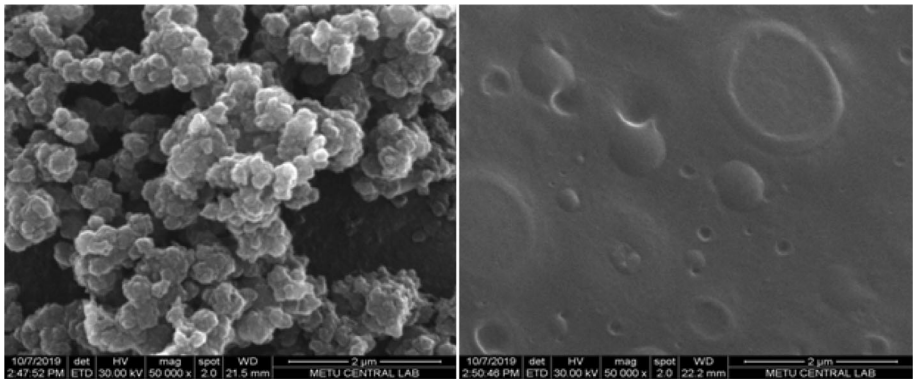


FIGURE 5 SEM images of pristine P(DTFBT) (left) and (B P(DTFBT)/laccase (right) surfaces

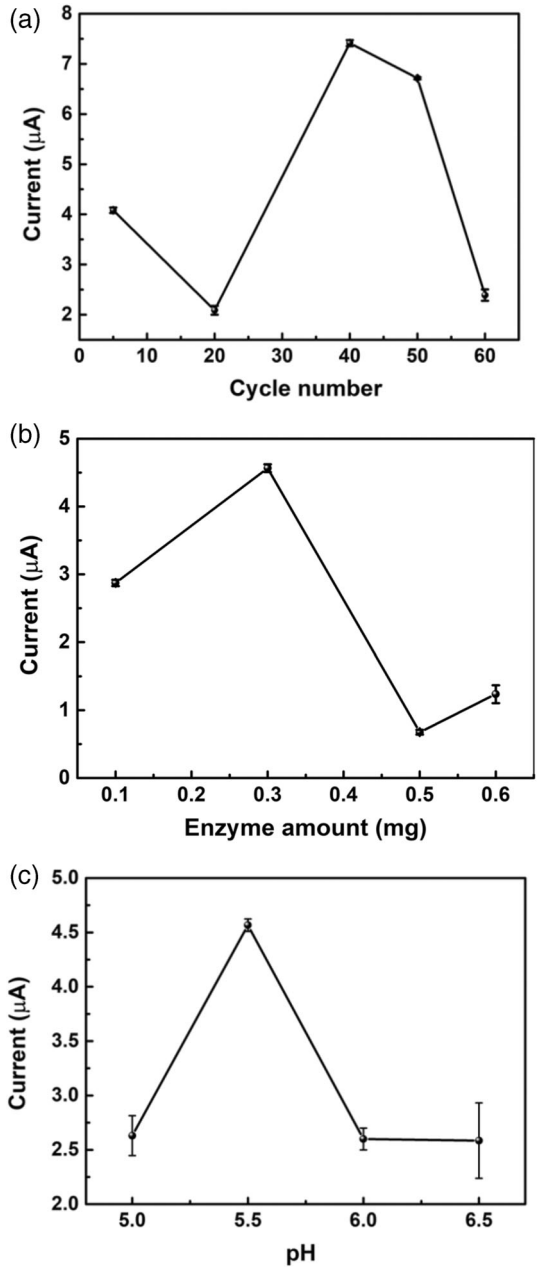


FIGURE 6 Effect of the (a) cycle number, (b) enzyme amount, and (c) pH on biosensor response (50 mM buffer solution; 25°C, −0.3 V). Error bars show standard deviation (SD) of three measurements

results of the good anchoring of the biomolecules on the polymer coated surface.

3.4 | Optimization and analytical characterization of the biosensor

The polymer cycle number, enzyme amount and pH influences were investigated through the amperometric behavior of the biosensor toward 0.05 M catechol. For

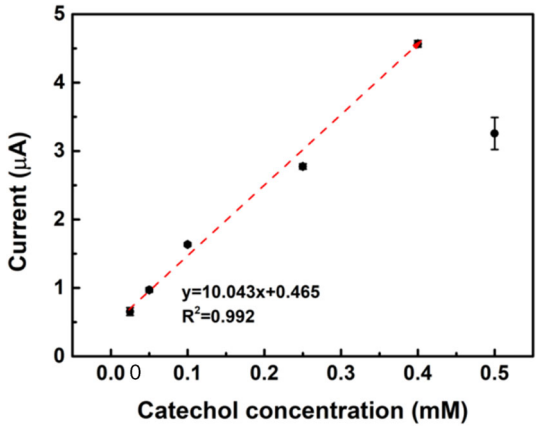


FIGURE 7 Calibration curve for catechol (in 50 mM acetate buffer, pH 5.5, 25°C, −0.7 V). Error bars show standard deviation of three measurements [Color figure can be viewed at wileyonlinelibrary.com]

cycle optimization works, varying scan numbers were used to produce polymer on electrode surface (5, 20, 40, 50, and 60) and their sensor responses were followed. The highest response was obtained from the 40 cycles of P(DTFBT) layer deposition and it was used as the optimum number for the following optimization steps (Figure 6a). With very thin or thick polymer films may arise improper enzyme immobilization steps and diffusion problems between the polymer coated electrode and the biomolecule, respectively. In the following optimization step, five different amounts of laccase (Lac; 0.1, 0.3, 0.5, and 0.6 mg) were dissolved in the same buffer solution and immobilized on the optimized polymer surface with GA (1%). The highest and stable sensor responses were shown with 0.3 mg enzyme amount (Figure 6b). The pH influence was also investigated in the pH range between 5.0 and 6.5. As shown in Figure 6c, the currents of the biosensor reached the maximum value at pH 5.5. Therefore, pH 5.5 was selected as the optimal pH value for subsequent measurements in 50 mM acetate buffer solution.

The amperometric calibration plot was obtained by plotting current (μA) versus catechol concentration to determine catechol at different concentrations from 0.025 to 0.4 mM by the P(DTFBT)/Laccase biosensors (Figure 7). It is noticeable that a linear relation was observed in the range from 25 to 400 μM catechol in acetate buffer. Figure 7 shows the linear calibration curve of amperometric responses against the concentration of catechol. The linear equation is represented by $y = 10.043\,x + 0.465$ with a correlation coefficient of $R^2 = 0.992$, a sensitivity of 166.74 μA/(mM cm^2) and a detection limit of 0.014 mM (S/N = 3). Moreover, Kmapp and Imax values were estimated as 0.11 mM and 3.42 μA,

TABLE 2 Comparison of several different laccase based electrodes with the proposed biosensor toward catechol

Biosensor architectures	Linear range (μM)	LOD (μM)	Sensitivity ($\mu AmM^{-1} cm^{-2}$)	References
GCE/Lac/PANI	3.2–19.6	2.07	70	6
Cu-Ordered mesoporous carbon (OMC)/chitosan (CS)	0.67–15.75	0.67	NR	29
SPEs/Lac	NR	10	3.02–0.216	30
Tramates versicolor (TvL) based sensor	0.2–1	NR	17.45	31
Fe3O4@Au core-shell nanoparticles/Lac	5.0–70.0	2 μM	NR	32
PM1/Lac	5.0–175.0	9.86	153.6	9
AuNP-MoS2-Lac/GCE	2–2000	2	16.3 μA/mM	33
GE/BOTT/Lac	0.5–25.0	0.38	110.81	10
P(DTFBT)/GE/Lac	25–400	14	166.74	This work

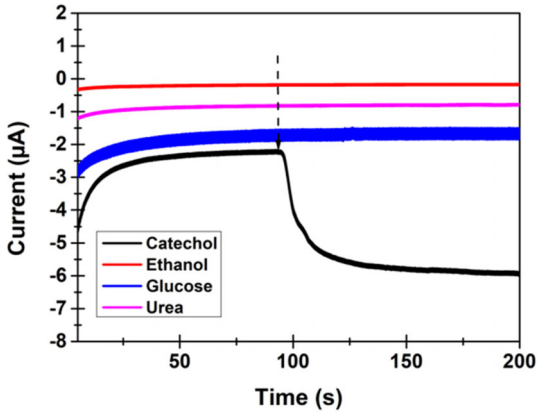


FIGURE 8 Effect of interfering species on biosensor responses (in pH 5.5 50 mM acetate buffer, 25°C, −0.7 V) [Color figure can be viewed at wileyonlinelibrary.com]

respectively. Table 2 summarizes the performance of several Laccase based biosensors toward catechol. It was observed that the P(DTFBT)/Laccase biosensor showed a relatively low detection limit, a wide linear range and high sensitivity. The good analytical results of the proposed sensor may be associated due to the fact that P(DTFBT) polymer provided a great environment for immobilization of Laccase, hence promoting the reaction between the enzyme and the substrate.

The anti-interference ability of the developed biosensor was investigated by the amperometric response of P(DTFBT)/Laccase toward catechol. At a potential of −0.3 V, catechol, glucose, urea and ethanol (0.05 M) were sequentially added to the acetate buffer solution (50 mM pH 5.5) under stirring. As shown in Figure 8, P(DTFBT)/Laccase biosensor displayed sensitive responses to the addition of catechol on the contrary the interfering reagents give no significant amperometric responses. The results indicated that the prepared biosensor had good selectivity property.

To investigate the repeatability of the biosensors, repeated experiments were carried out for P(DTFBT)/Laccase biosensor in buffer solution using certain amount of catechol. The standard deviation (SD) of the amperometric current responses was 0.051, which indicated good signal repeatability of the biosensor. In a period of 2 h, seven measurements were done and the biosensor still retained 1.15% of the initial value, which indicates the proper surface design for biomolecule localization. These results suggested that the proposed novel sensor could be proposed for detecting catechol.

4 | CONCLUSION

In summary a designed polymer, P(DTFBT), was an amperometric electrochemical catechol biosensor based on Lac and conjugated polymer, P(DTFBT) has been developed for the first time. The fabricated P(DTFBT)/Laccase electrode presents advantages, including biocompatibility with the Lac enzyme, high electron-transfer efficiency, and enhanced analytical signal for catechol. The proposed biosensor shows a wide linear response range, low detection limit and high sensitivity values for catechol detection, 25–400 μM, 14 μM, and 166.74 μA/(mM cm^2), respectively. Moreover, the optimized biosensor exhibited good selectivity and stability, and enabled successful detection of catechol in water samples. Furthermore, electrochemical and spectroelectrochemical studies of the polymer obtained by electrochemical polymerization of fluorine-containing monomer 4,7-bis (4-hexylthienyl)-5-fluoro-2,1,3-benzothiadiazole were carried out. After polymer was obtained, scan rate, spectroelectrochemical and kinetic studies were performed in monomer free electrolyte solution. Optical contrasts, switching times and band gap were determined. It was concluded that the polymer obtained by electrochemical

oxidation of the monomer has a low band gap and shows multichromic properties. Moreover, these great electrochemical properties bring good electrochemical detection ability of biosensor for catechol detection. Hence, in this study, we aimed to create a more sensitive sensor for the selective detection and sensitive determination of catechol. The biosensor based on the designed polymer architecture can be extended to other enzymes and other phenolic pollutants if desired.

AUTHOR CONTRIBUTIONS

Yasemin Udum: Conceptualization (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); resources (lead); software (lead); supervision (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Melek Aktas Gemci:** Investigation (equal). **Duygu Cevher:** Investigation (equal). **Saniye Soylemez:** Data curation (equal); formal analysis (equal); methodology (equal). **Ali Cirpan:** Visualization (equal); writing – original draft (equal). **Levent Toppare:** Visualization (equal); writing – original draft (equal).

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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