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Enhancing biosensor properties of conducting polymers via copolymerization: Synthesis of EDOT-substituted bis(2-pyridylimino) isoindolato-palladium complex and electrochemical sensing of glucose by its copolymerized film

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ABSTRACT

1,3-Bis(2-pyridylimino)isoindoline derivative bearing 3,4-ethylenedioxythiophene (**EDOT-BPI**) and its palladium complex (**EDOT-PdBPI**) were synthesized and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, UV–Vis spectroscopies and via mass spectrometric analysis. Polymerization of **EDOT-PdBPI** and copolymerization with 4-amino-N-(2,5-di(thiophene-2-yl)-1*H*-pyrrol-1-yl)benzamide (**HKCN**) were carried out by an electrochemical method. In addition, P(EDOT-PdBPI-*co*-HKCN) modified graphite rod electrode was improved for amperometric glucose sensor based on glucose oxidase (GOx). In this novel biosensor matrix, amino groups in **HKCN** were used for the enzyme immobilization. On the other hand, **EDOT-PdBPI** used to mediate the bioelectrocatalytic reaction. Amperometric detection was carried out following oxygen consumption at −0.7 V vs. the Ag reference electrode in phosphate buffer (50 mM, pH 6.0). The novel biosensor showed a linear amperometric response for glucose within a concentration range of 0.25 mM to 2.5 mM (LOD: 0.176 mM). Amperometric signals at 1 mM of glucose were 17.9 μA under anaerobic conditions. Amperometric response of the P(EDOT-PdBPI-*co*-HKCN)/GOx electrode decreased only by 13% within eight weeks. The P(EDOT-PdBPI-*co*-HKCN)/GOx electrode showed good selectivity in the presence of ethanol and phenol. This result shows that, modification of the proposed biosensor by copolymerization of amine functionalized monomer, which is indispensable to the enzyme immobilization, with palladium complex bearing monomer, which is mediate the bioelectrocatalytic reaction, have provided to give perfect response to different glucose concentrations.

1. Introduction

The chemistry of isoindolines has been the key for the development of phthalocyanines as well as related macrocycles and chelating ligands (Tamgho et al., 2013; Bekaroğlu, 2000; Torres, 2000). Among the isoindoline-based chelating ligands, bis(2-pyridylimino)isoindolines (*BPI*) have been the focus of interest because they are readily synthesized and easily modified (Hanson et al., 2011; Kim et al., 2012; Selvi et al., 2005; Tran et al., 2014; Wen et al., 2011). *BPIs* have been shown to function as neutral, nondeprotonated and also as uninegative ligands. The ligands are capable of occupying three sites about a metal ion and forming either 1:1 or 2:1 (ligand:metal) complexes depending on the coordination number and geometry of the metal ion (Bakthavachalam and Reddy, 2013; Balogh-Hergovich et al., 2005; Dietrich et al., 2005; Meder et al., 2005; Pap et al., 2011a). Most of the published papers about bis(2-pyridylimino)isoindolines and their metal complexes focused on structural characterization and main application areas of these compounds are homogeneous catalysis and biomimetics (Csonka et al., 2015; Kaizer et al., 2008, 2007; Pap et al., 2011b; Sauer et al., 2012). Here we present first time a new bis(2-pyridylimino)isoindolato-palladium complex bearing electropolymerizable EDOT (3,4-(ethylenedioxy)thiophene) substituent and show it may be used for glucose sensing as heterogeneous catalytic system.

Most of enzymatic glucose biosensors are based on the immobilized glucose oxidase (GOx), which catalyzes the oxidation of glucose to gluconic acid and H_2O_2, and the concentration of

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glucose can be detected via electrochemical detection of the enzymatically released H_2O_2. However, the weak immobilization strength of GOx limits the sensing characteristics of glucose biosensor (Chou et al., 2016). Enzyme immobilization onto the electrode surface is a crucial step in assembling amperometric biosensors (Malhotra et al., 2006). Frequently, entrapment in different substrate materials (Bongiovanni et al., 2001), covalent binding (Gerard et al., 2002), physical adsorption onto a solid supporting matrix (Gerard et al., 2002; Cosnier, 2000) are used as the common techniques in enzyme immobilization. The physical absorption; entrapment and other methods suffer from leaching problems of the enzyme. However this problem can be significantly overcome by using chemical cross-linking method of immobilization via glutaraldehyde. Conductive polymers can be used as an immobilization matrix for biologically active molecules in biosensor (Ramanavicius et al., 2005; Teles and Fonseca, 2008). When approached the functionalized unique conducting polymers, which have a variety of functional groups such as amino or carboxyl groups for cross-linked via glutaraldehyde, have received great attention for their ability to form highly stable biodetection systems through the strong covalent bonds between the polymeric platforms and biomolecules (Ekiz et al., 2010; Gerard et al., 2002). Poly(3,4-ethylenedioxythiophene) (PEDOT) has received tremendous attention in the past decade and has become one of the most investigated conducting polymers. To obtain polymers combining PEDOT with improved properties, synthetic efforts have been devoted toward the preparation of substituted EDOT-based monomers (Aydogan et al., 2014; Groenendaal et al., 2003; Karadağ et al., 2014; Kirchmeyer and Reuter, 2005; Soganci et al., 2014b). On the other hand, an interesting feature of the *BPI* is that its metal complexes have been shown to mimic catalase enzymes which can decompose hydrogen peroxide to oxygen and water in a protonated and deprotonated form to protect organisms from reactive oxygen species which can lead to early aging in cells (Kaizer et al., 2009). Considering the catalase activity of metal complexes of *BPI*s and functionality of conductive polymers as an immobilization matrix for glucose in biosensors, bis(2-pyridylimino) isoindoline **EDOT-BPI** modified with EDOT was synthesized, the corresponding new Pd complex **EDOT-PdBPI** obtained in this study. The complex **EDOT-PdBPI** was successfully electropolymerized on the electrode surface by oxidation. Electrochemical and spectroelectrochemical properties of **EDOT-PdBPI** were also investigated. Furthermore, we evaluated the glucose sensing properties of electron-conducting polymer films of **EDOT-PdBPI**.

2. Experimental

2.1. Chemicals

The experimental studies were carried on with high purity chemicals. D-Glucose, glucose oxidase (GOx, from Aspergillus niger, 200 U/mg) were purchased from Sigma. **EDOT-Pht** and **HKCN** were synthesized according to the published procedures (Söyleyici et al., 2013; Yıldız et al., 2011).

2.2. Equipment

In order to pursue electrochemical synthesis, performing cyclic voltammetry and sensor studies an Ivium potentiostat/galvanostat was used in all electrochemical measurements. The spectra were collected with a Diode Array UV–Vis spectrophotometer (Agilent 8453) with a PC interface. Spectrophotometer was used to conduct the spectroelectrochemical experiments of polymer. The indium tin oxide (ITO) coated glass plates of thickness of 0.7 mm with resistance of 8–12 $\Omega\ sq^{-1}$ were purchased from Delta Technologies Limited, USA, and used as such for spectroelectrochemical studies. All electrochemical experiments were carried out in a one component cell. The copolymer films were deposited on ITO plates and graphite rode ($19.6 \times 10^{-2}\ cm^2$) electrochemically used as working electrode, a Pt counter electrode and Ag/Ag^+ reference electrodes.

2.3. Synthesis of *EDOT-BPI*

EDOT-Pht (0.500 g, 1.678 mmol), 2-amino-4-methyl-pyridine (0.455 g, 4.205 mmol) and anhydrous $CaCl_2$ (0.047 g, 0.424 mmol) were suspended in 1-hexanol (16 mL) and heated at reflux for 24 h. After cooling to room temperature the reaction product was isolated as a yellow solid which was then washed with water and dried over P_4O_{10}. The product was crystallized from methanol. Yield 0.484 g (58%). Mp: 215 °C. FT-IR: ν_{max} 3278.30 (NH), 3084.70 (CH, aromatic), 2913.43 (CH, aliphatic) $cm^{-1.}$ ^1H NMR ($CDCl_3$): δ 13.91 (NH), 8.48 (aromatic H, 2H), 7.98–6.94 (aromatic H, 7H), 6.42 (thiophene H, 2H), 4.64–4.25 (CH and CH_2, 5H), 2.41 (CH_3, 6H) ppm. ^{13}C NMR (APT, $CDCl_3$): δ 161.39 (C-4), 160.45 (C-8), 153.42 (C-7), 149.26 (C-10), 147.53 (C-12), 141.33 (C-17), 141.00 (C-20), 138.04 (C-2), 128.90 (C-1), 124.00 (C-6), 123.64 (C-11), 121.46 (C-5), 119.66 (C-9), 106.52 (C-3), 100.17 (C-18), 100.08 (C-19), 71.70 (C-15), 66.65 (C-14), 65.69 (C-16), 21.01 (C-13) ppm. UV–Vis ($CHCl_3$): λ_{max} 410, 387, 367, 297, 265 nm. MS (LC-MS): *m/z* 498 $[M]^+$ (theoretical *m/z* value= 497.57).

2.4. Synthesis of *EDOT-PdBPI*

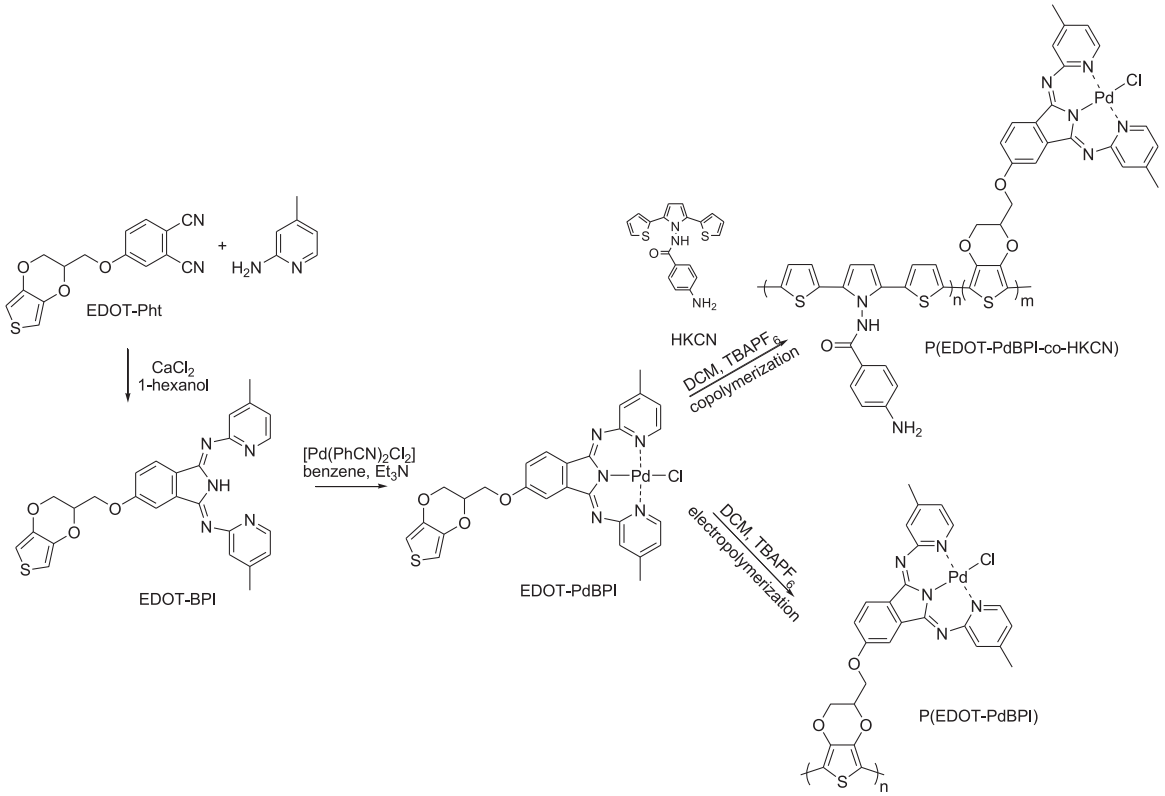
EDOT-BPI (0.100 g, 0.200 mmol), and $[Pd(PhCN)_2Cl_2]$ (0.085 g, 0.221 mmol) were suspended in dry benzene (5 mL). To this suspension NEt_3 (0.022 g, 0.221 mmol) was added and the reaction mixture was subsequently stirred at room temperature for 15 h. The crude palladium complex and NEt_3HCl were isolated by filtration, the ammonium salt was removed by extraction with water, and the reaction product was recrystallized from CH_2Cl_2/n-hexane, giving **EDOT-PdBPI** as a deep yellow-ochre solid. Yield 0.058 g (45%). Mp: 285 °C. FT-IR: ν_{max} 3095.68 (CH, aromatic), 2988.64 (CH, aliphatic) $cm^{-1.}$ ^1H NMR ($CDCl_3$): δ 9.57 (aromatic H, 2H), 7.79–6.81 (aromatic H, 7H), 6.35 (thiophene H, 2H), 4.50–4.21 (CH and CH_2, 5H), 2.33 (CH_3, 6H) ppm. ^{13}C NMR (APT, $CDCl_3$): δ 161.27 (C-4), 153.42 (C-8), 152.71 (C-12), 151.66 (C-10), 151.18 (C-7), 141.32 (C-17), 140.98 (C-20), 140.02 (C-2), 130.88 (C-1), 126.49 (C-6), 123.69 (C-11), 121.33 (C-5), 118.95 (C-9), 106.66 (C-3), 100.18 (C-18), 100.16 (C-19), 71.72 (C-15), 66.64 (C-14), 65.68 (C-16), 20.66 (C-13) ppm. UV–Vis ($CHCl_3$): λ_{max} 464, 432, 355, 328, 260 nm. MS (LC-MS): *m/z* 639.80 $[M+H]^+$ (theoretical *m/z* value= 638.43).

2.5. Polymerization of *EDOT-BPI* and *EDOT-PdBPI*

Electrochemical polymerization of **EDOT-BPI** and **EDOT-PdBPI** were studied using cyclic voltammetry and chronoamperometry. The synthetic route for P(EDOT-PdBPI) is illustrated in Scheme 1. The reaction was performed with a three-electrode method, with an ITO as a working electrode, platinum as a counter electrode, and an Ag/AgCl electrode as the reference electrode, respectively. The potential limit for **EDOT-BPI** and **EDOT-PdBPI** was between, −0.2 and 1.5 V, −1.0 and 1.8 V, respectively and the scan rates were 100 mV s^{-1}.

2.6. Biosensor fabrication

Initially, the graphite electrode was polished on wet emery paper and rinsed with distilled water. Electrochemical copolymerization of P(EDOT-PdBPI-*co*-HKCN) was potentiodynamically carried out on a graphite electrode in 0.1 M $TBAPF_6$/DCM



Scheme 1. Synthetic route for **EDOT-BPI**, **EDOT-PdBPI**, P(EDOT-PdBPI) and P(EDOT-BPI-*co*-HKCN).

(tetrabutylammonium hexafluorophosphate/dichloromethane) medium containing 1.0 mg/mL **EDOT-PdBPI** and 4.0 mg/mL **HKCN** according to the literature (Soganci et al., 2014a). The synthetic route for the P(EDOT-PdBPI-*co*-HKCN) is illustrated in Scheme 1. For the immobilization of GOx, 5 μL enzyme solution (2.0 mg of enzyme was dissolved in 5 μL buffer solution) (which equals to 50 units) and 1.0% of 5 μL GA in potassium phosphate buffer (50 mM, pH 7.0) were dropped on the electrode. The electrodes were allowed to dry at the ambient conditions for 2 h. Prior to measurements, electrode surface was rinsed with distilled water to eliminate the unbounded residues. In this way, the novel synthesized conducting copolymer, EDOT-PdBPI-*co*-HKCN, where glucose oxidase was successfully immobilized via cross-linking due to the free amino functional groups of the polymeric structure.

2.7. Measurements

For biosensor application, CV experiments were performed in 10 mL Na-phosphate buffer (0.05 M, pH 6.0) with the potential scans between −0.5 V and +1.0 V at the scan rate of 100 mV s^{-1}. Chronoamperometric measurements using the mediated biosensor were also carried out in 10 mL Na-phosphate buffer (0.05 M, pH 6.0). During this measurement, enzymatic reaction proceeds on two half reactions namely a reduction and a following oxidative step. In the first step, GOx catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone, which is spontaneously hydrolyzed to gluconic acid and in the second step, the reduced GOx is reoxidized by oxygen to yield H_2O_2 (Bankar et al., 2009). The decrease in the amount of oxygen is monitored. On the other hand, for analytical characterization of sensor, the current responses at −0.7 V vs. Ag^+/AgCl reference electrode were followed as the current densities (Gorton, 1995). Before and after glucose addition, the measuring current densities differences were recorded and the current vs. time plot has been obtained. Each measurement was carried out at least 3 times and each data using the graphs were given as the mean and standard deviation. Buffer was refreshed after each measurement.

3. Results and discussions

3.1. Syntheses of *EDOT-BPI* and *EDOT-PdBPI*

The bis(2-pyridylimino)isoindole derivative **EDOT-BPI** was prepared according to the synthetic route first described by Siegl et al. by reaction of a 2-aminopyridine derivative with an ortho-phthalodinitrile (Scheme 1) (Siegl, 1977). 1-hexanol was used instead of 1-butanol as the solvent unlike Siegl's method. The preparation of the corresponding square-planar palladium(II) complex **EDOT-PdBPI** was carried out in benzene using $[Pd(PhCN)_2Cl_2]$ as the Pd(II) precursor and triethylamine as auxiliary base. The structures of the **EDOT-BPI** and **EDOT-PdBPI** were confirmed by FT-IR NMR, UV–Vis and MS spectroscopic techniques (Figs. S1-S10). The coordination of the *BPI* ligand to palladium(II) can be conveniently monitored by ^{1}H NMR spectroscopy. The most characteristic change in the NMR spectra is the coordination shift of the 6-pyridyl protons from ca. δ 8.4 ppm in the free ligand to ca. δ 9.5 ppm in the palladium complexes, and the disappearance of the NH resonance of the isoindole unit (Fig. S8) (Siggelkow et al., 2004). Also, the IR spectra of **EDOT-PdBPI** confirmed the formation of the complex with the deprotonated isoindoline ligand, due to disappearance of the N-H signal seen in **EDOT-BPI** at 3278 cm^{-1} (Fig. S8). In the electronic spectra, the band at 464 nm can be assigned to a ligand-to-metal charge transfer transition (Kaizer et al., 2007).

3.2. Electrochemical properties of P(EDOT-BPI) and P(EDOT-PdBPI)

The cyclic voltammogram (CV) curves of the **EDOT-BPI** and **EDOT-PdBPI**, respectively in DCM/BFEE and DCM solution

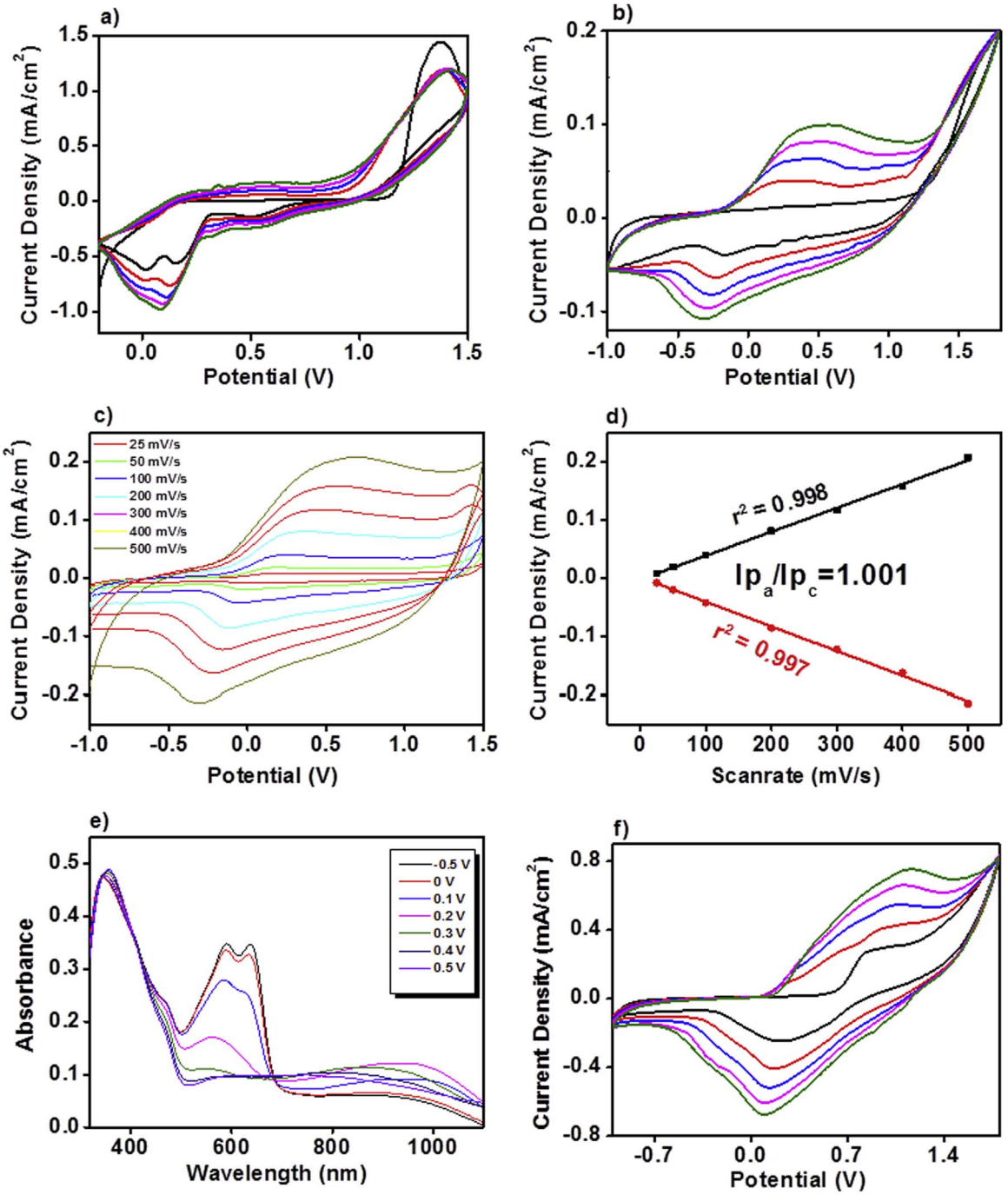


Fig. 1. Cyclic voltammograms **(a) EDOT-BPI** and **(b) EDOT-PdBPI** in DCM/$TBAPF_6$ at scan rate of 100 mV s^{-1}. **(c)** Cyclic voltammogram P(EDOT-PdBPI) on ITO-coated glass at different scan rates **(d)** A plot of scan rates against current density during cyclic voltammetry of P(EDOT-PdBPI) thin film **(e)** Optoelectrochemical spectrum of P(EDOT-PdBPI) film in $TBAPF_6$/DCM **(f)** Cyclic voltammograms of P(EDOT-PdBPI-*co*-HKCN) on graphite electrode in 0.1 M $TBAPF_6$/DCM at scan rates of 100 mV s^{-1}.

containing 0.1 M $TBAPF_6$ at a potential scan rate of 100 mV s^{-1} are shown in Fig. 1a and b. As the CV scan continued, the current density gradually increased with increasing scanning cycles, and polymer films could be observed on the ITO working electrode. **EDOT-BPI** was electrochemically polymerized in DCM/borontrifluoride diethyl etherate (BFEE) (3:1 v/v) because of polymerization was not efficient in DCM. As a superior solvent and electrolyte, BFEE has been proved to be a convenient electrolyte to polymerize the fused aromatic molecules and their derivatives (Guzel et al., 2015). As previously reported, due to Lewis acidity of BFEE reduces the polymerization potential of many aromatic molecules, usually used to obtain polymer film. As shown in Fig. 1a, the CV of **EDOT-BPI** tended oxidation and reduction peaks at 1.1 and 0.3 V. In the case of **EDOT-BPI**, although the formation of a tenuous polymer film was observed even with the naked eye, the electrodeposition on the electrode surface could not be achieved steadily. Hence, P(EDOT-BPI) could not be examined in further electrochemical studies conducted within the scope of this work. Fig. 1b represents the cyclic voltammograms of the growth of **EDOT-PdBPI**. The oxidation and reduction peaks of P(EDOT-PdBPI) were observed at 0.34 and −0.23 V, respectively, which are different from the potentials of P(EDOT-BPI).

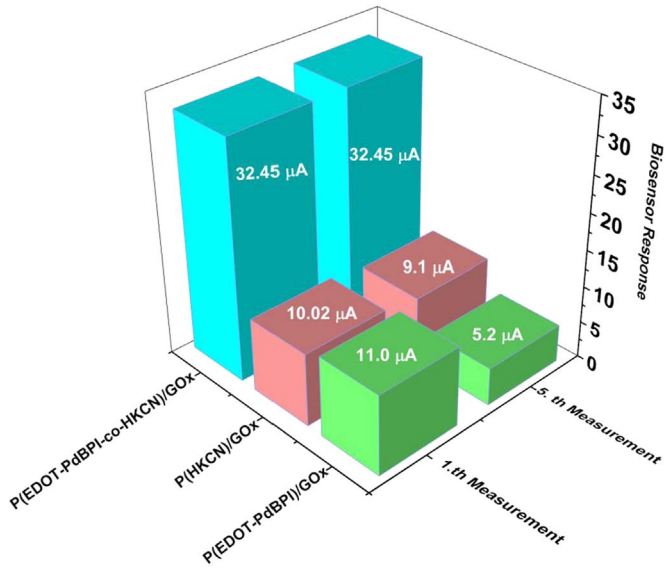


Fig. 2. Amperometric biosensor responses of P(HKCN)/GOx in sodium acetate buffer, 50 mM, pH 4.5, −0.7 V, [Glc]: 2.5 mM, P(EDOT-PdBPI)/GOx in sodium phosphate buffer, 50 mM, pH 6.5, −0.7 V, [Glc]: 2.5 mM, P(EDOT-PdBPI-*co*-HKCN)/GOx in sodium phosphate buffer, pH 6.0, −0.7 V, [Glc]: 2.5 mM.

3.3. Scan rate dependence of the peak currents of P(EDOT-PdBPI)

Fig. 1c shows the redox properties of electrochemically prepared P(EDOT-PdBPI) thin film observed using CV at potential between −1.0 and 1.5 V in 0.1 M $TBAPF_6$/DCM solution with different scan rates of 25, 50, 100, 200, 300, 400, 500 mV s^{-1}, respectively. Nevertheless, the current densities were directly proportional to the scan rates, there was an anodic shift in corresponding oxidation peak at increasing scan rate that indicated the polymeric film was electroactive and well adhered to the electrode surface. The linear relationship between the peak current with scan rates in Fig. 1d also demonstrated that the electrochemical processes were reversible in even at high scan rates (Içli et al., 2010), (Soganci et al., 2016). Besides this, when analyzed the oxidation and reduction peaks of the polymer, it was determined as 0.29 and −0.05 V, respectively at 100 mV s^{-1}.

3.4. Spectroelectrochemical properties of P(EDOT-PdBPI)

Spectroelectrochemistry was used to elucidate the electronic structure and optical behavior of as-prepared polymers upon oxidation on ITO glass. Fig. 1e presents the absorption spectra of the prepared P(EDOT-PdBPI) films under various applied potentials. As seen from the Fig. 1e, P(EDOT-PdBPI) film shows two absorption peak at 590 and 607 nm in its neutral form, and the corresponding band gap is calculated as 1.79 eV. When the applied potential was increased, the absorption of the P(EDOT-PdBPI) at about 600 nm was decreased similar to PEDOT (Elschner et al., 2010; Soganci et al., 2014b; Wang, 2009).

3.5. Characterization of the P(EDOT-PdBPI-co-HKCN)/GOx enzyme electrode

3.5.1. Electrochemical properties of the P(EDOT-PdBPI-co-HKCN)

To further explore its application as an enzyme electrode, the electrochemical deposition of P(EDOT-PdBPI-*co*-HKCN) was tested by CV. First of all, the monomer, **EDOT-PdBPI**, **HKCN** (1:4, w/w), was electrochemically copolymerized on the previously cleaned bare graphite electrode, and then washed with distilled water to remove impurities. Electrocopolymerization of the EDOT-PdBPI-*co*-HKCN was achieved via CV by sweeping the potential between −1.0 and 1.5 V in 0.1 M $TBAPF_6$/DCM at a scan rate of 100 mV s^{-1}. The CV curves were depicted in Fig. 1f. When the comparing CVs of **HKCN** (Timur, 2014) and copolymer, it has been confirmed that the copolymerization was successful. On the other hand, for the preparing enzyme electrode, chronoamperometric measurement was performed. The copolymer P(EDOT-PdBPI-*co*-HKCN), film was deposited onto graphite electrode in same solvent medium at 1.5 V during 60 s. When analyzed Fig. S11, it has determined that, total charge density of electrode surface as 5.43 mC/cm^2.

3.5.2. Influence of the modified electrodes

The influence of electrode surface on the amperometric response of enzyme sensor was investigated. For these purpose, P(HKCN)/GOx, P(EDOT-PdBPI)/GOx, P(EDOT-PdBPI-*co*-HKCN)/GOx electrodes were prepared. Electrochemical polymerization and copolymerization of P(HKCN)/GOx, P(EDOT-PdBPI)/GOx, P(EDOT-PdBPI-*co*-HKCN)/GOx were potentiodynamically carried out on a graphite electrode in 0.1 M $TBAPF_6$/DCM medium containing 5.0 mg/mL **HKCN**, 5.0 mg/mL **EDOT-PdBPI**, 1.0 mg/mL **EDOT-PdBPI** and 4.0 mg/mL **HKCN**, respectively. For the immobilization of GOx to each electrode, 5 μL enzyme solution (2.0 mg of enzyme was dissolved in 5 μL buffer solution) (which equals to 50 units) and 1.0% of 5 μL GA in potassium phosphate buffer (50 mM, pH 7.0) were dropped on the electrode. Relative response values of P(HKCN)/GOx, P(EDOT-PdBPI)/GOx and P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme sensors were achieved maximum at pH 4.5, 6.5, 6.0, respectively, in the presence of 2.5 mM glucose. When comparing the response of modified electrodes, it has been determined that the highest value for P(EDOT-PdBPI-*co*-HKCN)/GOx. After five measurements, there is a 50% decrease of the I (μA/cm^2) for the experiments performed with the P(EDOT-PdBPI)/GOx electrode. The decrease of current response is originated from the P(EDOT-PdBPI)/GOx has not any functional group to fix of the enzyme on the electrode surface. Comparative results are shown in Fig. 2.

3.5.3. Effect of pH

pH was optimized since working conditions also affects the biosensor performance. To determine the optimum pH of the proposed biosensor, different buffers were prepared in the range of 4.0–7.5. pH dependence of the responses was investigated over a pH range between 4.0 and 7.5 with 50 mM sodium acetate buffer at 4.0–5.5 and 50 mM phosphate buffer at 6.0; 7.5 at 25 °C. The best result was achieved at pH 6.0 sodium phosphate buffer solution as given in Fig. 3a.

3.5.4. Calibration of enzyme electrode

In order to evaluate the influence of P(EDOT-PdBPI-*co*-HKCN) in the electrocatalytic action of GOx, the amperometric responses of the P(EDOT-PdBPI-*co*-HKCN)/GOx electrode was examined at different concentrations of glucose (0.25–100 mM) under standard conditions. The amperometric responses of P(EDOT-PdBPI-*co*-HKCN)/GOx electrode and calibration plots of current vs. glucose concentrations for the enzyme electrodes are shown in Fig. 3b. The amperometric response of the P(EDOT-PdBPI-*co*-HKCN)/GOx electrode showed a linear relation with glucose concentration from 0.25 mM to 2.5 mM in the inset of Fig. 3b with the following parameters:

$$y = 10.871x + 5.959\left(r^2 = 0.9983\right)$$

After these results, a middle value has been chosen in the region detected as linear (1.0 mM) and this value has been utilized for repeatability of the analysis results for P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme sensor. Comparison of analytical performance of poly (2,5-dithienyl pyrrole) derivatives based biosensors have

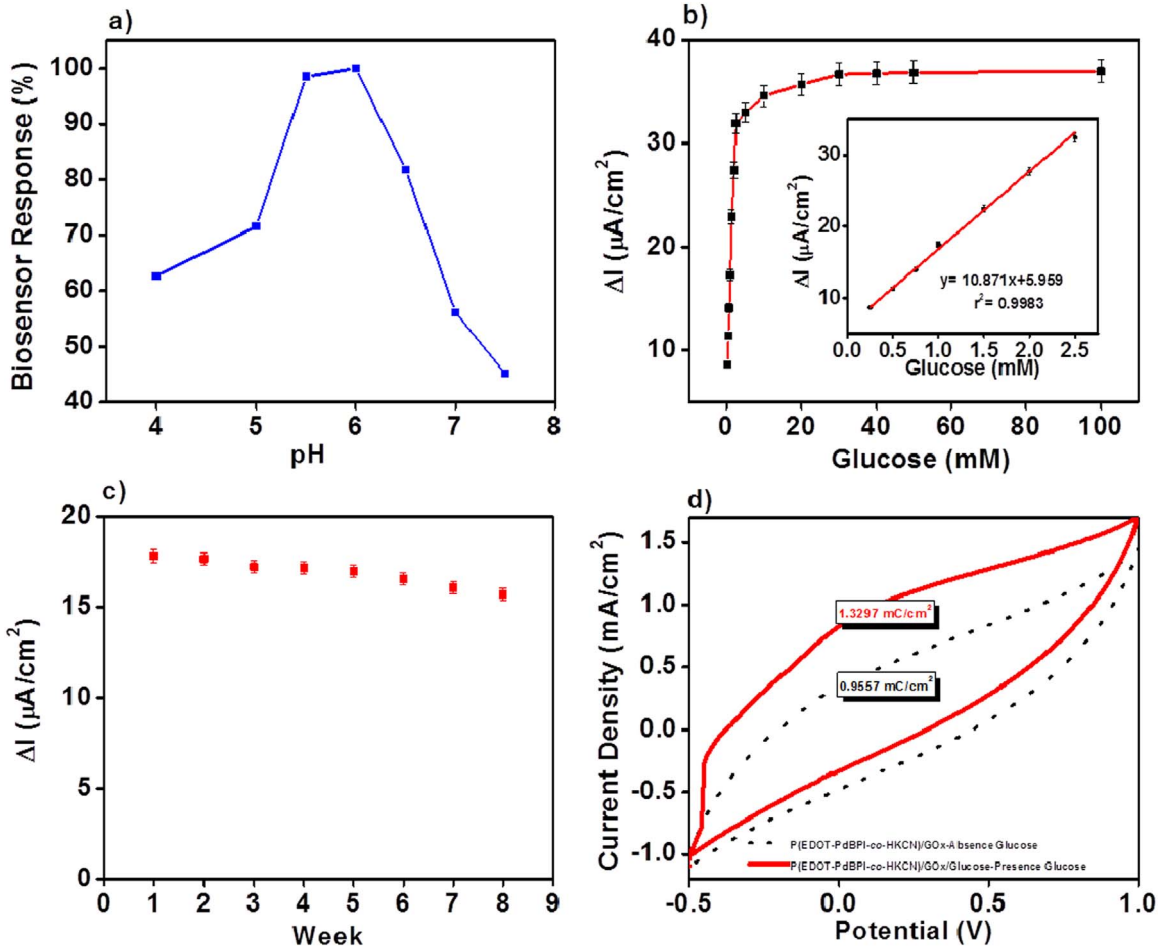


Fig. 3. **(a)** Effect of pH on P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme electrode in 50 mM Na-acetate buffer and Na-phosphate buffer. **(b)** Calibration curve for glucose (in pH 6.0, 50 mM PBS, −0.7 V). Error bars show the standard deviation of three measurements. **(c)** Stability of the P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme sensor. **(d)** CVs of P(EDOT-PdBPI-*co*-HKCN)/GOx/Absence Glucose (dashed line) and P(EDOT-PdBPI-*co*-HKCN)/GOx/Presence Glucose (solid line) (pH 6.0 Na-phosphate buffer, 50 mM at 100 mV s^{-1} scan rate).

shown in Table 1.

3.5.5. Analytical characterization, interference study and real sample analysis

For P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme sensor constructed in optimized operating conditions, 15 measurements have been taken by using glucose concentration (1.0 mM) which is in the range of linear determination. Standard deviation and variation coefficient have been calculated as (S.D) 0.581 mM (n:15) and (cv) 3.26% (n:15) respectively using calibration graphics which were plotted in consistence with the measures obtained. LOD (limit of detection) value was calculated as 0.176 mM (n: 15) for P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme sensor. Also, limit of quantification, LOQ was calculated as 0.533 mM for P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme sensor.

The stability of the electrode towards oxidation of glucose is evaluated by placing the P(EDOT-PdBPI-*co*-HKCN)/GOx in an electrolyte consisting of 0.1 M PBS with 1 mM of glucose under chronoamperometric conditions as described before. The anodic current response towards oxidation of glucose is monitored periodically as shown in Fig. 3c. When not in use, the electrode is suspended above the 0.1 M PBS at 4 °C in a refrigerator and after storage for 8 weeks, the response of the electrode is about 87% of the initial value. This is noticeable that the biosensor can be used for long-time studies with its favorable immobilization platform. Thus, we decided that enzyme sensor shows good reproducibility in the linear range.

The ability of the sensor to discriminate the interfering species having electrochemical activity similar to the target analyte is one of the most important analytical factors for an amperometric biosensor. Ethanol, phenolic compounds and other oxidizable compounds generally coexist with glucose in real samples and they likely can interfere the detection of glucose. The interfering effects of, 0.1 mM ethanol, 0.1 mM phenol in the existence of 1 mM glucose are investigated. As shown in Table S1, the response on P(EDOT-PdBPI-*co*-HKCN)/GOx is not obviously affected by addition of 1 mM of ethanol, 1 mM of phenol, furnishing evidence that GOx has good selectivity to glucose and the interfering substances have no obvious effects on the performance of the modified electrode. These results represent that the constructed P(EDOT-PdBPI-*co*-HKCN)/GOx amperometric glucose biosensor has good anti-interference ability and a high selectivity.

The feasibility of the biosensor for real sample detections as a potential detector was investigated by analyzing several commercial beverages. The target sample was injected to the reaction cell without pretreatment and the concentration of glucose was calculated using the calibration curve. Glucose contents of the samples were calculated from the calibration curve by measuring them in amperometric measurements instead of glucose without any pretreatment. The comparison of the results obtained from product label and P(EDOT-PdBPI-*co*-HKCN)/GOx biosensor was shown in Table 2. As shown in Table 2, the determined glucose levels are close to the values obtained by enzymatic catalytic spectrophotometry. The results are in a good agreement with our

Table 1
Comparison of analytical performance of poly(2,5-dithienyl pyrrole) derivatives based biosensors.

Electrode	Immobilization Matrix	Biological Material	Working Potential	Linear Range for Glucose (mM)	LOD (mM)	ΔI_{max} (μA)	Stability	Reference
GOD	SNS-NH_2	GOx	−0.7 V (vs. Ag/AgCl)	1–10	0.903	6.3	NR	(Ayranci et al., 2015)
GOD	BEDOA-6	GOx	−0.7 V (vs. Ag/AgCl)	0.025–1.25	0.025	5.5	NR	(Kesik et al., 2013)
GOD	PPBDT	GOx	−0.7 V (vs. Ag/AgCl)	0.05–2.0	0.05	3.5	42 days	(Emre et al., 2011)
GOD	TBT	GOx	−0.7 V (vs. Ag/AgCl)	$0.25–1 \times 10^{-6}$	0.03	6.1	Longer than 2 weeks	(Ekiz et al., 2010)
GOD	SNS-COOH–Lys	GOx	−0.7 V (vs. Ag/AgCl)	0.01–2.40	0.019	3.6	28 days	(Demirci et al., 2012)
GOD	DTP-NH_2	GOx	−0.7 V (vs. Ag/AgCl)	0.01–1.0	0.005	NR	NR	(Azak et al., 2013)
GOD	**EDOT-PdBPI-co-HKCN**	**GOx**	**−0.7 V (vs. Ag/AgCl)**	**0.25–2.5**	**0.176**	**32.6**	**Longer than 8 weeks**	**This Work**

NR: Not Reported

Table 2
Glucose analysis results for P(EDOT-PdBPI-*co*-HKCN)/GOx enzyme sensor and spectrophotometric method in real samples.

Sample	Glucose[a] ($g\ L^{-1}$)		
	Spectrophotometric Method	**P(EDOT-PdBPI-*co*-HKCN)/GOx**	**Recovery, %**
Coke	0.503 ± 0.002	0.505 ± 0.03	99.62
Juice	0.555 ± 0.001	0.574 ± 0.01	96.80

[a] Data were calculated as the average of 3 trials ± S.D.

data showing that the novel biosensor can be utilized for practical sample testing with a reliable accuracy and precision.

Beside these, cyclic voltammetry was performed in Na-phosphate buffer solution (0.05 M, pH 6.0) using a graphite electrode, P(EDOT-PdBPI-*co*-HKCN)/GOx in the absence and presence of 2 mM of glucose (Fig. 3d). The charge density of the P(EDOT-PdBPI-*co*-HKCN)/GOx/Presence Glucose is greater than that of the P(EDOT-PdBPI-*co*-HKCN)/GOx/Absence Glucose. After addition of 2 mM of glucose, increasing of charge density is due to glucose oxidase (GOx) enzyme, which catalyzes the oxidation of glucose.

4. Conclusions

Here we present first time synthesis, characterization and electropolymerization of a new bis(2-pyridylimino)isoindolato-palladium complex bearing electropolymerizable EDOT (3,4-(ethylenedioxy)thiophene) substituent. In addition, P(EDOT-PdBPI-*co*-HKCN) modified graphite rod electrode was improved for amperometric glucose sensor based on glucose oxidase (GOx). In this novel biosensor matrix, amino groups which are arising from the **HKCN** were used for the enzyme immobilization. On the other hand, **EDOT-PdBPI** used to mediate the bioelectrocatalytic reaction. For comparison, three different enzyme electrodes (P(HKCN), P(EDOT-PdBPI) and P(EDOT-PdBPI-*co*-PHKCN)) were constructed and it was found that copolymer electrode has the best biosensing properties.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.020.

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