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Development of a novel biosensor based on a polypyrrole–dodecylbenzene sulphonate (PPy–DBS) film for the determination of amperometric cholesterol

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ABSTRACT

Herein a novel amperometric biosensor based on a conducting polymer with anionic dopant modified electrode was successfully developed for detection of cholesterol. Polypyrrole is deposited on a platinum surface and the sodium dodecylbenzene sulphonate (DBS) ion-doped polypyrrole film was electrochemically prepared by scanning the electrode potential between −0.8 and +0.8 V at a scan rate of 20 mV/s. The present electrochemical biosensor was optimized in terms of working potential, number of cycles, concentrations of monomer, and anionic dopant. Cholesterol oxidase (ChOx) was physically entrapped in PPy–DBS to construct an amperometric cholesterol biosensor. Amperometric determination is based on the electrochemical detection of H_2O_2 generated in the enzymatic reaction of cholesterol. Kinetic parameters, operational and storage stabilities, pH, and temperature dependencies were determined. *K*m and *I*max were calculated as 0.11 μM and 0.967 nM/min, respectively. The operational stability results showed that 90.0% of the response current was retained after 30 activity assays. Morphology of electrodes was characterized by SEM and AFM. Additionally, contact angle measurements were made with 1 μL water of polymer film and enzyme electrode. As a result, the cholesterol biosensor suggested in this study is easy to prepare and is highly cost-effective. This composite (PPy–DBS) can supply a biocompatible and electrochemical microenvironment for immobilization of the enzyme, making this material a good candidate for the fabrication of highly sensitive and selective cholesterol biosensors.

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Biosensor; cholesterol; cholesterol oxidase; polypyrrole; sodium dodecylbenzene sulphonate

Introduction

Cholesterol is a fundamental diagnostic parameter for coronary heart disease, arteriosclerosis, and other clinical (lipid) disorders. It is used to identify the risks involved in thrombosis and myocardial infarction. The clinical analysis of cholesterol in serum samples is important to diagnose and prevent such clinical disorders as hypertension, cerebral thrombosis, and heart attack. Hence, it is important to develop a reliable and sensitive biosensor which can permit a suitable and rapid determination of cholesterol. Ideally, the total cholesterol concentration should be less than 200 mg/dL (<5.17 mM). The borderline high cholesterol is defined as 200–239 mg/dL (5.17–6.18 mM), and 240 mg/dL (≥6.21 mM) and above is accepted as high.

Different analytical methods have been used for the determination of cholesterol such as colorimetric, spectrometric and electrochemical methods. Among these methods, electrochemical detection has attracted significant attention due to its speed, simplicity, and low cost. Thus, amperometric biosensors are more attractive due to their low detection limit as enzyme stabilization can easily be achieved. Especially, the enzyme based cholesterol sensors have gained special focus due to their long term stability, high sensitivity, low cost, fast response, and wide linear range. Therefore, they hold a leading position among the presently available biosensor systems (Moraes et al. 2009, Ram et al. 2001).

Most of the literature on cholesterol biosensors has focused on diagnosing disorders (Bongiovanni et al. 2001, Moraes et al. 2009, Muhammed et al. 2009), mostly on such cardiovascular diseases as heart disease, atherosclerosis, hypertension, coronary artery disease, and cerebral thrombosis (Ansari et al. 2008, Nauck et al. 2000, Yu and Ju 2002).

In the fabrication of cholesterol biosensor for the estimation of free cholesterol and total cholesterol, mainly cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) have been employed as the sensing elements (Arya et al. 2008, Feng and Liu 2015, Rahman and Asiri 2015, Umar et al. 2009). Electrochemical transducers have been effectively utilized for the estimation of cholesterol in the system (Arya et al. 2007, Charpentier and Murr 1995, Singh et al. 2006, Yïldïrïmoğlu et al. 2009, Zhou et al. 2006). Based on the number and reliability of optical methods, a variety of optical transducers have been used for cholesterol sensing, namely monitoring: luminescence, change in color of dye, fluorescence, and the others (Arya et al. 2008).

Different analytical methods have been used for the determination of cholesterol for instance colorimetric, spectrometric and electrochemical methods (Ahmad et al. 2012, Krug et al. 1994, Valentini et al. 2004, Yao et al. 1995). Among these methods, electrochemical detection of cholesterol has achieved significant attention due to the rapid determination, simplicity, and low cost. Thus, amperometric biosensors are

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more attractive due to their low detection limit and enzyme stabilization can be easily achieved. An amperometric enzyme electrode has also been used for the determination of cholesterol, but the disadvantage is the long-term stability of the enzyme. It has been mentioned in the literature that free cholesterol is determined by enzyme electrode, which is immobilized on the conducting polymer matrix (polypyrrole (PPy) or polyaniline) (Srivastava et al. 2014, Soylemez et al. 2014).

Amperometric biosensors are also used for the accurate measurement of cholesterol in a rapid manner without any need for sample pretreatment. ChOx contains flavin adenine dinucleotide (FAD) as the active redox centre. The amperometric detection of cholesterol can be based on enzymatic reaction employing ChOx and this reaction is followed by electrochemical oxidation on the electrode surface.

$$\text{Cholesterol} + O_2 \rightarrow \text{Cholest-4-en-3-one} + H_2O_2$$
$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e-$$

Several detection schemes have been proposed for enzymatic monitoring of cholesterol. Production of hydrogen peroxide during the above-mentioned enzymatic reaction can be measured using an electrochemical reaction (Çete and Bal 2013).

Conductive polymer electrodes in biosensor application have started to become widespread. The immediate advantage of conductive polymer electrodes is the lower cost of the raw materials and the economical production steps. Among various conducting polymers, PPy as an intelligent material plays an important role in the electrochemical biosensors for the purpose of increased electrochemical activity and sensitivity, owing to its good biocompatibility, conductivity, stability, and efficient polymerization at neutral pH as well as easy synthesis. PPy films can easily be formed from aqueous solutions by chemical or electrochemical routes, and have a high degree of selectivity due to their inherent size-exclusion property. A recent review on the applications of polymers in electrochemical biosensors could be found in the literature (Cete et al. 2006, Çete et al. 2007, Dolmaci et al. 2012, Gorgulu et al. 2013, Teles and Fonseca 2008), in which PPy is highlighted.

Polymers present themselves as advantageous materials for the following reasons (Guiseppi-Elie et al. 1995). Firstly, there is considerable flexibility in the available monomeric structures. Secondly, many of the available monomers are compatible with the conditions required for enzyme activity such as neutral aqueous solutions. Thirdly, electroactive polymers are capable of being reversibly doped and undoped, a feature which may be exploited to develop enzyme-based biosensors. Finally, these materials can be deposited electrochemically, a method that offers a simple approach for enzyme immobilization. Now there exists a growing literature on the use of electroactive polymers and its derivatives for sensor fabrication (Kim et al. 2000, Shin et al. 1996, Soylemez et al. 2013a, Soylemez et al. 2013b).

In the present paper, we report the results of a systematic study on the technical development and optimization of an amperometric biosensor observed by physically entrapped cholesterol oxidase in PPy–DBS films. DBS was used as anionic dopant. Several parameters such as working potential, concentrations of pyrrole and DBS, cyclic number were optimized and substrate concentration was studied. Besides, effect of pH, temperature, operational and storage stabilities were determined. Furthermore, AFM, SEM, and contact angle techniques were used for PPy–DBS electrodes morphology.

Materials and methods

Equipment and reagents

The electrochemical experiments were carried out by using EpsilonEC electrochemical analyzer with a three-electrode cell. The working electrode was a Pt plate (0.5 cm^2). Auxiliary and reference electrodes were Pt wire and Ag/AgCl electrode (3.0 M KCl), respectively. The pH values of the buffer solutions were measured using an ORION Model 720 A pH/ionmeter. Temperature control was achieved with Grant GD120 thermostat. Cholesterol oxidase (EC 1.7.3.3 from *Aspergillus niger* purified from the microorganism and with an activity of 10 unit mL^{-1}) and cholesterol were purchased from Sigma. The supporting electrolyte sodium perchlorate, pyrrole, and sodium dodecylbenzene sulphonate (DBS) were supplied by Aldrich. All other chemicals were obtained from Sigma. All the solutions were prepared using twice-distilled water.

Preparation of PPy–DBS film electrode

The surface of the Pt plate electrode was cleaned (Gros et al. 2000) and covered with PPy–DBS film by electropolymerization of pyrrole in an anionic dopant. The Pt plate electrode was immersed in 10.0 mL solution containing 0.15 M pyrrole and 5.0 mL of sodium DBS. The solution was purged with argon to remove the oxygen. Pyrrole electropolymerization was carried out on the Pt electrode surface by cyclic voltammetric scans between −0.8 and +0.8 V at a scan rate of 20 mV/s (vs Ag/AgCl electrode (3.0 M KCl)). PPy was obtained after 4 cycles (Figures 1 and 2). After electropolymerization, PPy–DBS film was rinsed with deionized water to remove the unreacted pyrrole monomer.

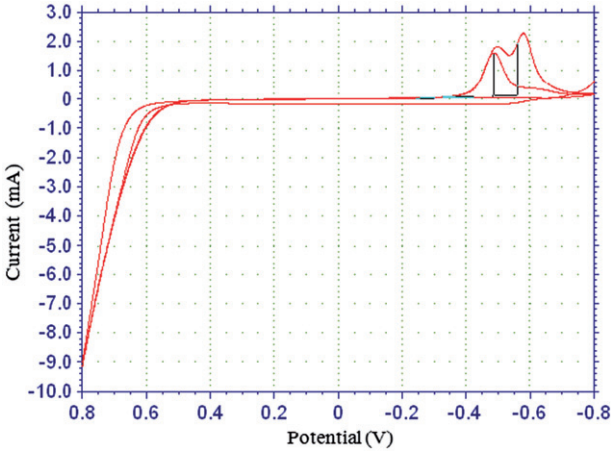


Figure 1. Growth of PPy–DBS films in aqueous solution of 0.15 M pyrrole and 0.2 M DBS at a scan rate of 20 mV/s vs Ag/AgCl electrode (3.0 M KCl).

The sensitivity of PPy–DBS film electrode to hydrogen peroxide

The response current of electrode covered with PPy was determined as follows. A buffer solution of pH 7.0 (10 mL, 25° C) was added to the cell. The solution included 0.1 M phosphate to adjust pH and 0.1 M sodium perchlorate as a supporting electrolyte. The electrodes were maintained at 0.4 V for approximately 4 h and steady current (*i*a) was saved. The different concentration of hydrogen peroxide was added to the cell. The currents acquired at 0.4 V were recorded every 200 s. The difference in current values was plotted against the hydrogen peroxide concentration. Linear region of current values was determined (Figure 8).

Immobilization of cholesterol oxidase on PPy–DBS film

Pt/PPy–DBS–ChOx film electrode was used against the Pt electrode, which was used as the counter electrode. The reference electrode was composed of Ag/AgCl. Immobilization of cholesterol oxidase was carried out by the physical entrapment approach. The concentrations of pyrrole and DBS were 0.15 M and 0.1 M, respectively. 0.5 mL of ChOx enzyme (10 U/mL) is added into the solution. The solution was purged with argon to remove the oxygen. Electropolymerization was carried out on the Pt electrode surface by cyclic voltammetric scans between −0.8 and 0.8 V at a scan rate of 20 mV/s (vs Ag/AgCl electrode (3.0 M KCl)). PPy was obtained after 4 cycles. After the fabrication of cholesterol oxidase entrapped PPy film onto the PPy–DBS working electrode was finished, the electrode was rinsed with deionized water to remove the unreacted pyrrole monomer and free cholesterol oxidase. Immobilized enzyme electrode was kept in a refrigerator at the 4 °C in phosphate buffer when it was not in use (Nauck et al. 1997).

The sensitivity of PPy–DBS–ChOx enzyme electrode to hydrogen peroxide

The response currents of enzyme electrode were determined as follows. A buffer solution of pH 7.0 (10 mL, 25 °C) was added to the cell. The solution included 0.1 M phosphate to adjust pH and 0.1 M sodium perchlorate as a supporting electrolyte. The enzyme electrodes were kept at 0.4 V for approximately 5 h and steady current (*i*a) was saved. The different concentration of cholesterol was added to the cell. The currents acquired at 0.4 V were recorded every 200 s. The difference in current values was plotted against the cholesterol concentration.

Amperometric measurements

Quantification of cholesterol was achieved via electrochemical detection of enzymatically released H_2O_2.

$$\text{Cholesterol} + O_2 \rightarrow \text{Cholest-4-en-3-one} + H_2O_2$$
$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e-$$

Enzyme electrode was immersed into phosphate buffer (0.1 M) at pH 7.0. The solution contained 0.1 M sodium chloride as supporting electrolyte. Electrode was brought to equilibrium by keeping it at 0.4 V [vs. Ag/AgCl electrode (3.0 M KCl)]. Steady-state current (*i*a) was recorded. Cholesterol solution was added to the cell from stock solution and the system was stirred. Current (*i*b) values obtained at 0.4 V were recorded and current values ($\Delta i = ib - ia$) were plotted against the cholesterol concentration.

Results and discussion

In this study, we prepared a new hydrogen enzyme-based biosensor for the quantification of cholesterol. All parameters were examined. The electrochemical responses of the biosensor electrode to cholesterol were investigated. Immobilization of cholesterol oxidase upon of PPy–DBS film was carried out. Besides, we determined pH, temperature, operational and storage stabilities.

SEM (scanning electron microscope) studies of PPy–DBS film

In these experiments, SEM JEOL Model 6060 LV was used. The morphology of electrode was characterized by SEM (Figures 2 and 3). The SEM image of PPy–DBS is illustrated in Figure 2. The SEM image (Figure 2) acquired for the Pt/PPy–DBS

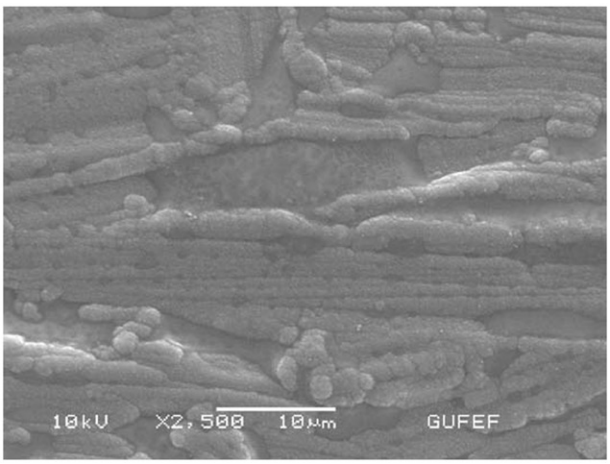


Figure 2. The SEM of Pt/PPy–DBS electrode.

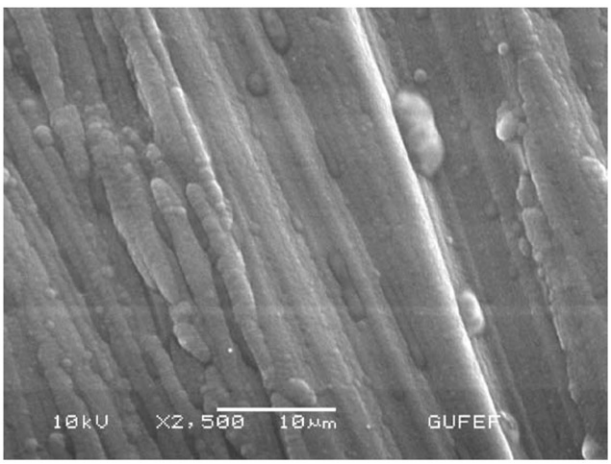


Figure 3. The SEM of Pt/PPy–DBS–ChOx.

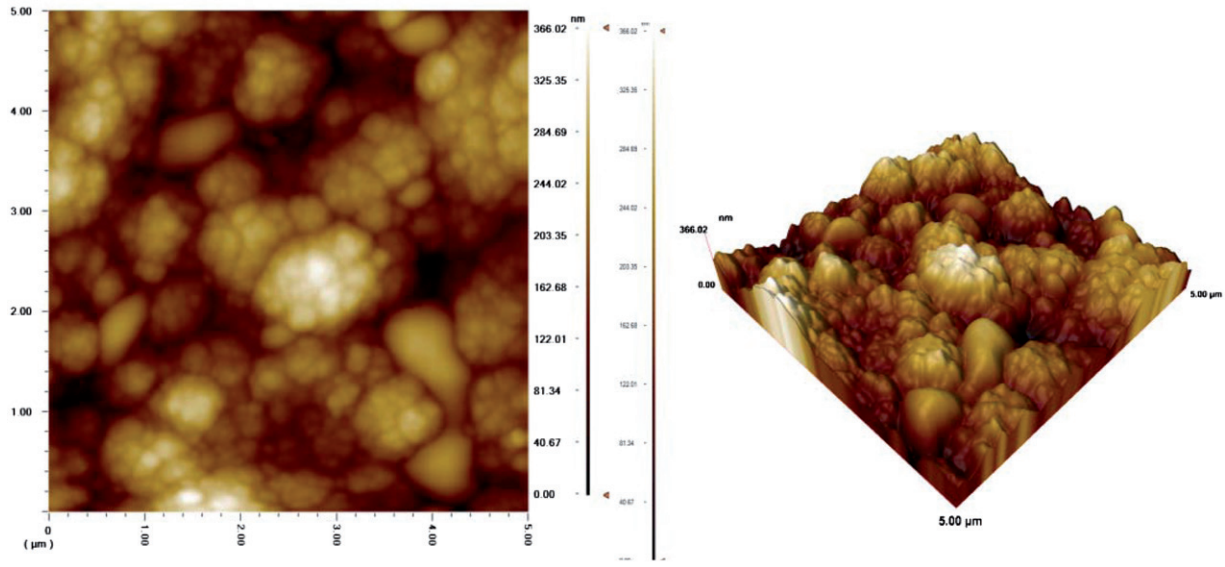


Figure 4. The AFM of Pt/PPy–DBS–ChOx.

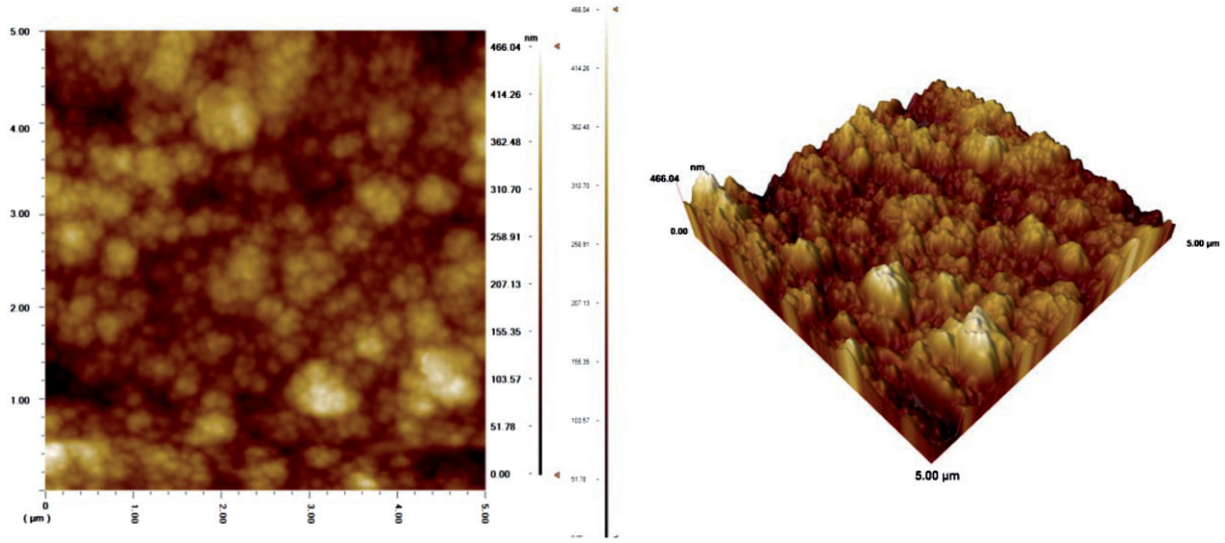


Figure 5. The AFM of Pt/PPy–DBS electrode.

electrode demonstrates fine microspheres resulting in the porous surface. The SEM image of Pt/PPy–DBS–ChOx is given in Figure 3.

AFM (atomic force microscope) studies of PPy–DBS film

In these experiments, AFM JEOL Model 6060 LV was used. The morphology of electrode was characterized by AFM (Figures 4 and 5). The AFM image of PPy–DBS is illustrated in Figure 3. The AFM image (Figure 3) acquired for the Pt/PPy–DBS electrode demonstrates fine microspheres resulting in the porous surface. The AFM image of Pt/PPy–DBS–ChOx is given in Figure 4.

Contact angle studies of PPy–DBS film

In these experiments, contact angle Kruss model DSA 100 was used. The hydrophilicity of electrode was characterized by



Figure 6. The contact angle of Pt/PPy–DBS electrode.

contact angle (Figures 6 and 7). The contact angle picture of PPy–DBS is shown in Figure 6. The contact angle image (Figure 6) acquired for the Pt/PPy–DBS electrode demonstrates it to be a hydrophobic surface. The contact angle of Pt/PPy–DBS–ChOx is given in Figure 7. It has shown that



Figure 7. The contact angle of Pt/PPy–DBS–ChOx.

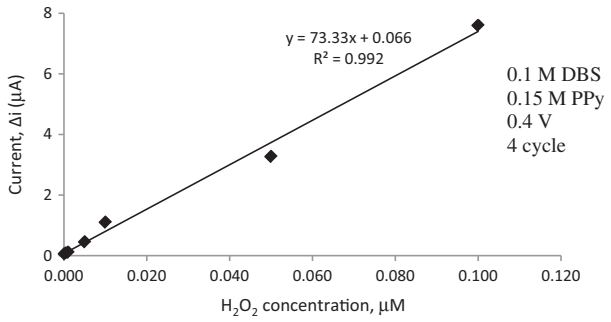


Figure 8. Amperometric response measurements of Pt/PPy–DBS electrode.

enzyme electrode is more hydrophilic than free enzyme electrode. This state can be attributed to hydrophilic groups of protein.

Amperometric response measurements of Pt/PPy–DBS electrode

As the PPy–DBS coated electrode was designed to be utilized as a biosensor, the determination of its sensitivity to hydrogen peroxide is a result of enzymatic reactions. The results of amperometric response currents detected for Pt/PPy–DBS electrodes are illustrated in Figure 8. This figure illustrates that the PPy–DBS electrode gives a great linear response.

The determination of working potential

After preparing Pt/PPy–DBS electrodes, oxidation of the hydrogen peroxide was examined at a different potential (Figure 9). For this purpose, four different potentials (0.4, 0.5, 0.6, and 0.7 V) were examined. The biggest current value was achieved at 0.70 V. At high potentials, interferences caused by exogenous substances present in body fluids (e.g., ascorbic acid) (Zhang et al. 2007). Therefore, 0.4 V was selected as working potential.

The pyrrole concentration experiments

The electrodes sensitivity to hydrogen peroxide was studied to explain the important of pyrrole concentration (Figure 10). For this reason, several pyrrole concentrations (0.05, 0.1, 0.15, and 0.2 M) were examined. The lowest correlation coefficient of electrode was found at 0.05 M pyrrole concentration. It was shown that high pyrrole concentration was not suitable for

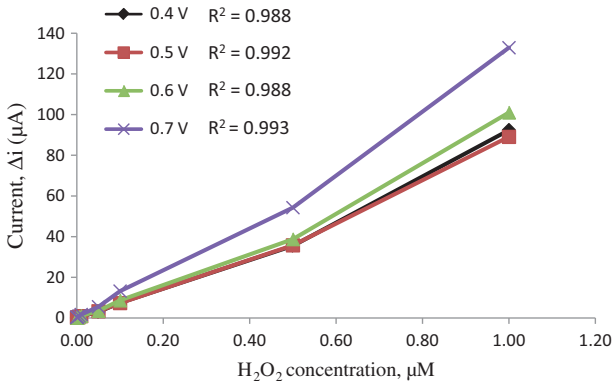


Figure 9. The effects of different potentials on amperometric response measurements.

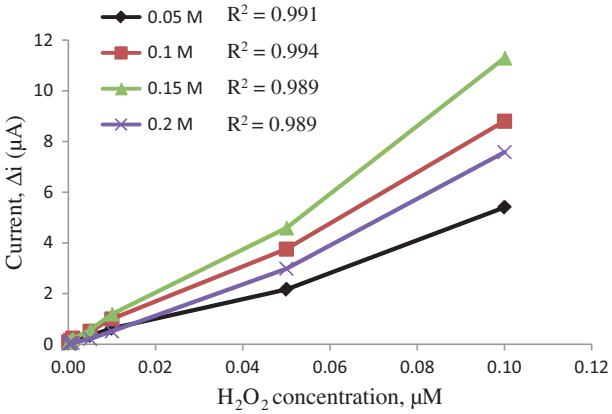


Figure 10. The effects of different pyrrole concentrations on amperometric response measurements.

polymer film. Surface of these electrodes prepared by 0.15 M pyrrole was smoother and stronger than the other electrodes. Therefore, the pyrrole concentration was used as 0.15 M in the next trial.

The effect of DBS concentration

In this study, DBS was used as an anionic dopant. The sensitivities of electrodes to hydrogen peroxide were determined to clarify the role of DBS concentration. Three different DBS concentrations (0.05, 0.1, 0.15 M) were examined (Figure 11). Figure 11 shows that correlation coefficients of film electrodes covered in 0.05 and 0.15 M DBS were lower than 0.10 M. The best linear response was appeared with the use of 0.05 M sodium DBS. All of the experiments were carried out with Pt/PPy–DBS electrodes prepared by maintaining the DBS concentration at 0.1 M.

The effect of cycle number of PPy–DBS film

Film thickness was determined using cycle numbers. The effects of film cycle number on the sensitivity of the electrodes against hydrogen peroxide concentration were examined for different cycles (Figure 12). Figure 12 indicates that the electrodes prepared by 2, 4, 6, and 12 cycles have high linearity. When cycle numbers increase, film thickness grows up. Sensitivity decreases with increasing film thickness because it

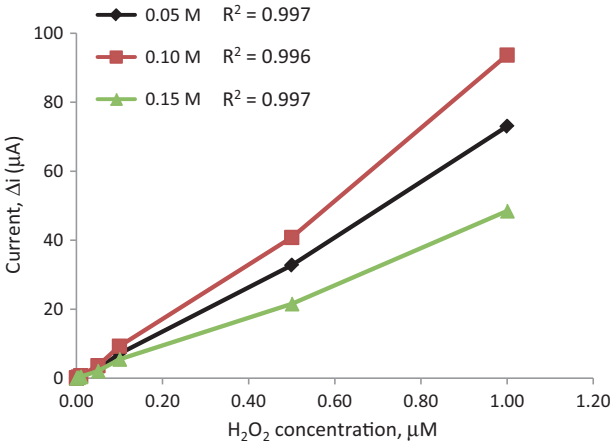


Figure 11. The effects of different concentrations of sodium dodecylbenzene sulphonate on amperometric response measurements.

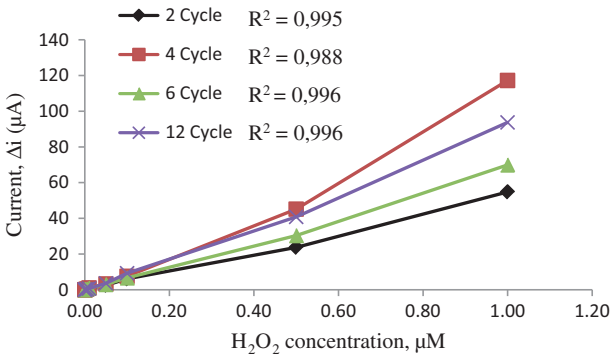


Figure 12. The effects of cycle number of polypyrrole film on amperometric response measurements.

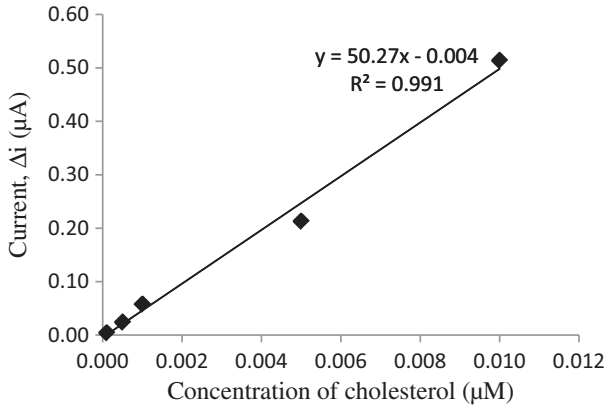


Figure 13. Amperometric response measurements of Pt/PPy–DBS–ChOx electrode.

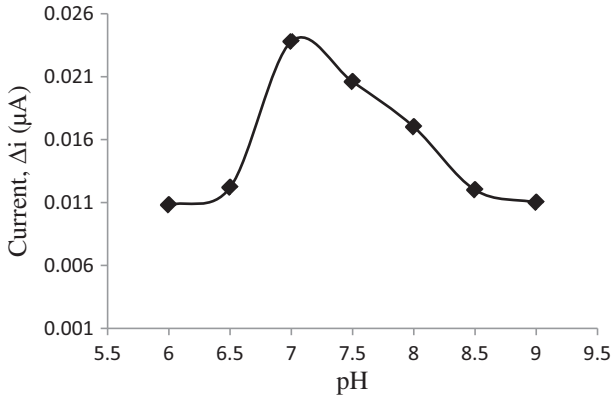


Figure 14. The effect of pH on the response of the biosensor (in the phosphate buffer (pH 7.0) containing 50 μM cholesterol at 0.4 V working potential).

is proportional to conductivity. The highest response currents were obtained for electrodes prepared by 4 cycles. Moreover, this electrode has much more mechanical strength and reproducibility. The observed higher value of amperometric response current may be attributed to the thin film. Pt surface was too thick for the higher cycles. Therefore, amperometric response currents were poor. For the electrodes prepared by 4 cycles, the surface of the electrode was smoother and more durable than the others.

Amperometric response measurements of PPy/DBS–ChOx

Since the PPy–DBS coated electrode prepared was designed to be utilized as a biosensor, the determination of its sensitivity to hydrogen peroxide is a result of enzymatic reactions. The results of amperometric response currents detected for Pt/PPy–DBS–ChOx electrodes are illustrated in Figure 13. The figure shows that the enzyme electrode was very sensitive to cholesterol. This enzyme electrode can be used to determine cholesterol in various samples.

pH dependence of the response of biosensor

The pH of buffer has a great influence on enzyme activity and the catalytic capacity for hydrogen peroxide reduction. The change in ionization state of amino acid side chain can perform critical functions in the enzyme active site; thus, the enzyme activity should change with the pH of buffer. The effect of the buffer pH on the amperometric response of biosensor was examined by using phosphate buffer solutions in a range of $6.0 \leq$ pH ≤ 9.0 in the presence of 0.05 mM of cholesterol. The effect of solution pH on the cholesterol response at the proposed biosensor is illustrated in Figure 14. The graph shows that the best pH for cholesterol biosensor is 7.0. This value is possible. At extreme pH values, the enzyme was denaturated. Therefore, pH 7.0 is selected by considering both the sensitivity and the stability of the biosensor and consequently further experiments were performed at pH 7.0. Besides, the phosphate buffer (0.1 M) was used as the buffer system for all other experiments.

For cholesterol biosensor, optimum pH was reported to be 7 in the previous studies (Abdelwahab et al. 2010, Khan et al. 2008, Singh et al. 2004, Singh et al. 2011, Turkarslan et al. 2009, Zhao et al. 2008). However, pH values different from 7.0 were also used in literature (pH 7.5; 6.5; 6.8; 6.0) (Ahmad et al. 2010, Basu et al. 2007, Brahim et al. 2001, Singh et al. 2006). The difference in pH values was attributed to the fact that the used polymer and the type of immobilization were different, environment changes in the enzyme's active site can cause different interactions between enzyme and immobilization materials (Ahmadalinezhad and Chen 2011, Gholivand and Khodadadian 2014, Tiwari and Gong 2008). Furthermore, enzymes become stronger after immobilization. Immobilized enzymes can show high activities under different pH

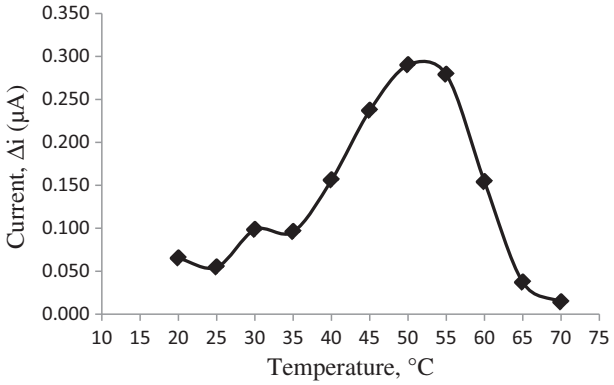


Figure 15. The effect of temperature on the response of the biosensor (at pH 7.0, 50 μM cholesterol at 0.4 V working potential).

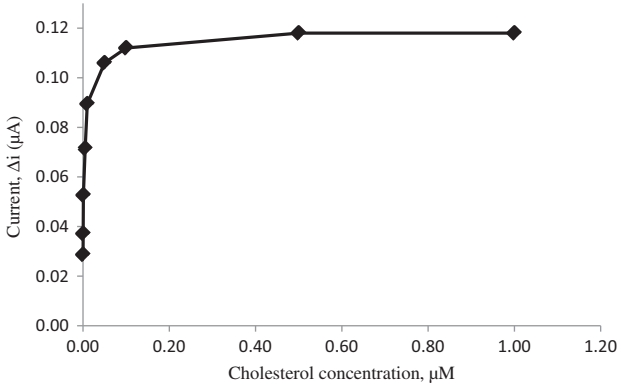


Figure 16. The effect of cholesterol concentration on the response of the biosensor (at 0.1 M pH 7.0 the phosphate buffer, 25 °C, 0.4 V working potential).

conditions when compared with free enzyme and may form the stable substrate–enzyme complex.

Temperature dependence of the response of biosensor

Temperature exerts a marked influence on the enzymatic activity as well as on the performance of the amperometric cholesterol biosensor employed in this work, the sensitivity of which increases at lower temperatures. The effect of this parameter on the sensor response was evaluated in the range 20–70 °C. As illustrated in Figure 15, the current response gradually increased with increasing temperature and reached a maximum at 50 °C. This value is higher than those in many other studies (Basu et al. 2007, Chauhan et al. 2010, Singh et al. 2004, Singh et al. 2006, Turkarslan et al. 2009). This was attributed to the fact that the used polymer and immobilization materials and the type of immobilization were different. Additionally, immobilized enzymes can show high activity under temperatures different from physiological conditions compared to that of free enzymes. Enzyme can be denaturated after a long incubation period at a temperature of 50 °C. However, for practical convenience, all other experiments were carried out at 25 °C.

Effect of substrate amount on the response of biosensor

The value of the Michaelis–Menten kinetic parameter (*K*m), which shows the enzyme–substrate kinetics, was determined by analyzing the slope of enzymatic reaction. *V*max is the maximum rate for enzymatic reaction. The effect of the substrate concentration on the reaction rate, catalyzed by immobilized ChOx, was studied using varying initial concentration (0.1 μM–1 mM) of cholesterol substrate (Figure 16). PPy–DBS film, used in this study, increased the affinity of enzyme to cholesterol. *K*m(app) constant and *I*max, a specific parameter of enzymes, was found out using 1/[ACh] – 1/Δi graph (Lineweaver–Burke plot; Figure 17). With the increase in substrate concentration, there was an increase in amperometric current signal. Kinetic parameters *K*m(app) and *I*max for the enzyme biosensor were detected at constant temperature (25 °C) and pH 7.0 while varying the substrate concentration. *K*m(app) and *I*max were calculated as 0.11 μM and 0.967 nM/min, respectively. *K*m can be understood from the *K*m(app) value, which is lower than the *K*m(app) values in literature (Khan et al. 2008, Matharu et al. 2007, Singh et al. 2004, Singh et al. 2011).

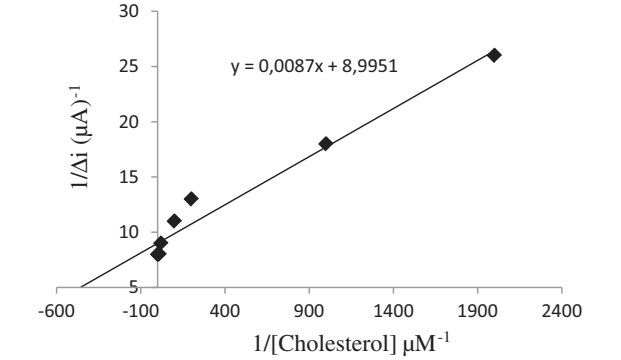


Figure 17. The effect of cholesterol concentration on the response of the biosensor (Lineweaver–Burk plot at 0.1 M pH 7.0 phosphate buffer, 25 °C, 0.4 V working potential).

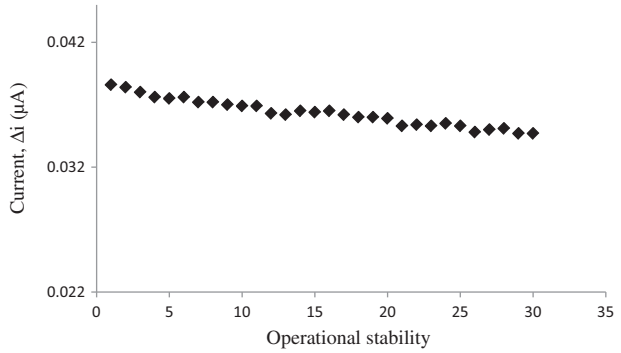


Figure 18. Operational stability of the biosensor in pH 7.0 phosphate buffer, 50 μM cholesterol 25 °C, 0.4 V working potential.

Operational stability and storage stability

In order to test the operational stability of the prepared enzyme electrode, the current changes, obtained after subsequent use, were plotted against the number of measurements (Figure 18) . By the end of the 30 measurements, the biosensor had lost 10.0% of its initial activity. The operational stability was examined by applying activity assay (under optimum

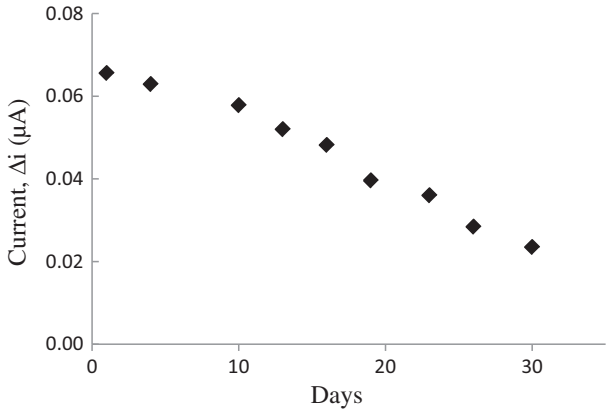


Figure 19. Storage stability of the biosensor in pH 7.0 phosphate buffer, 50 μM cholesterol 25 °C, 0.4 V working potential.

Table 1. Interfering substances on the amperometric response of the biosensor.

Interfering substances	Concentration	Response current of interfering substances (μA)
Glucose	5 mM	0.0064
Uric acid	0.1 mM	0.0450
Paracetamol	0.1 mM	0.6950
Ascorbic acid	0.1 mM	0.6150

conditions) for 30 times in the same day at a constant temperature, pH, and substrate concentration.

Response of the enzyme electrode, prepared under optimum conditions, was measured for a period of 48 days at a constant cholesterol concentration (50 μM). Results of nine measurements during this period are plotted in Figure 19. In general, an enzyme is not stable in aqueous solution during storage and the activity is gradually reduced (Hendji et al. 1993). The reusability was tested because of its importance for repeated applications in a batch reactor. The main advantage of reusability is to reduce the cost of the treatment. The electrode showed 36% of the initial amperometric response at the end of the 30 days.

Interference effects

The influence of electrochemical interferents on the current response of cholesterol was evaluated at Pt/PPy–DBS–ChOx biosensor. A few common substances found in serum or urine were studied for any interfering effect on the analysis of cholesterol. Known concentrations of ascorbic acid, uric acid, glucose, and paracetamol (acetoaminophen) were added. The results are shown in Table 1, which represents the amperometric responses of cholesterol (50 μM), in the presence of glucose (5 mM), uric acid (0.1 mM), paracetamol (0.1 mM), ascorbic acid (0.1 mM) in PBS (pH 7.0) solution. These interferences were almost removed by diluting the solution in cell. Thus, Pt/PPy–DBS–ChOx biosensor exhibited high selectivity for the determination of cholesterol.

Conclusion

In this study, we report a new enzyme-based amperometric biosensor for the determination of cholesterol. ChOx was physically entrapped onto a PPy–DBS film surface. All parameters were examined. Sodium DBS was used as an anionic dopant. The experimental results showed clearly that the biosensor exhibited good performance in the determination of cholesterol. It has a very low detection limit and an acceptable response time for a biosensor. Herein prepared cholesterol biosensor can be used in a large concentration range. Hence, cholesterol determination can be made in a variety of different samples with our biosensor. The biosensor had a minimum detectable concentration of 10 nM. It was seen that the cholesterol biosensor was highly sensitive, selective and its operational stability was found to be very good. Thus, cholesterol determination can be made with our biosensor repeatedly. The cholesterol biosensor suggested in this study is easy to prepare and is highly cost-effective. This composite (PPy–DBS) can supply a biocompatible and electrochemical microenvironment for immobilization of the enzyme, making this material a good candidate for the fabrication of highly sensitive and selective cholesterol biosensors.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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