J Mater Sci (2009) 44:954–961
DOI 10.1007/s10853-008-3184-y

Poly (pyrrole-co-*N*-methyl pyrrole) for application to cholesterol sensor

K. Singh · T. Basu · Pratima R. Solanki · Bansi Dhar Malhotra

Received: 21 August 2008 / Accepted: 11 December 2008 / Published online: 13 January 2009

Abstract Cholesterol oxidase (ChOx) has been electrochemically entrapped onto *p*-toluene sulphonate (PTS) doped poly (pyrrole-co-*N*-methyl pyrrole) (1:1) on indium-tin-oxide (ITO) glass plates. This ChOx-copolymer-PTS/ITO bioelectrode has been characterized using cyclic voltammetry (CV), Fourier transform infrared (FTIR) spectroscopy, UV-visible spectroscopy, scanning electron microscopy (SEM) techniques. The cholesterol bioelectrode shows response time of 19 s, linearity from 50 to 400 mg/dL as a function of cholesterol concentration. It exhibits optimum pH range between 6.5 and 7.5, shelf-life of up to 6 weeks at 4 °C and shows almost undisturbed response in presence of interferents like ascorbic acid, uric acid and glucose.

Introduction

Cholesterol biosensor has recently attracted much attention due to their application in clinical diagnosis of heart ailments. Cholesterol (cholest-5-en-3β-ol) is a ubiquitous component and much of it is located in the membranes. The main function of cholesterol is to modulate fluidity of the membrane by interacting with its complex lipid components. The cholesterol level varies in different cell membranes and undergoes changes during different physiological conditions. The high blood cholesterol level is associated with atherosclerosis, nephrosis, diabetes mellitus, myxedema, jaundice and the low cholesterol level is the root cause of hyperthyroidism, cerebral thrombosis, anaemia and maladsorption [1–3]. The determination of cholesterol is presently based on spectrophotometric technique [4] that is complicated and expensive and requires expertise [5]. It has been found that biosensors can be helpful for clinical diagnostics due to their good selectivity, fast response and miniaturized size [6].

Prior to the fabrication of a biosensor, immobilization of a desired biomolecule in a matrix is considered to be very important. In this context, a large number of materials such as self-assembled monolayers and multilayers, Langmuir–Blodgett films, sol–gel films, screen printed electrode, nanomaterials and conducting polymers have been used for the immobilization of desired biomolecules including cholesterol oxidase [7–15]. Among these, conducting polymers have been considered as very important. This has been attributed to their various advantages such as biocompatibility, ease of preparation, chemical inertness and redox characteristics. Among the various conducting polymers, polypyrrole has attracted much attention [16–20] for biosensor application. Vidal et al. have fabricated a cholesterol biosensor by the entrapment of ChOx within the over oxidized polypyrrole (PPy) film. Using immobilized ChOx and potassium ferricyaide $K_3[Fe(CN)_6]$ on dodecylbenzene sulphonate ion doped PPy films, Brahim et al. have entrapped ChOx within a composite of poly(2-hydroxethyl methacrylate) (p(HEMA))-tertraethyleneglycol diacrylate (TEGDA)/PPy membrane for cholesterol

K. Singh
Amity Institute of Engineering and Technology,
Amity University, Noida, UP, India

T. Basu
Amity Institute of Nano Technology, Amity University,
Noida, UP, India

P. R. Solanki · B. D. Malhotra (✉)
Biomolecular Electronics and Conducting Polymer Research Group, National Physical Laboratory, Dr K. S. Krishnan Marg,
New Delhi 110012, India
e-mail: bansi.malhotra@gmail.com

biosensor fabrication and obtained enhanced stability. Singh et al. have reported a cholesterol biosensor based on PPy films and have shown that the electrode shows stability of 1 month [21]. Solanki et al. have used poly (aniline-co-pyrrole) film as matrix for cholesterol biosensor fabrication. The electrode shows sensitivity of 93.35 μA/mM/cm^2 [22]. Dhand et al. have reported cholesterol biosensor based on nano structured conducting polymer film and found Michaelis–Menten constant (K_m) to be 0.62 mM [23]. Türkarslan et al. [24] 2008 have immobilized cholesterol oxidase (ChOx) in conducting polymers like poly (pyrrole) (PPy) and poly (3, 4-ethylenedioxythiophene) (PEDOT) and Michaelis–Menton (K_m) values are found to be 7.9 and 1.3 mM for PPy and PEDOT, respectively. The steady state amperometric measurements of free cholesterol have been performed by an enzyme electrode which is developed by electrostatic immobilization of the cholesterol oxidase in poly(vinylferrocenium) perchlorate ($PVF + ClO_4^-$) film that has been coated on a Pt electrode. The linear working range and the sensitivity values are found to be 0.1–0.5 mM and 140 μAM^{-1} cm^{-2}, respectively [25]. Cholesterol oxidase (ChOx) has been covalently linked with Langmuir–Blodgett (LB) monolayers of polyaniline (PANI)-stearic acid (SA) prepared onto indium-tin-oxide (ITO) coated glass plates via glutaraldehyde (Glu) chemistry. The linearity has been obtained from 25 to 400 mg/dL of cholesterol concentration with sensitivity of 88.9 nA mg^{-1} dL [26].

In this manuscript, copolymer of pyrrole and *N*-methyl pyrrole has been electrochemically synthesized on indium-tin oxide (ITO) electrode using *p*-toluene sulphonate as an electrolyte. The copolymer is characterized using cyclic voltammetry, Fourier transform infrared (FTIR) spectroscopy and UV–visible spectroscopy. The electrochemical entrapment of enzyme (ChOx) in the conducting polymer films allows uniform and reproducible immobilization of enzymes over a small area as well as control of the coating thickness [27].

Experimental

Reagents

Pyrrole and *N*-methyl pyrrole (Merck) were purified by fractional distillation and are stored at 4 °C. *p*-Toluenesulphonate (PTS) and cholesterol oxidase (ChOx; EC 1.1.36, from pseudomonas fluorescens) with the specific gravity of 24 units (U)/mg, and horseradish peroxide (HRP, EC 1.11 1.7) with specific activity of 120 U/mg solid were procured from Sigma (USA). All other chemicals were of analytical grade.

Solutions preparation

Cholesterol oxidase (24.0 U/mL) and horseradish peroxidase (120 U/mL) were freshly prepared prior to being used in phosphate buffer saline (PBS) (50 mM, pH 7.0, 0.9% NaCl). A cholesterol stock solution (400 mg/dL) was prepared using Triton X-100 as a surfactant and was kept at 4 °C. This solution was further diluted to make various concentrations. *o*-dianisidine (1%) was prepared afresh in the de-ionized water.

Polymerization of poly-(pyrrole- co-*N*-methyl pyrrole)-*p*-toluene sulphonate films

The polypyrrole and poly *N*-methyl pyrrole homopolymer films were electrochemically prepared onto ITO coated glass plates in a three-electrode cell containing 0.1 M pyrrole and 0.1 M *N*-methyl pyrrole, respectively, in 0.1 M PTS solution in deionized water using a Potentiostat/Galvanostat (Model No 273A Princeton Applied Research). The monomers were doubly distilled prior to polymerization. The cell configuration consisted of Ag/AgCl as the reference electrode, platinum (Pt) foil as the counter electrode and indium-tin-oxide (ITO) as the working electrode. For the synthesis of poly(pyrrole-co-*N*-methyl pyrrole)-*p*-toluene sulphonate, a solution mixture containing 10 mL of 0.1 M PTS solution and 1:1 ratio of pyrrole and *N*-methyl pyrrole were stirred in a sonicator until a homogeneous solution was obtained. The electrochemical polymerization was carried out at 0.9 V for 600 s. All the electropolymerized poly (Py-co-NMP)/PTS/ITO electrode were washed with PBS buffer and dried at room temperature.

During the electrochemical polymerization, copolymer shows higher onset potential than that of polypyrrole. The electrical conductivity of polypyrrole, *N*-methyl pyrrole and poly (Py-co-NMP)/PTS/ITO was calculated using four points probe method and was found as 2.5×10^{-2}, 3.4×10^{-3} and 1.5×10^{-2} s/cm^2, respectively (Table 1).

Preparation of ChOx/poly (Py-co-NMP)/PTS/ITO bioelectrode

The ChOx/poly (Py-co-NMP)/PTS/ITO bioelectrodes were prepared onto desired ITO coated glass plates by a single step using 50 μL of cholesterol oxidase (ChOx) in a three-electrode cell containing 1:1 ratio of pyrrole (Py) and *N*-methyl pyrrole (NMP), 0.1 M PTS (electrolyte) at the same potential of 0.9 V for 600 s that were used for polymer electrodes. The ChOx is entrapped in the copolymer electrode during electro-polymerization of the monomers. The resulting bioelectrodes were washed with the phosphate buffer (50 mM, pH 7.0) to get readout of unreacted monomers and unbound enzyme from the

Table 1 Anodic peak potential, anodic peak current and conductivity for polymers and co-polymers

S. No.	Polymer	Onset potential (mV)	Conductivity (S/cm)	Anodic potential, Ep (mV)	Anodic peak current (I_p)μ amp
1	Poly pyrrole/PTS/ITO	550	2.5×10^{-2}	1193	6.8
2	Poly(Py-co-NMP)/PTS/ITO (1:1)	600	1.5×10^{-2}	1257	5.6
3	Poly *N*-methyl pyrrole/PTS/ITO	810	3.4×10^{-3}	1132	2.6

film. These ChOx/poly(Py-co-NMP)/PTS/ITO bioelectrodes were stored at 4 °C when not in use.

Equipments

Ultraviolet-visible (UV–vis) absorbance was measured for copolymer electrodes as well as bio-electrode by using Shimadzu (Model 160A) spectrophotometer. Cyclic voltammetric studies were carried out using Potentiostat-Galvanostat (Model No 273A Princeton Applied Research). Fourier-transform-infrared (FTIR) studies were done using Perkin Elmer Spectrum BX and SEM photographs were taken using scanning electron microscope (LEO 440). Water purification system (Model Milli Q) was used to obtain deionized water.

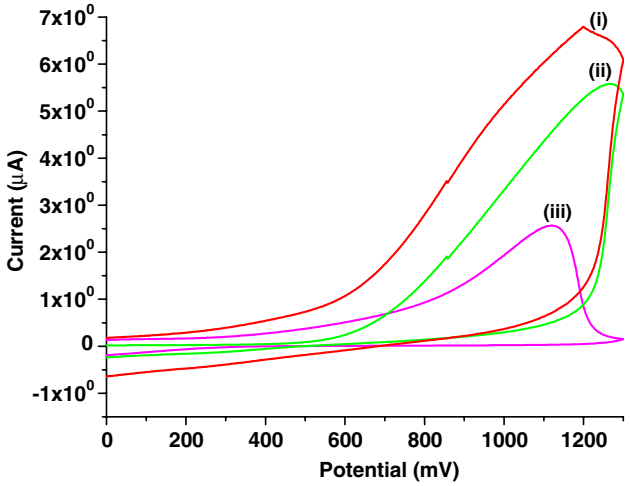


Fig. 1 Cyclic voltammograms of polymers and co-polymers: (i) polypyrrole/PTS/ITO, (ii) poly (pyrrole-Co-*N*-methyl)/PTS/ITO (1:1) and (iii) poly-*N*-methyl polymer/PTS/ITO electrode

Results and discussion

Characterization of ChOx/co-polymer/PTS/ITO bioelectrode

Cyclic voltammetric (CV) studies of polymer films

Figure 1 shows cyclic voltammograms of polypyrrole/PTS/ITO (curve i), poly *N*-methyl pyrrole/PTS/ITO (curve iii) and co-polymer/PTS/ITO electrode (curve ii) in PBS (50 mM) at scan rate of 50 mV/s in the range of −0.2 to 1.2 V. The copolymer (curve ii) shows lower anodic peak current than poly pyrrole (curve i) and higher anodic potential than polypyrrole (Table 1). It may be attributed to the restriction of π conjugation by the presence of methyl group in *N*-methyl pyrrole which decreases electronic conductivity [28, 29].

UV–visible spectroscopic studies

Figure 2 (A) exhibits UV–visible spectra of copolymer and polymer electrode, (B) for bioelectrode and polymer electrode. Polypyrrole/PTS/ITO (curve a) film shows a peak at 540 nm (Fig. 2A) due to inter-band charge transfer associated with the excitation of aromatic ring to non-aromatic ring [30]. The poly *N*-methyl pyrrole (Fig. 2A, curve c) shows a peak at 561 nm. The copolymer (curve b) in Fig. 2B shows two peaks at 515 and 680 nm. The peak at 515 nm belongs to poly pyrrole block and 680 nm is attributed to poly *N*-methyl pyrrole block. The appearance of two peaks in copolymer signifies both poly pyrrole and *N*-methyl pyrrole moieties are present in the copolymer. However, both the peaks in copolymer are shifted from the original peak position of the homopolymers indicating the formation of a block copolymer [-$(A)_n$–$(B)_{m-}$] (Scheme 1). The peak corresponding to *N*-methyl pyrrole unit is shifted to 680 from 561 nm, indicating a red shift and the peak corresponding to pyrrole unit exhibits a blue shift, i.e. from 540 to 515 nm. The observed shift in peak position of π–π^* transition in the conducting copolymer indicates the formation of block copolymer rather than a polymeric blend because all polymers are in the identical oxidation state that is conducting state as shown by the conductivity value. The blue shift in pyrrole block reveals that the extent of conjugation of pyrrole is hindered in the copolymer due to presence of *N*-methyl pyrrole unit and the red shift in *N*-methyl block reveals an increase in conjugation length due to the presence of pyrrole unit [31, 32].

Figure 2B shows UV–visible spectra of poly(Py-co-NMP)PTS/ITO and ChOx/(poly(Py-co-NMP)PTS/ITO

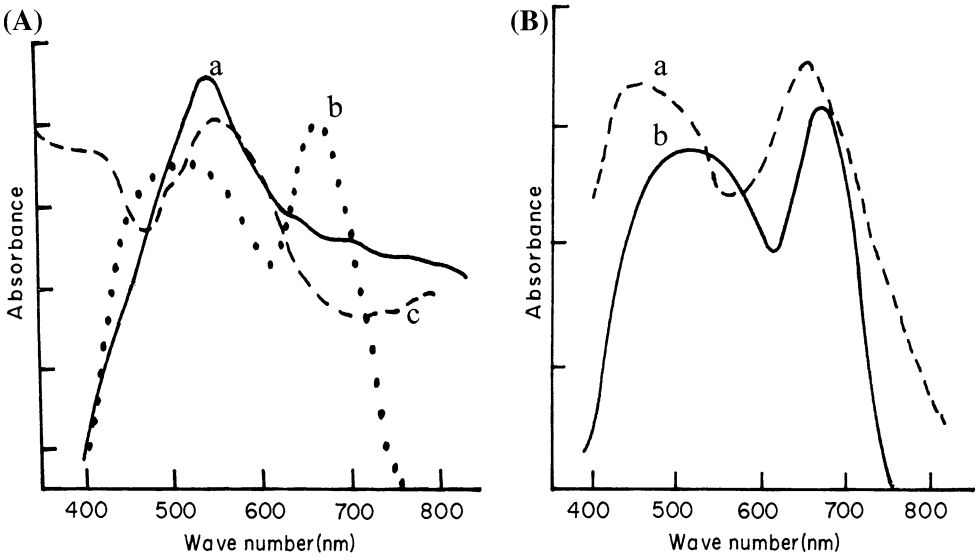
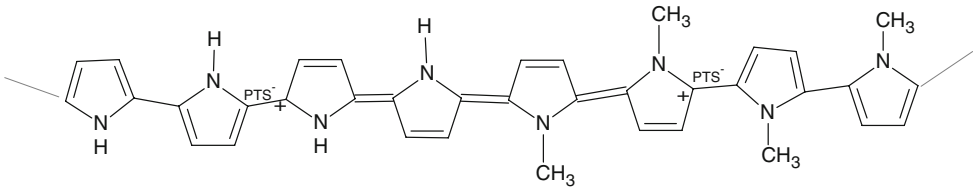


Fig. 2 UV–visible spectra of **A** poly (Py-co-NMP)/PTS consisting of curve (a) polypyrrole/PTS/ITO, (b) copolymer, (c) poly *N*-methyl pyrrole and **B** showing (a) poly(Py-co-NMP)PTS/ITO, (b) ChOx/poly(Py-co-NMP)PTS/ITO bioelectrode

Scheme 1 Structure of block poly (pyrrole-co-*N*-methyl pyrrole)/PTS film



bioelectrode. The peak corresponding to pyrrole moiety in the copolymer (curve a) shows a red shift in the bioelectrode (curve b) indicating higher extent of conjugation, derived from an enhanced interaction with cholesterol oxidase and the peak due to *N*-methyl pyrrole unit shows no change in peak position in the bioelectrode which may be attributed to lesser interaction of *N*-methyl pyrrole unit with the enzyme due to steric hindrance from the methyl group.

FTIR spectroscopic studies

In order to confirm the formation of copolymer, FTIR spectra have been recorded for the conducting copolymer and are compared with those of conducting homopolymers, that is doped and half oxidized state. The homopolymer show characteristics peaks as reported in the literature [32]. The copolymer contains peaks due to N–H vibration frequency at 3,385 cm^{-1}, assigned to poly pyrrole unit and C–H (*N*-methyl) at 2,945 cm^{-1} assigned to poly *N*-methyl pyrrole unit indicating the formation of co-polymer containing pyrrole and *N*-methyl pyrrole unit rather than a polymeric blend (data not shown). Figure 3 shows FTIR spectra obtained for poly (Py-co-NMP)/PTS/ITO (curve a) and ChOx/poly (Py-co-NMP)/PTS/ITO (curve b) bioelectrode.

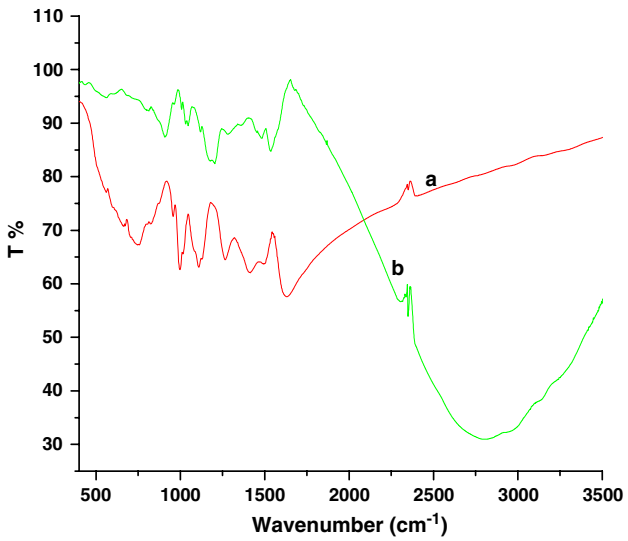


Fig. 3 FTIR spectra of **a** poly(Py-co-NMP)/PTS/ITO and **b** ChOx/(poly(Py-co-NMP)/PTS/ITO bioelctrode

The peak seen in the range of 1,640–1,690 cm^{-1} of ChOx/Poly (Py-co-NMP)/PTS bioelectrode is attributed to the amide linkage of ChOx molecules and broad peak in the region of 3,000 cm^{-1} is due to N–H stretching of amide bond of cholesterol oxidase [33].

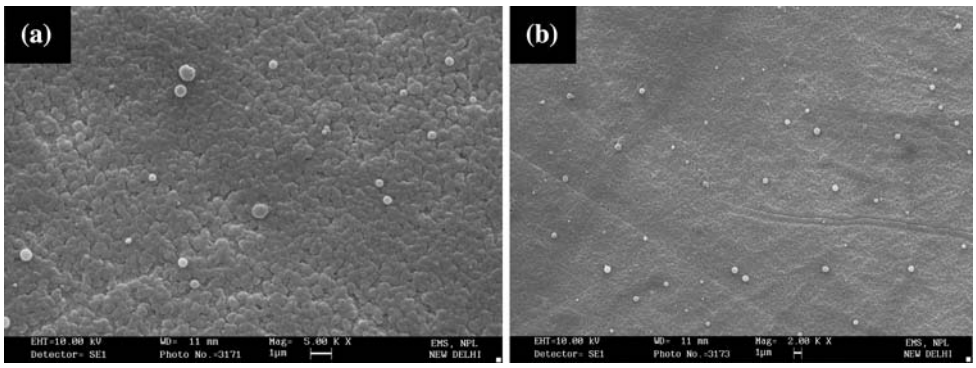


Fig. 4 SEM pictures of **a** poly(Py-co-NMP)/PTS/ITO electrode and **b** ChOx/poly(Py-co-NMP)/PTS/ITO bioelectrode

Scanning electron microscope (SEM) studies

Copolymer texture and enzyme immobilized electrode surfaces are shown by SE micrographs (Fig. 4a, b) which shows a uniform and compact globular structure with uniform surface coverage. The SEM picture obtained for the poly (Py-co-NMP)/PTS copolymer film (image a) show fine micro-spheres that result in protruded globular appearance on the surface indicating porous nature of the surface [22]. The incorporation of large dopant anion PTS during electrochemical polymerization results in increased porosity in the Film. The SEM image of ChOx/poly(Py-co-NMP)/PTS bioelectrode (image b) also reveals globular structure with reduced porosity arising due to the presence of entrapped cholesterol oxidase [27, 34] or smoothening of the surface indicating entrapment of ChOx due to large anion doping facilitates increased loading of enzyme loading in copolymer film.

Electrochemical studies

A three-electrode assembly comprising of ChOx/poly(Py-co-NMP)/PTS/ITO as working electrode, Ag/AgCl as the reference electrode and Pt Foil (2×2 cm^2) as the counter electrode has been used for electrochemical studies. The CV of ChOx/poly (Py-co-NMP)/PTS/ITO bioelectrode (curve a) and poly (Py-co-NMP)/PTS/ITO (curve b) electrode is compared in Fig. 5. The dramatic decrease in anodic peak current in the enzyme electrode reveals that enzyme electrode exhibits a slow redox process. However, the magnitude of current increases with scan rate up to 50 mV/s, indicating it is a diffusion controlled process. Thus, all the biosensor measurements have been done at 50 mV/s.

Scheme 2 shows the proposed mechanism of ChOx immobilization by an in situ electro polymerization which is based on physical entrapment within the polymer matrices. During electro polymerization negatively charged enzymes are entrapped as a dopant in the copolymer matrix. A large number of positive charges are generated

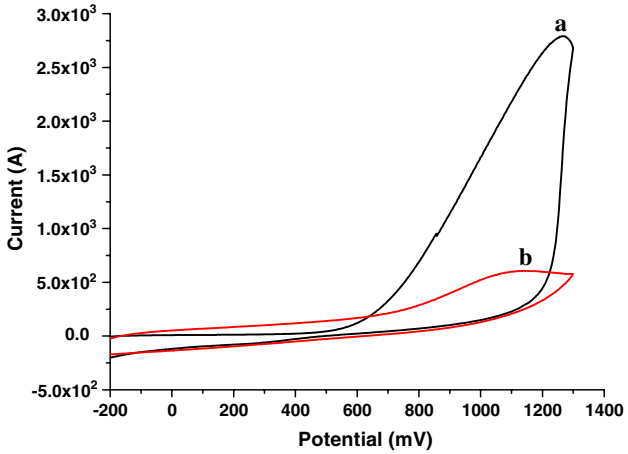
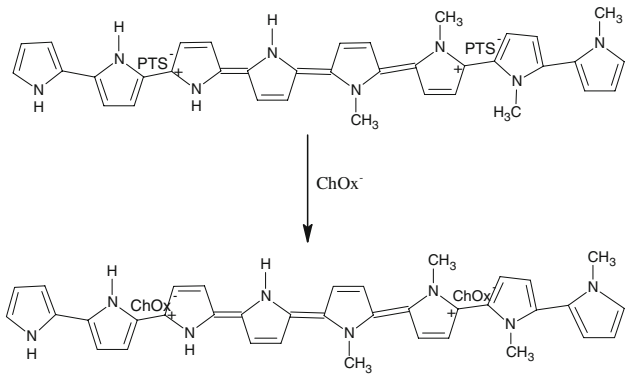


Fig. 5 Cyclic voltammogram of poly(Py-co-NMP)/PTS/ITO electrodes and ChOx/poly(Py-co-NMP)/PTS/ITO bioelectrode



Scheme 2 Entrapment of ChOx on poly(Py-co-NMP)/PTS/ITO electrode

on the nitrogen atoms in the polymeric chains of poly (Py-co-NMP) film due to doping with *p*-toluene sulphonate ions. The negatively charged cholesterol oxidase appears to electrostatically attract positively charged nitrogen atoms in the copolymer chains resulting in stronger binding of cholesterol oxidase with the polymeric chains [22, 27].

Enzyme activity measurements of ChOx/(poly(Py-co-NMP)/PTS/ITO bioelectrode

For cholesterol oxidase enzyme activity measurements, a reaction mixture consisting of cholesterol, horseradish peroxidase and *o*-dianisidine (0.1%) in deionized water have been used. The absorption intensity values for different concentration of cholesterol in the reaction mixture have been recorded at 500 nm by dipping enzyme electrodes for about 2 min at room temperature. The activity of the cholesterol oxidase in ChOx/poly (Py-co-NMP)/PTS has been calculated on the basis of Eq. 1 and has been found to be 0.125 U cm^{-2}.

$$\alpha_{app}^{eng}\left(\text{U cm}^{-2}\right) = AV/\epsilon St \quad (1)$$

where A = difference in absorbance at 500 nm before and after the incubation of a ChOx/poly(Py-co-NMP)/PTS, V = total volume (3 mL), ε = millimolar extinction coefficient of oxidized *o*-anisidine (7.5), t = reaction time.

One unit of enzyme activity is defined here as the activity that results in the conversion of 1 μm of cholesterol into cholestenone per min. Figure 6 shows the plot of absorbance at 500 nm with concentration in mg/dL. A linear range is observed between 50 and 400 mg/dL. The reaction of cholesterol with dye is shown below.

$$\text{Cholesterol} + O_2 \rightarrow \text{Cholesterone} + H_2O_2 \quad (2)$$

$$H_2O_2 + o\text{-anisidine}_{(red)} \rightarrow 4H_2O + \text{Quinonemine dye}_{(oxi)}(\text{coloured product}) \quad (3)$$

Electrochemical response studies of cholesterol bioelectrode

The cyclic voltammetric studies have been carried out between −200 and 1.2 V on bioelectrode as a function of cholesterol concentration. It is observed that the anodic current increases with increased concentration of cholesterol. A linear relationship is obtained between the current and cholesterol concentration in the range of 50–400 mg/dL with a regression equation of $\Delta i = 0.6406C + 0.943$. The electrode can detect cholesterol up to 25 mg/dL. The substrate concentration can be measured by monitoring the change in amperometric current as per the biochemical reaction (Eqs. 2–3) (Fig. 7 and Scheme 3). The value of apparent Michaelis–Menten constant (K_m (app)) has been estimated using the Lineweaver–Burke plot and has been found to be 0.62 mM and correlation coefficient is found to be 0.9857. The low value of K_m suggests that the matrix prepared in our case is facilitating the enzymatic reaction. It has been found that the K_m value is less than (e.g. 2 mM, 1.5 mM) [35, 36] that of the other cholesterol biosensors. The response time is 19 s. The low response time demonstrates that porous morphology of copolymer is suitable for entrapment of cholesterol. Table 2 shows the copolymer bioelectrode characteristics.

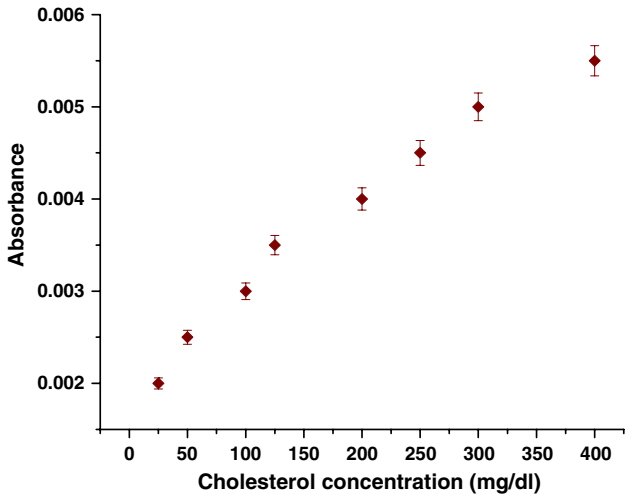


Fig. 6 Plot of UV absorbance versus cholesterol concentration

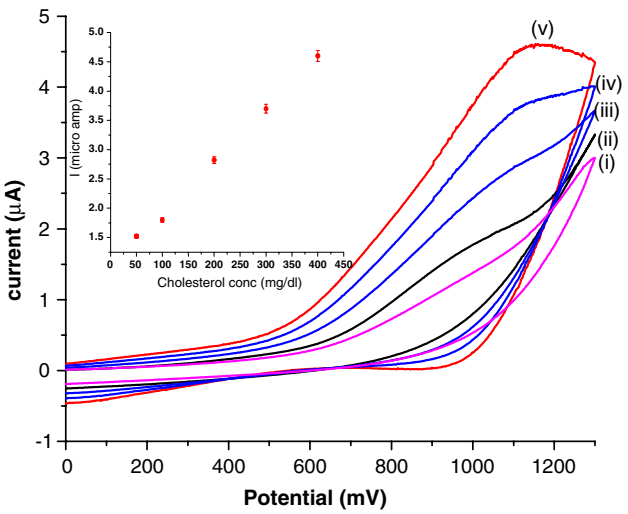
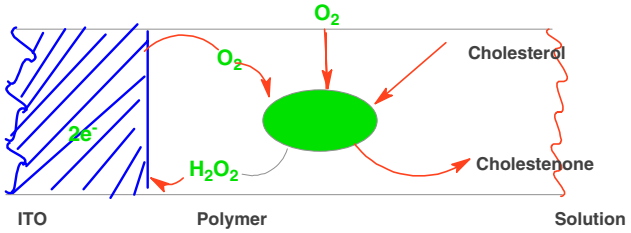


Fig. 7 Cyclic voltammetric curves obtained for bioelectrode as a function of cholesterol concentration as (i) 50, (ii) 100, (iii) 200, (iv) 300 and (v) 400 mg/dL. Inset plot of anodic peak current versus cholesterol concentration



Scheme 3 Electrochemical mechanism for enzymatic reaction sequences for the detection of cholesterol

Table 2 Linear regression, correlation coefficient, standard deviation, sensitivity and K_m of the bioelectrode

Electrode	Linear regression	Correlation coefficient	Standard deviation (SD)	Sensitivity (mA/mol/cm^2)	K_m (mM)
ChOx/poly(Py-co-NMP)/ PTS/ITO	0.9431	0.9857	0.0724	0.6406	0.62

Effect of pH

The effect of pH (6.0–8.0) on the response measurements of electrochemically entrapped ChOx/poly(Py-co-NMP)/PTS/ITO bioelectrode is shown in Fig. 8. The maximum response is observed in pH range of 6.8–7.3. An increase in response in this range at a given concentration of enzyme indicates that net increase in enzyme charge gives rise to higher amount of immobilized enzyme. This implies that the enzyme entrapment in the polymer backbone during polymer growth via electrostatic interaction and supports the enzyme immobilization mechanism as described by Scheme 2. So pH 7.0 has been selected for response studies. Cholesterol oxidase has isoelectric point at about 5 pH. At pH below 5, enzyme remains in the protonated form and loses its negative charge (e^-), at high pH, substrate ($SubH^+$) losses proton to form Sub and H^+. In both the cases, enzyme activity decreases.

Interferent studies

Metabolites such as urea, ascorbic acid and glucose present in the serum may interfere in the estimation of cholesterol concentration. The values of amperometric response obtained in the presence of interferents such as urea (2 mM), ascorbic acid (2 mM) and glucose (5 mM) on the response of the bioelectrode as shown in Table 3 reflect

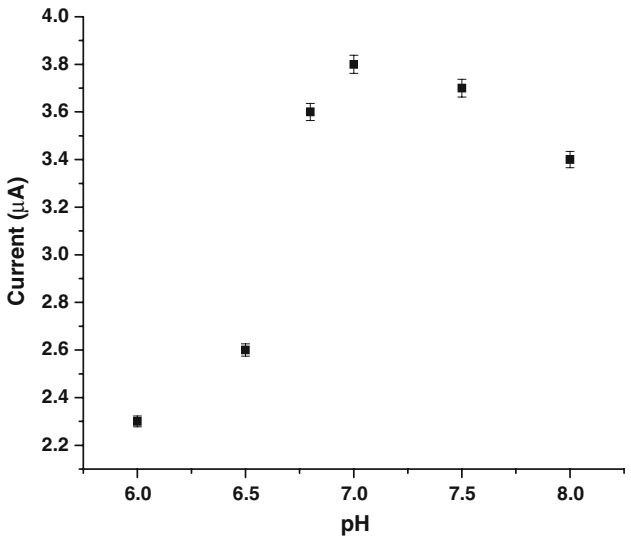


Fig. 8 Effect of pH on amperometric response of the cholesterol electrode

Table 3 Effect of intereferents on the amperometric response of the bio electrode

Cholesterol concentration (mg/dl)	Cholesterol	Cholesterol + urea	Cholesterol + ascorbic acid	Cholesterol + glucose
200	3.7	3.5	3.5	3.2

that urea and ascorbic acid have no inference on the electrode efficiency, whereas glucose decreases the response current to some extent.

Storage stability

The storage stability of ChOx/poly(Py-co-NMP)/PTS/ITO bioelectrode has been experimentally determined by measuring the current response at 200 mg/dL cholesterol solution up to 45 days at an interval of 15 days. These electrodes are kept at 4 °C when not in use. It is observed that there is not much decrease in the response current within 15 days and even after 45 days 85% of enzyme activity is observed.

Conclusions

The (1:1) copolymer of pyrrole and *n*-methylpyrrole has been electrochemically prepared and characterized by cyclic voltammetry, UV–visible spectroscopy and FTIR spectroscopy indicating that it is a block copolymer. Cholesterol oxidase has been successfully immobilized on the electrochemically synthesized Py-NMP copolymer by electrochemical entrapment technique. The cholesterol electrode has low response time of 19 s with good stability at 4 °C. This cholesterol biosensing bioelectrode shows selectivity in presence of interferents like ascorbic acid, glucose and urea and high sensitivity and linearity. Efforts should be made to utilize this conducting polymer bioelectrode for estimation of cholesterol in clinical samples.

Acknowledgements Tinku Basu is thankful to Dr. Ashok K.Chauhan, Founder President of Amity University, U.P., India, for his constant encouragement and motivation to perform the innovative work in the development of Science and Technology. We thank Director, National Physical Laboratory, New Delhi, India, for the facilities. PRS is grateful to the Council of Scientific Industrial Research (CSIR), India, for the award of the Senior Research Associate (SRA).

References

1. Nauck M, Marz W, Wieland H (2000) Clin Chem 46:436
2. Singh S, Chaubey A, Malhotra BD (2004) Anal Chim Acta 502:229
3. Arya SK, Datta M, Malhotra BD (2008) Biosens Bioelectron 23:1083
4. Zak B (1957) Am J Clin Pathol 27:583
5. Karube I, Hara K, Matsuoka H, Suzuki S (1982) Anal Chim Acta 139:127
6. Tan X, Lia M, Cai P, Luoa L, Zou X (2005) Anal Biochem 337:111
7. Vidal JC, Garcya E, Castillo JR (1999) Sens Actuators B 57:219
8. Wang HY, Mu SL (1999) Sens Actuators B 56:22
9. Ram MK, Bertoncello P, Ding H, Paddeu S, Nicolini C (2001) Biosens Bioelectron 16:849
10. Li JP, Peng TZ (2002) Chin J Chem 20:1038
11. Garcya E, Vidal JC, Aramendıa MT, Castillo JR (2004) Electroanalysis 16:494
12. Nakaminami T, Ito S, Kuwabata S, Yoneyama H (1999) Anal Chem 71:1068
13. Gobi KV, Mizutani F (2001) Sens Actuators B 80:272
14. Yao T, Takashima K (1998) Biosens Bioelectron 13:67
15. Brahim S, Narinesingh D, Guiseppi-Elie A (2002) Biosens Bioelectron 17:53
16. Vidal JC, Ruiz EG, Castillo JR (2001) Electroanalysis 13:229
17. Vidal JC, Espuelas J, Ruiz EG, Castillo JR (2002) Anal Lett 35:837
18. Singh S, Chaubey A, Malhotra BD (2004) Anal Chim Acta 502:229
19. Kumar A, Rajesh , Chaubey A, Grover SK, Malhotra BD (2001) J Appl Polym Sci 82:3486
20. Brahim S, Narinesingh D, Elie AG (2001) Anal Chim Acta 448:27
21. Singh S, Chaubey A, Malhotra BD (2004) J Appl Polym Sci 91:3769–3773
22. Solanki PR, Singh S, Prabhakar N, Pandey MK, Malhotra BD (2007) J Appl Polym Sci 105:3211
23. Dhand C, Singh SP, Arya SK, Datta M, Malhotra BD (2008) Anal Chim Acta 602:244
24. Türkarslan O, Kayahan SK, Toppare L (2008) J Solid State Electrochem. doi:10.1007/s10008-008-0590-7
25. Ozer BC, Ozyoruk H, Celebi SS, Yıldız A (2007) Enzy Micro Tech 40:262
26. Matharu Z, Sumana G, Arya SK, Singh SP, Gupta V, Malhotra BD (2007) Langmuir 23:13188
27. Garcia-Moreno E, Ruiz MA, Barbas C, Pinagarron JM (2001) Anal Chim Acta 448:9
28. Diaz AF, Kanazawa KK (1982) In: Miller JS (ed) Extended linear chain compounds. Plenum, NY, p 119
29. Gonzalez-Tejera MJ, Garcia MV, de la Blanca ES, Redondo MI, Raso MA, Carrillo I (2007) Thin Solid Films 515:6805
30. Singh R, Naurala AK, Tandon RP, Rao SUM, Panwar VS, Mansingh A, Chandra S (1996) Synthetic Met 79:1
31. Sarac AS, Dogru E, Ates M, Parlak EA (2006) Turk J Chem 30:401
32. Ustamehmtoglu B, Kelleboz E, Sarac AS (2003) Int J Poly Anal Charact 8:255
33. Wencheng S, Iroh JO (1999) Electrochimica Acta 44:3321
34. Solanki PR, Arya SK, Singh SP, Pandey MK, Malhotra BD (2007) Sens Actuators B 123:829
35. Singh S, Singhal R, Malhotra BD (2007) Anal Chim Acta 582:335
36. Arya SK, Prusty AK, Singh SP, Solanki PR, Pandey MK, Datta M, Malhotra BD (2007) Anal Biochem 363:210