# Application of Conducting Poly(aniline-*co*-Pyrrole) Film to Cholesterol Biosensor

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**ABSTRACT:** Cholesterol oxidase (ChOx) has been covalently immobilized onto poly(aniline-*co*-pyrrole), electrochemically deposited onto indium-tin-oxide (ITO) glass plates, using glutaraldehyde as a crosslinker. These poly (An-*co*-Py)/ChOx films have been characterized using UV–visible spectroscopy fourier transform infrared spectroscopy, scanning electron microscopy, and photometric and amperometric techniques, respectively. The poly(An-*co*-Py)/ChOx bioelectrodes have been utilized for cholesterol estimation in the range of 1–10 m*M*. The ChOx activity in poly(An-*co*-Py)/ChOx bioelectrode has been found to be the highest at pH 7.0 at 25°C. The sensitivity and stability of poly(An-*co*-Py)/ChOx bioelectrode have been experimentally determined as 93.35 μA/m*M* and 10 weeks at 4°C, respectively. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 3211–3219, 2007

**Key words:** polyaniline; polypyrrole; copolymer; cholesterol; cholesterol oxidase; biosensor; electrochemical polymerization

## INTRODUCTION

Cholesterol is known to play an important role in the membrane and outer layer of plasma lipoproteins. Determination of cholesterol concentration in blood and serum is a fundamental parameter for the prevention and diagnosis of a number of clinical disorders such as heart diseases, atherosclerosis, hypertension, cerebral thrombosis, and coronary and peripheral vascular diseases.[1–4] Many enzymatic and nonenzymatic methods[5–8] have been reported for the measurement of cholesterol. The nonenzymatic methods are highly time-consuming, require expert manpower, expensive chemicals, and are unsuitable for rapid and automated analysis of cholesterol serum. Enzymatic methods for the determination of cholesterol involve complicated procedures and high cost because of the use of expensive enzymes in each assay.[9,10] Attempts have been made to develop sensitive, selective, rapid, reliable, and low cost biosensors for the determination of cholesterol in serum and blood.[11–14] The stability of the enzymatic electrodes for biosensor application can be achieved by immobilization of enzyme on suitable matrices. Immobilization of cholesterol oxidase (ChOx) on various matrices such as conducting polymers,[15–17] sol-gels,[18–20] self-assembled monolayers (SAM),[21,22] carbon paste,[23] cellulose acetate,[24] nanoparticles,[25] graphite–teflon composite matrix,[26] etc. have been carried out using various immobilization procedures such as physical adsorption,[27,28] entrapment,[27] covalent linkage,[21,29] etc.

Conducting polymers have recently attracted much attention as suitable matrices for the immobilization of biomolecules,[30–35] since these can efficiently transfer electric charge produced during a biochemical reaction. Conducting polymers such as polypyrrole (PPy) and polyaniline (PANI) are attractive matrices since they contribute towards the speed, sensitivity, versatility, considerable flexibility, bio-compatibility, and improvement in the shelf-life of biosensing electrodes due to electrostatic binding between biomolecule and conducting polymers.[16,36,37] The exclusion properties of PPy films provide selectivity for biosensors against interferents present in biological media, particularly electroactive endogenous anionic species such as ascorbate and urate.[38] PANI is considered an attractive polymer having two redox potentials to facilitate enzyme-polymer charge transfer. The majority of the studies have been restricted to homopolymers only, prepared either by chemical or electrochemical techniques.[34,35]

Vidal et al.[39] have reported an amperometric cholesterol biosensor based on the electrochemically polymerized pyrrole on a layer of Prussian-blue (PB) onto the Pt electrode for electro-catalytic detection of $H_2O_2$ produced during the enzymatic reaction of

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cholesterol and ChOx. The influence of the formation of SAMs on the Pt surface, stability of the PB layer, and the formation of an outer layer of Nafion (Nf) as a means of improving selectivity were studied. The shelf-life of this cholesterol biosensor was found to be about 25 days. Singh et al. have reported electrochemical coimmobilization of cholesterol esterase and ChOx onto PPy films to estimate total cholesterol.[29] The sensitivity, apparent $K_m$, and the shelf-life of these PPy/ChEt/ChOx electrodes were found to be as 0.15 μA/m*M*, 9.8 m*M*, and about 4 weeks at 4°C, respectively. A mediator-based amperometric cholesterol biosensor has been developed by entrapment of the ChOx enzyme and charge transfer mediator using both artificial (ferrocene derivative) and natural (flavin nucleotide) in a PPy layer.[17] ChOx and potassium ferricyanide physically immobilized onto electrochemically prepared dodecylbenzene sulfonate (DBS)-doped PPy films were used to estimate cholesterol.[40] These Chox/DBS-PPy/ITO electrodes showed linearity in the range of 2–8 mM of cholesterol and the electrodes were stable for about 3 months. A cholesterol biosensor developed by depositing layer-by-layer nanothin films of polystyrenesulfonate and ChOx onto microperoxidase-11 (MP-11), which was covalently linked with Au-alkanethiol electrodes showed linearity in the range of 0.2–3.0 m*M*.[41] Lin and Yang[42] immobilized ChOx (COD) on the surface of polyacrylonitrile (PAN) hollow fiber dialyzer using glutaraldehyde as a cross-linker. This bioelectrode showed poor storage stability retaining 53% of its initial activity of ChOx in 30 days. A bi-enzymatic biosensor has been fabricated using horseradish peroxidase and ChOx enzymes physically entrapped onto the surface of a pyrolitic graphite electrode for free cholesterol estimation.[43] An amperometric cholesterol biosensor based on covalent immobilization of cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) onto electrochemically prepared PANI films.[15] The linearity and shelf-life of this ChEt/ChOx/PANI electrode was obtained as 50–500 mg/dL and 6 weeks, respectively. An optical biosensor has been reported based on the coimmobilization of cholesterol esterase, ChOx, and peroxidase onto electrochemically prepared PANI film (PANI/ChEt/ChOx/POD).[16] The linearity range of this (PANI/ChEt/ChOx/POD) electrode has been observed as 50–500 mg/dL and shelf-life as 6 weeks, respectively. All these cholesterol biosensors suffer from either low range of linearity and poor stability.

Composites, copolymers, and double layers having conductive matrix can be prepared when two monomers having conductive homopolymers are polymerized, and the conductive product with different properties than those of homopolymers could be obtained.[44]

The commercial exploitation of the conducting polymer based biosening devices is linked with their ease of processability. The processability can be enhanced either by making substitution into the aromatic nucleus or copolymerizing in such a way that there is variation in the torsion angle between adjacent phenyl rings of the polymer.[45] Desired properties of PANI, such as mechanical strength, can be enhanced by mixing it with a polymer that has good mechanical properties. PANI is a unique polymer that has a nitrogen heteroatom incorporated between phenyl rings along with polymer chain. This structure provides flexibility and allows the existence of three different oxidation states that are leucoemeraldine, emeraldine, and pernigraniline. Leucoemeraldine and pernigraniline forms of PANI are not stable and they will return to the state of emeraldine under atmospheric environment. Moreover, copolymerization is known to be an easy and powerful method for obtaining polymer with desired properties, and are thus widely used in the production of commercial polymers including fundamental investigations of structural-property relationships.[46–49] Aniline and pyrrole can be copolymerized by oxidative chemical copolymerization.[50,51] Electrochemical copolymerization of pyrrole and aniline has been reported using various solvents and acetonitrile in the presence of an organic acid trifluoroethanoic acid ($CF_3COOH$), tetraethyl ammonium tetrafluoroborate, or tetramethyl ammonium trifluoro methane sulfonate as electrolyte.[52,53] In the present article we have electrochemically synthesized copolymer film of aniline and pyrrole for the immobilization of ChOx. These poly(An-*co*-Py)/ChOx bioelectrodes have been characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), UV–visible spectroscopy and utilized for the estimation of cholesterol by amperometric and optical techniques, respectively.

## EXPERIMENTAL

Cholesterol oxidase (ChOx), (4 U/mL, EC 1.1.36, from pseudomonas species), and peroxidase (40 U/mL, EC 1.136, from pseudomonas fluorescens) enzymes were procured from Sigma Chemical, USA. Potassium phosphate (monobasic and dibasic salt), *o*-dianisidine, and other chemicals were of analytical grade. Aniline (Sigma) and pyrrole (Aldrich) were distilled prior to being used.

Potentiostat/Galvanostat-273A (Princeton Applied Research) was used for electrochemical synthesis and amperometric studies of copolymer poly(An-*co*-Py) in potentiostatic mode. Indium-tin-oxide (ITO) coated glass plates (Balzers), sheet resistance 15 Ω/cm, were used as substrates for deposition of desired polymeric film that were used as working electrodes.

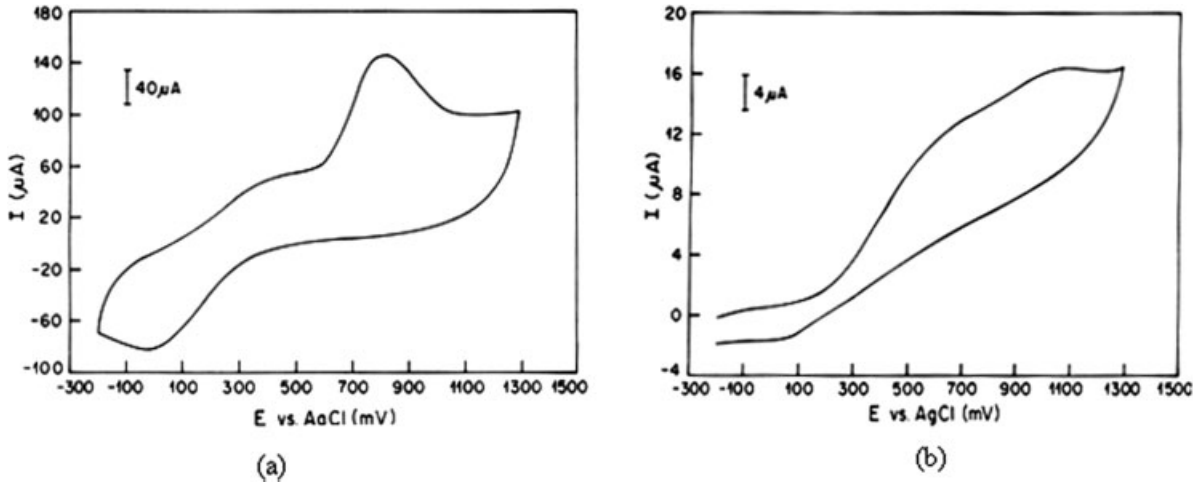


**Figure 1** (a) Cyclic voltammogram of poly(An-*co*-Py) film in phosphate buffer (50 m*M*, pH 7.0) and (b) Cyclic voltammogram of poly(An-Py)/ChOx bioelectrode in phosphate buffer (50 m*M*, pH 7.0).

Platinum foil (counter electrode) was obtained from the Johnson-Mathey. Millipore water purification system (Milli Q1075) was used to obtain deionized water. UV–visible spectrophotometer (Shimadzu 160A) was used to measure the ChOx activity. FTIR studies were recorded using Perkin–Elmer Spectrum BX and SEM photographs were recorded using SEM (LEO 440).

For synthesis of poly(An-*co*-Py) copolymer, a mixture of 20 mL of 0.1*M* sulfuric acid and 1 : 1 ratio of aniline and pyrrole monomer solution was stirred until homogeneous mixture was obtained. ITO glass plates were used as substrates for copolymer film deposition and the electrochemical polymerization was carried out at 0.8 V. The electrical conductivity of poly(An-*co*-Py) film was found to be $3.1 \times 10^{-2}$ S/cm while the conductivity of PPy and PANI has been calculated as $2.8 \times 10^{-2}$ and $2.0 \times 10^{-2}$ S/cm measured by four points probe method. The poly(An-*co*-Py) film was dried at room temperature and was washed with buffer prior to being used.[54] The cyclic voltammetric (CV) studies of copolymer film were done between −0.2 and 1.3 V.

The enzyme solutions of ChOx (4.0 U/mL) and peroxidase (40.0 U/mL) were prepared afresh in phosphate buffer (50 m*M*, pH 7.0). Cholesterol solution was prepared using Triton X-100 as surfactant. First cholesterol was dissolved in 10 mL of Triton X-100. It was gently heated until it became clear and transparent solution.

Poly(An-*co*-Py)/ChOx bioelectrodes were prepared by covalent immobilization of ChOx by using glutaraldehyde as the crosslinking agent. The poly(An-*co*-Py)/ChOx bioelectrodes were kept overnight for drying and were thoroughly washed with phosphate buffer solution to get rid off any loosely bound enzyme prior to be used.

## RESULTS AND DISCUSSION

### Cyclic voltammetric studies

Figure 1(a) shows the CV response of a poly(An-*co*-Py) films, the prominent oxidation peaks seen at 0.8 V (PPy) and 0.3 V (PANI) in phosphate buffer (50 mM, pH 7.0) observed at the scan rate of 20 mV/s confirm the synthesis of copolymer. The lower value of amperometric current observed for poly(An-*co*-Py)/ChOx bioelectrode has been attributed to the slow electron transfer rate in the electrode due to nonconducting nature of the ChOx enzyme [Fig. 1(b)].

The surface concentration of anions in the poly (An-*co*-Py) electrode is calculated using Brown-Anson model equation[15]

$$I_p = \frac{n^2F^2I^*AV}{4RT} \quad (1)$$

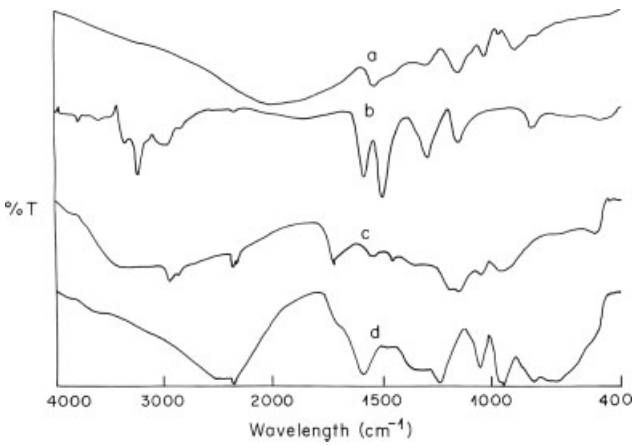


**Figure 2** FTIR of (a) polypyrrole (PPy), (b) polyaniline (PANI), (c) poly-(An-*co*-Py)/ChOx, and (d) poly-(An-*co*-Py) bioelectrodes.

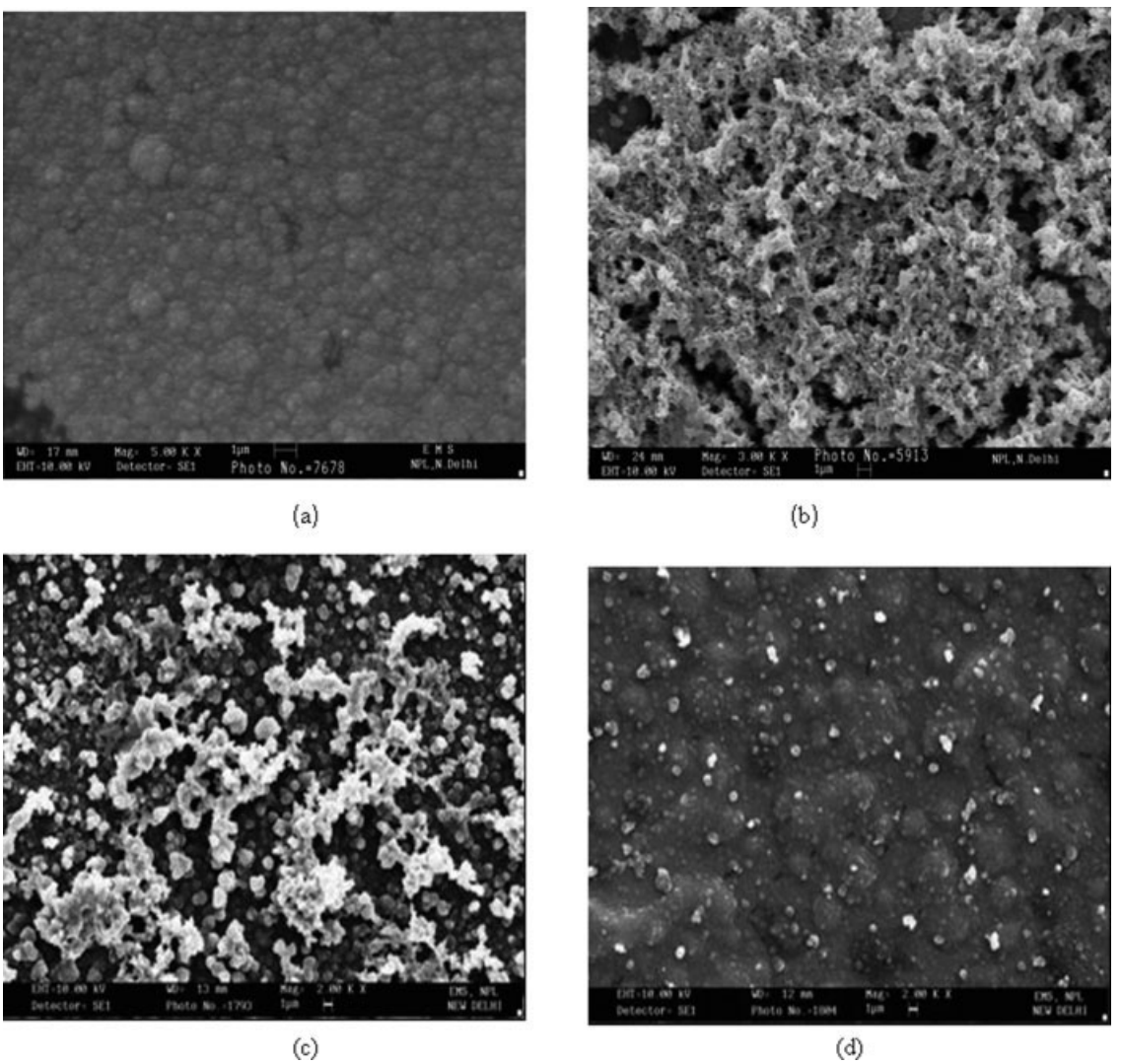


**Figure 3** (a) Scanning electron micrographs of an electrochemically synthesized polyaniline (PANI) film. (b) Polypyrrole (PPy) film. (c) Copolymer poly(An-*co*-Py) film. (d) Copolymer (poly(An-*co*-Py) containing immobilized ChOx.

where $n$ is the number of electrons transferred (2), $F$ is Faraday constant (96,584 C/mol), $I^*$ the surface concentration of the poly(An-Py) films (mol/cm$^2$), $A$ is the surface area of the electrode (1 cm$^2$), $V$ the scan rate ($20 \times 10^{-3}$ V/s), $R$ the gas constant (8.314 J/mol K), and $T$ is the absolute temperature (298 K). The calculated surface concentration ($I^*$) of anions in poly(An-*co*-Py) films is $1.9 \times 10^{-9}$ mol/cm$^2$.

### FTIR studies of PPy, PANI, poly(An-*co*-Py), and poly(An-*co*-Py)/ChOx bioelectrodes

FTIR spectra were recorded for PPy, PANI, poly(An-*co*-Py)/ChOx, and poly(An-*co*-Py) [Fig. 2(a–d)], respectively. Characteristic peaks seen at 1535, 1488, 1301, 1165, 960, 892, and 739 cm$^{-1}$ indicate the presence of PANI chain back-bone Figure 2(b). The peaks

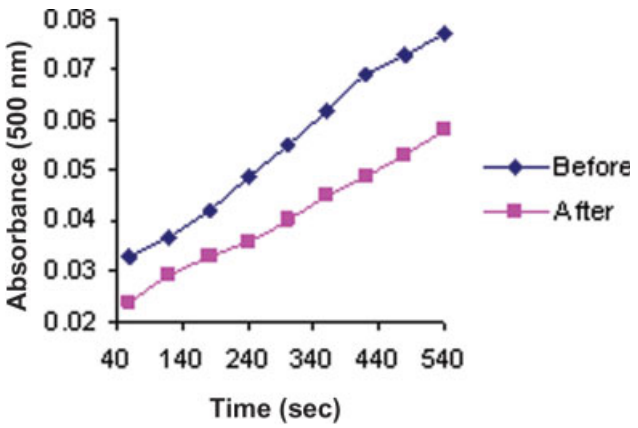


**Figure 4** Photometric response of poly(An-*co*-Py)/ChOx bioelectrode as a function of time. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

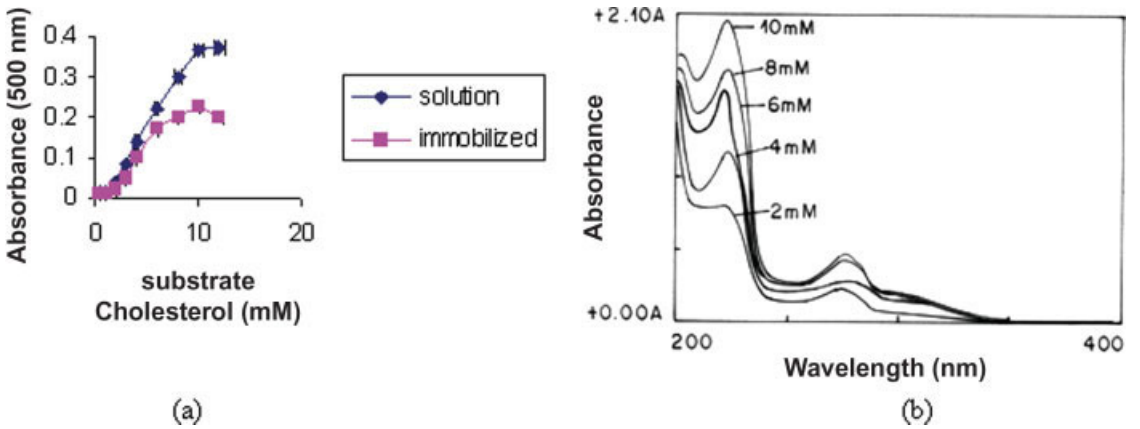


**Figure 5** (a) Photometric response of poly(An-*co*-Py)/ChOx bioelectrode in immobilized solution phase as a function of cholesterol concentration (m*M*), in phosphate buffer (50 m*M*, pH 7.0). (b) UV spectra for poly(An-*co*-Py)/ChOx bioelectrode as a function of cholesterol concentration (1–10 m*M*) in phosphate buffer (50 m*M*, pH 7.0). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

at 1535 and 1488 $cm^{-1}$ reveal the C=C vibration of quinoid benzoid rings. The peaks at 1584 and 1496 $cm^{-1}$ indicate the presence of C=N and C—N vibration in PPy [Fig. 2(a)]. The 1541 and 1718 $cm^{-1}$ peaks seen in Figure 2(c) are due to amide I and amide II linkage of the ChOx enzymes. The broad peak obtained at ~3300 $cm^{-1}$ indicates the N—H stretching vibration of both aniline and pyrrole Figure 2(d).[51]

### SEM studies for polyaniline, polypyrole, poly (An-*co*-Py), poly(An-*co*-Py)/ChOx bioelectrodes

Figure 3 shows the SEM images of PANI, PPy, and poly(An-*co*-Py) films with and without ChOx. PANI film shows rough or spongy type structure, whereas PPy film exhibits granulated structure.[49] The SEM picture of copolymer film shows uniform distribution of fibers and granules arising due to pyrrole-*co*-aniline molecules [Fig. 3(c)].[44] The immobilization of ChOx enzyme onto the poly(An-*co*-Py) electrode decreases the roughness and granular nature of surface. It appears that enzyme has been stationed at pores of the poly(An-*co*-Py) electrode indicating smoother surface [Fig. 3(d)].

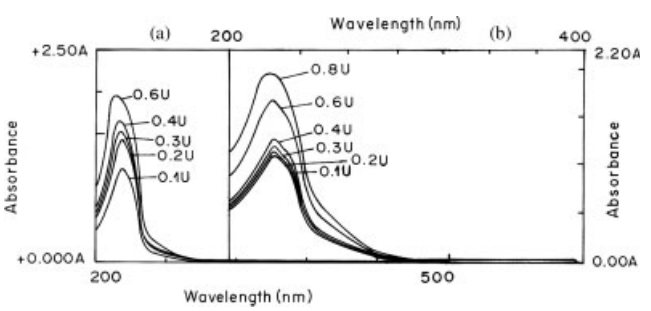


**Figure 6** UV spectra of poly(An-*co*-Py) as a function of different units of ChOx enzyme.

### Photometric studies for poly(An-*co*-Py)/ ChOx bioelectrode

The following biochemical reaction occurs as a result of interaction of ChOx with cholesterol:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{ChOx}} \text{Cholestenone} + H_2O_2 \quad (2)$$

$$H_2O_2 + \text{o-dianisidine}_{(\text{red})} \xrightarrow{POD} 4H_2O + \text{Quinonemine dye}_{(\text{oxi})}(\text{colored product}) \quad (3)$$

The enzyme activity of ChOx was assayed in free and in the immobilized phase by measuring the absorbance of colored compound formed as a result of biochemical reaction [eq. (3)]. In the free phase, the absorbance was measured by adding 10 μL of ChOx in the reaction mixture consisting of 3.0 mL phosphate buffer (50 m*M*, pH 7.0), 0.1 mL cholesterol solution (4 m*M*), 50 μL peroxidase (40 U), and 0.05 mL of *o*-dianisidine (1%) at 500 nm. The enzyme activity of poly(An-*co*-Py)/ChOx bioelectrode was determined by incubating the electrode in the reac-

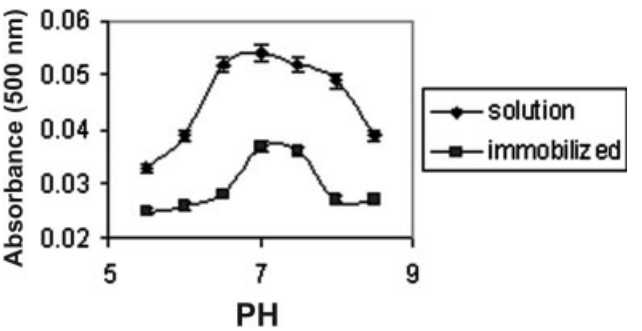


**Figure 7** Photometric response of poly(An-*co*-Py)/ChOx bioelectrode as a function of pH in solutional immobilized phase.

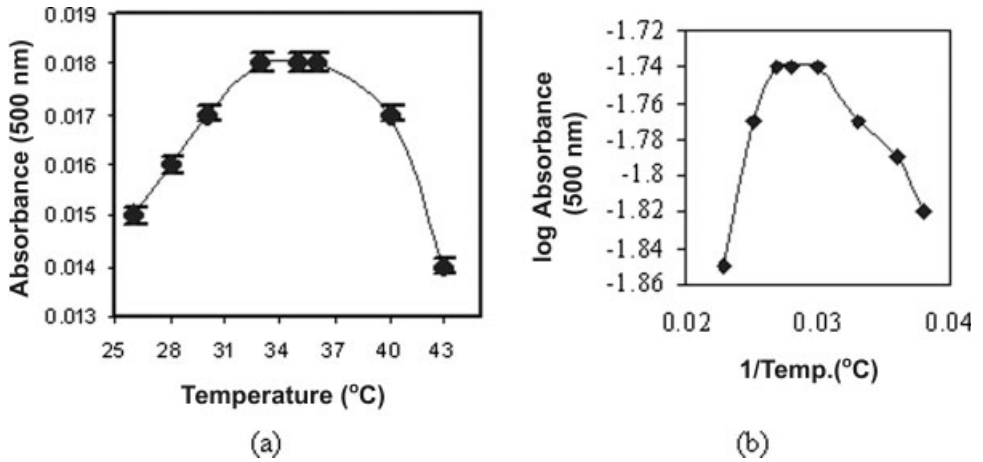


**Figure 8** (a) Photometric response of poly(An-*co*-Py)/ChOx bioelectrode as a function of temperature in the presence of cholesterol (4 m*M*) and phosphate buffer (50 m*M*, pH 7.0). (b) Arrehenius plot for the effect of temperature on the response of poly(An-*co*-Py)/ChOx bioelectrode in the presence of cholesterol (4 m*M*) and phosphate buffer (50 m*M*, pH 7.0).

tion mixture for 60 s. Figure 4 shows the photometric response of poly(An-*co*-Py)/ChOx bioelectrode as a function of reaction time. The decreased absorbance obtained for the poly(An-*co*-Py)/ChOx bioelectrode compared to poly(An-*co*-Py) electrode might be due to the fact that some of the active sites of enzymes get engaged in making covalent bonds with glutaraldehyde resulting in the reduced availability of number of sites for reaction with substrate.

### Effect of substrate concentration for poly(An-*co*-Py)/ChOx bioelectrode

The photometric response of the poly(An-*co*-Py)/ChOx bioelectrode in immobilized and solution phase was studied with different concentration of cholesterol varying from 1 to 10 m*M* [Fig. 5(a)]. Velocity (change in absorbance per minute) is almost linearly proportional to the cholesterol concentration upto about 10 m*M* where after it reaches a saturation point. The apparent $K_m$ value of the poly(An-*co*-Py)/ChOx bioelectrode was obtained as 4 m*M*. Figure 4(b) shows the UV spectra of different cholesterol concentrations and the peak obtained at 240 nm corresponds to cholestenone formation [eq. (2)].

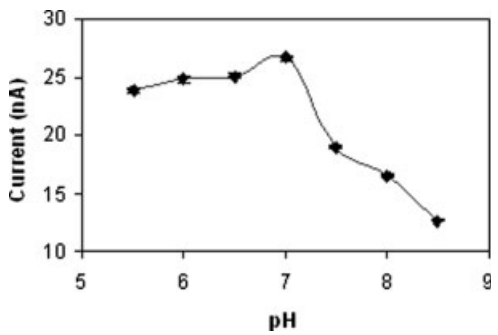


**Figure 9** Amperometric response of poly(An-*co*-Py)/ChOx bioelectrode as a function of pH using cholesterol (4 m*M*) in phosphate buffer (50 m*M*, pH 7.0).

### Enzyme loading on poly(An-*co*-Py) film

Figure 6(a,b) exhibits the UV spectra of ChOx obtained in solution and immobilized phase indicating increased activity of ChOx with increase in enzyme units (U/ml).

### Study of pH variation on the poly(An-*co*-Py)/ChOx bioelecrode

pH can affect the activity of enzyme by changing the structure or by changing the charge on substrate

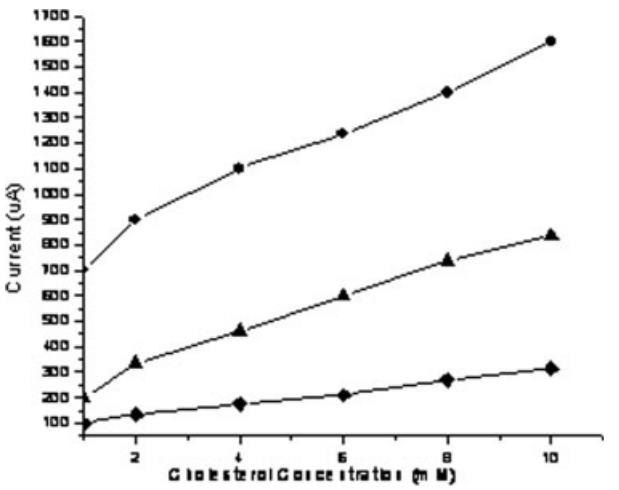


**Figure 10** Amperometric response of poly(An-*co*-Py)/ChOx (-●-), PANI (-▲-), and PPy (-◆-) bioelectrodes as a function of cholesterol concentration (1–10 m*M*) using phosphate buffer (50 m*M*, pH 7.0).

**TABLE I**
**Effect of Interferents on the Amperometric Response (mA/cm$^2$) of Poly(An-*co*-Py)/ChOx bioelectrode**

| Electrode | Cholesterol concentration (4 m*M*) | Cholesterol (4 m*M*) + Lactate (2 m*M*) | Cholesterol (4 m*M*) + uric acid (0.2 m*M*) | Cholesterol (4 m*M*) + glucose (5 m*M*) |
|---|---|---|---|---|
| Poly(An-*co*-Py) | 1.10 | 1.01 | 1.40 | 1.32 |

binding or catalysis. Figure 7 shows the absorbance change between the pH range of 5.5–8.5 for poly(An-*co*-Py)/ChOx bioelectrode and in solution phase resulting in a bell-shaped profile. Absorbance increases with increase in pH range (pH 5.5–7.0) giving rise to a broad peak, whereas the value of absorbance decreases with the increase in pH beyond 7.5. It appears that at the low pH (5.5), negatively charged enzyme ($Enz^-$) protonates and loses its negative charge ($e^-$): $Enz^- + H^+ \rightarrow EnzH$. At high pH (8.5), positively charged substrate ($SH^+$) ionizes and loses its positive charge: $SH^+ \rightarrow S + H^+$. Interaction of $SH^+$ and $Enz^-$ at extreme pH values will result in the reduction of the effective concentration of $Enz^-$ and $SH^+$, which in turn will reduce the value of the absorbance. The optimum pH (Fig. 7) is obtained between 7.0 and 7.5 in free and in immobilized state.

### Effect of temperature on poly(An-*co*-Py)/ChOx bioeletrode

The thermal stability of poly(An-*co*-Py)/ChOx bioelectrode enzyme has been studied by measuring the absorbance at different temperatures ranging from 26 to 43°C [Fig. 8(a)] in the presence of cholesterol (4 m*M*) and phosphate buffer (50 m*M*, pH 7.0). It has been observed that the rate of reaction increases with temperature up to 33°C and the optimum temperature range was obtained at 33–36°C owing to increased kinetic energy of the reacting molecules. Figure 8(b) shows variation of log (absorbance) as a function of reciprocal temperature (Arrehenius plot). The activation energy of poly(An-*co*-Py)/ChOx bioelectrode has been calculated using the following equation.

$$\frac{d(\log k)}{dt} = \frac{E_a}{2.303RT} \tag{4}$$

Slope $= E_a/2.303$RT, where $E_a$ is the activation energy, $R$ the gas constant, and $T$ is the temperature. The calculated activation energy observed at lower temperature (36°C) was about 73 kJ/mol and beyond this temperature the activation energy is much higher than the lower temperature revealing that poly(An-*co*-Py)/ChOx bioelectrode has the highest activity in the range of 26–36°C. The storage stability of the poly(An-*co*-Py)/ChOx bioelectrode was found to be about 10 weeks when stored at 4°C.

**TABLE II**
**Characteristics of Conducting Polymer Based Cholesterol Biosensors**

| Electrode used | Sensing element | Immobilization techniques | Linearity (m*M*) | Transducer used | Shelf-life (days) | Sensitivity (μA/m*M*) | Reference |
|---|---|---|---|---|---|---|---|
| Pt Prussian Blue polypyrrole | Cholesterol oxidase, chytochrome P450seck 201E | Entrapment | 0.025–0.35 | Amperometric | 25 | 0.441 | 39 |
| Polypyrrole/ PVS | Cholesterol oxidase, cholesterol esterase | Electrochemical entrapment | 1–8 | Optical | 30 | – | 55 |
| Polypyrrole/ PVS | Cholesterol oxidase, cholesterol esterase | Electrochemical entrapment | 1–8 | Amperometric | 60 | 0.15 | 30 |
| Polypyrrole/ DBS | Cholesterol oxidase, potassium ferricyanide | Physical adsorption | 2–8 | Amperometric | 90 | – | 40 |
| Polyaniline | Cholesterol oxidase, cholesterol esterase | Covalent linkage | 1.29–12.93 | Amperometric | 45 | $2.9 \times 10^{-5}$ | 15 |
| Polyaniline | Cholesterol oxidase, cholesterol esterase, peroxidase | Covalent linkage | 1.29–12.93 | Optical | 45 | 1.62 | 16 |
| Polypyrrole | Cholestrol oxidase, Ferrocene monocarboxylic acid | Electrochemical entrapment | 0.3 | Amperometric | 10 | – | 56 |
| Poly (An-*co*-Py) | Cholesterol oxidase | Covalent linkage | 1–10 | Amperometric/ optical | 75 | $0.93 \times 10^2$ | Present work |

### Amperometric studies of poly(An-*co*-Py)/ChOx bioelectrode

Amperometric response is based on the measurement of current generated by dissociation of $H_2O_2$ produced in eq. (2)

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^- \quad (5)$$

Figure 9 shows the effect of pH on amperometric response of poly(An-*co*-Py)/ChOx bioelectrode in the presence of phosphate buffer (50 m*M*). The optimum pH is observed at pH 7.0. Figure 10 shows the effect of cholesterol concentration on the amperometric response of poly(An-*co*-Py)/ChOx, PANI/ChOx and PPy/ChOx bioelectrodes with the range of 1–10 m*M*. The sensitivity of these poly(An-*co*-Py)/ChOx, PANI/ChOx and PPy/ChOx bioelectrodes was found to be as 93.3, 69.0, and 32.2 μA/m*M*, respectively.

### Interference studies for poly(An-*co*-Py)/ChOx bioelectrode

The effect of interferents (uric acid, lactate and glucose) has been studied on the amperometric responses of poly(An-*co*-Py)/ChOx bioelectrode by adding the normal physical concentration (uric acid (0.2 m*M*); lactate (2 m*M*); and glucose (5 m*M*) in the reaction mixture. It has been found that the presence of interferents have negligible effect on the current obtained (Table I).

Table II gives the comparison of the characteristics of cholesterol biosensor based on copolymer [poly (An-*co*-Py)], present work and homopolymers viz PPy and PANI as reported in literature.

## CONCLUSIONS

It has been shown that ChOx can be immobilized onto electropolymerized poly(An-*co*-Py) film. The sensitivity of poly(An-*co*-Py)/ChOx bioelectrode has been achieved as 93.3 μA/m*M*, which is much higher than PANI/ChOx (69.0 μA/m*M*) and PPy/ChOx (32.2 μA/m*M*). These poly(An-*co*-Py)/ChOx bioelectrodes have response time of 30 s with the stability of 70 days. The presence of interferents such as glucose, uric acid, and lactate in solution does not alter the observed amperometric response. Experiments are being conducted to estimate the cholesterol concentration in serum as well as blood samples using the poly(An-*co*-Py)/ChOx bioelectrode.

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