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Langmuir–Blodgett film based on MEH-PPV for cholesterol biosensor

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ABSTRACT

Cholesterol oxidase (ChOx) has been immobilized onto conducting poly[2-methoxy,5-(2′-ethyl-hexyloxy)-1,4-phenylene vinylene] (MEH-PPV)/stearic acid (SA) Langmuir–Blodgett film transferred onto octadecanethiol (ODT) modified gold plate. The ChOx/MEH-PPV/SA LB film bioelectrode exhibits has been characterized by FT-IR, contact angle, and atomic force microscopy. The response of the ChOx/MEH-PPV/SA LB film bioelectrode carried out using differential pulse voltammetry (DPV) studies reveal linearity from 1.29 to 12.91 mM of cholesterol concentration and response time as 30 s. This ChOx/MEH-PPV/SA bioelectrode exhibits values of correlation coefficient as 0.9939, standard deviation as 0.0029 μA and limit of detection as 1.66 mM. UV–visible spectrophotometer studies reveal that 5.2×10^{-3} U of ChOx are actively working per cm^2 area of ChOx/MEH-PPV/SA LB film bioelectrode and this bioelectrode is thermally stable upto 55 °C with reusability of about 60 times.

1. Introduction

The application of conducting polymer based ultra thin films including self assembled monolayers (SAM) [1] and Langmuir–Blodgett (LB) films [2–4] to biosensors has recently aroused much interest. The various advantages including control of geometrical arrangement and thickness of desired molecular films have made this technique important for development of desired biosensor with improved characteristics [5,6]. Compared to SAMs, thickness of an LB film can be controlled via layer-by-layer deposition on desired substrates. Besides this, loading of a biomolecule (e.g enzyme) can be controlled by altering the number of layers used for deposition. However, conducting polymers cannot be used to obtain a stable LB monolayer because of either energetically unfavorable orientation of a polymer chain at the air–water interface or due to lack of dipole moment associated with the polymer backbone. It has recently been shown that addition of fatty acids such as arachidic acid, stearic acid etc. to a given conducting polymer can assist the LB deposition onto a solid substrate [7].

LB films of conducting polymers such as polyaniline [2], polythiophene [3] etc. have recently been employed for biosensor application. Singhal et al. have used LB films of poly(3-dodecyl thiophene) and poly(*N*-vinyl carbazole) for fabrication of glucose (Glu) biosensor [3], and urea (U) biosensor [4]. Matharu et al. have recently reported a polyaniline (PANI)-stearic acid (SA) LB film based cholesterol biosensor [2]. This LB bioelectrode has been found to have stability for about 10 weeks and linearity from 0.65 to 10.34 mM of cholesterol concentration. However, preparation of PANI LB film is difficult due its nonsolubility in most organic solvents. Further it requires a cross-linking agent for biomolecule immobilization.

poly[2-methoxy,5-(2′-ethyl-hexyloxy)-1,4-phenylene vinylene] (MEH-PPV) has been reported to be an interesting matrix for fabrication of molecular electronic devices [8,9]. The alkoxy groups present in the polymer provide solubility in given organic solvent [10]. It has been shown that thin film of MEH-PPV can be used for molecular electronics applications [11–15]. It may be noted that MEH-PPV has a high density of hole-traps (10^{15} cm^{-3}) [16] that is likely to be advantageous for immobilization of desired biomolecules without use of any additional reagent.

Among the various biochemical analytes, estimation of cholesterol has recently demanded much attention. Cholesterol is an essential structural constituent of cell membranes and balanced quantity of cholesterol in blood provides durability and integrity to the cell architecture. However, its elevated levels are associated with atherosclerosis, nephrosis, diabetes milletus, myxedema, jaundice and decreased levels may result in hyperthyroidism, anaemia and maladsorption [17,18]. Therefore, regular estimation of cholesterol is necessary. In this context, biosensor can provide easy and reliable method for its regular estimation. Efforts have been made to prepare cholesterol biosensor based on different conducting polymer matrices. However cholesterol biosensors containing

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advantageous features of conducting polymer and LB technique has not yet been much explored.

We report results of studies relating to preparation and characterization of conducting polymer LB film of MEH-PPV with immobilized cholesterol oxidase (ChOx) for application to cholesterol biosensing.

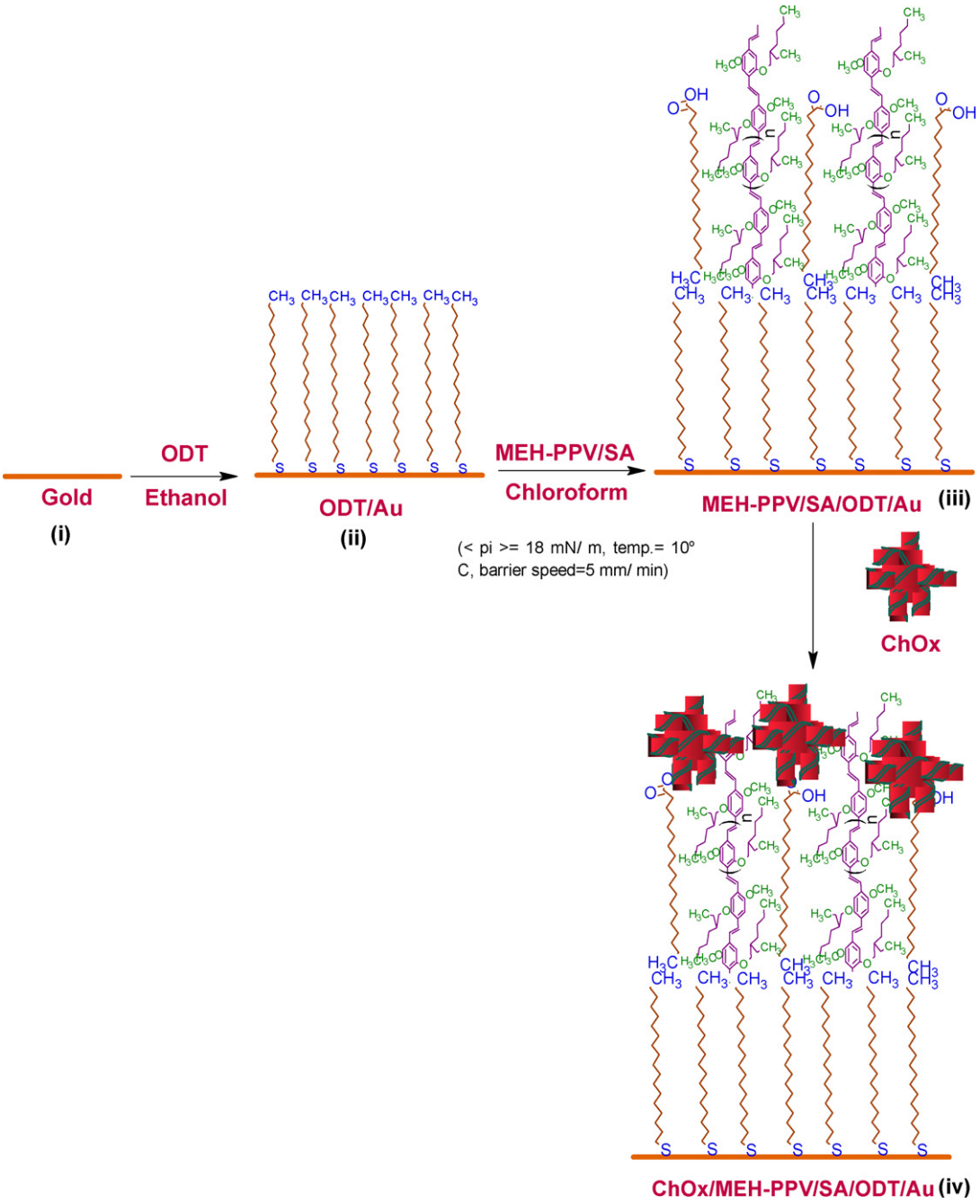
2. Experimental

2.1. Chemicals and reagents

ChOx (E.C.1.1.3.6, from pseudomonas fluorescens) with specific activity of 24 unit per milligram ($U\,mg^{-1}$), horseradish peroxidase (HRP, E.C.1.11.1.7) with specific activity of $200\,U\,mg^{-1}$ solid, *o*-dianisidine, cholesterol, chloroform, ethanol, stearic acid (SA), cadmium chloride ($CdCl_2$) and MEH-PPV were procured from Sigma–Aldrich, USA. Octadecanethiol (ODT) was procured from Merck, India. All other chemicals used were of analytical grade and used as received. Stock solution of cholesterol was prepared in 10% Triton X-100 and stored at 4 °C. This stock solution was further diluted in de-ionized water to make different concentrations of cholesterol solution [19].

2.2. Preparation of LB films of MEH-PPV/SA

ODT modified gold plate is used as a substrate to transfer MEH-PPV/SA LB monolayers (Scheme 1, ii). The ODT modified gold plate has been utilized to obtain the optimum adhesion of



Scheme 1. Schematic showing preparation of ChOx/MEH-PPV/SA LB film bioelectrode (i) gold surface; (ii) formation of ODT self-assembled monolayer on gold surface; (iii) LB film of MEH-PPV/SA and (iv) immobilization of ChOx onto MEH-PPV/SA LB film electrode.

LB monolayers of MEH-PPV onto a gold surface as self-assembled monolayer (SAM) of ODT on the gold surface provides similar organic environment that in turn may provide stability to the transferred organic monolayers. Prior to SAM formation, gold plates are pre-cleaned with acetone, ethanol, and with copious amount of de-ionized water. Further, gold plates are cleaned with piranha solution (H_2SO_4:H_2O_2; 7:3) for about 5 min followed by rinsing in de-ionized water. ODT SAM formation has been carried out as reported earlier [19].

For LB monolayer preparation, spreading solution comprising of 0.1 mg ml^{-1} of MEH-PPV mixed with stearic acid (1:1) is prepared in chloroform and ultra-sonicated for about 2 h. Monolayer deposition is carried out in an LB trough (NIMA, UK). An optimized amount (50 μl) of the prepared solution is spread onto water subphase containing 2×10^{-4} M $CdCl_2$. $CdCl_2$ is added to the subphase to obtain desired degree of control over monolayer behavior and successful deposition of multilayers as addition of these divalent ions reduces repulsion between adjacent ionized groups of stearic acid onto the water surface and make the monolayer more stable (NIMA, UK Manual). The molecules present in a monolayer are compressed by a movable barrier to record the surface pressure-area isotherm. The pressure and temperature for monolayer transfer are optimized after which 20 monolayers are transferred onto the pre-cleaned ODT modified gold plate by vertical dipping at a speed of 5 mm min^{-1} (Scheme 1, iii).

2.3. Immobilization of cholesterol oxidase onto MEH-PPV/SA LB film electrode

ChOx is physisorbed onto MEH-PPV LB film electrode (Scheme 1, iv). This is done by spreading 20 μg of ChOx in phosphate buffer saline (PBS) solution (50 mM, pH 7.0, 0.9% NaCl) onto 0.5 cm^2 area of MEH-PPV/SA LB film electrode. After drying, this ChOx/MEH-PPV/SA LB film bioelectrode is kept at 4 °C overnight. The electrode is then washed thoroughly with buffer (PBS containing 0.05% Tween 20) to remove any unattached enzyme and is stored at 4 °C when not in use. The ChOx/MEH-PPV/SA LB film bioelectrode is characterized by Fourier transform infrared (FT-IR) spectroscopy (Perkin-Elmer Instruments, model spectrum BX using ATR accessory), atomic force microscopy (AFM) (Veeco DICP2 instrument loaded with SPM Lab Analysis Software) (see supporting information, Supplement 1b) and contact angle (CA) measurements (DSA 100, DSA/V 1.9, Kruss Gmbh Hamburg), respectively. Response measurements of ChOx/MEH-PPV/SA LB film bioelectrode have been carried out by differential pulse voltammetry (DPV) (Autolab Potentiostat/Galvanostat, Eco Chemie, Netherlands) and UV–visible spectrophotometer (UV-160 A, Shimadzu).

Preparation of ChOx/MEH-PPV LB film bioelectrode is shown in Scheme 1. Scheme 1 shows modification of Au surface by ODT SAM (ii). MEH-PPV/SA LB film is then deposited onto the modified Au surface (iii). The prepared LB film has been used for physisorption of ChOx for ChOx/MEH-PPV/SA LB film bioelectrode preparation (iv).

2.4. Differential pulse voltammetric (DPV) studies

The DPV studies of ChOx/MEH-PPV/SA LB film bioelectrode have been carried out in the range, −0.1 to 0.4 V by an Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands) using a three-electrode cell with ChOx/MEH-PPV/SA LB film bioelectrode as working electrode, Ag/AgCl as a reference electrode and platinum foil as a counter electrode in 10 ml of PBS solution (50 mM, pH 7.0, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe. All measurements have been carried out in triplicate sets to investigate the reproducibility of the system.

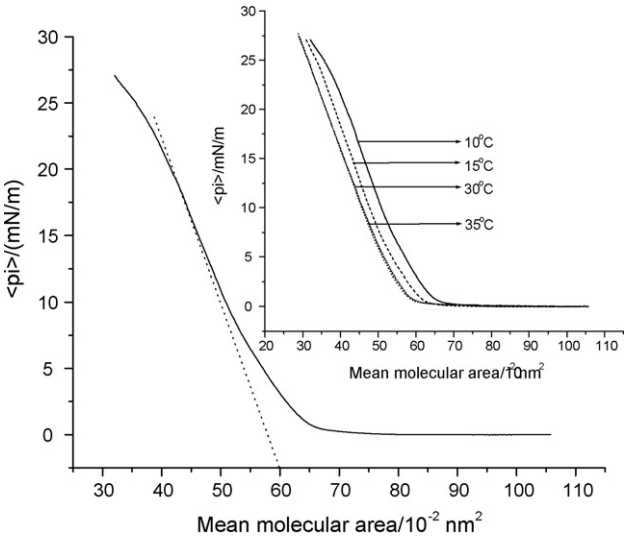


Fig. 1. The pressure-area isotherm of MEH-PPV/SA monolayer on air/water interface at temperature 10 °C and pressure 18 mN m^{-1}. Inset shows isotherms at different temperature from 10 to 35 °C.

2.5. UV–visible spectrophotometric studies of the ChOx/MEH-PPV/SA LB film bioelectrode

The UV–visible measurements have been carried out to investigate enzyme activity and thermal stability of ChOx/MEH-PPV/SA LB film bioelectrode. The LB film bioelectrode is dipped in 3 ml PBS solution (50 mM, pH 7.0, 0.9% NaCl) containing 20 μl HRP (1 mg ml^{-1} in PBS), 20 μl of *o*-dianisidine dye (1% in deionized water) and 100 μl of analyte (cholesterol solution) and is kept for about 4 min. The difference between the initial and final absorbance value of oxidized *o*-dianisidine at 500 nm due to the enzymatic reaction [19] after 4 min incubation is recorded and results of triplicate sets are plotted.

3. Results and discussion

3.1. LB film formation

Fig. 1 shows a typical surface pressure-mean molecular area isotherm of MEH-PPV/SA monolayer at subphase temperature of

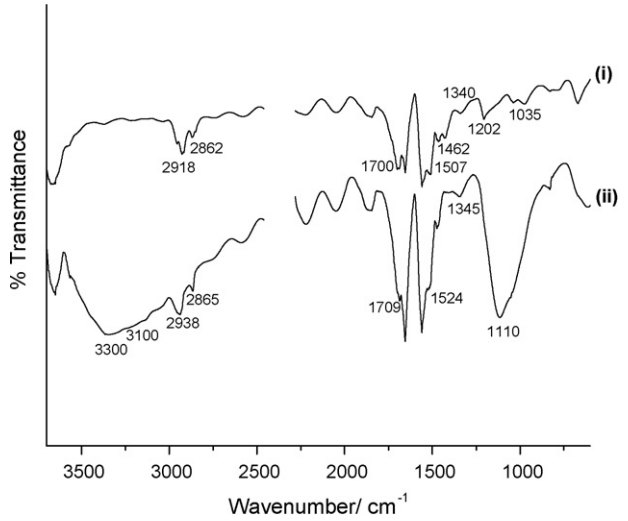


Fig. 2. FT-IR spectra of (i) MEH-PPV/SA LB electrode and (ii) ChOx/MEH-PPV/SA LB bioelectrode.

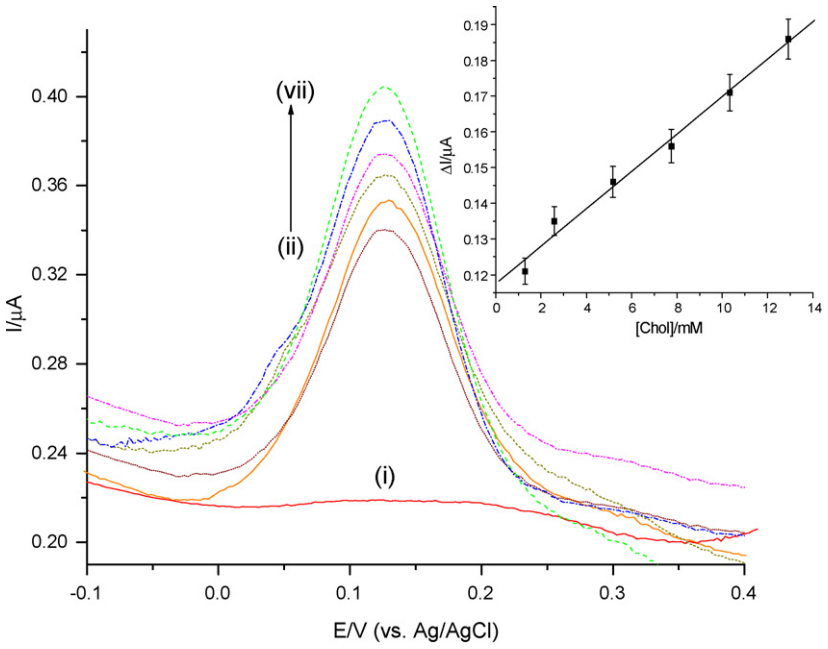


Fig. 3. DPV curve of ChOx/MEH-PPV/SA LB film bioelectrode as a function of cholesterol concentration in PBS solution (50 mM, pH 7.0, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (i) 0, (ii) 1.29, (iii) 2.58, (iv) 5.17, (v) 7.75, (vi) 10.34, and (vii) 12.91 mM. Linear regression curve of the ChOx/MEH-PPV/SA LB film bioelectrode (inset).

10 °C. The isotherm shows considerable curvature indicating the expanded monolayer behavior. In the region of surface pressure (16–22 mN m^{-1}), the isotherm has nearly constant slope and this part of the isotherm has been extrapolated to zero surface pressure to calculate the limiting mean molecular area that has been found to be 0.60 nm^2. The observed high mean molecular area further supports expanded nature of the MEH-PPV molecules in the monolayer at air/water interface. Pressure-mean molecular area isotherms of monolayer obtained at different temperatures are shown in the inset of Fig. 1. It is observed that lowering the subphase temperature result in shift of the isotherm towards higher mean molecular areas. This shows that polymer chains are comparatively more rigid at lower temperature thereby imposing restriction on close packing and results in increased mean molecular area. For the LB film formation pressure of 18 mN m^{-1} and temperature of 10 °C has been found to be optimum. The deposition curve at optimized parameters (data not shown) suggests Y-type LB film formation with approximate transfer ratio of 0.9–1.1.

3.2. Contact angle (CA) studies

CA measurements have been carried out using Sessile drop method. It has been observed that there is change in contact angle value from 98.4° for MEH-PPV/SA LB film electrode to 67.56° for the ChOx/MEH-PPV/SA LB film bioelectrode which suggests immobilization of ChOx (see supporting information, Supplement 1a). This can be understood to arise from a large number of carboxylic and amino groups present in ChOx that help to decrease the contact angle value.

3.3. FT-IR studies

The FT-IR spectra of MEH-PPV/SA LB film electrode and ChOx/MEH-PPV/SA LB film bioelectrode are shown in Fig. 2. The peaks seen at 2918 cm^{-1} and 2862 cm^{-1} observed for the MEH-PPV/SA LB film (Fig. 2(i)) are assigned to CH and CH_2 stretching, respectively. The 1507, 1462, 1340, 1202, 1035 cm^{-1} peaks are due to semi-circular phenyl stretch (C–C aromatic), anti-symmetric phenyl stretch, symmetric alkyl CH_2 deformation, phenyl oxygen stretch and alkyl-oxygen stretch, respectively. The peak seen near 1700 cm^{-1} for –COOH group shows presence of stearic acid. Presence of all these peaks indicates formation of mixed monolayer of MEH-PPV and stearic acid. In FT-IR spectra of ChOx/MEH-PPV/SA LB film bioelectrode (Fig. 2(ii)), additional strong broad bands seen at 3300, 3100 and 1110 cm^{-1} are assigned to amide A, amide B and C–N vibration in enzyme. Presence of these additional peaks reveal binding of ChOx on MEH-PPV/SA LB film surface.

3.4. Response studies of ChOx/MEH-PPV/SA LB film bioelectrode using differential pulse voltammetry (DPV)

3.4.1. Effect of cholesterol concentration

Fig. 3 shows differential pulse voltammogram of ChOx/MEH-PPV/SA LB film bioelectrode as a function of cholesterol concentration in PBS solution (50 mM, pH 7.0, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe. In most enzyme based biosensors, hydrogen peroxide (H_2O_2) oxidation is monitored in electrochemical detection. However, electro-oxidation of H_2O_2 requires higher anodic potential (about 0.6 V) and is affected by co-oxidizable substances, e. g. ascorbic acid (As), uric acid (UA) etc. that are usually present in biological samples [20]. To minimize the effect of interferent, $[Fe(CN)_6]^{3-/4-}$ has been used since its presence in close proximity with the bioelectrode in buffer allows detection of cholesterol at lower potential (0.13 V). In Fig. 3 the oxidation peak observed at 0.13 V corresponds to oxidation of $[Fe(CN)_6]^{4-}$. The increase in oxidation current with increasing concentration of cholesterol indicates enhanced enzymatic product. In the biochemical reaction, ChOx present on the matrix reacts with cholesterol and converts it into cholest-4-en-3-one. And during the re-oxidation of ChOx after enzymatic reaction, $[Fe(CN)_6]^{3-}$ gets converted into $[Fe(CN)_6]^{4-}$ by accepting an electron from the reduced ChOx thereby causing increase in the oxidation current of $[Fe(CN)_6]^{4-}$.

It can be seen (Inset of Fig. 3) that ChOx/MEH-PPV/SA LB film bioelectrode can be used to estimate cholesterol concentration from 50 mg dl^{-1} (1.29 mM) to 500 mg dl^{-1} (12.91 mM) with response time of 30 s. The values of correlation coefficient and standard deviation are found to be 0.9939 ($n = 6$) and 0.0029 μA, respectively. The

Table 1
Effect of interferents on the electrochemical response of ChOx/MEH-PPV/SA LB film bioelectrode.

Analyte/interferent	No analyte	Chol	Chol + UA	Chol + Glu	Chol + U	Chol + LA	Chol +As
Current(I/μA) for ChOx/MEH-PPV/SA LB film bioelectrode	0.219	0.368	0.365	0.355	0.356	0.361	0.362
Change in current (ΔI/μA) w.r.t. no analyte condition		0.149	0.146	0.136	0.137	0.142	0.143

response of ChOx/MEH-PPV/SA LB bioelectrode follows Eq. (1).

$$\Delta I(\mu A) = 0.11755(\mu A) + 5.24\,(\text{nA mM}^{-1}) \times [\text{Chol}](\text{mM}) \quad (1)$$

where ΔI and [Chol] represent the change in oxidation peak current and cholesterol concentration, respectively. The sensitivity of the bioelectrode estimated from slope of linear regression line is found to be 5.24 nA mM^{-1}.

The limit of detection (LOD) has been determined using $3\,s_{y/x}$/sensitivity (where $s_{y/x}$ is the standard deviation) [21] and is found to be 1.66 mM.

3.4.2. Effect of pH and interferents

To find the optimum pH, the DPV response of ChOx/MEH-PPV/SA LB film bioelectrode has been taken in PBS solution (50 mM, 0.9% NaCl, 5 mM $[Fe(CN)_6]^{3-/4-}$) of pH ranging from 6.0 to 8.0 at 30 °C. The investigation of ChOx/MEH-PPV/SA LB film bioelectrode using DPV studies at various pH values indicate that bioelectrode is most active around pH 7.0 (data not shown). All experiments have thus been carried out at pH 7.0.

The effect of interferents [UA (0.1 mM), Glu (5 mM), lactic acid (LA) (0.5 mM), As (0.05 mM), and U (1 mM)] on DPV response of ChOx/MEH-PPV/SA LB film bioelectrode for cholesterol measurement has been studied by taking solution containing 1:1 ratio of a fixed concentration of cholesterol (5.17 mM) and the interferent. Table 1 shows the effect of interferents on the electrochemical response of ChOx/MEH-PPV/SA LB film bioelectrode. It can be seen that these interferents (UA, LA and As) affect the response of the ChOx/MEH-PPV/SA LB film bioelectrode by less than 5%.

3.5. UV–visible studies

3.5.1. Effect of cholesterol concentration

The UV–visible measurements have been carried out to check the activity of ChOx onto the ChOx/MEH-PPV/SA LB film bioelectrode. The response has been taken with different cholesterol concentrations ranging from 25 mg dl^{-1} (0.65 mM) to 500 mg dl^{-1} (12.91 mM), and the difference between initial and final absorbance values of oxidized *o*-dianisidine due to the enzymatic reaction after 4 min incubation of the bioelectrode is recorded. The absorption rate is found to be proportional to the concentration of cholesterol in solution. Fig. 4 shows the variation of absorbance (A) obtained at 500 nm as a function of cholesterol concentration. It can be seen that electrode shows linear behavior upto 300 mg dl^{-1} (8 mM), after which the absorbance gets saturated with further increase in cholesterol concentration. The experiments carried out in triplicate sets have been found to be reproducible within 5% error.

The UV–visible response studies have been utilized for estimation of the apparent Michaelis-Menten $\left(K_m^{app}\right)$ constant using Lineweaver-Burke plot (curve not shown). The K_m^{app} value for the immobilized enzyme has been found to be 0.95 mM. The observed K_m^{app} value for immobilized enzyme onto ChOx/MEH-PPV/SA LB film bioelectrode is found to be lower than that of the free enzyme (3.71 mM) [2]. This shows enhanced affinity of the immobilized cholesterol oxidase to cholesterol attributed to better conformation of cholesterol oxidase onto well-aligned LB monolayers. Further, UV–visible response studies have been utilized to estimate the apparent enzyme activity (U cm^{-2}) using equation; a_{app}^{enz} (U cm^{-2}) = $AV/t\,\varepsilon\,s$, where A is the difference in absorbance before and after incubation, V is the total volume (3.00 cm^3), ε is the millimolar extinction coefficient (7.5 for *o*-dianisidine at 500 nm), t is the reaction time (min), and s is the surface area (cm^2) of the electrode [22]. The apparent enzyme activity has been found to be 5.2×10^{-3} U cm^{-2}, indicating that 5.2×10^{-3} U of ChOx is actively working per cm^2 area of ChOx/MEH-PPV/SA LB film bioelectrode.

3.5.2. Effect of temperature and stability

UV–visible response of ChOx/MEH-PPV/SA film bioelectrode as a function of temperature has been taken for a fixed cholesterol concentration (200 mg dl^{-1}, 5.17 mM). The reaction of enzyme (ChOx) in ChOx/MEH-PPV/SA LB film bioelectrode with analyte (cholesterol) has been carried out at temperature (T) varying from 20 to 55 °C in PBS solution (pH 7.0, 50 mM, 0.9% NaCl) containing HRP and *o*-dianisidine. The results suggest that reaction rate increases linearly upto about 55 °C. The absorbance of *o*-dianisidine as a function of temperature follows Eq. (2).

$$A(\text{abs}) = 0.00232(\text{abs}) + 5.38 \times 10^{-4}(\text{abs}\,^\circ\text{C}^{-1})T(^\circ\text{C}) \quad (2)$$

The higher thermal stability can be attributed to better enzyme (cholesterol oxidase) immobilization onto MEH-PPV/SA LB matrix that perhaps prevent uncoiling of enzyme subunits.

To find its storage stability, the response of ChOx/MEH-PPV/SA LB film bioelectrode has been tested at regular interval for about 3 months. This has been done by taking UV response of the bioelectrode at various intervals of time (0, 2, 7, 14, 30, 45 and 60, 90 days) with fixed concentration of cholesterol (200 mg dl^{-1}, 5.17 mM). The percent residual activity is measured by assigning 100% activity to the value observed in the initial stage (0 day). The bioelectrode exhibits 80.5% of the initial activity even after 3 months indicating the stability of the immobilized cholesterol oxidase to be about 3 months when stored at 4 °C.

The ChOx/MEH-PPV/SA LB film bioelectrode has been investigated for reusability by taking repeated UV–visible response of a single bioelectrode for a fixed cholesterol concentration (5.17 mM) at 30 °C. It has been found that this bioelectrode can be used for about 60 times without much loss in the activity. The excellent reusability can perhaps be attributed to the strong electrostatic

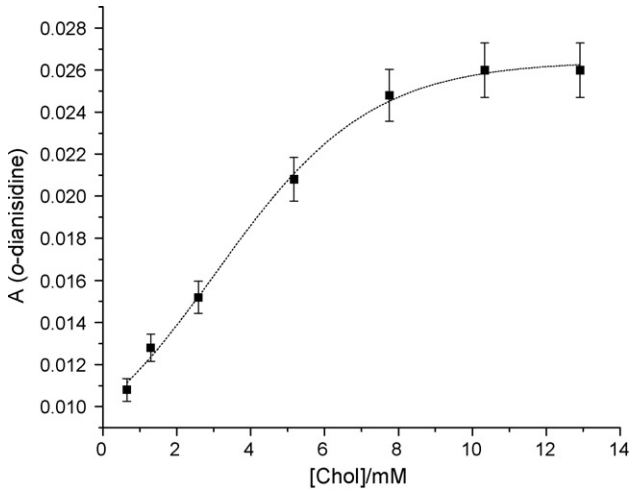


Fig. 4. Absorbance of *o*-dianisidine as a function of cholesterol concentration in PBS solution (50 mM, pH 7.0, 0.9% NaCl).

Table 2
Characteristics of the ChOx/MEH-PPV/SA LB film bioelectrode, along with those reported in literature.

Immobilization matrix	Sensing element	Method of immobilization	Transducer	Response time, detection limit [DL] and sensitivity [S]	Linearity	Shelf life	Reusability	Reference
3-Mercaptopropionic acid/Au ITO coated glass	ChOx	Covalent	Amperometry	30 s	upto 5.17 mM	–	–	[23]
	Cytochrome P450SCC(5 μg in 40 LB)	–	Electrochemical	3 min, [DL]: 11.6 mg dl^{-1}, –	100–750 μM	–	–	[24]
Au-Nps/Cysteamine (SAM)/Au	ChOx	Physical adsorption	Electrochemical	15 min, [DL]: 5×10^{-9} M, –	7.5×10^{-8} to 1×10^{-6} M and 1×10^{-6} to 5×10^{-5} M	–		[25]
Gold electrode	ChOx	Physical adsorption	Amperometry	<2 min [DL]: 60 μM [S]:0.13 μA mM^{-1}	0–2.1 mM	–	30 times	[26]
PANI/ITO	ChOx, ChEt, HRP	Covalent	Spectrophotometry	240 s, [DL]: 25 mg dl^{-1}, –	2.58–10.3 mM	6 weeks		[27]
Polypyrrole/pt disc electrode	ChOx, ChEt	Entrapment	Spectrophotometry	2 min	1–8 mM	4 weeks	10 times	[28]
DBS doped ppy/ITO	ChOx/$K_3Fe(CN)_6$	Physical adsorption	Amperometry	30 s	2–8 mM	12 weeks		[29]
BSA/polycarbonate/oxygen electrode	ChOx/ChEt	Cross-linking	Polarography	90 s	0.05–1.29 mM	8 weeks	30 times	[30]
Streptavidin/biotin/Thioctic acid(SAM)/Au nanowires	ChOx/ChEt	Covalent	Square wave Voltammetry	[S]: 69 nA mM^{-1},–	1–6 mM	10 days	20 times	[31]
MEH-PPV/SA/Au LB electrode	ChOx	Physical adsorption	Differential pulse voltammetry	30 s, 1.66 mM, 5.24 nA mM^{-1}	1.29–12.91 mM	3 months	60 times	Present work

interactions of the negatively charged ChOx molecules with the MEH-PPV molecules on the LB surface.

Characteristics of the ChOx/MEH-PPV/SA LB film bioelectrode along with those reported in literature have been summarized in Table 2. It can be seen that ChOx/MEH-PPV/SA LB film bioelectrode can detect cholesterol over a broad range of 1.29–12.91 mM with detection limit of 1.66 mM. This bioelectrode shows better shelf life (3 months) and high reusability (60 times) over other matrices. The observed lower response time of 30 s may be attributed to well-aligned LB matrix. However, efforts should be made to improve the sensitivity of this bioelectrode.

4. Conclusions

Cholesterol oxidase has been immobilized onto conducting polymer LB film of MEH-PPV and SA fabricated onto ODT modified gold coated glass plate. The cholesterol has been detected at lower potential (0.13 V). The bioelectrode has linearity in the range, 1.29–12.91 mM of cholesterol concentration. The values of correlation coefficient and standard deviation calculated from the analysis of the linear regression of DPV data have been found to be 0.9939 and 0.0029 μA, respectively. The ChOx/MEH-PPV/SA LB film bioelectrode exhibits sensitivity as 5.24 nA mM^{-1}, is stable up to 55 °C and can be used about 60 times without much loss in the activity indicating its excellent stability. Efforts should be made to improve the sensitivity of this bioelectrode and to utilize MEH-PPV/SA LB film electrode for estimation of other analytes like lipo-proteins, total cholesterol and triglycerides etc. Besides this, it would be interesting to undertake similar studies using other polyphenylene derivatives.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2008.12.023.

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