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PAPER

Realization of an efficient cholesterol biosensor using ZnO nanostructured thin film

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A zinc oxide (ZnO) nanostructured thin film synthesized by a vapour phase transport technique on a platinum coated silicon (Pt/Si) substrate has been successfully utilized for the detection of cholesterol. Amperometric and photometric studies reveal that the prepared bioelectrode ChOx/ZnO/Pt/Si is highly sensitive to the detection of cholesterol over a wide concentration range, 0.12–12.93 mM (5–500 mg dl^{-1}). The higher sensitivity is attributed to the large surface area of ZnO thin film for effective loading of ChOx besides its high electron communication capability. A relatively low value of the enzyme's kinetic parameter (Michaelis–Menten constant, 1.08 mM) indicates an enhanced affinity of the enzyme (ChOx) towards the analyte (cholesterol). The prepared bioelectrode is found to exhibit a long shelf life of more than 10 weeks, having negligible interference from the presence of other biomolecules present in human serum indicating potential application of the ZnO nanostructured thin film for cholesterol sensing.

Introduction

Estimation of cholesterol levels has aroused great interest from the scientific community as various clinical studies have shown a deep relationship of serum cholesterol with coronary heart diseases. Cholesterol is a building block of body tissue and an essential structural constituent in most vertebrate cell membranes. Cholesterol and its fatty acid are also important constituents of nerve brain cells. On the other hand, high serum cholesterol levels are related to various clinical disorders such as heart diseases, coronary artery disease, arteriosclerosis, hypertension, cerebral thrombosis *etc.*[1–5] Thus, maintaining an optimum level of cholesterol in human serum is of the utmost importance. This emphasizes the need for the development of a reliable and highly sensitive method for active and fast determination of cholesterol levels in blood serum. Amongst the various methods used for cholesterol detection, biosensors are widely used due to their high selectivity, rapid response, reproducibility and stability.[2,4,5] The key step in biosensor fabrication is the fabrication of a suitable matrix over which enzyme can be immobilized without much loss of its bioactivity.[4,6–9] Thus developing an efficient matrix having a compatible microenvironment for the biomolecules with good electron transfer capability is desirable to realize an efficient biosensor with enhanced sensitivity and rapid response. A variety of matrices such as metal oxides, self assembled monolayers, conducting polymers and nanocomposites have been used as a platform for immobilization of biomolecules.

Nanostructured metal oxides such as zinc oxide, cerium oxide, tin oxide, tellurium oxide, *etc.* have recently been preferred as efficient matrices because of their ability to promote faster electron transfer between the electrode and the active site of desired enzyme, and their ease of fabrication.[9] Amongst these nanostructured matrices, zinc oxide (ZnO) is found to exhibit unique properties including high catalytic activity, high isoelectric point (IEP = 9.5), wide band gap (3.37 eV), biocompatibility and good electron communication features.[2,4,6,7,9–15] Furthermore nanostructured thin film is advantageous due to the availability of a large surface area for effective enzyme loading. A high IEP of the matrix is important for the immobilization of enzymes having low IEP like cholesterol oxidase (ChOx, IEP = 4.7) by physical adsorption technique *via* electrostatic interaction. Few reports are available on the fabrication of cholesterol biosensors using ZnO thin films and nanostructures as shown in Table 1. It may be seen from Table 1 that the ZnO thin films and nanostructures for cholesterol sensing have been grown using various physical and chemical deposition techniques. However, to the best of our knowledge, there has not been any report on the exploitation of ZnO thin film grown by the vapour phase transport technique, which is known to provide highly stable nanostructured thin film.[16,17] It is important to point out that all essential features of efficient biosensors *i.e.* linearity over a wide range of analyte concentrations, low K_m value, rapid response, low detection limit, long shelf life, reproducibility and high sensitivity are difficult to obtain in one biosensor structure together. In the present work, our main focus is to develop a cholesterol biosensor based on a ZnO nanostructured matrix possessing

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Table 1 Summary of reported results obtained by various workers on the response parameters of ZnO based matrices for cholesterol sensing. The results obtained in the present work are also summarized for comparison

Immobilization matrix	Linearity	K_m	Enzyme activity	Surface concentration	Response time	Sensitivity	Shelf life (weeks)	Reference
Sol–gel derived ZnO	5–400 mg dl^{-1}	0.98 mg dl^{-1} = 0.0253 mM		1.86×10^{-8}	10 s	5.9×10^{-4} A mg^{-1} dl = 2.284 μA $μM^{-1}$	12	Solanki *et al.* 2009
Rf-sputtered ZnO film	25–400 mg dl^{-1}	2.1 mM	2.3×10^{-3}		—	—		Saha *et al.* 2007
Chemically synthesized ZnO	1–15 μM = 0.038–0.579 mg dl^{-1}	2.57 mM			10 s	61.7 μA $μM^{-1}$ cm^{-2}	4	Umar *et al.* 2009
ZnO nanorods grown chemically	10^{-6} to 10^{-2} mM = 0.038–386 μg dl^{-1}				10 s			Israr *et al.* 2010
ZnO nanoparticles	5–300 mg dl^{-1}	8.63 mg dl^{-1} = 0.223 mM	5.2×10^{-3}	9.02×10^{-8}	—	1.41×10^{-4} A mg^{-1} dl	8	Khan *et al.* 2009
ZnO nanoparticles	1–500 nM	4.7 mM			5 s	23.7 μA mM^{-1} cm^{-2}		Umar *et al.* 2009
ZnO nanowalls	10^{-6} to 10^{-3} M				5 s			Israr *et al.* 2011
ZnO nanostructures	0.12–12.93 mM	1.08 mM	4.8×10^{-2}	2.4×10^{-7}	5 s	153 μA mM^{-1} cm^{-2}	10	Present work

most of the above mentioned sensor features with improved response characteristics. In the present work the ZnO nanostructured matrix has been synthesized by a vapour phase transport technique. The cholesterol sensing response characteristics of the prepared bioelectrode (ChOx/ZnO/Pt/Si) have been studied using amperometric and photometric techniques and show good linearity over a wide concentration range of cholesterol with enhanced response.

Experimental

Materials

Cholesterol oxidase (ChOx) from *Streptomyces* species, horseradish peroxidase and cholesterol were procured from Sigma-Aldrich. Sodium phosphate monobasic anhydrous and sodium phosphate dibasic dihydrate were obtained from Sisco chemical, India. ZnO powder (99.9% purity) and potassium ferricyanide were acquired from Merck & Co. Graphite (99.99% purity) was procured from Alfa Aesar. All the chemicals were used without further purification. Deionized water (resistivity 18.2 MΩ cm) was used in the preparation of aqueous solutions.

Measurement and apparatus

The cyclic voltammetry (CV) studies were carried out on a Gamry potentiostat/galvanostat (Reference 600) using a three-electrode cell configuration with Ag/AgCl electrode as a reference electrode, ZnO nanostructured sample as the working electrode and platinum foil as a counter electrode. The cyclic voltammetry studies were performed in 10 ml of phosphate buffer saline (PBS) solution (50 mM, pH 7.0, 0.9% NaCl) containing 5 mM $K_3[Fe(CN)_6]$. The apparent enzyme activity was studied by photometric assay using a Perkin Elmer (lambda 35) UV–visible spectrophotometer.

Preparation of solutions

Phosphate buffer saline (PBS) 50 mM, pH 7.0 (0.9% NaCl) solution was prepared by adjusting the proportion of monobasic sodium phosphate solution with dibasic sodium phosphate solution and subsequently adding 0.9% NaCl to the solution. ChOx (1 mg ml^{-1}) and HRP (1 mg ml^{-1}) solutions were freshly prepared in PBS buffer of pH 7.0. The stock solution of cholesterol was prepared in 10% Triton X-100 and stored at 4 °C. The solution of *o*-dianisidine dye (1%) was freshly prepared in deionized water.

Preparation of ZnO nanostructured thin film by vapour phase transport technique

ZnO thin film was grown on platinum (Pt) coated Si (100) substrates (Pt/Si) using a vapour phase transport process. ZnO and graphite powders were mixed well at a weight ratio of 2 : 1 and used as the source. A schematic of the vapour phase transport process indicating varying temperature zones in the tube furnace is shown in Fig. 1. A quartz boat containing the powder mixture was placed in a horizontal quartz tube in the central hot zone of the temperature controlled furnace which was maintained at a temperature of 900 °C. The Pt/Si substrate was placed on an alumina plate in the zone where the temperature would be around 600 °C *i.e.* at a distance from the centre zone along the direction of flow of Ar gas. The platinum film (100 nm) was deposited on the Si substrate by rf sputtering using Pt metal target under Ar ambient, and a 10 nm thin buffer layer of titanium (Ti) was deposited prior to Pt to improve adhesion. The central zone of the furnace was maintained at 900 °C for 1 h and then cooled down to room temperature with Ar gas flowing

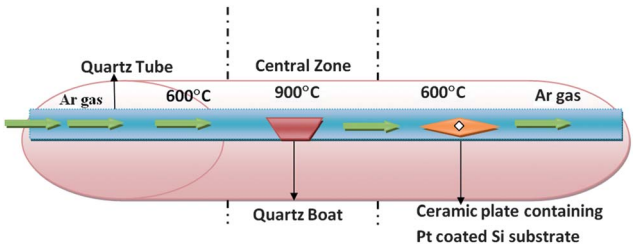

Fig. 1 Schematic of the vapour phase transport technique.

through the quartz tube. The Ar gas was flowing at a flow rate of 30 sccm when the temperature was rising and subsequently was maintained at 50 sccm when a temperature of 900 °C was attained in the central zone. During the process graphite acts as a catalyst which vapourises ZnO powder at a lower temperature of 900 °C and settles down on the substrate surface as a nanostructured thin film at a temperature of 600 °C resulting in a ZnO/Pt/Si electrode. A deposition time of 1.0 hour and flow rate of 50 sccm are optimum to obtain a high quality ZnO thin film having well defined oxidation and reduction peaks in the CV curves.[16–18]

Immobilization of enzyme

The ChOx enzyme having a low IEP of 4.7 was immobilized on the surface of the ZnO based electrode (ZnO/Pt/Si) by physical adsorption technique. 10 μL of ChOx solution (1 mg ml^{-1}) prepared in PBS was spread over the surface of the ZnO/Pt/Si electrode and dried at room temperature for about an hour. The electrode was then kept overnight for enzyme immobilization at a temperature of 4 °C and subsequently washed with buffer solution. A schematic of the prepared bioelectrode (ChOx/ZnO/Pt/Si), which is stored at 4 °C when not in use, is shown in Fig. 2.

The ZnO thin films were characterized for their crystallographic structure using X-ray diffraction technique [Bruker D8 Discover] and surface morphology using scanning electron microscope (SEM) [Quanta FEI 200]. Optical phonon modes and phase purity were studied using Raman spectroscopy [Renishaw In Via reflex micro Raman spectrometer] and Fourier transform infrared spectroscopy (FTIR) [Perkin Elmer Frontier].

Results and discussion

Physical properties

The X-ray diffraction pattern of the as deposited ZnO thin film is shown in Fig. 3. The nanostructured thin film is found to be polycrystalline having ZnO crystallites oriented along (100), (002), and (101) planes. The positions of the XRD peaks match very well with those of bulk ZnO, confirming the growth of polycrystalline ZnO thin film with wurtzite hexagonal structure. All XRD peaks were broad, and the grain size estimated using Scherrer's relation is found to be about 14 nm. The values of lattice parameters are estimated to be $c = 5.22$ Å and $a = 3.26$ Å which are found to be slightly greater than the corresponding bulk values ($c = 5.20$ Å and $a = 3.25$ Å) indicating the presence of some stress in the synthesized ZnO thin film. The SEM micrograph of 120 nm thin ZnO based electrode as shown in the inset

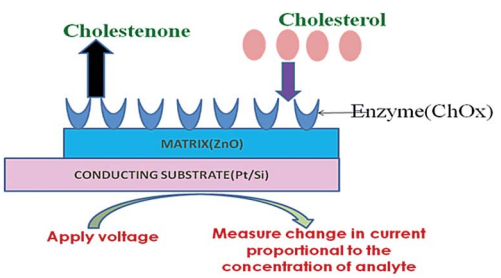

Fig. 2 Schematic of the ChOx/ZnO/Pt/Si bioelectrode.

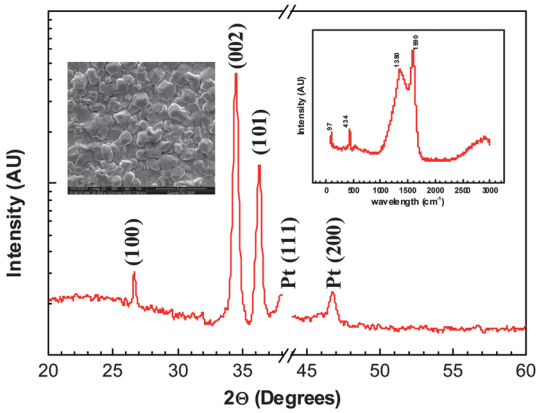

Fig. 3 XRD spectra of as grown ZnO/Pt/Si electrode; inset shows (left) SEM image and (right) Raman spectra of the as grown ZnO/Pt/Si electrode.

(a) of Fig. 3 shows the growth of thin films having hexagonal disc shaped crystallites with rough and porous microstructures, which facilitate the effective loading of ChOx and provide a suitable environment for the immobilized ChOx enzyme.

Room temperature Raman spectroscopy studies on the ZnO nanostructured thin film deposited on the Pt/Si substrate were carried out under backward scattering configuration with incident light normal to the surface of the sample. Well defined Raman modes were observed at 97, 434, 1380 and 1590 cm^{-1} as shown in the inset (b) of Fig. 3. The peaks observed at 97 cm^{-1} and 434 cm^{-1} correspond to the E_2 (low) and E_2 (high) characteristic modes of ZnO wurtzite phase respectively,[19] while the modes observed at 1380 and 1590 cm^{-1} correspond to K point symmetry and E_{2g} symmetry modes for graphite.[20] These results indicate that some amount of graphite is also present along with ZnO in the prepared samples.

The FTIR spectra of the ZnO/Pt/Si electrode and the ChOx immobilized ChOx/ZnO/Pt/Si bioelectrode are shown in Fig. 4. It may be seen that there are strong absorption bands at 588 cm^{-1} and 666 cm^{-1} for the Zno/Pt/Si electrode, corresponding to the Zn–O stretching vibration modes and C=H bending mode respectively.[6,9] The results confirm the formation of ZnO along

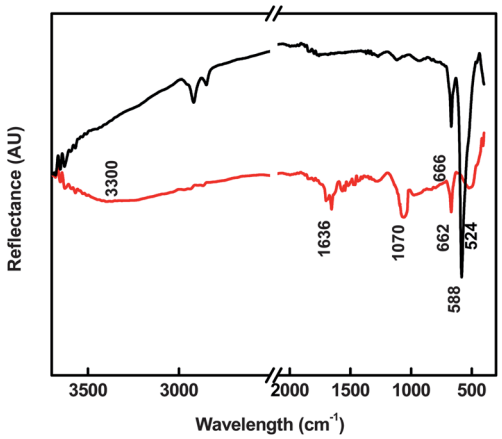

Fig. 4 FTIR spectra of (black) ZnO/Pt/Si electrode and (red) ChOx/ZnO/Pt/Si bioelectrode.

with the presence of C in the matrix. Similar results on the presence of carbon/graphite have been observed in the Raman analysis of the ZnO/Pt/Si electrode. Upon immobilization of ChOx (ChOx/ZnO/Pt/Si), the bioelectrode shows a weak band at 524 cm^{-1} due to the Zn–O stretching which is found to be shifted compared to the one obtained for the ZnO/Pt/Si electrode (588 cm^{-1}). The observed shifting in this mode may be attributed to the electrostatic interaction between the ZnO and ChOx that might have disturbed the stretching vibrational modes of Zn–O.[9] On the other hand, the C=H bending mode is found to be present almost at the same position (662 cm^{-1}), indicating that the graphite present in the nanostructured matrix does not participate in the electrostatic interaction with ChOx. A few extra bands are also observed at 1070 cm^{-1}, 1636 cm^{-1} and 3300 cm^{-1} in the FTIR spectra of the ChOx/ZnO/Pt/Si bioelectrode and are assigned to C–O stretching, carbonyl stretch amide I and amide B of the enzyme ChOx respectively.[9,21] The obtained results clearly highlight the successful immobilization of ChOx on the surface of the ZnO matrix by electrostatic interaction.

Cyclic voltammetric studies

Immobilization. Cyclic voltammograms of the Pt/Si substrate, ZnO/Pt/Si electrode and ChOx/ZnO/Pt/Si bioelectrode have been recorded using a three electrode cell in a PBS solution containing 5 mM $K_3[Fe(CN)_6]$ as mediator and the variation is shown in the inset (i) of Fig. 5. It is important to note that the prepared ZnO matrix (Zno/Pt/Si) shows a low peak oxidation current (314 μA) compared to the corresponding value obtained for the Pt/Si substrate (504 μA). This observed decrease in oxidation current may be related to the semiconducting nature of the prepared matrix. The immobilization of ChOx over the surface of the ZnO/Pt/Si electrode results in an increase in the peak oxidation current to 427 μA (inset (i) of Fig. 5) revealing that ZnO thin film provides a suitable 3d platform for ChOx immobilization resulting in enhanced electron communication between ChOx and the electrode through the mediator. However, the separation between peak oxidation current and reduction current was found to increase from 0.14 V to 0.16 V for the ChOx/ZnO/Pt/Si bioelectrode (inset (i) of Fig. 5) and may be attributed to some potential drop across the ChOx due to its non conducting nature. The activity of the biomolecules is found to be highly dependent on the pH of the surrounding medium. Hence, in the present work, the effect of the pH of the buffer solution on the activity of the prepared bioelectrode (ChOx/ZnO/Pt/Si) has been studied. The peak oxidation current in the CV of the ChOx/ZnO/Pt/Si bioelectrode has been recorded by varying the pH of the buffer solution, which is found to be maximum at a pH of 7.0 and reduces on either increasing or decreasing its value. Hence, ChOx is found to be exhibiting maximum activity in a buffer solution of pH 7.0. As a result, the pH of the buffer solution was kept fixed at 7.0 throughout the experiments.

CV investigations at various scan rates (10–100 mV s^{-1}) have been performed on the ChOx/ZnO/Pt/Si bioelectrode to determine the concentration of ionic species on its surface and the variation is shown in Fig. 5. As the scan rate increases, the anodic peak potential shifts towards the positive values and the cathodic peak potential towards the negative values, suggesting a quasi reversible process. The anodic and cathodic peak currents are found to be proportional to the square root of the scan rate (ν) over the range 10 to 100 mV s^{-1} indicating that it is a diffusion controlled process.[21] The linear regression equations fitted to the peak anodic and cathodic current variation are mentioned below:

$$I_{pa} = 0.033\nu^{1/2} + 0.098 : R = 0.998 \quad (1)$$

$$I_{pc} = -0\ .027\nu^{1/2} - 0.052 : R = 0.993 \quad (2)$$

where R is the regression coefficient, having a value close to 1, which indicates that the variation of I_{pa} and I_{pc} as a function of $\nu^{1/2}$ is perfectly linear (shown in the inset (ii) of Fig. 5). The anodic peak potential is found to vary linearly with the logarithm of ν. According to the Laviron's theory,[21,22] the total surface concentration of ionic species is found to be 2.4×10^{-7} moles cm^{-2} which is relatively higher than the corresponding values reported for the ZnO based matrix by other workers for cholesterol biosensors. This is due to the higher loading of the ChOx enzyme on the nanostructured surface of the Zno matrix.[4,9]

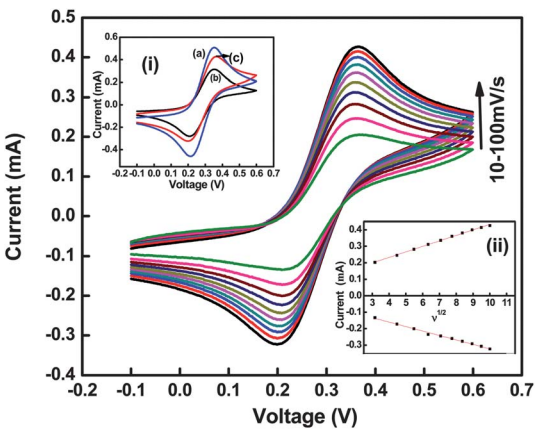

Fig. 5 Effect of scan rate on CV's of the ChOx/ZnO/Pt/Si bioelectrode. Inset shows (i) CV response of (a) Pt/Si substrate (b) ZnO/Pt/Si electrode (c) ChOx/ZnO/Pt/Si bioelectrode and (ii) variation of peak oxidation and reduction currents with square root of the scan rate.

Electrochemical response studies. The working mechanism of the cholesterol biosensor during measurements could be explained by taking into account the dual role of ChOx where it provides not only a good specificity but also works as a catalyst to initiate the chemical reaction. The enzyme (ChOx) catalytic reaction between a cholesterol molecule and the oxygen present in the electrolyte solution produces 5cholest-3-one and hydrogen peroxide as products of the chemical reaction. However, 5cholest-3-one is highly susceptible to undergo the spontaneous process of isomerization to form 4cholest-3-one as a stable molecule.

$$\text{cholesterol} + O_2 \xrightarrow{\text{ChOx}} \text{5cholest-3-one} + H_2O_2 \xrightarrow{\text{Isomerization}} \text{4cholest-3-one} + H_2O_2$$

The H_2O_2 produced in this reaction further dissociates as follows

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$$

These released electrons are being detected by the electrode. However, the dissociation of H_2O_2 takes place at a very high potential. However, when mediator ($K_3[Fe(CN)_6]$) is present in the electrolyte, the electron transfer takes place through the mediator at a low potential (<0.4 V) as observed in the present case. The schematic of the reaction in the presence of mediator ($K_3[Fe(CN)_6]$) is shown in Fig. 6.

Fig. 7 shows the cyclic voltammograms of the ChOx/ZnO/Pt/Si bioelectrode in PBS containing $K_3[Fe(CN)_6]$ as a redox couple for varying concentration of cholesterol. It may be seen that the peak oxidation current keeps on increasing with successive addition of cholesterol from 0.12 to 12.93 mM. This may be due to the fact that ZnO acts as a good receptor of the electrons generated during reoxidation of ChOx and transferred to the electrode *via* Fe^{3+}/Fe^{2+} conversion resulting in an increased electrochemical response. As the concentration of cholesterol in the electrolyte solution increases, a greater number of electrons are generated resulting in an enhanced peak oxidation as well as reduction currents for the ChOx/ZnO/Pt/Si bioelectrode (Fig. 7). The response time of the bioelectrode based on the ZnO nanostructured matrix is found to be very fast (5 s) which may be because of the fast electron communication feature of the prepared ZnO nanostructured matrix. The value of the response time of the bioelectrode was measured from CV studies by taking the CV response after a regular interval of time starting from 1 s to 10 s (with a step of 1 s) by dissolving the desired concentration of analyte (cholesterol) in the PBS buffer solution. The response was found to increase with an increase in time from 1 s to 5 s and thereafter tends to remain constant. Variation of peak oxidation current with time for 5.2 mM analyte concentration is shown in the inset (c) of Fig. 7. The peak oxidation current keeps on increasing with time until 5 s and thereafter saturates to a constant value. Therefore, 5 s is considered the response time in the present study. Peak oxidation current is found to increase linearly with increasing cholesterol concentration (0.12–12.93 mM) as shown in the inset (a) of Fig. 7. The range of cholesterol concentrations chosen in the present work is much beyond the physiological range (2.5–7.5 mM). The sensitivity of the prepared bioelectrode (ChOx/ZnO/Pt/Si) towards cholesterol, estimated from the variation of peak oxidation current with cholesterol concentration curve, is about 153 μA mM^{-1} cm^{-2}, which is found to be much higher than the corresponding values reported for cholesterol biosensors based on ZnO matrices (Table 1). The high sensitivity observed in the present work may be attributed to the exploitation of the ZnO nanostructured matrix and effective loading of ChOx on its surface.

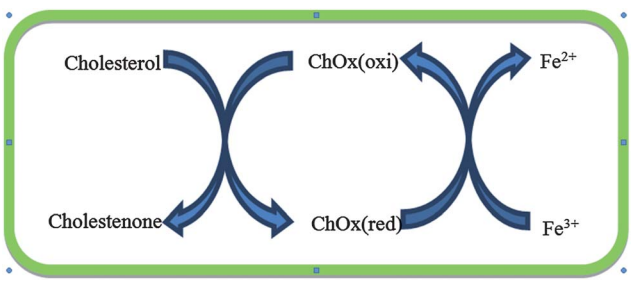

Fig. 6 Schematic of reaction occurring at the bioelectrode with cholesterol in the presence of mediator.

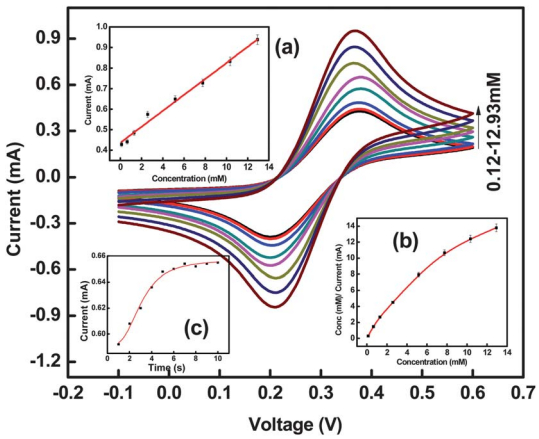

Fig. 7 CV response of the ChOx/ZnO/Pt/Si bioelectrode as a function of cholesterol concentration. Inset (a) variation of peak oxidation current with cholesterol concentration, (b) Hane's plot and (c) variation of peak oxidation current with time.

Michaelis–Menten kinetic parameters

The Michaelis–Menten kinetic parameter (K_m) of the enzymatic reaction has been estimated using Hane's plot (*i.e.* graph between [analyte concentration] and [analyte concentration/current]).[23] The Hane's plot is shown in the inset (b) of Fig. 7 and the value of K_m is estimated to be 1.08 mM for the prepared bioelectrode. The relatively lower value of K_m reflects the enhanced activity of ChOx enzyme towards its analyte (cholesterol). The increased interaction between the enzyme's active sites and cholesterol is due to the favourable conformational changes obtained in the structure of ChOx on being immobilized on the surface of the ZnO nanostructured matrix, which are facilitated by the suitable biocompatible microenvironment provided by the ZnO thin film.

Photometric response

To carry out the photometric enzyme assay of immobilized ChOx, the bioelectrode (ChOx/ZnO/Pt/Si) was dipped in 3 ml PBS solution containing 20 μL horseradish peroxidase (HRP), 20 μL *o*-dianisidine dye and 100 μL of analyte (cholesterol). The difference between the initial and final values of absorbance at a fixed wavelength (500 nm) after 3 min incubation of the bioelectrode was recorded as a function of cholesterol concentration. The absorbance is found to be increasing linearly with an increase in cholesterol concentration up to 300 mg dl^{-1} and thereafter saturates as shown in Fig. 8. These results show that the enzyme activity increases with an increase in cholesterol concentration. The apparent enzyme activity, *i.e.* the amount of enzyme bound on the surface of the ZnO matrix, has been calculated using the equation $a_{app}^{enz}(U\ cm^{-2}) = AV/\varepsilon ts$, where A is the difference in absorbance before and after incubation, V is the total volume (3.17 cm^3), ε is the millimolar extinction coefficient (7.5 for *o*-dianisidine at 500 nm), t is the reaction time (min) and s

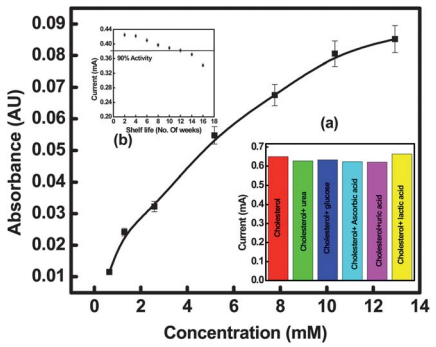

Fig. 8 Change in absorbance of ChOx/ZnO/Pt/Si bioelectrode with cholesterol concentration. Inset shows (a) shelf life studies and (b) effect of interferents on the response of the bioelectrode ChOx/ZnO/Pt/Si.

is the surface area (cm^2) of the prepared bioelectrode. The estimated value of apparent enzyme activity on the surface of the bioelectrode was estimated to be 4.8×10^{-2} U cm^{-2}. The obtained value is much higher than that obtained by other workers (2.3×10^{-3} U cm^{-2} (ref. 6), 5.2×10^{-3} U cm^{-2} (ref. 4)) using ZnO based matrices for cholesterol detection (Table 1).

The selectivity of the prepared ChOx/ZnO/Pt/Si bioelectrode has also been studied by comparing the magnitude of the peak oxidation current (CV response) when adding normal concentrations of interferents such as glucose (5 mM), uric acid (0.1 mM), lactic acid (0.5 mM), ascorbic acid (0.05 mM) and urea (1 mM) separately to the normal concentration of cholesterol (5.2 mM) (Fig. 8, inset (a)). It is observed that the response of the prepared bioelectrode (ChOx/ZnO/Pt/Si) is not significantly affected by the presence of these interferents, giving a maximum interference of about 4% with lactic acid suggesting that the prepared bioelectrode based on the ZnO matrix is highly selective towards cholesterol. The shelf life studies of the prepared ChOx/ZnO/Pt/Si bioelectrode (inset (b) of Fig. 8) shows that it retains more than 90% activity even after 10 weeks (which is found to be much longer than that obtained by other workers (4 to 8 weeks) for ZnO matrices, which may be attributed to the synthesis of a highly stable ZnO nanostructured matrix in the present case using the vapour phase transport process).

Conclusions

In summary, we have fabricated a cholesterol biosensor by immobilizing ChOx on the surface of a ZnO nanostructured matrix prepared by a vapour phase transport technique. The ChOx/ZnO/Pt/Si bioelectrode exhibits high sensitivity (153 μA mM^{-1} cm^{-2}) and a linear response over the cholesterol concentration range 0.12 to 12.93 mM, with high stability and good selectivity. The low value of the Michaelis–Menten constant (1.08 mM) indicates an enhanced affinity of ChOx towards cholesterol. The results highlight the potential application of the prepared bioelectrode based on nanostructured ZnO for cholesterol biosensing.

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