Analytical Biochemistry 383 (2008) 194–199

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Polyaniline–carbon nanotube composite film for cholesterol biosensor

Chetna Dhand [a,b], Sunil K. Arya [a,b], Monika Datta [b], B.D. Malhotra [a,*]

[a] Biomolecular Electronics and Conducting Polymer Research Group, National Physical Laboratory, Dr. K. S. Krishnan Marg, New Delhi 110012, India
[b] Department of Chemistry, University of Delhi, Delhi 110007, India

ARTICLE INFO

Article history:
Received 14 May 2008
Available online 15 September 2008

Keywords:
Cholesterol
Cholesterol oxidase (ChOx)
Polyaniline (PANI)
Carbon nanotubes (CNTs)
Biosensor

ABSTRACT

Nanocomposite film composed of polyaniline (PANI) and multiwalled carbon nanotubes (MWCNT), prepared electrophoretically onto indium tin oxide (ITO)-coated glass plate, was used for covalent immobilization of cholesterol oxidase (ChOx) via *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) chemistry. Results of linear sweep voltammetric measurements reveal that ChOx/PANI–MWCNT/ITO bioelectrode can detect cholesterol in the range of 1.29 to 12.93 mM with high sensitivity of 6800 nA mM^{-1} and a fast response time of 10 s. Photometric studies for ChOx/PANI–MWCNT/ITO bioelectrode indicate that it is thermally stable up to 45 °C and has a shelf life of approximately 12 weeks when stored at 4 °C. The results of these studies have implications for the application of this interesting matrix (PANI–MWCNT) toward the development of other biosensors.

Since the discovery of carbon nanotubes (CNT)[1], there has been enormous interest in exploring and exploiting their unique properties for various applications [1–3]. A promising application of CNT is their use in biosensors and nanoscale electronic devices [4,5]. The results reported by various researchers suggest that CNT can be used to promote electrons transfer reactions of desired biomolecules such as dopamine [6,7], β-nicotinamide adenine dinucleotide (NADH) [8], norepinephrine [9], cytochrome *c* [10], ascorbic acid [11], and cholesterol [12,13]. However, poor solubility of CNT in most solvents is currently a major barrier for developing such CNT-based devices [14,15]. The challenge of solubilizing CNT can be addressed through their covalent modification [15,16] or noncovalent functionalization [14,17]. In particular, "wrapping" of CNT with polymeric chains can be useful for improving their solubility without impairing their physical properties [18]. The fabrication of conducting polymer (CP)/CNT composites has gained much interest recently. It has been demonstrated that CP/CNT composites possess properties of each of the constituents with a synergistic effect [19,20]. Among various conducting polymers, polyaniline (PANI) is unique due to its relatively facile synthesis, conductivity, and environmental stability [21–23]. In addition, it is the first commercialized conducting polymer and has found applications in Schottky devices [24], electrochromic devices [25], and chemical sensors [26]. However, this polymer has limited biosensor applications because PANI is neither electrochemically active nor conducting in neutral solution, and it reveals its redox characteristics only in an acidic medium (pH < 3) [27]. This prevents the coupling of this conducting polymer to a biological system.

For fabrication of a biosensor, the electrical contact of redox biomolecules, including enzymes with electrode surface, is a challenge. Most bioelectrocatalytic systems require mediated electron transfer between redox enzymes and conductive supports, and this process can be enhanced by increased electrical conductivity of the electron mediating system. Thus, it should be interesting to develop PANI/CNT composite systems that reveal redox activity at neutral pH and to investigate these composites as matrices for bioelectrocatalytic activation of enzymes [28]. Liu et al. used PANI/poly(aminobenzene sulfonic acid)-modified single-walled carbon nanotubes (PABS–SWCNT) multilayer film for detection of reduced NADH at lower potential [29]. They showed that PABS–SWCNT inside a multilayer film can be used to dope PANI effectively and shift its electroactivity to the neutral pH environment. Granot et al. used charge transfer properties of pyrene sulfonic acid-functionalized SWCNT embedded in PANI (PANI/SWCNT) to mediate bioelectrocatalyzed oxidation of glucose in the presence of glucose oxidase (GOx) [28]. Ali and coworkers recently reported a nonoxidative approach to electrochemical detection of dopamine with high sensitivity and selectivity using a PANI/CNT matrix in physiologically relevant solutions [30].

In this article, we report the fabrication of a mediator-free cholesterol biosensor based on electrophoretically [31,32] deposited

* Corresponding author.
E-mail address: bansi.malhotra@gmail.com (B.D. Malhotra).

[1] *Abbreviations used:* CNT, carbon nanotube; NADH, β-nicotinamide adenine dinucleotide; CP, conducting polymer; PANI, polyaniline; PABS–SWCNT, poly(aminobenzene sulfonic acid)-modified single-walled carbon nanotube; GOx, glucose oxidase; MWCNT, multiwalled carbon nanotube; ITO, indium tin oxide; ChOx, cholesterol oxidase; HRP, horseradish peroxidase; NHS, *N*-hydroxysuccinimide; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl carbodiimide); CCVD, catalytic chemical vapor deposition; PBS, phosphate-buffered saline; FT–IR, Fourier transform infrared; EIS, electrochemical impedance spectroscopy; LSV, linear sweep voltammetric; AA, ascorbic acid; UA, uric acid; Glu, glucose; LA, lactic acid.

0003-2697/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2008.08.039

PANI–multiwalled carbon nanotubes (MWCNT) composite film on indium tin oxide (ITO)-coated glass, with cholesterol oxidase (ChOx) covalently immobilized on the composite surface. The ChOx/PANI–MWCNT/ITO bioelectrode was found to be highly sensitive to cholesterol and exhibits a fast response time of 10 s.

Materials and methods

Chemicals and reagents

ChOx (EC 1.1.3.6, from *Pseudomonas fluorescens*) with specific activity of 24 U mg^{-1}, horseradish peroxidase (HRP, EC 1.11.1.7) with specific activity of 200 U mg^{-1}, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-dimethylaminopropyl carbodiimide) (EDC), and *o*-dianisidine were procured from Sigma–Aldrich (USA). The MWCNT were prepared via the catalytic chemical vapor deposition (CCVD) technique [33]. The CNTs were purified and functionalized using boiling acid treatment [34]. PANI was synthesized by chemical oxidative polymerization using ammonium peroxidisulfate as oxidant [35]. All other chemicals were of analytical grade and used as received. Deionized water (resistance ~ 18.2 MΩ) from the Millipore water purification system was used for the preparation of desired aqueous solutions.

Preparation of solutions

The solution of ChOx (24 U/ml) and HRP (200 U/ml) was prepared freshly in phosphate buffer (50 mM, pH 7.0) prior to being used. Stock solution of cholesterol was prepared in deionized water having 10% Triton X-100 and was stored at 4 °C. This stock solution was further diluted to make different concentrations of cholesterol solution. *o*-dianisidine (1%) solution was prepared freshly in deionized water.

Fabrication of composite film and immobilization of ChOx

Thin film of PANI–MWCNT composite was deposited by the electrophoretic technique [36] on ITO-coated glass plate to serve as a matrix for immobilization of ChOx. For electrophoretic film fabrication, colloidal suspension of PANI–MWCNT was prepared by mixing 100 μl of PANI/formic acid (1 mg ml^{-1}) solution with 10 μl of CNT dispersion (1 mg ml^{-1} in acetonitrile) in 9.9 ml of acetonitrile. Electrophoretic deposition of PANI-MWCNT composite was carried out using the set-up shown in Fig. 1. Variation of voltage and time was also investigated for desired uniform film formation. Washing of film prepared in this way was accomplished using deionized water followed by drying. ChOx was covalently immobilized onto the electrophoretically deposited composite film via amide bond formation between the NH_2 group of PANI and the COOH group of ChOx using EDC as the coupling agent and NHS as the activator [31]. For ChOx immobilization, the optimal binding was achieved when 30 μl of solution of 5 μg ChOx mixed with 0.4 M EDC and 0.1 M NHS was dropped on 1 cm^2 of PANI–MWCNT film and kept in a humid chamber for 3 h. Thus, fabricated ChOx modified bioelectrode (ChOx/PANI–MWCNT/ITO) was then washed with phosphate buffer saline (PBS) solution (50 mM, 0.9% NaCl, pH 7.0) containing 0.05% Tween-20 to remove any unbound enzyme and was stored at 4 °C when not in use.

Characterization

The fabricated electrodes (PANI–MWCNT/ITO and ChOx/PANI–MWCNT/ITO) were characterized using Fourier transform infrared (FT–IR) and electrochemical impedance spectroscopy (EIS) techniques, respectively. FT–IR spectra were recorded using a PerkinElmer instrument (model spectrum BX using ATR accessory) to check the binding of ChOx with PANI–MWCNT composite film. EIS studies in the frequency range of 0.01 to 10^5 Hz, amplitude 5 mV, were conducted on an Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands). The measurements were carried out in PBS (50 mM, 0.9% NaCl, pH 7.0) containing 5 mM $Fe(CN)_6^{3-/4-}$ (redox probe) using a three-electrode cell with Ag/AgCl as a reference electrode and Pt foil as a counter electrode.

Linear sweep voltammetric measurements

Linear sweep voltammetry (LSV) was carried out on an Autolab Potentiostat using a three-electrode cell with Ag/AgCl as a refer-

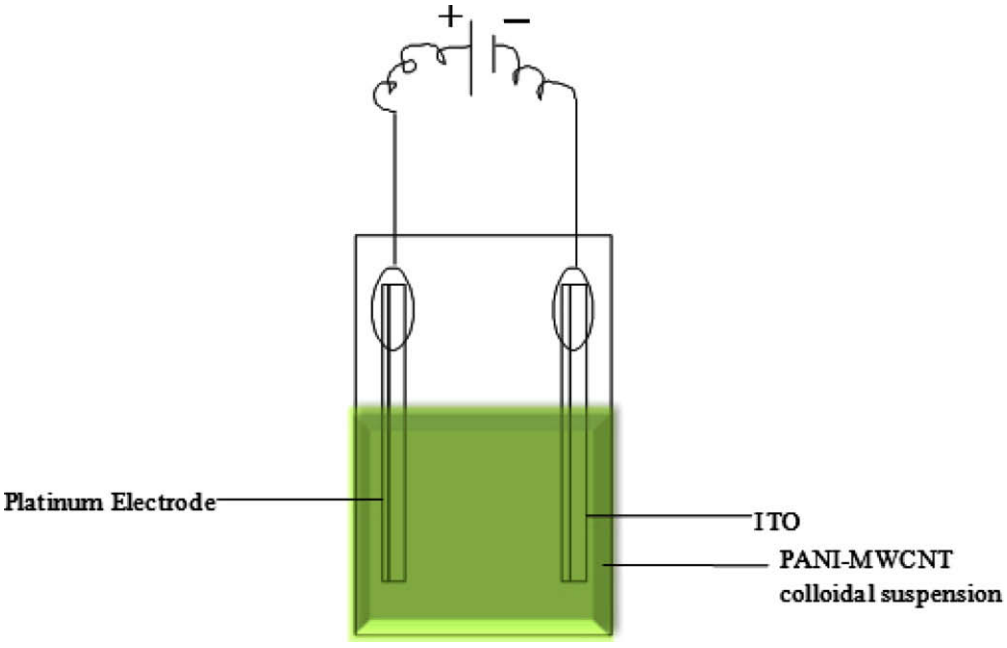


Fig. 1. Schematic of the setup used for the electrophoretic deposition.

ence electrode and Pt foil as a counter electrode. Cholesterol estimation studies using ChOx/PANI–MWCNT/ITO bioelectrode were conducted in the range of –0.3 to 0.8 V in PBS (50 mM, 0.9% NaCl, pH 7.4) solution. The bioelectrode was kept for 10 s in cholesterol solution for the enzymatic reaction prior to recording of the LSV spectra.

LSV studies were carried out to estimate the effect of interferents such as ascorbic acid (AA), uric acid (UA), glucose (Glu), lactic acid (LA), and urea. Artificial conditions were achieved by mixing the interferents AA (0.05 mM), UA (0.1 mM), Glu (5 mM), LA (0.5 mM), and urea (1 mM) with cholesterol solution (7.76 mM) in a 1:1 ratio, and the spectra were recorded under conditions similar to those used for cholesterol estimation.

Photometric studies

Photometric measurements were conducted using a UV–visible spectrophotometer, Phoenix-2200DPCV. Photometric experiments were carried out as a function of pH, temperature, and cholesterol concentration using PBS buffer (50 mM, 0.9% NaCl, pH 7.4). These measurements were also used to estimate the enzyme activity. To carry out photometric enzymatic assay of the immobilized ChOx, ChOx/PANI–MWCNT/ITO bioelectrode was dipped in 3 ml of PBS solution containing 20 µl of HRP (1 mg dl^{-1}), 20 µl of *o*-dianisidine dye, and 100 µl of cholesterol. The difference between the initial and final absorbance values at 500 nm after 3 min incubation of cholesterol were recorded and plotted.

Results and discussion

Electrophoretic deposition of composite film

In the PANI–MWCNT composite film formation using electrophoretic technique, good quality films are obtained when positively charged composite in acidic colloidal suspension is dragged toward the negatively biased ITO-coated glass electrode at an applied voltage of 120 V for 30 s. This results in the formation of uniform, homogeneous, and 200-nm-thick films via grain-by-grain deposition of colloid particles on the electrode surface. No deposition is observed on the positively biased Pt electrode, revealing that all the MWCNTs get completely wrapped by positively charged PANI fibers in colloidal suspension. During the composite film formation, gentle shaking or brief sonication of the colloidal solution for a few seconds is found to be necessary to achieve desired dispersion leading to the deposition of homogeneous film.

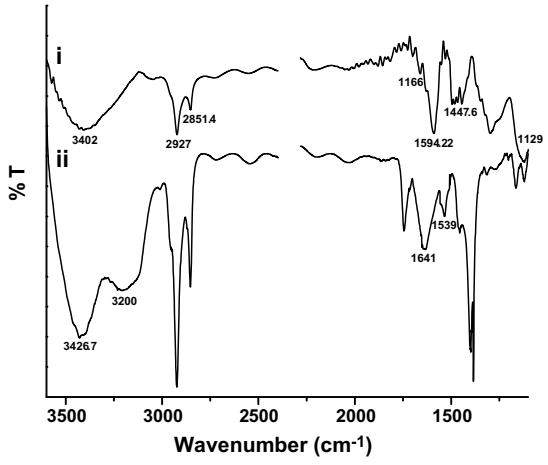


Fig. 2. FT–IR spectra of MWCNT/PANI/ITO electrode (i) and ChOx/PANI–MWCNT/ITO bioelectrode (ii).

FT–IR studies

Fig. 2 shows FT–IR spectra obtained for PANI–MWCNT/ITO electrode (curve i) and ChOx/PANI–MWCNT/ITO bioelectrode (curve ii). The FT–IR spectrum of electrophoretically deposited PANI–MWCNT composite (curve i) shows benzenoid and quinoid ring stretching bands (C=C) present at 1447.6 and 1594.2 cm^{-1}. The presence of peaks at 1129 and 3402 cm^{-1} is attributed to B–N^{+}=Q stretching and –N–H stretching vibrations of PANI in the composite. The peak at 1704 cm^{-1} is attributed to –C=O stretching vibrations and indicates the presence of carboxyl group (–COOH). The presence of these peaks reveals the existence of both PANI and MWCNT on the ITO electrode. In the FT–IR spectrum of ChOx/PANI–MWCNT/ITO bioelectrode (curve ii), ChOx binding is indicated by the appearance of additional absorption bands at 1641 and 1539 cm^{-1} assigned to the carbonyl stretch (amide I band) and –N–H bending (amide II band), respectively [37]. Also, a broad band seen around 3200 cm^{-1} is attributed to amide bond present in ChOx.

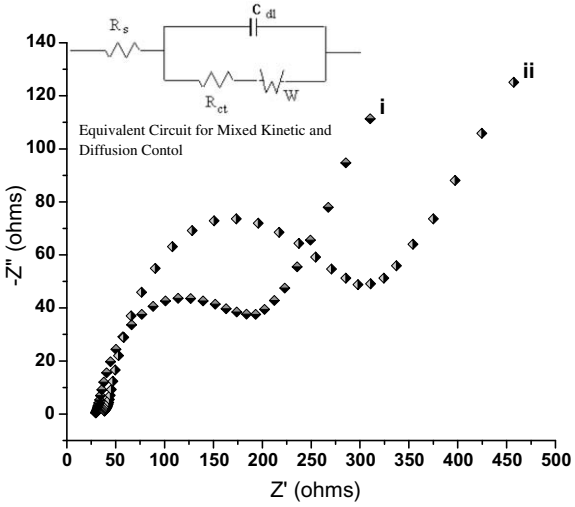


Fig. 3. Impedance spectra of PANI–MWCNT/ITO electrode (i) and ChOx/PANI–MWCNT/ITO bioelectrode (ii).

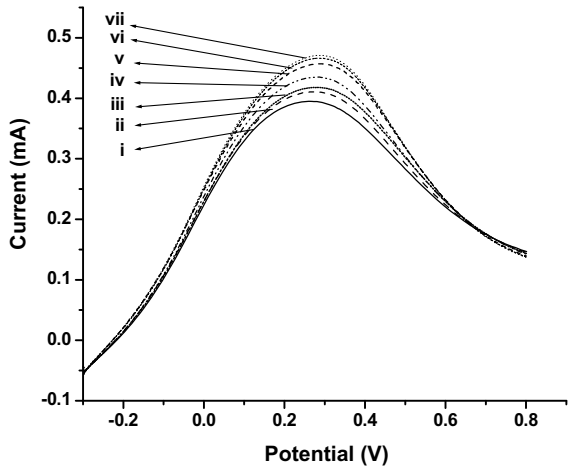
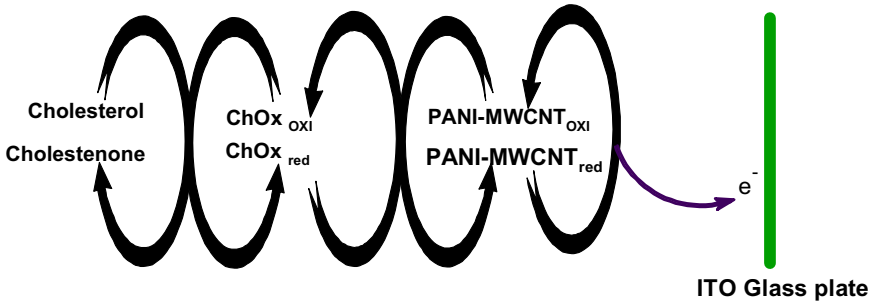


Fig. 4. Linear sweep voltammograms recorded for ChOx/PANI–MWCNT/ITO bioelectrode as a function of different concentrations of cholesterol: (i) 0 mM; (ii) 1.3 mM; (iii) 2.6 mM; (iv) 5.2 mM; (v) 7.8 mM; (vi) 10.34 mM; (vii) 12.93 mM.



Scheme 1. Schematic illustration of biochemical reaction at ChOx/PANI–MWCNT/ITO bioelectrode.

EIS studies

EIS analysis was carried out to investigate the ChOx immobilization onto the PANI–MWCNT matrix (Fig. 3). A Nequist plot was used to find the change in the charge transfer resistance (R_{CT}) after immobilization of ChOx on PANI–MWCNT/ITO matrix. It can be seen (curve i) that the value of R_{CT} obtained as 212 Ω for PANI–MWCNT/ITO electrode increases to 325 Ω for ChOx/PANI–MWCNT/ITO bioelectrode (curve ii). This increase in R_{CT} is attributed to the fact that most biological molecules, including enzymes, are poor electrical conductors at low frequencies (at least < 10 KHz) and cause hindrance to the electron transfer. These results indicate binding of ChOx onto PANI–MWCNT composite.

Response studies using LSV technique

Fig. 4 shows the linear sweep voltammograms of ChOx/PANI–MWCNT/ITO bioelectrode obtained as a function of cholesterol concentration. An anodic peak is observed around 0.28 V in LSV, and the anodic current is found to increase continuously with cholesterol concentration. It is found that when the current difference between the particular cholesterol concentration and zero concentration obtained at 0.28 V is plotted (data not shown), the bioelectrode can be used to detect cholesterol in the range of 1.29 to 12.93 mM with a fast response time of 10 s.

The anodic peak around 0.28 V corresponds to the oxidation of polyaniline present in the matrix, and its increase with an increase in the cholesterol concentration suggests that ChOx gets electrically contacted by the PANI–MWCNT modified electrode [28]. It may be noted that no peak is observed relating to the oxidation of H_2O_2 in the range of 0.5 to 0.7 V. This indicates that the biochemical reaction perhaps occurs as shown in Scheme 1, where PANI–MWCNT matrix directly accepts electrons from the reduced ChOx. Direct acceptance of electrons by the matrix is attributed to enhanced charge transport in the PANI–MWCNT film due to electron hopping through the conductive MWCNTs that mediate electrons transfer via redox polymer [28]. Enhanced charge transport in this matrix was recently revealed via cyclic voltammetry and EIS studies [36].

It was found that the ChOx/PANI–MWCNT/ITO bioelectrode in the range of 1.29 to 10.34 mM for cholesterol follows the equation [Current (mA) = 0.4 (mA) + 0.00678 (mA mM^{-1}) × Cholesterol Con-

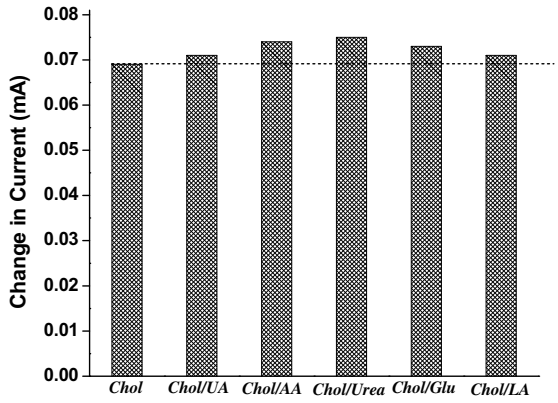


Fig. 5. Effect of various interferents on the response of ChOx/PANI–MWCNT/ITO bioelectrode. Chol, cholesterol.

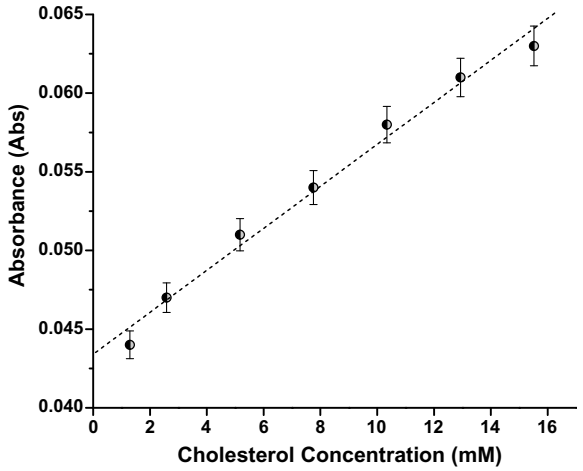


Fig. 6. Photometric response of ChOx/PANI–MWCNT/ITO bioelectrode as a function of cholesterol concentration.

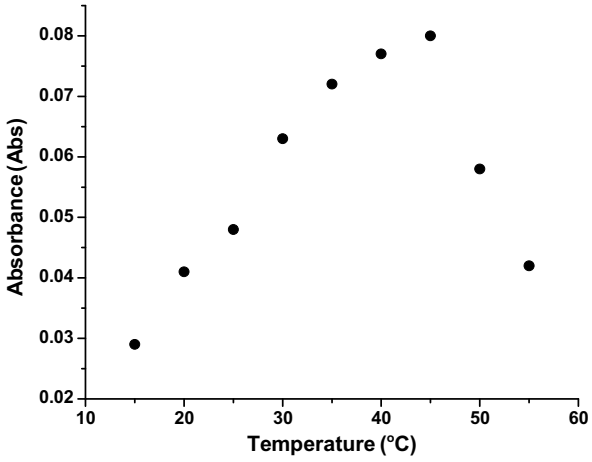


Fig. 7. Photometric response of ChOx/PANI–MWCNT/ITO bioelectrode as a function of temperature.

Table 1
Characteristics of electrophoretically deposited PANI–MWCNT-based ChOx/PANI–MWCNT/ITO bioelectrode along with those reported in the literature

Immobilization Matrix	Sensing element	Method of immobilization	Linearity (mM)	Transducer used	Sensitivity	Shelf life	Reference
MWCNT	ChOx	Physical entrapment	0.5–6	Amperometric	0.559 μA $cm^{-2}mM^{-1}$	-	[12]
Polyaniline	ChOx	Covalent	0.65–10.34	Spectrophotometric	7.76×10^{-5} Abs mg^{-1} dl	11weeks	[31]
Fe_3O_4 Nanoparticls	ChOx	Covalent	1.3–5.2	Spectrophotometric	–	15 days	[38]
Thiotic acid/Gold	ChOx/ChEt	Biotin-Avidin	1–6	Squarewave voltammetric	69 nA mM^{-1}	10 days	[39]
Gold electrode	ChOx	Physical Adsorption	0–2.1	Amperometric	0.13 μA mM^{-1}	–	[40]
MWCNT/Screen Printed Carbon Electrode	ChOx, ChEt, HRP, K4Fe(CN)6	Physical adsorption	2.58–10.34	Amperometric	0.0059 μA mg^{-1} dl	2 months	[13]
PANI-MWCNT	ChOx	Covalent	1.29–12.93	Amperometric and Spectrophotometric	6800 nA mM^{-1}	12 weeks	Present work

centration (mM)] with 3 μA and 0.994 as standard deviation and correlation coefficient, respectively. Furthermore, the bioelectrode in this range exhibits a high sensitivity of 6800 nA mM^{-1}. This is attributed to the high electron communication feature of the PANI–MWCNT composite. The enhanced bioelectrocatalytic oxidation of cholesterol by composite is due to the effective charge that facilitates electrical contact between the redox site of ChOx and the electrode. All of the experiments were carried out in triplicate sets, and results reveal reproducibility of the system within 2% error.

Interferent studies

Fig. 5 shows the effect of interferents on the observed response of ChOx/PANI–MWCNT/ITO electrode. In the figure, the first bar (cholesterol) shows the change of current obtained with 7.76 mM cholesterol when compared with current obtained for 0 mM cholesterol concentration. The remaining bars show the change in current corresponding to the mixture of cholesterol and interferents in a 1:1 ratio. The straight line parallel to the X axis shows the variation in observed current in the presence of desired interferents, revealing a maximum of 8% interference. The percentage interference (% inter) was calculated using Eq. (1) for various interferents:

$$\%\ \text{inter} = \frac{|I_{\text{Chol}} - I_{\text{Inter}}|}{I_{\text{Chol}}}, \quad (1)$$

where, I_{chol} is the change of current obtained with 7.76 mM cholesterol when compared with current obtained for 0 mM cholesterol concentration and I_{inter} is the change in current corresponding to the mixture of cholesterol and interferents in a 1:1 ratio.

Photometric studies of bioelectrode for enzyme activity measurement

Fig. 6 shows the photometric response of the ChOx/PANI–MWCNT/ITO bioelectrode as a function of cholesterol concentration. The value of absorbance was found to increase with increased cholesterol concentration (1.29–15.51 mM). The results were used to estimate the amount of immobilized enzyme units using the equation $a_{\text{app}}^{\text{enz}}$ (U cm^{-2}) = $AV/\varepsilon ts$, where A is difference in absorbance before and after incubation, V is the total volume (3.14 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 (1 cm^2) of the electrode. The value of immobilized ChOx units was estimated to be 7.5×10^{-3} U cm^{-2}, indicating that 7.5×10^{-3} units of enzyme actively participate in the biochemical reaction.

Thermal, pH, and shelf life studies of bioelectrode

Photometric studies were carried out to find the optimal temperature range, pH, and stability for the bioelectrode. The activity of the ChOx/PANI–MWCNT/ITO bioelectrode was investigated as a function of temperature (15–55 °C). Fig. 7 reveals the bioelectrode to be best active in the range of 30 to 45 °C. The pH studies carried out in the range of 6.0 to 9.0 (data not shown) suggest that bioelectrodes exhibit maximum activity at around pH 7.4.

To investigate the stability of the ChOx/PANI–MWCNT/ITO bioelectrode, photometric experiments were carried out at regular intervals of 1 week for approximately 12 weeks with the cholesterol concentration of 7.76 mM at 30 °C. Studies revealed that the ChOx/PANI–MWCNT/ITO bioelectrode retains more than 95% of activity even after 12 weeks (data not shown).

The results obtained in the current studies, along with those reported in the literature, are summarized in Table 1. The advantage of PANI–MWCNT composite film over the other matrices is clearly visible from this table revealing that the bioelectrode can detect cholesterol over a broad range with high sensitivity. Table 1 indicates that this electrochemically active matrix not only enhances the bioelectrocatalytic oxidation of cholesterol indicated by high sensitivity of 6800 nA mM^{-1} but also exhibits a fast response time of 10 s with better stability of 12 weeks.

Conclusions

We have fabricated the cholesterol biosensor based on electrophoretically deposited PANI–MWCNT composite film onto ITO-coated glass plate. ChOx was covalently immobilized onto PANI–MWCNT film using EDC–NHS chemistry. The results of response measurements carried on this bioelectrode using LSV reveal a detection range of 1.29 to 12.93 mM for cholesterol with high sensitivity of 6800 nA mM^{-1} and a fast response time of 10 s. Photometric studies reveal the stability of the bioelectrode as 12 weeks with the enzyme activity of 7.5×10^{-3} U cm^{-2}. Attempts should be made to use this electrophoretically deposited PANI/MWCNT matrix for the immobilization of other biomolecules.

Acknowledgments

We thank Vikram Kumar, Director of the National Physical Laboratory (New Delhi, India), for the facilities. Chetna Dhand is thankful to the Council of Scientific and Industrial Research (CSIR, India) for the award of a Senior Research Fellowship. We thank B. P. Singh for providing MWCNT. We acknowledge the financial support received from the Department of Science and Technology (DST), Government of India (DST/TSG/ME/2007/06), India–Japan Project (DST/INT/JAP/P-21/07), and Department of Biotechnology, Government of India (DBT/GAP070832).

References

[1] S. Iijima, Helical microtubules of graphitic carbon, Nature 354 (1991) 56–58.
[2] P.M. Ajayan, Nanotubes from carbon, Chem. Rev. 99 (1999) 1787–1799.
[3] M.S. Wang, L.M. Peng, J.Y. Wang, Q. Chen, Electron field emission characteristics and field evaporation of a single carbon nanotube, J. Phys. Chem. B 109 (2005) 110–113.
[4] Q. Zhao, Z. Gan, Q. Zhuang, Electrochemical sensors based on carbon nanotubes, Electroanalysis 14 (2002) 1609–1613.

[5] T. Nishino, T. Ito, Y. Umezawa, Carbon nanotube scanning tunneling microscopy tips for chemically selective imaging, Anal. Chem. 74 (2002) 4275–4278.
[6] P.J. Britto, K.S.V. Santhanam, P.M. Ajayan, Carbon nanotube electrode for oxidation of dopamine, Bioelectrochem. Bioenerg. 41 (1996) 121–125.
[7] S. Hocevar, J. Wang, R.P. Deo, M. Musameh, B. Ogorevc, Carbon nanotube modified microelectrode for enhanced voltammetric detection of dopamine in the presence of ascorbate, Electroanalysis 17 (2005) 417–422.
[8] M. Musameh, J. Wang, A. Merkoci, Y. Lin, Low-potential stable NADH detection at carbon-nanotube-modified glassy carbon electrodes, Electrochem. Commun. 4 (2002) 743–746.
[9] J. Wang, M. Li, Z. Shi, N. Li, Z. Gu, Electrocatalytic oxidation of norepinephrine at a glassy carbon electrode modified with single wall carbon nanotubes, Electroanalysis 14 (2002) 225–230.
[10] J. Wang, M. Li, Z. Shi, N. Li, Z. Gu, Direct electrochemistry of cytochrome *c* at a glassy carbon electrode modified with single-wall carbon nanotubes, Anal. Chem. 74 (2002) 1993–1997.
[11] Z. Wang, J. Liu, Q. Liang, Y. Wang, G. Luo, Carbon nanotube-modified electrodes for the simultaneous determination of dopamine and ascorbic acid, Analyst 127 (2002) 653–658.
[12] M. Guo, J. Chen, J. Li, L. Nie, S. Yao, Carbon nanotubes-based amperometric cholesterol biosensor fabricated through layer-by-layer technique, Electroanalysis 16 (2004) 1992–1998.
[13] G. Li, J.M. Liao, G.Q. Hu, N.Z. Ma, P.J. Wu, Study of carbon nanotube modified biosensor for monitoring total cholesterol in blood, Biosens. Bioelectron. 20 (2005) 2140–2144.
[14] A. Star, J.F. Stoddart, D. Steuerman, M. Diehl, A. Boukai, E.W. Wong, X. Yang, S.W. Chung, H. Choi, J.R. Heath, Preparation and properties of polymer-wrapped single-walled carbon nanotubes, Angew. Chem. Intl. Ed. 40 (2001) 1721–1725.
[15] J. Chen, M.A. Hamon, H. Hu, Y. Chen, A.M. Rao, P.C. Eklund, R.C. Hadden, Solution properties of single-walled carbon nanotubes, Science 282 (1998) 95–98.
[16] P.J. Boul, J. Liu, E.T. Mickelson, C.B. Huffman, L.M. Ericson, I.W. Chiang, K.A. Smith, D.T. Colbert, R.H. Hauge, J.L. Margrave, R.E. Smalley, Reversible sidewall functionalization of buckytubes, Chem. Phys. Lett. 310 (1999) 367–372.
[17] Z. Yang, X. Chen, C. Chen, W. Li, H. Zhang, L. Xu, B. Yi, Noncovalent-wrapped sidewall functionalization of multiwalled carbon nanotubes with polyimide, Polym. Compos. 28 (2007) 36–41.
[18] M.J.O. Connell, P. Poul, L.M. Ericson, C. Huffman, Y. Wang, E. Haroz, C. Kuper, J. Tour, D. Ausman, R.E. Smalley, Reversible water-solubilization of single-walled carbon nanotubes by polymer wrapping, Chem. Phys. Lett. 342 (2001) 265–271.
[19] E. Kymakis, G.A.J. Amaratunga, Single-wall carbon nanotube/conjugated polymer photovoltaic devices, Appl. Phys. Lett. 80 (2002) 112–114.
[20] K.H. An, S.Y. Jeong, H.R. Hwang, Y.H. Lee, Enhanced sensitivity of a gas sensor incorporating single-walled carbon nanotube–polypyrrole nanocomposite, Adv. Mater. 16 (2004) 1005–1009.
[21] K.C. Persaud, P. Pelosi, Sensor arrays using conducting polymers for an artificial nose, in: J.W. Gardner, P.N. Bartlett (Eds.), Sensors and Sensory Systems for an Electronic Nose, Kluwer Academic, Boston, 1992, pp. 237–256.
[22] A.G. MacDiarmid, J.C. Chiang, M. Halpern, W.S. Huag, S. Mu, L.D. Somasiri, "Polyaniline": Interconversion of metallic and insulating forms, Mol. Cryst. Liq. Cryst. 121 (1985) 173–180.
[23] T.A. Skotheim, R.L. Elsenbaumer, J.R. Reynolds, Handbook of Conducting Polymers, Marcel Dekker, New York, 1997.
[24] S.S. Pandey, M.K. Ram, V.K. Srivastava, B.D. Malhotra, Electrical properties of metal (indium)/polyaniline Schottky devices, J. Appl. Polym. Sci. 65 (1997) 2745–2748.
[25] A. Bessiere, C. Duhamelb, J.C. Badot, V. Lucas, M.C. Certiat, Study and optimization of a flexible electrochromic based on polyaniline, Electro. Chim. Acta 49 (2004) 2051–2055.
[26] J. Janata, M. Josowicz, Conducting polymers in electronic chemical sensors, Nat. Mater. 2 (2003) 19–24.
[27] A.F. Diaz, J.A. Logan, Electroactive polyaniline films, J. Electroanal. Chem. 111 (1980) 111–114.
[28] E. Granot, B. Basnar, Z. Cheglakov, E. Katz, I. Willner, Enhanced bioelectrocatalysis using single-walled carbon nanotubes (SWCNTs)/polyaniline hybrid systems in thin-film and microrod structures associated with electrodes, Electroanalysis 18 (2006) 26–34.
[29] J. Liu, S. Tian, W. Knoll, Properties of polyaniline/carbon nanotube multilayer films in neutral solution and their application for stable low-potential detection of reduced β-nicotinamide adenine dinucleotide, Langmuir 21 (2005) 5596–5599.
[30] S.R. Ali, Y. Ma, R.R. Parajuli, Y. Balogun, W.Y.C. Lai, H. He, A nonoxidative sensor based on a self-doped polyaniline/carbon nanotube composite for sensitive and selective detection of the neurotransmitter dopamine, Anal. Chem. 79 (2007) 2583–2587.
[31] C. Dhand, S.P. Singh, S.K. Arya, M. Datta, B.D. Malhotra, Cholesterol biosensor based on electrophoretically deposited conducting polymer film derived from nano-structured polyaniline colloidal suspension, Anal. Chim. Acta 602 (2007) 244–251.
[32] K. Tada, M. Onoda, Nanostructured conjugated polymer films by electrophoretic deposition, Adv. Funct. Mater. 12 (2002) 420–424.
[33] R.B. Mathur, S. Pande, B.P. Singh, T.L. Dhami, Electrical and mechanical properties of multi-walled carbon nanotubes reinforced PMMA and PS composites, Polym. Compos. (2008) doi: 10.1002/pc.20449.
[34] C. Du, N. Pan, High power density supercapacitor electrodes of carbon nanotube films by electrophoretic deposition, Nanotechnology 17 (2006) 5314–5318.
[35] J. Stejskal, R.G. Gilbert, Preparation of a conducting polymer [IUPAC technical report], Polyaniline, Pure Appl. Chem. 74 (2002) 857–867.
[36] C. Dhand, S.K. Arya, S.P. Singh, B.P. Singh, M. Datta, B.D. Malhotra, Preparation of polyaniline/multiwalled carbon nanotube composite by novel electrophoretic route, Carbon 46 (2008) 1727–1735.
[37] Z. Matharu, G. Sumana, S.K. Arya, S.P. Singh, V. Gupta, B.D. Malhotra, Polyaniline Langmuir–Blodgett film based cholesterol biosensor, Langmuir 23 (2007) 13188–13192.
[38] G.K. Kouassi, J. Irudayaraj, G. McCarty, Examination of cholesterol oxidase attachment to magnetic nanoparticles, J. Nanobiotechnol. 3 (2005) doi: 10.1186/1477-3155-3-1.
[39] S. Aravamudhan, A. Kumar, S. Mohapatra, S. Bhansali, Sensitive estimation of total cholesterol in blood using Au nanowires based micro-fluidic platform, Biosens. Bioelectron. 22 (2007) 2289–2294.
[40] A. Parra, E. Casero, F. Pariente, L. Vazquez, E. Lorenzo, Cholesterol oxidase modified gold electrodes as bioanalytical devices, Sens. Actuat. B 124 (2007) 30–37.