Fabrication of nanocrystalline CdS electrode via chemical bath deposition technique for application to cholesterol sensor

Hemant Dhyani\textsuperscript{1}, Saurabh Srivastava\textsuperscript{2}, Md. Azahar Ali\textsuperscript{2}, B.D. Malhotra\textsuperscript{2,3} and Prasenjit Sen\textsuperscript{1}

\textsuperscript{1}School of Physical Sciences, Jawaharlal Nehru University, New Delhi-110067, India
\textsuperscript{2}Department of Science and Technology Centre on Biomolecular Electronics, National Physical Laboratory, Dr. K. S. Krishnan Marg, New Delhi-110012, India
\textsuperscript{3}Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi, 110042, India

E-mail: "Prasenjit Sen" psen.jnu@gmail.com

Abstract. A nanocystalline CdS electrode has been fabricated by chemical bath deposition (CBD) technique onto hydrolyzed indium tin oxide (ITO) coated glass substrate at 78\textdegree{}C for the immobilization of cholesterol oxidase (ChOx). The prepared Nano-CdS based electrode has been characterized using UV-visible, X-ray diffraction (XRD), Fourier transform-infrared (FTIR) and scanning electron microscopy (SEM). The ChOx/Nano-CdS/ITO bioelectrode shows the detection range of cholesterol from 50 to 400 mg/dl with improved sensitivity of 1.35 µA/mgdll\textsuperscript{-1}/cm\textsuperscript{2}, low detection limit (6.1 mg/dl) and low $K_m$ (0.45mM) value indicating strong enzyme (cholesterol oxidase)-matrix (CdS) affinity.

1. Introduction
In recent years semiconductor quantum dots (Qdots) have attracted much interest in the different fields of science due to their unique optical, electronic and electrochemical properties. These properties especially large absorption spectra but narrow emission bands, excellent photostability, high quantum yield and size dependent photoluminescence make them desirable fluorescence probes for the sensing of biological samples [1-4]. Apart from optical sensing, other platforms comprising of quantum dots have recently been explored for the sensing of biological analytes using the electrochemical technique. In this context, it has been reported that the semiconductor Qdots can be used to obtain enhanced charge transfer in enzymatic electrochemical biosensor [5-9]. The Qdots deposited onto desired substrate, have been used for $\text{H}_2\text{O}_2$ estimation [10]. Vastarella et al. have fabricated enzyme-core/shell nanoparticle hybrid material and enzyme/semiconductor nanocluster for the amperometric biosensing [11]. Among the various semiconductors Qdots, the CdS nanocrystals have attracted considerable attention due to their good chemical stability, photo-electrochemistry, intrinsic properties of a narrow band gap and excellent electro-catalysts for the sulfide/polysulfide redox couple [12-14]. The good electron transport behavior of CdS quantum dots due to higher charge detaching efficiency [15] and high enzyme affinity to the hexagonal nanocrystalline surface area has recently been reported for electrochemical sensing [16,17]. There are several methods to deposit the semiconductor CdS onto a substrate such as electrodeposition, vacuum evaporation, photochemical

\textsuperscript{1} To whom any correspondence should be addressed.
deposition, spray pyrolysis, sputtering, Chemical bath deposition (CBD) etc. The CBD technique is an easy process to achieve high quality crystalline films by adjusting the temperature, reagent concentration and pH for large area industrial applications with low cost.

We report results of the studies relating to preparation of nanocrystalline CdS film onto indium tin oxide coated glass substrate by chemical bath deposition (CBD) technique for the immobilization of cholesterol oxidase used for cholesterol estimation.

2. Experimental

2.1. Fabrication of the nano-CdS/ITO electrode

The CdS quantum dots have been prepared and deposited simultaneously onto ITO substrate by chemical bath deposition (CBD) technique reported in literature [18] with some modifications. For the synthesis of CdS, 36.60mg cadmium chloride, 152.24mg thiourea and 1.2gm ammonium nitrate are dissolved separately in 10 ml water. After this we dissolved 701.25mg KOH in 25 ml water and then mixed the cadmium chloride and ammonium nitrate with KOH and raised the temperature of the bath at kept at 78°C with constant stirring. For deposition, the desired area of pre-cleaned ITO substrate is suspended into the bath and mixed with 10 ml solution of thiourea to form CdS. The observed yellowish color indicates formation of nanocrystalline CdS. After about 40 minutes the suspended electrode is taken out, washed with distilled water and stored in a refrigerator.

2.2. Immobilization of ChOx onto nano-CdS/ITO electrode

Cholesterol oxidase is immobilized via physical adsorption onto the surface of prepared Nano-CdS/ITO electrodes. 30 µl of ChOx (1mg/dl) solution is spread onto the Nano-CdS/ITO electrode surface and the electrode is then left inside a humid chamber for about 4h for immobilization of ChOx on electrode surface and stored in 4°C when not in use. The ChOx binds with the CdS quantum dots via electrostatic interaction.

2.3. Instrumentations

Surface morphology and structural properties of electrode (Nano-CdS/ITO) and bioelectrode (ChOx/Nano-CdS/ITO) has been characterized using scanning electron microscopy (SEM, Zeiss EVO 40) and Fourier transform infra-red (FT-IR) spectroscopy (Perkin–Elmer, model “Spectrum BX” using ATR accessory), UV–visible spectrophotometer (Model 2200DPCV, Phoenix) and X-ray diffraction(PAN alytical X’Pert PRO). Electrochemical investigations (CV and EIS) of the prepared electrodes have been carried out using an Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands) in a conventional three-electrode electrochemical cell consisting of Ag/AgCl as reference electrode and platinum foil as the counter electrode. Electrochemical impedance spectroscopy (EIS) studies have been performed in the frequency range, 0.01–10⁷ Hz with amplitude of 5 mV in PBS solution.

3. Results and discussion

3.1. UV-visible and X-ray diffraction

The UV-visible absorption spectra of the Nano-CdS/ITO electrode is shown in Fig. 1(a). the characteristic absorption peak assigned to the first excitonic state of the CdS electrode is located at 450 nm with the broad range of 425-500 nm. The blue-shift is observed for the absorption band of Nano-CdS as compared to the 520nm of the bulk CdS. This indicates quantum confinement effect and presence of CdS nanoparticles/crystallites in the fabricated electrode[19]. The X-ray diffraction (XRD) pattern of the Nano-CdS/ITO electrode is shown in the Fig.1(b). The XRD pattern indicates polycrystalline nature of the Nano-CdS film deposited onto ITO glass. The CdS exists in two crystallographic forms, hexagonal (Wurtzite) and cubic (Zincblend). The presence of the peaks with 2θ values at 30.55°, 45.56° and 50.93° may be associated with cubic planes (111), (220) and (311) or with the hexagonal lattice planes (101), (110) and (200), respectively. The extra peaks seen at 35.80
and 60.6 are due to the ITO substrates. The average grain size (D) of the CdS is found to be as 12 nm, calculated using Scherrer formula $D=\frac{0.94\lambda}{\beta \cos \theta}$, where $\lambda$ is the X-ray wavelength, $\beta$ is the full width at half maximum and $\theta$ is the Bragg angle.

Figure 1. (a) UV-Visible spectra of Nano-CdS/ITO electrode & (b) XRD pattern of Nano-CdS/ITO.

3.2. Scanning electron microscopy studies
The scanning electron micrographs (SEM) of the Nano-CdS/ITO electrode shows the uniform and continuous crystallization during the film formation (Fig. 2(a)). The homogeneous crystalline granular and nanoporous structure of CdS with size distribution of 50–100 nm can be seen on the ITO surface. The SEM image of the cholesterol oxidase (ChOx) immobilized onto the electrode surface Fig. 2(b) demonstrates the covered and packed granular structure indicating immobilization of ChOx via electrostatic interactions.

Figure 2. (a) SEM micrograph of Nano-CdS/ITO electrode & (b) ChOx/Nano-CdS/ITO bioelectrode.

3.3. Fourier transform infra-red spectroscopy studies
FT-IR spectra of Nano-CdS/ITO electrode is shown in Fig. 3(a). The peak seen at 671 cm$^{-1}$ in the fingerprint region, corresponds to Cd-S vibrational band. The peaks observed at 1187 cm$^{-1}$, 1363 cm$^{-1}$ and 1450 cm$^{-1}$ are assigned to O-C=O (symmetric and asymmetric stretching band) due to strong physical absorption of CO$_2$ and H$_2$O on the electrode surface. The spectra [Fig. 3(b)] shows the broad
peak at 1100 cm$^{-1}$ confirms the immobilization of ChOx with the peak found at 1630 cm$^{-1}$ is due to amide bonds showing the presence of ChOx[20].

Figure 3. FT-IR spectra of (a) Nano-Cds/ITO electrode and (b) ChOx/Nano-Cds/ITO bioelectrode

3.4. Cyclic voltammetry studies

![Figure 4](image1.png)  
**Figure 4.** Cyclic Voltammetric (CV) of bare ITO (a), Nano-Cds/ITO electrode (b) and ChOx/Nano-Cds/ITO bioelectrode at scan rate 20 mV/s in PBS (50mM, pH 7.0, 0.9% NaCl) containing $[\text{Fe(CN)}_6]^{3-/4-}$ (5mM).

![Figure 5](image2.png)  
**Figure 5.** Cyclic Voltammetric (CV) of ChOx/Nano-Cds/ITO bioelectrode at different scan rate 30-100 mV/s in PBS containing $[\text{Fe(CN)}_6]^{3-/4-}$ (5mM).

Fig. 4 shows results of cyclic voltammetric (CV) studies conducted on bare ITO (a), Nano-Cds/ITO electrode (b) and ChOx/Nano-Cds/ITO bioelectrode (c) at scan rate 20 mV/s with potential range -7.0V to +7.0V in PBS (50mM, pH 7.0, 0.9% NaCl) containing $[\text{Fe(CN)}_6]^{3-/4-}$ (5mM). It can be seen that the magnitude of the peak current for Nano-Cds/ITO electrode (3.29×10$^{-4}$A) decreases as compared to bare ITO (5.17×10$^{-4}$A) and the peak is shifted toward the higher potential. This is due to
the semiconducting CdS that obstructs the electron transfer between the CdS QDs electrode and the solution. The peak current is found to be higher for ChOx/Nano-CdS/ITO bioelectrode (c) as compared to that of the Nano-CdS/ITO electrode (b) indicating that Nano-CdS quantum dots perhaps act as mediator resulting in enhanced electron transfer between enzyme and electrode.

Fig. 5 shows cyclic voltammograms obtained for ChOx/Nano-CdS/ITO bioelectrode in PBS containing $[Fe(CN)_{6}]^{3−/4−}$ recorded at different scan rates (30–100mVs$^{−1}$). It can be seen that the anodic potential shifts towards positive side and the cathodic peak potential (Fig.6A) shifts in the reverse direction. The peak-to-peak separation potential $ΔE ~0.37$ V increases with increasing scan rate resulting in uniform facile charge transfer kinetics. The magnitude of current of both anodic peak (I$_a$) and cathodic peak (I$_c$) observed for ChOx/Nano-CdS/ITO bioelectrode increases linearly with the scan rate indicating quasi reversible diffusion controlled behavior. The values of the slope, intercept and correlation coefficient given as

$I_a [\text{ChOx/Nano-CdS/ITO}] = -1.3μA + 0.8μA (s/mV) \times \text{scan rate (mV/s)}$ with $r^2=0.993$ (Eq.1)

$I_c [\text{ChOx/Nano-CdS/ITO}] = -0.002μA -0.35μA (s/mV) \times \text{scan rate (mV/s)}$ with $r^2=0.998$ (Eq.2)

3.5. Cyclic voltammetry studies

![Figure 6](image6.png)

**Figure 6.** (A) Anodic peak current and cathodic peak current vs square root of scan rate of ChOx/Nano-CdS/ITO bioelectrode from CV studies, (B) CV response at different cholesterol concentration (50-400 mg/dl) at scan rate 20 mV/s in PBS (50mM, pH 7.0, 0.9% NaCl) containing $[Fe(CN)_{6}]^{3−/4−}$ (5mM) and (C) Sensor response curve plot between current vs cholesterol concentration.
The results of response studies of the ChOx/Nano-CdS/ITO bioelectrode for different cholesterol concentrations in phosphate buffer (50mM, pH 7.0, 0.9% NaCl) containing $[\text{Fe(CN)}_6]^{3-/4-}$ are shown in Fig. 6(B). The peak current of CV response increases with adding cholesterol indicating increase in the $\text{H}_2\text{O}_2$ concentration produced due to the interaction of ChOx with cholesterol during the biochemical reaction [Fig. 6C]. The ChOx/Nano-CdS/ITO bioelectrode shows linearity as 50–400 mgdl$^{-1}$ and the detection limit as 6.1 mg dl$^{-1}$. This biosensor shows high sensitivity as 1.35 $\mu$A/mgdl$^{-1}$/cm$^2$ with linear regression ($r^2$) 0.950. The Michaelis–Menten constant ($K_m$), that gives an indication of the enzyme-substrate kinetics, can be obtained from the Lineweaver–Burke plot. The $K_m$ value for the ChOx/Nano-CdS/ITO bioelectrode has been calculated using the formula $3\times \text{SD}/m$, where SD is standard deviation and m is the slope of the curve and it is found to be 17.0 mgdl$^{-1}$ indicating high affinity for cholesterol.

3.6. Effect of interferents of the ChOx/Nano-CdS/ITO bioelectrode

The effect of interferents (lactic acid, ascorbic acid, uric acid, glucose and urea) on the response of this cholesterol sensor has been evaluated by adding the solution containing (1:1) ratio of cholesterol (100mgdl$^{-1}$) and interferents such as glucose (5 mM), ascorbic acid (0.05 mM), uric acid (0.1 mM), lactic acid (0.5mM) and urea (1 mM) (data not shown). The results indicate negligible effect of these interferents on the CV response of ChOx/Nano-CdS /ITO bioelectrode.

4. Conclusions

We have fabricated the nanocrystalline CdS electrode using chemical bath deposition technique. The UV-Visible and X-ray diffraction studies confirms that the quantum confinement effect during the formation of CdS favors its deposition as homogeneous nanocrystals and helps in ChOx loading. FT-IR and SEM studies reveal the successful immobilization of ChOx onto Nano-CdS/ITO electrode surface. The ChOx/Nano-CdS/ITO bioelectrode exhibits linear range of 50–400 mg/dl and improved sensitivity 1.35 $\mu$A/mgdl$^{-1}$/cm$^2$. It has been found that the CdS Qdots based bioelectrode shows the detection limit of 6.1 mg/dl and low $K_m$(0.45mM) value indicating strong enzyme-matrix affinity. Efforts should be made to control the shape and size of Nano-CdS and to utilize this electrode for estimation of total cholesterol, triglycerides and low density lipoproteins (LDL) etc.

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