Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Mediator-Free Biosensor Using Chitosan Capped CdS Quantum Dots for Detection of Total Cholesterol

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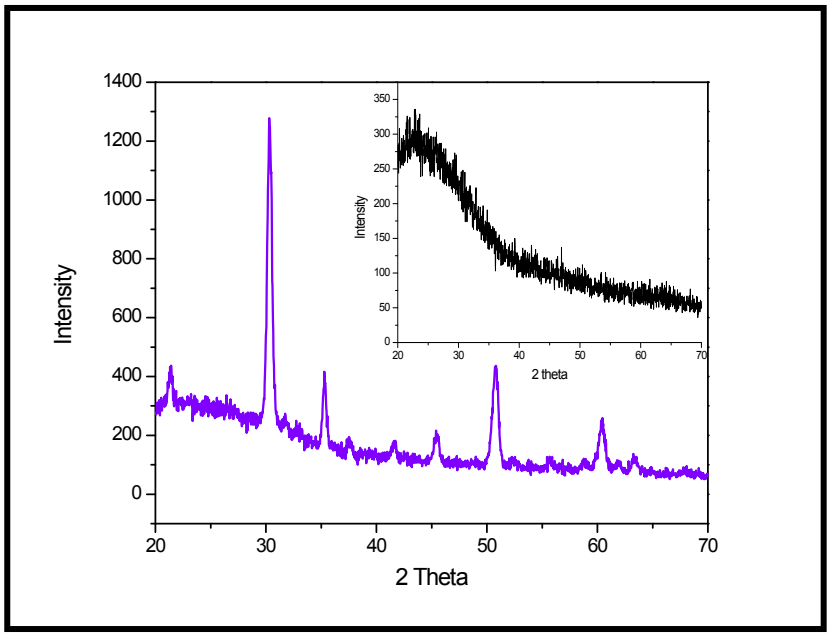

Figure S1: XRD pattern of Chitosan film (inset) and CHIT-CdS (QDs) composite film.

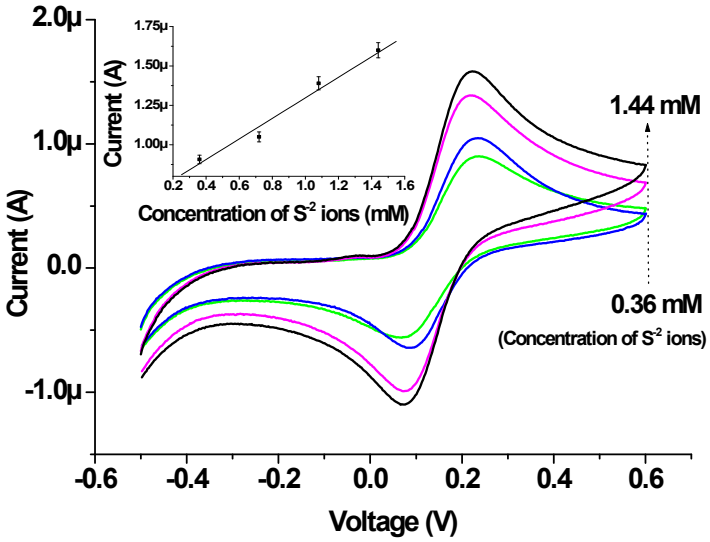

Figure S2:CVof the ChEt-ChOx/CHIT-CdS/ITO as a function of S^{-2} ions (inset: anodic current vsconcentration of S^{-2} ions)

EIS measurements carried out for variously modified electrodesare shown in Fig.S3in the frequency range 0.0–$5\times10^{3}\Omega$ in PBS containing ferro/ferricynide. The modified electrodes impedance can be presented as the sum of the real (Z'), and imaginary ($-Z''$) components that originate mainly from the resistance and capacitance of the cell, respectively. It shows the Faradaic impedance spectra, presented as Nyquist plots obtained from real (Z'') and imaginary ($-Z''$) components of CHIT/ITO (curve; green), ChEt-ChOx/CHIT/ITO (dark yellow), CHIT-CdS/ITO (blue) and ChEt-ChOx/CdS-CHIT/ITO (red) electrodes.

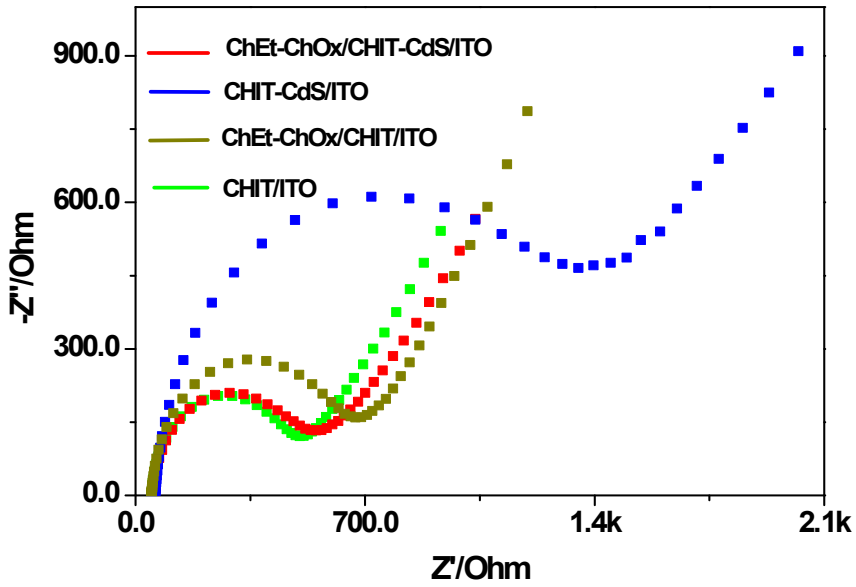

Figure S3-Impedance spectra of CHIT/ITO(green),ChEt-ChOx/CHIT/ITO (dark yellow), CHIT-CdS/ITO (blue), and ChEt-ChOx/CHIT-CdS/ITO (red) in PBS (7.4 pH) containing $[Fe(CN)_6]^{3-/4-}$ as a redox species.

The charge-transfer resistance (R_{ct}), depends on the insulating features at the electrode/electrolyte interface. The values of R_{ct} derived from the diameter of semicircle of impedance spectra are obtained as $4.35\times10^{+2}$ Ω for chitosan modified ITO electrode (CHIT/ITO) which is lower compared to that of CHIT-CdS/ITO electrode (1.30kΩ). This is due to the incorporation CdS quantum dots which provide insulating/semiconducting surfaces that enhance the resistance properties of the film. In the case of ChEt-ChOx/CHIT/ITO electrode, the R_{ct} value increases due to enzyme's intrinsic insulating property which increases film resistance. However, after incorporation ChEt-ChOx onto CHIT-CdS/ITO electrode, the value R_{ct} is found to be $4.51\times10^{+2}\Omega$. The low R_{ct} value of this ChEt-ChOx/CHIT-CdS/ITO as compared to CHIT-CdS/ITO electrode further confirms that CdS has favorable orientation with enzymes active sites for electron transportation between solution and electrode interface.

Figure S4 demonstrates typical CV of ChEt-ChOx/CdS-CHIT/ITO bioelectrode with scan rate varying from 30 to 90 mVs^{-1} in PBS. With increase in the scan rate, there is increase in both the cathodic and anodic peak currents accompanied with small shift and increased peak-to-peak separation. Inset to Fig.S4 shows the cathodic and anodic peak currents,linear dependence with the scan rate from 30 to 90mVs^{-1} indicating a surface controlled diffusion and quasi-reversible process.This reveals that the electron transfer between enzyme and electrode could be easily performed and it was a surface confined electrochemical process. The values of the slope, intercept and correlation coefficient given in the inset to Fig.S4.The surface concentration of ChEt-ChOx/CdS-CHIT/ITO bioelectrode have been estimated from the plot of peak current vs potential using the equation (Brown–Anson model) as

$$I_p = n^2F^2I^*Av/4RT \qquad (1)$$

Where n is the number of electrons transferred, $F$is the Faraday constant (96,584 C/mol), I^* is the surface concentration (mol/cm^2), A is surface area of the electrode (0.25 cm^2), v is the scan rate (20 mV/s), $R$is gas constant [8.314 J/(mol K)], and T is absolute temperature (298 K). The values of surface concentration for ChEt-ChOx/CdS-CHIT/ITO bioelectrodehave been found to be as 5.6 × 10^{-7}mol/cm^2.

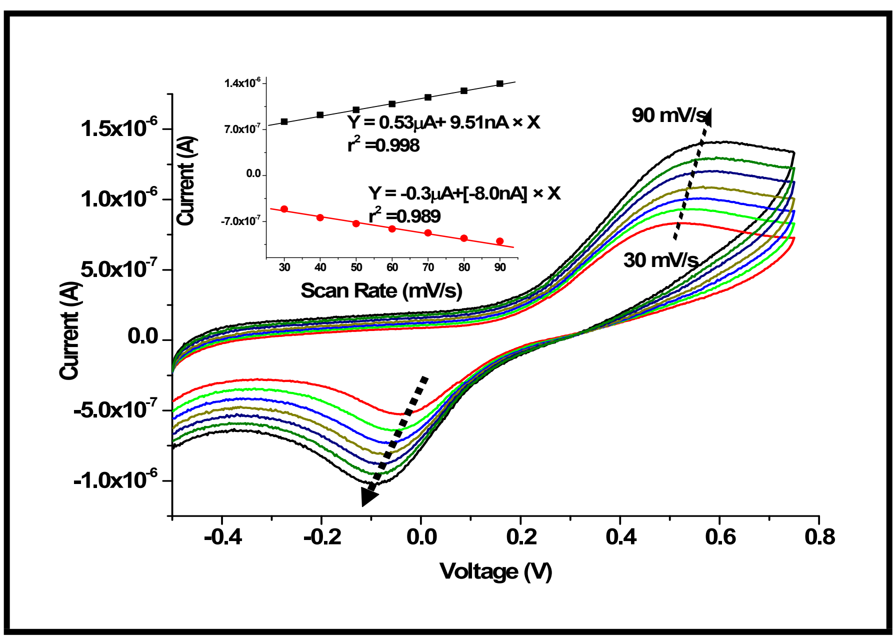

Figure S4: Cyclic voltammetric (CV) of ChEt-ChOx/CHIT-CdS/ITO bioelectrode at different scan rate 30-90 mV/s in PBS (50mM, pH7.4, 0.9% NaCl) solution.

Table: Response of cholesterol sensor reported in present work compared to those reported in literature.

Matrix of Nanomaterials	Immobilization method	Linearity	Sensitivity	K_m Value	Reference
Fe Nanoparticle	ChOx, Covalent	50-200 mg/dl		0.45 mM	[1]
CHIT-MWCNT	ChOx, Entrapment		59.93 µA/mg/dl	0.24 mM	[2]
Ti-Au Nanoparticles	ChEt-HRP-ChOx, Physical adsorption	0.97-7.8 mM	29.33µA/mM	0.64 mM	[3]
Au Nanowaire	ChOx-ChEt, covalent	0.01-0.060 mM	0.85µA/mM	17.1 mM	[4]
Pt-Au/ZnO	ChOx, physical adsorption	0.1-759.3 µM	26.8 µA/mM	1.84 mM	[5]
CHIT-SnO_2	ChOx, physical adsorption	0.26-10.36 mM	34.7 µA/mg/dl	3.8 mM	[6]
NiO-CHIT	physical adsorption	10-400 mg/dl	0.808µA/mg/dl	0.67 mM	[7]
CHIT-CdS QDs	ChEt-ChOx, Covalent	1.29-12.93 mM	0.384 µA/mM	0.39 mM	Present work

References:

[1] G. K.Kouassi, J.Irudayaraj,G.McCarty, *J. Nanobiotechnol.***2005**,*3*, 1.

[2]X.Tan,M. Li, P.Cai,L.Luo,X.Zou,*Anal. Biochem.***2005**,*337*, 111.

[3] A. Ahmadalinezhad, A.Chen,*Biosens.Bioelectron.***2011**,*26*, 4508.

[4] S.Aravamudhan, N.S.Ramgir,S.Bhansali,*Sens. Actuators B***2007**,*127*, 29

[5] C. Wang, X. Tan, S. Chen, R. Yuan, F. Hu, D. Yuan, Y.Xiang,*Talanta***2012**,94, 263– 270

[6] A. A. Ansari, A. Kaushik, P. R. Solanki, B. D. Malhotra, *Electroanalysis***2009**,21, 965-972

[7] J. Singh, P. Kalita, M. K. Singh, B. D. Malhotra, *Appl. Phys. Lett.***2011**,98, 123702