

## Development of a cholesterol biosensor based on polyaniline-Ag nanocomposites

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In the present study, chronoamperometry was employed to prepare polyaniline- p- toluene sulphonic acid- silver (PANI-pTSA-Ag) nanocomposite film on indium tin oxide (ITO) coated glass plate for covalent immobilization of cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) via N-ethyl-N'- (3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to fabricate a cholesterol bioelectrode (ChEt-ChOx/ PANI-pTSA-Ag /ITO). The scanning electron microscopy (SEM), atomic force microscopy (AFM) and electrochemical techniques were employed for characterizing PANI-pTSA-Ag /ITO electrode and ChEt-ChOx/ PANI-pTSA-Ag /ITO bioelectrode. We observed the high sensitivity of 36.3 $\mu$ A/ mg/dl with a fast response time of 10 seconds in the range of 20 to 400 mg/dl for the bioelectrode. The linear regression analysis of bioelectrode revealed standard deviation and correlation coefficient of 11.6  $\mu$ A and 0.992, respectively. The low value of Michaelis-Menten constant ( $K_m$ ) estimated as 6.99mg/dl (0.107mM) indicates high affinity of cholesterol esterase and cholesterol oxidase enzymes to cholesterol. We observed that the ChEt-ChOx/ PANI-pTSA-Ag /ITO bioelectrode exhibits uniform activity for 50 days under refrigerated conditions. Attempts have been made to utilize this electrode for estimation of total cholesterol in blood serum samples.

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