Title of Ph. D. thesis: Construction and evaluation of a creatinine biosensor based on c-MWCNT/PANI composite film coated Pt electrode.

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ABSTRACT

The the present work was aim to construct a highly sensitive electrochemical amperometric biosensor used for determination of creatinine in serum and urine, based on of covalent co-immobilization of creatinine amidohydrolase (CA), creatine amidinohydrolase (CI) and sarcosine oxidase (SO) onto c-MWCNT/PANI composite film electrodeposited on the surface of Pt electrode using N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxy succinimide (NHS) chemistry.

A creatinine biosensor was fabricated using Enzymes/c-MWCNT/PANI/Pt as working electrode, Ag/AgCl as reference electrode, and Pt wire as auxiliary electrode connected through potentiostat. The optimal current response was obtained at 0.2 V, and hence 0.2 V was selected as the optimum working potential for all amperometric determination of creatinine. The enzyme electrode was characterized by scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and electrochemical impedance spectroscopy (EIS).

The optimum pH and temperature of present enzyme electrode was 7.5 and 35 °C respectively. The biosensor detected creatinine levels as low as 0.1 µM, estimated at a signal-to-noise ratio of 3, within 5 s. The optimized biosensor showed a linear response range of 10 to 750 µM creatinine with sensitivity of 40 µA/mM/cm^2. A Lineweaver–Burk plot gave the apparent K_m value of 0.26 mM for immobilized enzymes. The method showed a good reproducibility as the analytical recoveries of added creatinine (0.5 mg/dl and 1.0 mg/dl) in serum sample were 98.47 % and 97.91 %. The results of within- and between-batch coefficients of variation (CVs) for serum creatinine determination were less than 3.6 % and 4.1 %, respectively, showing a good repeatability of the method. A good correlation (r = 0.989) was obtained between present biosensor and standard chemical spectrophotometric method for measurement of creatinine. Among the various serum metabolites and metal ions at their physiological concentrations, none had practically any significant interference except Cd^{+2} and Ni^{+2} ions, which caused a slight stimulation in biosensor response. The fabricated biosensor was successfully employed for determination of creatinine in human serum. The biosensor maintained 85 % of the initial activity after 180 days of regular 150 uses, when stored dry at 4 °C.