ABSTRACT

DESIGN AND OPTIMIZATION OF AN AMPEROMETRIC HORSERADISH PEROXIDASE(HRP) BIOSENSOR FOR CARBAMATE ANALYSIS

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The study presented in this thesis focused on the design and optimization of a horseradish peroxidase (HRP) biosensor for the detection and quantification of four carbamate pesticides in wastewater streams.

Glassy Carbon Electrode (GCE) peroxidase biosensors were prepared using three different approaches. In one method, sensors containing the ferrocenemonocarboxylic acid (FcCOOH) mediator was physically entrapped within the enzyme matrix. This was done by adding glutaraldehyde solution to the peroxidase enzyme mixed with bovine serum albumin (BSA) and the ferrocenemonocarboxylic acid mediator. Biosensors containing glutaraldehyde, HRP and BSA were also prepared, but with the mediator dissolved in the solvent medium, rather than entrapped in the biosensor film. The third method involved covalent attachment of the ferrocenemonocarboxylic acid mediator to the enzyme, followed by entrapment of the complex in the sensor film.

All biosensors were optimized with respect to pH, solvent composition, mediator concentration and enzyme content, electrode potential, film thickness and stirring speed. Calibration curves for optimized biosensors utilizing FcCOOH in solution were found to be linear over a hydrogen peroxide (H_2O_2) concentration range of $0.125 \times 10^{-5} - 1.0 \times 10^{-3}$ M with a detection limit of 0.20×10^{-5} M. The sensitivity of the sensor was found to be 12.76×10^{3} μA M^{-1} with response times to changes in H_2O_2 concentration ranging between 15 – 20 seconds. These sensors also demonstrated a high degree of usefulness after 20 days with an overall decline in catalytic response of approximately 44%.

Calibration curves for optimized biosensors containing FcCOOH covalently attached to the peroxidase enzyme were found to be linear over a H_2O_2 concentration range of 2.5×10^{-5} - 4.0×10^{-3} M with a detection limit of 0.36×10^{-3} M. The sensitivity of the sensor was found to be 55.22×10^{7} μA M^{-1} . 'Single use' biosensors showed a gradual decrease in amperometric response falling to 52% of its original value after 24 days. Biosensors used repeatedly over a 30 day period showed a 50% decline in their catalytic response after 21 days of storage.

All biosensors constructed, were used to examine their efficiency and accuracy of analysis by monitoring the inhibitory effects of carbamate inhibitors on the catalytic efficiency of the peroxidase enzyme.

Biosensors utilizing FcCOOH in solution exibited detection limits for both carbaryl and methiocarb of 2.67nM and 2.46 nM respectively, almost five times lower than that for carbaryl utilizing immobilized cholinesterase.

All biosensors demonstrated good detection limits for all inhibitors investigated. For example, the detection limits for both carbaryl and methiocarb were 15.27 nM 13.57 nM respectively for biosensors containing ferrocene chemically attached to the peroxidase enzyme. The detection limits were improved when the two inhibitors were investigated using biosensors in which the ferrocene mediator was dissolved in solution. Here, the detection limits were 2.67 nM and 2.46 nM for carbaryl and methiocarb respectively. In investigating the effects of sample matrix on the catalytic response of the biosensors, water obtained from the mouth of the Caroni Arena Water Treatment Plant catchment was spiked with different concentrations of methiocarb. Water from the Santa Cruz river and chlorinated tap water were also analyzed, against standard deionised water. The results did indicate however, that waters from the mouth of the catchment did have the most significant effect (13% reduction) on the electrochemical response of the peroxidase biosensors.

Keywords: Horseradish peroxidase; Glassy Carbon Electrode; Biosensor; Ferrocenemonocarboxylic acid; Glutaraldehyde; Bovine Serum Albumin; carbamates.