A Reagent-less Enzymatic Amperometric Alcohol Biosensor Using the Vertically Aligned Carbon Nanofiber (VACNF)

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A reagentless amperometric enzymatic biosensor was constructed on a carbon substrate for the detection of ethanol. Yeast alcohol dehydrogenase (YADH), an oxidoreductase, and its cofactor nicotinamide adenine dinucleotide (NAD⁺) were immobilized by adsorption and covalent attachment to the carbon substrate. Carbon nanofibers grown by plasma enhance chemical vapor deposition (PECVD) were chosen as the electrode material due to their excellent structural and electrical properties.

Electrochemical techniques were employed to test the function and performance of the constructed biosensor. Characterization of the electrode was performed using NADH. This allowed the function of the electrode to be examined as well as to determine the oxidation peak potential of NADH. Subsequently, amperometric measurements were conducted on for the detection of ethanol, methanol, and isopropanol to determine the response in electrical current as a result of an increase in analyte concentration. The storage stability, reusability, and response time of the biosensor was also examined.

The cyclic voltammogram of NADH pictured in Figure 1, shows the oxidation peak is at 0.75V. Figure 2 illustrates the amperometric measurements obtained from electrodes on which the enzyme is covalently attached and adsorbed, respectively. Steady state is achieved in six seconds for both biosensors.

The linear concentration range for the modified electrode used in these experiments with the enzyme adsorbed to the surface is approximately 1.75 mM to 6 mM and with the enzyme covalently attached to the surface is approximately 2 mM to 8 mM. Studies of other alcohol biosensors have displayed concentration ranges of 45 µM – 4 mM for a carbon paste electrode [1] and 1.5 M to 8.5 M for a multi-walled carbon nanotube electrode [2].

Figure 1 Cyclic voltammetry of 1mM NADH with the unmodified carbon nanofiber electrode.

Figure 2. (a) Amperometric measurements of ethanol with modified carbon nanofiber electrode (covalent attachment). (b) Amperometric measurements of ethanol using modified carbon nanofiber electrode (adsorption).
