STUDIES ON AMPEROMETRIC SENSORS

by

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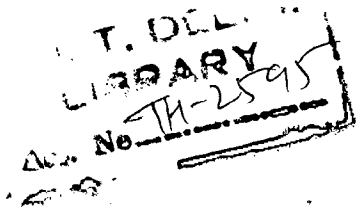

CERTIFICATE

This is to certify that the thesis entitled **"Studies on Amperometric Sensors"** being submitted by **Jyotsna Sharma** to the **Indian Institute of Technology, Delhi** for the award of the degree of **Doctor of Philosophy** in **Chemistry** is a record of bonafide research work carried out by her. She has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to me knowledge, has reached the requisite standard.

The results contained in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

Dated: 22 June '98

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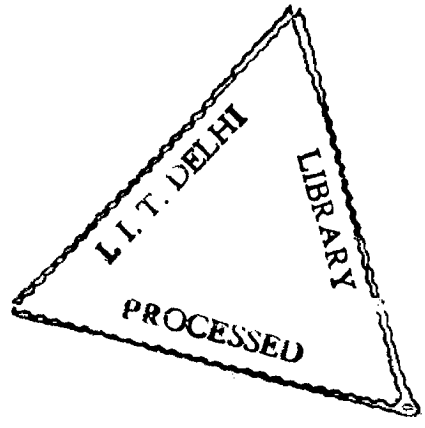
In this work as in life, whatever is beneficial comes from the Almighty, and so, in all humility, I dedicate it at the Lotus Feet!

Jyotsna

(JYOTSNA SHARMA)



ABSTRACT

A biosensor is a device incorporating a biological sensing element (*viz.* enzymes, antibodies, nucleic acids etc.) either intimately connected to or integrated within a transducer. The transducer converts the chemical signal to an easily processible analytical signal. With an electrode as a transducer, biosensors may be classified as- potentiometric and amperometric. The investigations reported in this thesis are based on amperometric measurements.

Amperometric biosensors (also called enzyme electrodes) typically involve oxidoreductase enzymes that use either O_2 (oxidases) or $NADH/NAD^+$ (dehydrogenases) as electron acceptors. While direct electrochemistry of enzymes is hard to achieve on conventional electrode surfaces, electron transfer mediators (redox couples that effectively shuttle electrons between the enzyme active site and the electrode) have provided a route to establishing electrical communication between the enzymes and electrode surfaces.

The objective of the work reported in this thesis was to examine the utility of p-benzoquinone (BQ) as a mediator in various substrate-enzyme reactions.

Detailed cyclic voltammetric studies have shown that a pyrolytic graphite electrode on which both BQ and glucose oxidase (GOX) were immobilized functioned as a good sensor for glucose. Glucose could be linearly detected in the range 1-50 mM.

Organic sulphur compounds adsorb on gold surfaces to form self-assembled monolayers (SAM) which are expected to be useful in designing biosensors wherein enzymes could be immobilized. A gold electrode modified with a SAM of bis(4-pyridyl) disulphide (PySSPy) was used to incorporate GOX through glutaraldehyde coupling. By incorporating ferrocenecarboxylic acid (FCA) as a mediator, glucose could be detected in the range 1-25 mM. Kinetic data on the reaction between FCA and GOX has been obtained from cyclic voltammetry.

In order to examine whether BQ will be effective as a mediator for another enzyme, investigations have been carried out with D-amino acid oxidase using D-lysine as the substrate. By immobilizing both the enzyme and the mediator at a glassy carbon electrode, catalytic currents were observed and D-lysine could be determined in the range 1-10 mM. Kinetic data has been reported.

The electrochemical oxidation of NADH is of interest in the development of amperometric biosensors for NAD^+ dependent dehydrogenases. The oxidation occurs at high overpotentials and lowering of this overpotential is necessary for the development of sensors based on dehydrogenase enzymes. A pyrolytic graphite electrode and a glassy carbon electrode modified with BQ have been found to oxidize NADH electrocatalytically at considerably lower potentials. The rate constant for the reaction between NADH and BQ has been determined in both the cases.

Efforts to develop a sensor for ethanol using alcohol dehydrogenase (ADH) have been made. Homogeneous solution studies with FCA and BQ as mediators have shown that electrocatalytic oxidation of ethanol occurred via a mediated mechanism. Ethanol was detected in the range 1-10 mM. The sensor investigations have been further extended by entrapping ADH and BQ in a conducting polymer, polypyrrole. The catalytic current was linearly related to the ethanol concentration in the range 1-8 mM.

The direct electrochemistry of ADH also yields a significant information for the development of an ethanol sensor. Detailed cyclic voltammetric studies on ADH at an edge plane pyrolytic graphite electrode and a gold electrode modified with PySSPy have shown evidence for the presence of cytochrome c. Addition of ethanol has been found to give rise to catalytic currents typical of a mediated electrochemical reaction. This provides a novel method of sensing ethanol without employing an external mediator.

It was considered interesting to examine whether BQ would mediate the oxidation of L-ascorbic acid (AH_2). The electrocatalytic oxidation of AH_2 has been examined at a BQ modified pyrolytic graphite electrode as well as in solution using BQ as a mediator. The second order rate constant for the reaction between BQ and AH_2 has been determined in both the cases.

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