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**THE DEVELOPMENT OF AMPEROMETRIC
BIOSENSORS FOR THE DETECTION OF
GLUCOSE, LACTATE AND ETHANOL**

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Abstract

Amperometric biosensors, also commonly known as enzyme sensors or enzyme electrodes, are a growing and very progressive area of research. Biosensors are analytical devices that contain a biological sensing element connected to a physical transducing element. The physical transducer "senses" the change in the biological element as it undergoes a chemical reaction. The physical transducer then converts chemical equivalents from the enzyme reaction in a dependent relationship to electrical equivalents that can be measured. Biosensors combine the power of electrochemistry with the specificity of enzymes to produce sensors that are specific to particular enzyme substrates. Some have wide specificities and others are quite narrow. Considering the wide range of enzymes available, the choice depends on the end use of these sensors.

The aim of the current study was to design biosensors for the detection of glucose, lactate and ethanol. The method for attaching enzymes to electrodes was based on the carbodiimide method. The carbodiimide method activates haeme which then is able to be covalently attached to enzymes. Enzyme-haeme conjugates were then allowed to absorb onto platinum electrodes by exploiting the knowledge that haeme can bind irreversibly to platinum by sharing *pi*-electrons with the *d*-orbitals of platinum. The enzymes involved were glucose oxidase, lactate dehydrogenase and alcohol dehydrogenase.

The use of flow injection analysis for evaluating biosensors was described and was found to be a fast, efficient method and the results were highly reproducible. In testing electrodes, the results of the present study showed it was possible to obtain current response that was dependent on the concentration of substrate when these enzyme electrodes were used. A particularly significant result in this study was the achievement of current responses that were dependent on substrate concentration in the absence of NAD^+ for lactate and alcohol dehydrogenases using the substrates lactate and ethanol respectively. There is however much work to be done to improve the success rate of making these enzyme electrodes. Several factors were found to cause variable results whilst making and using these enzyme electrodes, such as the absorption of unbound enzyme to the sensing surface of the electrode that may produce significant current response, the formation of aggregated haeme during the enzyme-haeme conjugation process and most importantly, and the ability to make successful enzyme-haeme conjugates to be absorbed onto the sensing surface of the electrodes.

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Abbreviations

ADH	Alcohol dehydrogenase
AO	Alcohol oxidase
BSA	Bovine serum albumin
CV	Cyclic voltammogram
DMF	Dimethyl formamide
DCC	Dicyclohexylcarbodiimide
GO	Glucose oxidase
HS	6-hydroxysuccinimide
LDH	Lactate dehydrogenase
LOD	Lactate oxidase
NAD ⁺	β -nicotinimide adenine dinucleotide
NADH	β -nicotinimide adenine dinucleotide (reduced form)
Pt	Platinum
R.O. water	Reverse osmosis water
TLC plates	Thin layer chromatography plates