



Polyvinylferrocenium modified Pt electrode for anaerobic glucose monitoring

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Abstract

An amperometric enzyme electrode for the determination of glucose under anaerobic solution conditions was developed by immobilizing glucose oxidase and then by adsorbing ferrocene in polyvinylferrocenium matrix coated on a Pt electrode surface. The amperometric response due to the electrooxidation of ferrocene that the reduced flavin adenine dinucleotide centers of glucose oxidase was measured at a constant potential. The response characteristics of the enzyme electrode were investigated. The effects of the thickness of the polymeric film, the amount of the enzyme immobilized, the amount of the mediator, the glucose concentration, the applied potential, operating pH and temperature on the response of the enzyme electrode were studied. The response time and the optimum pH were found to be 30–40 s and pH 7.4 at 25 °C, respectively. The linear response was observed up to 5.0 mM glucose concentration that the produced detectable current was 0.0075 mM glucose concentration. The activation energy (E_a) of immobilized enzyme reaction was calculated to be 41.3 kJ mol⁻¹ from the Arrhenius plot. The apparent Michaelis-Menten constant (K_{Mapp}) was found to be 6.05 mM glucose according to the Lineweaver-Burk graph of the Michaelis-Menten equation under the optimum conditions. The interference signal due to the most common electrochemical interfering species was also evaluated.

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Keywords: Polyvinylferrocenium; Ferrocene; Glucose; Enzyme electrode

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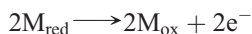
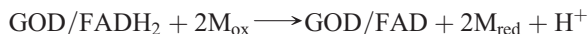
1. Introduction

Enzyme electrodes are becoming popular for the determination of specific substrates in the clinical and industrial analysis. The rapid development of enzyme immobilization technology has assisted further development in this analytical field. The combination of immobilized enzymes with electrochemical sensors has produced many low-cost devices that are rapid and sensitive to use.

The determination of glucose is important in food and fermentation industry and in clinical chemistry, and there have been many papers on this subject. Most were based on the reaction of glucose in the presence of the enzyme, glucose oxidase (GOD), according to:



Either the production of hydrogen peroxide or the consumption of the oxygen is measured at the electrode surface and related to the sample glucose concentration through a previously prepared calibration curve [1–3]. There are some disadvantages with this kind of glucose biosensor in vitro monitoring in whole blood and plasma; variations in the level of dissolved oxygen may cause fluctuations in the electrode response, and the dynamic range of dissolved oxygen. Another drawback of such conventional glucose biosensor is that the hydrogen peroxide generated is harmful to GOD and thus limits sensor performance. Many of these limitations can be overcome through the use of various redox active mediators to replace oxygen. The biosensor involves a two step procedure in which the enzyme takes part in first redox reaction with glucose and is in turn reoxidized by the mediator and finally the reduced mediator is electrochemically oxidized form.



In this scheme, GOD/FAD and GOD/FADH₂ represent the oxidized and reduced form of flavin adenine dinucleotide within GOD, respectively, and M_{ox}/M_{red} are the oxidized and reduced forms of mediator. Different kinds of electron mediator, such as hexacyanoferrates [4–6], glucose oxidase/Cu(II) complex [7], Os-complexes [8–10], quinon derivatives [11,12], organic dyes [13], conducting salts [14,15], cytochrome b562 [16], [60]fullerene [17], ferrocene [18–20] and ferrocene derivatives [22–30] has been described. Among them, ferrocene and its derivatives have some of the most promising characteristics. Different methods have been studied for the immobilization of ferrocenes on electrode surfaces to produce chemically modified electrodes. Covalent binding [22,23] or adsorbing [21,24] to platinum or carbon is frequently used. In other approaches, the ferrocene moieties were part of polymer backbone and these materials were deposited onto a surface or incorporated to a carbon paste electrode [18–20,24–30].

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