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Amperometric glucose biosensor based on gold-deposited polyvinylferrocene film on Pt electrode

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Abstract

The preparations and performances of the novel amperometric biosensors for glucose based on immobilized glucose oxidase (GOD) on modified Pt electrodes are described. Two types of modified electrodes for the enzyme immobilization were used in this study, polyvinylferrocene (PVF) coated Pt electrode and gold deposited PVF coated Pt electrode. A simple method for the immobilization of GOD enzyme on the modified electrodes was described. The enzyme electrodes developed in this study were called as PVF-GOD enzyme electrode and PVF-Au-GOD enzyme electrode, respectively. The amperometric responses of the enzyme electrodes were measured at constant potential, which was due to the electrooxidation of enzymatically produced H_2O_2. The electrocatalytic effects of the polymer, PVF, and the gold particles towards the electrooxidation of the enzymatically generated H_2O_2 offers sensitive and selective monitoring of glucose. The biosensor based on PVF-Au-GOD electrode has 6.6 times larger maximum current, 3.8 times higher sensitivity and 1.6 times larger linear working portion than those of the biosensor based on PVF-GOD electrode. The effects of the applied potential, the thickness of the polymeric film, the amount of the immobilized enzyme, pH, the amount of the deposited Au, temperature and substrate concentration on the responses of the biosensors were investigated. The optimum pH was found to be pH 7.4 at 25 °C. Finally the effects of interferents, stability of the biosensors and applicability to serum analysis of the biosensor were also investigated.

Keywords: Polyvinylferrocene; Gold deposition; Glucose; Electrochemical biosensor

1. Introduction

The area of biosensors, particularly enzyme-based amperometric electrodes, has received great attention in these last years. Amperometric biosensors combine the advantages of the electrochemical techniques with the high substrate specificity of the enzymes. The determination of glucose is one of the most popular and well-known biosensor applications. In particular, glucose is of special importance because of its involvement in human metabolic process. Diabetics do not produce enough insulin in their pancreases to control adequately the level of glucose in blood. Furthermore the detection of glucose is also important in food and fermentation industry, and there have been many papers on this subject. Glucose electrochemical biosensors based on the enzymatic oxidation mediated by glucose oxidase (GOD) have generated considerable interest. This enzyme catalyzes the oxidation of glucose to gluconolactone in the presence of oxygen. In this enzymatic reaction, coenzyme flavin adenin dinucleotide (FAD) is reduced to $FADH_2$. In the natural enzymatic reaction, molecular oxygen functions as an electron acceptor for $FADH_2$ and reoxidized $FADH_2$ to FAD, whereas O_2 is reduced to H_2O_2. The amperometric response has been obtained either using O_2 (Liu and Ju, 2003; Alonso et al., 2004) or H_2O_2 electrodes (Gulce et al., 1995a; Kumar et al., 1997; Liu et al., 1999; Xu and Chen, 2000; Yang et al., 2002; Armada et al., 2003; Uang and Chou, 2003; Yabuki et al., 2003; Chen and Dong, 2003; Sung and Bae, 2003) in the case of first generation amperometric glucose sensors. A decrease in the reduction current of O_2 or an increase in the oxidation current of H_2O_2 is measured with O_2 or H_2O_2 amperometric sensors, respectively. However, in these detections, the current depends on the partial pressure of O_2 in the systems, and the quantitative analysis at high concentration of glucose is difficult, because O_2 is consumed with proceeding the enzymatic reaction. Furthermore, when H_2O_2 concentration is measured, a highly catalytic electrode and a high oxidation potential are required. In such condition, certain electroactive

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species in the analyte solution can be oxidized, and the oxidation current interferes with the output of the sensor. To overcome these disadvantages, the second generation amperometric glucose sensors were proposed (Chuang et al., 1997; Saito and Watanabe, 1998; Koide and Yokoyama, 1999; Wu et al., 2000; Sharma et al., 2003; Yang et al., 2003; Gulce and Gulce, 2005). In these sensors, the redox active species, which are called mediators, are employed as artificial electron acceptors for $FADH_2$ in place of O_2 molecules. The electron acceptors of this type are not consumed and can repeatedly oxidize $FADH_2$. In the last years, new sensors based on the direct determination of H_2O_2, for selective, fast and sensitive glucose determination, without using membranes or redox mediators, have been proposed. They consist of the metallic micro- or nanoparticles (such as platinum (Ye et al., 2003; Tang et al., 2004; Zhou et al., 2005; Wang et al., 2005) dispersed in electrode surface, so they are third generation biosensor, palladium (Zhang et al., 1996; Lim et al., 2005), rhodium (Wang et al., 1994), ruthenium (Wang et al., 1995), copper (Male et al., 2004; Pan et al., 2005) and gold (Zhao et al., 1992; Ju et al., 1998; Celej and Rivas, 1998; Pan et al., 2003; Zhong et al., 2005)). Gold, a less expensive and environmentally benign noble metal in comparison with other metals, has been receiving increased attention as a novel catalyst material. The modification of the electrode surface has been carried out by electrodeposition (Zhao et al., 1992; Ju et al., 1998) or by evaporation of enzyme-gold colloids (Celej and Rivas, 1998; Pan et al., 2003; Zhong et al., 2005).

We have shown in an earlier study that the accumulation of metallic gold on the polyvinylferrocene (PVF) coated electrode surfaces during immersion into the AuX_4^- solution without the need for any electrolysis at cathodic potentials implies immediately that a chemical reduction takes places according to the following reaction (Kavanoz et al., 2004)

$$3PVF + AuX_4^- \rightarrow 3PVF^+ + Au + 4X^- \quad (1)$$

This observation provided an alternative possibility to deposit Au on the PVF coated electrode.

In this study, we report the use of Pt electrode modified with PVF, for the enhanced electrocatalytic detection of H_2O_2 and the development of glucose biosensors based on GOD immobilized onto these PVF modified electrodes. Two types of glucose biosensors were prepared. In the first type of the biosensors, the GOD enzyme was immobilized into the polymer matrix by immersing PVF coated Pt electrode in enzyme solution for several times and this enzyme electrode was called as PVF-GOD enzyme electrode. In the second type of the biosensors, the GOD enzyme was immobilized on the PVF coated and then gold deposited Pt electrode by the same strategy, and this enzyme electrode was called as PVF-Au-GOD enzyme electrode. The experimental conditions related to the preparations and characterizations of the biosensors have been studied in detail. The performances of the biosensors, i.e. linear range, sensitivity, selectivity, response time and stability were also described. The use of the glucose biosensor developed in this study for determining glucose in whole blood was demonstrated and the results are compared with the conventional clinical procedure for blood glucose.

2. Experimental

2.1. Reagents

PVF was prepared by the chemical polymerization of vinylferrocene (Alfa Products) (Smith et al., 1976). The purification of methylene chloride (Merck) was accomplished according to the method proposed in literature (Perrin and Armorego, 1980). The buffer solutions were prepared using NaH_2PO_4 (Analar, BDH) and NaOH (Merck). The solution containing GOD was prepared by dissolving of GOD (Sigma G 6125, from *Aspergillus niger*) in 250 μL of 0.01 M phosphate buffer solution of pH 7.4. Glucose (Merck) solution was prepared in 0.1 M phosphate buffer solution of pH 7.4. $KAuCl_4$ solution was prepared by dissolving $KAuCl_4$ (Merck) in 0.01 M KCl (Merck) solution. All aqueous solutions were prepared from triple-distilled water.

2.2. Preparation of the biosensors

The Pt electrode was immersed in a solution of PVF in methylene chloride for a certain time period and the solvent was then evaporated for the preparation of the PVF coated Pt electrode. This electrode was called as PVF electrode. The average thickness of the dry film was estimated from the charge, Q, consumed during complete electrooxidation of the film by stepping the potential from 0.0 to 0.7 V versus SCE in 0.1 M phosphate buffer solution as described by Bard (Peerce and Bard, 1980). The first biosensor developed in this study consists of the immobilization of the GOD enzyme on the PVF coated Pt electrode, and this enzyme electrode was called as PVF-GOD electrode. The PVF-GOD electrode was prepared by immersing the PVF coated Pt electrode in a solution of GOD. The polymer-coated electrode was kept in the enzyme solution for 30 min without stirring.

The second biosensor developed in this study consists of the immobilization of the GOD enzyme on the PVF coated and then gold deposited Pt electrode, and this enzyme electrode was called as PVF-Au-GOD electrode. The PVF coated Pt electrode was immersed in $KAuCl_4$ solution for some time without the application of any potential for the preparation of gold deposited PVF coated Pt electrode. The accumulation of metallic gold on the PVF coated Pt electrode surfaces during immersion into $KAuCl_4$ solution takes place by the chemical reduction of Au^{3+} to Au according to the reaction (1). The gold deposited PVF coated electrode was kept in the GOD enzyme solution without stirring for some times for the immobilization of GOD. The preparation of the PVF-Au-GOD electrode was produced in this way. The enzyme-attached electrodes were rinsed with 0.01 M phosphate buffer solution at the working pH for 5 min to remove the remaining enzyme on the electrode surface, and then stored in 0.01 M phosphate buffer solution at 4 °C.

2.3. Measurements and apparatus

The responses of the enzyme electrodes were determined with a jacketed electrochemical cell which kept the solution at the desired temperature. Oxygen was introduced into the solution in this cell at a constant flow rate to obtain an oxygen-