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Preliminary investigations on a glucose biosensor based on the potentiometric principle

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Abstract

In this study, an electron mediator and a simple immobilization process are adopted to fabricate a potentiometric glucose biosensor. The enzyme and the electron mediator are immobilized on the surface of tin oxide (SnO_2)/indium tin oxide (ITO) glass using a covalent bond method to develop a disposable potentiometric glucose biosensor. The SnO_2/ITO glass is a pH sensor fabricated by depositing SnO_2 thin films onto an ITO glass. The glucose oxidase (GOD) and the electron mediator (ferrocenecarboxylic acid, FcA) are co-immobilized on the SnO_2/ITO glass using 3-glycidyloxypropyltrimethoxysilane (GPTS). This work investigates the coimmobilization of GOD and FcA as a useful approach for enlarging the dynamic range to a glucose concentration of 360 mg/dl, and for improving linearity and sensitivity of the fabricated glucose biosensor. The experimental results indicate that the optimal weight ratio of GOD to FcA is 1:1. The output signal is associated with the pH of the measurement environment and the optimal pH value is pH 7.5.

Keywords: Glucose oxidase (GOD); 3-Glycidyloxypropyltrimethoxysilane (GPTS); Tin oxide (SnO_2); Electron mediator; Potentiometric biosensor

1. Introduction

Diabetes is still a metabolic disorder that is caused by a total or partial lack of insulin. Glucose fluctuations within the normal physiological range of 110 ± 25 mg/dl are considered to be acceptable; diabetics had values of 360 mg/dl or higher [1]. For almost four decades, researchers engaged in the development of glucose-sensing devices which monitored the glucose levels in biological fluids rapidly, accurately and continuously, especially to help type II diabetes mellitus patients to monitor their daily sugar levels [2].

Spectrophotometric approaches were laboratory methods, which were not useful in on-line monitoring. The inconvenience of spectrophotometric methods was overcome using analyzers based on electrochemical methods, in which biosensors were applied. Numerous biosensors were employed to measure glucose levels in biological samples. Among these biosensors, amperometric glucose biosensors were attracted significant interest. However, applying a high polarizing voltage ($E_{app} = 0.6$–0.8 V) oxidized interfering substances such as ascorbic acid and uric acid, which were commonly present in biological fluids, leading to nonspecific signals [3]. Several artificial redox mediators were investigated as electron acceptors, to reduce the applied potential in an amperometric glucose biosensor, and thereby solved this problem. Electron mediators were incorporated to reduce the potential applied to the working electrode below 0.8 V. Hence, some investigations demonstrated the effects of applying electron mediators [2,4–12]. Furthermore, base electrodes were modified to improve the performance of glucose biosensors [13–17]. Accordingly, in glucose biosensors based on the amperometric principle, polarizing voltage is the key factor leading to interference.

Since no extra potential was required to apply on potentiometric biosensors, interference caused by the polarizing voltage could be eliminated. The enzyme field effect transistor (EnFET), based on the ion-sensitive field effect transistor (ISFET), was

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first introduced by Caras and Janata [18]. Glucose oxidase typically hydrolyzes glucose according to the equations shown as follows [19]:

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\beta\text{-D-glucose oxidase}} \text{D-glucono-}\delta\text{-lactone} + H_2O_2 \quad (1)$$

$$\text{D-Glucono-}\delta\text{-lactone} \rightarrow \text{D-gluconate} + H^+ \quad (2)$$

In that system, the common electron acceptor oxygen (O_2) produced the product hydrogen peroxide (H_2O_2). ISFETs measured the glucose concentration by detecting the variation in pH caused by the generation of hydrogen ions by the dissociation of glucose acid. Due to the low dissociation constant ($pK_a \cong 3.8$) of glucose acid, ISFET glucose sensors were responsible for their low sensitivities [20]. The glucose concentration in human blood was typically around 110 mg/dl, reaching 360 mg/dl or more for diabetics. However, the concentration of oxygen in arteries and veins did not exceed 14.5 and 5.85 kPa, respectively. Since the concentration ratio of oxygen in real blood was unfavorable, the dynamic range of the biosensor was normally limited by the oxygen and did not exceed several mM. Additionally, hydrogen peroxide, one of the by-products of glucose oxidation, inhibited glucose oxidase, reducing the sensitivity and repeatability of a steady glucose biosensor measurement system [21].

Seo et al. [21] and Lee et al. [22] utilized a platinum (Pt) electrode actuator on an ISFET sensitive gate to electrolyze the hydrogen peroxide. Their sensors, coupled with the Pt electrode actuator, exhibited a wide dynamic range, from 18 to 180 mg/dl. Yin et al. [19] developed an amorphous tin oxide (SnO_2)/indium tin oxide (ITO) glass structure with a sensitive gate, coimmobilized with glucose oxidase and manganese dioxide (MnO_2) by a cross-linking method, they developed the sensor as a disposable glucose EnFET. MnO_2 was employed as a catalyst, as it catalyzed the decomposition of hydrogen peroxide to H_2O and O_2. The glucose EnFET doped with MnO_2 was found to be useful in extending the dynamic range up to a glucose concentration of 360 mg/dl and the output signal was enlarged to 50 mV.

The technology used to process ISFET was strongly related to the various MOSFET technologies [23]. The dimension of MOSFET was reduced to the nanoscale, affecting the size of the sensing area, and thereby, the sensitivity. For reasons of sensitivity, the sensing areas of most biosensors were limited to the micro scale [24]. The ISFET fabrication processes were incompatible with the fast-developing MOSFET technologies. This incompatibility represented an obstacle to the development of ISFEF.

In our study, the SnO_2/ITO glass pH sensor was fabricated by depositing SnO_2 thin films onto ITO glass, which was not limited by MOSFET technologies. This approach had numerous advantages, including high sensitivity, ease of fabrication and low cost [25,26]. The use of a low-cost substrate and the simple fabrication process reduced the cost of the potentiometric glucose biosensor made from an ITO substrate below that made from an ISFET substrate. To our knowledge, potentiometric glucose biosensors were based on the principles of the electrolyzation [21,22] or catalyzation [19] of H_2O_2, to improve their performance. An artificial redox electron mediator was used for the first time herein to develop a potentiometric glucose biosensor and thereby solve the problems of O_2 deficiency, and the H_2O_2 inhibition effect on glucose oxidases (GOD) problems. Ferrocene and its derivatives were some of the most efficient electron mediators [2,5]. Among the ferrocene derivatives, ferrocenecarboxylic acid (FcA) was the optimal alternative to be used in the fabrication, because of its hydrophilic characteristic, so it could be mixed with the enzyme in a single step without any pretreatment [27–29]. Moreover, we discovered that 3-glycidyloxypropyltrimeth-oxysilane (GPTS) [30,31] acted as a very effective and easily applied attachment medium to coimmobilize FcA and GOD on the SnO_2/ITO glass pH sensor.

In this study, a potentiometric glucose biosensor based on the SnO_2/ITO glass pH sensor was realized, by applying GPTS to coimmobilize enzyme and electron mediator on its surface. The following equations describe the reaction sequences [10]:

$$\text{Glucose} + \text{GOD(FAD)} \rightarrow \text{Gluconolactone} + \text{GOD}(FADH_2) \quad (3)$$

$$\text{GOD}(FADH_2) + 2FcA_{(ox)} \rightarrow \text{GOD(FAD)} + 2FcA_{(red)} + 2H^+ \quad (4)$$

Herein, ferrocenecarboxylic acid (FcA) is employed as an electron mediator. In the following reaction, the active center flavin adenine dinucleotide (FAD) of glucose oxidases oxidizes the glucose penetrating the membrane; electrons are then transferred from the reduced GOD($FADH_2$) to $FcA_{(ox)}$. The resulting product, H^+, is detected by the SnO_2/ITO glass pH sensor. Accordingly, applying this mediator to a potentiometric glucose biosensor and using GPTS to simplify the immobilization process was expected to produce excellent response characteristics when the biosensor was exposed to glucose in a physiological environment. Furthermore, because no extra potential was required to apply on the potentiometric glucose biosensor, other biological molecules presented in physiological fluids will not be capable of reacting at the electrode surface.

2. Experiment

2.1. Chemicals and materials

Glucose oxidase (GOD; EC 1.1.3.4, type VII, from aspergillus niger) with an activity value of 121 units/mg was purchased from the Sigma Company. 3-Glycidyloxypropyltrimethoxysilane (GPTS) and ferrocenecarboxylic acid (99%) were obtained from the Aldrich Company. All other reagents were of reagent grade and were used without further purification. Deionized (D.I.) water was used to make all of the electrolytes and the buffer solutions. Tin oxide thin films were formed using a radio frequency sputtering system (tin oxide target, 99.9%) at a substrate temperature of 150 °C. ITO glass (50–100 Ω/□; ITO coating thickness 23 nm) was supplied by the Wintek Corporation.