An Optical Glucose Biosensor Fabricated by Encapsulating Glucose Oxidase in Silica Gel via Sol-Gel method

Shuxian Chen[1], Zhaoxian Xiong[1]*, Helan Ye[1] and Xiaopeng Dong[2]
[1]*Department of Materials Science and Engineering, Xiamen University, Xiamen 361005, China*
[2]*Department of Electronic Engineering, Xiamen University, Xiamen 361005, China*

*Corresponding author. College of materials, Xiamen University, Xiamen 361005, PR China
Tel.: 0086 592 8818063
E-mail: zxxiong@xmu.edu.cn

Abstract

An optical glucose biosensor was fabricated by encapsulating glucose oxidase in TEOS-derived gel film along with $Ru(bpy)_3Cl_2$ as an luminescent oxygen transducer, for determining the concentration of glucose in the blood and urine samples. When the oxidation reaction of glucose by glucose oxidase occurred, an increase in the fluorescence intensity of $Ru(bpy)_3Cl_2$ was observed due to the oxygen consumption. A good response performance to glucose was exhibited for the biosensor with a wide linear range from 2.0 to 18.0mM, R=0.997. The detection limit of the biosensor was estimated to be 0.368mM, with response time < 50s. There was no apparent interference for the biosensor with fructose, urea and ascorbic acid. In addition, the enzymatic activity and long-term stability were also discussed in details.

1. Introduction

Glucose sensing is of great significance in clinical analysis for the diagnosis and therapy of diabetes [1]. A variety of glucose sensing strategies are known, among which methods based on the enzyme glucose oxidase (GOx) are most widespread. Optical enzymatic glucose biosensors may provide an effective approach to overcome problems caused by enzyme-free sensors because of the enzymatic specificity and high efficiency, which were less disturbed by interference species [2,3,4]. The most commonly used is the classical method based on the glucose oxidase (GOx) reaction:

$$\text{Glucose} + O_2 \xleftarrow{} \text{GOx} \xrightarrow{} \text{glucuronicacid} + H_2O_2$$

The fluorescence spectroscopy of $Ru(bpy)_3^{2+}$ is governed by metal-to-ligand charge transfer (MLCT) process, which complexes are suitable luminescent dyes for oxygen sensing based applications due to their response functions of luminescence to quenching by oxygen [5]. The consumption of oxygen occur on the oxidation reaction of glucose leads to the increasing of the fluorescence intensity, which can be detected.

The sol-gel method is a simple process to prepare glassy material at room temperature that can support the immobilisation of different reagents.The attachment of enzymes to an insoluble matrix is an essential step in the development of biosensors. Since 1990, when Braun etal [6]. reported for the first time entrapment of proteins in silica gel, the sol-gel process has become an attractive way of immobilization of biological material in biosensor construction and has been reviewed few times [7,8,9]. The fact that sol-gel technology enables the incorporation of biomolecules under room temperature conditions into silica xerogels has stimulated extensive research activities in the field of sol-gel based sensors.

In this paper, we describe our study on the sol-gel based glucose biosensor with optical detection. This sensor is based on a fluorescent oxygen-sensitive ruthenium-ligand complex entrapped in a sol-gel along with GOx.

2. Experimental

2.1. Materials

Trimethoxysilane (TEOS), hydrochloric acid , and glucose were purchased from Sinopharm Chemical Reagent Co. Ltd. Tris(2,2'-bipyridine)ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2$) was purchased from Simga-Aldrich. Glucsoe oxidase (GOx) from A. niger of activity 216U mg^{-1} and horseradish peroxidase (HRP) of activity 80U mg^{-1} were purchased from Toyobo Co.Ltd. Stock solution of

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GOx was prepared by dissolving 80mg of GOx in 5ml buffer phosphate solution of pH 6.86. 1mol/l glucose solution was prepared by dissolving the solid compound in 5ml deionized water. Lower concentration solution were obtained by dilution of this solution in bidistilled water.

2.2. Instrumentation

The fluorescence spectra and response curves were measured by a F7000 Fluorescence Spectrophotometer (Hitachi, Japan), equipped with a xenon lamp as a light source. The excitation and emission wavelengths were set to 473nm and 590nm, respectively. The sensing films were fixed inside a home-machined triangle cuvette. Activity of Enzyme were measured on a Ultraviolet Visible Spectrophotometer (Hitachi, Japan).

2.3. Preparation of sol-gel film

A stock sol-gel solution was prepared by mixing of 7.8ml of TEOS with 1.6ml of deionized water and 1.1ml of 0.01M HCl. It was stirred for 3 hours at room temperature until the transparent homogenous solution was obtained and then doped with 1.3ml of 0.1mM $Ru(bpy)_3Cl_2$. The sol was left overnight at 4^0C before used. To prepared the sol-gel film, 500μl of sol were mixed with 50μl of GOx solution in a polystyrene cuvette and stirred for 2min. The gel accured after about 10min, after which phosphate butter solution of pH 6.86 was added and replaced daily. This system was stored at 4^0C for aging for five days.

3. Results and discussion

3.1. Synthesis of the glucose-sensitive gel

Considering silica alkoxydes as starting materials, the sol-gel process is represented by the following global chemical equation, in which R is an alkyl group:

$$M(OR)_4 + xH_2O \longrightarrow M(OH)_4 + xROH$$

M = Si, Ti,A l,B, Zr,Ce,Sn

$$M\text{-}OH + RO\text{-}M \longrightarrow M\text{-}O\text{-}M + ROH$$

$$M\text{-}OH + HO\text{-}M \longrightarrow M\text{-}O\text{-}M + H_2O$$

$$x(M\text{-}O\text{-}M) \longrightarrow (M\text{-}O\text{-}M)_x$$

The encapsulation of enzyme in sol-gel materials requires stringent control over synthesis conditions because these dopant molecules tend to denature in environments with high alcohol concentrations and extreme pH values as well as elevated temperatures. Standard sol-gel synthesis reactions are typically acid catalyzed and generate alcohol [10]. The sol-gel process can be modified for chemical compatibility with the enzyme by using alcohol generated on hydrolyzation as its solvent but not adding any alcohol. Butter solutions are also added to the sol to raise the pH, which seems to indicate a more favourable microenvironment for the enzyme. TEM image of the gel prepared from the modified method was displayed in Fig.1. The surface of the film was rough, which was beneficial to the chemical reactions because of its large specific surface area.

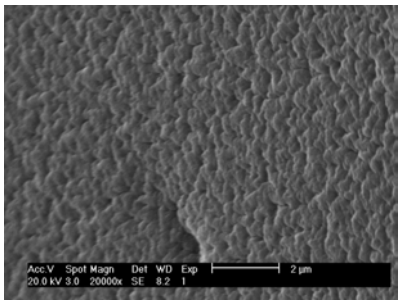

Fig.1 TEM image of the surface of gel film

3.2. Enzyme activity and response behavior of the glucose biosensor

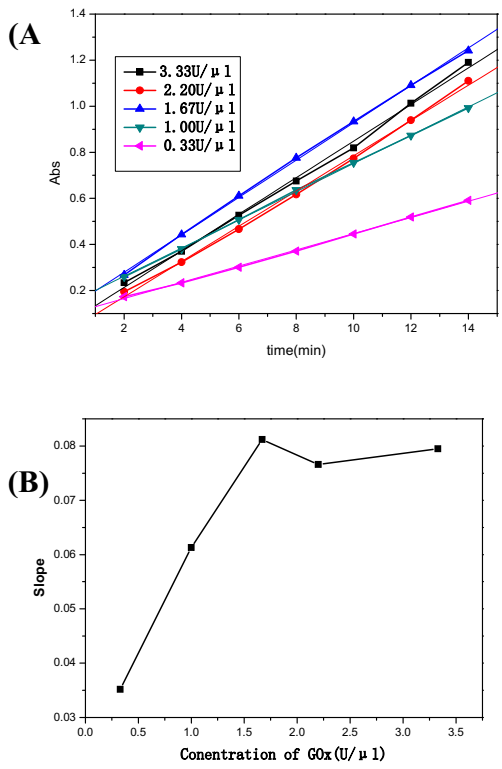

Fig.2 (A) Absorbency response of glucose biosensor towards different concentration of GOx at 500nm. (B) Effect of concentration of GOx on the activity of glucose biosensor.

The activity of GOx after entrapment in sol-gel was tested by the system of HRP, phenol and

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aminoant ipyrine base on irreversible chromogenic reactions. The increase in absorbance at 500nm with time was recorded and the initial slope taken as the parameter for activity. The effects of the amount of GOx on the sensor were examined. A range of GOx concentration from 0.33U μ L^{-1} to 3.33U μ L^{-1} was tested as showed in Fig.2A. Slopes of each curve were fited and showed in Fig.2B. The largest activity was achieved of a median concentration, 1.67U μ L^{-1}, of GOx. With a lower concentration of GOx, however, the activity was smaller because of insufficient enzyme amount. Moreover, a higher concentration of GOx also led to smaller response. The reason for this may be that the additional enzyme was actually immobilized but the concentration of oxygen outside the surface was limited.

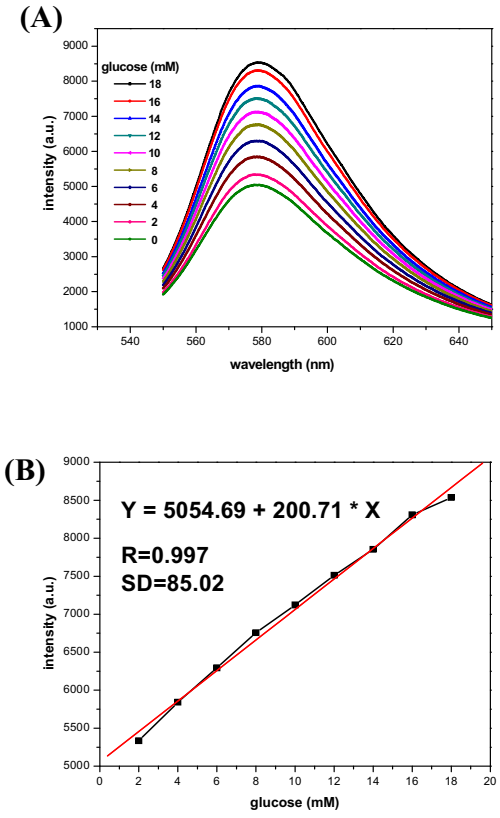

Fig.3 (A) Fluorescence response of glucose biosensor towards different concentration of glucose , excited at 473nm. (B) Correlation between the max fluorescence intensity and the concentration of glucose.

the fluorescence response of glucose biosensor arrays towards different concentrations of glucose from 0 to 18 mM were displayed in Fig.3A. The intensity of fluorescence increased along with the glucose concentration, which indicates a wide range of response to the glucose. Fig.3B summarized the maximum fluorescence intensity as a function of the glucose concentration. Experiments results showed that glucose concentration from 0 to 18mM has a linear relationship, Y=5054.69+200.71X, with the maximum fluorescence intensity with a high coefficient of 0.997. The limit of detection was calculated to be 0.368mM from 3 times signal to noise. The response time was less than 50sec. When the film was washed after the determination with buffer solution at pH=6.86, the recuperation time was around 30min.

It is well known that enzyme was high selectivity, as seen in Fig.4. No obvious interference in fluorescence intensity appeared when fructose, urea, and ascorbic acid were added to PBS with a pH value of 6.86, while the adding of glucose may lead to a high increasing of the maximum fluorescence intensity.

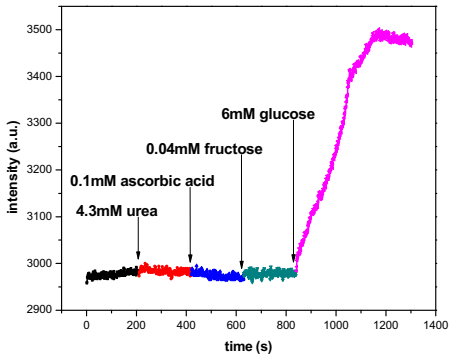

Fig.4 Test for the selectivity of the sensors by adding 4.3 mM urea, 0.1 mM ascorbic acid, 0.04 mM fructose and 6 mM glucose to pH 6.86 PBS.

To evaluate the stability of the system, the response slopes of the biosensor which described in Fig.3B were tested after 15days and 30days, as Fig.5 showed.. Compared with the original response slope, the response after a month decreased to 20%, which indicated that the stability of the sensor needs to be further improved.

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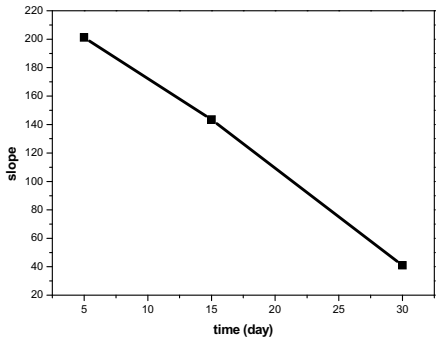

Fig.5 Test of stability on the glucose biosensor after 5days, 15days and 30days keeping.

4. Conclusion

An optical glucose biosensor was sucessfully fabricated by encapsulating glucose oxidase in TEOS-derived gel film along with $Ru(bpy)_3Cl_2$ as an luminescent oxygen transducer, for determining the concentration of glucose. A good response performance to glucose was exhibited for the biosensor with high sensitivity, good reproducibility and a fast response. Other biosensors with uricase and cholesterol oxidase are being developed with this similar technique.

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