Multifunctional sol-gel sensing membrane for fiber optic glucose sensor

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ABSTRACT

In this paper, Glucose oxidase (GOD) was immobilized on a novel silica membrane. The multifunctional sensing membrane was prepared by sol-gel method. GOD was immobilized on the aminated silica surfaces by glutaraldehyde cross-linking method. The fiber optical glucose sensor based on fluorescence quenching was designed and fabricated using lock-in amplifying technology to realize the detection of glucose concentration. The experimental results show that a linear range between phase delay φ and the glucose concentration of the solution was observed in the concentration range of 100 to 600 mg/dl and the detection limit is 50mg/dl, the sensor can meet the demand of clinical application. The response time of the sensing membrane was about 15s. The experimental results demonstrated that this biosensor with the multifunctional sensing membranes has high sensitivity, repeatability, good stability and fast response.

Keywords: multifunctional membrane; sol-gel; amination; fluorescence quenching

1. INTRODUCTION

Glucose monitoring has become increasing interest in medical diagnosis and in food industry[1]. In particular, the continuous monitoring of glucose in blood is required for the diagnosis of diabetes disease, control of insulin application and artificial pancreas function. There are several ways to detect the glucose concentration, most of them are based on enzyme catalysis, such as colorimetry[2], spectrophotometry[3], flow-injection chemiluminescence[4], and electrochemical detection[5]. However, these methods are time-consuming and are not satisfactory for on-line and real-time monitoring. Electrode method was a glucose concentration detecting method using enzyme electrode. However, this kind of electrodes also has the shortages of short life time, easy disturbance by electrical or magnetic field, and cannot be used continuously.

In recent years, fiber optic biosensor is one of the hot topics in biomedical science due to its applicability, unique biological compatibility. The fiber optic biosensors are effective ways to detect biomass concentration. The performances of a biosensor are largely dependent on the development of sensitive membrane. The sol-gel technique is often used for sensitive membrane, and current research is aimed at exploiting the multifunctional membrane for fiber optic biosensors. Based on sol-gel method, the multifunctional membrane was developed by embedding optical indicator and enzyme simultaneously in sol-gel matrix[6]. By using sol-gel entrapped enzyme, however, gelation process stresses and small gel

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pore size can result in a high mass-transfer resistance of biosensor, because of the long diffusion distance and the inaccessibility of substrate to the embedded enzyme. This immobilization method can affect enzymatic biocatalytic activities and can lower response time of biosensor.

A variety of immobilized approaches of glucose oxidase had been reported including encapsulation through sol-gel method, adsorption and covalent coupling [7-8]. These approaches, however, may suffer from diffusion barriers of the substrates through the membranes or leakage of enzymes from the membranes. In this paper, the purpose of this work is to improve the performance of biosensor by ameliorating enzymes immobilization technique. Functionalization of silica membranes is effective method to immobilize an enzyme on surface with a high retention of its biological activity. Amino groups were produced on silica membrane surface through 3-aminopropyltriethoxysilane and glucose oxidase was attached using the amino groups.

2. EXPERIMENTAL

2.1 Materials and Methods

Glucose oxidase (from Aspergillus niger, GOD, E.C.1.1.3.4, 100U/mg), $Ru(bpy)_3Cl_2$ and 3-aminopropyltriethoxysilane (APTES, 99.0%) were purchased from Sigma-Aldrich. Tetraethyl orthosilicate(TEOS), methanamide and glutaraldehyde (25%aqueous solution) were purchased from Shanghai Chemical Reagent Company. All solutions were freshly prepared in double-distilled water before use. All reagents were with analytical grade and used without further purification and double-distilled water was used throughout the experiment. Lock-in amplifier (SR830, Standford Research Systems, USA) was used for measuring the phase delay of the sensor head.

2.2 Glassy slide pretreatment

Ahome-made glassy slide (1.0×1.0cm diameter) was used as the base material for membrane-forming in the outmost surface of the glass slide. The glassy slide was treated by H_2O_2 /H_2SO_4 (3:7 volume ratio, 30wt%H_2O_2 solution, 98wt% H_2SO_4 solution) for 1h. The glassy slide was rinsed thoroughly with double-distilled water. The glass slide was immersed in the ammonium hydroxide solution containing ammonium hydroxide, H_2O_2 and double-distilled water (1:1:5 volume ratio, 25wt%ammonium hydroxide solution, 30wt% H_2O_2 solution) for 0.5h and washed thoroughly with double-distilled water, and dried in air at room temperature.

2.3 Preparation of sol-gel membranes

Silica membrane was derived from hydrolyzation and congregation of TEOS using hydrochloric acid (HCl) as catalyst, which was prepared by mixing suitable proportions of TEOS, ethanol, 0.05M HCl and methanamide. 150μl fluorescence indicator $Ru(bpy)_3Cl_2$ with concentration of 8mg/ml was added in the mixture. The mixture was stirred at room temperature for 5h until it became homogeneous. The mixture was placed in a freezer (4°C) for 3 days until gelation occurred. It was then spun on pretreated glassy-substrate to form the thin membranes with different thicknesses by a spin-coater. The thicknesses of the silica thin membranes were adjusted by the rotating speed of the spin-coater and the content of silica sol. After the membranes were formed it was put into sealed glass container for 2 days at 4°C. Then, the membranes were rinsed with 0.1M phosphate buffer with pH 6.5 and stored at 4°C until used.

2.4 Preparation of aminofunctional membranes

50μl 1% APTES was equably added on the above-prepared silica thin membranes and the aminofunctional membranes

was kept at 4°C for 2h, then washed with 0.1M phosphate buffer (pH 6.5). Aminofunctional membranes were activated by treating with 2.0% glutaraldehyde in 0.1M phosphate buffer (pH 7.0) for 2h and washed with double-distilled water and the same buffer.

2.5 Immobilization of GOD

The immobilization of GOD on aminofunctional membranes was achieved by covalent cross-linking method. The lyophilized GOD powder was dissolved in 0.1M phosphate buffer solution (pH7.0) and the GOD concentration is 5mg/ml. These aminofunctional membranes were placed in glucose oxidase solution, and the immobilization reaction was allowed to proceed at 4°C for 12h. The process for the immobilization of GOD is summarized in Figure 1. At the end of the procedure the membranes were rinsed with a phosphate solution with pH 6.5. The target membranes were stored at 4°C until used.

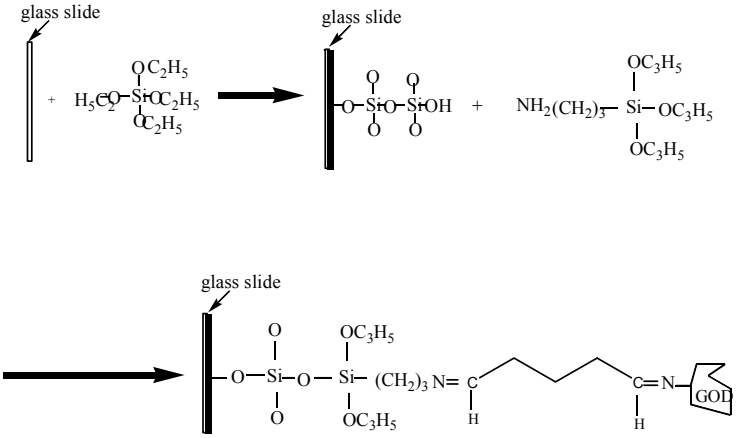

Figure 1. Schematic diagram of the process for the immobilization of GOD

2.6 Principle of biosensors

In this biosensing approach, glucose is easily oxidized by the enzyme bound to the membrane surface, and oxygen is simultaneously consumed [9], and an oxygen gradient will be created on the membrane due to the consumption of the dissolved oxygen caused by the glucose oxidation, which leads to a fluorescence change of the sensitive membrane because the fluorescence of the indicator ($Ru(bpy)_3Cl_2$) in the novel multifunctional sol-gel sensing membrane would be quenched by oxygen molecules. The glucose concentration can be determined by detecting the consumption of dissolve oxygen. The relationship between the fluorescence phase shift and the oxygen concentration can be mathematically described by equation [10-11] (1):

$$\frac{\tan\phi_0}{\tan\phi} = 1 + K_{sv}[Q] \quad \text{..... (1)}$$

Where Φ_0 and Φ are the fluorescence phase shifts of the sensing materials in the absence and in the presence, respectively, of the oxygen. $[Q]$ is oxygen concentration and K_{sv} is the Stern-Volmer constant. When φ is very small, we can take φ to be $\tan\varphi$. By collecting the data of phase delay φ the quantification of glucose is achieved. The apparatus is shown schematically in Figure 2 and Figure 3.

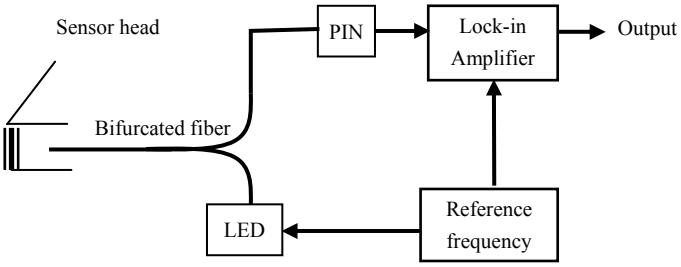

Figure 2. Schematic diagram of the sensor apparatus

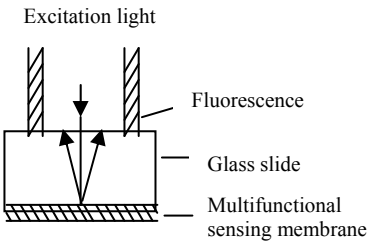

Figure 3. Schematic diagram of the sensor head

3. RESULTS AND DISCUSSION

3.1 Influence of the proportion of sol precursors

The physical and chemical properties of the final membranes were markedly influenced by proportion of precursor in the sol-gel process [12]. Our former research found that the high-quality membrane can be obtained[13], when the mole ratio between H_2O:Si is in the range of 3-4, the methanamide concentration in the sol is 16% and the volume ratios is TEOS : ethanol : 0.05M HCl =5 : 8 : 1.6.

The membrane was formed on the surface of the glass with different methods [14], such as dip coating method, the pulling method and the spin coating method. In three methods, we have used a spin-coating method. The thicknesses of the silica thin membranes were easily adjusted by the rotating speed of spin-coater and the content of silica sol. A certain amount of the sol will be put on the glass substrate, and the substrate will rotate in a certain speed, the sol will form a membrane by the rotating centrifugal force. Table 1, Table 2 and Table 3 show a performance of membrane with different the rotating speed of the spin-coater and the content of silica sol. The optimal properties of the membrane were found by using 80μl sol, 3000r/min rotating speed and 30s rotating time.

Table 1. Effect of different sol content on membrane properties

sol content(μl)	membrane properties
60	uneven
80	good, smooth surface, even
100	uneven, rough surface

Table 2. Effect of different rotating speed on membrane properties

rotating speed (r/min)	membrane properties
1000	uneven
3000	even
5000	uneven

Table 3. Effect of different rotating time on membrane properties in 3000 r/min

rotating time(s)	membrane properties
10	uneven
30	even
50	uneven

3.2 The standard curve of fiber optic glucose sensor

Figure 4 shows the glucose concentration-phase curve for fiber optic glucose sensor with the novel sensing membrane. The sensing membrane is normally based on the oxidation of glucose according to the reaction equation (2). Glucose concentration is evaluated by phase delay φ. A linear relationship between φ and glucose concentration was observed. A good linearity in the range of 100mg/dl to 600mg/dl was achieved, as shown in Figure 4 and the linear graph were defined by the equation of $y = 0.03133+0.0001x$ and $R^2 = 0.9868$, while the detection limit is 50mg/dl. Therefore the present synthesized GOD composite is promising for construction of glucose biosensing devices.

$$\beta\text{-D-glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{D-gluconic acid} + H_2O_2 \quad \ldots\ldots. (2)$$

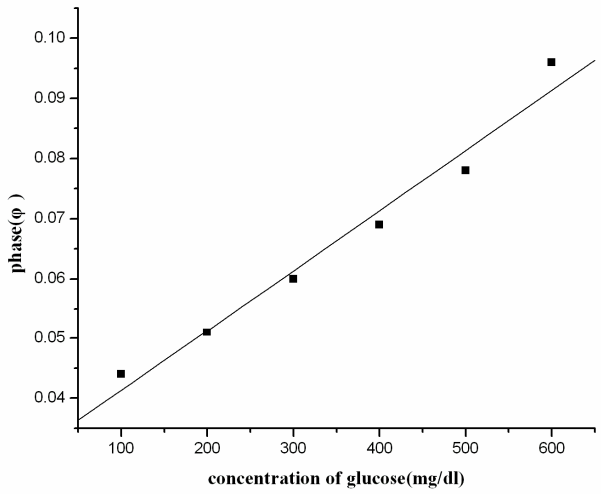

Figure 4. Standard curve of glucose concentration in 0.1M pH7.0 phosphate buffer at 25°C

3.3 Response time and repeatability of fiber optic glucose sensor

The response time of the sensor was tested by determining 50-600 mg/dl glucose in 0.1M pH 7.0 phosphate buffer solution. The sensor was stored at 4°C when not in use, and the detection was repeated for 4 times. The experimental results shown that the response time of the fiber optic glucose sensor with multifunctional membrane is about 15s, it is rather fast for a biosensor. Response time of the biosensor with multifunctional sol-gel sensing membrane was faster than those as reported[6,15,16]. As compared to the results from the GOD entrapped in the sol matrix, more GOD can be immobilized on the membrane surface using the present method. The accessibility of substrate molecules to the

immobilized enzyme was increased. Substrate molecules are easily oxidized by the enzyme bound to the membrane surface. It may be a promising strategy to improve the efficiency of enzymatic catalysis. Four consecutive $\Delta\varphi$ were measured by determining 100mg/dl glucose in 0.1M pH 7.0 phosphate buffer solutions, the relative standard deviation values are ±1.5%.

3.4 The stability of the fiber optic glucose sensor

The stability of biosensor was tested by determining 100mg/dl glucose in 0.1M pH 7.0 phosphate buffer solutions. The biosensor with multifunctional sol-gel sensing membrane was kept at 4°C and its performance was measured every 5 days. After storage for 30 days, only a slight decrease in response time was noticed. The $\Delta\varphi$ was shown considerable decrease to about 75% of its original value over the 50 days. This could be attributed to denaturation of GOD in the sensing membrane. In addition, hydrolysis of silica matrix should be taken into account for the GOD degradation. It can be concluded that this biosensor has good stability.

4. CONCLUSIONS

A membrane with amino groups was synthesized by using sol-gel method and GOD was covalently immobilized on membrane surface. The multifunctional sol-gel sensing membrane was used to develop simple and sensitive fiber-optic biosensors. By using lock-in amplifying technology, the fiber-optic biosensors were applied to the detection of the glucose concentration, which confirms that the biosensor with multifunctional sol-gel sensing membrane is feasible to practical application. This work will be attractive to the development of fiber optic microsensor and can be used for detecting glucose concentration in human body .

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