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Temperature controlling fiber optic glucose sensor based on hydrogel-immobilized GOD complex

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ABSTRACT

A novel fiber optic glucose biosensor based on Poly(*N*-isopropylacrylamide) (PNIPAAm)-immobilized glucose oxidase (GOD) complex (PIGC) was developed. PIGC was prepared by combining PNIPAAm with GOD immobilized on SiO_2 nanoparticles using in-situ complex method. The catalyzation of oxidation of glucose could be controlled based on the feature that PNIPAAm exhibited swelling and shrinking response to the temperature below and above low critical solution temperature (LCST), respectively. The biosensor can perform the controllable detection of glucose by changing temperature. The optimal detection conditions for this biosensor were achieved at pH 6.5, 30 °C and 10 mg of GOD amount. There is a good linear relationship between the phase delay difference φ and the glucose concentration in the range of 50–700 mg/dL. This biosensor has good repeatability, selectivity and can be used for the detection of practical samples.

1. Introduction

Diabetes mellitus, which afflicts millions of people in the world, is a group of metabolic disorders causing the high glucose concentration in the blood. It is one of the major causes of death and disabilities and has long term effects such as heart disease, cardiovascular disease, kidney failure and blindness [1]. It is crucial to detect and control the blood glucose for diabetic people to avoid diabetic emergencies and test the effectiveness of the medical treatments. Therefore, the development of a fast-responsive, reliable and low-cost method for the detection of glucose concentration is important to clinical medicine and human health.

Compared to many techniques that have been developed for glucose detection over the past few decades, such as high performance liquid chromatography (HPLC) [2,3], colorimetry [4], spectrophotometry [5], chemiluminescence [6], electrode [7,8], electrochemical sensor [9,10], quantum dots sensing [11] and optical sensing [12,13], fiber optic biosensors have many advantages such as high sensitivity, fast response, immunity from electrical interference, long distance sensing [14]. As a branch of fiber optic sensors, the enzyme-based fiber optic biosensors reveal potential applications in many fields with the optimization of its features. For example, if the enzyme performance can be controlled, the detection activities of the enzyme based fiber optic biosensors can be controlled, which will have important applications in many cases.

Stimuli-sensitive hydrogels have tunable network structures, good mechanical property and biocompatibility [15]. Their volumes will change with a slight variation of external stimuli, such as temperature, light, chemical environment, electric field, antigen, etc [16]. Thermo-responsive hydrogels, as an important kind of the stimuli-sensitive hydrogels, have a lower critical solution temperature (LCST), and will swell at the temperature lower than LCST and shrink at the temperature higher than LCST. Because of this characteristic, thermos-responsive hydrogels have been widely used as the carriers for the temperature controlled release of macromolecular drugs [17,18]. Poly(*N*-Isopropylacrylamide) (PNIPAAm) is a well known temperature sensitive polymer with the LCST of ~32 °C in aqueous solution [19]. If PNIPAAm is used to complex with enzyme, the enzyme performance can be manipulated by changing temperature. This kind of complex materials can be used as the sensing materials for the fiber optic biosensor to carry out controllable detection and for multi parameters fiber optic biosensors which can detect the concentrations of different species such as glucose and cholesterol at different temperatures.

In this work, PNIPAAm was combined with immobilized glucose oxidase (GOD) using SiO_2 nanoparticles as the carrier to form PNIPAAm-immobilized GOD complex (PIGC) with in-situ complex

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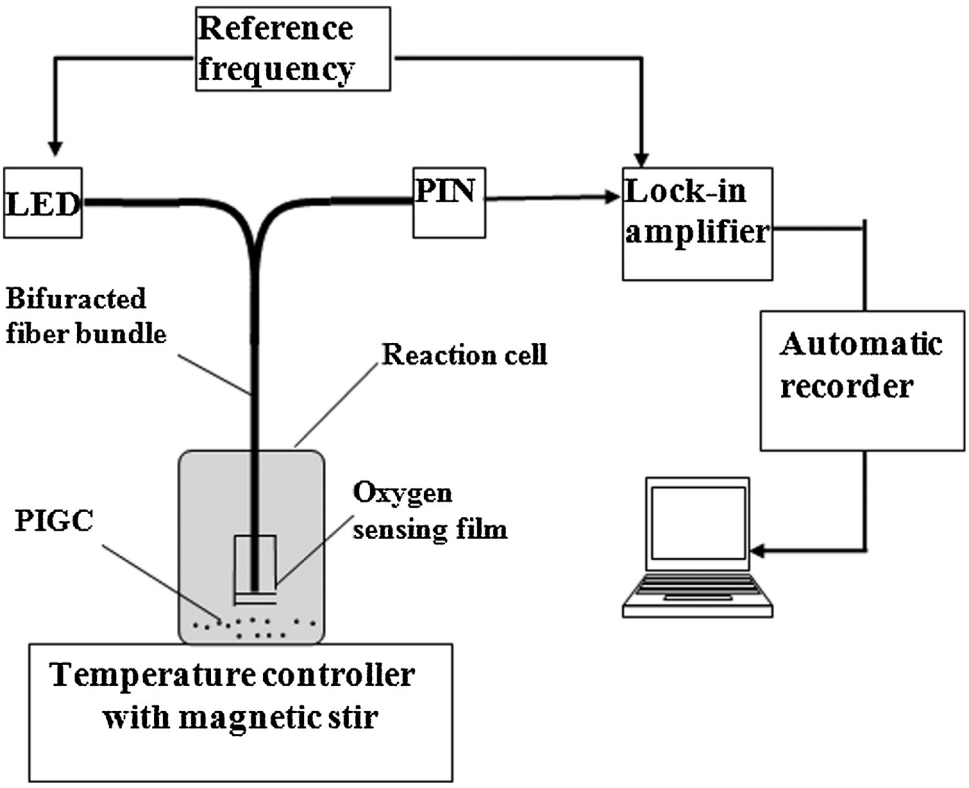

Fig. 1. Schematic diagram of the detecting system.

method. By changing temperature, the oxidation of glucose was controlled using PIGC as the catalyzer. A fiber optic glucose sensor to perform the controllable detection of glucose was fabricated and the sensor properties were studied using optical oxygen sensing film [20] and lock-in technology [21]. The sensor can detect glucose concentration effectively, indicating a promising prospect of practical application. To the best of our knowledge, this temperature controlling fiber optic glucose biosensor based on PIGC has never been reported before.

2. Experimental

2.1. Materials

GOD (E.C. 1.1.3.4, 100 U mg^{-1}) was obtained from Aspergillus-niger. Glucose, $Ru(bpy)_3Cl_2$ (99.0%) were purchased from Aldrich–Sigma. NIPAAm was purchased from Aldrich Chemical Co. Inc., USA and was recrystallized from benzene/n-hexane. Ammonium persulfate (APS), *N*,*N*′-methylenebisacrylamide (BIS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Sinopharm Chemical Reagent Co. All reagents were with analytical grade and used without further purification. Double-distilled water was used throughout the experiments. The oxygen sensing membrane was prepared as previously described [20].

2.2. Preparation of PIGC

SiO_2 nanoparticles were prepared using Stöber method [22]. GOD was immobilized on SiO_2 nanoparticles as described before [23]. The preparation procedure of PIGC was depicted as follows. 200 mg NIPAAm, 10 mg BIS were dissolved in 3 mL of immobilized enzyme solution (the immobilized enzyme content was 10 mg). The mixture was stirred at room temperature for 30 min under nitrogen air followed by the addition of 20 μL of TEMED and 80 μL of APS. The mixture was kept at −20 °C for 24 h and PIGC was formed. PIGC was then immersed in distilled water for 72 h and the water was refreshed every several hours in order to allow the unreacted chemicals to leach out. The complex was stored in distilled water at 4 °C.

2.3. preparation and principle of temperature controlling fiber optic glucose sensor

The detecting system consists of a lock-in amplifier, a LED with the excitation wavelength of 416 nm as the light source, a sensor head with an oxygen sensing membrane, a temperature controller, and a computer for data processing (see Fig. 1).

This sensor was based on the fluorescence quenching and consumption of oxygen. The oxidation of glucose catalyzed by GOD will consume oxygen in the solution. By detecting the fluorescence of $Ru(bpy)_3Cl_2$ quenched by oxygen, the oxygen concentration change was determined. Since a lock-in amplifier is used, the quenching could be described as

$$\frac{\tan\varphi_0}{\tan\varphi} = 1 + K_{sv}[Q] \quad (1)$$

Where φ_0 and φ are the phase delay difference of the sensor in the absence and presence of the oxygen, respectively, and K_{sv} is the Stern-Volmer constant. [Q] is the oxygen concentration. By detecting the data of phase delay difference φ the quantification of glucose is achieved.

2.4. Measurements

To detect the glucose concentration, measurements were performed with the setup shown schematically in Fig. 1. The sensor head was placed into a tiny reaction cell which contained glucose buffer solution and PIGC. An entire airtight reaction cell was introduced to eliminate the interference of oxygen from the open air.