Design and properties study of fiber optic glucose biosensor

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A new type of fiber optic glucose biosensor based on fluorescence quenching has been designed and its properties have been studied. Glucose can be oxidized by oxygen when glucose oxidase are used as the catalyst, therefore, the concentration of glucose can be measured by detecting the consumption of oxygen. For the detection of oxygen concentration, the ruthenium(II) complex, $Ru(bpy)_3Cl_2$, were used as the fluorescence indicator and its fluorescence lifetime were detected by lock-in technology. The detecting range of the sensor is $50-500$ mg/dl and its response time is 30 seconds, showing that this kind of sensors is possible to be used in clinical diagnosis and detection.

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The determination of glucose is very important in clinical diagnosis, especially in the diagnosis of diabetic patients. There are various kinds of methods for the determination of glucose based on the enzymatic reaction with glucose oxidase (GOD) as catalyst

$$\mathrm{D-glucose} + \mathrm{O_2} \xrightarrow{\mathrm{GOD}} \mathrm{D-gluconolactone} + \mathrm{H_2O_2}. \quad (1)$$

Glucose can be determined by measuring the production of hydrogen peroxide or the consumption of oxygen.

Since the first glucose sensor with an oxygen electrode was developed in 1962, research papers about glucose biosensors based on oxygen electrode have been developed extensively. However, this kind of glucose sensors have some deficiencies, such as poor duration, weak signal, easy to be affected by electronic or magnetic field and slow response. All these defects have caused the electro-chemical glucose biosensors to be difficult in their practical use.

Fiber optic biosensors have many advantages, such as high accuracy, immunity from the disturbance of electric and magnetic fields, fast response and low cost. Thus more and more attention have been paid on the research of fiber optic biosensors around the world. Sol-gel method are commonly used to immobilize GOD and fluorescence dye[1,2], which has good biological compatibility and fast response, but the sol-gel substrate are fragile and thus affect the properties of sensor. For the signal detection, researchers have tried to utilize photomultiplier to detect the change of electrical signals transformed from light signals[3]. However, such an electrical-optical transforming device is low in precision. Some institutes have utilized spectrometers in the system, but it is still too expensive for practical use.

In this paper , the design and fabrication of a fiber optic glucose sensor based on the enzymatic reaction of the oxidation of glucose have been achieved , and its properties have been studied. The ruthenium(II) complex $Ru(bPy)_3Cl_2$, whose fluorescence can be quenched by oxygen, was chosen as the fluorescence indicator, and cellulose acetate (CA) was used as the matrix to immobilize fluorescence indicator and GOD. Lock-in technology was used to detect the lifetime of the fluorescence indicator which changes with the consumption of dissolved oxygen in the solution where glucose is oxidized, and quantitative information about the glucose concentration can be obtained. CA membrane was reported to be the matrix to immobilize GOD in electro-chemical glucose sensors[4,5]. There is no report on fiber optic sensors with CA membranes as the matrix in which lock-in technology was used for the concentration determination of glucose solution. These kinds of sensors have the advantages of fast response, high accuracy, good duration, low cost and easy operation, therefore, it has wide application prospect.

For the oxidation of glucose shown in Eq. (1), the consumption of oxygen dissolved in the solution has quantitive relationship with the concentration of glucose. Therefore, the concentration of glucose can be determined by detecting the concentration of dissolved oxygen which can be mathematically described by Stern-Volmer equation

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K[\mathrm{O_2}], \quad (2)$$

where I_0 and I are the fluorescence intensities of the sensor in the absence and presence of the oxygen quencher in the sample solution and K is the Stern-Volmer constant which has certain value for a certain quencher. τ_0 and τ are the lifetime of the fluorescence in the absence and presence of the oxygen in the sample solution. $[\mathrm{O_2}]$ is oxygen concentration. By detecting I_0 and I or τ_0 and τ, oxygen concentration can be determined. Since fluorescence lifetime is the characteristic parameter of fluorescence substance and will not be influenced by its concentration and the exciting light intensity, the detecting accuracy and anti-disturbance ability of sensor can be improved greatly.

The method of phase delay is used to measure the fluorescence lifetime. Light signal from LED is supplied as sine modulated signal, so the fluorescence signal also appears a sine signal with a phase delay. The lifetime of fluorescence can be detected by the lock-in amplifier in terms of phase change. The relationship between $\tan\phi$ and τ is shown below

$$\tan\phi = \omega\tau, \quad (3)$$

where ω is the angular speed of sine modulating signal, $\omega = 2\pi f$, f is the frequency of sine modulating signal.

Lock-in technology is employed to lock and amplify fluorescence signal for the purpose of eliminating the influence of the exiting light. The principle is shown in Fig. 1.

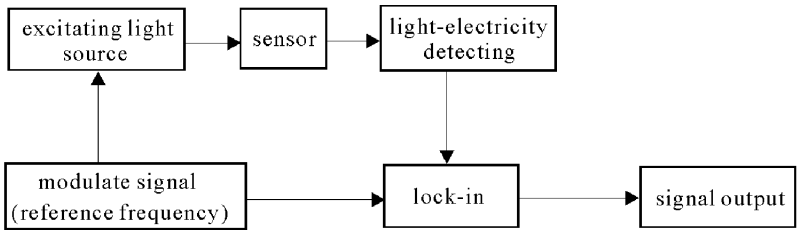

Fig. 1. Principle scheme for the detecting of fluorescence lifetime by lock-in technology.

From Fig. 1 we know that sine modulated signal provided by signal modulating source can modulate the light source to produce the sine exciting light signal. As a result, the fluorescence signal also changes in the way of sine signal but with a phase delay ϕ. The relationship between τ and ϕ is

$$\tan\phi = 2\pi f\tau. \tag{4}$$

Therefore, τ can be got by the data of phase delay ϕ. Eq. (5) can be obtained from Eqs. (2) and (4)

$$\frac{\tan\phi_0}{\tan\phi} - 1 = K[\mathrm{O_2}], \tag{5}$$

where $\tan\phi_0$ and $\tan\phi_0$ are the phase delay of the sensor in the absence and presence of the oxygen in the sample solution, $[\mathrm{O_2}]$ is the concentration of oxygen. Since phase delay is so small (less than 1 degree), $\tan\phi$ is approximately equal to ϕ, thus Eq. (5) can be transformed into

$$\frac{\phi_0}{\phi} - 1 = K[\mathrm{O_2}]. \tag{6}$$

The higher the concentration of glucose solution, the more the oxygen consumed, the less the concentration of dissolved oxygen. Therefore, the detection of glucose concentration is achieved by measuring phase delay ϕ. Since the detection object is phase difference between the signal to be detected and the reference signal, there is no any influence from stray light, and the sensor has good detecting accuracy and anti-disturbance ability.

GOD(type II-S, from Aspergillus niger)with a specific activity of 15000 – 25000 U per gram of solid was obtained from Sigma Co. (Munich, Germany). Horseradish Peroxidase was obtained from Faizyme laboratories Ltd. $\mathrm{Ru(bpy)_3Cl_2}$ was obtained from Aldrich Chem. Co.. CA, glucose, bovine serum albumin, and all other chemicals or biochemicals were of analytical-reagent grade, and are used without further purification.

All the fluorescence measurements were performed with a lock-in amplifier (SR830, Standford research systems, U.S.). A LED with the excitation wavelength of 416 nm was used as excitation light source.

To prepare CA membrane, 1.0 g of CA was added into 25 ml of acetone mixed with an appropriate proportion of distilled water. The mixture was stirred in an air-tight condition at room temperature. After CA was completely dissolved, the solution was stirred for another two hours. Then the solution was dipped on a clean culture. The CA membranes were obtained after acetone and water have been volatilized thoroughly. The thickness of the CA membrane can be controlled by controlling the quantity of solution in the culture. By controlling the volatilizing speed of acetone, the membrane can be made to be uniform.

During the preparation of CA membrane described above, an appropriate proportion of indicator, $\mathrm{Ru(bpy)_3Cl_2}$, was added in the mixture and the stirring time was kept a little longer, then the sensing membrane can be obtained.

To prepare the GOD membrane, the CA membrane(1×1 cm^2) reacted with sodium periodate, ethanediamine, glutaraldehyde, bovine serum albumin, glutaraldehyde respectively, thus a membrane with chemically activeated aldehyde groups on its surface was prepared. The membrane was to immerse in a 5 mg/ml of GOD in pH7.0 phosphate-buffer for about four hours and the GOD membrane can be obtained. The membrane was stored in a refrigerator at 4 °C. It can be used for a week.

Figure 2 shows the schematic of the fiber optic sensor. The GOD membrane and oxygen sensitive membrane were assembled on the sensor head. The glucose solutions with different concentration were prepared daily and water was used as the blank solution.

By detecting the phase delay ϕ using lock-in technology, the relationship between delay ϕ and glucose concentration can be obtained. All measurements were performed at room temperature(around 25 °C).

The thickness and uniformity of the cellulous acetate membrane are important for GOD membrane. The GOD membrane had the best catalyzing activity when the thickness of CA membrane was in the range of $30-50$ μm. If the volatilizing speed of acetone is too fast, the membrane will display poor evenness, which would affect the immobilization of GOD. In this experiment,

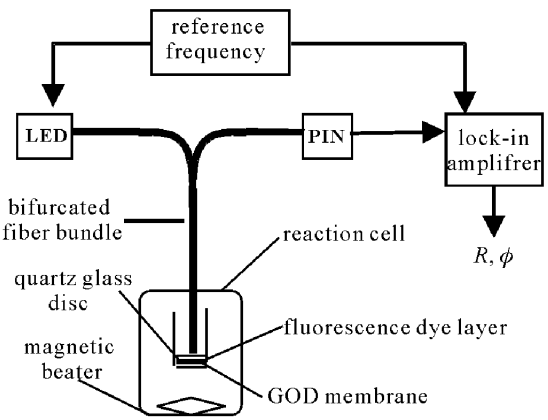

Fig. 2. The experimental scheme of fiber optic glucose sensor.

parchment paper was used to lower the volatilizing speed of acetone and satisfactory CA membrane was acquired for the immobilization of GOD. CA membrane need to be activated by reacting with sodium periodate, ethanediamine and glutaraldehyde solution, then the CA membrane with activated aldehyde groups can be obtained by reacting with bovine serum albumin molecule[4]. Finally GOD will be immobilized on the membrane through the coupling with BSA and GA.

$Ru(bpy)_3Cl_2$ has a high photostability and efficient yield of fluorescence with long lifetime, and oxygen displays remarkable fluorescence quenching effect on it, thus $Ru(bpy)_3Cl_2$ is one of the optimal indicators that are suitable for fiber optic biosensors based on fluorescence quenching[7]. CA is chosen as immobilizing substrate because it has good mechanic property, flexibility, low cost and other advantages over some substances such as polystyrene, silastic and agarose. The thickness of the oxygen sensitive membrane is also an influence factor for the sensor. If the fluorescence dye layer is too thin, it will be difficult to be assembled on the sensor head, and the amount of the fluorescence dye immobilized on the oxygen sensitive membrane will not be enough for the high detecting accuracy and duration of the sensor. If the membrane is too thick, the response of the sensor will be slow. The optimal thickness of the sensing membrane is in the range of $30 - 40$ μm and it can be used for 2 months.

Figure 3 shows the relationship between the concentration of glucose and the phase delay $|\phi|$ in pH6.0 phosphate buffer. From Fig. 3 we can know that $|\phi|$ has good linear relationship within the range of $50 - 500$ mg/dl. As we know, the normal blood glucose level of human is about 80 mg/ml, but for diabetic patients it can increase to $3 - 4$ times. Therefore, it will be possible for this sensor to be used in preclinical diagnosis.

Figure 4 shows the influence of the buffer pH to the sensor. The results show that the best pH range for the sensor is between pH6.0 and 7.0.

The influence of different assembled layers on glucose sensor property was studied. When there is no other additional layers acting as filter, GOD membrane contact directly with glucose solution, the sensor displays the fastest response(about 30 s). When the sensor head is assembled with an additional dialytic membrane with a thickness of 40 or 190 μm, the response time is 5 or 7 min, respectively. It can be explained in the following way.

Without additional layers, glucose molecule and oxygen molecule can easily diffuse into GOD membrane where the GOD catalyzed reaction take place; when equipped with additional layers, the diffusion of both glucose molecule and oxygen molecule is held back to some extent, thus the sensor display lower response. The

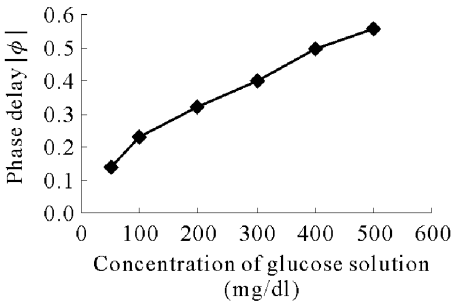

Fig. 3. Relationship between glucose concentration and phase delay ($f = 40$ kHz).

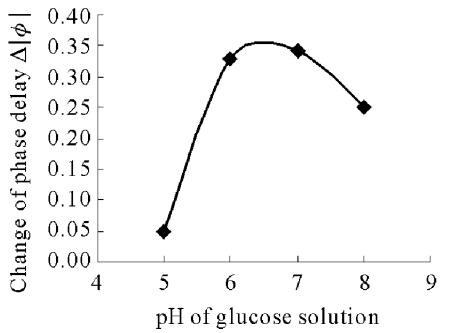

Fig. 4. Influence of buffer pH to the sensor ($f = 40$ kHz).

thicker the filter membrane is, the more difficult the molecule diffuses and the lower response the sensor displays. The results indicate that the response is controlled by the speed of dispersion of the reactant molecule.

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