

# A high performance glucose biosensor enhanced via nanosized SiO<sub>2</sub>

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## Abstract

A series of monodispersed nano-SiO<sub>2</sub> film enhanced glucose biosensors with different thickness were fabricated by using dip-coating method. The suitable thickness of nanosized SiO<sub>2</sub> film provided optimal environment for glucose oxidase to retain its bioactivity. A key factor to fabricate high sensitivity glucose biosensor was to enlarge the enzyme loading on the surface. The high surface area of the small nanosized SiO<sub>2</sub> particles in the thick film increased the surface enzyme loading, resulting in the high performance of the biosensor. But if the film is too thick, the performance of the sensor would decrease because the mass transfer of glucose and H<sub>2</sub>O<sub>2</sub> became difficulty. The electrochemical response of glucose with the 800 nm SiO<sub>2</sub>-biosensor revealed a linear behavior in the range of 0.005–2.5 mM glucose in pH 7.2 phosphate buffer solution. Such a glucose biosensor held its sensitivity as high as 71.1 μA mM<sup>-1</sup> cm<sup>-2</sup> and its detection limit as low as 0.3 μM. The good sensor-to-sensor reproducibility also indicates the simpleness and practicability of this kind of biosensor.

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**Keywords:** Glucose biosensor; Monodispersed nano-SiO<sub>2</sub>; Glucose oxidase; Immobilization; Film

## 1. Introduction

Glucose biosensors which utilize immobilized oxidase for the conversion of the target analytes into electrochemically detectable products are one of the most widely used detection methods for the determination of glucose in blood and food and some sugary drinks [1–3]. The methods of enzyme immobilization on electrodes include immobilization of enzyme in gels, cross-linked polymers, conductive salts or mixing into carbon paste or carbon-organic polymer hosts [4]. For example, sol-gel derived silicates have been proved to be highly compatible with enzymes [5–7]. Many kinds of nanometer materials such as TiO<sub>2</sub> [4], gold [8–11], silver and SiO<sub>2</sub> nanoparticles [12–15], have been used to construct nano-biosensors. With the development of material science, it provides more opportunities to immobilize various biomolecules which show high selectivity and sensitivity to the target analytes, and therefore fabricate biosensors with good performance. Very recently a variety of glucose biosensors with high sensitivity and excellent reproducibility using nano-technology had been reported [16–22], which made new developments of biosensors. Among these developments, the

nanoparticle enhanced glucose biosensors are one kind of fascinating biosensors.

Nanoparticles can play an important role in adsorption of biomolecules due to their large specific surface area and high surface free energy. As an instance, platinum nanoparticles with a diameter of 2–3 nm had been prepared and combined with single-wall carbon nanotubes (SWCNTs) to fabricate electrochemical sensors [20]. The response time and detection limit of this GC/CNT + Pt<sub>nano</sub> + GOx electrode were determined to be 3 s and 0.5 μM of glucose, respectively. This biosensor held very high performance, and it exhibited linearity from 0.5 μM up to 5 mM with a sensitivity of 2.11 μA mM<sup>-1</sup> in glucose solution. Though there have been great achievements in the field of nano-biosensors, tailoring of the electrode surface to construct sensitive and cheap biosensors is still a challenging and continuous analytical research interest [23].

Nanosized silica have been found to be a kind of good biocompatible solid support for enzyme immobilization [13,14,24–28]. Intensive researches have been conducted to construct glucose biosensors by using nanosized silica [15,24,29,30]. But there scarcely have studies dealing with the relationship between the performance of this kind of biosensors and the thickness of silicon oxide layer. With these in mind, we fabricated silicon oxide enhanced biosen-

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sors with different film thickness and studied the effect of film thickness on the performance of the silicon oxide modified glucose biosensor. The nanoparticles here were used to immobilize biocomponents. This work attempted to achieve simple and practical biosensors which also possessed high performance.

## 2. Experimental

### 2.1. Chemicals and reagents

Glucose oxidase (GOx) was purchased from sigma company, the activity was 178.5 units  $\text{mg}^{-1}$ .  $\text{SiO}_2$  particles (99.9%) were purchased from Beijing Century Science and Technology Development Co. Ltd. The particle size was 13 nm nominally. Polyvinyl butyral (PVB),  $\beta$ -D-glucose and other chemicals used in this work were available with analytical reagent grade. Electrochemistry measurements were carried out in 0.1 M phosphate buffer solution (PBS) (pH 7.2), which was prepared by dissolving 0.061 mol di-sodium hydrogen phosphate and 0.039 mol sodium di-hydrogen phosphate in 1 l of double distilled water. Also 0.1 mol potassium chloride was added to the solution to increase conductivity of the system. Different stock concentrations of anhydrous  $\beta$ -D-glucose were prepared in the PBS and stored at 4 °C when not in use (mutarotation was allowed for at least 12 h before use). GOx was dissolved in 0.1 M phosphate buffer solution (pH 7.2) with a concentration of 4.5  $\text{mg mL}^{-1}$  and stored at 4 °C.

### 2.2. Instrumentation

The particle size of the silicon oxide was measured by using a Hitachi H-800 transmission electron microscope (TEM). The accelerating voltage of electron beam was 120 kV. XPS data were taken from a PHI 5300 ESCA system using Al  $K\alpha$  X-ray source. A power of 250 W and a pass energy of 35.75 eV were adopted during the experiment. The base pressure of the analysis chamber was better than  $5 \times 10^{-9}$  Torr. All spectra were calibrated using the binding energy of C 1s (284.8 eV) as a reference. AES spectra were obtained using a PHI 610 scanning auger microscopy system. The energy and beam current of the Ar ion beam were 3.0 keV and 0.2  $\mu\text{A}$ , respectively. The beam diameter was 1 mm and the sputtering rate was approximately 25.0  $\text{nm min}^{-1}$  corrected by a thermally oxidized  $\text{SiO}_2$  thin film. Cyclic voltammetry (CV) and amperometric measurements were performed by using a CHI 660B electrochemical workstation. A three electrode cell with platinum flake (50 mm  $\times$  4 mm  $\times$  0.2 mm) as a counter electrode and a saturated calomel electrode (SCE) as reference electrode served for electrochemical measurements. All experiments were conducted at  $27 \pm 2$  °C if no special announcement.

### 2.3. Sensor fabrication

Platinum wire with a diameter of 0.7 mm were polished with a polish paper (1200 mesh) and calcined with alcohol burner.

When it became cool, the Pt electrode was modified by dip-coating method. Briefly, the Pt electrode was dipped into mixture which was made by blending of 1.0 g commercial  $\text{SiO}_2$  powder with 10 mL 2% PVB solution of ethanol and then lifted up at a rate of 3 cm/min and dried in air. A 5  $\mu\text{L}$  drop of glucose oxidase (GOx) solution (4.5  $\text{mg mL}^{-1}$ ) was dried on the  $\text{SiO}_2$  modified Pt electrode. Then 3.5  $\mu\text{L}$  of glutaraldehyde (2.5%) was applied on the resulting electrode to cross-link the enzyme. At last, the enzyme modified electrode was coated with 1 layer of PVB by dip-coating method and was washed with PBS. The resulted Pt/ $\text{SiO}_2$ /GOx sensors were stored at  $-10$  °C when not in use.

## 3. Results and discussion

### 3.1. Electrochemical characterization

The surface area of the base Pt electrode was 7  $\text{mm}^2$ . The Pt electrode was calcined with alcohol burner before use. Calcining was an efficacious way to clean the surface of noble metal electrode. It had the same effect as carefully cleaning in acid and organic solvents and water [31]. Both the calcined electrode and the carefully washed electrode had almost four to six times increase of response current in 10 mM hydrogen peroxide solution compared with untreated Pt electrode (data not showing). XPS data showed clearly that after calcining the carbon atoms in the electrode surface were reduced from 52.5% down to 28.4% and the surface platinum atoms increased from 20.4% up to 36.8%. The increase of surface platinum atoms leads to increasing activity of the Pt electrode.

GOx cross-linked with glutaraldehyde was immobilized on each nano- $\text{SiO}_2$  modified electrode. The Pt/ $\text{SiO}_2$ /GOx electrodes and Pt/GOx electrodes were tested by using a CHI660B electrochemical workstation. Cyclic voltammetry measurements were done to estimate the over potential of the sensor in glucose solution (scan rate: 10–200  $\text{mV s}^{-1}$ ). In glucose solution, a striking change in the cyclic voltammetry curve occurs. A large electrochemical redox current flow at potential higher than 0.4 V was observed. But the electrochemical redox current became stable only when the applied voltage was in the range of 0.5–0.6 V (the upper inset in Fig. 1). Thus, during chronoamperometric measurements, the working electrode was poised at +0.60 V versus SCE [4,20]. In addition, the peak current versus the square root of sweep rate plot was linear from 10 to 200  $\text{mV s}^{-1}$  (the upper inset in Fig. 1 curves (a)–(g), and its inset), indicating that this was a surface diffusion controlled process. At each amperometric measurement, when the background current was stable, a certain amount of glucose was added into the system with stir (stirring rate:  $\sim 1000$  rpm). The response current rapidly reached to a new stable state. Fig. 1 displayed a typical amperometric response curve of the Pt/ $\text{SiO}_2$ /GOx electrode with the 3-layer  $\text{SiO}_2$  film. A stable and fast amperometric response could be observed with successive injections of 50  $\mu\text{l}$  glucose solution (50 mM) into PBS. The time required to reach stable response was less than 3 s. The resulting calibration plot for glucose over the concentration range

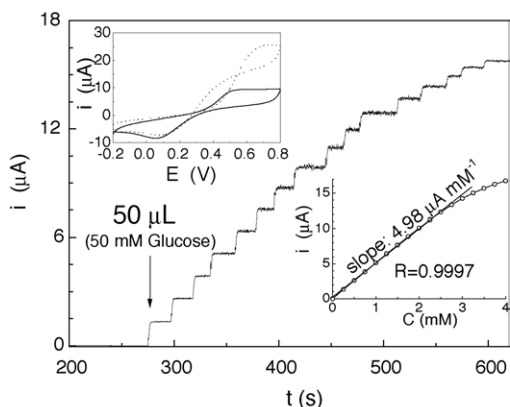


Fig. 1. Amperometric response of Pt/SiO<sub>2</sub>/GOx electrode with successive glucose injection (stirring rate: ~1000 rpm). The applied potential was 0.6 V vs. SCE. The upper inset shows the cyclic voltammetry measurements of Pt/SiO<sub>2</sub>/GOx electrode in 4 mM glucose solution with scan rate of 10, 20, 40, 60, 80, 100, and 200 mV s<sup>-1</sup>, respectively. The inset is the plot of peak currents at 0.6 V vs. square root of scan rate. The lower inset shows the calibration plot of the glucose concentration between 0.005 and 4 mM.

of 0.005–4 mM (considering the change of solution volume) was presented in lower inset. When glucose concentration was higher than 3.0 mM, the response curve began to deviate from linear. This deviation was caused by oxygen exhausting [32]. It revealed that such an electrode could work well in concentration range between 0.005 and 2.5 mM. The corresponding regression equation of the linear plot was:  $I (\mu\text{A}) = 0.023 + 4.98c$ ,  $N = 11$ ,  $S.D. = 9.91 \times 10^{-8}$ ,  $R = 0.9997$ , where  $c$  is the glucose concentration in mM. The sensitivity was thus estimated as  $71.1 \mu\text{A mM}^{-1} \text{ cm}^{-2}$  ( $4.98 \mu\text{A mM}^{-1}$ ).

### 3.2. Effect of pH

Investigation of the effect of the pH value on the performance of the biosensor is of great importance, because the activity of the immobilized GOx is pH dependent [15]. The pH dependence of the sensor was evaluated at 2 mM glucose solution over the pH range from 5.0 to 8.0 (Fig. 2). When the pH of the buffer was very low or very high, the GOx electrode exhibited low response current to glucose. The biosensor displayed an optimum sensitivity of the response at pH 7.2.

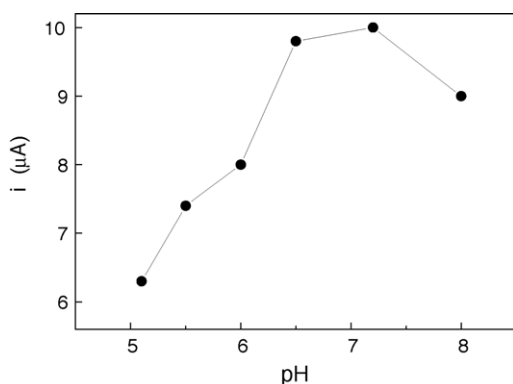


Fig. 2. Effect of pH on the response behavior of the biosensor.

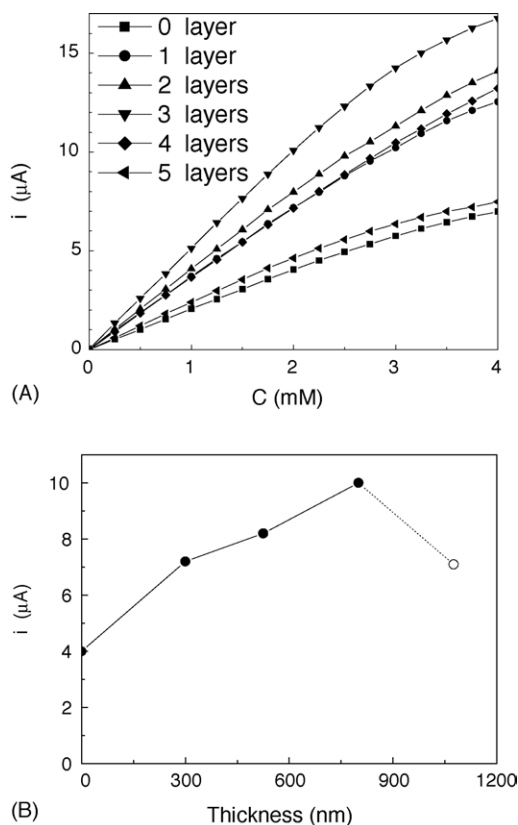
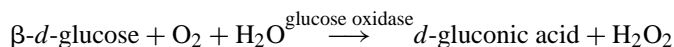


Fig. 3. (A) Current–concentration curves measured by different layers of SiO<sub>2</sub> modified biosensors. (B) The response currents of biosensors modified by 1, 2, 3 and 4 layers of SiO<sub>2</sub> measured in 2 mM glucose solution. The thickness of the 4 layers' film is estimate.

### 3.3. Effect of film thickness

With the increase of film thickness, the response current changed gradually. Fig. 3A showed the response current curves of biosensors modified by different layers of SiO<sub>2</sub> at different glucose concentration. The volume of enzyme on each biosensor was 5 μL (4 units) and the surface area of each base Pt electrode was 7 mm<sup>2</sup>. When layers of SiO<sub>2</sub> were less than three, the response current increased with the increase of SiO<sub>2</sub> layer. The 3-layer SiO<sub>2</sub> modified Pt/SiO<sub>2</sub>/GOx electrode had the highest performance. When layers of SiO<sub>2</sub> were more than four, the response current decreased with the increase of SiO<sub>2</sub> layer. The response curve of 5 layers of SiO<sub>2</sub> modified biosensor was almost the same as the one without SiO<sub>2</sub>. The apparent Michaelis–Menten constants ( $K_M^{\text{app}}$ ) can be determined following the Lineweaver–Burk type equation [33,34]:  $1/i = (K_M^{\text{app}}/i_{\text{max}})(1/C) + (1/i_{\text{max}})$ , where  $i_{\text{max}}$  and  $i$  are the current measured under substrate saturation and the steady-state current for a given substrate concentration ( $C$ ), respectively. The apparent  $K_M^{\text{app}}$  determined in the present studies worked out to be 14.8, 8.9, 7.8, 6.1, 9.4, and 12.9 mM, corresponded to 0, 1, 2, 3, 4, and 5 layers' sensor, respectively. The  $K_M^{\text{app}}$  of 3-layer-SiO<sub>2</sub> modified Pt/SiO<sub>2</sub>/GOx electrode was 6.1 mM, showing high biological affinity to glucose.

This kind of glucose biosensor is normally based on the oxidation of glucose according to the following reactions:



The response current produced from the decomposition of hydrogen dioxide on the electrode. More enzymes would produce more  $\text{H}_2\text{O}_2$  at the same glucose concentration under the same condition and thus produce higher response current. As to the biosensor modified by  $\text{SiO}_2$  films, with the increase of film thickness, the number of  $\text{SiO}_2$  particles increased, and the specific surface area of the sensor enlarged, thus the valid enzyme loading increased. There were more immobilized enzyme could participate in the reaction with glucose and produce more  $\text{H}_2\text{O}_2$ , resulted in higher response current. This was consistent with our previous work, where the response current of glucose biosensor increased with the decrease of particle size, which also could be explained as the increasing valid surface area of the biosensor accompanied with the decrease of particle size increased the enzyme loading on surface [35]. On the other hand, the mass transfer of glucose and  $\text{H}_2\text{O}_2$  was sensitive to the distance. With the increase of film thickness, the mass transfer became difficulty more and more. Thus, led to the speed of electrode reaction decreased drastically. These two contrary effects caused the result that of the 3 layers, one had the highest performance. In order to understand this behavior more rationally, the thickness of the films were measured by PHI 610 scanning auger microscopy system (SAM). The thickness of 1, 2 and 3 layers of films, calculated according to the sputtering time, were about 300, 525 and 800 nm, respectively. Fig. 3B showed the response currents of sensors modified by different layers of  $\text{SiO}_2$  at 2 mM glucose solution. The thickness of each layer was quite uniform (about 275 nm). So both the surface enzyme loading and the distance of mass transfer increased uniformly. These two contrary effects, as discussed above, caused the last results. It also showed that 800 nm was the appropriate thickness in this system. This thickness was unexpected large. The great number of  $\text{SiO}_2$  particles in the thick film had very high surface area and could undoubtedly increase the surface enzyme loading and thus achieved high performance biosensor.

### 3.4. Detection limit of glucose solution

Fig. 4 showed an amperometric response curve of a Pt/ $\text{SiO}_2$ /GOx sensor with the 3-layer  $\text{SiO}_2$  film at low glucose concentration. Stable and fast amperometric response could be observed with successive injections of glucose (50 mM) into PBS solution. The time required to reach stable response was less than 3 s. The lower inset showed the resulting calibration plot for glucose over the concentration range between 5 and 100  $\mu\text{M}$ . The corresponding regression equation of the linear plot was:  $I (\mu\text{A}) = 0.016 + 4.95c$ ,  $N = 11$ ,  $\text{S.D.} = 2.13 \times 10^{-9}$ ,  $R = 0.9999$ , where  $c$  is the glucose concentration in mM. The sensitivity was thus estimated as  $70.7 \mu\text{A mM}^{-1} \text{cm}^{-2}$  ( $4.95 \mu\text{A mM}^{-1}$ ). This showed that the sensor could work very well in concentration range between 0.005 and 0.1 mM. The detection limit thus was estimated as 0.3  $\mu\text{M}$  according to the signal-to-noise ratio of 3.

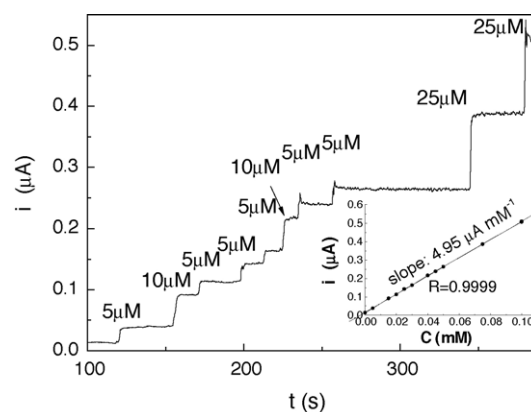


Fig. 4. Amperometric responses with successive glucose injection at low concentration. The applied potential was 0.6 V vs. SCE. The lower inset shows the calibration plot of the glucose concentration between 5 and 100  $\mu\text{M}$ .

This was almost the lowest detection limit so far in the field of nanoparticles enhanced glucose biosensor.

### 3.5. Repeatability and stability of the sensor

The stability of this kind of sensor was tested by measuring six glucose solutions (samples a–f). The final glucose concentration at each measurement was 2 mM. Fig. 5 showed the results of the six measurements. In Fig. 5A, two measurements were done by injection 0.4 mL stock glucose solution (50 mM) into PBS (samples a and b), two measurements were done by successively injection 0.2 mL stock glucose solution (50 mM) into PBS (samples e and f), and another two measurements were done by using 2 mM glucose solution prepared beforehand (samples c and d). The response current of each measurement was extracted and displayed in Fig. 5B (hollow up triangle). The relative departure of each measurement was less than 5%, showing good stability of the sensor. A 2 mM glucose solution was measured five times discontinuously, and the relative departure of each measurement was less than 3%, showing high stability Fig. 5B (black circle). The long-term stability of the biosensor was checked out by measuring 2 mM glucose solution day by day. After 20 days, it still kept 80% of its response activity compared with its first measurement. After 75 days, it still kept 60% of its response activity compared with its first measurement (the sensor was stored dryly at  $-10^\circ\text{C}$ ).

The sensor-to-sensor reproducibility was conducted to show the repeatability of the method to fabricate nano- $\text{SiO}_2$  enhanced glucose biosensors. Three 2 layers of  $\text{SiO}_2$  modified sensors and four 3 layers of  $\text{SiO}_2$  modified sensors were fabricated at different time. Every sensor was tested with successive injections of glucose solution into PBS. All sensors had stable and fast amperometric response with glucose injections. The resulting calibration plot for glucose over the concentration range 0.005–4 mM (considering the change of solution volume) was presented in Fig. 6. It revealed that all sensors could work well in concentration range between 0.005 and 2.5 mM with a correlation coefficient larger than 0.999. The relative departure of 2-layer-sensors and 3-layer-sensors at 2 mM glucose solution

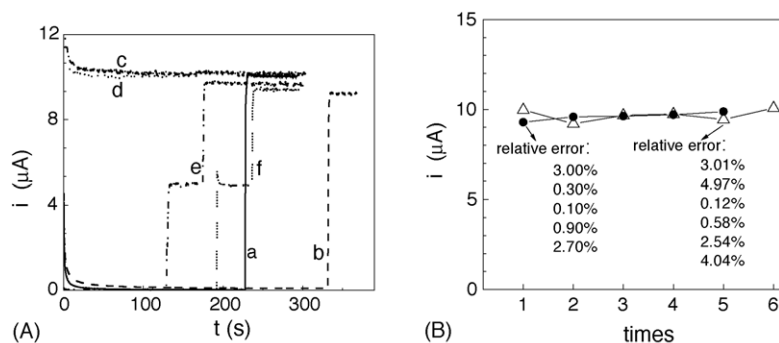


Fig. 5. (A) The  $i$ - $t$  curves of the sensor measured in six 2 mM glucose solutions. The measurements for sample a and b were done by injection of 0.4 mL stock glucose solution into PBS, the measurements for sample c and d were done by using prepared 2 mM glucose solution, and the measurements for samples e and f were done by successively injection of 0.2 mL stock glucose solution into PBS. (B) The response currents of the six measurements (hollow up triangle) shown in (A) and the five response currents of the same 2 mM glucose solution measured discontinuously (black circle). The relative departure of each measurement was calculated and showed.

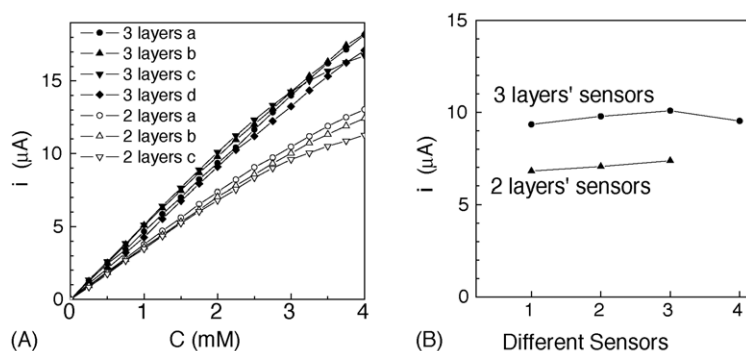


Fig. 6. (A) Current-concentration curves of sensors fabricated in different blocks. (B) The response currents of 2-layer sensors and 3-layer sensors at 2 mM glucose solution. The relative departure of these two kinds of sensors was less than 7% at glucose concentration of 2 mM.

both were calculated to be less than 7%, showing high repeatability.

#### 4. Conclusion

The effective enzyme loading on surface was a key factor to fabricate this first generation glucose biosensor. The high surface area of the small nanosized  $\text{SiO}_2$  particles in the thick film increased the surface enzyme loading and led to the high performance of the biosensor. The distance of mass transfer was another key factor to this kind of biosensor. The performance of the sensor will decrease if the film is too thick. The 800 nm biosensor had the highest performance and could work well in glucose concentration range between 0.005 and 2.5 mM with a response slope as high as  $71.1 \mu\text{A mM}^{-1} \text{cm}^{-2}$  and a detection limit as low as 0.3  $\mu\text{M}$ . This work showed that commercial nanosized monodispersed silicon oxide was suitable to immobilize glucose oxidase. The advantages of this biosensor fabricating method depended on the fact that it was very simple and cost effective, and it is also a very practical method to volume-produce glucose biosensors.

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