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Size dependence of SiO_2 particles enhanced glucose biosensor

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Abstract

A wide size range of SiO_2 particles were synthesized and were used as enzyme immobilization carriers to fabricate glucose biosensors. The size of the particles was in the range of 17–520 nm. These biosensors could be operated under physiological conditions (0.1 M phosphate buffer, pH 7.2). Particle size could affect the performance of SiO_2 modified glucose biosensors drastically. The smaller particles had higher performance. The smallest SiO_2 modified biosensor could work well in the glucose concentration range of 0.02–10 mM with a correlation coefficient of 0.9993. Its sensitivity was 2.08 μA/mM and the detection limit was 1.5 μM glucose.

Keywords: Glucose biosensor; Nanosized SiO_2; Glucose oxidase; Enzyme immobilization

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 [1,2]. 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 [3]. For example, sol–gel derived silicates have been proved to be highly compatible with enzymes [4–7]. Many kinds of nanometer materials such asTiO_2 [3], Au [8,9], Ag and SiO_2 nanoparticles [10–15], have been used to construct nano-biosensors. Recently a variety of glucose biosensors with high sensitivity and excellent reproducibility using nano technology have been reported [16–23], which made new development of biosensors. Among these developments, the nanoparticle enhanced glucose biosensor is one of the most fascinating biosensors.

Nanoparticles can play an important role in adsorption of biomolecules due to their large specific surface area and high surface free energy. Since Zhao and coworkers reported that glucose oxidase (GOx) was immobilized in Au sol and had enhanced performance [24], this kind of biosensors had been studied by many authors [3,10–15,21]. Jiang and coworkers studied the effect of nanosized SiO_2 and Au particles on the adsorbability and enzymatic activity of glucose oxidase. They found the amount of adsorbed enzyme increases with the decrease of the particle size [12]. Thus one could infer that with the decrease of particle size, it should get higher performance for nanoparticles enhanced biosensors. But their work was focused on the different mechanism of adsorption force of hydrophilic gold and hydrophobic silica nanometer particles, the sensitivity of sensors which containing SiO_2 particles was very low, and no linear relation was found between the response current and the concentration of the glucose solution. So it was necessary to study this phenomenon thoroughly to construct low-cost but high performance biosensor by using the cheap nano silica. The synthesis of SiO_2 particles had been studied by many authors since it was first reported by Stöberr et al. and developed by other authors [25–27]. These efforts made it easy to control the size of SiO_2 particles without the use of surfactants, which could change the surface state of SiO_2 [12]. On the other hand, silicon dioxide had been found to be a good biocompatible solid support for enzyme immobilization [11,12,15]. Thus SiO_2 particles were ideal carriers in present study.

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The aim of this work was to investigate the rule of fabrication simple and high performance glucose biosensor. In this work, a wide size range of SiO_2 particles were synthesized. These particles were used as enzyme immobilization carriers to fabricate a series of glucose biosensors. The amperometric measurement clearly showed that smaller particle size had higher performance in detection of glucose. These biosensors could be operated under physiological conditions (0.1 M phosphate buffer, pH 7.2). Amperometric detection of glucose with these biosensors resulted in a rapid and stable response in the glucose concentration range of 0.02–10 mM. The 17 nm SiO_2 biosensor had the best performance. It could achieve a sensitivity of 2.08 μA/mM and a detection limit of 1.5 μM glucose (signal-to-noise ratio of 3).

2. Experimental

2.1. Chemicals and reagents

Glucose oxidase was purchased from Sigma Company, the activity was 178.5 U/mg. Polyvinyl butyral (PVB), β-D-glucose, tetraethoxysilane (TEOS) and other chemicals used in this work were available with analytical reagent grade. PVB solution was prepared by dissolving 2 g PVB in 100 ml ethanol. All the other solutions were prepared with double distilled water. GOx was dissolved in 0.1 M phosphate buffer solution (PBS) (pH 7.2) with a concentration of 4.5 mg ml^{-1} and stored at 4 °C. The PBS 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. The PBS contained KCl (0.1 M) to increase conductivity of the system. Different stock concentrations of anhydrous β-D-glucose were prepared in the PBS and stored at 4 °C (mutarotation was allowed for at least 12 h before use).

2.2. Instrumentation

The particle size was measured by using Hitachi H-800 transmission electron microscopy (TEM). The accelerating voltage of electron beam was 120 kV. Cyclic voltammetry (CV) and amperometric measurements were performed by using a CHI 660B electrochemical workstation. A three electrode cell with platinum flake (50 mm × 4 mm × 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 ± 2 °C.

2.3. Synthesis of SiO_2 nanoparticles

The synthesis of SiO_2 particles was according to the method described by Stöberr et al. and developed by other authors [25–27]. The detailed process, taken the preparation of 220 nm SiO_2 as an example, was as follows: 18 ml ethanol was mixed with 1.3 ml NH_4OH (25% NH_3) and 1.0 ml H_2O, added 0.40 ml TEOS ($TEOS_1$) with stir and kept at 27 °C for 2 h (t_1) to reaction. Then added 0.60 ml TEOS ($TEOS_2$) with stir and kept 2 h (t_2). After ageing for 10 h, the nanoparticles were separated from the solution with centrifugal machine. The separated particles were washed twice with ethanol by ultrasonicating and by centrifuging. The resulted particles were dispersed in ethanol solution of 2% PVB with ultrasonic.

2.4. Enzyme electrode fabrication

Platinum wire with a diameter of 0.7 mm was polished with polish paper (1200 mesh) and calcined with alcohol burner. When it became cool, the Pt electrode was dipped in mixture which was made by blending of 150 mg SiO_2 powders with 10 ml 2% PVB solution of ethanol for 1 min and dried in air. A 5 μl drop of glucose oxidase solution (4.5 mg ml^{-1}) was dried on the SiO_2 modified Pt electrode. Then 3 μl of glutaraldehyde (2.5%) was applied on the resulting electrode to cross-link the enzyme. At last, the enzyme modified electrode was coated with one layer of PVB by dip-coating method and was washed with PBS. The enzyme-modified electrodes were stored at 4 °C.

3. Results and discussion

3.1. The size of synthesized SiO_2 particles

Through the hydrolysis and condensation of TEOS in ethanol with ammonia as catalyst, and by control the reaction time and the amount of TEOS, ethanol, ammonia and water, SiO_2 particles with different sizes could be achieved [25–27]. Table 1 displayed the sizes of the synthesized SiO_2 particles and corresponding synthesis parameters. The particle size could be calculated from TEM micrographs, which were shown in Fig. 1. The scale bar were 50 nm (A), 100 nm (B), 200 nm (C), 200 nm (D), and 500 nm (E), respectively. The estimated sizes of 90% of the resulted SiO_2 particles were 17 ± 3 nm, 90 ± 18 nm, 220 ± 25 nm, 280 ± 35 nm and 520 ± 60 nm, respectively (20 particles were measured to get average size of each sample).

3.2. Effect of enzyme loading

The amount of enzyme loading could affect the response current of this kind of glucose biosensor [16]. Fig. 2 showed the response curves of SiO_2 biosensors with different enzyme loading. For each glucose concentration, with the increase of enzyme loading, the response current increased gradually. Glucose biosensors without mediator are normally based on the oxidation of glucose according to the following reactions: β-D-glucose + $O_2 + H_2O \longrightarrow^{\text{glucose oxidase}}$ D-gluconic acid + H_2O_2. The response current produced from the decomposition of hydrogen dioxide on the electrode. More enzymes would produce more H_2O_2 at the same glucose concentration under

Table 1
Particle sizes and synthesis parameters of the synthesized SiO_2 particles

Particle size (nm)	Ethanol (ml)	NH_4OH (ml)	H_2O (ml)	$TEOS_1$ (ml)	t_1 (h)	$TEOS_2$ (ml)	t_2 (h)
17	20	0.3	0.2	0.1	3	0.1	3
90	20	0.65	0.5	0.4	2	0.4	2
220	18	1.3	1.0	0.4	2	0.6	2
280	13	4.0	3.1	0.4	2	0.6	2
520	16	2.7	2.1	1.0	6	–	–

$TEOS_1$ means the TEOS added at the first time and $TEOS_2$ the second time, t_1 represents the reaction time after the addition of $TEOS_1$ and t_2 corresponds to $TEOS_2$.

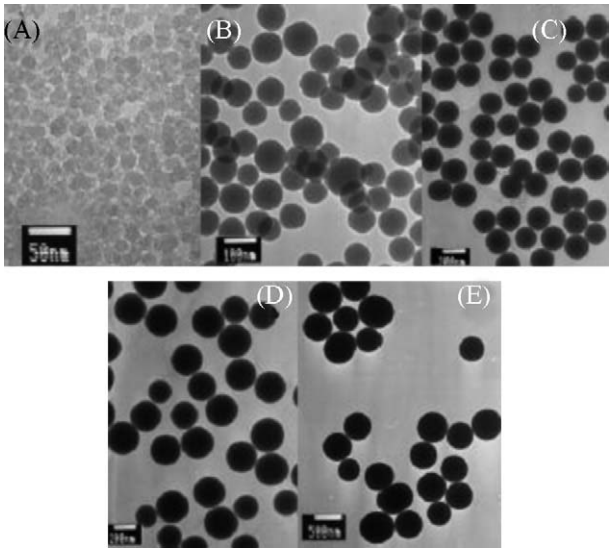

Fig. 1. TEM micrograph of synthesized SiO_2 particles.

the same condition. The anodic peak current produced from the base electrode was proportional to the square root of the potential scan rate in the range of 10–400 mV s^{-1} in the hydrogen dioxide solution (data not showing), indicating that the decomposition of hydrogen dioxide on the electrode was a surface diffusion controlled process. Thus the higher concentration of H_2O_2 will produce higher response current due to higher diffusivity. When the loading of enzyme was more than 5 μl, the response currents tended to be saturated. Farther increase of enzyme loading would be a waste of this expensive reagent. So in the following experiments the enzyme loadings were all 5 μl.

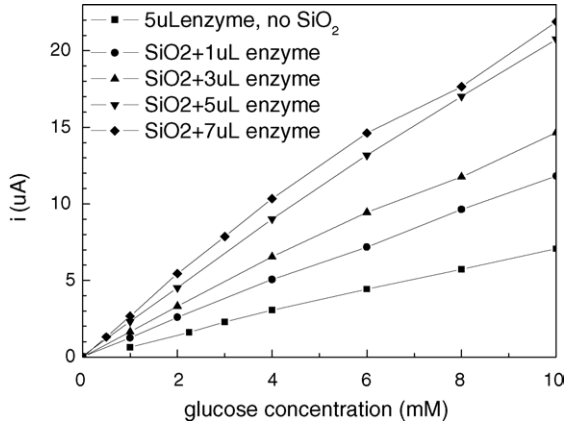

Fig. 2. The response curves of SiO_2 biosensors with different amount of enzyme loading in glucose solution. The underneath one (black square) represent the biosensor without SiO_2 as a comparison.

3.3. Effect of pH

The influence of the buffer pH on the performance of glucose biosensors has been studied by many authors [3,13,22]. 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 [13]. The pH dependence of the sensor modified by 17 nm SiO_2 was evaluated over the pH range from 5.0 to 8.0 as the sensor was immerged into 10 mM glucose solution (Fig. 3). When the pH of the buffer was very low or very high, the GOx electrode exhibited low response current to glucose. An optimum response current could be observed at pH 7.2.

3.4. Cyclic voltammetric behavior of enzyme electrode

CV measurement was done four times as shown in Fig. 4A with a potential scan rate of 100 mV s^{-1}. Except for the first response curve, the curves were almost overlapped, showing very high stability. In the following study, only the fourth response curve of each glucose concentration was shown for briefness. The resulted CV curves with different concentrations of glucose measured by the biosensor modified with 17 nm SiO_2 were shown in Fig. 4B (only the curves of 0, 2 and 10 mM were shown for briefness). The results showed

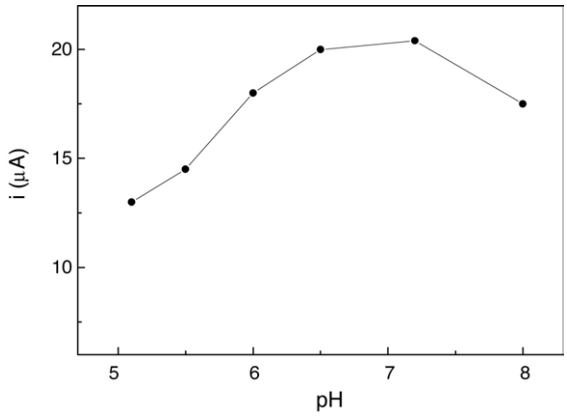

Fig. 3. Effect of pH on the response behavior of the biosensor.

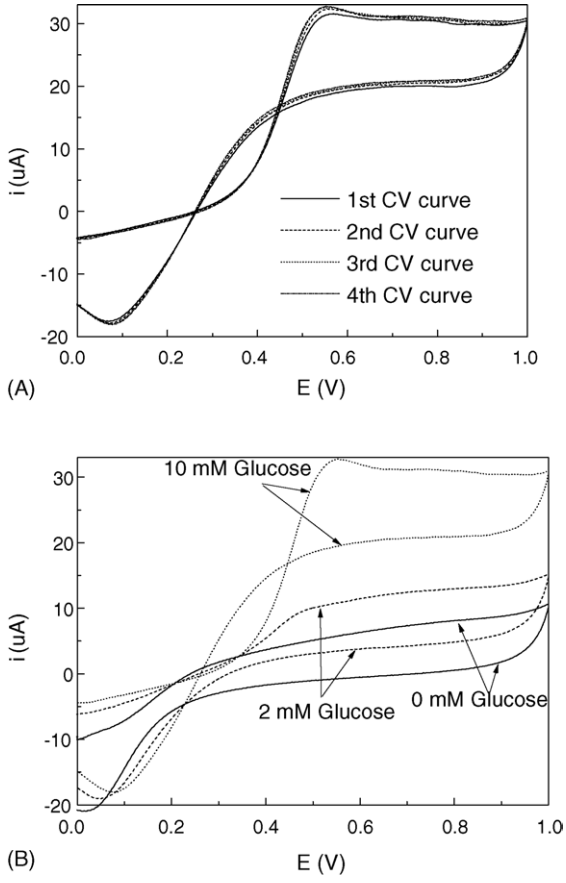

Fig. 4. (A) Four times CV measurements in 10 mM glucose solution of the 17 nm SiO_2 biosensor. Except for the first response curve, the curves were almost overlapped. (B) The CV curves of different concentration of glucose measured by 17 nm SiO_2 biosensor. From down to up, the glucose concentrations were 0, 2 and 10 mM, respectively. The other glucose concentrations were not shown here for clarity. The arrows were guide to the eyes (scan rate: 100 mV s^{-1}).

that the nanoparticles modified electrode held high electron-transfer efficiency. 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. This behavior was particularly apparent at higher glucose concentration. But the electrochemical redox current became stable only when the applied voltage was higher than 0.55 V. With the increase of glucose concentration, the redox current increased monotonously. In fact, after a study of the data of oxidation current or reduction current at 0.6 V versus SCE of each concentration, we found that the oxidation current or reduction current increased linearly with the concentration of glucose. We would show the equivalence of CV measurement method and the chronoamperometric method in the next section.

3.5. Amperometric response of glucose sensor

During chronoamperometric measurements, the working electrode was poised at +0.60 V versus SCE. When the background current was stable, a certain amount of glucose was added into the system with stirring (about 1000 rpm). The response current rapidly reached to a new stable state. Fig. 5 displayed a typical amperometric response curve of a Pt/SiO_2/GOx electrode with the SiO_2 size of 17 nm. A stable and fast amperometric response could be observed with successive injections of glucose into PBS solution. The time required to reach stable response was less than 5 s. The upper inset showed the amperometric response with larger amount of glucose injection (1 mM glucose each time). The response time was still less than 5 s, showing the rapid response character. The lower inset was the calibration plot of the glucose concentration range of 0.02–0.4 mM. The corresponding regression equations of the linear plot were: I (μA) = 0.0097 + 1.993c, R = 0.9997 (n = 11), where c is the glucose concentration in mM. The detection limit was estimated as 1.5 μM (signal-to-noise ratio of 3).

Different stock concentrations of glucose were measured to get the response current curves. The resulting calibration plot for glucose over the concentration range of 0.02–10.0 mM was presented in Fig. 6 (black square). The corresponding equation of the linear plot was: I (μA) = 0.163 + 2.078c, R = 0.9993 (n = 7), where c is the glucose concentration in mM. This relation revealed that such an electrode could work well in glucose concentration range of 0.02–10.0 mM with a sensitivity of 2.078 μA/mM. Fig. 6 also gave the calibration plot of data from CV curves. Each CV curve had two data at 0.6 V, the higher one was the oxidation current and the lower one was the reduction current (Fig. 4). The corresponding equations of the linear plot were: I (μA) = 6.378 + 2.566c, R = 0.9999 (n = 7), and I (μA) = −0.238 + 2.082c, R = 0.9994 (n = 7), respectively, where c is the glucose concentration in mM. The plot of

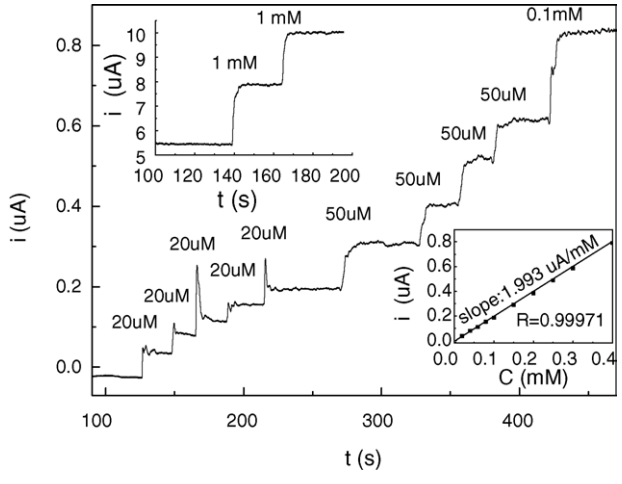

Fig. 5. Amperometric response curve with successive glucose injection of the biosensor modified with 17 nm SiO_2. The applied potential was 0.6 V vs. SCE. The upper inset showed the amperometric response with larger amount of glucose injection. The lower inset showed the calibration plot of the glucose concentration between 20 and 400 μM. The slope of the calibration plot was about 1.993 μA/mM and the correlation coefficient was 0.9997.

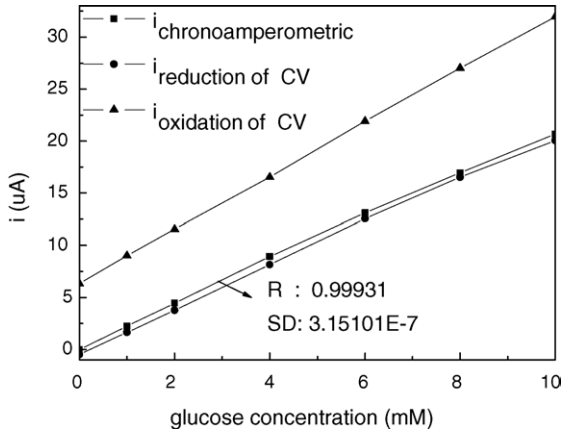

Fig. 6. The response currents of 0, 1, 2, 4, 6, 8 and 10 mM glucose. The black square represented data got from chronoamperometric measurements; the black circles represented the reduction currents of CV curves at 0.6 V vs. SCE whilst the black triangle represented the oxidation currents at the same voltage.

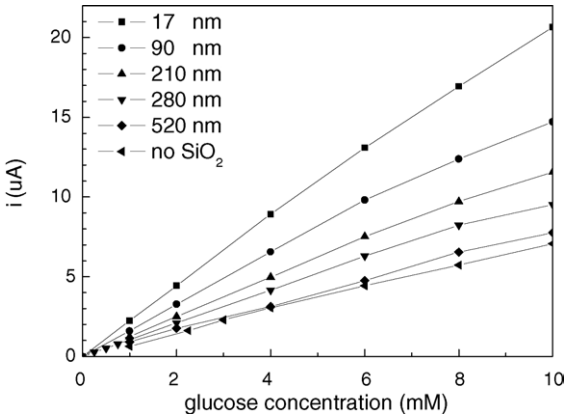

Fig. 7. Response currents of biosensors modified by different sized SiO_2.

reduction currents was almost in the same position compared with the amperometric response data. So the CV measurement here had equivalent effect with chronoamperometric method.

Fig. 7 showed the response current of biosensors modified with different sized SiO_2. The volume of enzyme at each biosensor was 5 μl (about 4 U) and the surface area of each base Pt electrode was 7 mm^2. The response curve of 520 nm SiO_2 modified biosensor was almost the same as the one without SiO_2. This indicated that large particle had very little effect on the performance of the modified biosensor. With the decrease of particle size, the valid surface of the modified electrode increased, and the surface enzyme loading increased, there were more immobilized enzyme could participate in the reaction with glucose. Thus more H_2O_2 gained and increased the response current. On the other hand, the active center of enzyme was buried in the entangled enzyme chain, with the decrease of the particle size, it might create suitable microenvironment which benefits the exposition of the active center, and increases the activity of enzyme [15].

3.6. Reproducibility and long-term stability of the glucose sensor

The preparation of the biosensor was reproducible. A glucose solution (10 mM) was measured with five 17 nm SiO_2 biosensors, which were prepared under the same conditions. The relative standard deviation was below 6%. The long-term stability of the biosensor modified by 17 nm SiO_2 was tested in 10 mM glucose solution. This glucose sensor was used intermittently and stored at 4 °C. After 25 days, it maintained 85% of its original activity and still displayed an excellent response to glucose.

4. Conclusions

In brief, particle size could affect the performance of SiO_2 particles modified glucose biosensors drastically. With the decrease of particle size, the response current increased gradually. The 17 nm SiO_2 modified biosensor had very high performance. Its sensitivity was 2.08 μA/mM and the detection limit was 1.5 μM glucose. Present work provided a rule to construct high performance biosensors simply by using smaller particles. It also proved that the CV measurement was equal to the chronoamperometric method. We believe these two points are suitable for other amperometric biosensor system.

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