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A high performance glucose biosensor enhanced via nanosized SiO_2

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Abstract

A series of monodispersed nano-SiO_2 film enhanced glucose biosensors with different thickness were fabricated by using dip-coating method. The suitable thickness of nanosized SiO_2 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_2 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_2O_2 became difficulty. The electrochemical response of glucose with the 800 nm SiO_2-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 $\mu A\,mM^{-1}\,cm^{-2}$ 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.

Keywords: Glucose biosensor; Monodispersed nano-SiO_2; 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_2 [4], gold [8–11], silver and SiO_2 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_{nano} + 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 $\mu A\,mM^{-1}$ 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 mg^{-1}. 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), β-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 β-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 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 Kα 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 5×10^{-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 μA, respectively. The beam diameter was 1 mm and the sputtering rate was approximately 25.0 nm min^{-1} corrected by a thermally oxidized 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 × 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 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 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 μL drop of glucose oxidase (GOx) solution (4.5 mg mL^{-1}) was dried on the SiO_2 modified Pt electrode. Then 3.5 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 1 layer of PVB by dip-coating method and was washed with PBS. The resulted Pt/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 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-SiO_2 modified electrode. The Pt/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 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 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: ∼1000 rpm). The response current rapidly reached to a new stable state. Fig. 1 displayed a typical amperometric response curve of the Pt/SiO_2/GOx electrode with the 3-layer SiO_2 film. A stable and fast amperometric response could be observed with successive injections of 50 μl 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