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Bioelectrochemistry 70 (2007) 342–347
Bioelectrochemistry
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A simple method to fabricate a chitosan-gold nanoparticles film and its application in glucose biosensor

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Received 11 December 2005; received in revised form 12 April 2006; accepted 2 May 2006
Available online 16 May 2006

Abstract

A novel film of chitosan-gold nanoparticles is fabricated by a direct and facile electrochemical deposition method and its application in glucose biosensor is investigated. $HAuCl_4$ solution is mixed with chitosan and electrochemically reduced to gold nanoparticles, which can be stabilized by chitosan and electrodeposited onto glassy carbon electrode surfaces along with the electrodeposition of chitosan. Then a model enzyme, glucose oxidase (GOD) is immobilized onto the resulting film to construct a glucose biosensor through self-assembly. The resulting modified electrode surfaces are characterized with both AFM and cyclic voltammetry. Effects of chitosan and $HAuCl_4$ concentration in the mixture together with the deposition time and the applied voltage on the amperometric response of the biosensor are also investigated. The linear range of the glucose biosensor is from $5.0\times10^{-5}\sim1.30\times10^{-3}$ M with a Michaelis–Menten constant of 3.5 mM and a detection limit of about 13 μM.

Keywords: Chitosan; Gold nanoparticles; Electrochemical deposition; Glucose oxidase; Biosensor

1. Introduction

Metal nanoparticles, such as silver (Ag) [1], gold (Au) [2–4], platinum (Pt) [5] and palladium (Pd) [6] nanoparticles have attracted much interest in the construction of biosensors due to their unique chemical and physical properties. Gold nanoparticles, in particular, have been widely used to construct biosensors because of their excellent ability to immobilize biomolecules and at the same time retain the biocatalytic activities of those biomolecules. Many kinds of biosensors, such as enzyme sensor [7–9], immunosensor [10] and DNA sensor [11], with improved analytical performances have been prepared based on the application of gold nanoparticles.

Biopolymer chitosan is a polysaccharide derived by deacetylation of chitin. It has primary amino groups that have pK_a values of about 6.3 [12]. At pH below the pK_a, most of the amino groups are protonated, making chitosan a water-soluble polyelectrolyte. When the pH is raised above the pK_a, the amino groups are deprotonated, and chitosan becomes insoluble. Chitosan is inexpensive and displays an excellent film-forming ability, biocompatibility, nontoxicity, high mechanical strength, and a susceptibility to chemical modifications. The stabilization of gold nanoparticles with chitosan has been extensively reported [13,14]. As chitosan in solution is protonated and positively charged, it can be adsorbed onto the surfaces of gold nanoparticles, stabilizing and protecting the nanoparticles, and further construct biosensors [15]. In our previous work, we have developed two kinds of biosensors based on the excellent properties of chitosan and gold nanoparticles [16,17]. Gold nanoparticles, which were prepared in advance through the reduction of $HAuCl_4$ with citrate, can be self-assembly onto electrodeposited chitosan films and then immobilize enzymes effectively. And also they can be mixed with chitosan and enzymes to construct biosensors through simple one-step electrodeposition. However, in both of these systems, gold nanoparticles need to be prepared previously, which prolongs the whole time of biosensor preparation and makes the procedure a bit complicated.

Recently, several methods for the formation of gold nanoparticles on the surface of electrodes directly through the electrochemical reduction of $HAuCl_4$ have been reported. Mena et al. compared different strategies for the construction of amperometric enzyme biosensors using gold nanoparticle-modified

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doi:10.1016/j.bioelechem.2006.05.002

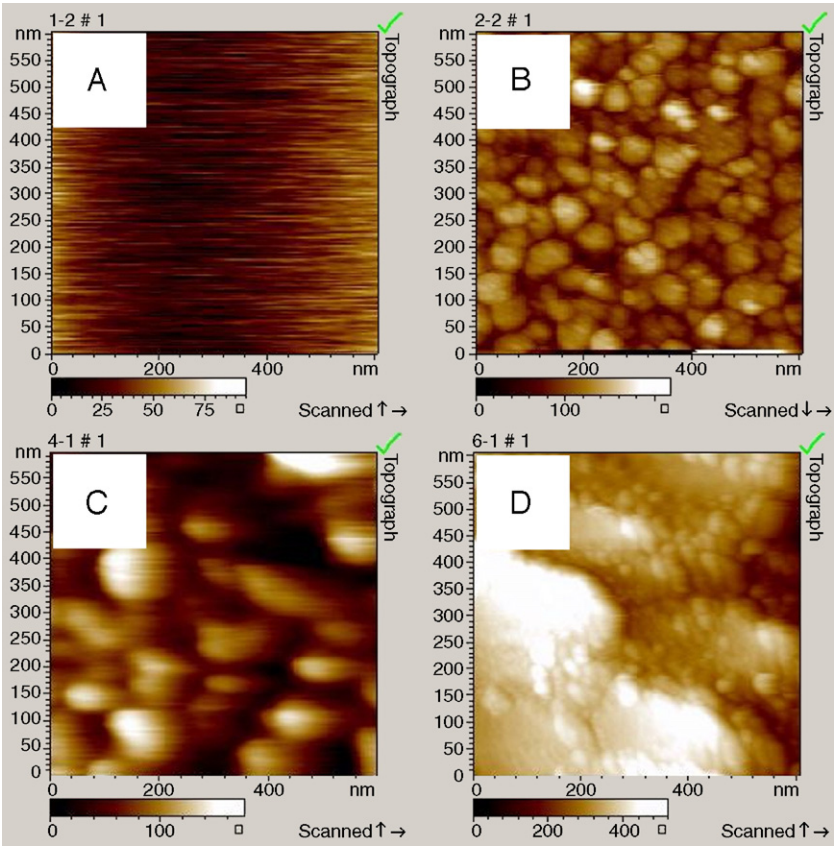

Fig. 1. AFM images of bare gold substrate (A), electrochemically deposited gold nanoparticles (B), chitosan (C), chitosan-gold nanoparticles (D) on gold surfaces.

electrodes [18]. Compton et al. investigated electrochemical detection of As(III) at a gold nanoparticle-modified glassy carbon (GC) electrode which was fabricated by the eletrochemical deposition of Au nanoparticles onto GC [19]. By this means, one can synthesize gold nanoparticles on the surface of electrode directly in a short of time, and the sizes of the nanoparticles can be controlled by different conditions of electrochemical deposition with the advantageous properties being kept.

In this work, we investigated a simple method for fabricating a chitosan film containing gold nanoparticles. $HAuCl_4$ solution is mixed with chitosan and electrochemically reduced to gold nanoparticles directly, and the produced gold nanoparticles were stabilized by chitosan and electrochemically deposited onto the glass carbon electrode under a certain voltage along with chitosan. The whole procedure cost only about 10 min. Then a model enzyme, glucose oxidase (GOD), was assembled on the chitosan-gold nanoparticles modified electrode. Characteristics and performance of both the film and the resulting biosensor were studied in detail.

2. Experimental

2.1. Reagents and apparatus

Chitosan from crab shells (85% deacetylated) and GOD (E. C. 1.1.3.4,type II-S, units g^{-1}) were purchased from Sigma. $HAuCl_4 \cdot 4H_2O$ (Au%>48%) was obtained from Aldrich. Chitosan solution was prepared by adding chitosan flakes to water and gradually dropping 2.0 M HCl to the solution to maintain the pH near 3, and then filtered. The filtrate was adjusted to about pH 5.0 using 2.0 M NaOH. Glucose stock solutions were allowed to mutarotate overnight at room temperature before use. All other reagents were of analytical grade and commercially available. Twice-distilled water was used throughout.

All electrochemical measurements were performed on a CHI 750A electrochemical workstation (Shanghai Chenhua Apparatus

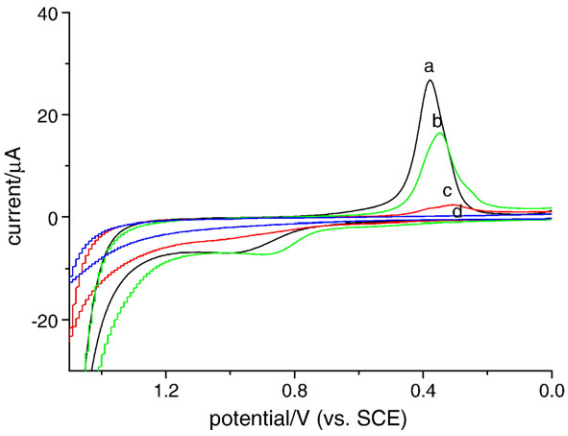

Fig. 2. Cyclic voltammogram at a gold electrode (a), gold nanoparticles (b) and chitosan-gold nanoparticles film (c) modified GC and bare GC (d) electrodes at a scan rate of 50 mV s^{-1} in 0.1 M pH 7.4 PBS. The chitosan-gold nanoparticles film was electrodeposited from 4.0 ml 0.04 wt.% chitosan solution (250 mg/L $HAuCl_4$) for 4 min.

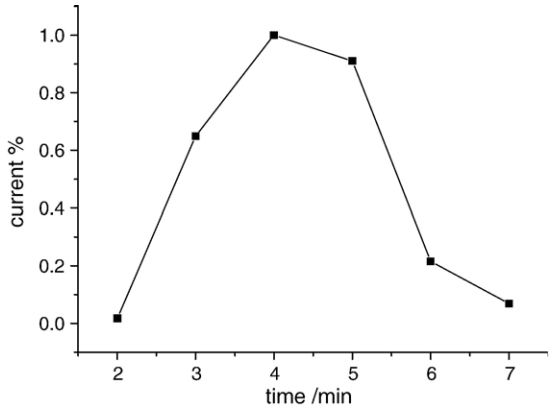

Fig. 3. The effect of electrodeposition time on the resulted biosensor response to 1.0 mM glucose in pH 7.4 PBS. Electrodeposition conditions: chitosan solution containing 200 mg/L $HAuCl_4$, electrodeposition time 60–420 s, applied potential −2.0 V.

Corporation, China). All experiments were carried out using a conventional three-electrode system with a glass carbon (GC) disk electrode (2.0 mm diameter) as a working electrode, a platinum foil as an auxiliary electrode, and a saturated calomel electrode as a reference electrode. Before each modification, glass carbon disk electrodes were abraded with fine SiC paper and polished carefully with 0.3 and 0.05 μm alumina slurry and sonicated in water and absolute ethanol. Cyclic voltammetric experiments were carried out in quiescent solutions with the scan rate of 50 mV s^{-1}. Amperometric experiments were carried out under stirred phosphate buffer solution (PBS, 0.1M, pH 7.4) with an applied potential of 0.70 V at ambient laboratory temperature (25±2 °C). Atomic force microscopy (AFM) studies were performed in ambient conditions using a Molecular Imaging Pico SPM in tap mode with a 10 μm scanner.

2.2. Procedure

Electrochemical deposition of chitosan-gold nanoparticles and immobilization of GOD.

The electrochemical deposition of chitosan-gold nanoparticles onto the electrode surface was performed following a potentiostatic procedure with a conventional three-electrode system. The polished GC was immersed into solution of chitosan–$HAuCl_4$ and a fixed potential in the range of −0.5–−3 V was applied for a certain time, depending on the experiments. Chitosan-gold nanoparticles film was deposited on the GC electrode surface as a result. After deposition, the electrode was removed from the solution and rinsed with water, and then the electrode was dried in air at room temperature for about 3 h. After that, the modified electrode was immersed in 10 mg ml^{-1} GOD for 10 h at 4 °C. For comparison, the enzyme electrode without gold nanoparticles or chitosan was prepared similarly, but in the absence of $HAuCl_4$ or chitosan. All the resulting electrodes were stored at 4 °C when not in use.

Gold nanoparticles, chitosan and chitosan-gold nanoparticles films electrochemically deposited on gold slides in corresponding solutions were used to study the morphologies by AFM.

3. Results and discussion

3.1. Electrochemical deposition of chitosan-gold nanoparticles

In our previous work [16,17], we added gold nanoparticles to the chitosan solution while stirring and found that they can be evenly distributed within the solution without aggregation. Actually, the mixture of chitosan and gold nanoparticles can be stored for more than 1 month, which means chitosan is a good stabilizer for gold nanoparticles. Based on this fact, we investigate the preparation of gold nanoparticles through electrochemical reduction of $HAuCl_4$ in the presence of chitosan, which is expected to stabilize the produced gold nanoparticles, and at the same time chitosan was electrodeposited onto electrodes surface to form a film containing gold nanoparticles.

Fig. 1 exhibits the AFM images of the bare gold and the substrate modified with different materials: chitosan film, gold nanoparticles, and chitosan-gold nanoparticles film, respectively. As can be seen from Fig. 1B, gold nanoparticles are obviously formed on the gold substrate, which manifested that $HAuCl_4$ can be electrochemically reduced to gold nanoparticles. The size of the formed gold nanoparticles is about 50 nm, and the roughness of the formed gold nanoparticles layer is about 10 nm. The electrodeposited chitosan film (Fig. 1C) shows a porous structure, and the roughness of the formed chitosan layer is about 10 nm, which is dependent on the applied voltage, the deposition time, and the chitosan concentration as reported previously [20]. It can be seen from Fig. 1D that the produced gold nanoparticles are homogenously distributed within the electrodeposited chitosan film, while the size of the nanoparticles is about 40 nm, which is smaller than that of Fig. 1B. Simultaneously, the surface of the film is rougher than both of the chitosan and gold nanoparticles films. The reason of the difference can be attributed to the synthesis of the gold nanoparticles on the surface of electrode. It is well known that the free amino groups in chitosan contribute polycationic, chelating, and film-forming properties, along with ready solubility in weak acid. It is similar in behavior to hydrophilic polymers that can adsorb on metal particle surfaces and form complexes [14]. In

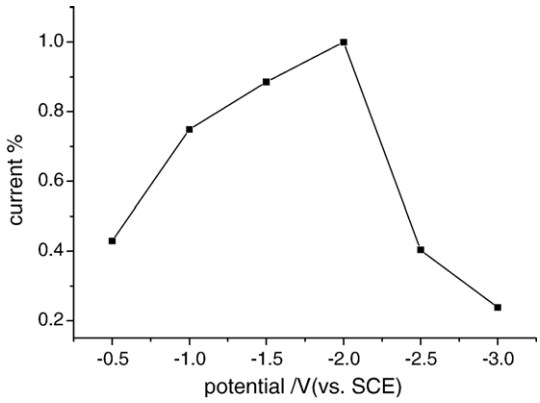

Fig. 4. The effect of applied potential on the resulted biosensor response to 1.0 mM glucose in pH 7.4 PBS. Electrodeposition conditions: 0.05% chitosan solution containing 200 mg/L $HAuCl_4$, electrodeposition time 240 s, applied potential is from −0.5–−3.0 V.

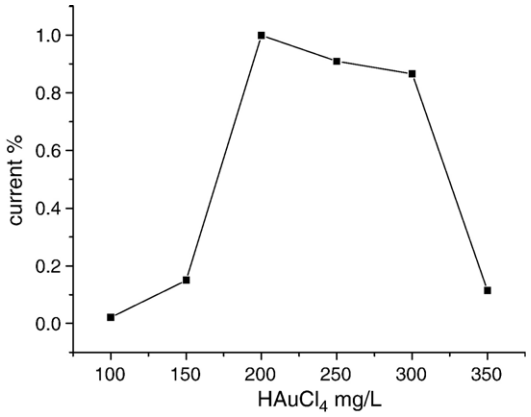

Fig. 5. The effect of $HAuCl_4$ concentration on the resulted biosensor response to 1.0 mM glucose in pH 7.4 PBS. Electrodeposition conditions: 0.04% chitosan containing 100–350 mg/L $HAuCl_4$, electrodeposition time 240 s, applied potential − 2.0 V.

the presence of chitosan, the produced gold nanoparticles will be covered by chitosan and thus prevent it from growing bigger, as chitosan can be readily adsorbed onto gold nanoparticles. Moreover, chitosan adsorbed onto gold nanoparticles can also stabilize the formed gold nanoparticles, and along with the electrodeposition of chitosan, gold nanoparticles can be entrapped into the chitosan film.

3.2. Cyclic voltammogram of the modified electrode

Fig. 2 shows the cyclic voltammogram (CV) of the gold electrode, bare GC electrode, gold nanoparticles, chitosan-gold nanoparticles modified GC electrodes in the 0.1 M pH 7.4 PBS. From Fig. 2d, we can see that the CV of the bare GC has no peak from 0–1.2 V. But from Fig. 2a, b, c we can see the obvious redox peaks of gold at about 0.9 V and 0.3–0.4 V. For the bare gold electrode, the accurate value of redox is 1.008 V and 0.379 V (Fig. 2a). For gold nanoparticles modified GC electrode, the value of the redox peaks of gold is 0.900 V and 0.348 V(Fig. 2b). For chitosan-gold nanoparticles modified GC electrode (Fig. 2c), the value of the reduction peak turns to 0.310 V. It can be seen that the Ep value is more and more positive along with the sizes of gold particles decrease. Simultaneously the peak current of which decreases sharply compared to the gold nanoparticles modified electrode which may be due to the formed chitosan film. These results manifest that gold is present in the modified GC electrode, which resulted from the reduction of $HAuCl_4$, and they exist in the form of gold nanoparticles as verified with AFM above.

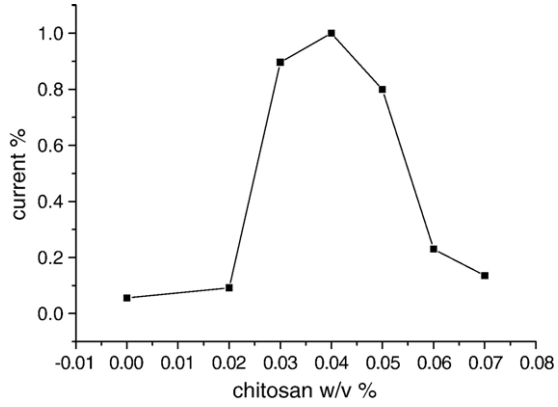

Fig. 6. The effect of chitosan concentration on the result biosensor response to 1.0 mM glucose in pH 7.4 PBS. Electrodeposition conditions; chitosan solution in different concentration containing 200 mg/L $HAuCl_4$, electrodeposition time 240 s, applied potential −2.0 V.

3.3. Optimization of chitosan-gold nanoparticles biosensor performance

It has been reported that the thickness of the deposited chitosan film and the amount of gold nanoparticles were dependent on the applied voltage, the deposition time and the chitosan concentration, and on the other hand, the area of the gold surface is correlated to the concentration of the $AuCl_4^-$ solution, deposition time and the deposition voltage. In this section, we explored the effect of the four deposition conditions on the response of the biosensor.

First, the effect of deposition time on the properties of the deposited film is studied (Fig. 3). The time we choose is 2, 3, 4, 5, 6, 7 min respectively which is from a 0.05% w/v chitosan solution containing 250 mg/L $HAuCl_4$ with the applied voltage of −2.0 V. The response current increases with the increase of deposition time and reaches the maximum when the deposition time is 4 min. Further increasing the deposition time, the response current decreases due to the thicker film-forming.

Second, we explored the effect of deposition voltage (Fig. 4). The deposition voltage is −0.5, −1.0, −1.5, −2.0, −2.5, −3.0 V respectively which is from a 0.05% w/v chitosan solution containing 250 mg/L $HAuCl_4$ with the deposition time of 4 min. Similarly, the response current increases with the increase of deposition voltage and reaches the maximum when the deposition voltage is −2.0 V, then decreases.

Third, we studied the effect of the concentration of $HAuCl_4$ and chitosan solution (Figs. 5, 6). The response currents reach the maximum when 200 mg/L $HAuCl_4$ or 0.04% chitosan solution is chosen. On the whole, we can see that the response of the biosensor increases firstly and then decreases with the increase

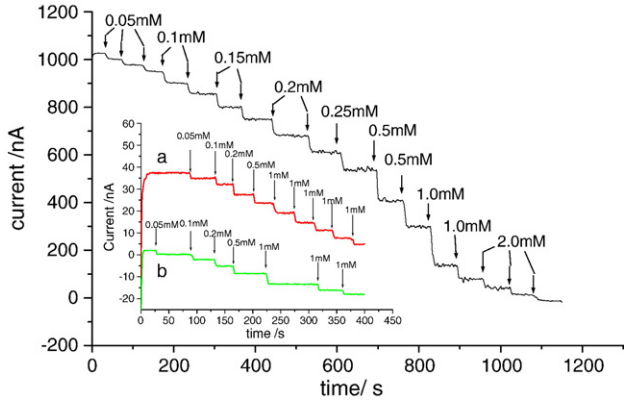

Fig. 7. Successive amperometric response of the chitosan-gold nanoparticles-GOD electrode to glucose in 0.1 M PBS (pH 7.4) at 0.7 V. The inset a and b is the successive amperometric response of the chitosan-GOD and gold nanoparticles-GOD electrodes respectively. The glucose addition each time is from 0.050 to 2.0 mM as indicated.

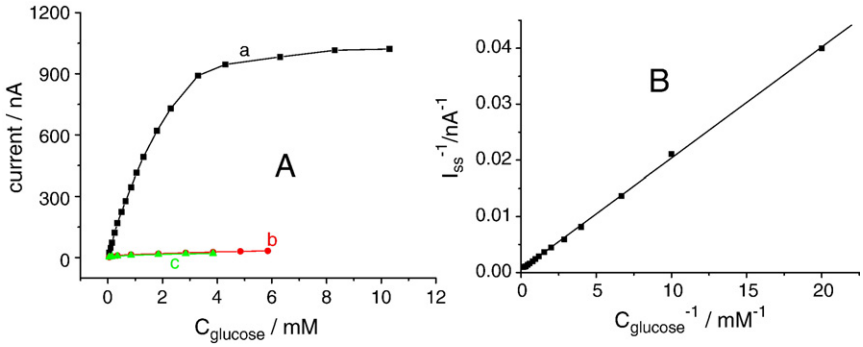

Fig. 8. A: Calibration curves of the biosensors based on (a) chitosan-gold nanoparticles film, (b) chitosan and (c) gold nanoparticles. B: The Lineweaver–Burk plot of $1/i_{ss}$ vs. $1/C$ for the biosensor based on chitosan-gold nanoparticles film.

of chitosan and $HAuCl_4$ concentrations. So for the deposited conditions we finally choose 0.04% w/v chitosan solution containing $HAuCl_4$ 200 mg/L with the applied voltage −2.0 V and the deposition time of 4 min.

3.4. Effect of chitosan and gold nanoparticles on the enzyme immobilization

Fig. 7 is the typical steady-state responses of the GC electrode modified with chitosan film (insert a) and gold nanoparticles (insert b) and chitosan-gold nanoparticles at the working potential of 0.7 V. Compared with the electrodes modified with mono-component, we can see that the response of the GC electrode modified with chitosan-gold nanoparticles is much larger than that of the electrodes modified with only chitosan or gold nanoparticles and also the linear range is much wider. It indicates that when there is no gold nanoparticles or chitosan, the deposited chitosan or gold nanoparticles can only adsorb a small quantity of GOD and results in a small response to glucose. In other words, the advantages of the chitosan-gold nanoparticles film is that the electrochemically generated gold nanoparticles are stabilized by chitosan and evenly dispersed within the chitosan film, which forms an excellent platform for enzyme immobilization combining the advantages of chitosan and gold nanoparticles. On the other hand, during the process of the film forming, the gold nanoparticles reduced on the electrode surface improves the condition of the electrode surface, which induces chitosan to be deposited with the nanoparticles easily, simultaneously increase the effective area surface. That is to say, the resulting film don't permit only to increase the retention of GOD on the electrode surface, but also to increase the effective area surface when the chitosan-gold nanoparticles are used. The two aspects make the chitosan-gold nanoparticles film better than each of them used alone on the detection of glucose.

3.5. Amperometric determination of glucose with the biosensor

From Fig. 7, we can see that a subsequent addition of glucose to the stirring PBS provokes a remarkable increase in the oxidation current at the biosensor based on chitosan-gold nanoparticles, and the time required to reach the 95% steady state response is within 10 s. Fig. 8A shows the calibration curves of the three kinds of enzymes. The proposed biosensor presents a linear response to glucose concentration within the range from 50 μM to 1.30 mM ($r=0.996$, $n=10$) with a detection limit of 13 μM at a signal-to-noise ratio of 3.

When glucose concentration is higher than 3.0 mM, a plateau current was observed, showing the characteristics of the Michaelis–Menten kinetics. The apparent Michaelis–Menten constant (K_m^{app}) can be calculated from the electrochemical version of the Lineweaver–Burk plot [21]:

$$\frac{1}{i_{ss}} = \frac{K_m^{app}}{i_{max}}\frac{1}{C} + \frac{1}{i_{max}} \quad (1)$$

where i_{ss} is the steady-state current after the addition of substrate, i_{max} is the maximum current measured under saturated substrate condition, and C is the bulk concentration of the substrate. The K_m^{app} value, which gives an indication of the enzyme substrate kinetics for the biosensor, was determined by an analysis of the slope and intercept for the plot of the reciprocals of the steady-state current vs. glucose concentration. The Lineweaver–Burk plot of $1/i_{ss}$ vs. $1/C$ for the biosensor based on chitosan-gold nanoparticles film was shown in Fig. 8B. The K_m^{app} value for the biosensor was estimated to be 3.5 mM, which is much smaller than the biosensor prepared by sol-gel organic–inorganic hybrid material (20 mM) [22] and smaller than that biosensor prepared by covalent attachment of glucose oxidase to an Au electrode modified with gold nanoparticles (4.3 mM) [23]. The smaller K_m^{app} value means that the immobilized GOD possesses higher enzymatic activity and the proposed electrode exhibits a higher affinity to glucose.

The reproducibility of the proposed biosensor has also been studied. The relative standard deviation (RSD) of the biosensor response to 0.10 mM glucose was 3.3% for 10 successive measurements.

4. Conclusion

This study describes a fast and easy way for the fabrication of chitosan-gold nanoparticles film using electrochemical deposition method. We can control the characteristics of the film by controlling the deposition condition, and the whole procedure costs only several minutes. In addition, the resulted chitosan film

containing gold nanoparticles can be used to construct biosensor through assembling enzymes on the surface of the film, and the immobilized enzymes keep good bioactivity. The film fabrication technique demonstrated in this work and its application on biosensor are readily applicable to the fabrication of other chitosan-metal nanoparticles film and biosensors based on biomaterial.

Acknowledgment

The financial supports from the National Natural Science Foundation of China (No. 20575029, 90206037, 20521503, 20435010) and the key project of the Science and Technology Research of MOE are gratefully acknowledged.

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