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Electrochemically deposited nanocomposite film of CS-Fc/Au NPs/GOx for glucose biosensor application

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ABSTRACT

A simple and controllable electrodeposition method is described to fabricate a homogeneous chitosan-ferrocene/Au nanoparticles/glucose oxidase (CS-Fc/Au NPs/GOx) nanocomposite film. The morphologies and electrochemistry of the nanocomposite film were investigated by using scanning electron microscopy (SEM) and electrochemical techniques including electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV), respectively. The ferrocene group (Fc) covalently bounded chitosan hybrid (CS-Fc) can effectively avoid the leakage of Fc and retain its electrochemical activity. Further immobilization of Au NPs into the CS-Fc matrix enhances both the charge-transport properties of the composite and bioaffinity to enzyme. Biosensor based on this CS-Fc/Au NPs/GOx film has advantages of fast response, excellent reproducibility and high stability. This biosensor shows a linear response to glucose in the concentration range from 0.02 to 8.66 mM with a detection limit of 5.6 μM at a signal-to-noise ratio of 3. The present method offers a facile way to fabricate biosensors and bioelectronic devices.

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1. Introduction

Amperometric biosensors based on glucose oxidase (GOx), the most effective tool for the determination of glucose, are of great importance in chemical, biological, clinical, and many other fields (Lopez-Avila and Hill, 1997; de Melo et al., 2002; Xu et al., 2002; Terry et al., 2005). One of the key issues in developing sensitive and stable amperometric biosensor is the effective immobilization of enzymes. An ideal immobilization process should be cheap, quick, enzyme friendly, and be applicable to a large range of biomolecules (Jia et al., 2007). With regard to the matrix for enzyme immobilization, an important requirement is their biocompatibility and inertness, as a result, the native structure of the enzyme is not interfered and the biological activity of the enzyme remains unchanged. Therefore, polysaccharide-based supports are the most common choice for the construction of biosensors (Cosnier et al., 2002; Jawaheer et al., 2002). Chitosan (CS), a polysaccharide biopolymer, displays a remarkable combination of physicochemical properties including an excellent membrane-forming ability, good biocompatibility, high permeability towards water, good adhesion, non-toxicity, and high mechanical strength. These properties make CS a promising matrix for enzyme immobilization (Ng et al., 1998; Wang et al., 2003; Cruz et al., 2000; Qian and Yang, 2006; Wang et al., 2005b). Moreover, a large number of CS's hydroxyl and amino groups are reaction sites for chemical modifications and suitable for anchoring a large variety of organometallic complexes, which makes CS a good candidate as a precursor for molecular immobilization (Vincent and Guibal, 2003; Alonso-Sande et al., 2006; Abd-El-Aziz, 2002). Ferrocene (Fc) and its derivatives have been used as mediators in the development of enzyme-based biosensors (Chuang et al., 1997; Sulak et al., 2006; Deng et al., 2007; Hodak et al., 1997), due to their properties of relatively low molecular mass, good electrochemical reversibility and stability at low potential (Fernandez and Carrero, 2005; Qiu et al., 2007a,b). However, the Fc and its derivatives modified electrodes are unstable and difficult to be controlled because of their weak adsorption on electrode surface. Covalent attachment of Fc group to the CS longer chain could offer a way for building novel redox active hybrid, which combines redox activity and biocompatibility, opening wide application in biocatalysis and biosensors. This strategy can effectively prevent leakage of Fc from the matrix and retain the biocompatibility of CS (Wu et al., 2006; Yang et al., 2007). In particularly, the modification of polymers with electron-transfer mediators constitutes a simple, cheap and effective strategy for constructing amperometric biosensors (Zhang and Gorski, 2005a,b).

However, the application of CS biopolymer in the development of enzyme-based biosensors is still very limited due to its poor conductive properties. An effective solution is the introduction of gold nanoparticles (Au NPs) into polymer matrices (Kang et al., 2007; Du et al., 2007a; Luo et al., 2004; Tangkuaram et al., 2007; Wang and Wang, 2004; Daniel and Astruc, 2004). The immobilization of

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Au NPs can be realized via residual amino groups in CS, which provides a natural environment for bimolecular immobilization, thus the bioactivity and natural conformation of the immobilized biomolecules can be retained. On the other hand, the immobilized Au NPs can enhance the electrical conductivity of the CS-based hybrid films, increasing the sensitivity of biosensor (Xue et al., 2006).

Recently reported methods in construction of enzyme-based electrochemical biosensors using CS are simply coating a mixture of enzyme and CS solution on electrodes (Du et al., 2007b; Xu et al., 2006). Obviously, the formed enzyme layer usually lacks of favorable microstructures, and the enzyme molecules immobilized by physical or chemical adsorption also have to be confronted with the problem of enzyme leakage, leading to poor stability of the biosensors. Therefore, it is clear that development of controllable techniques for fabrication of CS enzyme-modified electrodes is desirable. Electrochemical deposition has been reported as an effective method for enzyme immobilization (Jia et al., 2007; Wang et al., 2005a) and CS film formation (Wu et al., 2002; Fernandes et al., 2003). The electrochemically deposited composite films tightly attach to the electrode surface. In this method, other substances such as Au NPs (Bharathi et al., 1999) and redox mediators (Wu et al., 2006) can be effectively incorporated into the film to form biocomposites. In addition, this electrochemical method is facile and versatile, and it is suitable for selective deposition of films with controllable thickness.

Herein, we report a simple electrochemical approach to controllable fabrication of a biosensor with CS-Fc/Au NPs/GOx biocomposite film. Due to the excellent biocompatibility and nontoxicity of CS-Fc and Au NPs, the composite film provided a biocompatible microenvironment around the enzyme and maintained the high biological activity of the immobilized biomolecules. The morphology and the electrochemistry of the composite film were imaged by scanning electron microscope and electrochemical techniques such as electrochemical impedance and voltammetry, respectively. The results showed that this immobilization technique was effective in preventing the leakage of both enzyme and mediator. Furthermore, the Au NPs improved the conductivity of biocomposite film and facilitated electron transfer. Thus, the present biosensor exhibited good analytical performances such as sensitivity, precision, accuracy, response time, stability and reproducibility towards the quantification of glucose.

2. Experimental

2.1. Chemicals and reagents

Glucose oxidase (GOx, from *Aspergillus niger*, EC 1.1.3.4. 150,000 units g^{-1}), β-D(+)-glucose, chitosan (CS, 85% deacetylation), sodium cyanoborohydride ($NaCNBH_3$, 95%), ferrocenecarboxaldehyde (FcCHO, 98%) and hydrogen tetrachloroaurate ($HAuCl_4{\cdot}4H_2O$) were purchased from Sigma–Aldrich (St. Louis, USA) and used as received. Au NPs were prepared according to the literature (Siebrands et al., 1993; Qiu et al., 2007c), the particle size was about 12 nm. All other chemicals were of analytical grade and used without further purification. All the solutions were prepared using doubly distilled water.

2.2. Preparation of Fc covalently bound CS derivatives

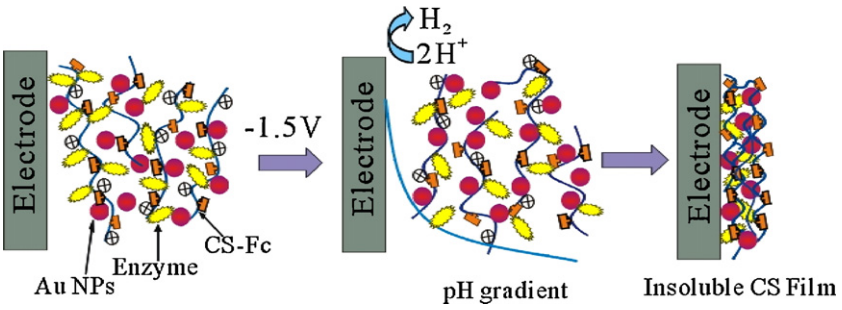
Fc covalently bound CS was prepared according to the reported method with a little modification (Yang et al., 2007). Briefly, CS (75.0 mg) was dissolved in 0.1 M acetic acid solution (15 mL). FcCHO (20 mg) was dissolved in methanol (15 mL) and added to the CS solution. After the mixture was stirred at room temperature for 2 h, $NaCNBH_3$ (100 mg) was dropped and the reaction mixture was stirred for another 24 h. The reaction was quenched by precipitation with 5% NaOH and the yellow product was exhaustively washed with ethanol and water. The product was dried in air and finally redispersed in 0.2 M acetate buffer (pH 5.0) using sonication.

2.3. Electrochemical deposition of CS-Fc/Au NPs/GOx biocomposite film

Glassy carbon electrode (GCE, 3 mm diameter, CH Instruments, Inc.) was wet polished down to a mirror-like finish with 1.0, 0.3 and 0.05 μm alumina slurry, respectively, and rinsed thoroughly with doubly distilled water between each polishing step. The electrode was successively sonicated in 1:1 nitric acid, acetone and doubly distilled water, and then allowed to dry at room temperature. The polished GCE was immersed in the deposition solution containing 2.0 mL CS-Fc (0.5 mg mL^{-1}), Au NPs (0.06 mg mL^{-1}) and GOx (4.0 mg mL^{-1}), and a constant potential of −1.5 V was applied for 120 s. At this potential, H^+ is reduced to H_2 at the electrode. This reaction leads to increased local pH value in the vicinity of the electrode surface. When the pH was higher than 6.3, CS became insoluble, and CS hydrogel incorporated Au NPs and enzyme were electrodeposited at the electrode surface (Fernandes et al., 2003; Luo et al., 2005; Yi et al., 2005). The obtained film was denoted as CS-Fc/Au NPs/GOx/GCE. The procedure of CS-Fc, Au NPs and enzyme deposition is illustrated in Scheme 1. The modified electrode was removed from the solution and rinsed with water, then dried in air at room temperature for about 3 h. For comparison, enzyme electrode without Au NPs was also prepared using the same procedure. All the resulting electrodes were stored at 4 °C when not in use.

2.4. Characterization

Scanning electron microscopy (SEM) images were collected on a Quanta 200 scanning electron microscope (FEI, USA). The accelerating voltage was 20 kV. Fourier transform infrared spectra (FTIR) were recorded on a Nicolet 5700 FTIR spectrometer (Nicolet, USA). All the electrochemical experiments were performed on an Autolab PGSTAT30 electrochemical workstation (Eco Chemie, Netherlands).



Scheme 1. Illustration of the electrochemical fabrication of CS-Fc/Au NPs/GOx/GCE.

A three-electrode system was used including an Ag/AgCl (3 M KCl) reference electrode, a platinum wire auxiliary electrode, and the modified electrode as the working electrode. The electrolyte solutions were purged with N_2 for at least 10 min to remove O_2 and kept under N_2 atmosphere during measurements. The impedance spectra were recorded within the frequency range of 10^{-2} to 10^6 Hz. The amplitude of the applied sine wave potential in each case was 5 mV.

3. Results and discussion

3.1. Morphology of the CS-Fc/Au NPs/GOx biocomposite film

CS is positively charged in acidic conditions, it can be mixed with negatively charged Au NPs to form a stable solution for electrodeposition. When the solution pH is higher than 6.3, CS became insoluble (Bharathi et al., 1999). The behavior allows CS to be deposited onto electrodes by increasing the local pH at conducting substrates. At a constant electrodeposition potential of −1.5 V, H^+ is electrochemically reduced to H_2 at the cathode, gradually increasing the local pH near the electrode surface, thus CS-Fc became insoluble to be deposited on the electrode surface with entrapped Au NPs. In this process, the evolved hydrogen bubbles could on the other hand function as dynamic template for porous structured film formation (Jia et al., 2007). Fig. 1A shows the SEM image of the electrodeposited CS-Fc/Au NPs film. As expected, the hybrid film displayed a three-dimensional structure, and Au NPs were uniformly dispersed in the hybrid film without obvious aggregation, indicating that a nanocomposite film composed of CS-Fc and Au NPs was formed through one-step electrodeposition process. When GOx was added sequentially to CS-Fc/Au NPs solution, GOx molecules were immobilized on the CS-Fc/Au NPs chains by the strong interactions between Au NPs and amine groups in the enzymes (Gole et al., 2001). This behavior allows CS-Fc/Au NPs incorporated with GOx to be deposited onto electrodes by increasing the local pH at conducting substrates. Fig. 1B shows the SEM image of the CS-Fc/Au NPs/GOx film. The formed CS-Fc/Au NPs/GOx membrane retained the three-dimensional structure on the electrode surface. It can be noted that GOx was well-fixed and regularly distributed in the CS-Fc/Au NPs/GOx hybrid film along with Au NPs, no aggregation of them was observed. However, when a film was constructed simply by manual dropping CS-Fc/Au NPs/GOx solution on electrode surface (Fig. 1C), three-dimensional structure of the film was not observed. These results suggested that the electrochemical deposition technique provided a simple and controllable way to construct three-dimensional CS films. The three-dimensional structure of the composite films can provide a friendly microenvironment for GOx incorporation and make the immobilized enzyme

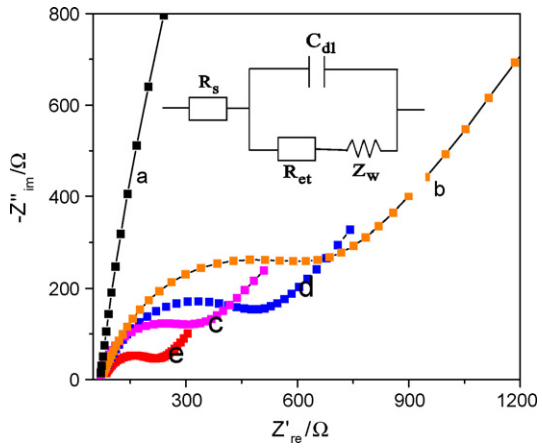


Fig. 2. The electrochemical impedance spectra of different electrodes: (a) bare GCE, (b) CS/GCE, (c) CS-Fc/GCE, (d) CS-Fc/GOx/GCE, (e) CS-Fc/Au NPs/GOx/GCE. *Inset*: Equivalent circuit used to model impedance data in the presence of redox couples. Supporting electrolyte, 0.1 M PBS (pH 7.0) + 0.1 M KCl + 5.0 mM $Fe(CN)_6^{4-/3-}$ solution.

easy to be accessed by its substrate as compared to the planar film. Moreover, the uniformly distributed Au NPs could provide a conductive pathway of electron-transfer. Therefore, the present three-dimensional nanocomposite film is more favorable for the construction of biosensors.

3.2. Electrochemical impedance characterization

Electrochemical impedance spectroscopy (EIS) is an effective method for probing the interfacial properties of modified electrodes and often used for understanding chemical transformations and processes associated with the conductive supports (Dong et al., 2001; Bardea et al., 2000; Feng et al., 2000). The semi-circle diameter in the impedance spectrum is equal to the electron-transfer resistance, R_{et}, which controls the electron-transfer kinetics of the redox probe at the electrode interface. Fig. 2 shows the EIS results of a bare GCE, CS/GCE, CS-Fc/GCE, CS-Fc/GOx/GCE, and CS-Fc/Au NPs/GOx/GCE in the presence of redox probe $Fe(CN)_6^{4-/3-}$. They were measured at the formal potential of the electrochemical probe. It could be seen that the bare electrode exhibited an almost straight line, characteristics of a diffusion limiting process (Fig. 2a). After deposition a layer of CS (Fig. 2b), the EIS of the modified electrode showed a higher interfacial resistance (650 Ω), which indicates the successful formation of CS film. For the film of CS-Fc (Fig. 2c), R_{et} decreased to 330 Ω, which is smaller than that of the CS film due to the increased conductivity of CS-Fc film and facilitated electron-transfer rate. However, R_{et} increased to 500 Ω after

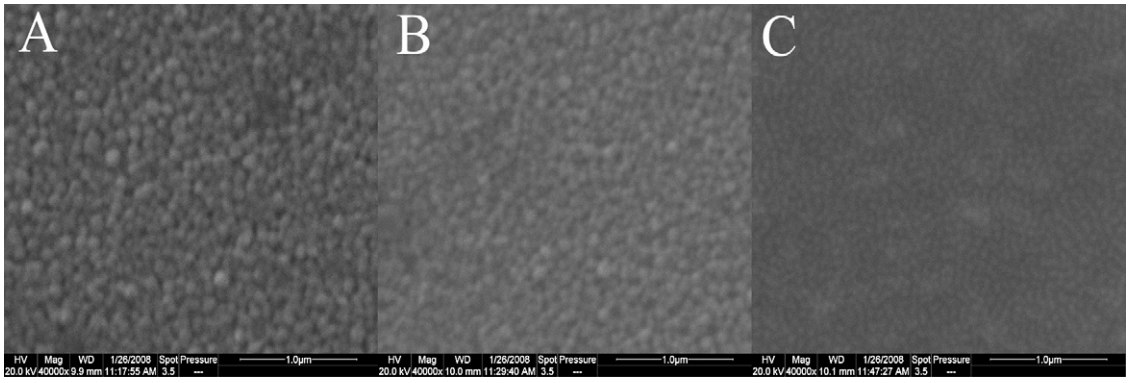


Fig. 1. Typical SEM images of (A) CS-Fc/Au NPs, (B) CS-Fc/Au NPs/GOx films obtained by electrodeposition method and (C) CS-Fc/Au NPs/GOx film obtained by dropping method.

electrodeposition of CS-Fc/GOx film (Fig. 2d). This was attributed to the immobilization of GOx on the CS-Fc/GOx film that hinders the access of the redox probe to the electrode. When Au NPs was added, the R_{et} of the resulted CS-Fc/Au NPs/GOx film surprisingly decreased to 230 Ω (Fig. 2e), indicating that the Fc and Au NPs form high electron conduction pathways between the electrode and electrolyte, and also promote the electron-transfer of the redox probe. To give more detailed information about the impedance of the modified electrode, a modified Randles equivalent circuit (inset Fig. 2) was chosen to fit the measured results. The two components of the scheme, R_s and Z_w, represent the bulk properties of electrolyte solution and diffusion of the applied redox probe, respectively. The other two components of the circuit, C_{dl} and R_{et}, depend on the dielectric and insulating features at the electrode/electrolyte interface. The EIS change of the modified process indicated that the CS-Fc/Au NPs/GOx nanocomposite film was successfully co-electrodeposited on electrode surface.

3.3. Electrochemical behavior of the modified GCE

Cyclic voltammograms (CVs) of the CS-Fc/Au NPs/GOx film electrode showed well-defined redox couple at 0.303 and 0.348 V at 50 mV/s (Fig. 3), which was attributed to the reduction and oxidation of the immobilized Fc group. The peak-to-peak separation (ΔE) was 45 mV and nearly independent of the scan rates, which shows that the electron-transfer is relatively fast and reversible. In addition, the CS-Fc/Au NPs/GOx modified electrode was found to be extremely stable during continuous potential cycling between 0 and 0.6 V for 1 h. These results indicated that the covalently bounded Fc in CS chain could prevent mediator from leakage efficiently. The linearity of i_p against $\nu^{1/2}$ plot (inset Fig. 3) suggested the electron-transfer model of Randles–Sevcik, which assumes semi-infinite linear diffusion, the diffusion coefficient of charge transfer (D) was determined in accordance to the equation $i_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} \nu^{1/2}$ (Shan et al., 2007; Calvo et al., 1996). Where n is the number of electrons, A is the electrode area (in cm^2), ν is the scan rate (in V/s), D is the diffusion coefficient of charge transfer, and C is the concentration of Fc redox centers in the film. The product $D^{1/2}C$ was calculated 2.0×10^{-9} mol cm^{-2} $s^{-1/2}$, which is nearly identical to other redox polymers (Sirkar and Pishko, 1998). Electron-transfer under diffusion control suggests that three-dimensional charge propagation

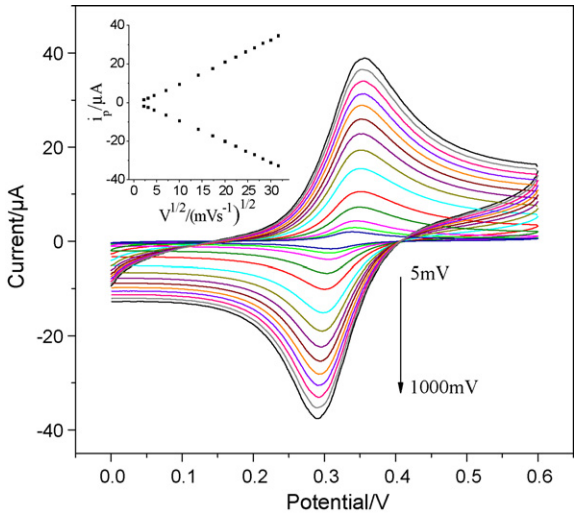


Fig. 3. CVs of the CS-Fc/Au NPs/GOx film electrode in 0.1 M PBS (pH 7.0 containing 0.1 M $KClO_4$) at different scan rates (from inner to outer: 5, 10, 20, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mV s^{-1}). *Inset*: Plots of peak currents vs. $\nu^{1/2}$.

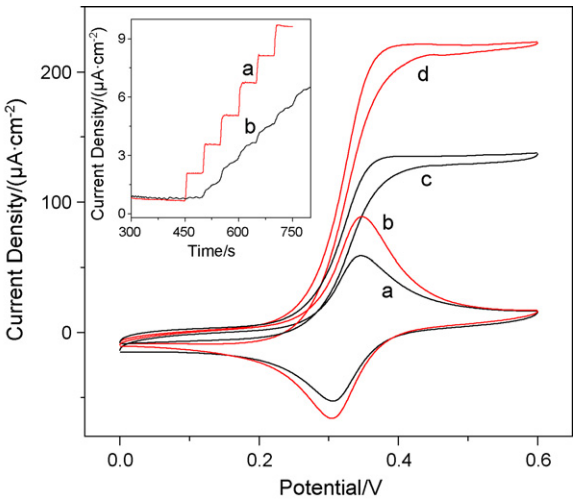


Fig. 4. CVs of GCE modified with CS-Fc/GOx (a and c) and CS-Fc/Au/GOx (b and d) at 20 mV s^{-1} in 0.1 M PBS (pH 7.0, containing 0.1 M $KClO_4$), in the absence (a and b) and the presence (c and d) of 15 mM glucose. *Inset*: Amperometric responses of CS-Fc/Au NPs/GOx (a) and CS-Fc/GOx (b) modified GCE towards glucose at +0.4 V upon each addition of 0.1 mM glucose in PBS (pH 7.0).

in the CS-Fc/Au NPs/GOx film is operative with this redox polymer. In the system reported here, diffusion of the mediator and chain reptation are not possible, thus charge propagate must through electron-hopping between neighboring the metallic conductive Au NPs and polymer chain segments containing ferrocene redox couples (Yang et al., 2007; Sirkar and Pishko, 1998).

3.4. Electrochemical response of CS-Fc/Au NPs/GOx composite film to glucose

The ability of the CS-Fc/Au NPs/GOx polymer in the electrocatalytic oxidation of glucose was evaluated by cyclic voltammograms (CVs). Fig. 4 shows the electrocatalytic response of both the CS-Fc/GOx and CS-Fc/Au NPs/GOx modified electrodes to glucose. The CS-Fc/GOx modified electrode exhibited a pair of redox peaks of Fc (Fig. 4a). Further incorporation of Au NPs in the film, the anodic peak and cathodic peak currents of the CS-Fc/Au NPs/GOx modified electrode increased considerably (Fig. 4b). Upon addition of glucose to the PBS solution, the anodic peak current increased noticeably, while the cathodic peak current decreased, indicating an obvious electrocatalytic oxidation of glucose at the CS-Fc/GOx (Fig. 4c) and CS-Fc/Au NPs/GOx (Fig. 4d) modified electrodes. However, the electrocatalytic current on the CS-Fc/Au NPs/GOx modified electrode was significantly improved, which was about 2.5 times higher as compared to CS-Fc/GOx modified electrode. These facts suggested that the present Au NPs in the CS matrix and ferrocene branched CS could facilitate electron communication between the heme site of the immobilized enzyme and the electrode surface. Similar behavior was observed for GOx immobilized in CS modified with electron-transfer mediators (Yang et al., 2007). Thus, it is expected that the CS-Fc/Au NPs/GOx film enhances the catalytic capacity of GOx for oxidation of glucose. To further discern the role of Au NPs on the enzyme immobilization, the current–time curve responses of the biosensor with and without Au NPs were also measured. Inset of Fig. 4 displays the typical current–time curves on CS-Fc/Au NPs/GOx (a) and CS-Fc/GOx (b) film electrodes for successive addition of 0.1 mM glucose in PBS (pH 7.0). Noticeably, the amperometric response of the CS-Fc/Au NPs/GOx (inset Fig. 4a) electrode was larger than that of the CS-Fc/GOx (inset Fig. 4b). The CS-Fc/GOx modified electrode stably responding to glucose was 15 s, and its detection sensitivity was 7.14 mA mM^{-1} cm^{-2}. These parameters were considerably improved with Au NPs incorporated

in the matrix. The response of the CS-Fc/Au NPs/GOx electrode to glucose decreased to <5 s and the sensitivity increased to 14.3 mA mM^{-1} cm^{-2}. The improved performance of the CS-Fc/Au NPs/GOx film can be attributed to the presence of Au NPs in the hybrid film providing the biocompatible microenvironments to maintain the activity of enzyme and enhancing electron transport, in turn, the reaction efficiency. This typical enzyme-dependent catalytic process can be expressed as follows (Cass et al., 1984):

$$\text{Glucose} + \text{GOx(FAD)} \rightarrow \text{Gluconolactone} + \text{GOx(FADH}_2\text{)} \quad (1)$$

$$\text{GOx(FADH}_2\text{)} + 2\text{CS-Fc}^+ \rightarrow \text{GOx(FAD)} + 2\text{CS-Fc} + 2\text{H}^+ \quad (2)$$

$$\text{CS-Fc} \leftrightarrow \text{CS-Fc}^+ + \text{e}^- \quad (3)$$

3.5. Solution pH on the biosensor response

The pH value of detection solution affected the performance of the biosensor. It is generally known that the bioactivity of GOx and the stability of CS are solution pH dependent. Alkaline solution will decrease the enzyme activity. On the contrast, strong acidic medium will decrease both the stability of CS and the bioactivity of GOx, leading to an obvious decrease in performance of the biosensor. We explored the effect of pH between 5.59 and 9.18, the present biosensor showed a maximum response at pH 7.0 PBS, which is in good agreement with the previous studies (Qian et al., 2004; Yang et al., 2003).

3.6. Amperometric determination of glucose

The amperometric response of the present biosensor to successive addition of glucose was evaluated at +0.4 V. The results are displayed in Fig. 5. The time required to reach the 95% steady-state response was 5 s. The response rate is much faster than that of 30 s reported in the CS-Fc/GOx film electrode (Yang et al., 2007), 10 s in chitosan–gold nanoparticles-GOx film (Du et al., 2007a), and 7 s in biocomposite film composed of chitosan, gold nanoparticles and GOx (Luo et al., 2004). Such a short response time further proves that the electrodeposition-derived CS-Fc/Au NPs material is a promising platform for the construction of biosensors. The amperometric response increased linearly to glucose concentration in the range from 0.02 to 8.66 mM with a detection limit of 5.6 μM (S/N = 3) (inset Fig. 5a). A substantially higher sensitivity is obtained with this polysaccharide redox film as compared with other glucose biosensor (Yang et al., 2007). When glucose concentration was high, a plateau current appeared, showing the characteristics of Michaelis–Menten kinetics. The apparent Michaelis–Menten constant (K_M^{app}), which gives an indication of the enzyme-substrate kinetics for the biosensor, can be obtained from the electrochemical version of the Lineweaver-Burk equation (Shu and Wilson, 1976; Kamin and Wilson, 1980):

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max}C}$$

where I_{ss} is the steady-state current, C the concentration of glucose, K_M^{app} the apparent Michaelis–Menten constant and I_{max} is the maximum current. From the curve of the $1/I_{ss}$ vs. $1/C$ based on the experimental data from Fig. 5, the K_M^{app} value of the biosensor was estimated to be 3.5 mM, which was much lower than those reported previously (20 mM (Wang et al., 1998), 4.3 mM (Zhang et al., 2005), and 6.6 mM (Kandimalla et al., 2006)). The smaller K_M^{app} value means that the immobilized GOx possesses higher enzymatic activity and the proposed biosensor exhibits a higher affinity to glucose.

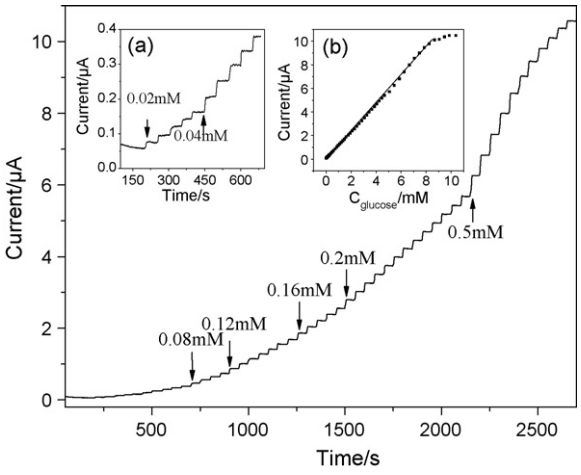


Fig. 5. Amperometric responses of CS-Fc/Au NPs/GOx modified GCE at detection potential of +0.4 V to successive addition of glucose in a stirred 0.1 M PBS (pH 7.0, containing 0.1 M $KClO_4$). *Inset*: (a) The magnified curve from 200 to 700 s and (b) calibration curve.

3.7. Reproducibility and stability of the biosensor

The reproducibility of the proposed biosensor was also studied. The relative standard deviation (RSD) of the biosensor response to 1.0 mM glucose was 3.1% for seven successive measurements. The RSD for detection of 1.0 mM glucose with five sensors prepared under the same conditions was 4.9%. The biosensor was stored dry at 4 °C and measured at intervals of one week. After 1 week, the biosensor retained 90% of its original sensitivity, and it remained about 81% of its original current after 4 weeks. The excellent reproducibility and stability of the biosensor are due to the good biocompatibility and conductivity of the CS-Fc/Au NPs/GOx composite. On the one hand, the formation of CS-Fc conjugate not only prevents the Fc mediator from leakage, but also provides a favorable microenvironment to maintain the activity of the enzyme. On the other hand, the presence of Au NPs improves the electron-transfer and prevents leakage of enzyme molecules from the CS-Fc/Au NPs/GOx film structure efficiently.

3.8. Effects of interferences

The effect of potential interfering species such as uric acid and ascorbic acid on the response of the present glucose biosensor was evaluated in 0.1 M pH 7.0 PBS containing 1.0 mM glucose. 0.1 mM uric acid (UA) and 0.1 mM ascorbic acid (AA) could produce an increase in the biosensor response by 4.3% and 6.0%, respectively. These increases were due to the oxidation of the interferents at the detection potential of +0.4 V. Nafion polymer as an effectively preselective barrier can electrostatically repel the negatively charged AA or UA, but does no affect neutral glucose molecules (Kristensen et al., 1987; Burmeister and Gerhardt, 2001). So the observed interferences can be eliminated by coating a Nafion layer on the CS-Fc/Au NPs/GOx modified electrode.

3.9. Determination of glucose in serum

The biosensor modified a layer of Nafion membrane was used for glucose assay of serum samples. The sample was diluted to its half concentration by mixing it with 0.1 M pH 7.0 PBS. Five parallel determinations were carried out. The glucose level was determined to be 6.45 mM close to the 6.54 mM determined by spectrophotometry, showing a good accuracy. The recoveries for the assays of 1.0–8.0 mM glucose were between 95% and 101% for ten measurements. The results were satisfactory and agreed closely with those detected by the hospital.

4. Conclusion

It can be concluded that the redox polymer, CS-Fc, could provide very suitable microenvironments for enzyme entrapment and retain its activity by controllable electrodeposition method. The proposed glucose biosensor exhibited high sensitivity, good stability and reproducibility. Furthermore, our results demonstrated that Au NPs played an important role similar to a conducting wire, which made it easier for the electron-transfer to take place. Compared with the CS-Fc/GOx electrode, the CS-Fc/Au NPs/GOx electrode had larger maximum current response, higher sensitivity and a wider linear response range. Therefore, the proposed feasible construction method is promising for the development of glucose biosensors, especially in preparation of biosensors for routine monitoring of glucose in real blood.

Acknowledgments

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