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A practical glucose biosensor based on Fe_3O_4 nanoparticles and chitosan/nafion composite film

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ABSTRACT

A practical glucose biosensor was developed by combining the intrinsic peroxidase-like activity of Fe_3O_4 nanoparticles (Fe_3O_4 NPs) and the anti-interference ability of the nafion film. Glucose oxidase (GOD) was simply mixed with Fe_3O_4 NPs and cross-linked on the Pt electrode with chitosan (Cs) medium by glutaraldehyde, and then covered with a thin nafion film. The biosensor showed high sensitivity (11.54 $\mu A\,cm^{-2}\,mM^{-1}$), low detection limit (6×10^{-6} M), and good storage stability. A linear calibration plot was obtained in the wide concentration range from 6×10^{-6} to 2.2×10^{-3} M. The modified electrode could virtually eliminate the interference during the detection of glucose. Furthermore, the biosensor was successfully applied to detect glucose in human serum sample. This fabrication of glucose biosensor was of considerable interest due to its promise for simple procedure and optimizing conditions in practical application.

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 detection methods for the determination of glucose in blood and food (Bakker, 2004; Prodromidis and Karayannis, 2002). Since Clark and Lyons proposed the initial concept of glucose enzyme electrodes in 1962 (Clark and Lyons, 1962), different approaches have been explored in the operation of glucose enzyme electrodes. By immobilizing glucose oxidase (GOD) on the electrode surface, it is available to employ such amperometric biosensors for the determination of glucose for their simplicity, high selectivity and intrinsic sensitivity.

The immobilization of enzymes is one of the crucial factors in biosensor preparation (Ren et al., 2005a,b; Fan et al., 2001). Many methods have been used to immobilize enzymes and to improve the enzymatic activity. Nowadays, nanoparticles can offer many advantages, such as large surface-to-volume ratio, high surface reaction activity, and strong adsorption ability to immobilize desired biomolecules (Dyal et al., 2003; Cai et al., 2006). There are growing interests in the application of nanoparticles to immobilize enzyme, as well as to exploit electrically contacted enzyme electrodes or protein-functionalized electrodes. Moreover, nanoparticles have the unique ability to promote electron transfer between electrodes and the active site of the enzyme due to their suitable size and physical properties (Orozco et al., 2009). Many kinds of metal nanoparticles such as Ag (Ren et al., 2005a,b), Au (Xiao et al., 2003; Xu et al., 2006), Pt (Bahshi et al., 2008), and semiconductor nanoparticles (CdSe/ZnS) (Gill et al., 2008) have been reported to immobilize enzyme to prepare modified electrodes.

Among a wide range of biomedical and technological applications (Kurlyandskaya and Levit, 2007; Zhang et al., 2008; Mavre et al., 2007), magnetic particles have been considered as an interesting material for the immobilization of desired biomolecules resulted from many superior properties: biocompatibility, superparamagnetic property, as well as better contact between biocatalyst and its substrates (Katz et al., 2004; Rossi et al., 2004; Lu and Chen, 2006; Huang et al., 2008). Electrochemical detection of hybridized DNA strands have been achieved with a magnetic nanoparticle modified electrode (Zhu et al., 2006). Nanoparticles of various ferric oxides (hematite, magnetite, amorphous Fe_2O_3, β-Fe_2O_3 and ferrihydrite) incorporated into carbon paste have exhibited electrocatalytic properties towards hydrogen peroxide reduction (Hrbac et al., 2007). Kaushik et al. have fabricated a new glucose biosensor and a urea sensor based on iron oxide nanoparticles-chitosan nanocomposite (Kaushik et al., 2008, 2009). Wang et al. have developed a novel amperometric glucose biosensor by immobilizing ferritin antibody on the surface of Fe_3O_4 nanoparticles (Fe_3O_4 NPs)/chitosan (Cs) composite film modified glassy carbon electrode (GCE) for determination of ferritin (Wang and Tan, 2007). However, magnetic particles have only been studied to be used to immobilize the linked enzymes in these reports. Furthermore, the mechanism of the electron transfer between the electrode and the substrate molecule, as well as the role of Fe_3O_4 NPs playing in

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the electron transfer of these biosensors have not been explained clearly.

Recently, Gao et al. (2007) have proved that Fe_3O_4 NPs in fact possessed an intrinsic enzyme mimetic activity similar to that natural peroxidase. It showed that Fe_3O_4 NPs not only offered a good microenvironment for retaining the bioactivity of enzymes, but also acted as natural peroxidase to oxidize organic substrates. Wei et al. have further confirmed that the Fe_3O_4 NPs possessed intrinsic peroxidase-like activity and developed this new biosensor in the application of detection of H_2O_2 and glucose by colorimetric method (Wei and Wang, 2008). The results that Fe_3O_4 NPs were used to enhance electrocatalytic and bioelectrocatalytic transformation have been emphasized with the aim to highlight a new concept for biosensors.

In this paper, we synthesized the Fe_3O_4 NPs for biosensor application and explained the mechanism of the electron transfer between the electrode and the substrate molecule. The fabrication, characterization and analytical performance of the modified biosensor based on Fe_3O_4 NPs to develop an electrochemical glucose sensor were also described in the paper. Moreover, the Pt/GOD/Fe_3O_4/Cs/nafion modified Pt electrode was used to achieve unique features of electrocatalytic reduction and elimination of interference. The new glucose sensor was used for the determination of glucose in human serum. Therefore, the new biosensor modified with Fe_3O_4 NPs, chitosan and nafion film have shown potential of robust, easy-to-make, durable analytical applications in both biological and environmental detection.

2. Materials and methods

2.1. Chemicals and reagents

Glucose oxidase was extracted from *Aspergillus niger* (Sigma company, 118 U/mg). β-D-Glucose was purchased from Sigma and nafion was from DuPont company. L (+)-Ascorbic acid (AA) and sodium hydroxide (NaOH) were products of Beijing Shiji Company. Chitosan was from Guo Yao Chemical Reagent Company. Phosphate buffers containing 0.1 M KCl consisted of KH_2PO_4 and Na_2HPO_4 (0.2 M, pH 6.8). All reagents were used without further purification and prepared with redistilled water.

2.2. Preparation of Fe_3O_4 nanoparticles

The Fe_3O_4 NPs were prepared via a previously reported coprecipitation method (Mikhaylova et al., 2004; Vereda et al., 2007). Firstly, 0.32 M ferric chloride and 0.31 M ferrous chloride were mixed in 0.45 M HCl solution diluted by deoxygenated water. Secondly, the mixed solution of ferrous and ferric salts was then added into 250 mL of 0.3 M oxygen-free sodium hydroxide solution under vigorous stirring at room temperature in a nitrogen atmosphere. The formed black Fe_3O_4 colloidal particles were separated by centrifugation and further washed with water for three times. The Fe_3O_4 NPs were then re-dispersed in water and stored at room temperature. (Referred to as the Fe_3O_4 nanoparticle stock solution with a total concentration of 25 mM.)

2.3. Preparation of enzyme electrodes

The platinum electrode with a diameter of 1 mm was first boiled in nitric acid for 8 min and washed in redistilled water. The aqueous solution of GOD (10 μL, 1 U/μL) and 10 μL Fe_3O_4 NPs solution were added to 20 μL glutaraldehyde (2%) to form a mixture and then dried on the Pt electrode. 400 μL Fe_3O_4 NPs was added to 2 mL chitosan solution (2%) in a 5 mL beaker. And then the platinum electrode was dipped into the mixture for 5 min and dried in air. Finally, 2 mL nafion (2.5%) was drop-coated on the Pt/GOD/Fe_3O_4/Cs electrode. These electrodes were stored at 4 °C for 24 h before measurement.

The enzyme electrode without Fe_3O_4 NPs and the enzyme electrode modified by Fe_3O_4 NPs while without nafion were used as comparison and prepared by the same method as described above.

2.4. Apparatus and measurements

Transmission electron microscope (TEM) of Fe_3O_4 nanoparticles was obtained with a JEM-2100 electron microscope, operating at 160 kV. Cyclic voltammetry and electrochemical impedance spectroscope (EIS) were performed using a CHI 604B electrochemical workstation (Shang Hai Chen Hua company) in 5 mM $K_3Fe(CN)_6$ and 2.5 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ (1:1) containing 0.1 M KCl. A conventional three-electrode system was used throughout the electrochemical experiment at room temperature with a modified electrode as working electrode, a bare Pt electrode as auxiliary electrode, and a glassy carbon electrode as reference electrode against which all potentials were measured in this paper.

The sensitivity of the glucose biosensor was tested by measuring the current response using a three-electrode cell consisting of a modified electrode as working electrode, a bare Pt electrode as auxiliary electrode and a reference electrode of Ag/AgCl. Measurements were conducted in a 5 mL phosphate buffer (pH 6.8) cell at 35 °C. A fixed potential of 0.4 V was applied to this electronic cell. Firstly, the three-electrode cell was put into phosphate solution at 35 °C. When background current reached a constant value, different concentrations (from 3×10^{-6} to 1.1×10^{-2} M) of β-D-glucose solution were added. Then response currents were noted down, and background currents were deducted. At last, the correlation between the current response and the concentrations of glucose solution was obtained.

3. Results and discussion

3.1. TEM characterization of the Fe_3O_4 NPs, GOD and Fe_3O_4 composite

Fig. 1A shows the typical transmission electron microscope (TEM) of the Fe_3O_4 NPs, which indicates that the sample is composed of a large quantity of well-dispersed spherical nanoparticles. The average size of these particles estimated from the TEM image is about 20 nm. The selected area of the same single nanoarchitecture in the inset of Fig. 1A can be indexed to the crystal lattice structure of Fe_3O_4 NPs. Fig. 1B shows the TEM image of the mixture of GOD and Fe_3O_4 NPs solution which indicates that the composite of Fe_3O_4 NPs with GOD are also well-dispersed.

3.2. Evaluation of the electrochemical performance of the electrodes

3.2.1. Cyclic voltammetric characterization

Cyclic voltammetric experiment is used to evaluate the electrochemical performance of the electrodes. Fig. 1C shows cyclic voltammograms (CVs) of different modified electrodes in 5.0 mM potassium ferricyanide solution. For the bare Pt electrode, the well-defined oxidation and reduction peaks caused by the Fe^{3+}/Fe^{2+} redox couples are noticed in forward and reverse scan (curve (a)). After the electrode is modified by Cs, an obvious decrease in reduction and oxidation peaks is observed (curve (b)). This may be due to the properties of the chitosan, as the organic membrane, which can block the electron transfer between solution and the Pt electrode. When Fe_3O_4 NPs are embedded into the Cs membrane, the

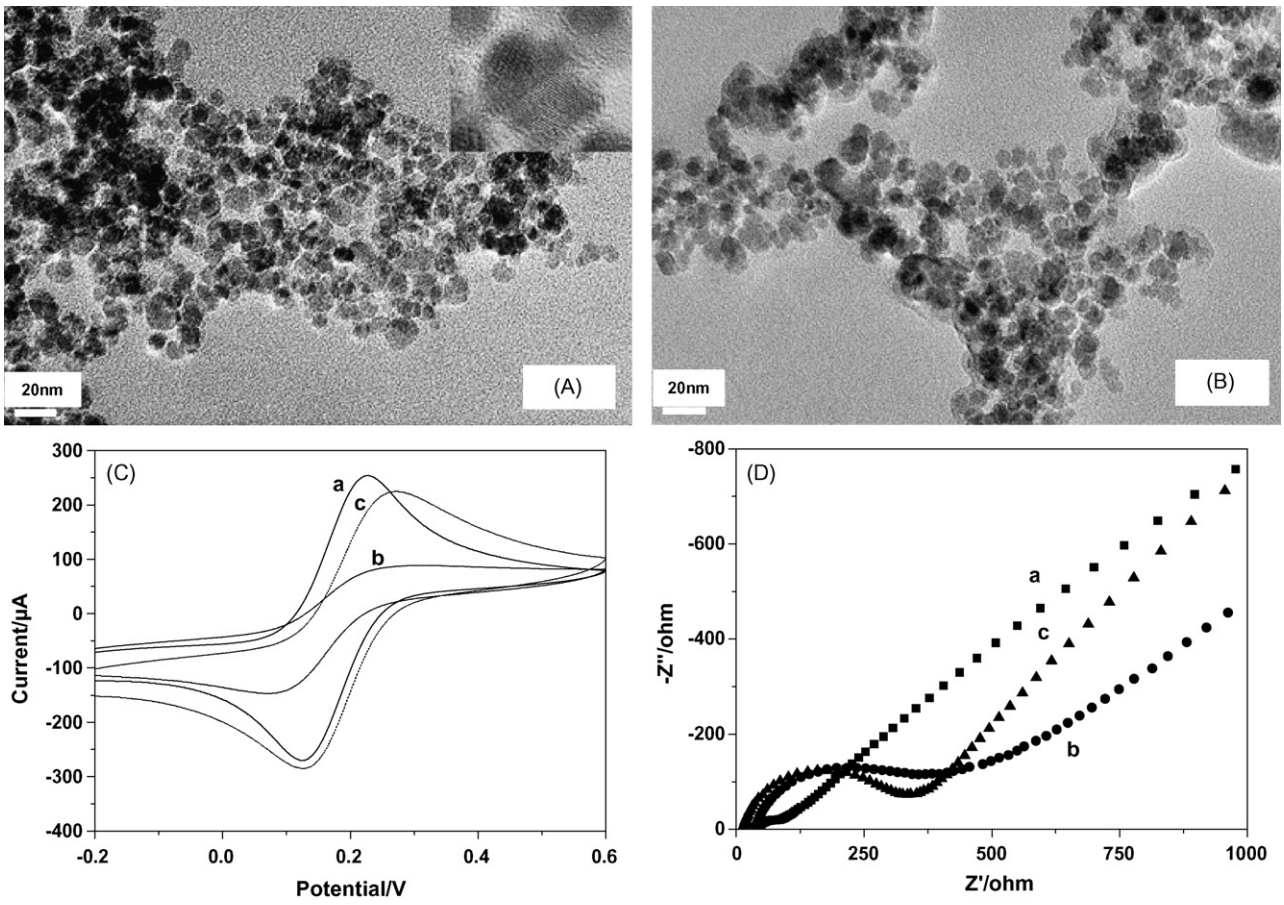

Fig. 1. (A) TEM image of the Fe_3O_4 NPs, the inset shows the crystal lattice of the selected-area Fe_3O_4 NPs. (B) TEM image of the Fe_3O_4 NPs with GOD. (C) Cyclic voltammograms (CVs) of different modified electrodes in 5.0 mM ferricyanide solution: (a) bare Pt electrode; (b) the electrode with Cs film; (c) the electrode with Cs film added GOD and Fe_3O_4 NP (scan rate is 50 mV/s). (D) The Nyquist plots of the EIS of bare Pt(a), Pt/Cs (b), Pt/GOD/Fe_3O_4/Cs(c), in 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 M KCl.

current response of CVs could be increased (curve (c)), indicating the enhancing effect of the Fe_3O_4 NPs on the electric conductivity of the enzyme electrode.

3.2.2. Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) can provide useful information on the impedance changes of the electrode surface during the fabrication process. The semicircular portion at higher frequencies corresponds to the electron-transfer-limited process and its diameter is equal to the electron-transfer resistance, which controls the electron-transfer kinetics of the redox probe at the electrode interface. Meanwhile, the linear part at lower frequencies corresponds to the diffusion process (Feng et al., 2005). Fig. 1D displays the Nyquist plots of the EIS of bare Pt (a), Pt/Cs (b), Pt/GOD/Fe_3O_4/Cs (c) in 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 M KCl. Cs displays an excellent film-forming ability, good adhesion, biocompatibility and susceptibility to chemical modification. It can perhaps overcome the existing problem of aggregation of Fe_3O_4 nanoparticles. However, the Nyquist diameter of the Pt/Cs (curve (b), the value of resistance is 458 Ω) is much larger than that of the bare Pt (curve (a), the value of resistance is 137 Ω), which suggests that the Cs coated on the electrode could increase the resistance of the electrodes (Miao and Tan, 2000). Whereas comparing with the Pt/Cs, the interfacial resistance of the Pt/GOD/Fe_3O_4/Cs (curve (c), the value of resistance is 217 Ω) is observed less than that of the Pt/Cs (b). The results show that the Fe_3O_4 NPs modified electrode can decrease the resistance of the electrodes and hold high electron-transfer efficiency.

3.3. The current response to glucose and the apparent Michaelis–Menten constant

Fig. 2A shows the current response curves of GOD electrodes with Fe_3O_4 nanoparticles in different concentrations of glucose. It can be seen that the electrode with Fe_3O_4 NPs and Cs can significantly enhance the current response. In particular, as the concentration of glucose reaches 4 mM, the current response of the GOD electrode containing Fe_3O_4 NPs and Cs (Pt/GOD/Fe_3O_4/Cs) (curve (a)) is seven times higher compared with the enzyme electrode without Fe_3O_4 NPs (Pt/GOD//Cs) (curve (c)). The relative standard deviation (RSD) of the current response of the Pt/GOD/Fe_3O_4/Cs electrode is 3.2%. Nafion is always used as a composite film to improve the anti-interference of electrodes because the negatively charged group of nafion layer can repulse the negatively charged ascorbic acid from the surface of the composite electrode. However, nafion would block electron transfer between electrode and glucose, and then tend to reduce current response. During our research, we surprisingly found that the current response of Pt/GOD/Fe_3O_4/Cs/nafion electrode declined only a little after modified with nafion film (curve (b)). The relative standard deviation of the current response of the Pt/GOD/Fe_3O_4/Cs/nafion electrode is 2.7%.

The resulted calibration plot for glucose over the concentration range of 6×10^{-6} to 2.2×10^{-3} M is presented in Fig. 2B. The linear plot reveals that such electrode can work well in glucose solution with a sensitivity of 11.54 μA cm^{-2} mM^{-1}, and the correlation coefficient is 0.996. An estimated detection limit of 6×10^{-6}M at a signal-to-noise ratio of 3 is much lower than that of 0.37 mM in

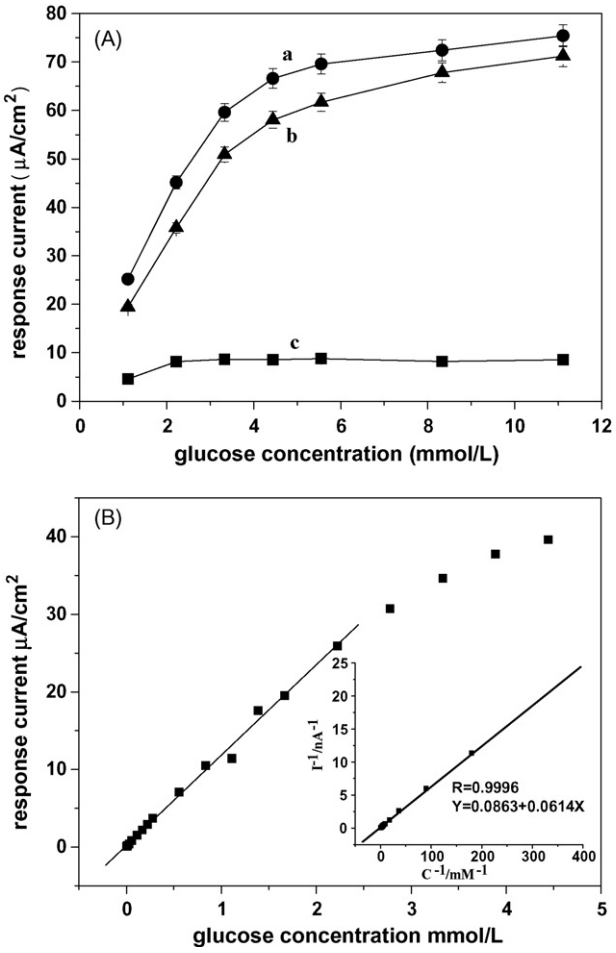

Fig. 2. (A) The current response curves of GOD electrodes with and without Fe_3O_4 nanoparticles or Cs in different concentration of glucose: (a) Pt/GOD/Fe_3O_4/Cs, (b) Pt/GOD/Fe_3O_4/Cs/nafion, (c) Pt/GOD/Cs. (B) The linear calibration plot corresponding to the current response of different concentration of glucose.

glucose sensor based on gold nanoparticle–chitosan composite film (Feng et al., 2009) and 0.015 mM by gold nanoparticles modified glucose sensor (Wang et al., 2008). The apparent Michaelis–Menten constant (K_M), which gives an indication of the enzyme–substrate, can be obtained from the Michaelis–Menten equation (Kong et al., 2009): $V = V_{max}$ [S]/(K_M + [S]) where V is rate of reaction, V_{max} is the maximum rate of reaction measured under saturated substrate condition, and [S] is the bulk concentration of the substrate. The value of the apparent Michaelis–Menten constant (K_M) is calculated to show suitability of the enzyme in the hybrid nanobiocomposite matrix. From the inner of Fig. 2B, K_M value is found to be 0.611 mM for the immobilized GOD by using Line weaver–Burke plot (1/I versus 1/[C]). The value of K_M is much lower than the reported 14.4 mM (Zou et al., 2008), 6.3 mM (Qiu et al., 2008) by ferrocene-modified multiwalled carbon nanotubes glucose biosensor and 3.73 mM by glucose biosensor based on prussian blue/chitosan hybrid film (Wang et al., 2009). These results show that the biosensor possesses higher biological affinity to glucose.

3.4. Discussion of the mechanism of the electron-transfer behavior

The electron-transfer behavior of the GOD-electrode occurs as the following:

$$\text{GOD-FAD} + 2e^- + 2H^+ \leftrightarrow \text{GOD-FADH}_2 \quad (1)$$

$$\text{Glucose} + \text{GOD-FAD} \rightarrow \text{gluconolactone} + \text{GOD-FADH}_2 \quad (2)$$

$$\text{GOD-FADH}_2 + O_2 \rightarrow \text{GOD-FAD} + H_2O_2 \quad (3)$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 + 2e^- \quad (4)$$

As is well known, β-D-glucose, the substrate of GOD, will result in the reductive form of GOD (GOD-$FADH_2$) in the enzyme-catalyzed reaction. In the process, it is reported that ferrocene could act as the electron-transfer mediators to communicate electrically with GOD and electrode (Escorcia and Dhirani, 2007). In our enzyme-catalyzed system, the electrons generated from the biochemical reactions would transfer to the Pt/GOD/Fe_3O_4/Cs electrode through the Fe (II)/Fe (III) couples (Lin and Leu, 2005). The results of the CVs also prove that Fe_3O_4 NPs could provide a conductive path through the composite membrane matrix. So the Fe_3O_4 NPs, acting as electron-transfer mediators, can help in enhancing the current response of enzyme electrode and then increasing the sensitivity of the biosensor. From Eqs. (3) and (4), it can be seen that the response current could be produced from the decomposition of hydrogen dioxide on the electrode with electrocatalytic properties of Fe_3O_4 NPs. It has been proved that Fe_3O_4 NPs with the valence-state of Fe (II) and Fe (III) was identified as the catalyst for H_2O_2 detection. The reduction of H_2O_2 proceeding as two-electron, two-proton reduction step which gives the product of H_2O, as well as the reductive valence-state of Fe (II) on Fe_3O_4 is oxidized to Fe (III) (Rossi et al., 2004). These processes are shown in Fig. 3A. The proposed biochemical reaction during the glucose detection is shown in Fig. 3B. In the enzyme-catalyzed process, Fe_3O_4 NPs could catalyze the reaction of H_2O_2 decomposed into H_2O (Eq. (4)), then enhance the electron transfer to the electrode. So the current response of enzyme electrode could be improved. In order to prove this process, we also afforded the contrast electrode only modified with the Fe_3O_4 NPs and Cs (Pt/Fe_3O_4/Cs) to detect glucose. The current response of the contrast electrode is close to the background current. It shows that the Fe_3O_4 NPs cannot catalyze H_2O_2 and enhance the current response without the process in which GOD catalyzes β-D-glucose forming H_2O_2 in the present of oxygen. Besides these specific physics-chemical characters of iron in the electron-transfer of the electrode, Fe_3O_4 NPs not only act as electron-transfer mediators and natural peroxidase, but also play an important role in the preparation of immobilized enzymes due to their desirable characteristics: large pore size and volume, and good electron conductivity (Lee et al., 2005). Fe_3O_4 NPs also create suitable microenvironment which benefit the exposition of the active center, and increase the activity of enzyme (Zhang et al., 2005). In conclusion, with the excellent characteristics of Fe_3O_4 NPs as an electron-transfer mediator and artificial peroxide, the

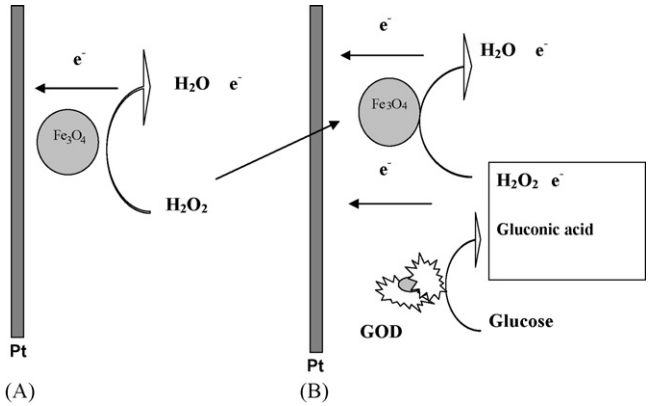

Fig. 3. Proposed mechanism of the electron transfer between nanocomposite and the Pt electrodes and the immobilization of glucose oxidase on nanocomposite matrix.

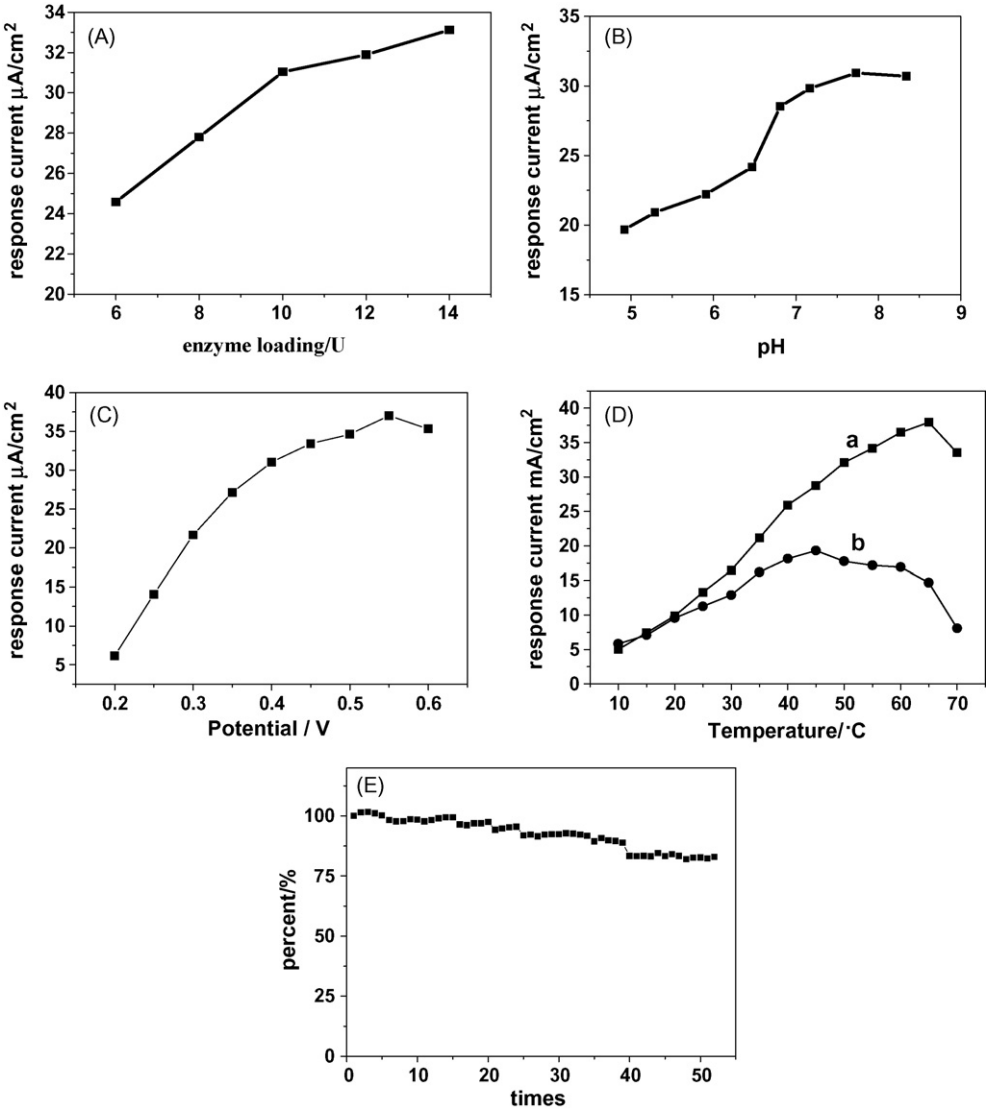

Fig. 4. Optimization of the biosensor: (A) response curves of the modified biosensor with different amounts of enzyme loading. (B) The current response of the biosensor at 2.2 mM glucose with increasing pH. (C) The current response of the biosensor at 2.2 mM glucose with increasing potential. (D) The current response of the biosensor at 2.2 mM glucose with increasing temperature ((a) the modified electrode with nafion film; (b) the modified electrode without Fe_3O_4 NPs and nafion film). (E) The stability of the biosensor was investigated by amperometric measurements.

new electrode modified by Fe_3O_4 NPs can obviously amplify the current response of enzyme electrode and as a result increase the sensitivity of the biosensor.

3.5. Optimization of the biosensor

3.5.1. Effect of enzyme loading

The amount of enzyme loading can affect the response current of this kind of glucose biosensor. Fig. 4A shows the response curves of the modified biosensor with different enzyme loading. For each glucose concentration, with the increase of enzyme loading, the response current increases gradually. More enzymes would produce more H_2O_2 at the same concentration of glucose under the same condition (Luo et al., 2004). It shows that Fe_3O_4 NPs could immobilize more enzymes on the electrode. However, From the Fig. 4A, we find that when the loading of enzyme is more than 10 U, the response current tends to be saturated. Further increment of enzyme loading would be a waste of this expensive reagent, so in the following experiments the enzyme loading is set as 10 U.

3.5.2. Effect of pH, working potential and temperature on the biosensor

Investigation of the effect of pH value on the performance of the biosensor is of great importance, because the activity of the immobilized GOD is pH dependent (Yang and Zhu, 2005). From the Eq. (1), as the proton is critical in redox behavior of GOD-FAD, the decrease of GOD response at high pH is possibly due to the decreases of proton concentration and bioactivity of the immobilized GOD. Fig. 4B shows that the pH dependence of the sensor response is evaluated at 2.2 mM glucose over the pH range from 5.0 to 8.3. The optimum response is achieved at pH 7.2. In order to keep consistent with the neutral condition of human blood, we chose phosphate buffers (pH 6.8) to ensure higher sensitivity of the biosensor. Fig. 4C shows the effect of working potential on the performance of the biosensor. The working potential is stepped from 0.2 to 0.6 V in 2.2 mM glucose. The steady-state current response increases obviously with the working potential from 0.2 to 0.4 V, and then increases smoothly from 0.4 to 0.6 V. Therefore, 0.4 V is selected as the working potential for amperometric detection of glucose concentration.

The results of the effect of temperature on biosensor are presented in Fig. 4D. It shows that the current response of the modified biosensor is enhanced with increasing temperature. The current response reaches a maximum at approximately 65 °C (curve (a)), and then goes down as the temperature turn higher. This phenomenon may be attributed to that the enzyme is denatured at high temperature. In contrast, the modified electrode without Fe_3O_4 NPs and nafion films shows that the response declines when temperature is higher than 45 °C (curve (b)). The result indicates that enzyme bioconjugated with Fe_3O_4 NPs and the cover of nafion films has good thermodynamic stability and life span. The modified glucose biosensor would be used in some special condition. In order to keep consistent with the temperature of human body, 35 °C is selected for this work.

3.5.3. Stability of the enzyme electrode

The stability of the biosensor is investigated by amperometric measurements in the presence of 2.2 mM glucose. Fig. 4E shows that the current response of biosensor is retained about 83% of its original response after 52 times uninterrupted detection. In addition, the long-term stability is also tested by measuring the glucose concentration after a month. It is revealed that the current response of the sensor maintains 84% of the initial current response. This means that Fe_3O_4 NPs and nafion films ensure well stability of the biosensor. With the help of Fe_3O_4 NPs and nafion films, GOD can keep bioactivity on the Pt electrode.

3.6. The interference study

The effect of several possible interfering substances, such as AA, uric acid (UA), sucrose, lactose on the glucose biosensor based on the Pt/GOD/Fe_3O_4/Cs/Nafion electrode is investigated at 0.4 V versus Ag/AgCl in phosphate buffer solution of pH 6.8. The addition of 0.3 mM sucrose, lactose and UA do not cause any observable interference to the detection of glucose.

The normal physiological level of glucose is 3–8 mM, which is much higher than that of AA (0.1 mM). However, the electron-transfer rate of AA is higher than that of glucose. The coexistence of AA with glucose in the physiological system might significantly influence the practical glucose detection. The signal for a fixed concentration of glucose is compared with the current value obtained in the presence of the variable concentrations of the interfering species AA. The biosensor is evaluated with 0.1 mM AA in the detection of 2.2 mM glucose. Table 1 compares the interferences of the modified electrode and the electrode without nafion. The current response of the Pt/GOD/Fe_3O_4/Cs/nafion electrode in the above solution is equal to the glucose solution without AA. However, an increase of the current response by 10% at the 0.1 mM AA is observed for the electrodes without nafion film. It is nafion film covered on the surface of the modified electrode that plays an important role in holding back the disturbance from interfering species. It indicates that the modified biosensor can give credible current signal though the interfering species reach high concentrations.

Table 1
Comparison of the interference performance of electrodes with and without nafion.

Interfering substances	Concentration (mM)	Pt/GOD/Fe_3O_4/Cs	Pt/GOD/Fe_3O_4/Cs/nafion
		RSD (%)	RSD (%)
Sucrose	3	7.5	2.3
Lactose	3	4.4	1.6
Uric acid	0.3	34	1.5
AA	0.1	11.3	0.3

Table 2
Glucose concentrations in serum samples tested by the proposed method and Automatic Biochemical Analyzer.

Serum sample	Clinical assay concentration (mM)	Proposed method ($n = 3$) concentration (mM)	RSD (%)
Sample 1	4.37	4.24	−2.97
Sample 2	6.19	6.08	−1.78
Sample 3	9.42	9.64	2.23

3.7. Application of the biosensor to serum samples

In order to investigate the possible application of this new biosensor in clinical analysis, the modified electrode was also tested in real human serum and the results were compared with those obtained by Automatic Biochemical Analyzer as shown in Table 2. The glucose in serum was firstly detected by Automatic Biochemical Analyzer, and then 400 μL of serum sample was diluted to 4 mL with PBS solution (pH 6.8). The response currents were noted down. The concentration of the glucose in blood sample was calculated from the calibration curve. From the Table 2, regardless of normal or high blood glucose level, we can see a good agreement between the data determined by two methods. It proved that the modified electrodes ascertained the practical application of the proposed biosensor in clinical analysis.

4. Conclusion

We have proposed a novel strategy for developing a composite electrode consisting GOD/Fe_3O_4/Cs/Nafion, which showed remarkably enhanced sensitivity and selectivity for glucose in the presence of excess AA, UA as inference. The novel glucose biosensor showed relatively rapid response, high sensitivity, broad linear range, low detection limit, good reproducibility, and long-term stability. The wide detection range and high sensitivity may be assigned to the amplification of the magnitude of current response since Fe_3O_4 NPs could catalyze the reaction of H_2O_2. This would account for the potential application of several characteristic enhancements in the determination of glucose in blood, drugs and food. Moreover, this biosensor virtually eliminated the interference and could be useful to detect glucose in samples containing other analytes. Therefore, this novel biosensor could be readily extended to the detection of other clinically important antigens by using Fe_3O_4 NPs to develop other simple and practical biosensors.

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