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Electrochemical performances of C/Fe nanocomposite and its use for mediator-free glucose biosensor preparation

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Abstract

C/Fe nanocomposite (CFN) was synthesized by a procedure similar to an exfoliation/adsorption process to intercalate Fe^{3+} into graphite oxide (GO) layers and would be reduced in a H_2 atmosphere. The results of X-ray diffractometry (XRD) and transimission electron microscopy (TEM) show that the form of CFN is carbon nanotube–Fe nanoparticle composite with α-Fe distributed on the nanotube wall. Paste electrode has been constructed using CFN mixed with paraffin. The electrochemical characteristics of such carbon–Fe nanocomposite paste electrode (CFNPE) has been compared with that of carbon paste electrode (CPE) and evaluated with respect to the electrochemistry of potassium ferricyanide, ascorbic acid and cysteine by cyclic voltammetry. CFNPE can accelerate the electron-transfer to improve the electrochemical reaction reversibility. To fabricate the third-generation glucose biosensor, glucose oxidase (GOD) was immobilized on CFNPE surface with Nafion covered after a pretreatment. Oxygen, ascorbic acid and uric acid have no interference with the glucose detection. The biosensor displays a remarkable sensitivity and stability and the results used in the determination of glucose in the human serum samples are satisfactory.

Keywords: C/Fe nanocomposite; C/Fe nanocomposite paste electrode; Glucose oxidase; Glucose biosensor

1. Introduction

Nanometer-sized carbon–metal composites have attracted the research interest because of their unique physical and chemical properties and the importance of these materials as catalysts [1–3]. Commercial carbon–metal nanocomposites containing Pt, Pd, Os, Pt-Os, Pt-Ru or Pt-Ru-P are commonly used as electrooxidation catalysts [3–5] in direct methanol fuel cells to enhance direct methanol fuel cells performance for commercial device application. The synthesis of C/Fe nanocomposite (CFN) has also been reported. Cao et al. [6] synthesized macroscopic three-dimensional arrays of Fe nanoparticles supported in aligned carbon nanotubes. Fe ion acts as the catalyst of carbon nanotube formation. In a reduction process, Fe is enveloped in nanotubes. Sun and Nava [7] have reported C/Fe nanoparticles which have been successfully synthesized using a modified graphite arc-discharge method. Carbon nanotubes–Fe nanoparticle compositions [8] have been fabricated using several ferrocenyl-aryl-acetylenic compounds. It has been reported that carbon nanotubes–Fe-alumina nanocomposites can be synthesized using H_2/CH_4 as reduction atmosphere [9]. But there are few researches to explore the electrochemical characteristics and application of this kind of material in the field of analytical chemistry. In present work, we investigated the electrochemical properties of C/Fe nanocomposite and used them to fabricate the mediator-free glucose biosensor—a third-generation enzyme sensor.

By far, three generation enzyme sensors have been reported. The first generation uses the natural mediator to transfer the electron. The sensor response is greatly af-

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fected by the amount of the mediator (for example, oxygen). Artificial mediators have been utilized during the fabrication of the biosensors to solve this problem. This kind of enzyme sensors is the second-generation one. The mediator can be polymerized on the electrode surface before enzyme immobilization [10–17], electrochemically codeposited with enzyme [18–24] or mixed with the enzyme in carbon paste [25–26]. In authors' laboratory, the enzyme sensors have been prepared with the mediator dissolved in solution using nano-Au particles to immobilize horseradish peroxidase [27–28]. However, the mediator could easily be destroyed to affect the sensor response. Mediate-free enzyme sensors—the third-generation biosensor—could settle the above problem. Palmisano and Zambonin [29] have explored a third-generation glucose biosensor based on overoxidized poly(pyrrole)/tetrathiafulvalene-tetracyanoquinodimethame (TTF-TCQN), with the electrons directly transfered between glucose oxidase (GOD) and the TTF-TCQN surface without any mediators.

In this work, we used an exfoliation/adsorption process to intercalate Fe^{3+} into graphite oxide (GO) layers, followed by reduction in a H_2 atmosphere to synthesize carbon–Fe nanocomposite. X-ray diffractometry (XRD) and transmission electron microscopy (TEM) were utilized to identify the form and microstructure of carbon and Fe in the composite. The electroactivity of C/Fe nanocomposite paste electrode (CFNPE) was investigated in phosphate buffer solution (PBS) (pH 7.0), $Fe(CN)_6^{3+}$, ascorbic acid and cysteine solution, compared with the carbon paste electrode (CPE). GOD biosensor which is mediate-free was explored based on CFNPE. Oxygen, ascorbic acid and uric acid did not show interference with the glucose detection.

2. Experimental

2.1. Apparatus and materials

XRD was performed with a D5000 X-ray diffractometer (SIEMENS German) using CoKα radiation. A H-800 transmission electron microscope (HITACHI, Japan) was used to observe the physical state of nanocomposite. All cyclic voltammograms and chronoamperometry data were acquired using a computer-based potentiostate/galvanostate (model 283) (EG&GP Princeton Applied Research, Princeton, NJ, USA). The three-electrode system consists of CFNPE or CFNPE immobilized with GOD, a saturated calomel reference electrode and a platinum wire auxiliary electrode.

Glucose oxidase and β-D-glucose were purchased from Sigma. Nafion is from Aldrich. Other chemicals were of analytical reagent grade. All solutions were prepared with doubly distilled water.

The supporting electrolyte was 0.067 mol/l phosphate buffer solution containing 0.1 mol/l KCl (PBS), which was prepared with KH_2PO_4 and Na_2HPO_4. The accurate concentration of glucose solution was determined by titration with 1.0 mol/l glucose solution prepared.

2.2. Preparation of CFN

The parent sample of graphite oxide was prepared by oxidation of graphite powder with $KMnO_4/H_2SO_4$ according to the method of Hummers and Offeman [30] and Lerf et al. [31]. The oxidized sample was washed with 5% aqueous HCl until the solution was free of sulfate and chloride ions. Then it was dried at 55 °C under vacuum to remove adsorbed water, and finally pulverized and dried under vacuum for 2 days at room temperature.

The dried graphite oxide (2 g) was put into 100 ml of water in a closed vessel and stirred at ambient temperature for 48 h to acquire a GO suspension. The suspension was diluted to 0.2% (w/v) with water. About 400 ml of the suspension (0.2%) was placed in a 1000 ml vessel, and the pH was adjusted to 3.0 using 0.1 M HCl. Then 1.2 ml of 8 M $FeCl_3$ was instilled. The suspension was kept stirring at ambient temperature for 2 h. The composite was dried under vacuum at 50 °C for 48 h. The dry composite was reduced in a H_2 atmosphere during 12 h at 600 °C, giving rise to carbon–Fe composite powders.

2.3. Fabrication of CFNPE

CFNPE was used as the matrix electrode. It was prepared according to the procedure reported elsewhere [32] with minor modifications. In a typical process, paraffin (140.4 mg) was dissolved in ether and dried in air for about 10 min. After evaporation of the excess of ether, the paraffin paste was mixed with an appropriate amount (281.2 mg) of CNF to form a paste and was squeezed into a PVC tube of 6 mm i.d. to a depth of 1 cm. Inside the tube the mass was in contact with a conducting graphite rod, which was in turn connected with an electric wire to complete the measurement circuit.

The preparation process of carbon paste electrode is similar to that of the CFNPE, only with CFN replaced by graphite powder.

2.4. Immobilization of GOD on CFNPE and CPE

CFNPE and CPE were subjected to cyclic voltammetric scanning in 0.02 mol/l $Fe(CN)_6^{3-}$ aqueous solution at potential ranging from −400 mV to 800 mV until the background current was stabilized and rinsed with water. The GOD was immobilized on CFNPE and CPE surface in the presence of BSA. The process is as follows. Two hundred microliliters of PBS (pH 7) containing 4 mg of GOD was mixed with 14 mg of BSA. Subsequently a 25-μl sample of the mixture was pipetted onto the CFNPE and CPE surfaces, respectively, and air-dried at room temperature. Then 10 μl of 0.5% Nafion was dripped on the GOD-modified electrode and air-dried at room temperature. The electrode was thoroughly washed af-

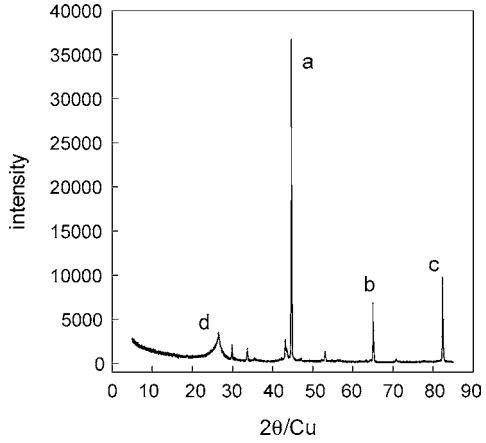


Fig. 1. XRD pattern of the CFN.

ter preparation, and when it was not used the electrode was stored in PBS (pH 7) at 4 °C.

3. Results and discussion

3.1. Characteristics of CFN

Fig. 1 shows X-ray diffraction profile of CFN. Three diffraction peaks of a, b and c are similar with those of α-Fe [8,9]; while peak d is consistent with that of carbon nanobute. The results display that Fe in nanocomposite is in the form of α-Fe and that of carbon might be carbon nanotube.

In order to confirm the form of carbon, the experiment of TEM was examined as shown in Fig. 2. One notices that the form of carbon is really nanotube, and the fuscous nanoparticle enveloped in the nanotube wall is α-Fe. The form of CFN is really carbon nanotube–Fe nanoparticle composite.

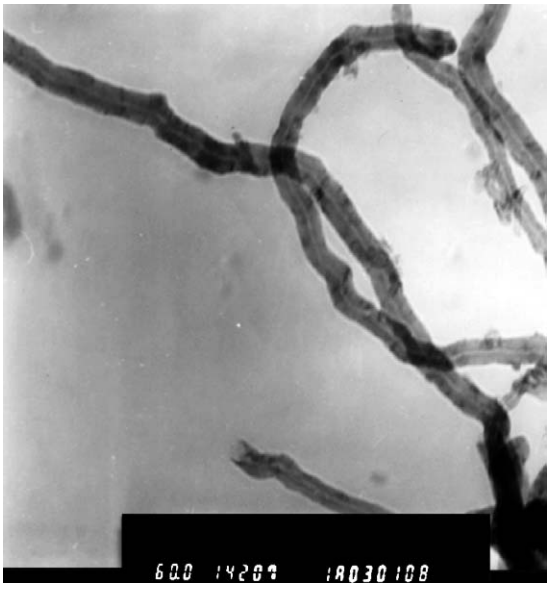


Fig. 2. TEM of the CFN.

Because H_2 was used for the reduction process at 600 °C for 12 h, the result of element analysis shows that the oxygen content in nanocomposite is quite low.

3.2. Electrochemical activity of CFNPE

The CFN paste electrode was used to investigate the electrochemistry of a wide range of chemical species. Using potassium ferricyanide cyclic voltammetric current recorded as function of scan rate shows a linear I_p versus $v^{1/2}$ relationship covering the 10–200 mV/s range. This indicates that the current is controlled by a semi-infinite linear diffusion.

The electrochemical characteristics of CFNPE were studied by cyclic voltammetry in 0.067 mM phosphate buffer solution (pH 7.0) and at a scan rate of 50 mV/s. The advantages of these new electrodes were compared with conventional CPE.

Fig. 3a shows the cyclic voltammogram obtained with CFNPE in PBS. At −622 mV, an oxidation peak appears. There is no peak on Fig. 3b obtained with CPE at potential range from 0 to −1200 mV. The results indicate that CFN can be oxidized, that is to say, CFN consists of nanoparticles with electrochemical activity.

Several different chemicals were utilized to investigate the electrochemical properties of CFNPE, compared with that of conventional CPE. Fig. 4 shows cyclic voltammograms obtained at 50 mV/s for ferricyanide, ascorbic acid, and cysteine at CFNPE (solid line) and CPE (dash line) without pretreatments. Ferricyanide (Fig. 4a) displays a couple of redox peaks. The oxidation and reduction potential on the CFNPE shifts negatively 139 and −93 mV, respectively, compared with that on CPE. For ascorbic acid (Fig. 4b), there is no electrochemical response on the conventional CPE, while a quasi-reversible redox process occurs at CFNPE. The oxidation and reduction potentials are 290 and 105 mV, respectively. For cyclic voltammetric experiments of cysteine (Fig. 4c), a high degree reversible reaction was observed at CFNPE

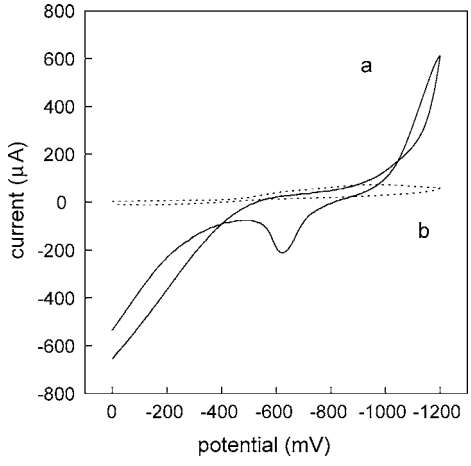


Fig. 3. Cyclic voltammograms obtained with the CFNPE (solid line) and CPE (dash line) in PBS (pH 7.0). Scan rate: 50 mV/s.

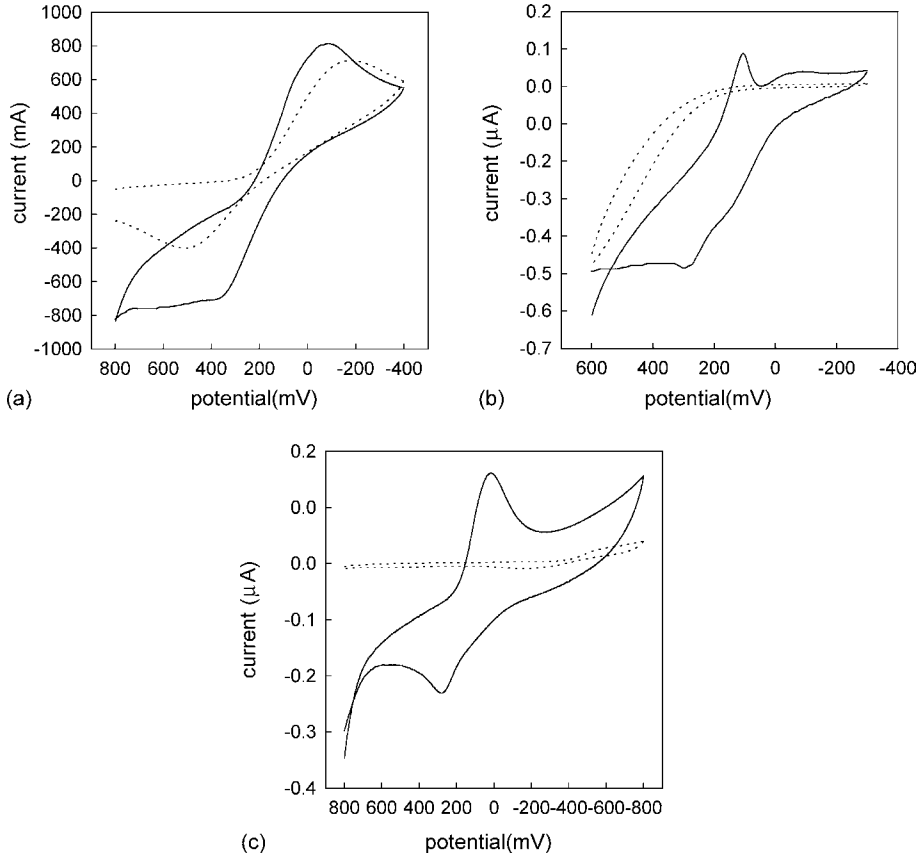


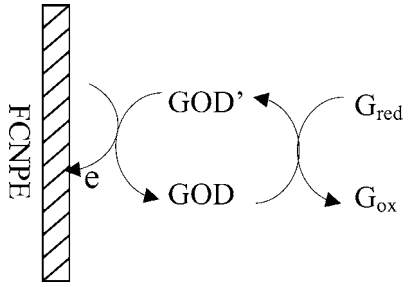
Fig. 4. Cyclic voltammograms obtained with the CFNPE (solid line) and CPE (dash line) in 20 mM $Fe(CN)_6^{3-}$ (a), 10 mM ascorbic acid (b) and 10 mM cysteine (c). Scan rate: 50 mV/s.

(ΔE_p = 265 mV) and the redox peaks at CPE turn to be very small. In fact, the electrochemical oxidation of cysteine was investigated at different concentrations, ranging from 0.01 to 10 mM, at CFNPE. There is a linear relationship between the cysteine concentration and oxidation current. All experimental results indicate that CFNPE can accelerate the electron-transfer to improve the electrochemical reaction reversibility, as compared with the conventional CPE.

3.3. The mediator-free glucose biosensor based on the CFNPE

3.3.1. Voltammetric characteristics of glucose biosensor

Fig. 5a shows the cyclic voltammograms obtained with the CFNPE-immobilized GOD in an unstirred 0.067 mol/l PBS (pH 7) with (solid line) and without (dash line) 10 mM glucose in the presence of O_2. As can be clearly seen, a couple of redox peaks appear, which attribute to the oxidation and reduction of Fe in CFN after the pretreatment. The reduction current increases greatly in the presence of glucose, while that of oxidation decreases. When the solutions are free of O_2, the magnitude of catalytic reduction response is similar with that in the presence of O_2 (Fig. 5b). These phenomena indicate that the electrons directly transfer between GOD and the CFNPE surface and O_2 has no effect on the electron-transfer.



Scheme 1.

According to reference [29,33], the possible mechanism may be as follows (Scheme 1):

$$GOD + G_{red} \rightarrow GOD' + G_{ox} \quad (1)$$

$$CFN + GOD' \rightarrow CFN_{ox} + GOD \quad (2)$$

$$CFN_{ox} + e^- \rightarrow CFN \quad (3)$$

where G_{red} and G_{ox} represent glucose and its oxidized form, respectively. GOD' represents the reduction form of GOD. CFN_{ox} represents the oxidation form of CFN.

In experiments, we found GOD immobilized on CPE whose preparation process is same as that of GOD immobilized on CFNPE, is easy to break off in solution. No reodx

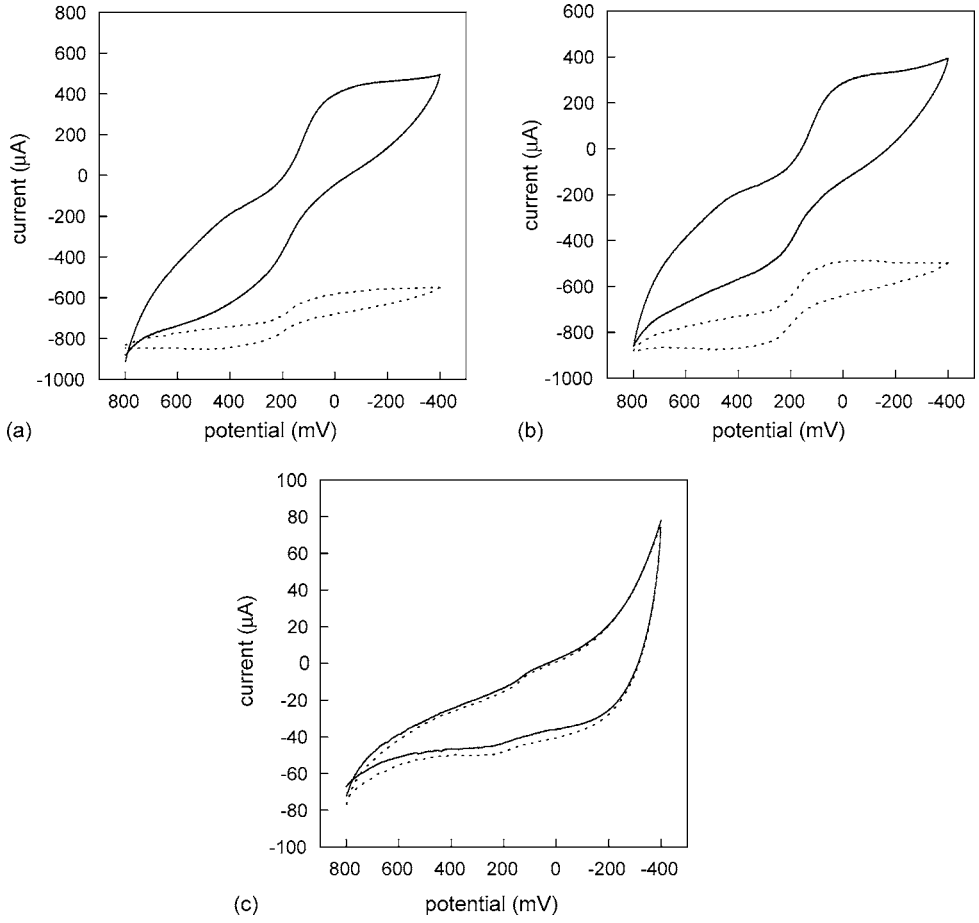


Fig. 5. Cyclic voltammograms obtained with the GOD biosensor based on the CFNPE in 10 mM glucose (solid line) and PBS (dash line) solution with oxygen (a), without oxygen (b) and without the pretreatment and with oxygen (c). Scan rate: 50 mV/s.

peaks appear on the cyclic voltammogram and no redox current change was observed in the presence of glucose. The results show that GOD immobilized on CPE using this way cannot form the glucose biosensor.

Fig. 5c shows the effect of the pretreatment on the glucose biosensor. The CFNPE was immobilized with GOD without the pretreatment in 20 mM potassium ferricyanide. There were no obvious redox peaks observed on the cyclic voltammograms. And the reduction current did not increase after the addition of glucose. The result shows that the pretreatment is necessary for the preparation of the glucose biosensor.

XRD showed that Fe in CFN is the form of α-Fe so the pretreatment transfers α-Fe to iron ion. It is a common knowledge that iron ion is prone to complex with enzyme and protein. According to the experimental results, it is suggested that GOD was immobilized on the CNFPE after the pretreatment by covalently complex action between GOD and iron ion.

3.3.2. *Effect on the Nafion*

Fig. 6 compares the effect of the amount of Nafion on the response of the glucose biosensor. It is observed that the amperometric response is rather small when only small amount

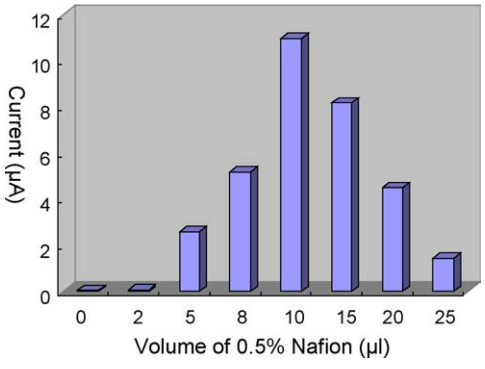


Fig. 6. Effect of the different amount of Nafion on the response using amperometry.

or even no Nafion was covered on GOD film, which seems to be attributed to the easy dissolution of GOD in solution. The response decreases with the increase of Nafion when more than 10 μl of 0.5% Nafion is added. It is found that the optimum amount was10 μl of 0.5% Nafion.

Moreover, the GOD biosensor without Nafion suffers from the interference of ascorbic acid and uric acid. The response due to glucose on the GOD membrane-covered Nafion is

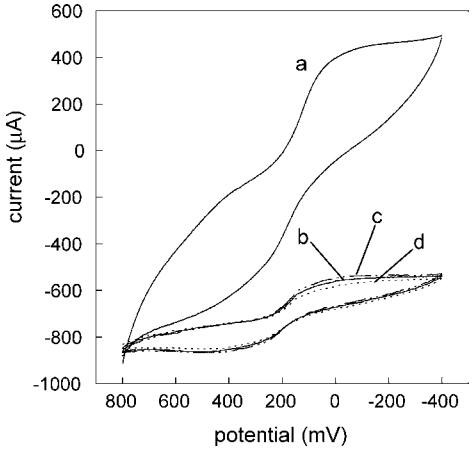


Fig. 7. Cyclic voltammograms obtained with the GOD biosensor based on the CFNPE in 10 mM glucose (a), PBS (b), ascorbic acid (c) and uric acid (d). Scan rate: 50 mV/s.

much greater comparing to those due to ascorbic acid and uric acid (Fig. 7). In glucose detection, the interference of these species can actually be ignored.

3.3.3. Detection of glucose

The reduction peak current was investigated at the glucose biosensor using different concentrations of glucose from 3.33×10^{-5} to 1×10^{-3} mol/l (pH 7). With the concentrations increasing, the peak potential appears to decrease slightly. The peak current increases with the increase of glucose concentration.

The effect of applied potential on glucose biosensor response was investigated. With the increase of applied potential from −50 to 250 mV, the glucose biosensor shows excellent sensitivity. As applied potential is less than 50 mV, the sensitivity actually levels off. A potential of 50 mV is the optimum applied potential for amperometric measurements.

Through experiments, it is found that the linear range covers from 6.67×10^{-6} to 1×10^{-2} mol/l with a detection limit of 3.17×10^{-6} mol/l, as shown in Fig. 8. The linear regression equation is I (μA) = 3.094 log C (M) + 17.79 with a correlation coefficient of 0.9934. The response is saturated at the glucose concentration of 3.33×10^{-2} mol/l.

The repeatability of response current of the GOD electrode was investigated at a glucose concentration of 0.67 mM. The variation coefficient (R.S.D.) is 3.3% for five successive assays. Sensors prepared independently show a good reproducibility with a R.S.D. of 4.2% as obtained for the response current at 0.67 mM glucose. The results indicate that the glucose biosensor has good stability and repeatability.

The stability of the glucose biosensor was investigated by monitoring the biosensor response with 6.67 mM glucose everyday over a month. The response current of biosensor decreased slightly after 20 days. After more than 20 days, the biosensor response tends to decline. After one month, the electrode had about 81.7% of its original sensitivity. The

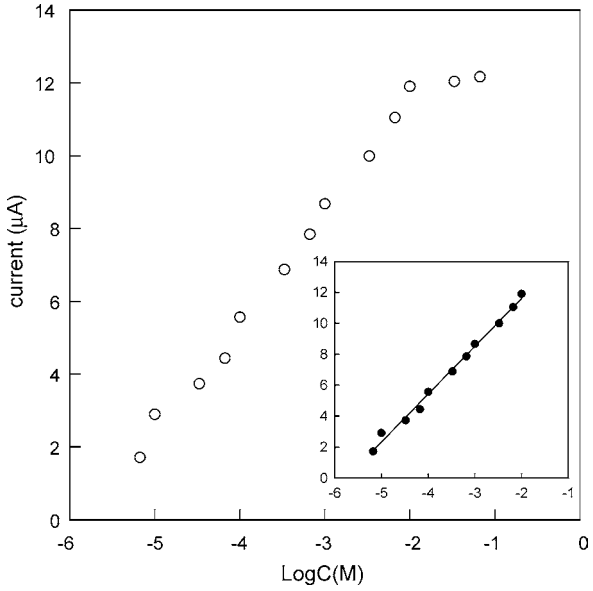


Fig. 8. Steady state calibration curve of GOD electrode in PBS (pH 7) at reduction potential of 50 mV (vs. SCE).

Table 1
Determination of glucose in human serum samples

Sample number	Spectrophotometry method (mmol/l)	Biosensor method (mmol/l)[a]	R.S.D. (%)
1	2.12	1.96	3.1
2	2.88	2.71	2.9
3	3.43	3.52	3.7

[a] Mean of five measurements.

results show that the GOD immobilized on the CFNPE can retain good activity for long time.

The glucose biosensor based on CFNPE is shown to be useful in the determination of glucose in human serum samples. The results are acceptable as compared with the traditional method (shown in Table 1).

4. Conclusion

The present study investigated the microstructure and electrochemical activity of CFN in detail. The XRD and TEM measurements indicate the formation of the nanocomposite materials with microstructure in the carbon nanotubes and α-Fe nanoparticle enveloped in nanotube wall. The CFNPE described in this paper provides a sensitive tool for investigating the electrochemitry of small molecular (potassium ferricyanide, ascorbic acid and cysteine), and it can be used as the substrate of the mediator-free glucose biosensor. The CFNPE has better electron-transfer property, and the reversibility electrochemical reaction involving small molecules could be improved by it, compared with that on the CPE.

GOD was covalently immobilized on the CFNPE surface after the pretreatment and then covered by Nafion to form a third-generation glucose biosensor. Without the mediator,

the GOD biosensor possesses high enzymatic activity and high affinity for glucose. Oxygen, ascorbic acid and uric acid have no interference with the glucose detection. Under optimized experimental conditions, broad linear range and low detection limit were obtained. The GOD immobilized on the CFNPE can retain good activity. On the other hand, its ease of fabrication, nice stability and reproducibility are obvious advantages and the results used in the determination of glucose in human serum samples are satisfactory.

Acknowledgements

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