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Glucose biosensor based on platinum nanoparticles supported sulfonated-carbon nanotubes modified glassy carbon electrode

H.J. Wang, C.M. Zhou, F. Peng, H. Yu*

The School of Chemical and Energy Engineering, South China University of Technology, Guangzhou 510641, PR China
*E-mail: cefpeng@scut.edu.cn

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Highly dispersed Pt nanoparticles supported on sulfonated multiwalled carbon nanotubes (Pt/sulfonated-MWCNTs) were used to modify glassy carbon (GC) electrode and then glucose oxidase was immobilized on the Pt/sulfonated-MWCNTs/GC electrode to construct a GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor. The electrochemical and detection performance were evaluated by cyclic voltammogram and chronoamperometry. The optimum detection conditions were determined and the stability was studied. The results show that the GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor has much higher detection sensitivity of 0.56 μA/mM and much larger linear range up to 6.4 mM at rather lower working potential of 0.5 V. It can keep more than 85% of its initial activity after continuously using one hour. The results show that the resultant GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor has high electrocatalytic activity and excellent detecting performance for glucose.

Keywords: glucose biosensor; chronoamperometry; Pt nanoparticles; sulfonated-MWCNTs

1. INTRODUCTION

Amperometric glucose biosensor is the most common used method for glucose detection, because of its advantages, such as simplicity and quickness. But there still exist some problems, such as narrow linear range, low sensitivity and stability, which can't satisfy the detection requirement with high precision. In order to improve the performance of the glucose biosensor, significant research and development efforts have been devoted to this field by many methods, such as the addition of redox mediators [1], conducting polymer [2-5], nanoparticles [6-11], etc. Among the various methods, the most attracting one at present, is to enhance the electron transfer and improve the electrocatalytic property of the biosensor by using carbon nanotubes (CNTs) [12-15] or the incorporation of CNTs and metal nanoparticles [16-21], which has nano-scaled dimension and conductibility and catalytic properties. Despite its advantages, the barrier of dispersing CNTs, which is the key for making CNTs-based biosensors, affects the immobilization of enzyme and limits its performance. Hitherto, the CNTs

used in above method is either pristine CNTs or simply purified CNTs. The latter is introduced a small quantity of oxygenous groups, such as carboxyl, carbonyl and hydroxyl, etc., which still can't satisfy the requirement of high dispersity of CNTs in solutions, and then affect the distribution of particles immobilized on the surface of CNTs. Thereby, the exertion of resulting biosensor modified by CNTs is restricted.

In this paper, carbon nanotubes with sulfonic groups, which were reported in our previous works [22, 23], were first used to construct a high sensitive glucose biosensor based on Pt nanoparticles supported on sulfonated multiwalled carbon nanotubes modified glassy carbon (GOD/Pt/sulfonated–MWCNTs/GC biosensor) with a very simple method. With this type of the carbon nanotubes, the Pt nanoparticles can be dispersed rather uniformly on the surface of the nanotubes [23, 24]. The results show that GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor has high detection sensitivity of 0.56 μA/mM and can work at much lower working potential of 0.5 V. The linear range of the GOD/Pt/sulfonated- MWCNTs/GC biosensor for glucose detection reaches 6.4 mM with the correlative coefficient of 0.993. The glucose biosensor prepared by this method can keep more than 85% of its initial activity after continuously using one hour.

2. EXPERIMENTAL PART

2.1. Reagents and solutions

Glucose oxidase (GOD, 133,600 units/g, type X-S, from Aspergillus niger), β-D(+)-glucose (97%) and Nafion (5 wt% in ethanol) were purchased from Sigma. Multi-walled carbon nanotubes (MWCNTs, 10-20 nm outer diameters) were kindly provided by Chengdu Organic Chemicals Co. Ltd.. All the other reagents used are analytic grade. 0.1 M phosphate buffer solution (PBS) as electrolyte solution was prepared from sodium dihydrogenphosphate and disodium hydrogenphosphate. All the reagents and material were used as received without further purification. All solutions were prepared with deionized water of a resistivity not less than 18 MΩ·cm (Milli-Q, USA). Experiments were performed at room temperature (25±1 ^{o}C).

2.2. Apparatus

The cyclic voltammogram and chronoamperometric experiments were carried out using a computer-controlled Autolab PGSTAT30 potentiostat/galvanostat with Gpes software (Eco Chemie, Netherlands). A conventional three electrode electrochemical cell was used in this work with a bare or modified glassy carbon electrode as working electrode (2 mm diameter), a platinum electrode as counter electrode and a Ag/AgCl electrode with saturated KCl as reference electrode, respectively. During the chronoamperometry experiment, a stirrer (DF-101B, Shanghai magnetic apparatus company) was used to keep the solution uniform. A precise pH meter (pHS-3C, LeCheng wiring company, Zhejiang province) was used to measure the pH value of different buffer solutions.

2.3. Preparation of modified GC biosensor

The preparation of Pt/sulfonated-MWCNTs was expatiated in our previous paper detailedly [22, 23]. Before modification, the glassy carbon (GC) electrodes was polished by 1400# and 0.5 μm alumina, and then washed with HCl, NaOH, ethanol and deionized water by ultrasonic, sequently. 1 mg Pt/sulfonated-MWCNTs was dispersed in 0.5 mL 0.5 wt% Nafion solution, and then ultrasonicated to form a homogeneous dispersed system. 5 μL of the dispersed solution was then casted on the washed surface of the GC electrode and dry at a desiccator for 1 hour to obtain the Pt/sulfonated-MWCNTs modified GC (Pt/sulfonated-MWCNTs/GC) electrode. For comparing, the same procedure was conducted to construct another two modified GC electrodes, MWCNTs and Pt/MWCNTs modified GC (MWCNTs/GC and Pt/MWCNTs/GC) electrodes, respectively. Then 5 μL of GOD (1mg/mL) was casted on the modified GC electrodes and dried for 1 hour at room temperature to obtain the resultant GOD/Pt/sulfonated-MWCNTs/GC, GOD/Pt/MWCNTs/GC and GOD/MWCNTs/GC glucose biosensors, respectively. The three GC glucose biosensors were stored in 0.1 M PBS (pH 7) at 4 ℃ in refrigerator when not in use.

3. RESULTS AND DISCUSSION

3.1. Comparison of cyclic voltammogram of three GC electrodes

Fig.1 is the cyclic voltammogram (CV) curves of the Pt/sulfonated-MWCNTs/GC, the Pt/MWCNTs/GC and the MWCNTs/GC electrodes in PBS (pH 7). It can be seen that the responding current of the Pt/MWCNTs/GC electrode is larger than that of the MWCNTs/GC electrode, especially at higher potential and lower potential, which exhibits the catalytic activity of Pt immobilized on the MWCNTs. The responding current of the Pt/sulfonated-MWCNTs/GC electrode is much larger than that of the other two electrodes. Moreover, several oxidation and reduction peaks appear on the CV curve of the Pt/sulfonated-MWCNTs/GC electrode, which is probably attributed to the oxidation and reduction of the sulfonic groups attached onto the surface of MWCNTs.

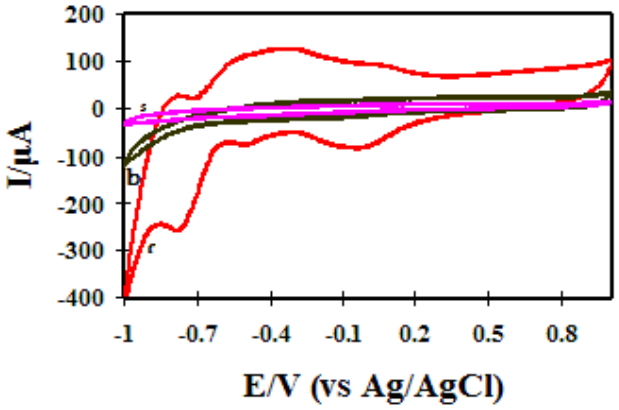

Figure 1. Cyclic voltommagram of MWCNTs/GC (a), Pt/MWCNTs/GC (b) and Pt/sulfonated MWCNTs/GC (c) electrodes in PBS (pH 7), scan rate: 100mV/s

3.2. Determination of optimum measurement condition of different modified glucose biosensors

In chronoamperometric measurement, pH value and working potential are the important factors, which influence the performance of the glucose biosensor. So, the optimum measurement condition should be determined first.

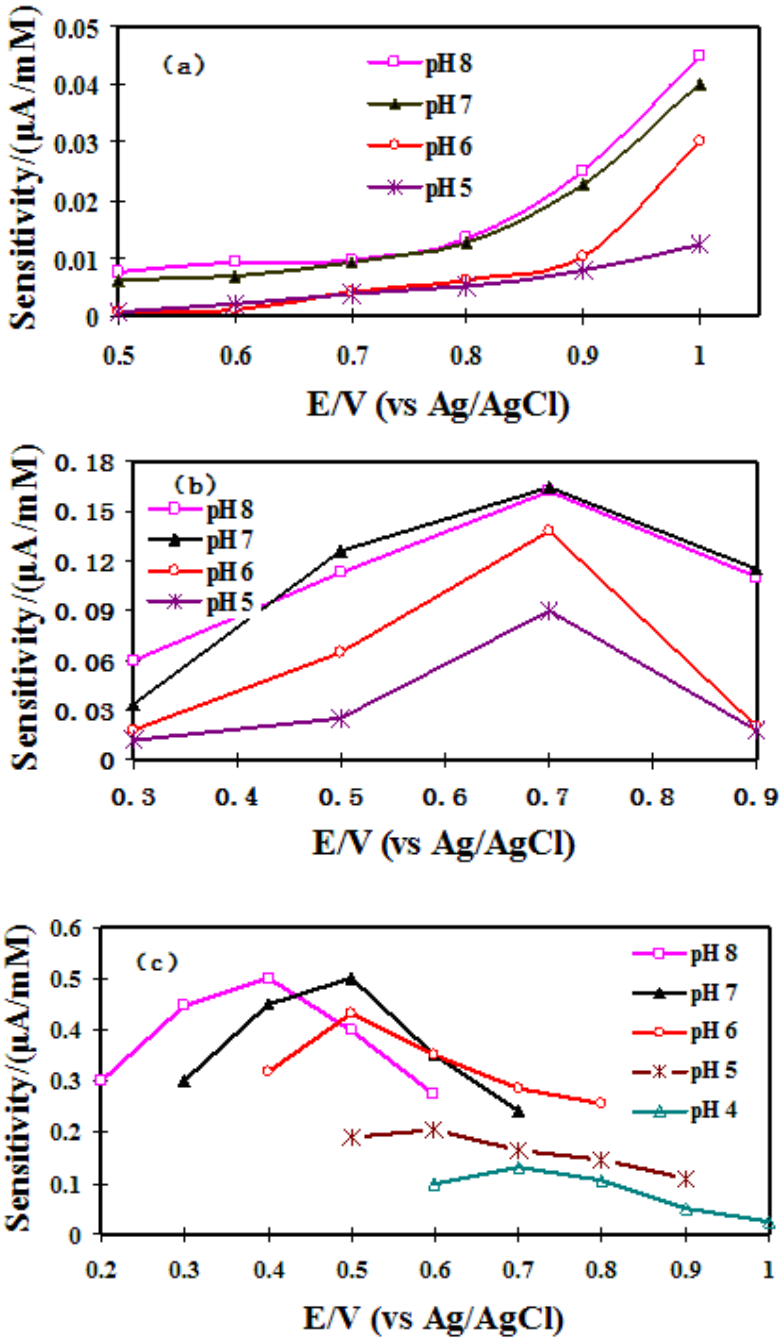

Figure 2. Relationship between amperometric responding to 0.4 mM glucose and working potential of chronoamperometry in different pH solutions with GOD/MWCNTs/GC (a); GOD/Pt/MWCNTs/GC (b) and GOD/Pt/sulfonated-MWCNTs/GC (c) biosensors

Fig.2 is the relationship between the responding current of chronoamperometry and the working potential at different pH buffer solutions with once 0.4 mM glucose addition. For the GOD/MWCNTs/GC glucose biosensor, the responding current increases with the increase of working potential in the range of 0-1 V in different pH buffer solutions (as shown in Fig.2a). But too high working potential can cause the coexisting substance to be oxidized; therefore the optimum working potential was chosen at 0.9 V. For the GOD/Pt/MWCNTs/GC glucose biosensor, all responding current reach the maximum at the working potential of 0.7V to all the buffer solution with different pH values (as shown in Fig.2b), naturally, the optimum working potential was chosen at 0.7V. The responding current increases with the increase of the pH value of the buffer solution for both the two kind biosensors from pH 5 to pH 8, which is due to the fact that the activity of the GOD can exert well at nearly neutral range. Considering the stability of the GOD and only little increase of responding current by increase the pH value from 7 to 8, the optimum pH value of the buffer solution was chosen at pH 7 for both the GOD/MWCNTs/GC and the GOD/Pt/MWCNTs/GC glucose biosensors. For the GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor, as shown in Fig.2c, the minimum working potential for the biosensor responding to glucose is rather lower than that of the other two biosensors (Fig.2a and Fig.2b) and lowers with the increase of the pH value of the buffer solution.

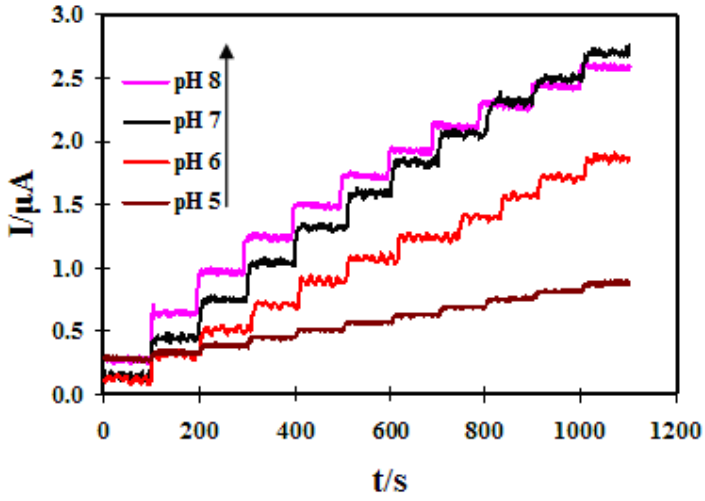

Figure 3. Chronoamperometry of GOD/Pt/sulfonated-MWCNTs/GC biosensor to glucose at optimum working potential of different pH buffer solutions (ten times addition with 0.4 mM one time)

For the GOD/Pt/sulfonated-MWCNTs/GC, different pH buffer solution corresponds to a different optimum working potential, at which responding current reaches the maximum. So, further experiments were needed to determine the optimum working potential and pH value for the GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor. Fig.3 is the chronoamperometric curves of the GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor to glucose at the respective optimum working potential of different pH buffer solutions (ten times addition with 0.4 mM one time). The linear fit is shown in Fig.4 and the results are summarized in Table 1. It can be seen that the responding current increases with the increase of pH value at the respective optimum working potential (Fig.3 and Fig.4).

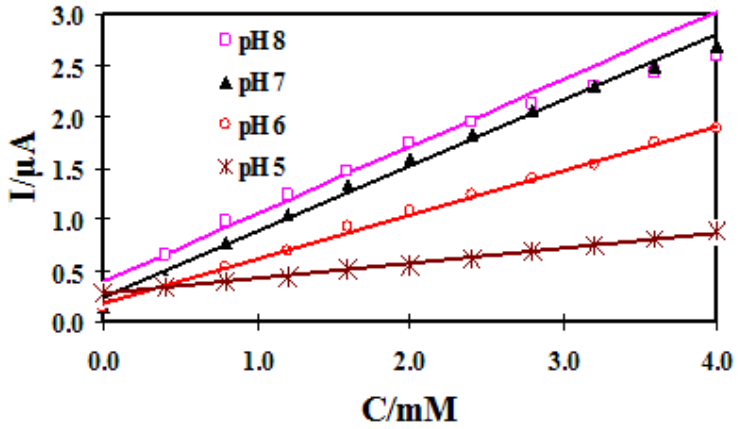

Figure 4 Linear fit of responding current and concentration of glucose for GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor in Fig.3

While the linear range and the correlation decrease with the increase of pH value (Fig.4 and Table 1). Although the optimum working potential at pH 8 is lower than that at pH 7, the sensitivity is almost the same with that at pH 7 and the linear range is much lower than that at pH 7, which indicates that higher pH value makes the stability of the biosensor decrease because of the deactivation of GOD at higher pH value. Considering the detecting sensitivity, linear range and the activity of the glucose oxidase synthetically, the optimum pH value of the buffer solution for Pt/sulfonated-MWCNTs modified glucose biosensor was chosen at 7 and the optimum working potential was 0.5 V accordingly.

Table 1. Results of linear fit for Pt/sulfonated-MWCNTs modified glucose biosensor responding to glucose

pH value	Working potential (V)	Linear range (mM)	Sensitivity (μA/mM)	Correlative coefficient
5	0.6	>4.0	0.15	0.998
6	0.5	>4.0	0.44	0.998
7	0.5	>4.0	0.64	0.997
8	0.4	~2.8	0.65	0.993

3.3. Comparison of detection performance of glucose biosensor with different modification

Fig. 5 is the chronoamperometric curves of the three modified glucose biosensors. From Fig. 5, we can obtain the calibration curves of glucose concentration shown in Fig.6 and the detecting results were summarized in Table 2. The results show that the responding current of the GOD/Pt/sulfonated-MWCNTs/GC biosensor to glucose is much larger than that of the GOD/Pt/MWCNTs/GC and the GOD/MWCNTs/GC biosensors at their respective optimum working potentials and pH values. The detecting sensitivity of the GOD/Pt/sulfonated-MWCNTs/GC biosensor to glucose (0.56 μA/mM) is twice larger than that of the GOD/Pt/MWCNTs/GC biosensor and more than five times larger than that

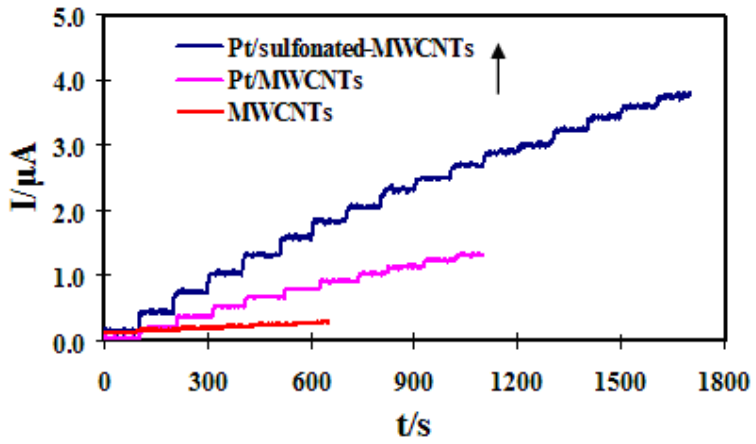

Figure 5. Chronoamperometry of GOD/Pt/sulfonated-MWCNTs/GC, GOD/Pt/MWCNTs/GC and GOD/MWCNTs/GC biosensors to glucose at their respective optimum detecting conditions

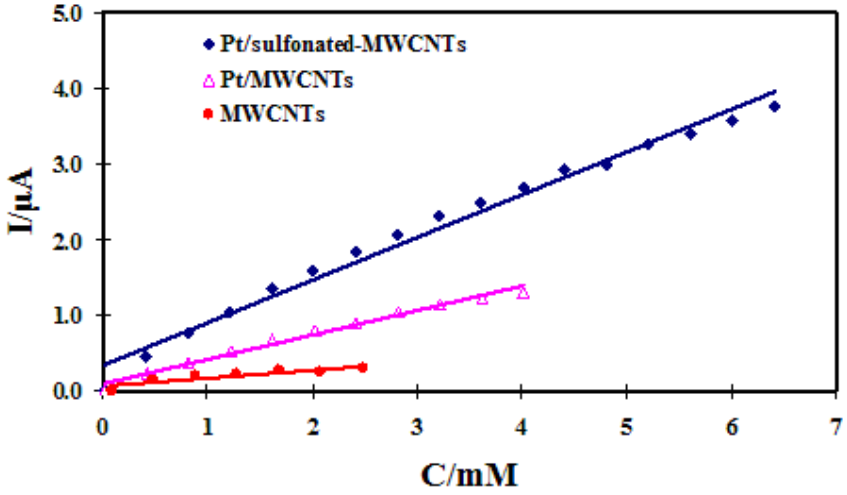

Figure 6 Linear fit of chronoamperometric responding current of GOD/Pt/sulfonated-MWCNTs/GC, GOD/Pt/MWCNTs/GC and GOD/MWCNTs/GC biosensors to glucose concentration in Fig. 5

of the GOD/MWCNTs/GC biosensor (0.10 μA/mM). Moreover, the working potential of the GOD/Pt/sulfonated-MWCNTs/GC biosensor (0.5V) is the lowest, which is favorable to avoid the oxidation of coexisting substance and thus has the higher detecting accuracy. The linear range of the GOD/Pt/sulfonated-MWCNTs/GC biosensor to glucose is the largest, which is up to 6.4 mM.

Table 2. Comparison of detection performance of Pt/sulfonated-MWCNTs, Pt/MWCNTs and MWCNTs modified glucose biosensor to glucose

Modification	Working potential (V)	Linear range (mM)	Sensitivity (μA/mM)	Correlative coefficient
MWCNT	0.9	0.08-2.4	0.10	0.969
Pt/MWCNT	0.7	0.02-4.0	0.32	0.993
Pt/ sulfonated –MWCNT	0.5	0.01-6.4	0.56	0.993

3.4. Comparison of stability of glucose biosensor with different modification

The stability of biosensors is also a very important factor, which influence the application and detecting veracity of the biosensor. The chronoamperometric responses to 0.4 mM glucose for the three modified glucose biosensors at their respective optimum working potential were recorded over a continuous 60 minutes period. Fig. 7 shows the comparison of the stability of the GOD/MWCNTs/GC, the GOD/Pt/MWCNTs/GC and the GOD/Pt/sulfonated-MWCNTs/GC glucose biosensors. Here, the activity remained percentage was defined as the ratio of the activity remained after using a period of time to the initial activity, which is the quantitive expression of the stability. The response of the GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor remains stable throughout the entire experiment, with more than 85% of its initial activity after continuously using one hour. In contrast, the GOD/MWCNTs/GC glucose biosensor displays a rapid decay of the response, with almost losing all its activity after continuously using only about half an hour, which indicates a nearly complete inhibition of the oxidation process.

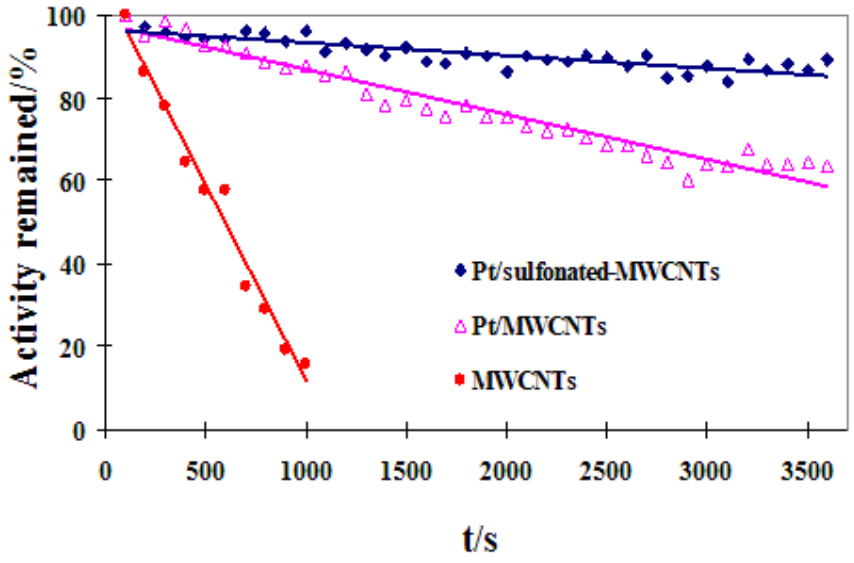

Figure 7. Stability of GOD/Pt/sulfonated-MWCNTs/GC, GOD/Pt/MWCNTs/GC and GOD/MWCNTs/GC glucose biosensors

The detecting performance of the GOD/Pt/MWCNTs/GC biosensor to glucose is only a little better than of the GOD/MWCNTs/GC biosensor. But the detecting performance of the GOD/Pt/sulfonated-MWCNTs/GC biosensor to glucose is much better than of the GOD/Pt/MWCNTs/GC biosensor. All these results show that the GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor prepared in this work has excellent detecting performance for glucose and good stability. It is probably due to the fact that Pt can be dispersed more uniformly on the surface of sulfonated MWCNTs than on pristine MWCNTs and Pt can exert its catalytic property more fully [23, 24]. So, it suggests that the dispersity of Pt particles on the surface of MWCNTs has great effect on the detecting performance of biosensor.

4. CONCLUSIONS

The GOD/Pt/sulfonated-MWCNTs/GC glucose biosensor prepared in this work shows excellent detecting performance for glucose with the optimum pH value of 7 and working potential of 0.5 V. The detecting sensitivity of the GOD/Pt/sulfonated-MWCNTs/GC biosensor, which is 0.56 μA/mM, is twice larger than that of the GOD/Pt/MWCNTs/GC biosensor and more than five times larger than that of the GOD/MWCNTs/GC biosensor. The linear range of the GOD/Pt/sulfonated-MWCNTs/GC biosensor to glucose is up to 6.4 mM. The stability of this biosensor is good with more than 85% of its initial activity reserved after continuously using one hour.

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