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Amperometric Biosensor with Al_2O_3/Al Foil Electrodes Modified by Pt Nanofuzz for Glucose Detection

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Electrodes of Al_2O_3/Al foils modified with Pt nanofuzz, which has a morphology resembling "fuzz," were fabricated by a novel method with a simple replacement reaction. Glucose oxidase was immobilized by cross-linking via glutaraldehyde, so as to form a new type of biosensor. The microstructure and composition of the electrodes were characterized by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), and the electrocatalytic ability of the sensor was determined from capacitance-voltage (C-V) curves. The sensors exhibited a high sensitivity of 56.79 $\mu A \cdot mM^{-1}cm^{-2}$ at +0.6 V (vs SCE). A linear range of response was found from 0.25 to 8 mM of glucose, with response time <10 s. The detection limit of the sample was estimated to be 12.5 $\mu M/cm^2$ (S/N = 3), and the Michaelis-Menten constant was calculated to be 7.35 mM. The optimal amount of glucose oxidase on the response current of the sensors was 20 U per electrode, and the optimal pH value was 6.86. The stability and selectivity of the sensors were also evaluated. By using uricase and cholesterol oxidases, this technique may be applied to other biosensors for the detection of uric acid and cholesterol.

1. Introduction

Much attention has been focused on glucose biosensors since Updike and Hicks fabricated the first type of glucose oxidase biosensor.[1] Different immobilization methods,[2–6] various immobilization materials,[7,8] and modified electrodes have been applied to electrochemical biosensors.

To improve the performance of the biosensor, various nanocomposites including semiconductors, metals, and polymers, were recently used to modify electrodes, which has led to high surface-to-volume ratios and biocompatibility. Different types of semiconductive nanocomposites were used, such as carbon nanotubes,[9,10] titanate nanotubes,[11] ZnO nanotubes,[12] and NiO nanospheres.[13] Metallic nanocomposites, *e.g.*, platinum nanoparticles[14] and nanotubes,[15] have a high catalytic activity for hydrogen

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peroxide electrooxidation. Moreover, Pt-Au hybrid film modified electrodes were also applied for the detection of dopamine, ascorbic acid, and uric acid.[16]

Conducting polymers such as PPy with high specific surface areas have been developed as potential candidates for biosensor applications.[17,18] Recently, a polypyrrole nanotube array sensor has been reported for enhanced adsorption of glucose oxidase in glucose biosensors,[19] and polypyrrole nanofibers were electrodeposited onto pencil graphite electrodes to form enzymeless biosensors.[20]

In this work, porous Al_2O_3/Al foil with high apparent surface area and modified with Pt nanofuzz, which has a morphology resembling fuzz, with high catalytic activity were applied, so as to improve the performance of biosensors. Firstly, Al_2O_3/Al foils were utilized as the electrode substrate. Although porous Al_2O_3/Al foil has been used as the anode of conventional aluminum electrolytic capacitors, there is no report on its application as the substrate of electrobiosensors. Secondly, electrodeposition with cyclic voltammetry is usually applied for the preparation of a Pt-nanomaterial-modified electrode.[14,15] However, in this work platinum nanofuzz was grown on the Al_2O_3/Al foil electrodes by a novel method of a simple replacement reaction instead of electrodeposition. This novel chemical method in this work is simpler and more timesaving compared with electrodeposition. With the immobilization of glucose oxidase, finally, a new type of biosensor with high sensitivity was fabricated and characterized.

2. Material and Methods

2.1 *Chemical reagents and electrode substrates*

Chemical reagents were used, including sulfuric acid, ascorbic acid, fructose, urea, chloroplatinic acid, bovine serum albumin (BSA), glutaraldehyde (GTA), and d-glucose, all of which were of analytical grade. Glucose oxidase (Aspergillus niger, E.C. 1.1.3.4, 126 U/mg) was directly applied. Aluminum oxide foil (Wenlin Machinery Plant, Zhejiang) was washed with ethanol and deionized water in an ultrasonic tank before use. Britton-Robinson buffer solutions (BR) at different pH values were prepared by mixing the stock solutions of 0.04 M orthophosphoric acid, acetic acid and boric acid, and adjusting the pH with 0.2 M NaOH.

2.2 *Preparation of biosensor*

A 50-nm-thick film of Au was sputtered onto Al_2O_3 foil by plasma sputtering. Then, it was dipped into a solution of 2 mM H_2PtCl_6 and 0.5 M H_2SO_4 for about 4 min. Meanwhile, the golden surface of the foil finally turned black. This is a novel method with a simple replacement reaction, that is, Au is replaced by Pt. Then, the electrode was washed with ethanol and deionized water. Then, different amounts of GOx (from a stock solution of GOx, 0.35% GTA and 1% BSA) were carefully dropped onto the surface of Pt nanofuzz/Al_2O_3/Al foil. After 4 h, the resulting electrode was rinsed with deionized water to remove the unbound GOx and then dried in air. The biosensor was stored at 4°C and used for further experiments. A photograph of the sensor is shown in Fig. 1(b) inset. The reaction area of the sensor is about 5 mm × 5 mm.

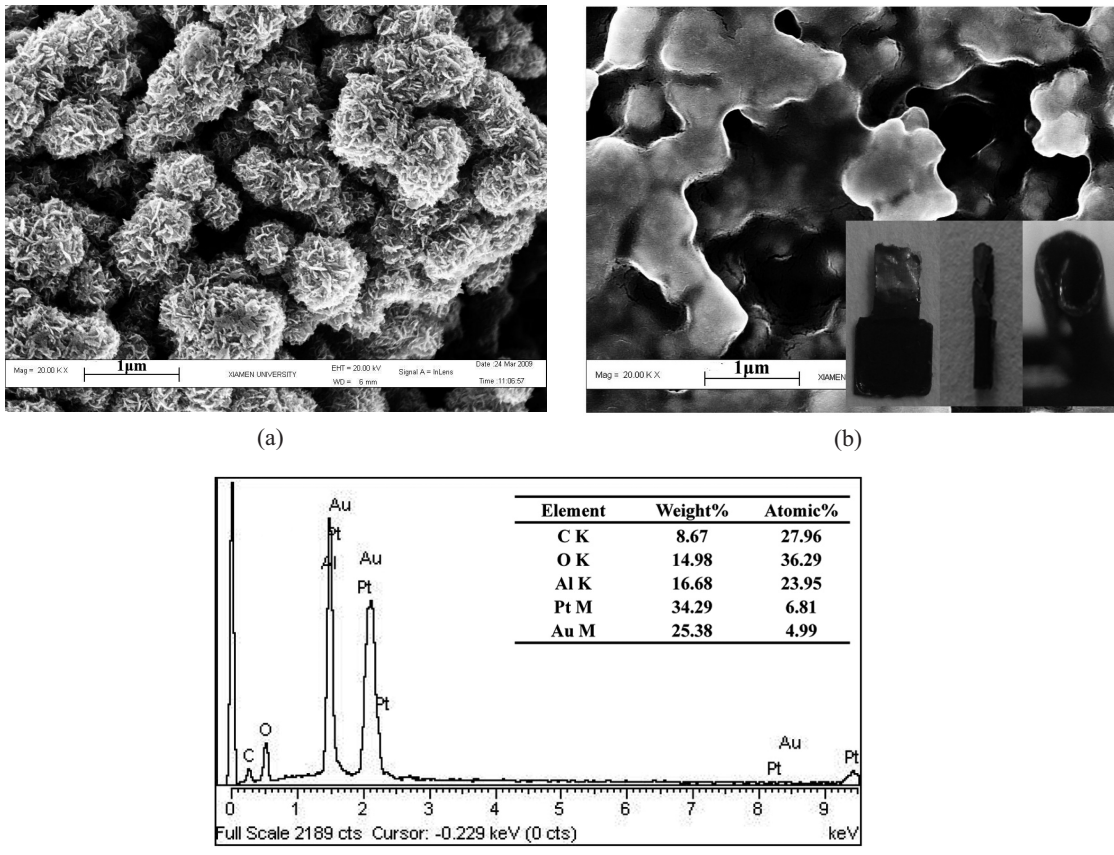

Fig. 1. SEM images of Pt nanofuzz/Al_2O_3/Al foil (a), and the sensor after cross-linking via GTA (b). The inset is a photograph of the biosensor. EDS spectrum of Pt nanofuzz/Al_2O_3/Al foil (c).

2.3 *Instrumentation and characterization*

Cyclic voltammetry (CV) was conducted using an EG&G 263A electrochemistry workstation. All the electrochemical experiments were carried out in a conventional three-electrode cell. Platinum foil was used as the counterelectrode and a saturated calomel electrode (SCE) was used as the reference electrode. CV was performed from −0.8 to 1.5 V at 10 mV/s. The electrochemical measurements were conducted in 0.025 mol/L pH 6.86 phosphate buffer solutions. Response current measurements were conducted by the addition of a certain amount of 1 M glucose stock solution to pH 6.86 0.05 M PBS at 0.6 V, while the sensor was used as a working electrode. Unless otherwise stated, the temperature was maintained at 25±2°C during the experiment. The morphology of the electrodes were observed by scanning electron microscopy (Leo 1530, Germany), and energy dispersive X-ray spectroscopy (EDS) patterns were obtained using Inca Energy 300 X- Model 7426 (Oxford, UK).

3. Results and Discussion

3.1 *Characterization of Pt nanofuzz/Al_2O_3/Al foil electrode*

Porous Al_2O_3/Al foil was used as a substrate material and its surface was relatively smooth. After dipping into a solution of 2 mM H_2PtCl_6 and 0.5 M H_2SO_4, the foils formed a nanofuzz morphology, as shown in Fig. 1(a). The formation of Pt nanofuzz resulted from the morphology of Al_2O_3/Al foil coated with Au by the simple replacement reaction described before. After cross-linking via GTA, the Pt nanofuzz/Al_2O_3/Al foil was obviously covered with a GTA/BSA/GOx layer and the biosensor was finally complete (Fig. 1(b)). As the foil of the sensor was flexible, it was easy to roll (Fig. 1(b) inset).

The presence of Pt on the surface of the foil was confirmed by EDS analysis for the Pt nanofuzz/Al_2O_3/Al foil, as displayed in Fig. 1(c). The atomic ratio of O to Al was 1.51, which was close to the stoichiometric proportion of Al_2O_3. Au atoms were also observed, which indicated that part of the Au remained on the surface of the foil.

It is well known that hydrogen peroxide, which is produced by the catalytic reaction (eq. (1)), is able to reach the electrode and be detected (eq. (2)). To study the electrocatalytic ability of the sensor, CV of the sensor was carried out in the absence and presence of 1 mM of glucose in pH 6.86 PBS at a scan rate of 10 mV/s (Fig. 2). With the addition of glucose, the oxidation current was clearly increased in the range from +0.4 to +0.9 V, indicating a wide potential range and high electrocatalytic ability for the sensor.

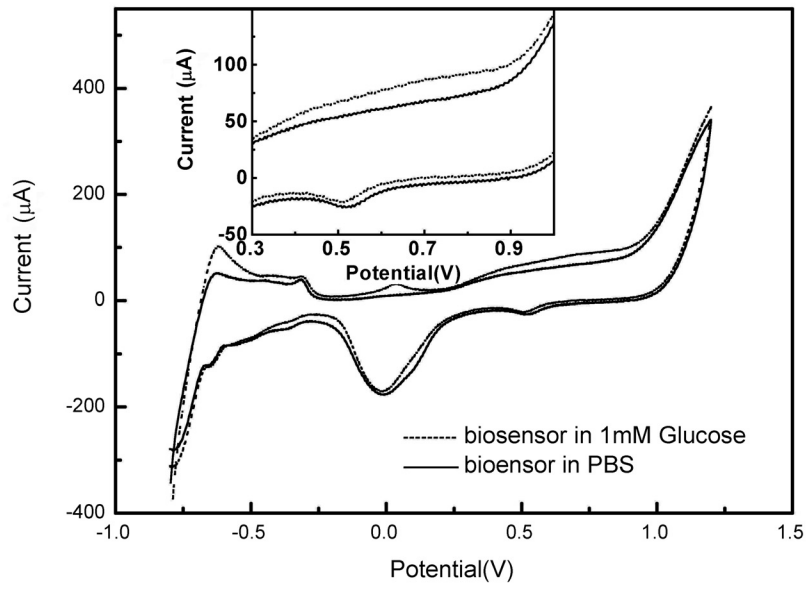

Fig. 2. Cyclic voltammetry of biosensor in the absence and presence of 1 mM glucose in PBS at pH 6.86 at a scan rate of 10 mV/s.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic Acid} + H_2O_2 \quad (1)$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

3.2 *Effects of amount of glucose oxidase on response current of the sensor*

The effects of the amount of GOx on the sensor were examined. Different amounts of GOx (10 U 20 U, 25 U and 30 U) were dropped onto the surface of the Pt nanofuzz/Al_2O_3/Al foil to form the biosensor configuration. The highest response currents of 5, 10, 15, and 20 mM glucose in PBS were achieved with a median amount, 20 U, of GOx (Fig. 3). With a lower amount of GOx (10 U), however, the response current was lower because of insufficient enzyme activity. Moreover, a larger amount of GOx (25 U and 30 U) also led to smaller response. The reason for this may be that the additional enzyme was actually immobilized but the glucose was intercepted as it arrived at the outer surface of the immobilized layer. As the enzyme layer became thicker, most of the reaction occurred at the outer surface where the concentration of oxygen was the highest. The resulting hydrogen peroxide escaped easily into the solution and was not captured effectively at the electrode.

3.3 *Optimum pH of sensor*

The effect of the pH on the response current was investigated in B-R buffer solution with pH ranging from 5.0 to 8.0. The response current of the sensor in glucose (1 mM) PBS increased from pH 5.0 to 6.86 and then decreased at a higher pH of 8.0 (Fig. 3 inset). Compared with the maximum activity of free GOx at a pH of 5.6,[21,22] the response current in this work reached its maximum at pH 6.86, which may be due to the

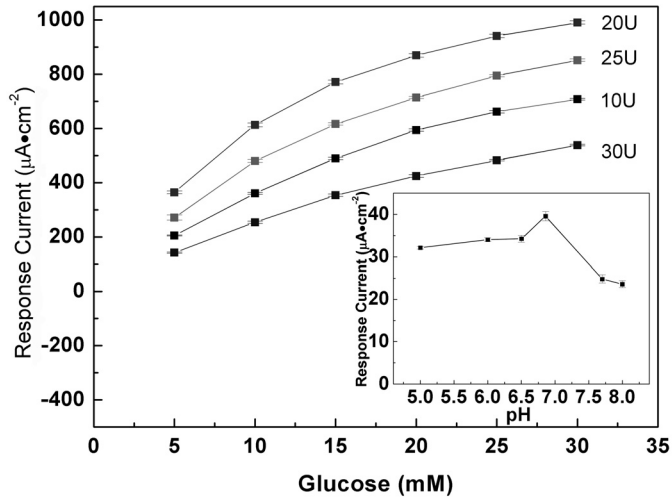

Fig. 3. Effects of amount of added GOx, and pH (inset) on the response current of the sensor.

immobilization of GOx in the GTA/BSA system making GOx more active at pH 6.86. This occurs because the immobilization converts typically free amines (lysine) into a neutral form.

3.4 *Response behavior of glucose biosensor*

The typical response current of the sensor under optimal conditions is illustrated in Fig. 1 (supplementary material) for the successive addition of 5 mM glucose in PBS with a pH of 6.86 at 0.6 V. The current increased along with the glucose concentration from 0 to 30 mM, which indicates a wide range of response to the glucose. However, the increment of the current was narrowed gradually, which indicates a lower response current at higher concentrations of glucose.

The response current of the sensor was investigated for successive additions of 1 mM (Fig. 4(a)) and 0.25 mM of glucose (inset in Fig. 4A) to further evaluate the performance of the biosensor. With high sensitivity and rapid response to glucose, in addition to response times of less than 10 s (95% of the steady-state current), the sensor exhibits a low diffusion resistance and high electronic conductivity for the electrode. The corresponding calibration curve was linear in the range from 0.25 to 8 mM ($R^2 = 0.997$), as shown in Fig. 4(b), with a sensitivity of 56.79 $\mu A \cdot mM^{-1}cm^{-2}$. The detection limit of the biosensor was 12.5 $\mu M/cm^2$ (S/N ratio = 3).

The apparent Michaelis-Menten constant (K_M^{app}), which is generally used to evaluate the biological activity of immobilized GOD, could be calculated using the Michaelis-Menten constant equation (eq. (3)).[23]

$$\frac{1}{i_{SS}} = \left(\frac{K_M^{app}}{i_{max}}\right)\left(\frac{1}{C}\right) + \frac{1}{i_{max}} \tag{3}$$

Here, C is the concentration of the substrate, i_{ss} is the steady-state current, and i_{max} is the maximum current measured under substrate saturation. According to the experimental data from the inset in Fig. 4(b), K_M^{app} was calculated to be 7.35 mM and i_{max} was 434.1 μA. The value of K_M^{app} in this work was lower than those of the native GOx in solution (27.0 mM)[24] and polypyrrole films (37.6 mM).[25] The lower value of K_M^{app} represented a higher enzymatic activity of immobilized GOx. Therefore, it is suggested that oxidation of the GOx (by oxygen) for the sensor is faster.

The performance of the biosensor in this work is compared, in Table 1, with the parameters in other works published recently. The main advantage of our sensors is that the sensitivity is higher than those in other published works.

3.5 *Selectivity and stability of biosensor*

It is well known that common interferents such as fructose, urea, and ascorbic acid may interfere with the response current of glucose biosensors. As seen in Fig. 5, no obvious interference in current appeared when 0.4 mM fructose was added to PBS containing 5.6 mM glucose with a pH value of 6.86, while the addition of 4.3 mM urea led to a nominal 4.79% current increment compared with that of 5 mM glucose.

(a)

(b)

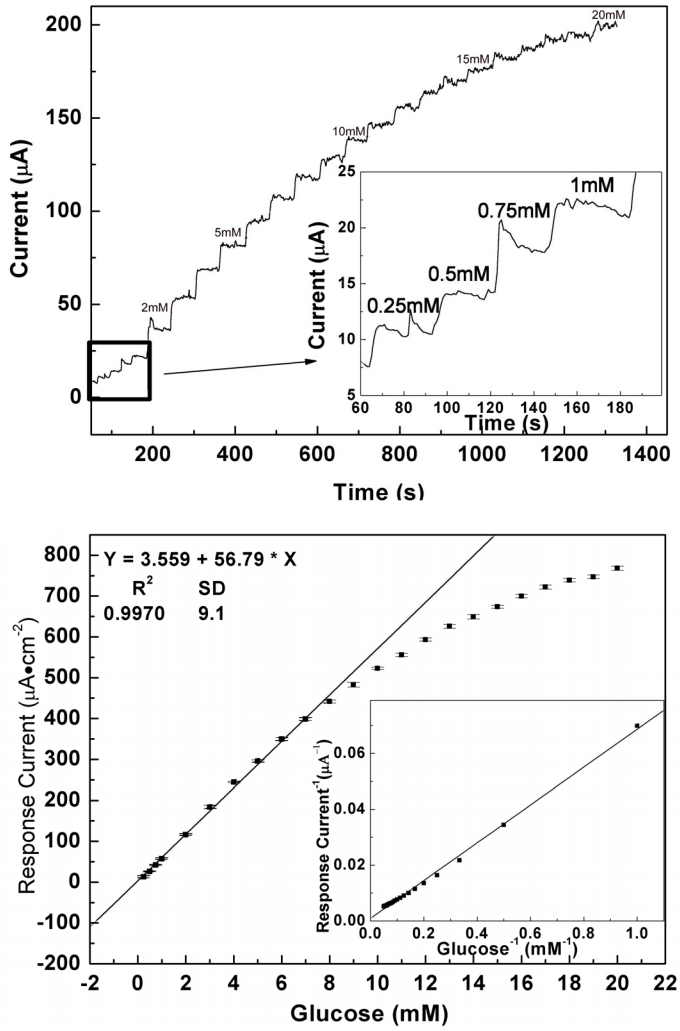

Fig. 4. Response current patterns with successive addition of 1 mM glucose from 0 to 20 mM (a) and 0.25 mM glucose from 0 to 1 mM (inset). (b) Calibration plot and determination of apparent Michaelis–Menten constant (inset).

However, an 8.30% current increase was observed when 0.1 mM ascorbic acid was added. Horseradish peroxidase[26] or nafion polymer[27] will be applied in the sensor to improve the selectivity in a future work.

To evaluate the stability of the system, the response current of the biosensor in 1 mM glucose was tested after one week. Compared with the original response current, the

Table 1
Analytical performances referred from recent works.

Biosensor configuration	Sensitivity ($\mu AmM^{-1}cm^{-2}$)	Response time (s)	Linear range (mM)	Km (mM)	Reference
Pt nanofuzz/Al_2O_3/Al foil	56.79	<10	0.25–8	7.35	This work
PPy nanotubes	7.4	≤4	0.5–10	7.01	Ekanayake *et al*., 2007
NiO nanosphere	4.3	5	1.5–7	7.76	Li and Liu *et al*., 2008
ZnO nanotube	21.7	3	0.05–12	19	Kong and Chen *et al*., 2008
Ferrocene-modified multiwalled carbon nanotubes	10.56	<7	0.01–4.2	6.3	Qiu *et al*., 2008
Pt nanotube	0.1		2–14	—	Yuan *et al*., 2005

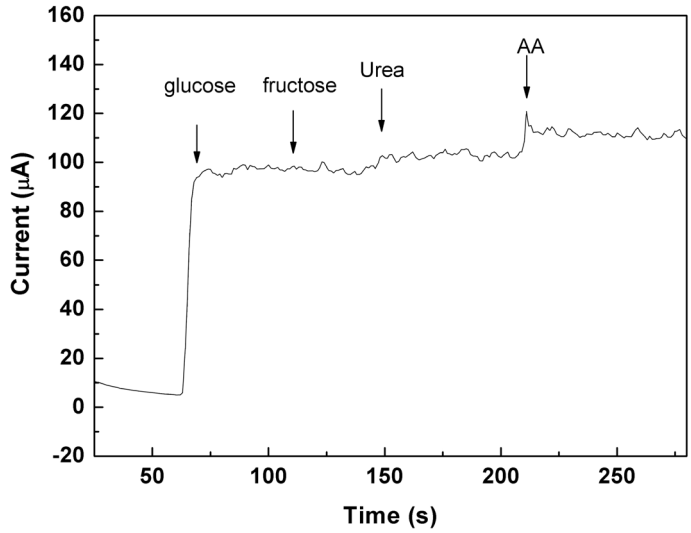

Fig. 5. Test results for the selectivity of sensors by adding 5.6 mM glucose, 0.4 mM fructose, 4.3 mM urea, and 0.1 mM ascorbic acid to pH 6.86 PBS in turn at +0.6 V.

response after one week decreased to 64.04%, which indicated that the stability of the sensor needs to be further improved.

The reproducibility (n = 10) was checked for several consecutive injections of a 1.0 mM glucose solution. A relative standard deviation of 3.5% was obtained, which indicates good reproducibility.

4. Conclusions

The electrodes of Al_2O_3/Al foils modified with Pt were fabricated by a simple replacement reaction, and had a high apparent surface area and a high catalytic activity. A novel type of biosensor was fabricated on the basis of the electrodes, and it exhibited high sensitivity, good reproducibility and a fast response to glucose under optimal conditions. Other biosensors with uricase and cholesterol oxidase are being developed by a similar technique.

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Cheng Lin achieved his BE (2003) at the School of Chemical Engineering, Nanjing University of Science and Technology and ME (2006) at the College of Chemistry and Materials Sciences, Fujian Normal University, and is now a Ph.D. candidate at the College of Materials, Xiamen University. His current fields of interest are biomaterials and biosensors.

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