Dendrimer-Encapsulated Pt Nanoparticles/Polyaniline Nanofibers for Glucose Detection

Lihuan Xu, Yihua Zhu, Longhua Tang, Xiaoling Yang, Chunzhong Li

Key Laboratory for Ultrafine Materials of Ministry of Education, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai 200237, China

Received 10 September 2007; accepted 8 February 2008
DOI 10.1002/app.28307
Published online 28 April 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A novel amperometric glucose biosensor based on self-assembling glucose oxidase (GOx) and dendrimer-encapsulated Pt nanoparticles (Pt-DENs) on nanofibrous polyaniline (PANI) was described. PANI nanofibers were synthesized via an interfacial polymerization method. A sulfonated polyelectrolytes-poly(sodium 4-styrenesulfonate) (PSS) was used to form the negative PANI/sulfonated polyelectrolyte complex, which had good disperse in aqueous solution. GOx was immobilized on the PANI/PSS surface by alternatively assembling a cationic Pt-DENs layer and an anionic GOx layer. The unique sandwich-like layer structure (Pt-DENs/GOx/Pt-DENs/PANI/PSS) formed by self-assembling provides a favorable microenvironment to keep the bioactivity of GOx and to prevent enzyme molecule leakage. The fabricated Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode exhibited excellent response performance to glucose with a detection limit of 0.5 μ*M*, wide linear range from 10 μ*M* to 4.5 m*M*, short response time within 5 s, improved sensitivity of 39.63 μA/(m*M* cm^2), and good stability (85% remains after 20 days). © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 1802–1807, 2008

Key words: polyaniline nanofibers; glucose oxidase; dendrimer-encapsulated Pt nanoparticles; biosensor

INTRODUCTION

Glucose biosensors play extremely important role in the field of bioanalysis for measuring blood glucose levels. The analytical power of electrochemical technique coupled to the specific biological recognition process is especially promising, which enables online and *in situ* monitoring analytical results, without real sample pretreatment.[1,2] Since the development of the first glucose biosensor, numerous attempts have been made to create sensitive, selective, reliable, and low-cost biosensors. However, the modified materials on the electrode surface and enzyme immobilization methods have important influence on the activity and the stability and other performance of biosensor,[3,4] and so it has significant theoretical meaning and practical value to probe new materials and immobilization methods.

Recently, conducting polymers have attracted much interest for application in biosensors.[5] Polypyrrole (PPY), poly-*o*-phenylenediamine, polyaniline (PANI), and polyphenol are known to posses many advantages which allow them to act as excellent material for immobilization of biomolecules and rapid electron transfer for the fabrication of efficient biosensors. Among them, PANI is considered to be an attractive polymer since this electronic material exhibits two redox couples in the right potential range to facilitate an enzyme–polymer charge transfer.[6] PANI doped with dodecylbenzenesulfonic acid has been reported for fabricating H_2O_2 biosensor after immobilized with horseradish peroxidase.[7] In addition, metal nanoparticles and conductive polymer composites showed good electrical conductivity, redox properties, and some other unique properties.[8,9] The composites of PANI and inorganic nanoparticles[10–14] have been synthesized. Kuang and coworkers have reported a glucose biosensor based on platinum microparticles dispersed in nanofibrous PANI.[15] And PANI film incorporating the negatively charged sulfonate-functionalized Au nanoparticles are prepared by electropolymerization, which is used as a matrix for the enhanced bioelectrocatalysis.[16] A biosensor based on carbon nanotube-doped PANI is also already prepared.[17]

On the other hand, operation of electrochemical biosensors requires a conjugation of the biochemical and electrochemical reactions, which make the

Correspondence to: Y. Zhu (yhzhu@ecust.edu.cn).
Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 20676038.
Contract grant sponsor: Key Project of Science and Technology for Ministry of Education; contract grant number: 107045.
Contract grant sponsor: Special Projects for key laboratories in Shanghai; contract grant numbers: 05DZ22302, 06DZ22008.

immobilization of biological recognition element becoming a focus. There are various methods for enzymes immobilization, such as adsorption,[18] cross-linking,[19] electrochemical copolymerization,[20] covalent attachment, and entrapment in polymeric gels or carbon paste.[21] Among them, electrostatic layer-by-layer (LbL) adsorption is one of the simplest and the most powerful methods for creating ordered, stable, and biocompatible multilayer films. Rivas and coworkers prepared a glucose biosensor based on the LbL self-assembling of GOx and chitosan derivatives on a thiolated gold surface.[22] A strategy for enzyme immobilization on LbL dendrimer-gold nanoparticle membrane has also been reported.[23] Our previous work has also reported two sensitive biosensors based on the dendrimer encapsulated Pt nanoparticles/enzyme multilayers[24] and the self-assembling glucose oxide and dendrimer-encapsulated Pt nanoparticles (Pt-DENs) on carbon nanotubes.[25] It allows control of the molecular architecture and suitable choice of template materials for enzyme immobilization, as is the case of poly(amidoamine) dendrimers (PAMAM). Pt-DENs have several advantages from the viewpoint of biosensing application. First, dendrimers provide multiple conjugation sites, which can be efficiently used as the bioconjugating units for the construction of spatially ordered enzyme nanostructure.[26] Second, the encapsulated Pt nanoparticles, the noble metal, also facilitate the electron transfer.[27]

In this article, PANI nanofibers were firstly synthesized via an interfacial polymerization method. Then, a novel glucose biosensor with high sensitivity and selectivity has been synthesized based on self-assembling GOx and Pt-DENs on nanofibrous PANI. The resulting biosensor exhibits excellent amperometric response to glucose and possesses biocompatible performance with the possibility of minimizing enzyme denaturing.

EXPERIMENTAL

Materials

Aniline was from Shanghai Chemical Reagent Company (China) and purified by distillation before use. Glucose oxidase (GOx, EC.1.1.3.4, TYPEVII, 150 U/mg) was purchased from Fluka, which was modified by the periodate-oxidized GOx (IO_4^--GOx) according to the literature.[28] Glucose and poly(sodium 4-styrenesulfonate) (PSS, MW $\sim$ 70,000) were purchased from Sigma-Aldrich. Pt-DENs were synthesized according to the previous literature.[29,30] The other chemicals were of analytical grade. Di-distilled water was used throughout the experiment. A 0.05*M* phosphate buffer solution (pH 6.0) was used in this article unless indicated otherwise.

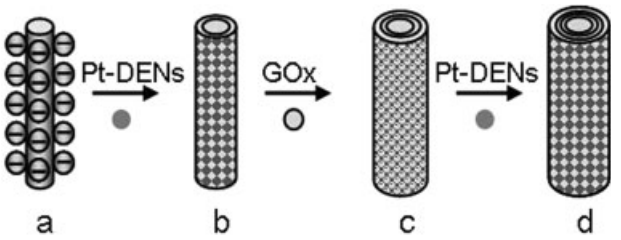

Figure 1 Schematics of the fabrication process of Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode. (a) The negatively charged PANI/PSS composite, (b) the adsorption of positively Pt-DENs, (c) the adsorption of negatively GOx, and (d) the adsorption of positively Pt-DENs.

Preparation

PANI nanofibers were synthesized according to the previous method.[31,32] Briefly, 10 mL predistilled aniline was dissolved in 100 mL CCl_4 (A.C.S. Reagent; Aldrich Chemical) while 5 m*M* ammonium peroxydisulfate (98%; Aldrich Chemical) was dissolved in water with HCl. When transferred to a 200 mL beaker, an interface formed between the two immiscible solutions. Green PANI soon formed at the interface, then slowly migrated into the water layer, and entire water phase was finally filled with dark emeraldine green PANI polymerized. The byproducts were removed from the aqueous phase using dialysis.

As-prepared PANI nanofibers (0.5 g) were second-doped with 50 mL PSS (3 mg/mL, 0.5*M* NaCl) for 24 h, and then centrifuged, washed, supernatant removed to obtain well-dispersive solution of the negatively charged PANI/PSS. Then, the positively Pt-DENs was absorbed onto PANI/PSS surface by self-assembly to form Pt-DENs/PANI/PSS. Using the same procedure, the negatively GOx (5 mg/mL) monolayer was adsorbed onto the Pt-DENs/PANI/PSS surface. The same operation was repeated to form the final Pt-DENs/GOx/Pt-DENs/PANI/PSS composite. Figure 1 illustrates the scheme for the fabrication of Pt-DENs/GOx/Pt-DENs/PANI/PSS composite by LbL assembly on PANI/PSS nanofibers. Each absorption step was allowed to adsorb for 30 min followed by washing twice with double-distilled water. After each deposition and washing step, the mixtures were centrifuged at 7000 rpm for 3 min and the supernatant was removed. The resulting structures were characterized by transmission electron microscopy.

Ten micro liters of the Pt-DENs/GOx/Pt-DENs/PANI/PSS composite aqueous solution (10 mg/mL) was dripped on the cleaned glassy carbon (GC) disk electrode surface (3-mm diameter), and the coating was dried at room temperature.

Instrumentation

The transmission electron microscopy (TEM) images were recorded on JEM-3010F. Electrochemical

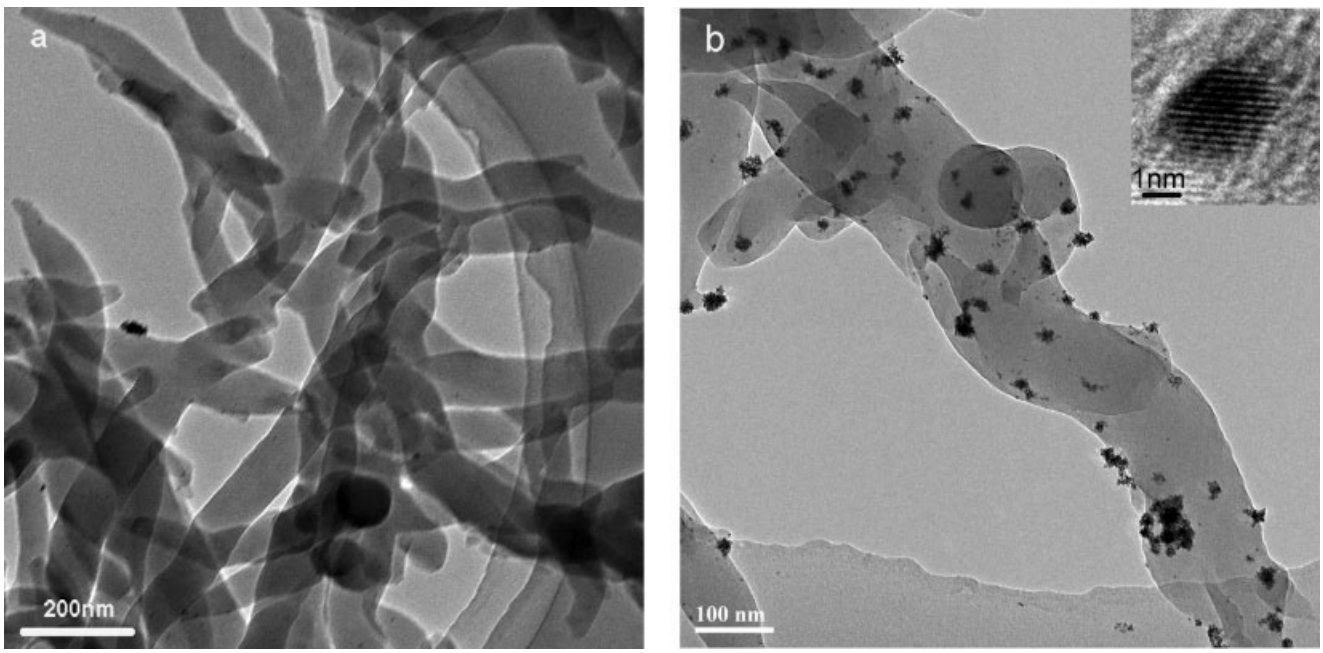

Figure 2 HR-TEM images of PANI nanofibers (a) and Pt-DENs/GOx/Pt-DENs/PANI/PSS composites (b). Inset: magnified HR-TEM image of single Pt-DENs.

measurements were carried out with an electrochemical workstation CHI 660C (Shanghai Chenhua Instrument Factory, China) connected to a personal computer using three-electrode cell, containing a modified GC disk electrode (3-mm diameter) as a working electrode, a Ag/AgCl (3*M* KCl) reference electrode and a platinum wire counter electrode. All potentials are referred to the Ag/AgCl reference electrode at room temperature (25°C).

RESULTS AND DISCUSSION

Morphology of PANI nanofibers and Pt-DENs/GOx/Pt-DENs/PANI/PSS composites

Figure 2 presents the typical HR-TEM images of the PANI nanofibers and Pt-DENs/GOx/Pt-DENs/PANI/PSS composites. It can be seen that PANI nanofibers with a diameter of about 100–120 nm are successfully synthesized by the interfacial polymerization [Fig. 2(a)], and platinum nanoparticles are relatively uniform distribution on the walls of PANI nanofibers [Fig. 2(b)]. The inset of Figure 2(b) shows that the roughly spherical Pt nanoparticles are prepared using the dendrimer as the template and the crystal lattice of Pt nanoparticles is also presented clearly. This indicates that the Pt-DENs with the positively terminal amino groups are deposited onto the surface of PANI nanofibers by the strong electrostatic and covalent interaction between sulfated groups of PSS and amino groups of Pt-DENs or between the terminal groups of aldehyde of GOx and amino groups of Pt-DENs, respectively.

Electrochemical and electrocatalysis characteristics of the enzyme electrode to hydrogen peroxide

As well known, the quantification of glucose can be obtained by electrochemical detection of the enzymatically liberated hydrogen peroxide from the electrode reaction. The process for GOx catalyzing the oxidation of glucose to gluconolactone is shown as follows:

$$\text{Glucose} + O_2 \overset{\text{GOx}}{\rightarrow} \text{Gluconolactone} + H_2O_2$$

Figure 3 shows the cyclic voltammograms of Pt-DENs/GOx/Pt-DENs/PANI/PSS composites

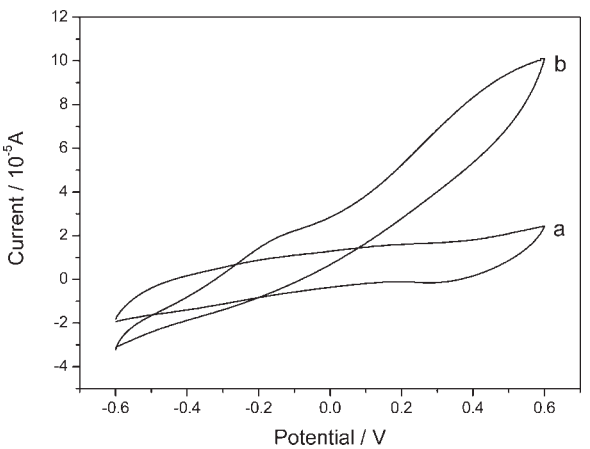

Figure 3 Cyclic voltammograms of Pt-DENs/GOx/Pt-DENs/PANI/PSS composites electrode in the absence (curve a) and presence of 1 m*M* H_2O_2 (curve b) in 0.05*M* phosphate buffer (pH 6.0). The scan rate was 50 mV/s.

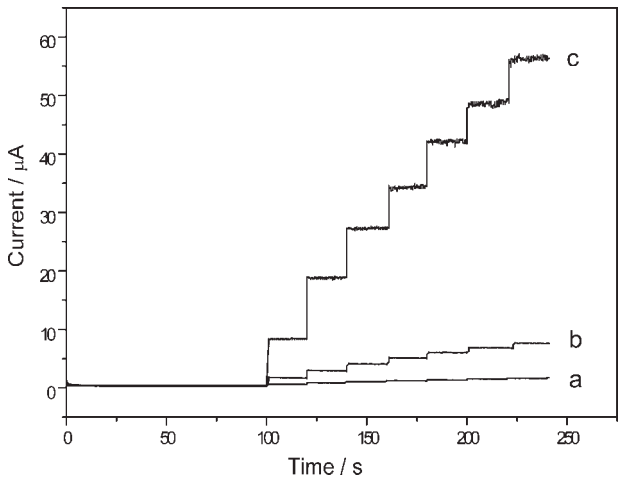

Figure 4 Current response of PANI (a), PANI/PSS (b), and Pt-DENs/GOx/Pt-DENs/PANI/PSS (c) modified electrodes to the successive addition of 1 m*M* hydrogen peroxide in PBS (pH 6.0) at an applied potential of 0.6 V.

modified GC electrode in the absence (curve a) and presence of 1 m*M* H_2O_2 (curve b) in 0.05*M* phosphate buffer (pH 6.0). It can be seen that the current response of the latter is larger than that of the former when the potential is over 0.1 V. It indicates that hydrogen peroxide molecules can permeate through the PANI composites film and carry out reaction on the working electrode, which results in obviously different cyclic voltammogram curves. It presents that Pt-DENs/GOx/Pt-DENs/PANI/PSS composites exhibit excellent electrocatalytic characterization for the measurement of H_2O_2 from the electrode reaction.

Figure 4 shows the typical chronoampermetric responses of electrodes, respectively, modified with pure PANI nanofibers, PANI/PSS, and Pt-DENs/GOx/Pt-DENs/PANI/PSS, to the successive addition of 1 m*M* H_2O_2 at an applied potential of 0.6 V. Clearly, the response current of Pt-DENs/GOx/Pt-DENs/PANI/PSS modified electrode is obviously higher than that of the others as being tested under identical experimental conditions. This indicates that the prepared Pt-DENs/GOx/Pt-DENs/PANI/PSS composites have facilitated the electron transfer between PANI nanofibers and electrode, which make the composites possess better electrocatalytic activity to H_2O_2. Therefore, the electrode modified with Pt-DENs/GOx/Pt-DENs/PANI/PSS composites is suitable for the measurement of glucose.

Optimization for prepared conditions of the enzyme electrode

Various experimental parameters, such as the applied potential and the pH value of the detection solution, which affects the amperometric determination of glucose, are studied. The effect of the

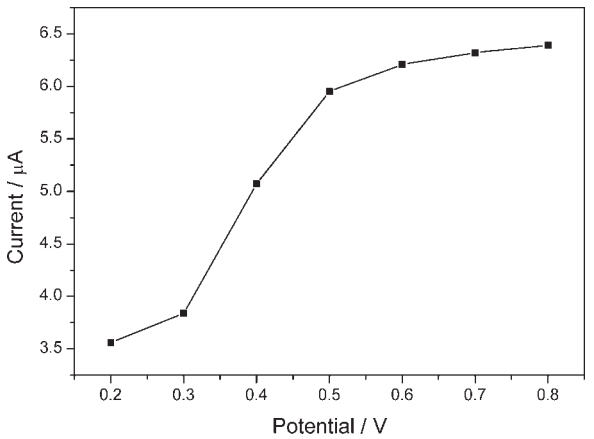

Figure 5 The effect of potential on the current response of Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode by the addition of 1.5 m*M* glucose at various levels of applied potential in PBS (pH 6.0).

operation potential on the amperometric response to glucose has been investigated, by using the Pt-DENs/GOx/Pt-DENs/PANI/PSS as the modified materials of electrode. Figure 5 shows the changes of the response current at various levels of applied potential, with the addition 1.5 m*M* glucose in PBS (pH 6.0). The result shows that the response current to glucose increases with the increase of applied potential. From 0.2 to 0.6 V, the response current is quickly increased. When the potential exceeds 0.6 V, the response current increases very slowly and more noise can be observed. So, a potential of 0.6 V is preferred in these experiments.

Figure 6 shows the effect of the pH value of the detection solution on the amperometric response of the GC electrode modified with the Pt-DENs/GOx/Pt-DENs/PANI/PSS. The maximum response current value can be observed at pH 6.0. The reason for

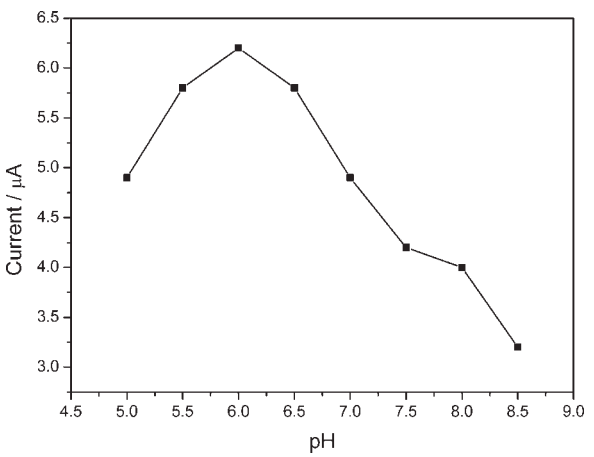

Figure 6 The effect of pH value on the current response of Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode by the addition of 1.5 m*M* glucose at various pH of PBS (pH 5.0–8.5) at an applied potential of 0.6 V.

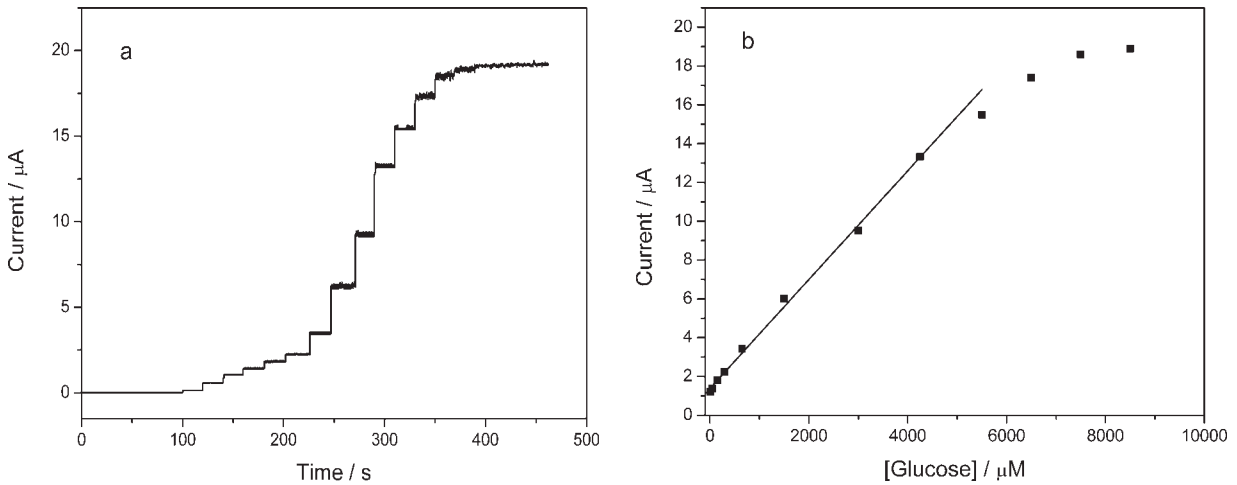

Figure 7 Amperometric response for Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode upon successive addition of glucose in 0.05*M* PBS (pH 6.0) at an applied potential of 0.6 V (a) and the calibration curve for glucose concentrations from 10 μ*M* to 4.5 m*M* (b).

the change is that the isoelectric point, I_p of GOx is 4.5, and therefore at pH $> I_p$, it is net negatively charged, while at pH $< I_p$, it is positively charged. In this article, GOx used as the component of the multilayer films is a negatively charged material. So a pH 6.0 of 0.05*M* phosphate buffer as support electrolyte is an optimal choice for glucose detection in this test.

In addition, the influences of the quantification deposited onto electrode surface and the assembly number of layers Pt-DENs/GOx/Pt-DENs/PANI/PSS composites are also investigated. No significant improvement in response current is observed when the volume of Pt-DENs/GOx/Pt-DENs/PANI/PSS composites is increased beyond 10 μL. More importantly, the more the deposited quantification, the worse is the film formation. Theoretically, the increasing number of self-assembly layers of Pt-DENs and GOx can increase response current, but in fact, the conductivity of PANI would decrease through multiple washing, resulting in the sensitivity reduction with the increasing of bilayers. In this article, the sandwich-like layer structure (Pt-DENs/GOx/Pt-DENs/PANI/PSS) formed by self-assembling is used and the quantity of 10 μL is correctly measured.

Amperometric determination of glucose on the Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode

Amperometric measurements are performed in a 10-mL electrochemical cell. Figure 7(a) shows the typical amperometric responses of the Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode for the successive addition of various concentrations of glucose at the applied potential of 0.6 V. A subsequent addition different concentration levels of glucose to the stirring PBS provokes a remarkable increase in the oxidation current, and the time required to reach the 95% steady state response is within 5 s. The resulting calibration curve plot [Fig. 7(b)] displays linearly from 10 μ*M* up to 4.5 m*M* with a correlation coefficient of 0.999, which is similar to the other reports.[32–34] This shows that the prepared biosensor could work very well in the concentration range between 10 μ*M* and 4.5 m*M*. The electrode has a low detection limit 0.5 μ*M* with the signal-to-noise ratio of 3. The response time is less than 5 s calculated as the time necessary for reaching 95% of the maximum signal and a sensitivity of 39.63 μA/(m*M* cm^2). When glucose concentration is further high, a plateau current is observed, showing the characteristics of Michaelis–Menten kinetics. These results indicate that doped PANI is a kind of conducting polymer, and the electron transfer between the electrode and Pt particles attached on the PANI nanofibers can be carried out by oxidation and deduction processes under electric field. Also, since the GOx and Pt-DENs disperse evenly on the PANI nanofibers by self-assembly, it improves the dispersion and contact between PANI and Pt-DENs, even between GOx and Pt-DENs. As a result, the electrocatalysis process to glucose happens by Pt nanoparticles and the improved amperometric response to glucose is obtained.

Interference tests

The anti-interference test is demonstrated in Figure 8, which compares amperometric responses for three relevant electroactive species (uric acid, ascorbic acid, acetaminophen; concentration of each species, 0.3 m*M*) with Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode at a working potential of 0.6 V. The

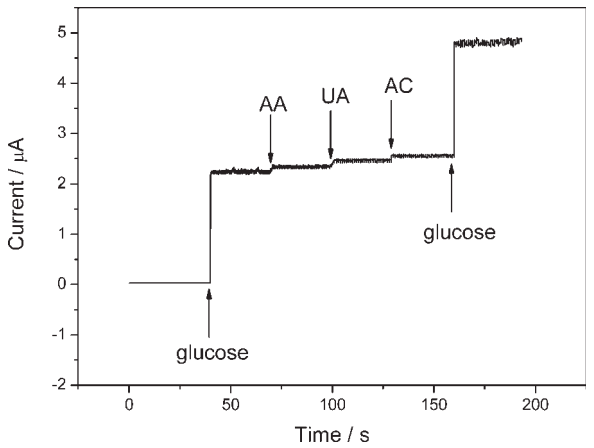

Figure 8 Current–time curves for the Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode exposed to 0.3 m*M* ascorbic acid (AA), 0.3 m*M* uric acid (UA), 0.3 m*M* acetaminophen (AC), and 0.3 m*M* glucose at applied potential 0.6 V.

successive addition of each interfering species brought out fairly discernible current response and a well-defined glucose response is obtained, which indicates that the biosensor based on Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode has good anti-interference ability.

The reproducibility of Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode is also estimated from the response to 1.5 m*M* glucose at five electrodes prepared under the same conditions. The results reveal that the biosensor has a satisfied reproducibility. A mean current response of 5.72 μA is obtained with a deviation of 4.4% for five times continuous determinations of the same sample. After the electrode is stored at 4°C under dry condition for 20 days, the steady-state response current only decreases by 15%. Such a result confirms that Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode is compatible with the immobilized enzyme and is helpful to maintain the bioactivity of GOx. The results indicate that the hybrid composites provide a good microenvironment for GOx immobilization, in which the nanofibrous PANI provides a high surface area for loading Pt-DENs and GOx, the PSS increases the dispersion of PANI, and the LbL adsorption technology prevents the leakage of the GOx effectively. These measures showed that the biosensor has long-term stability.

CONCLUSION

A novel amperometric glucose biosensor has been described, in which PANI nanofibers are first synthesized via an interfacial polymerization method and then the GOx and the cationic Pt-DENs are immobilized on the PANI/PSS surface by alternative LbL assembling process. The electrocatalytic activity toward H_2O_2 and the amperometric response toward glucose on the fabricated Pt-DENs/GOx/Pt-DENs/PANI/PSS electrode demonstrates that the electrostatic self-assembling method preserves the activity of enzyme molecules, improves the diffusion of target molecular, and prevents enzyme leaking. The biosensor shows a low detection limit, wide linear range, high sensitivity, good precision, and operational stability.

References

1. Liu, S.; Ju, H. Biosens Bioelectron 2003, 19, 177.
2. Lomillo, M. A. A.; Ruiz, J. G.; Pascual, F. J. M. Anal Chim Acta 2005, 547, 209.
3. Mu, S. J. Electroanal Chem 1994, 370, 135.
4. Singh, S.; Solanki, P. R.; Pandey, M. K.; Malhotra, B. D. Sens Actuators B 2006, 115, 534.
5. Gerard, M.; Chaubey, A.; Malhotra, B. D. Biosens Bioelectron 2002, 17, 345.
6. Mozhaev, V. V. Trends Biotechnol 1993, 11, 88.
7. Morrin, A.; Ngamna, O.; Killard, A. J.; Moulton, S. E.; Smyth, M. R.; Wallace, G. G. Electroanal 2005, 17, 423.
8. Sukeerthi, S.; Contractor, A. Q. Chem Mater 1998, 10, 2412.
9. Flitton, R.; Johal, J.; Maeda, S.; Armes, S. P. J Colloid Interf Sci 1995, 173, 135.
10. Park, J. E.; Park, S. G.; Korkitu, A.; Hatozaki, O.; Oyama, N. J. Electrochem Soc 2003, 150, A959.
11. Xia, H.; Wang, Q. Appl Polym Sci 2003, 87 1811.
12. Xia, H.; Wang, Q. Chem Mater 2002, 14, 2158.
13. Li, Z. F.; Swihart, M. T.; Ruckenstein, E. Langmuir 2004, 20, 1963.
14. Athawale, A. A.; Bhagwat, S. V. J Appl Polym Sci 2003, 89, 2412.
15. Zhou, H.; Chen, H.; Luo, S.; Chen, J.; Wei, W.; Kuang, Y. Biosens Bioelectron 2005, 20, 1305.
16. Granot, E.; Katz, E.; Basnar, B.; Willner, I. Chem Mater 2005, 17, 4600.
17. Luo, X.; Killard, A. J.; Morrin, A.; Smyth, M. R. Anal Chim Acta 2006, 575, 39.
18. Zheng, H.; Xue, H.; Zhang, Y.; Shen, Z. Biosens Bioelectron 2002, 17, 541.
19. Xiao, Y.; Ju, H.; Chen, H. Anal Biochem 2000, 278, 22.
20. Xu, J.; Chen, H. Anal Chim Acta 2000, 423, 101.
21. Wang, J.; Mo, J.; Li, S.; Porter, J. Anal Chim Acta 2001, 441, 183.
22. Miscoria, S. A.; Desbrieres, J.; Barrera, G. D.; Labbé, P.; Rivas, G. A. Anal Chim Acta 2006, 578, 137.
23. Crespilho, F. N.; Ghica, M. E.; Florescu, M.; Nart, F. C, Jr.; Oliveira, O. N.; Brett, C. M. A. Electrochem Commun 2006, 8, 1665.
24. Zhu, Y.; Zhu, H.; Yang, X.; Xu, L.; Li, C. Electroanal 2007, 19, 698.
25. Xu, L.; Zhu, Y.; Tang, L.; Yang, X.; Li, C. Electroanal 2007, 19, 717.
26. Yoon, C. H.; Kim, H. S. Anal Chem 2000, 72, 922.
27. Zhang, W.; Maye, M. M.; Leibowitz, F. L.; Zhong, C. J Anal Chem 2000, 72, 2190.
28. Zhao, M.; Crooks, R. M. Angew Chem Int Ed Engl 1999, 38, 364.
29. Zhang, Y.; Hu, N. Electrochem Commun 2007, 9, 35.
30. Crooks, R. M.; Zhao, M. J Am Chem Soc 2004, 126, 851.
31. Huang, J. X.; Virji, S.; Weiller, B. H.; Kaner R. B. Chem Eur J 2004, 10, 1314.
32. Yang, H.; Zhu, Y. Anal Chim Acta 2005, 554, 92.
33. Luo, X.; Xu, J.; Du, Y.; Chen, H. Anal Biochem 2004, 334, 284.
34. Shan, D.; He, Y.; Wang, S.; Xue, H.; Zheng, H. Anal Biochem 2006, 356, 215.