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Dendrimer-encapsulated Pt nanoparticles on mesoporous silica for glucose detection

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Abstract A hybrid system of mesoporous silica (MS) particle incorporated with poly(amidoamine) dendrimer-encapsulated platinum nanoparticles (Pt-DENs) was constructed in a neutral aqueous solution through electrostatic interaction. The MS/Pt-DENs composite particles immobilized with glucose oxidase (GOx) were used to modify a glassy carbon electrode for detecting the electrocatalytic response to the reduction of glucose. Pt-DENs can improve the conductivity of MS and enhance the electron transfer between redox centers in enzymes and electrode surfaces. The structure of composite particles and the performance of MS/Pt-DEN-modified electrodes were characterized by transmission electron microscopy, N_2 sorption characterization method, electrochemical impedance spectroscopy, cyclic voltammetry and amperometric measurements. The MS/Pt-DENs/GOx-modified electrodes, which had a fast response of GOx less than 3 s, could be used for the determination of glucose ranging from 0.02 to 10 mM. The detection limits were 4 μM at signal-to-noise ratio of 3.

Keywords Mesoporous silica · Dendrimer · Glucose · Glucose oxidase · Modified electrode

Introduction

The direct electrochemistry of enzymes has received increasing attention; studies of direct electron transfer between enzymes and electrode surface can also provide a

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platform for fabricating new kinds of mediator-free biosensors, enzymatic bioreactors and biomedical devices [1, 2]. However, enzymes exhibited a rather slow rate of heterogeneous electron transfer at conventional electrodes because of the deep burying of the electroactive prosthetic groups, the unfavorable orientations of enzymes at electrodes [3]. Therefore, the studies are focusing on selecting ideal electrode materials, such as carbon nanotubes [4], mesoporous materials [5–7] and biomaterials [8–10], to enhance the direct electron transfer between the enzymes and underlying electrodes.

Recently, a series of porous materials such as clay [11], montmorillonite [12], porous alumina [13] and zeolite [14] have been used as immobilization matrices. Mesoporous silica (MS) particles with controlled morphology are receiving increasing attention for their potential applications for immobilization as compartments, and their useful application as enzyme carriers has been suggested [7, 15]. However, like other inorganic mesoporous materials, MS has poor conductivity and is not suitable for the direct electrochemistry [16–18]. To improve this deficiency, metal nanoparticles such as gold, silver, platinum, etc., could be introduced to the electrode-modified materials [19–21]. These kinds of metal nanoparticles have excellent conductivity, catalytic property and biocompatibility. They can perform as "electronic wires" to enhance the electron transfer between redox centers in enzymes and electrode surfaces, and as catalysts to increase electrochemical reactions. However, these nanoparticles were intended to agglomerate. It is necessary to use protective agents such as small organic molecules or polymers to prevent aggregation. Highly branched dendritic macromolecules poly(amidoamine) (PAMAM) could also be used to modify electrode surface due to their good biocompatibility and adequate functional groups for chemical fixation. It was reported that the PAMAM

was capable of increasing the concentration of hydrophobic molecules at the electrode–solution interface, improving the sensitivity as well as the selectivity of certain specific electrochemical reactions [22, 23].

With this in view, the present work is undertaken to investigate the electrocatalytic activity of MS/poly(amido-amine) dendrimer-encapsulated platinum nanoparticles (Pt-DENs) hybrid material doped with glucose oxidase (GOx) toward the electrocatalytic oxidation of glucose. Cyclic voltammetry (CV) and amperometric measurements have been employed to investigate the catalytic activity of the microcomposite. The immobilized GOx showed a direct electrochemistry and exhibits a good electrocatalytic behavior to glucose. The prepared sensors can be used for the determinations of glucose.

Experimental section

Materials and reagents

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 [24], and glucose was from Sigma-Aldrich. Nafion (10% in methanol with equivalent weight of about 1100) was obtained from Aldrich. Pt-DENs and MS particles were synthesized according to the previous literature [25]. Tetraethyl or thosilicate and all other chemicals were purchased from Shanghai Chemical Reagent Limited Corporation. Phosphate buffer solutions (PBS, 0.1 M) with various pH values were prepared by mixing stock standard solutions of Na_2HPO_4 and NaH_2PO_4, and adjusting the pH with H_3PO_4 or NaOH. Doubly distilled and deionized water was used through this work.

Preparation of MS/Pt-DENs/GOx nanocomposites

MS particles (0.3 g) was dispersed in 10 mL of a Pt-DENs colloid solution (pH 7) and shaken for 1 h. The result solution was centrifuged at a speed of 6,000 rpm to get the product of MS/Pt-DEN hybrid materials. The hybrid material (10 mg) prepared above was dispersed in a solution of GOx (20 mg/mL, pH 6) and shaken for 1 h for enzyme absorption. The bioconjugates were then centrifuged and washed with distilled water three times.

Preparation of MS/Pt-DENs/GOx-modified electrodes

Glassy carbon electrodes (GCE, 3 mm diameter, Model CHI 104, CH Instruments) were polished with 1.0-, 0.3- and 0.05-mm alumina powder cleaned in a piranha solution (a 1:3 mixture of 30% H_2O_2 and concentrated H_2SO_4) and finally sonicated thoroughly in double-distilled water. Next, the polished electrode was sonicated in acetone and doubly distilled water and was then allowed to dry at room temperature. Five microliters of the MS/Pt-DENs/GOx bioconjugates prepared above were deposited on the surface of the pretreated GCE. The electrode was then left to dry and was stored for at least 24 h at 4 °C. The biosensor was stored under the same conditions when not in use. The MS/Pt-DEN-modified GCE and MS-modified GCE were fabricated using the same GCE by the same method.

Apparatus and characterization

Morphology and microstructure of the synthesized materials were examined by transmission electron microscopy (TEM, JEOL HF-2010 TEM) and N_2 sorption characterization method (Micromeritics ASAP 2010). All electrochemical experiments were performed on a CHI 660A electrochemical workstation (CH Instru. Co., Shanghai, China) at 20 °C (±2 °C). The working electrode was a modified GCE. A saturated calomel electrode (SCE) served as reference electrode, and a Pt wire served as counter electrode. The working solutions were deoxygenated with nitrogen gas for 15 min before measurement and a nitrogen atmosphere was maintained over the solutions during experiment.

Results and discussion

Physical characterization of MS and MS/Pt-DENs

Porous silica microspheres have already been used as templates for enzyme immobilization [6]. The presence of nanometer-sized pores and channels in MS particles offered a great opportunity for capturing biomacromolecules such as proteins via physical adsorption and pore diffusion, thus enabling very high substrate loading [7]. In this work, the same effect took place. MS microspheres were negatively charged at pH 7.0, whereas Pt-DENs were positively charged at the same pH. The electrostatic interaction caused the assembly of Pt-DENs on the surface of the oppositely charged MS microspheres; the unique structure of MS microspheres greatly facilitated this assembly process.

Figure 1 shows the TEM images of (a) MS, (b) Pt-DENs (inset: high-magnification view of Pt-DENs) and (c) MS/Pt-DENs. The Pt-DENs decorating the surface of the MS microspheres can be clearly observed in Fig. 1c. Figure 1c clearly demonstrates the spherical shapes and channel-like interior structures of the hybrid materials, and also confirmed that Pt-DENs were present in the MS microspheres.
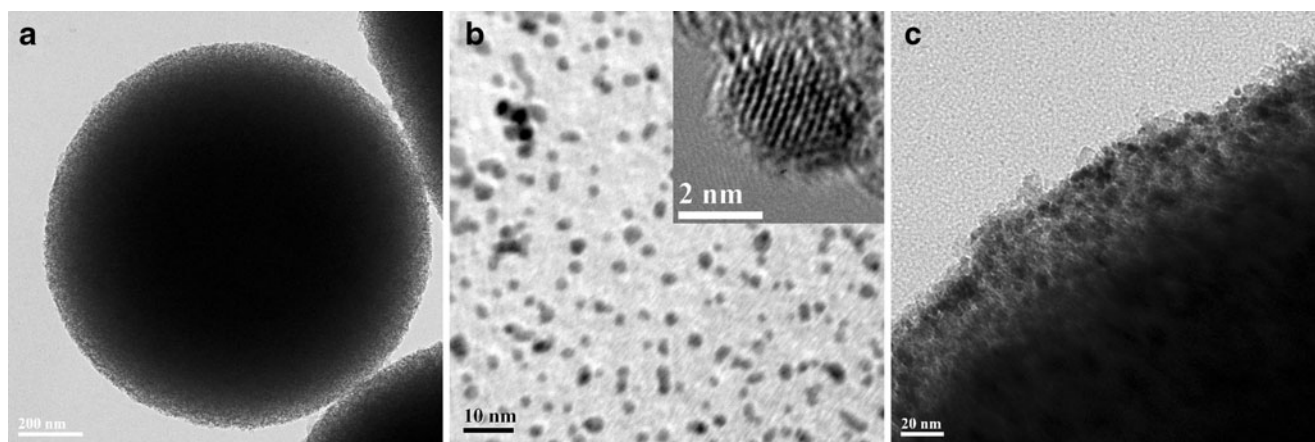
Fig. 1 TEM images of (**a**) MS, (**b**) Pt-DENs (*inset*: high-magnification view of Pt-DENs) and (**c**) MS/Pt-DENs

The Brunauer–Emmett–Teller (BET) method of nitrogen adsorption/desorption was further used to characterize the surface area, pore volume, and pore-size distribution of MS microspheres before and after Pt-DENs absorption. Compared to Fig. 2a, a narrower pore-size distribution and a sharp decrease in the number of larger pores were observed in Fig. 2b because of the infiltration of the Pt-DENs into the MS microspheres. Although the Pt-DENs could not penetrate into small pores because of steric difficulties, they could still infiltrate into some larger ones. With Pt-DENs filling in larger pores, these pores became smaller, resulting in a decrease in the whole pore volume as well as the BET surface area.

We have recorded FT-IR spectrum of MS/Pt-DENs/GOx shown in Fig. 3. As for silica, Si-OH stretching vibration peak was presented in 3,446 cm^{-1}, and the Si-O-Si asymmetrical stretching vibration peak appeared in 1087 cm^{-1} [26]. As for DENs, C = O stretching vibration peak was presented in 1735 cm^{-1}, C–O–C symmetrical stretching vibration peak appeared in 1060 cm^{-1}. Characteristic absorption peaks of GOx are located at 1655 cm^{-1} (amide I, 1,700–1,600 cm^{-1}) and 1,545 cm^{-1} (amide II, 1600–1500 cm^{-1}) separately [27]. The FT-IR spectrum of the MS/Pt-DENs/GOx shown in Fig. 3 not only exhibited all the characteristic absorption peaks of MS and Pt-DENs but also showed that the characteristic absorption peaks of amide I (1,655 cm^{-1}) and amide II (1,545 cm^{-1}). It was indicated that spatial structure of the GOx on the MS has been completely maintained.

Electrochemical activity of the MS/Pt-DENs/GOx-modified electrode

In this work, the MS/Pt-DEN hybrid material could play a role similar to that of ferrocene and graphite microparticles. Figure 3 showed the Nyquist plot of electrochemical impedance spectroscopy for both the MS-modified and

MS/Pt-DEN-modified GCEs. The semicircle portion at higher frequencies included in the impedance spectra corresponded to the electron-transfer-limited process. The diameter of the semicircle corresponded to the electrotransfer resistance (R_{et}), which controlled the electron-transfer kinetics of the redox
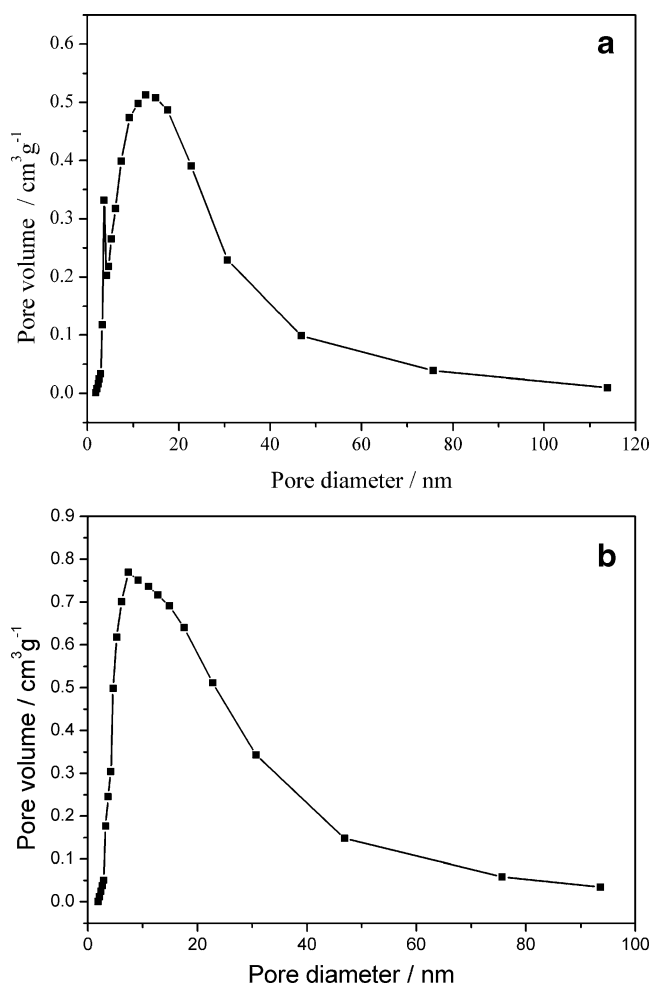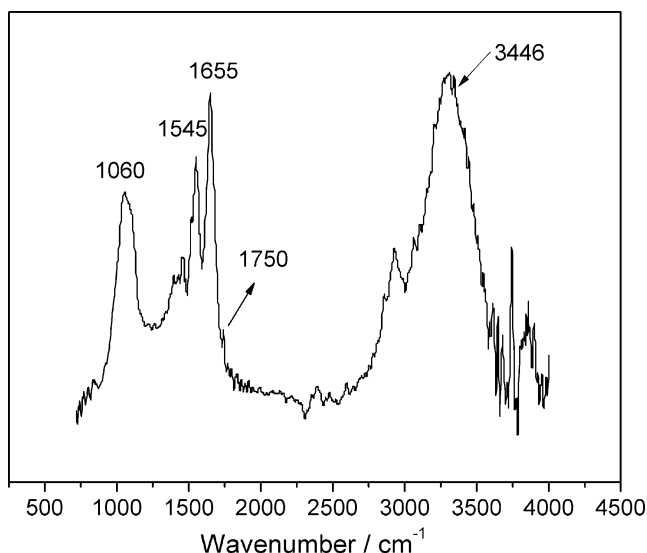

Fig. 2 Pore size distribution of (**a**) MS and (**b**) MS/Pt-DENs

Fig. 3 FT-IR spectra of MS/Pt-DENs/GOx hybrid microparticles

Fig. 5 Cyclic voltammograms of (a) bare GCE, (b) GOx/GCE, (c) Pt-DENs/GOx/GCE, (d) MS-GOx/GCE and (e) MS/Pt-DENs/GOx electrode in deoxygenated pH 6 PBS at a scan rate of 100 mV/s

probe at the electrode interface. MS/Pt-DEN-modified electrodes (Fig. 4b) showed large decreases in diameter compared to that of the MS-modified electrode (Fig. 4a), indicating much lower R_{et} values because of the contribution of assembled Pt-DENs. It is also proven that Pt-DENs can enhance the conductivity of MS microspheres.

Figure 5 displays the typical CVs for bare GCE, GOx/GCE, Pt-DENs/GOx/GCE, MS/GOx/GCE and MS/Pt-DENs/GOx/GCE electrodes in 0.1 M deoxygenated PBS (pH 6.0) over the potential range from 0 to −0.6 V at scan rate of 100 mV/s. No obvious redox peaks were observed at the GOx/GCE (b) because FAD in GOx, which is known to undergo redox reaction where two protons and two electrons are released or taken up, is deeply seated in a cavity and therefore it is not easily accessible for conduction of electrons to the electrode surface. After GOx incorporated with the Pt-
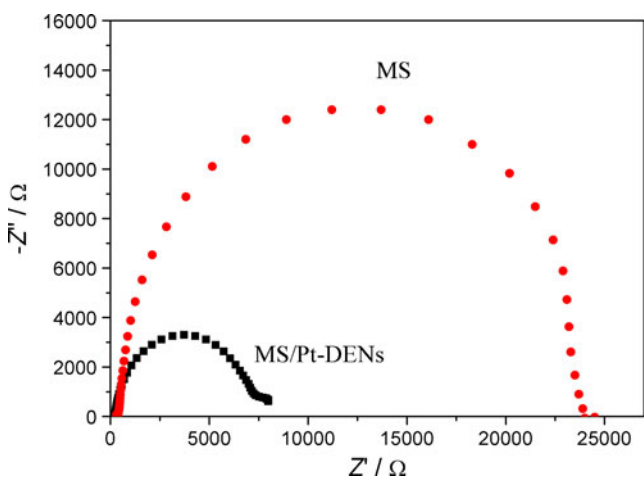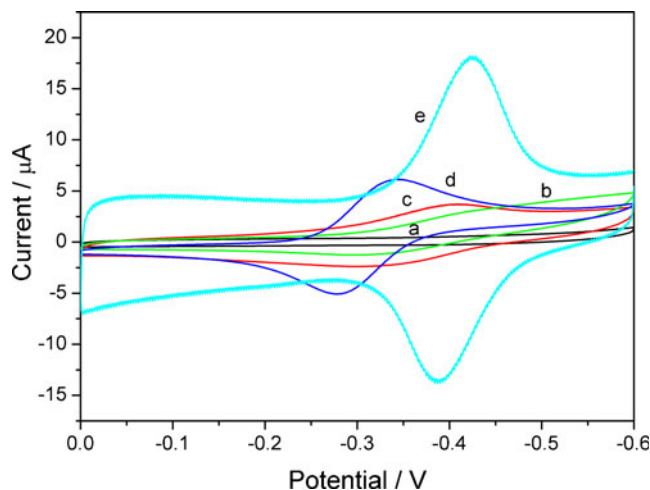
DENs, a couple of relatively small, but quasireversible redox peaks were observed at Pt-DENs/GOx/GCE (Fig. 5c), indicating the weak direct electron transfer between the immobilized GOx and the electrode. Compared to the redox peaks showed on the MS-GOx/GCE (Fig. 5d), the redox peaks observed on the MS/Pt-DENs/GOx/GCE was remarkable larger (Fig. 5e). The cathodic and anodic peak potentials were −0.391 and −0.422 mV, respectively. The formal potential was −407 mV near the standard electrode potential of −460 mV (vs. SCE) at pH 6.0 (25.8 °C) [28], which indicated that most GOx molecules preserved their native structures after the adsorption processes [29]. The small potential difference of cathodic and anodic peak potentials of 31 mV at the scan rate of 100 mV/s indicated a fast electron transfer process obtained for the electroactive center of FAD.

Fig. 4 Electrochemical impedance spectroscopy of (a) MS and (b) MS/Pt-DEN-modified GCE in 0.10 M KCl solutions with 5.0 mM [Fe(CN)6]$^{4−/3−}$
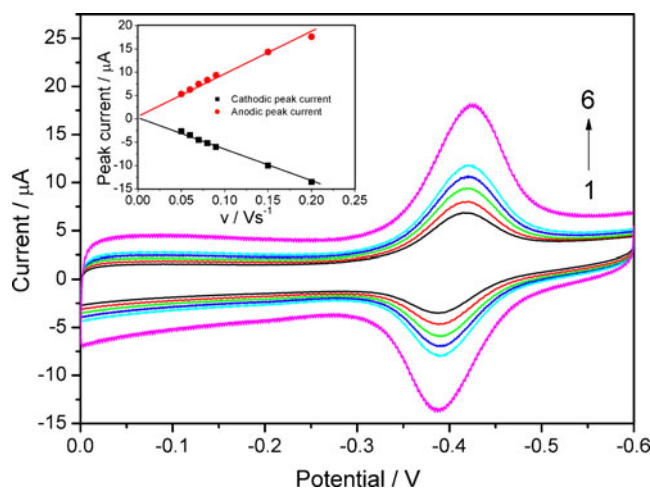

Fig. 6 Cyclic voltammograms of MS/Pt-DENs/GOx/GCE electrode at different scan rates, and the scan rate is 50, 60, 70, 80, 90, 150 and 200 mV/s, respectively
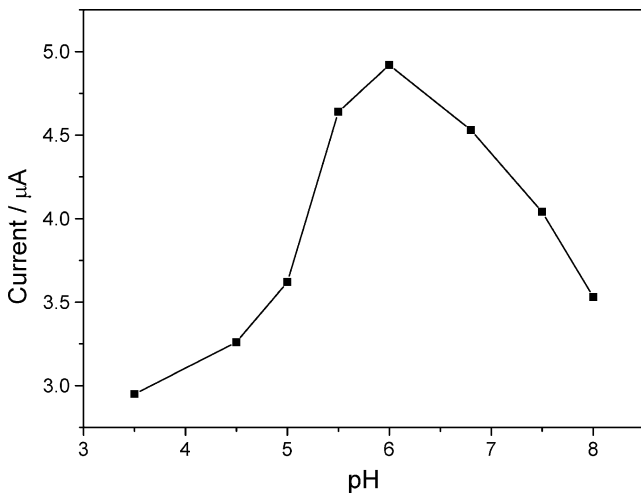
Fig. 7 Plot of peak current versus pH value

Fig. 9 Typical current–time plot for (a) MS/GOx and (b) MS/Pt-DENs/GOx electrode upon the successive addition of 0.01 mM glucose at –0.4 V in 0.1 M PBS

It was obvious that the direct electron transfer between the GOx and electrode was greatly enhanced after the GOx immobilized in MS/Pt-DENs matrix. The mechanism might be the interaction between GOx and MS was much stronger than that between GOx and GCE due to the presence of Si–OH groups presented in the MS particles [25] and also MS provided a biocompatible microenvironment for the entrapped GOx. Besides, Pt-DENs could act as "electrical wires" to would enhance electron transfer between the GOx and modified electrode.

The effect of scan rate on electrochemistry of the immobilized GOx is shown in Fig. 6. With increasing scan rate, the redox peak currents of the GOx increased linearly, and the peak-to-peak separation also increased, indicating a surface-controlled process.

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. During the preparation of the MS/Pt-DENs/GOx composite modified electrode, GOx is used as a negatively charged material for preparing the bioconjugates. The maximum response current value can be observed at pH 6.0 (Fig. 7). So, a pH 6.0 of 0.1 M phosphate buffer was used to support electrolyte for glucose detection in most cases.

Amperometric measurements for response to glucose

Figure 8 showed cyclic voltammograms of the MS/Pt-DENs/GOx/GCE, N_2-saturated, pH 6.0 PBS. When 1.0 mM glucose was added into the solution, the anodic peak current increased significantly compared to MS/GOx/
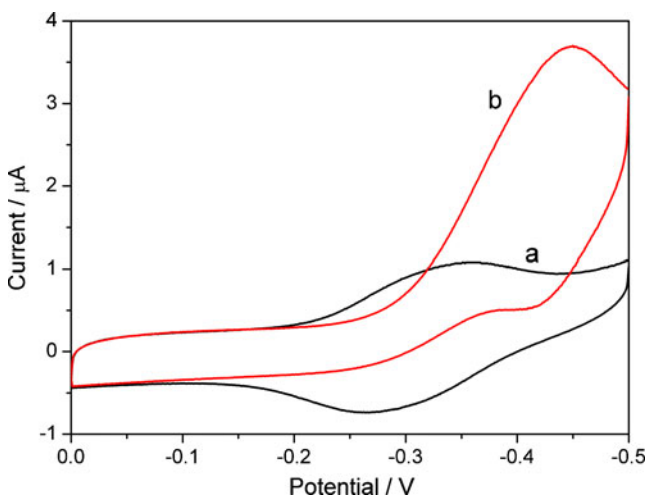

Fig. 8 Cyclic voltammograms of MS/Pt-DENs/GOx electrode at the scan rate of 0.1 V/s in 0.1 M (pH 6.0): (a) in the absence of glucose and (b) in the presence of 0.1 mM glucose
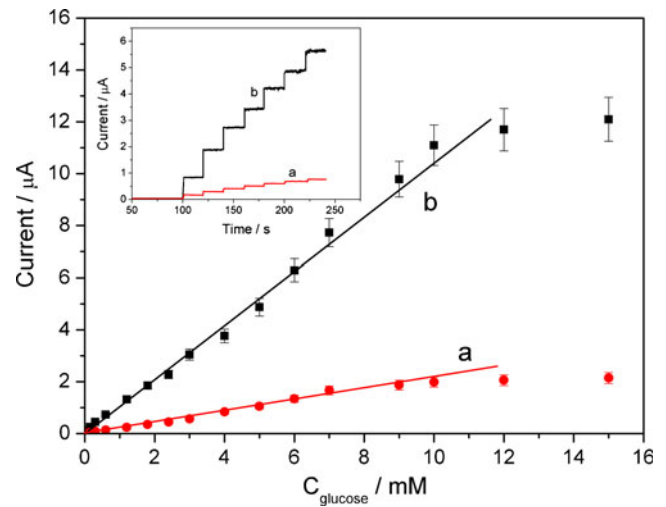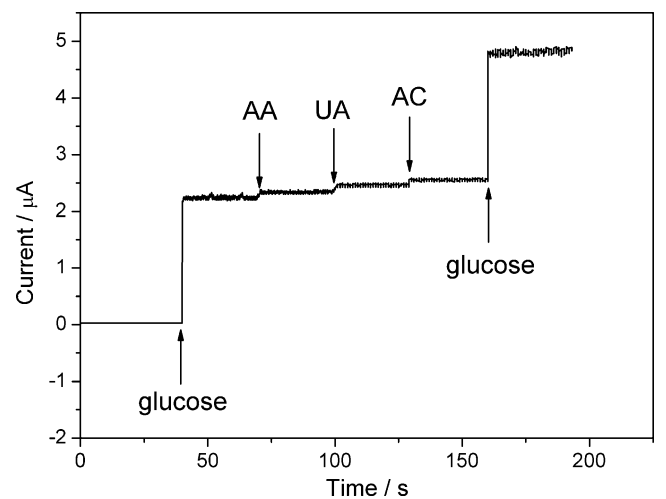

Fig. 10 Current–time curves for the MS/Pt-DENs/GOx electrode exposed to 0.1 mM ascorbic acid (AA), 0.1 mM uric acid (UA), 0.1 mM acetaminophen (AC) and 0.1 mM glucose at applied potential −0.4 V

GCE, which demonstrated that MS/Pt-DENs/GOx/GCE can electrocatalyze the glucose to H_2O_2 in N_2 saturated solutions. The reduction peak current also increased with the increasing glucose concentration, indicating that the glucose sensor with MS/Pt-DENs as matrix has high sensitivity.

The amperometric responses of the MS/GOx and MS/Pt-DENs/GOx composite-modified GCE upon the successive additions of glucose to 0.10 M pH 6.0 PBS at an applied potential of −0.4 V are shown in Fig. 9. The MS-modified GC electrode (Fig. 9a) showed a much smaller response to glucose than that of the MS/Pt-DENs/GOx-modified GCE (Fig. 9b) at the same glucose concentration. This indicated that MS/Pt-DENs/GOx had better response and performance toward the glucose than MS/GOx. Moreover, the electrocatalytic current of the MS/Pt-DENs/GOx-modified GCE clearly increases with each addition of glucose to the solution. The current reached its maximum value within 3 s after each addition of glucose, which suggests that the MS/Pt-DENs/GOx-modified GCE can respond rapidly to the change of the concentration of glucose. Thus, the MS/Pt-DENs/GOx-modified GCE showed an excellent electrocatalytic response to the reduction of glucose. The linear range of this biosensor to glucose concentration was 0.02–10 mM. The detection limit of the biosensor was 4 μM at a signal-to-noise ratio of 3, which was better than that reported by others [23, 25]. These results indicated that the GOx entrapped in the MS/Pt-DENs/GOx composite possesses electrocatalytic activity to glucose.

Stability and reproducibility of MS/Pt-DENs/GOx electrode

Reproducibility of the MS/Pt-DENs/GOx electrode was evaluated from the response for 0.1 mM glucose; the relative standard deviation is only 2.8% for 10 successive measurements. As for intro-electrode reproducibility, the GOx-modified electrode was stored in phosphate buffer solutions (pH 6.5) at 4 °C when not in use. The stability of the biosensor under storage was investigated by measuring the biosensor response with 0.1 mM of glucose over a 1-month period. The response current of the sensor decreased to 95.9% after storing for 15 days. After 30 days of storage, the biosensor retained about 73.5% of its original response. The stability of this biosensor is better than in some previous works [30, 31].

At the applied potential of −0.4 V, interferences were evaluated by three main interferents: 0.1 mM ascorbic acid, 0.1 mM uric acid and 0.1 mM acetaminophen. No appreciable signals were observed for the successive injections of interferents into the electrolyte solution (Fig. 10). On the other hand, the addition of 0.1 mM glucose provided MS/Pt-DENs/GOx electrode sensitivity close to that determined in the absence of interferents.

Conclusions

In this paper, we prepared the MS-Pt-DENs composite microspheres and immobilized GOx onto the modified GCE. The immobilized GOx retains its biological activity well and shows high catalytic activity to the reduction of glucose. Under the optimized experimental conditions, the catalytic currents are linear to the concentrations of glucose from 0.02 to 10 mM. The detection limits were 4 μM at signal-to-noise ratio of 3. The sensor shows a good reproducibility and stability. The fabrication method of biosensor opens a new opportunity for the development of simple and reliable other enzymes.

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