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Platinum nanoparticles functionalized nitrogen doped graphene platform for sensitive electrochemical glucose biosensing

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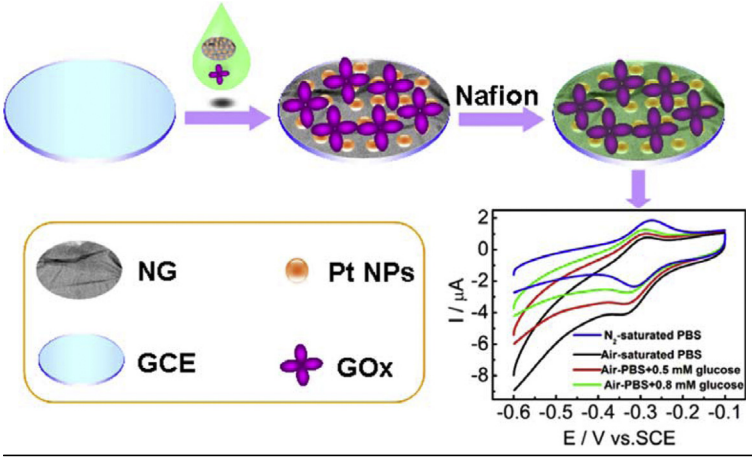
HIGHLIGHTS

- An efficient PtNPs@NG nanocomposite was prepared for the immobilization of enzyme.
- A novel electrochemical glucose biosensor was constructed based on this PtNPs@NG.
- The proposed glucose biosensor showed high sensitivity and low detection limit.
- The PtNPs@NG composite provided a promising platform for biosensing applications.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we reported an efficient platinum nanoparticles functionalized nitrogen doped graphene (PtNPs@NG) nanocomposite for devising novel electrochemical glucose biosensor for the first time. The fabricated PtNPs@NG and biosensor were characterized using transmission electron microscopy, high-resolution transmission electron microscopy, X-ray photoelectron spectroscopy, static water contact angle, UV–vis spectroscopy, electrochemical impedance spectra and cyclic voltammetry, respectively. PtNPs@NG showed large surface area and excellent biocompatibility, and enhanced the direct electron transfer between enzyme molecules and electrode surface. The glucose oxidase (GOx) immobilized on PtNPs@NG nanocomposite retained its bioactivity, and exhibited a surface controlled, quasi-reversible and fast electron transfer process. The constructed glucose biosensor showed wide linear range from 0.005 to 1.1 mM with high sensitivity of 20.31 mA M^{-1} cm^{-2}. The detection limit was calculated to be 0.002 mM at signal-to-noise of 3, which showed 20-fold decrease in comparison with single NG-based electrochemical biosensor for glucose. The proposed glucose biosensor also demonstrated excellent selectivity, good reproducibility, acceptable stability, and could be successfully applied in the detection of glucose in serum samples at the applied potential of −0.33 V. This research provided a promising biosensing platform for the development of excellent electrochemical biosensors.

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1. Introduction

Graphene, a two-dimensional carbon material with atoms arranged in a honeycomb lattice, has recently attracted enormous attention in constructing electrochemical biosensors due to its large surface area, excellent conductivity, high stability, strong mechanical stiffness, ease of functionalization and production [1–6]. Many studies have shown that the properties of graphene can be tuned by controlled its morphology and tailoring its electronic structure [7]. Specially, nitrogen doping in graphene could significantly increase the electron conductivity, improved the electron-donor properties and the binding ability of graphene [8,9], and enhanced the biocompatibility and sensitivity of graphene in biosensing applications [10]. Recently, nitrogen-doped graphene (NG) has been applied in electrocatalysis [11], solar cell [12], batteries [13], and electrochemical biosensor [14–16]. On the other hand, platimum nanoparticles (PtNPs) exhibited high electrocatalytic activity and have been extensively used for preparation of fuel cells and sensing applications [17,18]. PtNPs functionalized NG (PtNPs@NG) has become one of most attractive systems in catalysis research due to their remarkable catalytic capacity and free electron mobility [19,20]. Till now, PtNPs@NG has not been reported to immobilize proteins for electrochemical biosensing applications.

The fast and reliable determination of blood glucose levels was of considerable importance for diagnosis and therapy of diabetics [21]. The ultimate goal of glucose detection was to develop the third generation biosensor based on the direct electron transfer (DET) between the cofactor FAD of glucose oxidase (GOx) and electrode surface [22]. However, DET of enzyme at conventional electrodes was hard to realize because the FAD is deeply embedded in the protein shells [23]. Thus, various kinds of nanomaterials, such as metal nanoparticles [24], carbon nanomaterials [25–27], and semiconductor nanomaterials [28,29] have been explored to immobilize GOx for accelerating DET of redox enzyme on the electrode surface. Lin's group has reported the synthesis of NG for first immobilization of GOx and study of DET behavior of GOx at NG modified electrode, but the quantitative detection of glucose was performed by measuring H_2O_2 during the enzymatic ctatalysis [30], not DET of GOx. Hence it is necessary to develop NG-based third generation biosensor for highly sensitive detection of glucose.

In this paper, we reported the preparation of PtNPs@NG by adsorbing PtNPs on the synthesized NG. Then the resulted PtNPs@NG was used to modify the electrode for the immobilization of protein molecules. Based on DET of GOx at PtNPs@NG modified electrode, a novel electrochemical biosensor was proposed for highly sensitive detection of glucose for the first time (Fig. 1). PtNPs@NG nanocomposite provided a biocompatible microenvironment to retain native structure and bioactivity of the immobilized enzyme. A pair of obvious and well-defined redox peaks of GOx could be observed at PtNPs@NG modified glassy carbon electrode, showing the enhanced direct electron transfer between enzyme and electrode surface. Moreover, the constructed glucose biosensor showed high sensitivity, excellent selectivity, and good reproducibility. The assay results of serum samples with the proposed biosensor were in an acceptable agreement with the reference values. Therefore, the PtNPs@NG composite provided a promising and efficient platform for developing electrochemical biosensing system.

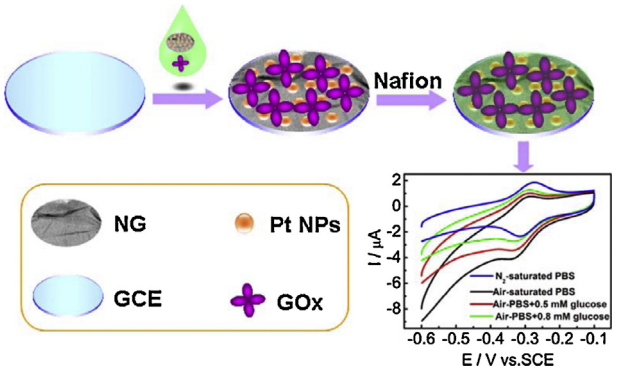

Fig. 1. Schematic illustration for fabrication of PtNPs@NG-based biosensor and electrochemical detection of glucose based on DET of GOx.

2. Materials and methods

2.1. Materials and reagents

GOx (EC 1.1.3.4, 108 U mg^{-1}, from *Aspergillus niger*) was supplied by Amresco. D-(+)-Glucose and Nafion were purchased from Sigma–Aldrich. Graphite powder (99.95%, 325 mesh) was purchased from Alfa Aesar. Hydrazine, sodium borohydride, and pyrrole monomer were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Hexachloroplatinic acid ($H_2PtCl_6{\cdot}6H_2O$) was purchased from Shanghai Sangon Biotechnology Co., Ltd (China). A stock solution of D-glucose was prepared and allowed to mutarotate at room temperature for 24 h prior to use. Phosphate buffer solution (PBS) was a mixture of 0.1 M Na_2HPO_4 and NaH_2PO_4 and its pH was adjusted with H_3PO_4 or NaOH solutions. All other chemicals and reagents are of analytical grade and were prepared using distilled water.

2.2. Apparatus

Electrochemical measurements were carried out on a CHI 852C electrochemical workstation (Co., CHI, Shanghai Chenhua, China). All experiments were performed with a three-electrode system using a glassy carbon electrode (GCE, $D = 3$ mm) as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The cyclic voltammetric experiments were carried out at a scan rate of 100 mV s^{-1} in an electrochemical cell filled with 5.0 mL of PBS. All pH measurements were performed with S-25 digital pH-meter with glass combination electrode. UV–vis spectra were recorded by UV-2550 spectrophotometer (Shimadzu Co., Japan). Transmission electron micrographs (TEM) were obtained with a Philips Tecnai-12 electron microscope (Holland) at an acceleration voltage of 120 kV. High-resolution transmission electron micrographs (HRTEM) were obtained with a FEI Tecnai G2 F30 S-TWIN field-emission transmission electron microscopy (USA) at an acceleration voltage of 300 kV. X-ray photoelectron spectroscopic (XPS) spectrum analysis was measured with an ESCALAB 250Xi spectrometer (USA). Electrochemical impedance spectroscopy (EIS) measurements were performed on an Autolab/PGSTAT30 (The Netherlands) in 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, and the amplitude of the applied sine wave potential was 5 mV. The impedance measurements were recorded at a bias potential of 190 mV within the frequency range from 10^{-1} Hz to 10^5 Hz and the number of frequency points of 50.

2.3. Preparation of reduced graphene oxide (rGO)

Graphene oxide was firstly prepared according to the Hummers and Offeman method [31]. Briefly, 5 g of graphite and 2.5 g of sodium nitrate were mixed into 15 mL of concentrated sulfuric acid. After the mixed solution was cooled to 0 °C in an ice-bath, 15 g of potassium permanganate was slowly added to the suspension. The addition rate was controlled carefully to prevent the

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temperature of the suspension from exceeding 20 °C. Next the ice-bath was removed and the suspension was stirred for 30 min, whilst the temperature was kept at 35 °C. Subsequently, 230 mL of water was slowly stirred into the brownish-grey paste, causing the temperature to increase to 98 °C. The diluted suspension was maintained at this temperature for 15 min, then diluted with 700 mL of warm water and treated with 3% hydrogen peroxide to reduce the residual permanganate and manganese dioxide. The suspension was filtered while it was still warm to avoid precipitation of mellitic acid formed as a side reaction. The as-formed yellowish-brown filter product was washed carefully with warm water and then treated with a resinous anion and cation exchanger to remove the remaining salt impurities. The dry form of graphene oxide was obtained by centrifugation and drying in vacuum at 40 °C. Reduction of graphene oxide was performed by adding 2 mL of hydrazine into the solution of 200 mg of graphene oxide in 200 mL water after sonicating until there were no granular substances, and then refluxed at 100 °C for 24 h. Finally, the black powder of rGO was obtained by filtration and dried in vacuum.

2.4. Preparation of nitrogen doped graphene (NG)

NG was synthesized according to previously described method [32]. Firstly, 40 mg of as-synthesized rGO was dispersed in 40 mL of deionized water with sonication. Then, 0.05 mL of pyrrole monnomer was added into the rGO suspension, and magnetically stirred for 24 h at room temperature. Next, 50 mL of ammoniium peroxydisulfate solution (3.79 mg mL^{-1}) was added to initiate the polymerization reaction for 24 h in an ice-bath. After the reaction, the solid sample was filtered and carefully washed with water and ethanol alternately. Finally, 0.1 g of the resultant PPy/rGO was transferred in a quartaz tube furnace and heated under Ar from room temperature to 600 °C at a rate of 1 °C min^{-1} and kept there for 2 h. A back powder of NG was obtained after the furnace was cooled to room temperature.

2.5. Preparation of Pt nanoparticles (PtNPs)

PtNPs were prepared according to the previous method with some modifications [33]. 1 mL of H_2PtCl_6 (5%) solution was added to 85 mL of deionized water, and the solution was heated from room temperature to 80 °C. 15 mL of sodium dihydrogen citrate (1%) was added to the solution under magnetic stirring, and kept the reaction mixture at 80 °C for 1 h. Three to four drops of sodium borohydride was added to the mixture, and then refluxed for 30 min. When the mixture changed from light yellow to dark brow, the mixture solution was removed from the heating element and immersed in cold water to stop the reaction. The PtNPs were obtained by this time, and stored in the refrigerator at 4 °C before using.

2.6. Preparation of PtNPs@NG and the GOx/PtNPs@NG/Nafion/GCE

1.0 mg of NG was firstly dispersed in 1.0 mL of deionized water with sonication to form 1.0 mg mL^{-1} suspension. Then 1.0 mg mL^{-1} of NG suspension was mixed with PtNPs solution at a 1:1 ratio, stirred for 12 h and then sonicated for 4 h. Under long-time mechanical stirring, PtNPs could be finally adsorbed on the NG to form PtNPs@NG composite. Next, 10.0 mg of GOx was added in 1.0 mL of PtNPs@NG suspension, and homogeneously mixed under gentle stirring for 15 min for the following use.

The GCE was firstly polished successively with 0.03 and 0.05 μm alumina slurry (Buhler) followed by rinsing thoroughly with distilled water, and then sonicated in 1:1 nitric acid–water (v/v), acetone and distilled water and finally dried in air. Then 5.0 μL of the resultant suspension was dropped on the surface of the

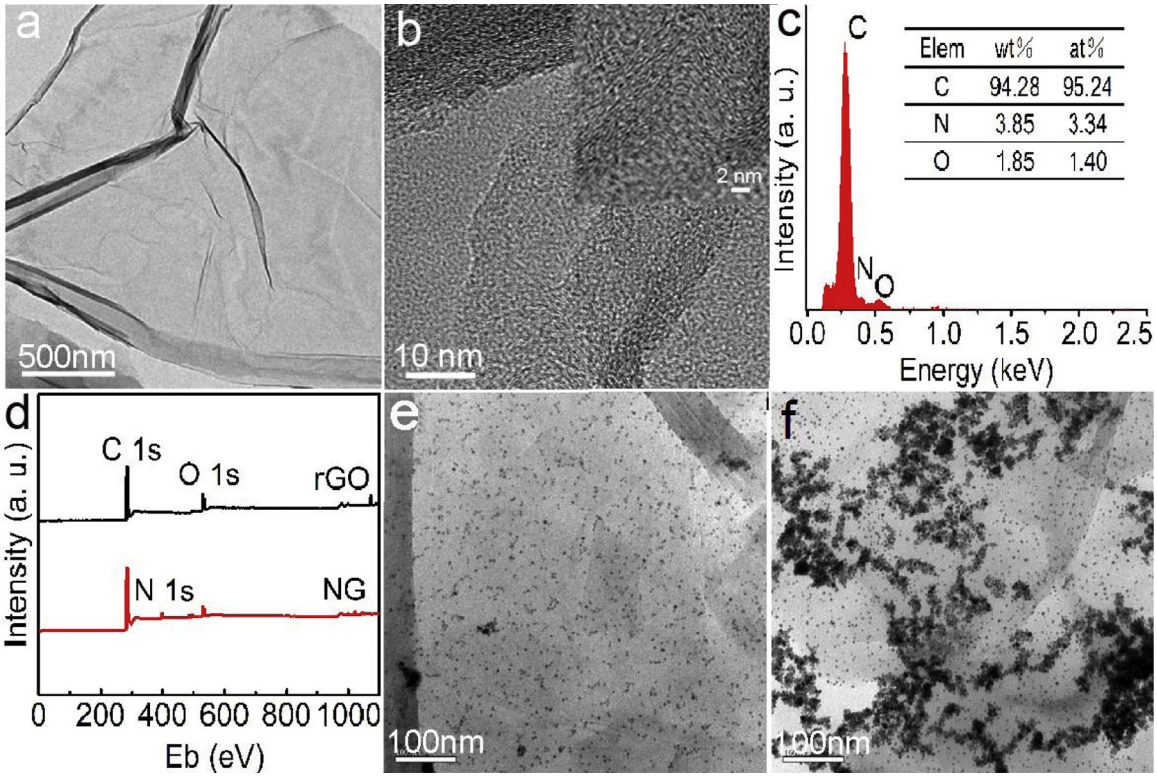

Fig. 2. (a) TEM image of NG, (b) HRTEM image of NG and its local enlargement (c), EDS spectrum and the elemental composition of NG, (d) XPS spectra of rGO and NG, (e) TEM image of PtNPs@NG, and (f) TEM image of GOx/PtNPs@NG.

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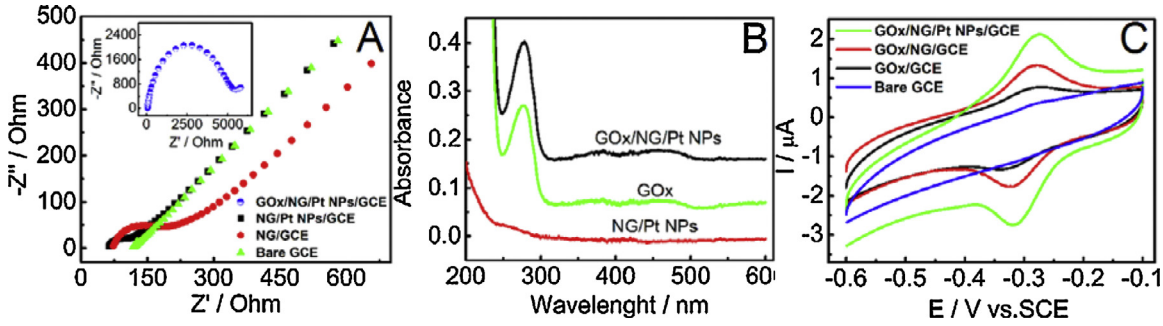

Fig. 3. EIS (A) for the bare, NG, PtNPs@NG and GOx/PtNPs@NG modified GCEs, UV–vis absorption spectra (B) of PtNPs@NG, GOx and GOx/PtNPs@NG and cyclic voltammograms (C) of the GCE/nafion, GOx/Nafion/GCE, GOx/NG/Nafion/GCE and GOx/PtNPs@NG/Nafion/GCE in 0.1 M pH 6.0 N_2-saturated PBS at a scan rate of 100 mV s^{-1}.

pretreated GCE and dried in a desiccator. After 5.0 μL of 0.5% Nafion was dropped on the GOx/PtNPs@NG/GCE surface, the GOx/PtNPs@NG/Nafion/GCE was finally obtained. The fabricated enzyme electrode was rinsed thoroughly with distilled water to wash away the loosely adsorbed enzyme molecules. When not in use, the enzyme electrodes were stored at 4 °C in a refrigerator.

3. Results and discussion

3.1. Characterizations of the GOx/PtNPs@NG/Nafion modified electrode

Fig. 2a shows the TEM image of NG, and the graphene planar sheets were observed clearly, indicating that the typical structure of graphene morphology was well retained after N-doping. The HRTEM image (Fig. 2b) of NG displayed the microstructure at the edge with distinguishable stacking structure, and the discontinuous graphitic layers suggested the formation of graphite fragments. The EDS spectrum (Fig. 2c) showed the N doping with an uncalibrated content of 3.85%, indicating the successful formation of NG. The chemical state of nitrogen before and after doping was investigated by XPS (Fig. 2d). No N signal was observed from the spectrum of rGO, while the spectrum of NG showed a clear N signal as expected. As seen form the TEM in Fig. 2e, PtNPs with the size of 5–7 nm were uniformly dispersed on the surface of NG with high density, which was advantageous to enhance the biocompatibility and sensitivity in biosensing applications. The TEM of GOx/PtNPs@NG in Fig. 2f displays obviously different surface morphology from PtNPs@NG. The aggregates of the loaded enzymes were observed clearly on the surface of PtNPs@NG, indicating the successful immobilization of enzyme in PtNPs@NG nanocomposite film.

The biocompatibility of a material for immobilization of biomolecules and preserving their bioactivity was positively related to its hydrophilicity [34], which could be characterized by measuring the contact angle of the substrate. The contact angles of rGO, NG, PtNPs@NG and GOx/PtNPs@NG were 66.9°, 56.5°, 48.3° and 38.1°, respectively (Fig. S1, Supporting Information). Compared with rGO and NG, PtNPs@NG showed an obvious decrease of contact angle, indicating the increased hydrophilicity, which was ascribed to the multiple defects from NG and the presence of PtNPs. Moreover, the excellent biocompatibility of PtNPs@NG composite was highly beneficial for protein immobilization. GOx/PtNPs@NG showed further decreased contact angle, which also confirmed the presence of enzyme in GOx/PtNPs@NG film.

Electrochemical impedance spectroscopy (EIS) was a powerful tool to study the interface property of the surface-modified electrode. The typical impedance spectrum is presented as Nyquist plot, and it consists of a semicircle part and a liner part. The semicircle part is ascribed to the electron-transfer limited process at higher frequencies and its diameter represents the electron transfer resistance (R_{ct}), which controls the electron transfer kinetics of the electrode interface. Fig. 3A shows the Nyquist plots of various modified electrodes in the frequency range of 10^{-1}–10^5 Hz. The bare GCE (pretreated freshly) displayed a straight line, and indicated a very small electron transfer resistance, which was very similar to the previous reports [35,36]. The R_{ct} of the NG/GCE was about 134 Ω, and its value was larger than that of the bare GCE, demonstrating that the NG film was formed on the surface of GCE. While NG is functionalized with PtNPs, the R_{ct} of the PtNPs@NG/GCE decreased to 64 Ω, indicating the presence of PtNPs on the NG and higher electron conductivity than NG. After GOx was trapped in the PtNPs@NG composite, the R_{ct} greatly increased to 5247 Ω. The possible reason was that the formed protein layer on the

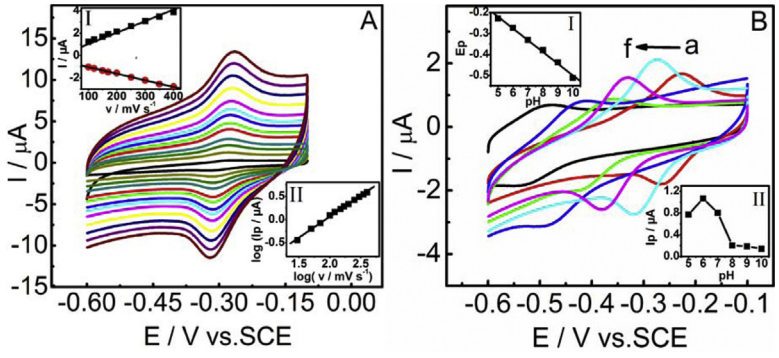

Fig. 4. (A) Cyclic voltammograms of the GOx/PtNPs@NG/Nafion/GCE in N_2-saturated 0.1 M pH 6.0 PBS at 10, 30, 50, 70, 100, 120, 150, 170, 200, 250, 300, 350 and 400 mV s^{-1} (from inner to outer). Inset I: plots of anodic and cathodic peak currents vs. scan rates, inset II: plot of logarithm of i_{pc} vs. logarithm of ν^{-1}; (B) Cyclic voltammograms of the GOx/PtNPs@NG/Nafion/GCE in N_2-saturated 0.1 M PBS with different pH values of (a–f) 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 at a scan rate of 100 mV s^{-1}. Inset I: plot of formal potentials vs. pH, inset II: plot of peak currents vs. pH.

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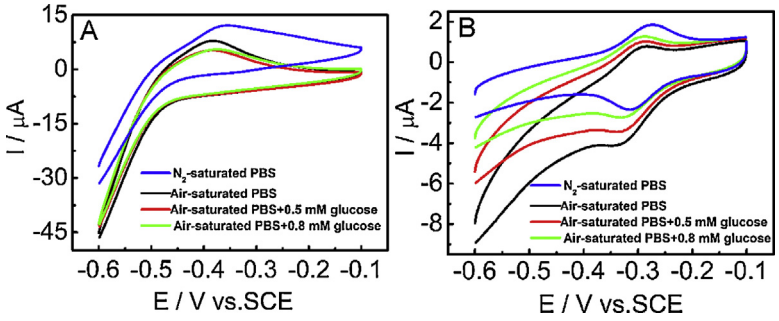

Fig. 5. Cyclic voltammograms of the PtNPs@NG/Nafion/GCE (A) and GOx/PtNPs@NG/Nafion/GCE (B) in 0.1 M pH 6.0 N_2-saturated PBS, air-saturated PBS, and air-saturated PBS including 0.5 and 0.8 mM of glucose at a scan rate of 100 mV s^{-1}.

electrode surface blocked significantly the diffusion of ferricyanide probe toward the electrode surface.

The UV–vis absorption spectra of PtNPs@NG, GOx and GOx/PtNPs@NG were shown in Fig. 3B. As seen from UV–vis absorption spectrum of PtNPs@NG, no obvious absorption peaks were observed in range of 240–500 nm. The UV–vis absorption spectrum of native GOx showed three absorption peaks. The intensive peak at 276 nm was ascribed to the characteristic of polypeptide chains, and other two weak peaks at 383 and 462 nm were attributed to the oxidized form of flavin group in protein structure. These absorption bands also were observed from the UV–vis absorption spectrum of GOx/PtNPs@NG. Moreover, the position and shape of three peaks were approximately the same as those of pure GOx, suggesting that the GOx immobilized in PtNPs@NG film retained its native structure.

3.2. Direct electrochemistry of the GOx/PtNPs@NG/Nafion/GCE

The cyclic voltammograms of different modified GCEs in 0.1 M N_2-saturated PBS at a scan rate of 100 mV s^{-1} were shown in Fig. 3C. Compared with the GOx/Nafion/GCE and the GOx/NG/Nafion/GCE, GOx immobilized on the PtNPs@NG/Nafion/GCE displayed a pair of more distinct and better-defined redox peaks at −0.274 V and −0.319 V, indicating that PtNPs@NG composite enhanced the direct electrochemistry of GOx. The larger reduction peak current of the GOx/PtNPs@NG/Nafion/GCE may be due to the larger specific surface and excellent conductivity of PtNPs@NG composite [37]. The peak potential separation was 45 mV, which was much smaller than that of 80 mV at GOx/graphene-chitosan modified GCE [27], indicating the faster DET between the electroactive center of GOx and GCE surface.

Fig. 4A shows the effect of the scan rate on the redox peaks current at the GOx/PtNPs@NG/Nafion/GCE. It could be seen that the anodic current (I_{pa}) and cathodic peak current (I_{pc}) linearly enhanced with the increasing scan rate from 10 to 400 mV s^{-1} (inset a of Fig. 4A). The I_{pa}/I_{pc} ratio was approximately equal to 1, indicating a quasi-reversible surface-controlled process. The logarithm plot of cathodic peak current versus logarithm of the scan rate showed a linear relationship (inset b of Fig. 4A). The slope was very close to the theoretical slope for thin layer electrochemical behavior [38]. The surface coverage the enzyme molecules on the surface of the GOx/PtNPs@NG/Nafion modified GCE was calculated to be 3.87×10^{-11} mol cm^{-2}, which was larger than those of 1.60×10^{-11} mol cm^{-2} at GOx/SnS_2/Nafion/GCE [29], 9.8×10^{-12} mol cm^{-2} at GOx/Au nanoparticles/carbon paste electrode [24], 7.82×10^{-12} mol cm^{-2} at GOx/Au-dihexadecyl phosphate modified electrode [39] and 2.86×10^{-12} mol cm^{-2} at bare GCE [40]. The charge-transfer coefficient and the apparent electron transfer rate constant (ks) for the proposed electrode were calculated to be 0.5 and 3.12 s^{-1}. The obtained value of ks was larger than those of 1.56 s^{-1} at boron-doped carbon nanotubes [41], 1.7 s^{-1} at multi-walled carbon nanotubes [42], 1.96 s^{-1} at Nafion–graphene GOx modified gold disk electrode [3], and 2.83 s^{-1} at graphene–chitosan modified GCE [27], indicating that PtNPs@NG facilitated the fast electron transfer between redox-active site of enzyme and the surface of electrode.

Fig. 4B shows the influence of the pH value on the electrochemical behavior of the GOx/PtNPs@NG/Nafion/GCE. The redox potentials of the GOx/PtNPs@NG/Nafion/GCE changed linearly as a function of the solution pH from 5.0 to 10.0 with a slope of −56.0 mV pH^{-1} (inset a of Fig. 4B). This slope was close to the theoretical value of −58.6 mV pH^{-1} reported previously [26], indicating same proton and same electron attending in the electron transfer process. In addition, the redox peaks current reached its maximum value at pH 6.0 (inset b of Fig. 4B), suggesting the optimal solution pH for the immobilization of GOx. Because of the participation of proton in the redox process of the loaded GOx, the decreased GOx response at higher pH value may be attributed to the decreased proton concentration, while the decrease of current response at lower pH value is possible due to the decrease of the bioactivity of immobilized enzymes [43].

3.3. Performance of the proposed glucose biosensor

Fig. 5 shows the cyclic voltammograms of the PtNPs@NG/Nafion/GCE (Fig. 5A) and GOx/PtNPs@NG/Nafion/GCE (Fig. 5B) in nitrogen- and air-saturated pH 6.0 PBS in the absence and presence of glucose. In air-saturated PBS, no peak was observed at PtNPs@NG/Nafion/GCE, though an increase in reduction current at potentials more negative than −0.4 V. However, the GOx/PtNPs@NG/Nafion/GCE clearly showed a great increase in

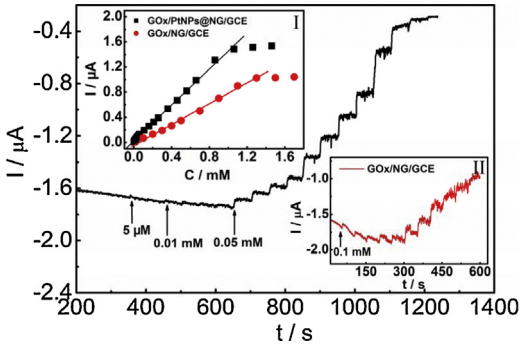

Fig. 6. Typical steady-state response of the GOx/PtNPs@NG/Nafion/GCE on successive addition of glucose into 0.1 M pH 6.0 air-saturated PBS at the applied potential of −0.33 V, inset: calibration curves of the GOx/PtNPs@NG/Nafion/GCE and GOx/NG/Nafion/GCE for glucose.

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Table 1
Comparison of analytical performance between the GOx/PtNPs@NG/Nafion/GCE and other modified electrodes.

Electrode	Applied potential (V)	Sensitivity ($mA\,M^{-1}\,cm^{-2}$)	Detection limit (mM)	Linear range (mM)	Reference
RGO/GOx/GCE	−0.44	1.85	–	0.1–27	[4]
RGO/Ag/GOx/GCE	−0.49	3.84	0.16	0.5–12.5	[44]
GOx/TiO_2-graphene/GCE	−0.60	6.2	–	0.0–8.0	[45]
ERGO-MWCNT/GOx/GCE	+0.35	7.95	0.0047	0.01–6.5	[46]
GOx/SnS_2/Nafion/GCE	−0.45	7.6	0.01	0.025–1.1	[29]
GOx/TPSP-ZnO/Nafion/GCE	−0.50	–	0.01	0.05–8.2	[37]
GOx/CNx-MWNTs/GCE	−0.50	13.0	0.01	0.02–1.02	[26]
Nafion/GOx/Ag-Pdop@CNT/GCE	−0.50	3.1	0.017	0.05–1.1	[47]
NCNT/GOx/GCE	+0.30	–	0.0012	0.002–0.14	[48]
Laponite/GOx/GCE	−0.45	4.8	0.01	0.02–1.9	[49]
Silica hybrid sol–gel/GOx/GCE	+1.0	10.8	0.019	0.06–4.4	[50]
GOx/Pt/FCNA/GCE	−0.08	6.0	0.3	0.5–8.0	[51]
GOx/PtNPs@NG/Nafion/GCE	−0.33	20.31	0.002	0.005–1.1	This work

RGO: reduced graphene oxide.
ERGO: electrochemically reduced graphene oxide.
MWCNT: multiwalled carbon nanotubes.
TPSP: tetragonal pyramid-shaped porous.
CNx-MWNTs: nitrogen-doped carbon nanotubes.
Pdop: polydopamine.
NCNT: nitrogen-doped carbon nanotubes.
FCNA: flower-like carbon nanosheet aggregation.
Pdop: polydopamine.
FCNA: flower-like carbon nanosheet aggregation.

reduction peak current and a simultaneous decrease in oxidation current. Upon addition of glucose to air-saturated PBS, no obvious change in current was observed for the PtNPs@NG/Nafion/GCE. While glucose was added into this system, the reduction peak current of GOx/PtNPs@NG/Nafion/GCE decreased obviously. Based on the response of the GOx/PtNPs@NG/Nafion/GCE to glucose, an enzyme biosensor in present work was proposed for highly sensitive detection of glucose.

Fig. 6 displays the typical amperometric response curve at the GOx/PtNPs@NG/Nafion/GCE on successive injection of glucose to the air-saturated stirring pH 6.0 PBS at −0.33 V applied potential. The GOx/PtNPs@NG/Nafion/GCE achieved 95% of the steady-state current only within 7 s. The current response increased linearly with the increase of glucose concentration from 0.005 to 1.1 mM with a high sensitivity of 20.31 $mA\,M^{-1}\,cm^{-2}$ and a correlation coefficient of 0.9989. The detection limit for glucose at the GOx/PtNPs@NG/Nafion/GCE was calculated to be 0.002 mM at signal-to-noise of 3. As a comparison, the GOx/NG/Nafion/GCE showed a linear range from 0.1 to 1.3 mM with a detection limit of 0.042 mM. The GOx/PtNPs@NG/Nafion/GCE showed 20-fold decrease in detection limit for glucose in comparison with the GOx/NG/Nafion/GCE. The present biosensor was also compared to the recent literatures involving in glucose biosensor, and the results were listed in Table 1. Compared with other types of enzyme electrodes, the present GOx/PtNPs@NG/Nafion/GCE showed obvious advantages such as much higher sensitivity and lower detection limit.

The apparent Michaelis–Menten constant (K_M^{app}) for the glucose biosensor was calculated to be 0.66 mM according to the Lineweaver–Burk equation [52]:

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_M^{app}1}{I_{max}C} \quad (1)$$

Here, I_{ss} was the steady-state current after the addition of substrate, C was the bulk concentration of substrate and I_{max} was the maximum current measured at the saturated substrate solution. The (K_M^{app}) value was much smaller than those of 4.4, 14.4, and 18.94 mM for immobilized GOx [27,53,54], indicating that the GOx immobilized in PtNPs@NG composite has higher biological affinity towards glucose.

3.4. Reproducibility and stability of the glucose biosensor

The reproducibility of the proposed biosensor was investigated by measuring 0.1 mM glucose for 5 times at the same enzyme electrode, the relative standard deviation (RSD) was 1.1%, suggesting good repeatability. After 60 successive detections, the response still retained 95% of the initial response, demonstrating the acceptable durability. The reproducibility of the enzyme electrode was also examined by determining 0.1 mM glucose at 5 different enzyme electrodes, and the RSD was 7.3%, indicating good fabrication reproducibility. The enzyme electrode was stored at 4 °C when not in use. After storage for two week, the current response of the biosensor decreased 2.1%. The peak current still retained 92% of its initial response after one month. These results demonstrated that PtNPs@NG composite could provide a biocompatible microenvironment to retain the bioactivity of the loaded enzyme.

3.5. Interference study and detection of glucose in the practical serum samples

The effect of possible interfering species on quantitative detection of glucose was studied using 0.05 mM ascorbic acid

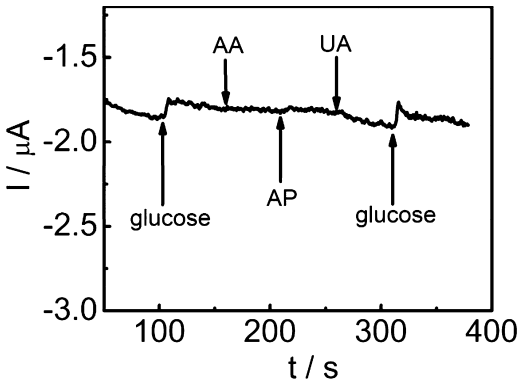

Fig. 7. Amperometric response of the GOx/PtNPs@NG/Nafion/GCE to 0.1 mM glucose, 0.05 mM AA, 0.05 mM AP and 0.025 mM UA in pH 6.0 PBS at −0.33 V applied potential.

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Table 2
Detection results of glucose concentrations in clinical serum samples

Sample	Proposed method (mM)	Reference method (mM)	Relative error (%)
1	4.34	4.46	−7.1
2	5.57	5.48	1.6
3	6.42	6.56	−2.1
4	6.72	6.93	−3.0
5	9.96	10.55	−5.6

(AA), 0.05 mM acetaminophen (AP) and 0.025 mM uric acid (UA) at the applied potential of −0.33 V. As shown in Fig. 7, a clear current response appeared upon the addition of 0.1 mM glucose to pH 6.0 PBS. While the successive injection of AA, AP and UA in the solution, leaded to no obvious increase of the current. When 0.1 mM glucose was added in the system again, the amperometric response was very close to the value detected in absence of the interferents, demonstrating the excellent selectivity of the proposed glucose biosensor toward glucose.

The practical application of the proposed glucose was examined by the detection of five human serum samples using the present method as well as the reference method (enzyme catalytic spectrophotometry). The human serum samples were received from Northern Jiangsu People's Hospital without any sample pretreatment except a dilution step. The reference values of glucose in serum sample were determined by this hospital using an Automatic Biochemical Analyzer. Prior to detection, serum samples were diluted appropriately with 0.1 mM pH 7.0 PBS, ensuring that glucose levels were in the linear response range. The results were shown in Table 2. The relative errors between the two methods were all less than 7.1%, demonstrating good accuracy of the present biosensor in the detection of the real samples.

4. Conclusion

In conclusion, an efficient PtNPs@NG nanocomposite was prepared for developing electrochemical biosensing platform for the first time. By the immobilization of GOx in the PtNPs@NG, a novel electrochemical biosensor was constructed for highly sensitive detection of glucose. The PtNPs@NG provided a favorable environment for the immobilized enzyme and enhanced the electron transfer between the enzyme molecules and electrode surface. Moreover, the constructed biosensor showed high sensitivity, wide linear range, excellent selectivity, and good reproducibility. The satisfactory results of the serum samples measured by the proposed biosensor showed the practical application of the biosensor. This work provided a promising PtNPs@NG platform for the immobilization of proteins development of excellent electrochemical biosensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2015.02.029.

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