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

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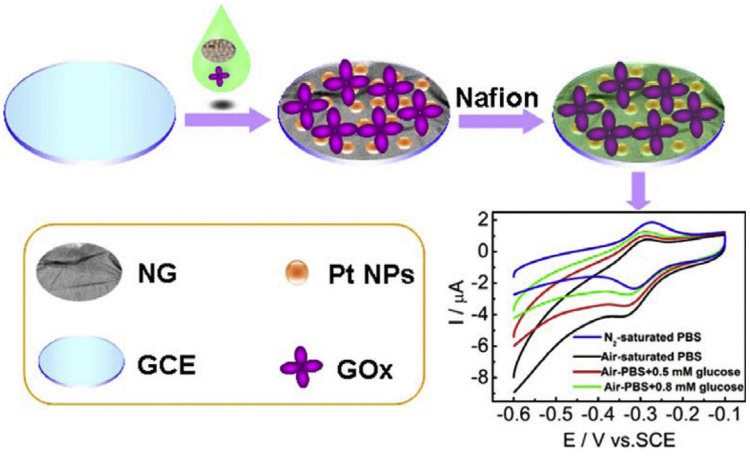
HIGHLIGHTS

- 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.

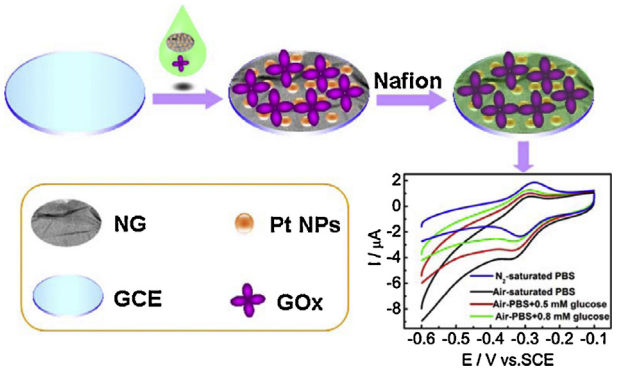

Fig. 1. Schematic illustration for fabrication of PtNPs@NG-based biosensor and electrochemical detection of glucose based on DET of GOx.

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