



An easy fabrication of glucose oxidase-dipeptide-reduced graphene oxide nanocomposite for glucose sensing



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ABSTRACT

We present an easy and fast way to fabricate glucose oxidase-diphenylalanine-reduced graphene oxide (GOx-FF-rGO) film by using pulsed potentiostatic method. Glassy carbon electrode (GCE) modified by the obtained GOx-FF-rGO nanocomposite film is systematically characterized for its application in glucose detection. Under optimized condition, the biosensor exhibits a wide linear response in the range from 0.1 to 30.0 mmol L⁻¹, with a low detection limit down to 0.01 mmol L⁻¹. Our results indicate that the pulsed potentiostatic deposition approach may lead to production of highly sensitive and stable electrochemical biosensors.

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1. Introduction

The direct electron transfer (DET)-based enzymatic biosensor is an ideal detection way for glucose, possessing several advantages such as low operation potential, reagent-less sensing and high selectivity [1–3]. But the active site of glucose oxidase (GOx), flavin adenine dinucleotide (FAD), is deeply embedded within a protective protein shell [4], the DET of enzymatically active GOx is extremely difficult. Numerous materials, including nano and mesostructured metal oxides [5,6], metal nanoparticles [7,8], conducting polymers [9–11], mesostructured silica [12], sol-gel matrix [13], carbon nanotubes [14–16] and graphene [17–21], have been used to modify the electrode for improving EDT. Despite recent studies show that GOx with DET property is not the real mediator-less biosensors [22,23], the introduction of some new materials and methods is still highly desirable for the design of DET-based biosensors and detection of glucose in the presence of oxygen.

Graphene is a two-dimensional carbon material that has very high electrical conductivity, and large surface area. Since its first discovery in 2004 [24,25], it has demonstrated tremendous application potentials in electrochemical biosensor because of its large surface area, excellent electrical conductivity and high electrocatalytic activity [26]. Based on this, various redox proteins

or enzymes have been coupled with graphene to construct electrochemical biosensor [27–30]. However, due to some inherent disadvantages, such as hydrophobicity and easy aggregation in aqueous solution, the applications of graphene for the construction of electrochemical biosensors have been limited. Therefore, DNA [27], ionic liquids [28], peptide [29] and small molecular weight polymers [30] have been introduced to form functionalized graphene composites. These composites can be synthesized by various methods [31–33]. As an alternative, a direct electrochemical reduction method is more green (without using any toxic solvents), cost-effective and it is more suitable for preparing less defective graphene sheets [34–37]. Recently, S. M. Chen and co-author presented a simple way for the immobilization of GOx on electrochemical reduction of graphene oxide (GO) film [21]. The results confirmed that the film showed high electrocatalytic activity to glucose in the presence of oxygen. In addition, our group has reported that reduce graphene oxide (rGO) nanosheets can be electrodeposited directly onto glassy carbon electrode through pulsed potentiostatic reduction of graphene oxide (GO) [38–40]. And we have confirmed that electrochemically reduced graphene oxide (ErGO) film modified GCE prepared by pulsed potentiostatic method showed enhanced electron transfer properties, and the agglomeration of rGO may be prevented.

In this work, we demonstrate a facile and efficient fabrication method for GOx-diphenylalanine (FF)-rGO composite film using a one-step pulsed potentiostatic electrodeposition in a graphene oxide mixture solution. Here, FF works as a modifier, which can increase the immobilization GOx on the graphene-based film due

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to its biocompatibility. Detailed electrochemical characterizations of the GOx-FF-rGO film modified GCE and its subsequent application in glucose sensing are presented. The proposed glucose biosensor exhibited high performance in terms of sensitivity, stability, selectivity, linear range and low limit of detection. Our results indicate that electrochemical approach used for the preparation of graphene-based composite film may fabricate high sensitive and stable electrochemical biosensors.

2. Experimental

2.1. Apparatus and Reagents

All the electrochemical measurements were carried out using a CHI 650A electrochemical analyzer (CHI instrumental, Shanghai, China) or a RST5000 electrochemical workstation (Zhengzhou Shiruisi Instrument Co., Ltd., Zhengzhou, China). A conventional three-electrode system was employed with a GCE ($d=3$ mm) or a modified GCE as the working electrode, a platinum (Pt) wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode (KCl saturation). Atomic force microscopy (AFM) images were obtained using a Dimension FastScan AFM (Bruker Co., Germany). Scanning electron microscopy (SEM) images were obtained under a Quonxe-250 scanning electron microscope (FEI Company, Czech). UV-vis absorbance spectra were recorded on the Model UV-2102 spectrophotometer (UNICO Company, Shanghai, China). All the pH measurements were made with a PHS-3C precision pH meter (Leici Devices Factory of Shanghai, China), which was calibrated with a standard buffer solution at 25 ± 0.1 °C every day.

GOx and D-(+)-Glucose were purchased from Sigma-Aldrich. Dopamine (DA), ascorbic acid (AA) and uric acid (UA) were obtained from XiYa reagent Co., Ltd (shandong, China). Graphite was purchased from Nanjing Xfnano Materials Tech Co., Ltd. (Nanjing, China). FF was acquired from Zhejiang Ontores Biotechnologies Co., Ltd (Zhejiang, China). A stock solution (2 mg mL^{-1}) of FF was prepared with ethanol and stored at 4 °C in the dark. All other reagents were of analytical grade and used directly without further purification. Doubly distilled water (DDW) was used for all preparations.

2.2. Preparation of GOx-FF-GO dispersion solution

GO was synthesized from graphite through the modified Hummers method [41]. GO (5 mg mL^{-1}) was added in DDW and ultrasonicated for approximately 60 min. FF was then added to the prepared GO dispersion solution to form a FF-GO solution. Finally, GOx (2 mg mL^{-1}) was added in FF-GO solution and ultrasonicated for about 1 h under ice-bath to form a uniform GOx-FF-GO dispersion solution.

2.3. Preparation of GOx-FF-rGO modified glassy carbon electrodes

Prior to modification, the bare GCE was polished successively with 0.3 and 0.05 μm Al_2O_3 powder and rinsed thoroughly with DDW between each polishing step. Subsequently, the GCE was sonicated in ethanol and double-distilled water for 2 min each before drying under N_2 blowing. After that, the cleaned GCE was immersed in GOx-FF-GO solution, and the GOx-FF-rGO film was deposited by pulsed potentiostatic method under constant stirring. The optimal parameters of electrodeposition were listed as follows: upper limit potential E_a , 0.1 V; lower limit potential E_c , -1.6 V; anodic pulse duration t_a , 0.6 s; cathodic pulse duration t_c , 0.3 s; experimental time t_{exp} , 80 s.

3. Results and Discussion

3.1. Characterization of GOx-FF-GO dispersion solution

UV-vis spectroscopy experiments were used to monitor the absorption band change of GO (Fig. 1). It is clear that the characteristic absorptions of GO were observed at 240 nm and 310 nm, but FF-GO gave an obviously decrease in absorbance, indicating that FF can bond to GO through the π - π interaction [42]. When GOx was added, obvious changes were also found. The result indicated that GOx have been bonded to FF-GO. Further AFM image showed the morphology of GO and GOx-FF-GO (Fig. 2). The results confirmed that the average thickness of GO sheets is approximately 1 nm; suggesting GO sheets were almost single-layer. But the average thickness of GOx-FF-GO increased to about 40 nm, and many winding attachments on the surface of GO was observed, which further reinforced that GOx and FF was successfully bonded on GO.

3.2. Pulse potentiostatic electrodeposition to fabricate GOx-FF-rGO/GCE

According to our previous work, GO sheets can be electrochemically reduced at $E = -1.2$ V (vs SCE) [40]. It make difficult to electrochemically reduce GOx-FF-GO because GOx and FF was bonded on GO. Here, we applied E_c , -1.6 V (vs SCE) to electrochemically reduce GOx-FF-GO.

The influence of the FF concentrations on the GOx-FF-rGO/GCE was also studied (Fig. S1). When the concentration of FF was lowered, FF can help to increase the immobilization GOx. However, with increasing concentration of FF, the immobilization GOx greatly decreased. The reason might be that too much FF adhesion to GO hinder electrodeposition of GOx-FF-GO. Therefore, 30 μg mL^{-1} was chose as the optimal concentration of FF solution.

3.3. Characteristics of GOx-FF-rGO film

Electrochemical impedance spectroscopy (EIS) can provide information on the impedance of the electrodes. EIS experiments were performed in 0.1 mol L^{-1} KCl solution containing 5.0×10^{-3} mol L^{-1} $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and the results were showed in Fig. S2. EIS spectra contain two portions i.e. semicircle at higher frequencies correspond to the electron transfer limited process and linear line relatively at lower frequencies correspond to the diffusion process. From Fig. S2 it is seen that there are no well-defined semicircle in

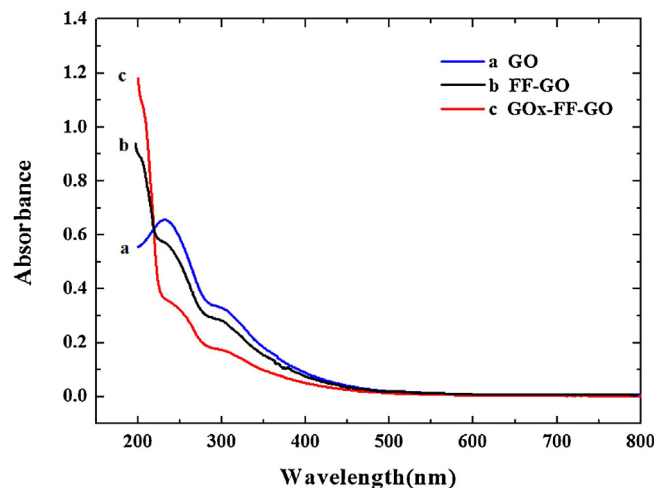


Fig. 1. UV-vis absorption spectra of GO (a), FF-GO (b) and GOx-FF-GO (c).

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