Research paper

Palygorskite-poly(o-phenylenediamine) nanocomposite: An enhanced electrochemical platform for glucose biosensing

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ABSTRACT

Palygorskite (Pal) may be a promising material for enzyme immobilization due to its large surface, high biocompatibility and stability. This attractive material combined with a conducting polymer, poly(o-phenylenediamine), was exploited as a platform for the immobilization of glucose oxidase (GOD) using glutaraldehyde as crosslinker, and thus a novel glucose biosensor was obtained. The results of electrochemical impedance spectroscopy (EIS) and SEM indicated the successful entrapment of GOD in the clay polymer nanocomposite (CPN) film. Amperometric detection of glucose was performed by holding the potential at the CPN electrode at 0.6 V for the oxidation of H_2O_2 generated in the enzymatic reaction. The apparent Michaelis–Menten constant (K_M^{app}) was calculated to be 5.25 mM, which is close to that of the free enzyme. The proposed biosensor exhibited a wide linear range, a low detection limit, a good reproducibility and accepted stability in the determination of glucose, providing a biocompatible platform for glucose biosensing.

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1. Introduction

Palygorskite (Pal) is among the most common cationic clays on the earth's surface, which has negatively charged magnesium aluminum silicate layers (Mousty, 2004). Owing to its unique properties, such as large specific surface area, environmental friendliness and nontoxicity, Pal finds applications in adsorption, pharmacy, ion exchanger, catalysts and cosmetics (Huang et al., 2007; Kong et al., 2009; Miao et al., 2007; Vaccari, 1998). The past decades have seen the development of Pal-based electrochemical sensors (Chen and Jin, 2010; Kong et al., 2011) and biosensors (Xu et al., 2007a,b). However, Pal is an insulating material, and thus modification of Pal by conductive materials is necessary to make it a good candidate for sensors. Among these conductive substances, metal nanoparticles (Chen et al., 2011; Liang et al., 2011), expanded graphite (Kong et al., 2010) and graphene (Kong et al., 2013) are commonly used as the modifiers of Pal.

As a new generation of functional polymer, conducting polymers have attracted considerable attention due to the fact that they have a high conductivity and stability in air and aqueous solutions (Wang and Mu, 1999). Glucose sensors based on conducting polymers, especially polypyrrole (Ozcan et al., 2008; Pesantez et al., 2012; Senel, 2011) and polyaniline (Kuczynska et al., 2010; Ramya and Sangaranarayanan, 2013; Zhou et al., 2005) have been proposed for the sensitive determination of glucose. However, to the best of our knowledge, poly(o-phenylenediamine) (PoPD), an important conducting polymer from the polymerization of o-phenylenediamine, has never been reported as the component of a glucose sensor.

Recently, Chirizzi and Malitesta (2011) have prepared a simple potentiometric biosensor for urea detection based on an electro-synthesized PoPD film, and the obtained sensor exhibits a sufficient sensitivity for practical determinations. So, it strongly attracts us to combine the merits of PoPD and Pal for biosensor construction. In the present work, Pal–PoPD nanocomposite was obtained by an in situ electrosynthesis of PoPD onto a Pt disk electrode in the presence of Pal, and then glucose oxidase (GOD) was crosslinked to the Pal–PoPD by glutaraldehyde for glucose biosensing. On one hand, PoPD makes the nanocomposite an ideal conducting material for biosensor; on the other hand, a large specific surface area and high adsorption ability of Pal is required for the support materials of enzyme immobilization. Herein, GOD was taken as a model enzyme because of its high stability, high catalytic ability, commercial availability and moderate cost (Muguruma et al., 2000; Shan et al., 2010). The crosslinked GOD was characterized by scanning electron microscopy (SEM) and electro-chemical impedance spectroscopy (EIS). The analytical performances of the Pal–PoPD nanocomposite immobilized with GOD were then examined for the determination of glucose via the amperometric oxidation of H_2O_2 generated in the enzymatic reaction.

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2. Experimental

2.1. Reagents and materials

Pal was obtained from Jiangsu NDZ Technology Company (Changzhou, China) and washed with distilled water to remove impurity and dried at room temperature. GOD (Type X-S, 100–250 U mg^{-1}) was from *Aspergillus niger* and *o*-phenylenediamine (*o*-PD) was obtained from Sigma-Aldrich. Glutaraldehyde solution (25%) was purchased from Kelong Chemical Reagent Company (Chengdu, China). All other chemicals were of analytical grade and used without further purification. The solutions were prepared by doubly distilled water. Clinical human serum samples were provided by the No. 2 People's Hospital of Changzhou.

2.2. Measurements and apparatus

SEM images were obtained with a model JSM-6360LA scanning electron microscope (JEOL, Japan). Electrochemical impedance spectroscopy (EIS) was recorded with a VersaSTAT 3 electrochemical instrument (Princeton Applied Research, USA). Other electrochemical measurements, including cyclic voltammetry and chronoamperometry experiments, were carried out on a CHI 660D electrochemical workstation (Huakeputian Instrument Co., Beijing, China). The pH values of phosphate buffer solutions (PBS) were determined by using a PHS-25 pH meter (Shanghai, China). The detection limit was calculated as the signal intensity to noise intensity ratio of 3 (signal-to-noise of 3).

2.3. Preparation of enzyme electrode based on Pal–PoPD nanocomposite

A Pt disk electrode (3 mm diameter) was polished with 0.05 μm alumina slurry and then sonicated in doubly distilled water before use. The Pal suspension (2 mg mL^{-1}) was prepared by dispersing Pal in doubly distilled water with stirring overnight, and then 40 mM *o*-PD and 1 M H$_2$SO$_4$ were added to this suspension. Electropolymerization of *o*-PD was carried out by cyclic voltammetry in a conventional three-electrode system comprised of Pt disk as the working electrode, Pt foil as the counter electrode and saturated calomel electrode (SCE) as the reference electrode. The repeated potential cycling was set between −0.25 and 1.20 V with a scan rate of 100 mV s^{-1}. After the electrolysis, PoPD coated Pal was obtained at the Pt disk surface. GOD was also dissolved in doubly distilled water with a concentration of 5 mg mL^{-1}. 10 μL of the GOD solution was dropped onto the surface of the Pal–PoPD nanocomposite by a microsyringe, and then the physical entrapment of GOD within the clay polymer nanocomposite (CPN) was achieved by crosslinking of the enzyme molecules with glutaraldehyde vapor for 15 min. Finally, the enzyme electrode was rinsed thoroughly with 0.1 M PBS to remove the GOD not firmly immobilized. The as-prepared glucose sensor was stored at 4 °C in a refrigerator when not in use.

3. Results and discussion

3.1. Electrosynthesis of Pal–PoPD nanocomposite

Electropolymerization of *o*-PD was carried out in the presence of Pal, and the resulting polymer can be served as the coating layer of Pal. The concentration of *o*-PD monomer affects the properties of the obtained Pal–PoPD nanocomposite, and it was discussed herein. Fig. 1 shows the 10th cycle on the cyclic voltammograms (CVs) showing the growth of the Pal–PoPD films at different *o*-PD concentrations in 1 M H$_2$SO$_4$. On each curve, there is a pair of obvious redox peaks appearing at −0.05 and −0.11 V, respectively, which is attributed to the oxidation and reduction of the PoPD. It is also found that the electroactivity of PoPD polymerized from 40 mM *o*-PD is a little higher than those from other
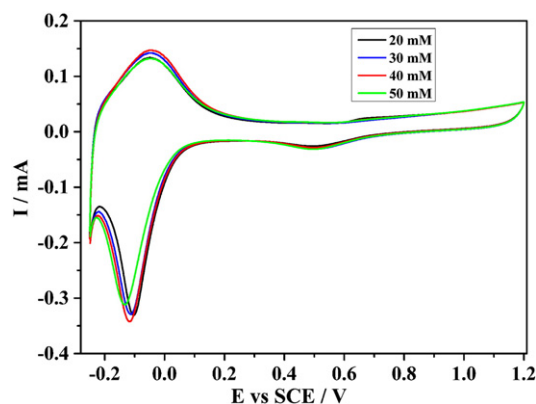

Fig. 1. The 10th cycle on the cyclic voltammograms showing the growth of the Pal–PoPD nanocomposite in 1 M H$_2$SO$_4$, and the *o*-PD monomer concentrations were 20, 30, 40, and 50 mM, respectively. Scan rate: 100 mV s^{-1}.

monomer concentrations. So 40 mM is chosen as the optimal *o*-PD monomer concentration for the synthesis of Pal–PoPD.

3.2. Characterization of the enzyme electrode

The electrochemical behaviors of the glucose sensor were characterized step-by-step by the EIS. Fig. 2 shows the Nyquist plots of the electroactive probes, [Fe(CN)$_6$]$^{3-}$/$^{4-}$, at the unmodified Pt disk electrode, Pal–PoPD and Pal–PoPD immobilized with GOD in 0.1 M PBS. Frequency sweeps extended from 0.1 to 10^5 Hz. As can be seen, there is a semicircle located in the high frequency range for all plots, and the diameter of the semicircle is equivalent to the electron transfer resistance (R_{et}). R_{et} represents the electron transfer kinetics of the electroactive probes at the electrode–solution interface (Liu et al., 2012). Comparing the EIS of the three electrodes, it is observed that the modification of the Pt disk electrode by the Pal–PoPD is accompanied by an increase in R_{et} from 100 Ω to 1300 Ω, which is due to the fact that the electron transfer resistance of the Pt disk electrode is significantly increased after its modification by the Pal–PoPD nanocomposite. After the immobilization of GOD to the Pal–PoPD nanocomposite, the R_{et} is further increased to 2000 Ω (curve c). The largest R_{et} at the Pal–PoPD immobilized with GOD suggests that the immobilized GOD acts as a barrier and hinders the electron transfer on the electrode–solution interface. So these EIS results clearly elucidate that Pal–PoPD and GOD were immobilized successfully on the Pt disk electrode after each modification process.
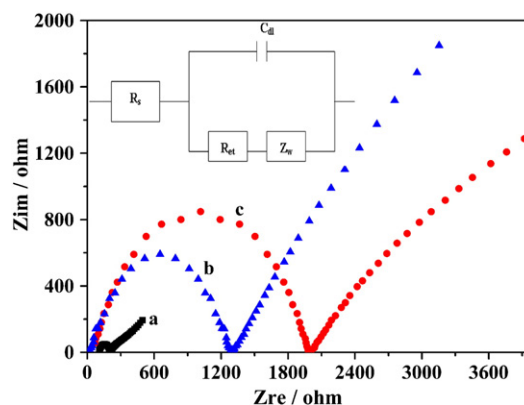

Fig. 2. EIS of the unmodified Pt disk (a), Pal–PoPD (b) and Pal–PoPD immobilized with GOD (c) electrodes in 0.1 M PBS with 5 mM [Fe(CN)$_6$]$^{3-}$/$^{4-}$ electroactive probes, respectively. Inset shows the equivalent circuit.