New amperometric glucose biosensor by entrapping glucose oxidase into chitosan/nanoporous ZrO_2/multiwalled carbon nanotubes nanocomposite film

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Abstract: A new nanocomposite material for construction of glucose biosensor was prepared. The biosensor was formed by entrapping glucose oxidase (Gox) into chitosan/nanoporous ZrO_2/multiwalled carbon nanotubes nanocomposite film. In this biosensing thin film, the multiwalled carbon nanotubes can effectively catalyze hydrogen peroxide and nanoporous ZrO_2 can enhance the stability of the immobilized enzyme. The resulting biosensor provides a very effective matrix for the immobilization of glucose oxidase and exhibits a wide linear response range from 8 µmol/L to 3 mmol/L with a correlation coefficient of 0.994 for the detection of glucose. And the response time and detection limit of the biosensor are determined to be 6 s and 3.5 µmol/L, respectively. Another attractive characteristic is that the biosensor is inexpensive, stable and reliable.

Key words: biosensor; nanocomposite; glucose oxidase; nanoporous ZrO_2; multiwalled carbon nanotubes; chitosan

1 Introduction

In recent years, much interest has been attributed to construct glucose biosensors, because the determination of glucose is very important for human metabolic diagnoses and agricultural applications [1]. For construction of biosensor, the method for immobilization of enzymes is very important. Some methods including cross-linking to a matrix, entrapment within the membrane and microencapsulation into polymer microspheres, hydrogels, are continuously developed [2−3]. However, some techniques are somewhat complex and the enzyme can be easily denatured and leached out from the matrix.

ZrO_2 is an inorganic ceramic material with very high mechanical strength and enhanced thermal stability in solution. Nanoporous ZrO_2 was used as a matrix for immobilization of enzyme [4−5]. However, the application of the inorganic ceramic material was limited because of its brittleness. To overcome this disadvantage, nanoporous ZrO_2 is often combined with organic polymers to form inorganic-organic materials because the inorganic-organic materials possess the advantages of organic component’s toughness and inorganic phase’s chemical and thermal stability [5].

Carbon-nanotubes (CNTs) lead to many new technical developments and applications due to large surface area, unique electronic properties, and relatively high mechanical strength [6]. It is found that CNTs have high electrocatalytic effect and fast electron-transfer rate [7−11]. Besides, CNTs can minimize the surface fouling onto electrochemical devices without mediator. The ability of CNTs to promote the electron transfer of hydrogen peroxide (H_2O_2) and NADH etc suggests that CNTs have great promise as oxidase-based and dehydrogenase-based amperometric biosensors [11−12].

Chitosan (CHIT), a natural biopolymer, exhibits excellent film forming ability, high permeability toward water. It has gained growing interest to be used in immobilizing biomolecules [13−14]. Nanocomposite of CHIT/CNTs was prepared [11−12] and used to covalently immobilize glucose dehydrogenase by cross-linking of glutaraldehyde (GA). But in the method, the unreacted excess GA must be removed from the mixture by multiple extractions with ethyl ether because GA can make enzyme denatured [11,15]. So the method of immobilizing enzyme using GA is very complex and noneffective.

In this study, a new nanocomposite was developed by dispersion of nanoporous ZrO_2 and multiwalled carbon nanotubes (MWNTs) in the matrix of biopolymer CHIT. By entrapping glucose oxidase (Gox) into the CHIT/MWNTs/ZrO_2 nanocomposite, a glucose biosensor was prepared.
2 Experimental

2.1 Apparatus and reagents

Scanning electron microscopy (SEM) image was obtained from JSM 6700 LV (Japan) operating at 5.0 kV. Cyclic voltammetry and amperometric measurements were performed with CHI 660A electrochemical station. A conventional three-electrode system using the modified glassy carbon (GC) electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum foil electrode as counter electrode was used. All applying potentials were measured and reported vs the SCE, and all experiments were carried out at room temperature.

Glucose oxidase (E.C 1.1.3.4 from Aspergillus niger, type VII-S; \(196 \times 10^3\) unit/g) and chitosan (CHIT, relative molecular mass of \(1 \times 10^6\); 75%−85% deacetylation) were produced in Sigma (St. Louis, Mo, USA). Nanoporous ZrO\(_2\) was supplied by Nano Material Application Engineering Technology Center (Zhejiang, China). MWNTs with 95% purity were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Nafion was obtained from Aldrich. \(\beta\)-D glucose was purchased from ICN Biomedicals Inc. (USA). Glucose stock solutions were allowed to rotate at room temperature overnight before use. All other reagents were of analytical grade. Phosphate buffer solution (0.067 mol/L, pH=7.0) was used as the supporting electrolyte in all measurements, and redistilled water was used throughout.

2.2 Preparation of CHIT/ZrO\(_2\)/MWNTs solution

MWNTs were functionalized according to Ref.[16]. Appropriate amount of nanoporous ZrO\(_2\) was dispersed in 0.3% (mass fraction) of CHIT (2% acetic acid), and the mass ratio of ZrO\(_2\) to CHIT was 1:100. The mixture was sonicated for 15 min after stirring for 1 h until a high dispersed colloidal solution was formed. Then the functionalized MWNTs (0.6–6.0 g/L) were dispersed into CHIT/ZrO\(_2\) solution by ultrasonic agitation to give a black suspension.

2.3 Preparation of CHIT/MWNTs solution

For preparation of CHIT/MWNTs solution, the functionalized MWNTs (6 g/L) were added into in 0.3% (mass fraction) of CHIT (2% acetic acid) by ultrasonic agitation to get uniformly dispersed solution.

2.4 Preparation of CHIT/ZrO\(_2\)/MWNTs/Gox Nafion glucose biosensor

Prior to the surface modification, the GC electrode was first polished with alumina slurry (followed by 0.3 \(\mu\)m and 0.05 \(\mu\)m), then rinsed thoroughly with redistilled water, and ultrasonically agitated successively in ethanol and redistilled water, each for 10 min. Enzyme solution with the Gox concentration of 30 g/L was prepared in phosphate buffer solution (0.067 mol/L, pH=7.0). The glucose biosensor was constructed by mixing 10 \(\mu\)L of Gox solution with 10 \(\mu\)L of CHIT/ZrO\(_2\)/MWNTs solution, and then dropping 6 \(\mu\)L of the composite solution on the GC electrode surface. The glucose biosensor was dried at 4 °C. Then 10 \(\mu\)L of 0.25% Nafion solution was dropped onto the enzyme electrode surface and dried in air. When not in use, the electrode was stored dry at 4 °C in refrigerator.

2.5 Preparation of CHIT/MWNTs/Gox glucose biosensor

The casting solution was obtained by mixing 10 \(\mu\)L of CHIT/MWNTs composite solution with 10 \(\mu\)L of enzyme solution (30 g/L). Then 6 \(\mu\)L of the resulting casting solution was pipetted onto the GC electrode surface. The glucose biosensor was dried at 4 °C.

3 Results and discussion

3.1 SEM image of CHIT/ZrO\(_2\)/MWNTs nanocomposite film

Homogenization within the nanocomposite film is a key factor in the construction of biosensor. Fig.1 shows the SEM image of CHIT/ZrO\(_2\)/MWNTs nanocomposite film. As can be seen, both of nanoporous ZrO\(_2\) and MWNTs distribute homogeneously in the CHIT film. MWNTs can act as high conductivity wires connecting nanocomposite film domains throughout the film. Besides, nanoporous ZrO\(_2\) and MWNTs can bind to glucose oxidase effectively[4−5,17]. So, this nanocomposite film offers an effective matrix to immobilize enzyme and is beneficial to the special response between enzyme and substrate.

Fig.1 SEM image of CHIT/ZrO\(_2\)/MWNTs nanocomposite film