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Adsorption of glucose oxidase at platinum-multiwalled carbon nanotube-alumina-coated silica nanocomposite for amperometric glucose biosensor

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ABSTRACT

Glucose oxidase (GOx) has been immobilized in platinum-multiwalled carbon nanotube-alumina-coated silica (Pt-MWCNT-ACS) nanocomposite modified glassy carbon electrode by adsorption to provide a novel amperometric glucose biosensor. The morphology, nature, and performance of the resulting GOx-Pt-MWCNT-ACS nanobiocomposite modified glassy carbon electrode were characterized by field emission scanning electron microscopy, energy dispersive X-ray spectroscopy, cyclic voltammetry, and amperometry. The influence of various experimental conditions was examined for the determination of the optimum analytical performance. The optimized glucose biosensor displayed a wide linear range of up to 10.5 mM, a high sensitivity of 113.13 mA M^{-1} cm^{-2}, and a response time of less than 5 s. The sensitivity for the determination of glucose at the GOx-Pt-MWCNT-ACS nanobiocomposite modified glassy carbon electrode is better than at common GOx-Pt-CNT nanobiocomposite modified electrodes. The proposed biosensor has good anti-interferent ability and long-term storage stability after coating with Nafion, and it can be used for the determination of glucose in synthetic serum.

1. Introduction

An electrochemical biosensor can be considered as the combination of an electrochemical transducer and a receptor, which normally measures the concentration of an analyte. The enzymatic reaction transforms the substrate into a reaction product that is detectable by the electrochemical transducer. Much effort has been made in including nanomaterials in electrochemical transducer in an attempt to construct a sensitive biosensor [1–3]. The incorporation of nanosized materials into a sensing device has been extensively investigated as a means of facilitating electrocatalysis [4,5]. Noble metal nanoparticles are of great interest because of their high catalytic activities for many chemical reactions and large surface-to-volume. In particular, the use of Pt nanoparticles is proven to be very useful for the improvement of electrochemical oxidation of hydrogen peroxide [6–9]. The favorable electrocatalytic detection of hydrogen peroxide by Pt nanoparticles has promoted their applications for developing oxidase-based biosensors with improved analytical performance. In recent studies, carbon nanotubes (CNTs) have been considered as a promising material for electrocatalyst support. The synthesis of Pt nanoparticles onto the surface of CNTs for a wide range of sensing applications has been reported in the literature [10].

CNTs are a new form of carbon materials discovered by Iijima in 1991 [11]. They have generated considerable interest in bioelectrochemistry because of their unique electrical conductivity, chemical stability, and mechanical strength associated with high aspect ratio [12–14]. The apparent electrocatalytic effects of CNTs can be attributed to the edge sites in CNTs [15,16]. The electrodes modified with CNTs have recently received much interest for the purpose of designing electrochemical sensors. CNT-based electrodes are prepared by mechanical abrasion onto graphite surface [17,18], as paste [19], and in the form of composite [20]. The major problem for the preparation of CNTs-based electrode is the insolubility of CNTs in all solvents due to the large intertube attraction energy. Methods for the dispersion of CNTs can be roughly classified as chemical and physical. Physical modification of CNTs includes a solubilization procedure using different solubilizers such as surfactants, polymers, and π-aromatic compounds. The dispersion of multiwalled carbon nanotubes (MWCNTs) in aqueous solutions by a noncovalent and inorganic method through alumina-coated silica (ACS) nanoparticle halos has been reported in our previous study [21]. A method for attaching MWCNT-ACS nanocomposite onto the surface of glassy carbon electrodes by a self-assembly process has been reported in the literature [22]. It has been demonstrated that the electrochemical properties of CNTs are not impaired by their association with ACS nanoparticles.

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For electrochemical biosensors, how to immobilize the biological recognition elements onto the surface of the electrochemical transducer is an important process. The methods for enzyme immobilization to the transducer include adsorption [23], encapsulation [24], entrapment [25], cross-linking [26], and covalent bonding [27]. Among these various enzyme immobilization protocols, adsorption is the simplest and involves minimal preparation. The bioactivities of the immobilized enzyme can be retained well because the adsorption needs no chemical reagents and seldom activation. Many substances adsorb enzymes on their surface, for example, alumina [28], silica [29], platinum nanoparticles [30], carbon nanotubes [31], and glass [32]. This work reports a glucose biosensor based on the adsorption of glucose oxidase (GOx) onto the surface of the Pt-MWCNT-ACS nanocomposite. The Pt nanoparticles were electrodeposited in the MWCNT-ACS nanocomposite in order to improve the electrocatalytic behavior for hydrogen peroxide which is released by the action of GOx upon glucose. The performance of the prepared glucose biosensor with respect to sensitivity, detection limit, linear range, and response time is presented and discussed. In the interference and storage ability studies, Nafion was coated on the top of the GOx-Pt-MWCNT-ACS nanobiocomposite modified glassy carbon electrode in the hope that the anti-interferent ability can be improved and the loss of the GOx can be prevented.

2. Experimental

2.1. Reagents

The reagents used were of analytical grade or the highest commercially available purity and were used as received without further purification. D-(+)-Glucose and GOx (Type X-S, *Aspergillus niger* (EC 1.1.3.4), 179 units mg^{-1}) were supplied from Sigma. K_2PtCl_6 were purchased from Acros. MWCNTs (TECO Nanotech Co., Ltd., Taiwan) used in this study was synthesized by an electric arc discharge method and of ∼99% purity. The MWCNTs are cylindrical with an inner diameter in the range 2–5 nm, an outer diameter in the range 20–40 nm, and a length of up to several micrometers. Positively charged ACS spheres (Ludox CL) were the product from DuPont. The pH value of the ACS solution was adjusted with 0.1 M HCl. All solutions were prepared with deionized water of resistivity of not less than 18 MΩ cm which was taken from a Milli-Q water purification system (Milli-Q, USA).

2.2. Apparatus

All electrochemical experiments in this work were performed using a computer-controlled Autolab PGSTAT30 Electrochemical Analyzer (Eco Chemie, Netherlands). Experiments were carried out using a conventional three-electrode system with a 3 mm diameter glassy carbon electrode as the working electrode, an Ag/AgCl (3 M KCl) as the reference electrode, and a Pt wire (all Metrohm., Switzerland) as the counter electrode. Field emission scanning electron microscopy (FESEM) images were obtained on a JSM-6700F (JEOL, Japan).

2.3. Preparation of GOx-Pt-MWCNT-ACS nanobiocomposite modified glassy carbon electrodes

The bare glassy carbon electrode was prepared by polishing with 0.3 and 0.05 μm alumina slurries and rinsing with deionized water several times. The maximum load of MWCNTs in a 1 wt% ACS solution has been reported in our previous study [21]. A 40 mg of MWCNTs were added to a 2 ml 1 wt% ACS solution (pH 2) and was ultrasonicated for about 2 h to form a homogeneous MWCNT-ACS solution. The self-assembly of MWCNT-ACS nanocomposite onto the surface of glassy carbon electrode was conducted by immersing a glassy carbon electrode in the MWCNT-ACS aqueous solution (pH 2) for 1 h. Then, the solvent was allowed to evaporate at room temperature in the air. The thickness of the prepared MWCNT-ACS nanocomposite was ca. 5 μm which was determined by SEM (not shown). Pt nanoparticles were electrodeposited in the MWCNT-ACS nanocomposite by a potentiostatic method at a potential of −0.22 V (vs. Ag/AgCl) in 0.5 M H_2SO_4 aqueous solution containing 1.3 mM K_2PtCl_6. The prepared Pt-MWCNT-ACS nanocomposite modified electrode was rinsed with deionized water several times. To prepare the GOx-Pt-MWCNT-ACS nanobiocomposite modified glassy carbon electrode, the Pt-MWCNT-ACS modified glassy carbon electrode was immersed in a 0.1 M phosphate buffer solution (pH 6) containing 10 mg mL^{-1} GOx for different times. The prepared GOx-Pt-MWCNT-ACS nanobiocomposite modified glassy carbon electrode was washed thoroughly with deionized water and transferred to 0.1 M phosphate buffer solution (pH 7) before use.

2.4. Amperometric measurements

In a typical measurement, 20 mL of sample was transferred to the cell. Amperometric detection was proceeded under batch conditions with stirring of 250 rpm. Constant-potential amperometry required the preconditioning (∼300 s) and operation of the electrode at a constant applied potential. When the current reached a baseline in the absence of substrate, every 2 min, substrate was added.

2.5. Determination of glucose in synthetic serum

The preparation of synthetic serum has been reported in the literature [33]. Synthetic serum was diluted with 0.1 M phosphate buffer solution (pH 7) in appropriate concentration. The amperometric determinations of glucose in synthetic serum were performed using the internal standard method.

3. Results and discussion

3.1. The morphology of GOx-Pt-MWCNT-ACS nanobiocomposite

The morphologies of the electrodeposition of Pt nanoparticles within MWCNT-ACS nanocomposite and the adsorption of GOx within Pt-MWCNT-ACS nanocomposite were studied by FESEM. Energy dispersive X-ray spectroscopy (EDS) was used to verify the Pt composition of the deposits after electrodeposition of Pt. The FESEM images of MWCNT-ACS nanocomposite before and after electroreduction of Pt nanoparticles are shown in Fig. 1a and b, respectively. The individual MWCNT is well dispersed within MWCNT-ACS composite with an outer diameter of 20–40 nm can be seen in Fig. 1a. Fig. 1b shows that Pt nanoparticles with a diameter of 10–15 nm are formed individually on the MWCNTs and some nanoparticles aggregated into large Pt particles. This configuration of Pt-MWCNT-ACS nanocomposite with a high dispersion of Pt nanoparticles may result in a good electrocatalytic behavior for the electrochemical oxidation of hydrogen peroxide. The deposition of Pt nanoparticles in MWCNT-ACS nanocomposite is supported by EDS observation. EDS patterns of the MWCNT-ACS nanocomposite before and after electrodeposition of Pt nanoparticles are shown in Fig. 2a and b, respectively. The Pt element can be seen after the electrodeposition of Pt. EDS result indicates that the nanoparticles electrodeposited within the MWCNT-ACS nanocomposite are platinum. The FESEM image of the adsorption of GOx in the Pt-MWCNT-ACS nanocomposite is shown in Fig. 1c. It can be seen that the surface of Pt-MWCNT-ACS nanocomposite was covered with GOx. The GOx-Pt-MWCNT-ACS nanobiocomposite was formed in a densely packed