

Short communication

Glucose biosensor based on multi-wall carbon nanotubes and screen printed carbon electrodes

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Abstract

This paper describes a disposable electrochemical biosensor for glucose monitoring. The sensor was based on multi-wall carbon nanotubes (MWCNTs) immobilized with glucose oxidase and upon screen printed carbon electrode. The effect of MWCNTs on the response of amperometric glucose oxidase electrode for glucose was examined. Results obtained, of interest for basic and applied biochemistry, represent a first step in construction of a MWCNT–enzyme electrode biosensor with potentialities for a successful application in the biosensor area.

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1. Introduction

Since the discovery of carbon nanotube (CNT) in 1991 (Lijima, 1991), it has been the target of numerous investigations due to their unique properties (Lijima, 1991; Zhao et al., 2002; Cohen, 2001; Baughman et al., 2002). The nanotube has helical arrangement of carbon hexagons on the tube surface along its axis (Jason et al., 1998). Carbon nanotube is a kind of inorganic material with a nano-structure, which was promising as an immobilization material due to its significant mechanical strength, high surface area, excellent electrical conductivity and good chemical stability (Yakobson and Smalley, 1997). CNTs have been used as modified electrodes to catalyze the electrochemical reaction of some biomolecules, such as dopamine, NADH, cytochrome c, etc. (Britto et al., 1996; Musameh et al., 2002; Wang et al., 2002a, 2002b; Zhao et al., 2003).

Jason et al. (1998) have shown that the catalytic activities of the immobilized β -lactamase I on or in carbon nanotubes had no drastic conformational change. The carbon nanotubes appeared to act as a benign host in its ability to encapsulate protein molecules within an environment, which offered some protection.

Jason et al. (1997) also reported the application of carbon nanotubes as an electrode material. Redox proteins such as cytochrome c and azurin were immobilized on and within carbon nanotubes and gave reproducible, well-behaved voltammetric responses.

The performance of carbon nanotubes paste electrode (CNTs-PE) prepared by dispersion of multi-wall carbon nanotubes (MWCNTs) within mineral oil was described (Maria and Gustavo, 2003). The resulting electrode showed excellent electrocatalytic activity toward ascorbic acid, uric acid and dopamine.

In this work we report the application of MWCNTs used in a glucose biosensor. On the carbon paste electrode, it is observed that catalyze ability of glucose oxidase was improved and the relative activity of glucose oxidase increased.

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This change made the novel biosensor more sensitive than the typical glucose biosensor.

2. Materials and methods

2.1. Reagents

Glucose oxidase (GOD, EC 1.1.3.4, lyophilized powder, 25 U/mg, from *Aspergillus niger*) was obtained from SIGMA. Glucose, potassium ferricyanide and potassium ferrocyanide were purchased from Aldrich. All other reagents were of the best quality available commercially.

A suspension of multi-wall carbon nanotubes (5 mg/ml) attached with carboxyl groups was a gift from Professor Xiao-Bin Zhang (Department of Materials Science and Engineering of Zhejiang University). The growth of carbon nanotubes was carried out in a horizontal tube furnace. Typically, 0.2 g of the so-prepared catalyst was uniformly sprayed on a quartz boat. At a furnace temperature of 1000 °C, the boat was placed in the central position of the furnace. Then a mixed gas of CH₄ and H₂ with total flow rate of 950 cm³/min (CH₄/H₂ = 900/50, v/v) was introduced into the furnace. The growth process lasted for 30 min. After this, the mixed gas supply was turned off and the quartz boat was cooled down to room temperature by the passage of nitrogen gas (400 cm³/min) (Li et al., 2004). The MWCNTs had an average length of 15 μm and a mean diameter of 15 nm.

Conductive carbon paste (JELCON CH-10) was purchased from JUJO. Conductive silver ink (Electrodag 479SS) and Ag/AgCl ink (Electrodag 7019) were purchased from Acheson.

2.2. Procedures

2.2.1. Preparation of screen printed three-electrode cell

The amperometric sensors used in the present investigation consisted of three screen printed electrodes deposited onto polyvinyl chloride (PVC). The silver ink was printed on PVC film and was cured 15 min at 93 °C. Then the prepared carbon paste was printed upon the printed silver layer to fabricate the working electrode and the counter electrode with some part of the silver ink layer uncovered. At last the Ag/AgCl paste was printed on PVC film as reference electrode. The final electrochemical system was dried for 5 min at 90 °C. The reaction area of working electrode was 4 mm². Fig. 1 shows the structure of three-electrode cell.

2.2.2. Construction of the MWCNTs modified CPE

The MWCNTs suspension was mixed with citrate buffer (pH 5.1, 0.1 mM), the mix ratios (MWCNTs suspension/citrate buffer, v/v) were 0, 10, 50 and 100%, respectively. A drop (2 μl) of above mixture was applied, respectively, onto the reaction region of carbon paste electrode, and then the electrodes were dried at room temperature.

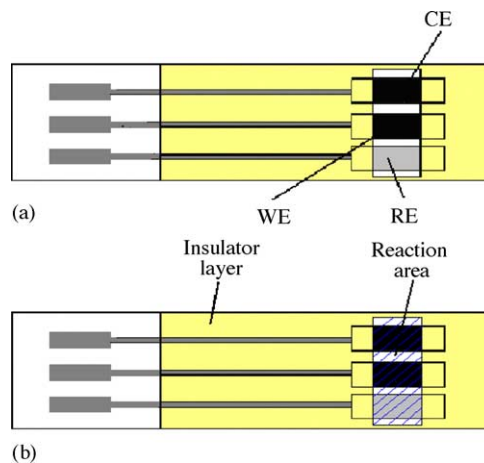


Fig. 1. Structure of the screen-printed planar electrochemical sensor: (a) WE (black): working electrode; CE (black): counter electrode; RE (gray): reference electrode. (b) Yellow area acting as insulator layer; diagonal line area acting as reaction area.

2.2.3. Construction of the enzyme electrode biosensor

2.2.3.1. Construction of the typical glucose biosensor. A GOD solution was prepared by dissolving 300 IU GOD, 60 mg potassium ferricyanide in 1 ml citrate buffer (pH 5.1, 0.1 mM). The enzyme solution was stirred slowly for 5 min and subsequently stored at room temperature for 30 min.

A drop of above enzyme solution (2 μl) was applied onto the reaction region of carbon paste electrode; then the electrodes were dried at room temperature. The typical amperometric glucose enzyme-biosensor was thus fabricated, for use as control electrode.

2.2.3.2. Construction of the novel MWCNTs glucose biosensor.

A drop (100 μl) of MWCNTs suspension was added to 1000 μl of the enzyme solution (Section 2.2.3.1), the mixing ratio (v/v) was 10%. After stirring slowly for 5 min and subsequently storing at room temperature for 30 min. The mixture (2 μl) was applied onto the reaction region of carbon paste electrode, then the electrodes were left to dry at room temperature.

2.2.4. Electrochemical measurements

2.2.4.1. Amperometric measurements. A potential of 300 mV versus Ag/AgCl reference electrode was applied to the working electrode of the two biosensors. Aliquots of 5 μl of glucose solution with differing concentrations were then applied onto the reaction region of two biosensors, respectively. The current responses of the typical biosensor and the MWCNTs biosensor were recorded.

2.2.4.2. Cyclic voltammetry measurements. A drop (5 μl of total volume) of 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] solution (1:1) was applied onto the reaction region of working electrode modified with different ratios of MWCNTs (2.2.2). Cyclic voltammetric experiments were performed at a scan rate of 100 mV/s.

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