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Short communication

Study of carbon nanotube modified biosensor for monitoring total cholesterol in blood

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Abstract

A carbon nanotube modified biosensor for monitoring total cholesterol in blood was studied. This sensor consists of a carbon working electrode and a reference electrode screen-printed on a polycarbonate substrate. Cholesterol esterase, cholesterol oxidase, peroxidase and potassium ferrocyanide were immobilized on the screen-printed carbon electrodes. Multi-walled carbon nanotubes (MWCN) were added to prompt electron transfer. Experimental results show that the carbon nanotube modified biosensor offers a reliable calibration profile and stable electrochemical properties.

Keywords: Total cholesterol; Biosensor; Carbon nanotube

1. Introduction

Normal human blood serum contains less than 200 mg/dl cholesterol, of which two third is esterified with fatty acids and one third is present as sterol (White et al., 1978). There is a strong positive correlation between high serum cholesterol level and arteriosclerosis, hypertension and myocardial infarction. So, the determination of cholesterol is important in clinical diagnosis.

As awareness of the importance of total cholesterol levels has increased, numerous methods for human blood cholesterol assays have been developed (Crumbliss et al., 1993; Charpentier and Murr, 1995; Martin et al., 2003; Tatsuma and Watanabe, 1991; Situmorang et al., 1999; Shumyantseva et al., 2004), including colorimetric spectrometric and electrochemical methods. Mainly enzymatic procedures are employed in clinical diagnosis due to their rapid, selective, sensitive nature and the great accuracy.

Former amperometric methods work at relatively high potentials so that many other species may be oxidized. To minimize the effect of interferences, techniques have been developed using the redox mediator ferricyanide to reduce hydrogen peroxide, making the product ferrocyanide detectable at relatively low potentials. However, this measuring system was affected by air oxidation of ferrocyanide that takes place as a competitive reaction during the enzymatic oxidation.

Carbon nanotubes are a novel type of carbon material and can be considered as the result of folding graphite layers into carbon cylinders. There are two groups of carbon nanotubes, multi-walled carbon nanotubes and single-walled carbon nanotubes (Zhao et al., 2002). Carbon nanotubes have been recognized as one of the most promising electrode materials since the first electrode application in the oxidation of dopamine in 1996 (Britto et al., 1996). Since then, the research has been focused on their electrocatalytic behaviors toward the oxidation of biomolecules and their performance has been found to be much superior to those of other carbon electrodes in terms of reaction rate, reversibility and detection limit. Rubianes (Rubianes and Rivas, 2003) have reported carbon nanotubes paste electrode could decrease the

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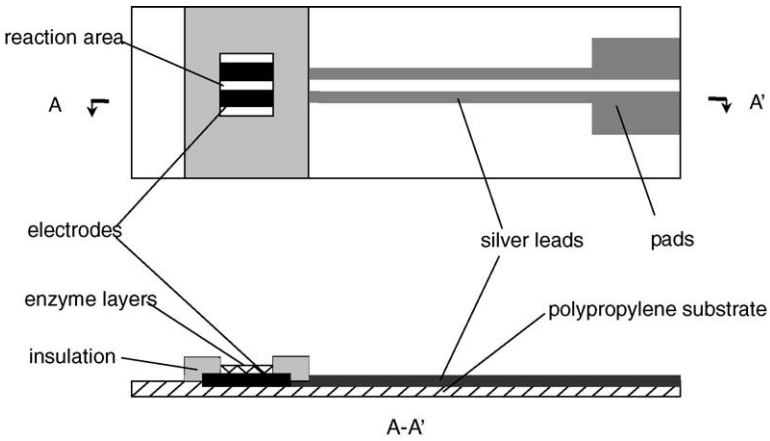

Fig. 1. The structure of the cholesterol biosensor.

overvoltage for the redox of ascorbic, uric acid and hydrogen peroxide (H_2O_2), while H_2O_2 is an important chemical material in biochemical assay, many substrate will convert to H_2O_2 to be determined on electrode surface. Davis et al. (Davis et al., 1997, 1998) have described the high surface area possessing abundant acidic sites that may offer special opportunities for the immobilization of enzymes in biosensors. Guiseppi-Elie (Guiseppi-Elie et al., 2002) has researched the direct electron transfer of glucose oxidase on carbon nanotubes. The similarity in length scales between nanotubes and redox enzymes suggests interactions that may be favorable for biosensor electrode applications. However, the detection mechanism of carbon nanotubes is not fully understood. A systematic study has not been reported so far. In this paper, we used carbon nanotubes to modify carbon paste electrode, which can promote electron transfer and enhance the response current.

2. Experiments

2.1. Reagents

All reagents, except multi-walled carbon nanotubes, were commercially available and of analytical-reagent grade chemicals. Cholesterol oxidase (COD), 20 U/mg, TOYOBO), cholesterol esterase (CEH, 100 U/mg, TOYOBO), peroxidase (POD), potassium ferrocyanide, trehalose, TritonX-100 were purchased from the respective companies. Carboxyl modified multi-walled carbon nanotubes were provided by the Department of Material Science, Zhejiang University.

2.2. Electrode preparation

The electrodes were fabricated using screen-printing technique, which is a common method for electrode fabrication (Nagata et al., 1995). Three masks were used to form sliver conducting lead wires, carbon film for electrochemical reaction and insulating film. The structure of the cholesterol sensor is shown in Fig. 1. The sensor consists of two carbon paste electrodes. One electrode was used as working electrode while the other was used as reference electrode. The function of the silver leads was to improve electric conductivity of the electrodes. The reaction area, where the carbon paste film was modified by carbon nanotubes and immobilized enzymes, was defined by the insulating film coated on the carbon paste film. There were 20 sensor bases on each plate. Once the sensor array was ready, sensor strips were cut out of the array to be measured. The size of the reaction area was 2 mm × 2 mm while the sensor strip was 35 mm × 10 mm

2.3. Electrode modification and enzyme immobilization

After dropping 2 μl the carboxyl modified multi-walled carbon nanotubes solution (5 mg/ml) onto the reaction area of the electrode, 1 μl CMC solution (20 mg/ml) was added. It was dried in hot air at 50 °C for 10 min to form a hydrophilic polymer layer. Subsequently, 70 unit cholesterol oxidase, 1.8 mg peroxidase, 8 mg potassium ferrocyanide and 6 mg trehalose were dissolved in 0.125 ml phosphate buffer of pH 7.0. Then 2 μl solution was added on the hydrophilic polymer layer to form a cholesterol oxidase layer. Finally, 200 unit cholesterol esterase, 3 mg trehalose, 1 μl TritonX-100 were dissolved in 0.125 ml phosphate buffer (pH7.0). And 2 μl of the solution was added to form a cholesterol esterase layer.

2.4. Measurement

All measurements were performed at 25 °C room temperature. Amperometric measurements were performed by a CHI660 Electrochemical Work Station from CH Instruments Inc., USA. The working potential is 300 mV. All reaction processes were recorded using an IBM PC compatible computer via a RS232 series port communicating to the electrochemical analytic station. The current values were read 180 s after the sample solution was dropped onto the reaction area.