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A New Application of Carbon Nanotubes Constructing Biosensor

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Abstract: Carbon nanotubes used for constructing biosensor was described for the first time. Single-wall carbon nanotubes (SWNTs) functionalized with carboxylic acid groups were used to immobilize glucose oxidase forming a glucose biosensor. The biosensor response can be determined by amperometric method at a low applied potential (0.40 V).

Keywords: Carbon nanotubes, biosensor, glucose oxidase, immobilized enzyme.

Due to novel structural, electronic, optical and mechanical properties, carbon nanotubes have attracted considerable attention[1-3]. Practical applications were found in many fields[4-5]. Immobilization of biomolecules on carbon nanotubes has been reported[6-7]. However, carbon nanotubes used for preparing biosensor has not been developed. In this paper, we described a new glucose biosensor based on covalent immobilization of glucose oxidase on SWNTs film functionalized with carboxylic acid groups.

The SWNTs were prepared by arc-discharge method[8]. The crude SWNTs were stirred in 6 mol/L HCl and washed to remove metal catalysts in the sample. After being dried under vacuum, 5 mg of the SWNTs sample was added into a 10 mL mixed acid (98%H_2SO_4 + 70%HNO_3, volumetric ratio 3:1) and ultrasonicated for 8 h at 50°C. The reaction mixture was centrifuged and the solid was washed repeatedly with water until pH value of the SWNTs suspension reached about 4. The SWNTs film on the electrode was prepared by dropping a 10 μL of the SWNTs suspension on a Pt electrode (3 mm×3 mm) and then dried under vacuum. The Pt/SWNTs electrode was activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in phosphate buffer solution (PBS) with pH 5.5 for 1 h, and then immersed in the PBS contaning 5 mg/mL glucose oxidase (GOD) at 4°C for 12 h. The resulting enzyme electrode was washed thoroughly with PBS, and stored at –10°C. The biosensor response was determined by amperometric method as described elsewhere[9].

The average length of the SWNTs treated with the mixed acid is about 100 nm. The FTIR spectrum (**Figure 1**) showed a peak at 1726 cm^{-1}, which is in accordance with the literature[2], indicating that the shortening process terminated the open ends of the SWNTs with carboxylic acid groups. The existence of –COOH groups provided precondition of covalent immobilization of enzyme. In addition, The Pt/SWNTs electrode showed stable electrochemical behavior and small background current (20 nA) at 0.4 V in PBS with pH 6.5.

Figure 1 FTIR spectrum of SWNTs-COOH

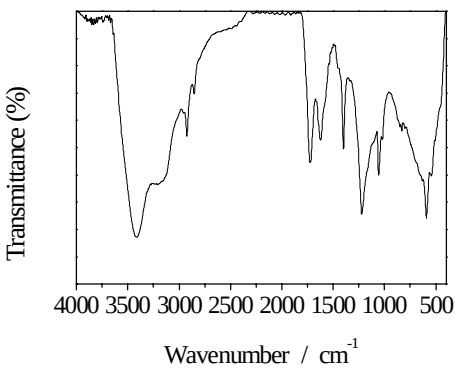
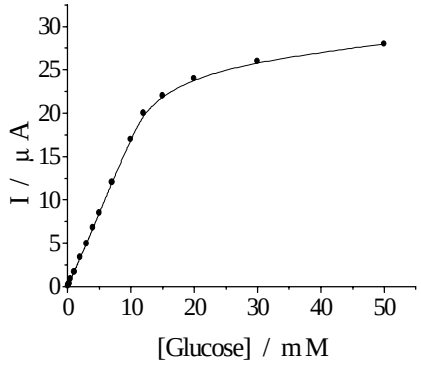


Figure 2 Calibration curves of the glucose biosensor in 0.1M PBS (pH6.5) at 0.40 V, 25°C



The Pt/SWNTs/GOD electrode has fast amperometric response (< 1 min) to glucose at applied potential 0.40 V. The response is influenced by the time of acid oxidation of SWNTs (optimum time is 8 h). **Figure 2** shows the biosensor response as a function of glucose concentration. The curve is linear with glucose concentration up to 12 mmol/L and reaches a plateau gradually at higher concentrations. The biosensor sensitivity (determined from the slope of the linear part) is 18.7 $mAM^{-1}cm^{-2}$. The apparent Michaelis-Menten constant of immobilized GOD was calculated to be 13.1 mmol/L according to the Lineweaver-Burk equation[10]. The value is close to the reported value for the free enzyme[11], illustrating the non-denaturating character of the enzyme anchoring procedure. In addition, the biosensor exhibited good operational and long-term stability (lifetime > 4 months), which is higher than the glucose sensor based on graphitic electrodes[12]. Further studies on the other characteristics of the biosensor and on the application of SWNTs to various biosensors are under progress.

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