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Functional Contrast and Kinetic Analysis of water-Dispersible Carbon Black Nanoparticles and MWNTs in Glucose Biosensors

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***Abstract*—Poly (sodium 4-styrenesulfonate) (PSS) was grafted onto carbon black surface by ambient surface-initiated atom radical transfer polymerization. And the carbon paste electrodes modified by CB-g-PSS and MWNTs were completed. CB-g-PSS and MWNTs had no effect on direct electron transfer based on the carbon paste electrode，but increased the redox speed of Fe $[(CN)^6]^{3-}$ / Fe$[(CN)^6]^{4-}$and led to stronger current response. The experimental results indicate that the sensitivity of modified by CB-g-PSS is 2.1 times of no modified, and MWNTs is 4 times. Measurable linear range is 1.1mmol/L~33.3mmol/L. For sensors modified by CB-g-PSS, testing of different concentration glucose: RSD<7%; sensors modified by MWNTs, RSD<7.5% (glucose < 15mmol/L), RSD<10% (glucose > 15mmol/L) respectively.**

I. Introduction

With the development of biochemical technology, studies on enzymatic biosensors have attracted many researchers' attention more and more in past years. These studies always focus on how to immobilize enzyme effectively, improve kinetic process of enzymatic reaction and increase the sensitivity. For solving these problems, many new materials and methods have been attempted to modify the surface of electrode continuously. Recently carbon black has been attached importance mostly. Using its nano-adsorption character, carbon black has been applied in gas sensors and humidity sensors, and has a good foreground[1-5]. But the reports of applying carbon black in enzymatic biosensors are infrequent.

In this study, glucose biosensors based on screen printed carbon electrodes were prepared by immobilized glucose oxidase, and modified with carbon black nanoparticles and MWNTs respectively. The experimental results are effective. They show that both carbon black particles and MWNTs can improve kinetic process of enzymatic reaction, shorten testing time and improve sensitivity.

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II. EXPERIMENT

A. Materials and instruments

GOx (>100 U/mg) were obtained from Sigma, USA. Primary carbon black particles were obtained from Cabot, USA. Poly (sodium 4-styrenesulfonate) (PSS) was made by College of Materials Science and Chemical Engineering, Zhejiang University, China. MWNTs were obtained from Shenzhen Nanotech Port, China. Stabilizer, $K_3Fe(CN)_6$, CMC and all other reagents are analytical reagent, and were not purified before using. Different concentration glucose were placed overnight at room temperature. Citric acid buffer is 0.2mol/L, PH=6.5. Deionized water was used in all experiments.

Testing instruments: CHI660A electrochemical work station was purchased from Chenghua, Shanghai. Medical ultrasonic cleanser was purchased from Kunshan Ultrasonic Instrument Corporation.

B. Synthesis of CB-g-PSS

Poly (sodium 4-styrenesulfonate) (PSS) was grafted onto carbon black surface by ambient surface-initiated atom radical transfer polymerization. Fig. 1 shows synthesis route of water-dispersible carbon black by aqueous SI-ATRP.

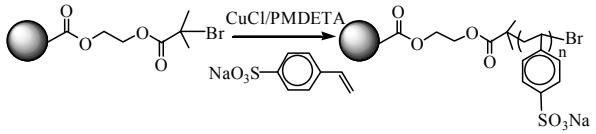


Fig. 1. Synthesis route of water-dispersible carbon black by aqueous SI-ATRP

Typically, CB-Br (50mg), CuCl (20 mg, 0.2mmol), PMDETA (0.04ml, 0.2mmol), NaSS (0.5004g, 2.43mmol), 1.5ml deionized water and 0.5ml methanol was placed in a 25mL dried two-necked flask. After evacuated and back-filled with argon three times, the flask was immersed in 30℃ for 12h. The polymerization was quenched by exposure to air, and the reaction solution immediately changed from deep brown to blue, which indicated that Cu (I) had been oxidized to Cu (II). At the end of reaction, the product was separated centrifugally and was washed with deionized water. Such centrifugation-redispersion cycles were repeated. The

product was dried in vacuum at 50℃ [4]. Appropriate CB-g-PSS powder was gotten, and made in 5mg/mL solution for using.

Transmission electron microscopy (TEM) is utilized to characterize morphologies of CB-g-PSS, as shown in Fig. 2. CB-g-PSS forms nano-sized agglomerates in water, the size is about 200~300nm.

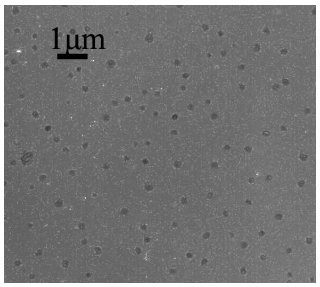


Fig. 2. TEM image of CB-g-PSS

C. Pretreatment of MWNTs

Because CNTs can not be dissolved in water, they need to be treated before using. MWNTs were added in mixture solution with oil of vitriol and aqua fortis (3:1). After supersonic for 24 hours, the solution was washed with deionized water and separated centrifugally until the pH was 7.0, as shown in Fig. 3. After drying, appropriate carboxylation MWNTs powder was gotten[6], and made in 5mg/mL solution for using.

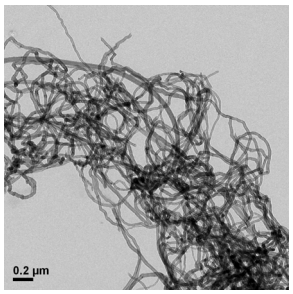


Fig. 3. TEM images of MWNTs

D. Structure of Electrodes

Fig. 4 shows the structure of screen-printed carbon paste electrodes and the microreactors on them. Two carbon paste electrodes are work electrode and reference electrode respectively. Through wires, they can be conducted with electrochemical work station. Reaction space is limited by chamber volume of microreactors. So sample capacity, enzyme quantity and reaction area can be controlled accurately.

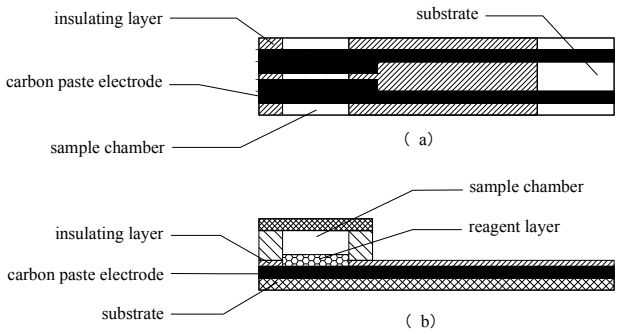


Fig. 4. (a) top view of electrodes; (b) section view of electrodes and microreactors

E. Preparation of biosensors

1) Preparation of unmodified biosensors

CMC, nano-SiO_2, $K_3Fe(CN)_6$ and stabilizer were added in citric acid buffer, mixed equably and printed on carbon paste electrodes. Then modified with appropriate enzyme, aired naturally, made chamber, waited for testing.

2) Preparation of biosensors modified with CB-g-PSS

CMC, nano-SiO_2, $K_3Fe(CN)_6$ and stabilizer were added in citric acid buffer, mixed equably and printed on carbon paste electrodes. Then dropped with 2uL, 5mg/mL CB-g-PSS, aired naturally, modified with appropriate enzyme, made chamber, waited for testing.

3) Preparation of biosensors modified with MWNTs

CMC, nano-SiO_2, $K_3Fe(CN)_6$ and stabilizer were added in citric acid buffer, mixed equably and printed on carbon paste electrodes. Then dropped with 2uL, 5mg/mL carboxylation MWNTs, aired naturally, modified with appropriate enzyme, made chamber, waited for testing.

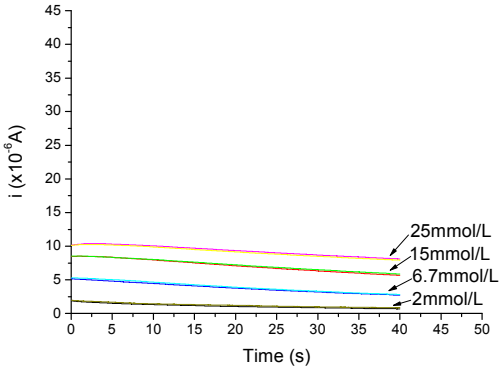
F. Method of experiment

Carbon paste electrode was connected to electrochemical work station. As capillarity, 1uL testing solution was soaked in the chamber. Work voltage was 0.3V. I-t curve was recorded for 40 seconds after power on.

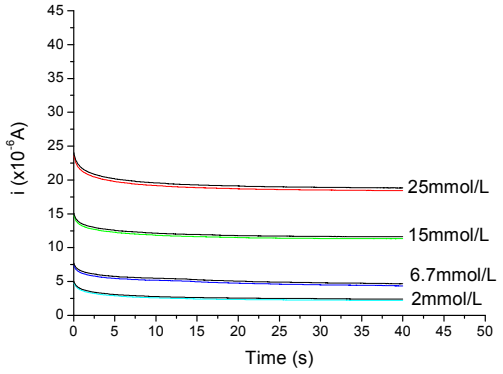
III. Results and Discussion

A. I-t curve of biosensors

2mmol/L, 6.7mmol/L, 15mmol/L and 25mmol/L glucose solutions were used to test for biosensors respectively. I-t curves are shown in Fig. 5.



（a）



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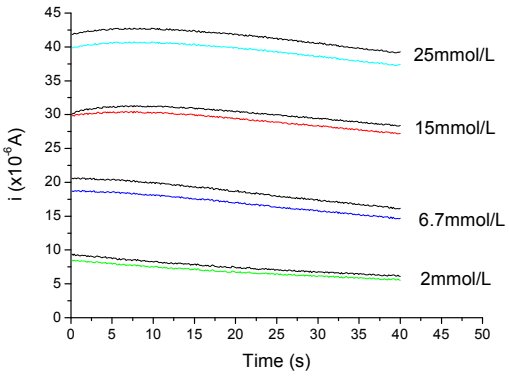

（c）

Fig. 5. (a) I-t curves of unmodified biosensors
(b) I-t curves of biosensors modified with CB-g-PSS
(c) I-t curves of biosensors modified with MWNTs

From Fig. 5, it can be seen that all biosensors have a short response stabilization time and go to balance quickly as soon as power on. Compared with I-t curves of biosensors modified with CB-g-PSS and MWNTs, I-t curves of unmodified biosensor are flat relatively. And I-t curves of low concentration glucose are flatter than high concentration. According to Cottrell equation, the order of the electric potential and responding electric current for diffusing controlling under ideal condition come down increasingly with time t-1/2 exponent[7]. So, all biosensors have good capacity of electron transport on the electrodes. The sensitivity modified with both CB-g-PSS and MWNTs are better than unmodified.

B. Correction curve of glucose Biosensor

1.1mmoL/L~33.3mmoL/L control solution samples were used to test for biosensors. Correction curves are shown in Fig. 6. It can be seen that the sensitivity of biosensors modified with CB-g-PSS is 2.1 times of unmodified, and the sensitivity of biosensors modified with MWNTs is 4 times of unmodified.

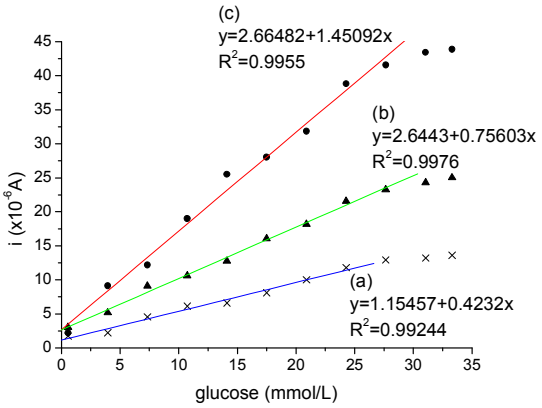

Fig. 6. (a) Correction curve of unmodified biosensors
(b) Correction curve of biosensors modified with CB-g-PSS
(c) Correction curve of biosensors modified with MWNTs

C. Repeatability and life of biosensors

Different concentration glucose were used to test the repeatability of biosensors modified with CB-g-PSS and MWNTs. Every control solution sample with glucose concentration between 1.1mmol/L~33.3mmol/L was tested by 20 sensors modified with CB-g-PSS. Experiments indicate RSD < 7%. Thus, these glucose biosensors modified with CB-g-PSS have good repeatability, and the measurable linear range is 1.1mmol/L~33.3mmol/L. At the same time, every control solution sample with glucose concentration between 1.1mmol/L~33.3mmol/L was tested by 20 sensors modified with MWNTs under same condition. When the concentration of control solution sample < 15mmol/L, RSD < 7.5%. When the concentration of control solution sample > 15mmol/L, RSD < 10%. Thus, these glucose biosensors modified with MWNTs have worse repeatability than modified with CB-g-PSS.

These biosensors were tested for 3 months. From the first day to the seventh day, the testing value was little higher than the standard value. From the eighth day to the sixtieth day, the testing value had no obvious change. After 60 days, the testing value changed gradually. The shelf life of these biosensors is about 2 months.

IV. CONCLUSIONS

In this study, Poly (sodium 4-styrenesulfonate) (PSS) was grafted onto carbon black surface by ambient

surface-initiated atom radical transfer polymerization. And the carbon paste electrodes modified by CB-g-PSS and MWNTs were completed. CB-g-PSS and MWNTs had no effect on direct electron transfer based on the carbon paste electrode，but increased the redox speed of Fe $[(CN)^6]^{3-}$ / Fe$[(CN)^6]^{4-}$and led to stronger current response. The experimental results indicate that the sensitivity of modified by CB-g-PSS is 2.1 times of no modified, and MWNTs is 4 times. Measurable linear range is 1.1mmol/L~33.3mmol/L. For sensors modified by CB-g-PSS, testing of different concentration glucose: RSD<7%; sensors modified by MWNTs, RSD < 7.5% (glucose < 15mmol/L), RSD < 10% (glucose > 15mmol/L) respectively. The study shows that correct modified method can make conductivity and nanometer property of nano-material better, and benefit to immobilize enzyme, improve kinetic process of enzymatic reaction and increase the sensitivity of biosensors.

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