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A Novel Cholesterol Oxidase Biosensor Based on Pt-nanoparticle /Carbon Nanotube Modified Electrode

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Abstract: A Pt-nanoparticle/carbon nanotube modified graphite electrode immobilized with cholesterol oxidase/sol-gel layer was developed for monitoring cholesterol. Using this electrode, cholesterol concentration (4.0×10^{-6} to 1.0×10^{-4} mol/L) could be determined accurately in the presence of ascorbic or uric acid, and the response time was rapid (< 20 s). This biosensor has high sensitivity and selectivity.

Keywords: Pt- nanoparticle, carbon nanotube, cholesterol oxidase, biosensor.

A variety of electrochemical biosensors for cholesterol detection have been proposed because of its importance in clinic and food analysis [1-2]. Classical devices for the detection were based on monitoring either the consumption of oxygen or the production of H_2O_2. The amperometric determination of H_2O_2 oxidation is sensitive and stable, but it requires a high anodic potential (over 0.6 V *vs* Ag/AgCl) and is affected by co-oxidable substances such as ascorbic acid and uric acid usually presented in biosamples[3-5]. So, a great deal of attention has been paid to avoid this interference in recent years [6-9]. However, there are few works about detecting H_2O_2 reduction in cholesterol biosensors[10-11].

Since the early 90s carbon nanotubes (CNTs) were discovered [12], scientists found that CNTs at electrode surface promoted the oxidation of biomolecules including dopamine, protein[13], and so on. Just recently, the interest in demonstration CNTs for biosensor applications is emerging [14-16]. However, no reference of CNTs electrode for cholesterol measurement was reported.

In our previous work, it was found that the Pt-decorated carbon nanotubes (CNT-Pt) could promote the electron transmission between hydrogen peroxide and electrode, and showed excellent electro-catalytic activity for reduction of H_2O_2[17]. Here a novel amperometric biosensor was prepared by immobilizing of cholesterol oxidase (ChOx) in sol-gel layer on a CNT-Pt modified electrode. The biosensor detects cholesterol based on H_2O_2 reductive current. The results showed that the ChOx/CNT-Pt biosensor was rapid in current response and selective against electroactive interference. It has been successfully used for determination of cholesterol in serum.

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Experimental

Cyclic voltammetry experiment was performed using CHI660 electrochemical workstation (CH Inc. USA) with a conventional three-electrode cell. A waxed graphite electrode ($0.283cm^2$) was used the substrate electrode, which firstly modified with CNT-Pt, and then covered with a ChOx/sol-gel layer [11]. A platinum wire and Ag/AgCl (1 mol/L KCl) were used as counter electrode and reference electrode, respectively. Cholesterol oxidase (ChOx, EC 1.1.3.6, C-8649, 18 U /mg), cholesterol and Triton X-100 were purchased from Sigma. All solutions were prepared from analytical grade reagents in double distilled water. Supporting electrolyte solution was 0.02 mol/L phosphate buffer solution containing 0.8 % (V/V) Triton X-100.

Results and Discussion

As shown in **Figure 1,** there is an obvious reduction peak at -0.18V for the biosensor in cholesterol solution and the addition of cholesterol causes the remarkable increase of the reduction current. In comparison of the CNT-Pt biosensor with conventional Pt sensor, there is only an anodic peak at about +0.6 V, illustrated high catalytic activity of CNT-Pt electrode. It is very important to lower the detecting potential, because biosensors will not suffer from co-oxidable substances at low potential.

Figure 2 presents chronoamperometric plot of the biosensor in a stirred solution with series addition of cholesterol. The balanceable current of the CNT-Pt/ChOx biosensor is obtained less than 20 s. The rapid response indicates that the CNT-Pt electrode exhibits excellent electrochemical activity in the biosensor and can enhance the efficiency of electron transfer between ChoX and electrode greatly[18-19].

Figure 1 Cyclic voltammograms of the CNT-Pt-ChOx biosensor in a: phosphate buffer (pH 7.0) + 0.8% Triton X-100, b-g: a + cholesterol (1×10^{-5},2×10^{-5}, 3×10^{-5}, 4×10^{-5}, 5×10^{-5}, 6×10^{-5}mol/L). Scan rate: 50mV/s

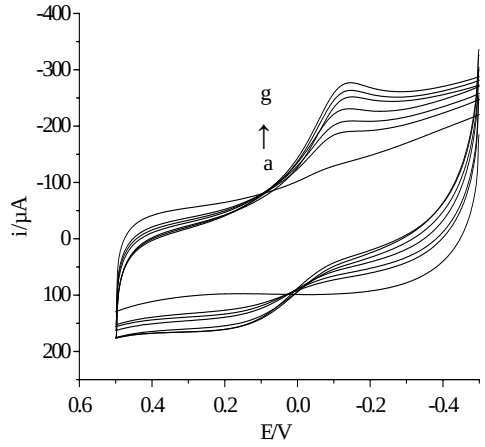
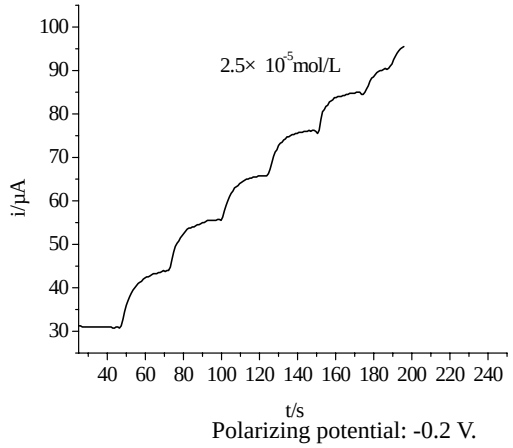


Figure 2 Chronoamperometric curve of the sensor with series addition of 2.5×10^{-5} mol/L cholesterol in 0.02 mol/L phosphate buffer (pH 7.0) + 0.8% Triton X-100.



Polarizing potential: -0.2 V.

Table 1 Determination of dissociative cholesterol in serum samples

Samples	Cholesterol found (10^{-4} mol/L)	Average (10^{-4} mol/L)	RSD (%)	Added (10^{-4} mol/L)	Recovery (%)
1	1.84, 1.86, 1.79, 1.72, 1.92	1.83	4.1	1.0	102.3
2	1.25, 1.27, 1.35, 1.31, 1.24	1.28	3.6	1.0	96.8
3	1.21 1.25 1.26, 1.26, 1.30	1.26	2.6	1.0	103.7

The storage stability of the fabricated biosensor has been studied by testing relative current response. The biosensor retained 98.2% of the initial activities to cholesterol after two weeks storage, 96.4% after four weeks and 94.3% after six weeks, respectively. The results showed that CNT-Pt nanoparticles were very effective as a matrix of enzyme sensors by taking advantage of the biocompatibility and huge surface of CNT-Pt nanoparticles and good electrocatalytic activity to hydrogen peroxide[20]. In order to examine the reproducibility of the sensor, sensitivities were measured (including 70 determinations) within 4 h, and RSD was found to be 2.5%.

The influence of some species on sensor response is investigated in 5×10^{-5} mol/L cholesterol solution. In particular, ascorbic acid and uric acid was focused on, since these compounds are easily oxidized at electrodes. It was found that 5×10^{-4} mol/L concentration of ascorbic acid, much higher than the normal level (5×10^{-5} mol/L) in serum, only resulted in a relative division of 2.2% on the current. Similarly, 2×10^{-3} mol/L uric acid only produced a division of 2.8%.

The dissociated cholesterol in serum has been determined by chronoamperometric method using the CNT-Pt/ChOx biosensor. The serum samples were diluted 10 times with the phosphate buffer containing 1.0% isopropanol and 0.8% Triton X-100, and then heated in a water bath of 40 °C for five minutes to dissolve the cholesterol in aqueous solution. The results of determination and standard addition are shown in **Table 1**. The relation standard deviations are among 2.6-4.1%, and the recoveries are in the range of 96.8-103.7%.

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Reference

1. A. Kumar, R. Malhotra, B. D. Malhotra, *et al*., *Analy. Chim. Acta*, **2000**, *43*(1-2), 414.
2. R. C. Srivastava, R. Sahney, S. Upadhyay, *et al., J. Membrane Science*, **2000**, *164* (1), 45.
3. M. K. Ram, P. bertoncell, H. Ding, *et al*., *Biosens. Bioelectron*., **2001**, *16* (9-12), 849.
4. J. L. Besombes, S. Cosnier, P. Labbé, *Talanta*, **1997**, *44* (12), 2209.
5. J. C. Vidal, E. Garcia, J. R. Castillo, *Sens. Actuators B,* **1999**, *57* (1-3), 219.
6. R. Garjonyte, A. Malinauskas, *Sens. Actuators B,* **1999**, *56* (1-2), 85.
7. I. L. Mattos, L. Gorton, T. Laurell, *et al*., *Talanta*, **2000**, *52* (5), 791.
8. Z. j. Liu, J. Q. Deng, D, Li, *Anal. Chim. Acta*, **2000**, *407* (1-2), 87.
9. R. S. Brown, J. H. T. Luong, *Anal. Chim. Acta*, **1995**, *310* (3), 419.
10. K. V. Gobi, F. Mizutani, *Sens. Actuators B,* **2001,** *80* (3), 272.
11. J. P. Li, T. Z. Peng, Y. Q. Peng, *Electroanalysis*, **2003**, *15* (12), 1031.
12. S.Iijima, *Nature*, **1991**, *354*, 56.
13. R. J. Chen, Y. Zhang, D. Wang, H. Dai, *J. Am. Chem. Soc.*, **2001**, *123* (16), 3838.
14. J. Wang, M. Musameh, *Anal. Chem*., **2003**, *75* (9), 2075
15. S. Sotiropoulou, N. A. Chaniotakis, *Anal. Bioanal. Chem*., **2003**, *375* (1), 103.
16. X. Yu, D. Chattopadhyay, I. Galeska, *et al*., *Electrochem. Commun*., **2003**, *5* (6), 408.
17. Y. N. Zhu, T. Z. Peng, J. P. Li, *Chem. J. Chin. Univ.*, in press
18. S. G. Wang, Q. Zhang, R. L. Wang, *et al*., *Electrochem. Commun*.,**2003**, *5* (6), 800.
19. C. X. Cai, J. Cheng, T. H. Lu, *Science China B*, **2003**, *33* (6), 511.
20. H. Tang, J. H. Chen, S. Z. Yao, *et al*., *Anal. Biochem*., **2004**, *331* (1), 89.

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