Electrochemical Studies of Cytochrome *c* on Electrodes Modified by Single-Wall Carbon Nanotubes

CHENG, Fa-Liang[a,b](程发良) DU, Shan[a](杜珊) JIN, Bao-Kang*,[a](金葆康)

[a] *School of Chemistry and Chemical Engineering, Anhui University, Hefei, Anhui 230039, China*

[b] *Department of Applied Chemistry, Dongguan University of Technology, Dongguan, Guangdong 523106, China*

Single-wall carbon nanotubes (SWNTs) modified gold electrodes were prepared by using two different methods. The electrochemical behavior of cytochrome *c* on the modified gold electrodes was investigated. The first kind of SWNT-modified electrode (noted as SWNT/Au electrode) was prepared by the adsorption of carboxyl-terminated SWNTs from DMF dispersion on the gold electrode. The oxidatively processed SWNT tips were covalently modified by coupling with amines (AET) to form amide linkage. Via Au—S chemical bonding, the self-assembled monolayer of thiol-unctionalized nanotubes on gold surface was fabricated so as to prepare the others SWNT-modified electrode (noted as SWNT/AET/Au electrode). It was shown from cyclic voltammetry experiments that cytochrome *c* exhibited direct electrochemical responses on the both electrodes, but only the current of controlled diffusion existed on the SWNT/Au electrode while both the currents of controlled diffusion and adsorption of cytochrome *c* occurred on the SWNT/AET/Au electrode. Photoelastic Modulation Infared Reflection Absorption Spectroscopy (PEM-IRRAS) and Quartz Crystal Microbalance (QCM) were employed to verify the adsorption of SWNTs on the gold electrodes. The results proved that SWNTs could enhance the direct electron transfer process between the electrodes and redox proteins.

Keywords single-wall carbon nanotubes, modified gold electrode, PEM-IRRAS, QCM

Introduction

Since the discovery of carbon nanotubes with their high surface area, high electrical conductivity, good chemical stability and significant mechanical strength[1] by Iijima in 1991,[2] many theoretical and experimental studies have been focused on their unique properties and potential applications.[3-9] As an intriguing new member in the carbon family, carbon nanotubes are usually concentric seamless cylinders with graphitic layers. According to their atomic structure, carbon nanotubes are divided into two types: multiwall carbon nanotubes (MWNTs)[2] and single-wall carbon nanotubes (SWNTs).[10,11] They are considered to have the ability to promote electron transfer reactions because of their subtle electronic properties when used in the electrochemical reactions. It has been reported that MWNTs could be mixed with bromoform, mineral oil, or liquid paraffin to fabricate carbon nanotube electrodes which were used to probe biochemical reactions[12,13] and in the electrocatalysis of oxygen.[14] Meanwhile, SWNTs were reported to be casted on Pt and Au electrodes to study its electrochemical properties, but no well-resolved voltammograms were obtained.[15] The electrochemical and electrocatalytic behavior of single-wall carbon nanotube (SWNT) film on a glassy carbon electrode was also investigated and the results showed that the SWNT-modified electrode might be used in biosensors to study the electrochemistry of biosystems.[16,17]

In this paper, the SWNTs were treated with nitric acid and potassium permanganate during the oxidation process. The carboxylic acid groups were introduced on the open ends of SWNTs.[18] The adsorption of carboxyl-terminated SWNTs from DMF dispersion on gold electrode was carried out and SWNT-modified electrode (noted as SWNT/Au electrode) was accordingly prepared. The oxidatively processed SWNT tips were covalently modified by coupling with amines (AET), using carbodiimide chemistry to selectively form amide linkage with carboxyl groups. Via Au—S chemical bonding, the self-assembled monolayer of thiol-functionalized nanotubes on gold surface was fabricated so as to prepare SWNT and AET doubly modified electrode (noted as SWNT/AET/Au electrode). These two types of SWNT-modified electrodes both showed stable promotion effect in the electrochemical reaction of cytochrome *c*, and the different reversible voltammetric responses were observed. Furthermore the Photoelastic Modulation Infared Reflection Absorption Spectroscopy (PEM-IRRAS) and Quartz Crystal Microbalance (QCM) were employed to study the adsorptive behavior of SWNTs on the metal electrode.

Experimental

Materials

Horse heart cytochrome *c*, type VI, was purchased from

* E-mail: bkjin@mail.hf.ah.cn; Tel.: +86-551-5107304; Fax: +86-551-5107342

Received January 15, 2002; revised and accepted December 26, 2002.

Project supported by the Natural Science Foundation of Anhui Province (No. 99046129) and the Major State Basic Research Development Program of Guangdong Province (No. c31902).

Sigma Chemical Co. and used without further purification. SWNT was a gift from Institute of Metal Research, Chinese Academy of Science (shenyang) and purified before use. The purified SWNTs were oxidized by nitric acid and potassium permanganate to introduce carboxyl group on the open ends of the SWNTs.[18] 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC, 98%) and 2-aminoethane-thiol hydrochloride (AET, 98%) were purchased from J&K Chemica Co. Solution of cytochrome *c* was prepared in 0.1 mol/L phosphate buffer solution (PBS, pH = 7.0) which was previously deaerated under high purified nitrogen. All other reagents are of analytical grade and were used without further purification. Quartz double-distilled water was used for all solutions. All experiments were performed at room temperature.

Equipments

Electrochemical measurement

Electrochemical measurements were carried out on an EG&G model 283 Potentiostat/Galvanostat from Princeton Applied Research Corporation (PARC, U.S.A). The electrochemical cell consisted of three-electrodes system where the SWNTs modified gold electrodes with a diameter of 1.5 mm were used as working electrode, an Ag/AgCl/KCl (3 mol/L) as the reference electrode and a platinum wire as a counter electrode. All potentials are given versus Ag/AgCl/KCl (3 mol/L) reference electrode in this paper.

Measurement of QCM

Seiko EG&G Model 917 Quartz Crystal Microbalance (QCM) was used to monitor the change of the resonant frequency that could reflect the mass change of the deposit on the quartz crystal surface. The whole instrument was driven by Seiko EG &G QCA 917 WinEchem electrochemical software. A gold disk electrode (Seiko EG&G) with area of 0.2 cm^2 on both side of 9 MHz AT-cut quartz crystal (AT-QCM) was used.

Measurement of PEM-IRR

ASPM-IRRAS measurements were performed on an Nexus-870 (Nicolet, U.S.A.) FT-IR instrument equipped with photoelastic modulation accessory. An MCT-A detector cooled with liquid nitrogen was used. For the infrared reflection absorption spectrum measurements (IRRAS), the polarization modulation method was employed. The monochromatic IR beam was reflected from the electrode surface at an angle of 83°, and the intensities of the *s*- and *p*-polarized IR beams, R_s and R_p, were measured simultaneously. Since only *p*-light interacts with the surface species, the IR reflection spectrum could be obtained from dividing the intensity of the reflected *p*-radiation by that of the reflected *s*-radiation.

Modification of the gold electrode

Preparation of SWNT/Au *electrode*

A certain amount of oxidatively processed SWNTs was dispersed with the aid of ultrasonic agitation in 10 mL of *N*, *N*-dimethylformamide (DMF) to give a black solution. The working electrode was carefully polished with emery paper (No. 2500) and ehamois leather containing Al_2O_3 slursy (0.05 μm) and then ultrasonically cleaned in acetone, ethanol and distilled water for 3 min respectively. The pretreated electrode was immersed in the solution of oxidized SWNTs in DMF soloution for a given time to have the particles of SWNTs adsorb on the electrode surface. The SWNT/Au electrode was withdrawn from the suspension and washed with distilled water for usage.

Preparation of SWNT/AET/Au *electrode*[19]

A certain amount of oxided SWNTs was dispersed with the aid of ultrasonic agitation in 10 mL of distilled water to give a black suspension. The carboxyl-terminated SWNTs were further thiol-derivated by reacting with $NH_2(CH_2)_2SH$ in aqueous suspension with the aid of a condensation agent, EDC, for 24 h at room temperature. After the suspension had been centrifugated, the supernatant was decanted and the remaining solid was washed with distilled-water repetitiously until the excess AET and EDC were got rid of. The self-assembled monolayer of thiol-functionalized nanotubes (SWNT/AET) was prepared by immersing the gold electrode in the suspension without AET for a given time. The SWNT/AET/Au electrode was withdrawn from the suspension and washed with distilled water for usage.

Results and discussion

Measurement of QCM

Fig. 1 illustrates the frequency response measured during the respective adsorption of SWNTs, SWNT/AET and AET on the surface of a gold electrode. At the start, they adsorb on the electrode surface gradually. With the mass of deposit increases, the resonant frequency at the same time decreases. But when the adsorption accomplishes saturation, the frequency would not change any longer. Here, the frequency shift values of SWNTs and SWNT/AET were both 280 Hz, while the the values of AET was 23 Hz.

According to the Sauerbery equation:[20]

$$\Delta F = -2.6 \times 10^6 F_0^2 \Delta M / A$$

where ΔF are F_0 frequency shift and fundamental frequency respectively, and ΔM and A mass change and electrode area, respectively. The adsorptive amounts of SWNTs

(SWNT/AET) and AET were about 273 ng and 22 ng respectively.

The mass of SWNTs particle is so larger than that of AET molecule that it took longer time to complete saturation for the adsorption of SWNTs (5000 s). In the same way, the frequency shift value was also larger for SWNTs (280 Hz) than for AET (23 Hz).

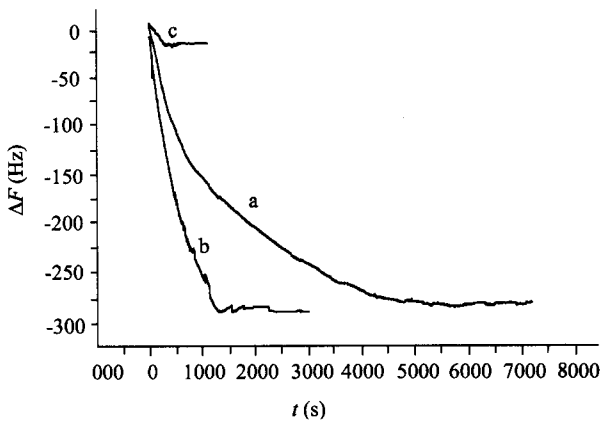

Fig. 1 Frequency response to SWNTs (a), SWNT/AET (b) and AET (c) on Au-QCM electrode.

The comparison between Fig. 1a and Fig. 1b indicated that the frequency shift values are almost equal, suggesting that the adsorptive amounts of SWNTs and SWNT/AET are almost equal. But less time was taken for SWNT/AET to reach saturation than for SWNTs, because SWNT/AET adsorbed easilier on gold surface via Au—S bonding. It could be anticipated that the stability of SWNT/AET/Au electrode is sure to be better than that of SWNT/Au electrode due to the strong interaction between gold surface and hydrosulfide group.

Measurement of PEM-IRRAS *of the modified electrodes*

In Fig. 2(a), the peak around 1743 cm^{-1} presented C = O stretching vibration of COOH group on the end of the

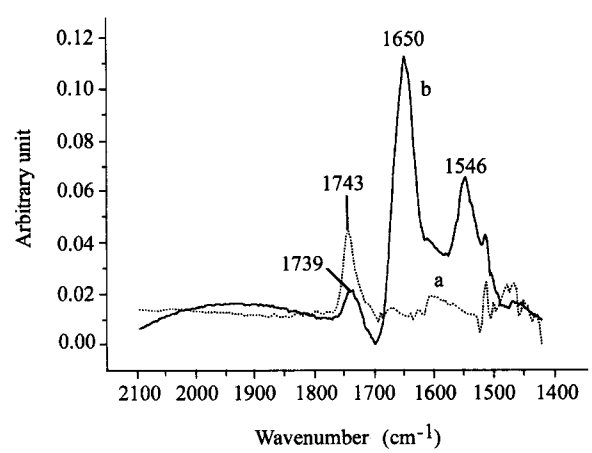

Fig. 2 PEM-IRRAS spectra of SWNT/Au electrode (a) and SWNT/AET/Au electrode (b).

oxidized SWNTs, suggesting that SWNTs adsorb on the gold surface. Comparing the results of Fig. 2(b), the intensity of the peak at 1739 cm^{-1} being much weaker confirmed the condensation between carboxylic termini of SWNTs and amino group of thiols. The strong absorption peaks at 1650 cm^{-1} and 1546 cm^{-1} were attributed to the I band (stretching vibration of C = O group, 1680—1630 cm^{-1}) and II band (bending vibration of – CONH – group and the stretching vibration of C—N group, 1570—1510 cm^{-1}) of the aliphatic second amides.

Electrochemical reaction of cytochrome c *on the modified electrodes*

The cyclic voltammogram of the SWNT/Au electrode in 0.1 mol/L PBS with pH 7.0 was shown in Fig. 3. It was clear that there was no peak on the CV. After addition of cytochrome *c* in the buffer, anodic and cathodic peaks were observed at 135 and 52 mV (at 50 mV/s) respectively (Fig. 3), which indicated that cytochrome *c* could take a rapid quasi-reversible one-electron transfer process at the SWNT/Au electrode.

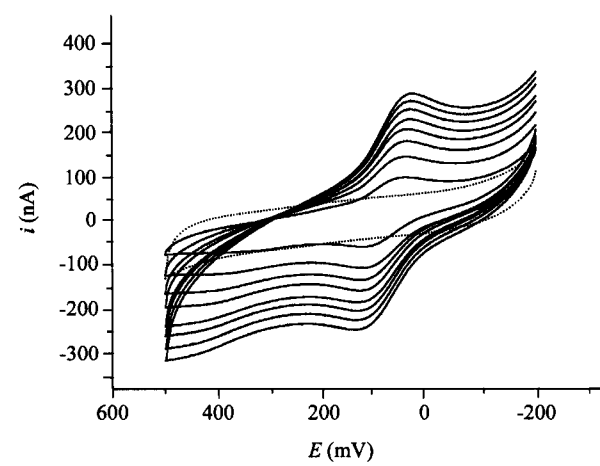

Fig. 3 Cyclic voltammograms at SWNT/Au electrode in the absence of cytochrome *c* at 50 mV/s (dotted line) and in the presence of 4 mg/mL cytochrome *c* at different scan rates in (solid line) 0.1 mol/L PBS (pH = 7.0). Scan rate from lower to higher: 10, 20, 30, 40, 50, 60, 70 and 80 mV/s.

The currents of the redox waves were found almost equal and to increase linearly with the square root of the scan rate (Fig. 4), indicating diffusion controlled process. Furthermore, with the increase of the scan rate, the peak separations varied from about 71 mV (at 10 mV/s) to 82 mV (at 80 mV/s) while the formal potential kept nearly invariant (Table 1). From the slope of the line in Fig. 4, the apparent diffusion coefficient D_0 of cytochrome *c* was calculated to be 1.8×10^{-7} cm^2/s (The geometric area of the gold electrode was applied to simulate calculation of D_0 because it was difficult to measure the surface area of the modified gold electrode). Using the theory of Nicholson[21], the rate constant of heteroge-

neous electron transfer was determined to be $(1.66 \pm 0.30) \times 10^{-3}$ cm/s. In addition, the cyclic voltammogram of cytochrome *c* was scanned continuously for 20 circles and the results showed that the SWNT/Au electrode had the stable promotion effect towards cytochrome *c* because the peak potentials and peak currents remained very stable after the second cycle (Fig. 5).

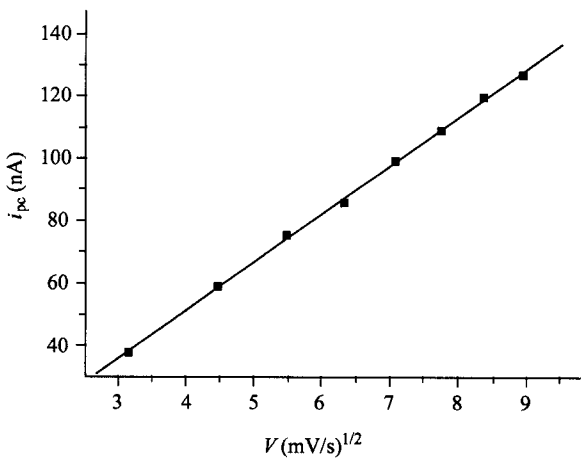

Fig. 4 Relationship between reduction peak current of 4 mg/mL cytochrome *c* and square root of scan rate, in 0.1 mol/L PBS at SWNT/Au electrode.

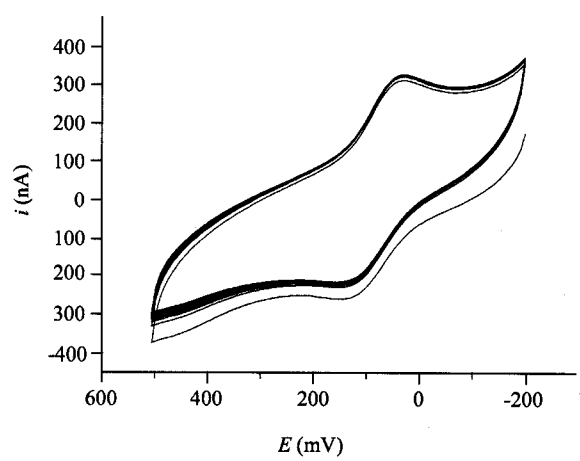

Fig. 5 Cyclic voltammogram of 4 mg/mL cytochrome *c* at SWNT/Au electrode in 0.1 mol/L PBS (pH = 7.0) at continuous scan for 20 cycles. Scan rate 100 mV/s.

Fig. 6 illustrated the cyclic voltammogram at SWNT/AET/Au electrode in the absence of cytochrome *c* and in the presence of 4 mg/mL cytochrome *c* in 0.1 mol/L PBS (pH = 7.0). No redox waves were observed in the blank solution while two couples of redox waves appeared at 48 mV and 205 mV (at 50 mV/s) respectively when the SWNT/AET/Au electrode was scanned in the buffer solution of cytochrome *c*, indicating that cytochrome *c* could take direct electron transfer reactions. This phenomenon is apparently different from that in previous reports. To prove the above waves should be the redox reactions of cytochrome *c*, the proteins of different concentrations were determined under the same conditions (Fig. 7). It is observed that the peak currents increased as more protein was added, which demonstrated our opinion.

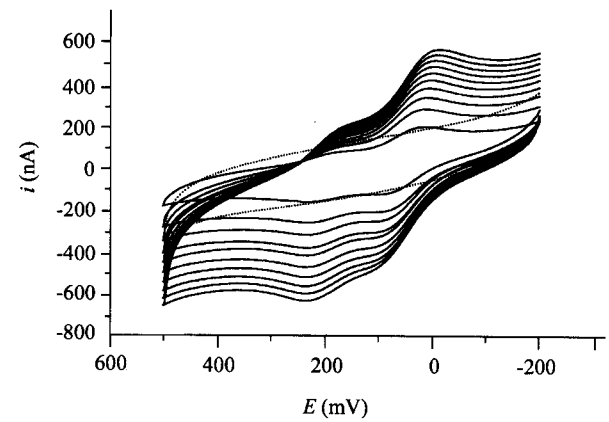

Fig. 6 Cyclic voltammograms at SWNT/AET/Au electrode in the absence of cytochrome *c* (dotted line) at 50 mV/s and in the presence of 4 mg/mL cytochrome *c* at different scan rates (solid line) in 0.1 mol/L PBS (pH = 7.0). Scan rate from lower to higher: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mV/s.

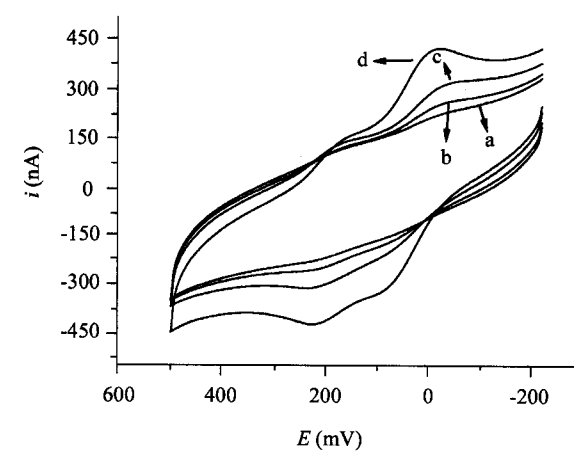

Fig. 7 Cyclic voltammogram of cytochrome *c* with different concentrations at SWNT/AET/Au electrode in 0.1 mol/L PBS (pH = 7.0). Scan rate: 50 mV/s. Concentration from lower to higher (mg/mL): 0.5 (a), 1.0 (b), 2.0 (c) and 4.0 (d).

The peak separation (ΔE_p) of redox peaks (at 204 mV) was 36 mV at 10 mV/s, and CV peak current response was linearly proportional to the scan rate in the range of 10 mV/s to 80 mV/s, which meant that there was adsorption of cytochrome *c* at the surface. Otherwise, the formal potential $E_0 = (E_{p.a} + E_{p.c})/2$ kept stable with the increase of scan rate, but the peak separation ΔE_p presented the trend of increase (Table 1), indicative of a quasi-reversible electron transfer process. The total concentration $\Gamma = 4.7 \times 10^{-11}$ mol/cm^2 of cytochrome *c* adsorbed could be calculated

Table 1 Relationship between peak separation, formal potential and scan rate[a]

Scan rate (mV/s)	10	20	30	40	50	60	70	80
ΔE_{p1} (mV)	71	71	70	71	71	75	76	82
$E_{1/2(1)}$ (mV)	83.5	85.0	83.0	83.5	83.5	82.5	82.0	83.0
$K_1^0/10^{-3}$ (cm²/s)	1.43	1.06	1.66	1.83	2.11	1.84	1.95	1.44
ΔE_{p2} (mV)	36	37	40	44	44	46	48	51
$E_{1/2(2)}$ (mV)	204	203	202	205	205	205	200	202
ΔE_{p3} (mV)	70	76	75	79	84	85	89	93
$E_{1/2(3)}$ (mV)	48.0	48.0	49.0	48.5	48.0	49.0	48.0	47.5
$K_3^0/10^{-3}$ (cm²/s)	1.52	1.46	1.78	1.70	1.35	1.39	1.30	1.22

[a] $E_{1/2} = (E_{p.a} + E_{p.c})/2$; $\Delta E_p = E_{p.c} - E_{p.a}$; $E_{p.a}$ and $E_{p.c}$ are anodic and cathodic peak potential, respectively; K^0 (heterogeneous electron transfer constant) could be calculated according to the following equation:[20] $\Psi = (D_0/D_R)^{\alpha/2} K^0/[D_0 \pi v (nF/RT)]^{1/2}$; D_0 and D_R are diffusion coefficient of oxidation state and reduction state, assuming $D_0 = D_R$; v is potential scan rate, α is transfer coefficient, n is the number of electron transferred in the reaction, F is Faraday constant (96.487 coulombs), R is the universal gas constant (8.314 J/K mol), and T is the Kelvin temperature; subscript "1" presents diffusion wave at SWNT/Au electrode; subscript "2" presents adsorption wave at SWNT/AET/Au electrode; Subscript "3" presents diffusion wave at SWNT/AET/Au electrode.

according to the equation:[22]

$$i_p = n^2 F^2 v \Gamma A / 4RT$$

where i_p is peak current, v potential scan rate, A electrode area, n the number of electron transferred in the reaction, F Faraday constant (96.487 coulombs), R the universal gas constant [8.314 J/(K·mol)], and T the Kelvin temperature.

The peak separation (ΔE_p) of the redox peaks at 48 mV was about 70 mV (at 10 mV/s), which was close to that of a quasi-reversible one-electron transfer process. The currents of the redox waves were almost equal and to increase linearly with the square root of the scan rate, indicative of a diffusion controlled process. Furthermore, with the increase of the scan rate, the peak separations varied from 70 mV (at 10 mV/s) to 102 mV (at 100 mV/s) while the formal potential $E_0 = (E_{p.a} + E_{p.c})/2$ kept stable (Table 1). The apparent diffusion coefficient of cytochrome *c* was calculated to be 3.0×10^{-7} cm²/s and using the theory of Nicholson.[21] The rate constant of heterogeneous electron transfer was determined to be $(1.4 \pm 0.22) \times 10^{-3}$ cm²/s. The reason why the adsorption peak potential is more positive than the diffusion controlled formal potential of cytochrome *c* is probably that the reduced species adsorbed much more strongly than the oxidized species on the SWNTs surface.

In a conclusion, cytochrome *c* exhibited direct electrochemical response on the both electrodes , but only the current of diffusion controlled existed on the SWNT/Au electrode, while both the currents of controlled diffusion and adsorption of cytochrome *c* occurred on the SWNT/AET/Au electrode. Moreover, the potential of diffusion peak at SWNT/AET/Au electrode shifted to negative by about 38 mV compared with that at SWNT/Au electrode, indicating that the oxidation process of cytochrome *c* occurred easily on the SWNT/AET/Au electrode.

The various immersion time of the bare electrode in the suspension with the presence of thiol-functionalized nanotubes (SWNT/AET) had effect on the voltammetric response of cytochrome *c*. When the immersion time got longer, the wave shape got more distinct and the peak currents got larger. The wave shape reached stability with the immersion time being 1.5 h, which was according with the measurement of QCM (Fig. 1). The detailed data of peak currents are listed in Table 2.

Table 2 Relationship between the peak currents of redox waves, peak seperation andimmersion time[a]

Immersion time (min)	i_{pa1} (nA)	i_{pc1} (nA)	i_{pa2} (nA)	i_{pc2} (nA)	ΔE_{p1} (mV)	ΔE_{p2} (mV)
10	114.2	123.3	21.7	14.7	93.0	119.0
30	155.1	173.0	60.3	29.8	87.0	74.0
90	168.9	179.6	61.8	35.5	83.5	44.0

[a] Subscript "1" presents diffusion wave; subscript "2" presents adsorption wave.

Conclusion

In this paper, two different SWNTs-modified gold electrodes were prepared and electrochemical behavior of cytochrome *c* at the modified electrodes was investigated by cyclic voltammetry, in which different phenomena were obtained. The experiments showed that cytochrome *c* exhibited direct electrochemical response on the both electrodes, but only the current of diffusion controlled existed on the SWNT/Au electrode. The modification of SWNTs on the electrodes was also verified by the measurements of QCM and PEM-IRRAS. The results of the experiments proved that SWNTs had favourable promotion effect on the electron transfer of cytochrome *c*.

References

1 Ebbesen, T. W. *Carbon Nanotube Preparation and Properties* CRC, Boca Raton, FL, **1997**, p. 715.
2 Iijima, S. *Nature (London)* **1991**, *354*, 56.
3 De Heer, T. W. A.; Chatelain, A.; Ugarte, D. *Science* **1995**,

270, 1179.

4 Treacy, M. M.; Ebbesen, T. W.; Gibson, J. M. *Nature* **1996**, *381*, 678.

5 Wang, X. K.; Lin, X. W.; Song, S. N.; David, V. P.; Ketterson, J. B.; Chang, R. P. H. *Carbon* **1995**, *33*, 949.

6 Saito, R.; Fujita, M.; Dresselhaus, G.; Dresselhaus, M. S. *Phys. Rev.* **1992**, *46*, 1804.

7 Ruoff, R. S.; Lorents, D. C. *Carbon* **1995**, *33*, 925.

8 Miintmire, J. W.; Dunlap, B. I.; White, C. T. *Phys. Rev. Lett.* **1992**, *68*, 631.

9 Kyotani, T.; Tasai, L. F.; Tomita, A. *Chem. Mater.* **1995**, *7*, 1427.

10 Iijima, S.; Ichihashi, T. *Nature* (*London*) **1993**, *363*, 603.

11 Bethune, D. S.; Kiang, C. H.; DeVeries, M. S.; Gorman, G.; Savoy, R.; Vazquez, J.; Beyer, R. *Nature* (*London*) **1993**, *363*, 605.

12 Britto, P. J.; Santhanam, K. S. V.; Ajayan, P. M. *Bioelectrochem. Bioenerg.* **1996**, *41*, 121.

13 Davis, J. J.; Coles, R. J.; Hill, H. A. O. *J. Electroanal. Chem.* **1997**, *440*, 279.

14 Britto, P. J.; Santhanam, K. S. V.; Rubio, A.; Alonso, J. A.; Ajayan, P. M. *Adv. Mater.* **1999**, *11*, 154.

15 Liu, C. Y.; Bard, A. J.; Wudl, F.; Weitz, I.; Heath, J. R. *Solid State Lett.* **1999**, *2*, 577.

16 Luo, H.; Shi, Z.; Li, N.; Gu, Z. *Anal. Chem.* **2001**, *73*, 915.

17 Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. *Anal. Chem.* **2002**, *74*, 1993.

18 Burghard, M.; Krstic, V.; Duesberg, G. S.; Muster, J.; Roth, S. *Synth. Met.* **1999**, *103*, 1540.

19 Liu, Z.; Shen, Z.; Zhu, Tao.; Hou, S.; Ying, L. *Langmiur* **2000**, *16*, 8570.

20 Sauerbery, G. G. Z. *Phys.* **1959**, *155*, 206.

21 Nichlson, R. S. *Anal. Chem.* **1965**, *37*, 1351.

22 Dong, S.; Che, G.; Xie, Y. *Chemically Modified Electrode*, 1st edn., Science Press, Beijing, **1995**, p. 53 (in Chinese).

(E0201154 SONG, J. P.)