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DIRECT PROTEINS ELECTROCHEMISTRY BASED ON IONIC LIQUID MEDIATED CARBON NANOTUBE MODIFIED GLASSY CARBON ELECTRODE

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1. ABSRACT

A novel glassy carbon electrode modified by a gel containing multi-walled carbon nanotubes (MWNTs) and ionic liquid of 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIPF_6$) is reported. The gel is formed by grinding of MWNTs and $BMIPF_6$. Such gel is then coated on the surface of a glassy carbon electrode. We have employed scanning electron microscopy, Fourier transform infrared spectrometry (FTIR) and cyclic voltammetry to characterize the modified electrode. The direct electron transfers of hemoglobin and catalase on the modified electrode have been observed and studied in detail electrochemically. Hemoglobin is verified to be adsorbed on the modified electrode with the retention of conformation, which has been proved by microscopic FTIR. The electrochemical response of the adsorbed hemoglobin on the modified electrode is very stable, and shows repeated changes in the different pH solutions. It also has shown electrocatalysis to the reduction of oxygen and trichloroacetic acid. Catalase adsorbed on the gel modified electrode still keep activity to hydrogen peroxide. This work provides a simple and easy approach to construct biosensors based on the carbon nanotubes and ionic liquids.

2. INTRODUCTION

Carbon nanotubes (CNTs), one of the new members of carbon family, are typical one-dimensional nanomaterials, and have unique mechanical, chemical and electrical properties (1,2). Such properties have made them very promising in various applications (2). The electrochemical sensing and bioelectrochemistry based on the CNTs have attracted increasing attentions due to their favorable characteristics as electrode materials (3,4). Different methods of development of CNTs electrode have been reported (5-14). The CNTs modified electrode prepared by drop coating is rather popular nowadays because of its simplicity (5,6). Aligned CNTs array electrode has also been developed by directly growing or chemically assembling CNTs on an electrode (7,8,9). CNTs /binder composite electrode is another way to fabricate various modified electrodes (10,11). Since the realization of direct electron transfers (ETs) on CNTs modified electrode with several proteins (3,4), the direct electrochemistry of some new proteins based on the CNTs has also been reported, for instance, microperoxidase MP-11 (7) and myoglobin (8,15). Furthermore, development of biosensors based on the CNTs has been the focus of this area (3,16,17). CNTs modified electrodes have been used to detect different biological species, such as H_2O_2 (6,10), NADH (10,12), glucose (12,18) DNA (19,20), and proteins (16, 17,20) with high sensitivity.

Ionic liquids (ILs), which are compounds that consist only of ions, are liquids at around room temperature. There has been great potential in applications due to the advantages such as no measurable vapor pressure, good thermal and chemical stability, high conductivity, and low toxicity (21,22). The applications of ILs in electrochemical studies have been widely carried out (23-25). Recently, the ILs modified electrodes have been reported (26,27). Wadhawan et al. reported that ILs droplet or film was deposited on the basal plane pyrolytic graphite electrode surface, and the voltammetric response of ferricyanide on such ILs modified electrode was studied (26). Wang et al. demonstrated an amperometric O_2 gas sensor based on supported ILs porous polyethylene membrane-coated electrode, which has wide detection range and high sensitivity (27). Fukushima et al. have recently demonstrated that pristine single-walled CNTs formed gels when mixing with imidazolium ion-based room-temperature ionic liquids by grinding (28). The combination of the ILs and CNTs may lead to a possibility

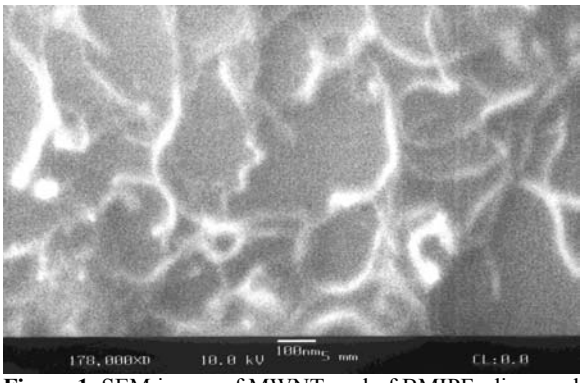

Figure 1. SEM image of MWNTs gel of $BMIPF_6$ dispersed in triply distilled water by sonication. The scale bar is 100 nm. Accelerating voltage: 10 kV.

to fabricate modified electrode. In this work, we have successfully prepared the multi-walled carbon nanotubes (MWNTs) gel of 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIPF_6$), and then coated the gel on a glassy carbon (GC) electrode to fabricate a new type of modified GC electrode. Such modified electrode is thermal stable with high conductivity. The construction of modified electrode is easy and simple. The electrochemical characteristics of the modified electrode in aqueous solution have been studied in detail. In addition, the direct ETs between hemoglobin (Hb) and catalase (Cat), and the modified electrode have been observed, and their adsorption, thermodynamic and kinetic behaviors have also been studied electrochemically. The adsorbed proteins in the gel can retain their activities. The modified electrode provides a platform for fabrication of biosensors, which shows promising application to detect various biomacromolecules.

3. MATERIALS AND METHODS

3.1. Chemicals and apparatus

MWNTs were produced by catalytic chemical vapor deposition (CCVD) method, and provided by the Department of Chemical Engineering of Tsinghua University of China as gifts. The details of synthesis were reported elsewhere (29,30). The purity of the MWNTs is above 95 %.

The ionic liquid of $BMIPF_6$ was synthesized according to the procedures described in the references (31,32). The $BMIPF_6$ has been characterized by ^1H NMR and IR, and purity is proved very high. Hemoglobin (from bovine) and catalase (from bovine liver, 2970 units/mg) were purchased from Sigma. $Ru(NH_3)_6Cl_3$ was obtained from Acros. Water was triply distilled with a quartz apparatus. Highly purity nitrogen was used for deaeration. All other reagents were of analytical grade.

A CHI 660A (CH Instruments, Inc.) electrochemical workstation with a three-electrode cell was employed to perform cyclic voltammetry (CV) and square wave voltammetry (SWV). The working electrode was a GC electrode or a modified GC electrode, the auxiliary and reference electrodes were platinum wire and saturated calomel electrode (SCE), respectively. The solutions were purged with highly purified nitrogen for at least 20 minutes prior to the experiments. Nitrogen atmosphere was maintained over the solutions during the experiments. All experiments were carried out at room temperature (18 +/- 2 ^oC).

The scanning electron microscope (SEM) image was obtained using Amary 1910 Field Emission Microscope. Microscopic Fourier transform (FT) IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer.

3.2. Fabrication of the modified electrode

12 mg MWNTs mixed with 0.2 mL $BMIPF_6$ was ground with an agate mortar for about 20 minutes, and then a black gel was formed (28). A GC disk electrode (diameter is 4 mm) was polished with alumina, and then was washed in triply distilled water and ethanol, respectively. The CNTs gel was placed on a smooth glass slide. The GC electrode was rubbed over the gel, and the gel was mechanically attached to the surface. Then the gel on the electrode surface was smoothed with a spatula to leave a thin gel film on the GC electrode surface (ca. 50-100 μm thick estimated by the optical microscopy, Olympus, BX-51), thus the gel modified GC electrode (denoted as MWNTs-Gel/GC electrode) was fabricated.

MWNTs modified GC electrode (called MWNTs/GC electrode) was prepared by drop coating suspension of MWNTs in ethanol (0.2 mg/mL) on the polished GC electrode. The method is similar to that reported previously (5). 15 μL of the MWNTs suspension in ethanol produced by sonication was dropped on the GC electrode, and then dried in air.

4. RESULTS AND DISSCUSSION

4.1. Characterization of the modified electrode

The MWNTs gel of the $BMIPF_6$ was dispersed in triply distilled water by sonication, and the image of SEM (Figure 1) indicates the MWNTs are untangled after treated with the $BMIPF_6$, which is consistent with previous report (28). Fukushima et al. pointed out that the mechanism of the gel formation is mainly because of cross-linking of the nanotube bundles, mediated by local molecular ordering of the ionic liquids rather than by entanglement of the nanotubes. The molecular ordering results from the "cation-π" interaction between imidazolium and nanotubes (28). The gel can be coated on the GC electrode surface to form a stable film. Without the ILs, MWNTs cannot be directly assembled to the electrode surface through rubbing by hand. Due to the very low volatility of the ionic liquid, the gel can be kept for above three months in air after formation by grinding. The SEM image of the surface of MWNTs-Gel/GC electrode is shown in Figure 2a. The surface of the gel is not smooth. After the MWNTs-Gel/GC measured in the 0.1 M blank phosphate buffer solution (pH 7.0), washed in water, and the surface of MWNTs-Gel/GC electrode was examined by SEM again (Figure 2b). The surface is obviously different from the initial state. Porous structures are formed, which may result from the ionic liquid partly dissolving in the buffer though the solubility is

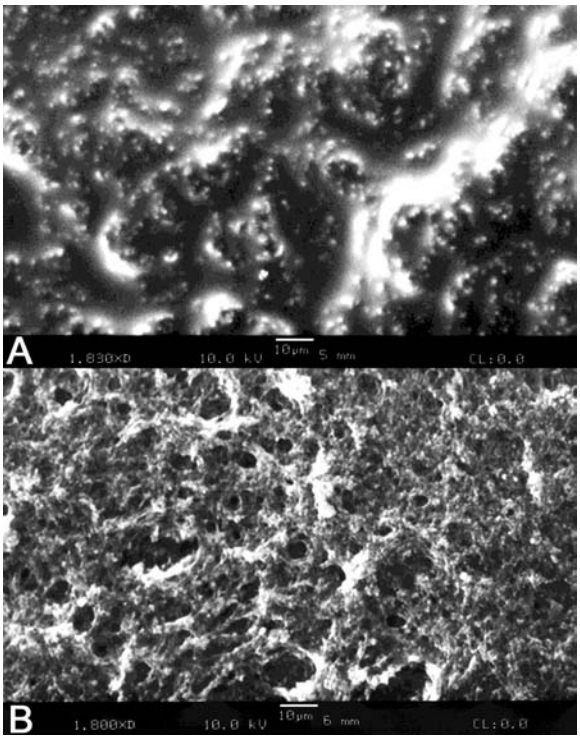

Figure 2. SEM images of (a) the MWWTs-Gel cast on the GC electrode without measuring in the solution, (b) the MWWTs-Gel after measured in the 0.1 M phosphate buffer solution (pH 7.0) and washed in triply distilled water. Scale bar is 10 μm. Accelerating voltage: 10 kV.

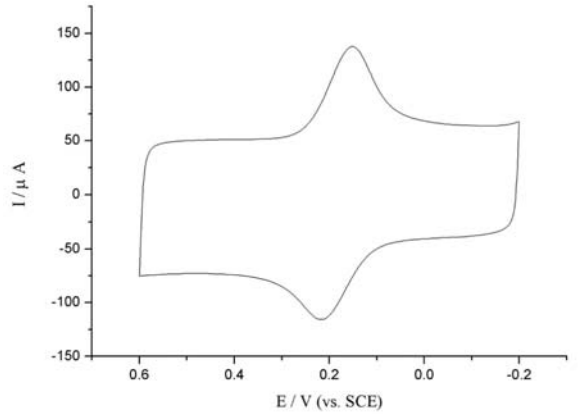

Figure 3. Cyclic voltammogram of the MWNTs-Gel/GC electrode in 1 mM $K_3Fe(CN)_6$ + 0.1 M KCl solution. Sweep rate: 0.05 V/s.

very low (33). The CNTs film on the electrode surface is not destroyed by the slight dissolving of the ILs. Due to the partly dissolving of the ionic liquids and the different compositions of the ILs and the aqueous solution, the voltammetric responses of the MWNTs-Gel/GC electrode are unstable in the solution at the beginning. However, after cyclic sweeps for 20 minutes, the electrochemical signals turn to be rather stable. Hence, all voltammograms of the MWNTs-Gel/GC electrode are recorded after it reaches equilibrium within the tested aqueous solution.

Figure 3 shows the cyclic voltammogram of $Fe(CN)_6^{3-}$ on the MWNTs-Gel/GC electrode. A pair of well-defined redox peaks is observed with the formal potential $E^{o'}$ [$E^{o'} = (E_{pa} + E_{pc})/2$] of 0.184 V (vs. SCE). The peak separation is 64 mV at the sweep rate equal to 50 mV/s, which reveals that this redox reaction is fast ET on the MWNTs-Gel/GC electrode. The result is consistent with the previous report of fast ET on the MWNTs electrode (34). The peak currents increase linearly with square root of the sweep rate in the range of 0.02 to 0.7 V/s.

In addition, the voltammetric behavior of $Ru(NH_3)_6^{3+}$ on the MWNTs-Gel/GC electrode is examined. The modified electrode also gives a pair of redox peaks in 1 mM $Ru(NH_3)_6Cl_3$ + 0.1 M KCl solution. The voltammograms are not shown here. The peak currents are proportional to the square root of the sweep rate in the range of 0.01 to 0.5 V/s. The separation of the redox peaks of $Ru(NH_3)_6^{3+}$ is 58 mV at the sweep rate of 50 mV/s which indicates the ET on the MWNTs-Gel/GC electrode is also fast. The formal potential of $Ru(NH_3)_6^{3+}$ is determined to be –0.182 V (vs. SCE).

4.2. Electrochemical investigation of hemoglobin on the MWNTs Gel/GC electrode

Hemoglobin (Hb), noted for a heme protein transporting oxygen in blood in vertebrate, is a model molecule for studying ET of heme proteins. Its electrochemical behaviors have been studied extensively (35-42). When the MWNTs-Gel/GC electrode is removed from the 0.1 M phosphate buffer (pH 7.0) containing no Hb after several cyclic sweeps, and then measured in the 0.5 mM Hb + 0.1 M phosphate buffer (pH 7.0), a pair of well-defined redox peaks can be observed (Figure 4a). The formal potential $E^{o'}$ for Hb is –0.360 V (vs. SCE), which is close to the reported value (35-37). The electrochemical response remains unchanged after consecutive 50 cycles. Figure 4b is the cyclic voltammogram without Hb in the buffer solution. The relationship of peak currents of Hb with the sweep rate has been analyzed. The peak currents increase linearly with v in the range of 0.01 to 0.5 V/s, which shows the electrochemical reaction of Hb is adsorption controlled. This is different from what we obtained previously for small molecules, such as $Fe(CN)_6^{3-}$ and $Ru(NH_3)_6^{3+}$. The baseline of Hb current is lower than the background current of the blank solution is resulted from the adsorption of Hb on the MWNTs-Gel/GC electrode, which changes the double layer of the modified electrode. The peak potential separation for Hb is nearly constant as sweep rate is lower than 0.2 V/s. The separation of the peak potential will be increased when the sweep rate increases further, reduction and oxidation peak potentials shift negatively and positively, and this is in agreement with the onset of limiting kinetic effects as sweep rate increases. According to the Laviron equation (43,44), the apparent standard rate constant k_s can be calculated and is equal to 2.3 s^{-1} at 18 °C.

After the voltammetric experiments, the MWNTs-Gel/GC electrode is removed from the Hb solution, washed in triply distilled water, and then measured in 0.1 M phosphate buffer (pH 7.0) containing no

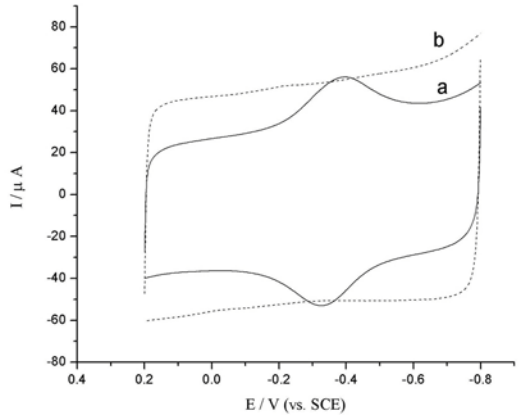

Figure 4. Cyclic voltammogram of the MWNTs-Gel/GC electrode (a) in 0.5 mM Hb + 0.1 M phosphate buffer solution (pH 7.0), (b) in 0.1 M phosphate buffer solution (pH 7.0) containing no Hb. Sweep rate: 0.1 V/s.

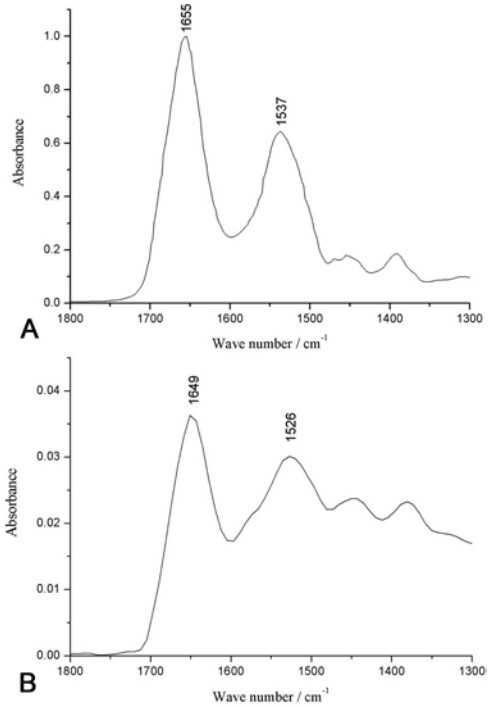

Figure 5. Microscopic FTIR spectra of (a) pure Hb and (b) dry Hb-MWNTs-Gel film

Hb. Compared with the voltammogram in Figure 4, the cyclic voltammogram does not alter and is still very stable. This indicates that Hb has possibly been adsorbed in the MWNTs-Gel film and the saturation has been reached rapidly. The MWNTs-Gel/GC electrode that with adsorbed Hb is called Hb-MWNTs-Gel/GC electrode. The electrochemical response of the Hb-MWNTs-Gel/GC electrode is very stable. Periodical scanning does not affect the electrochemical signals. After storing at 4 ^{o}C for three weeks the peak currents only decreased 2 % of the initial responses. In order to prove the adsorption of the Hb on MWNTs gel modified electrode and test the conformation changes of Hb, microscopic FTIR experiments have been performed. Shapes of the amide infrared bands are sensitive to conformational changes in the polypeptide backbone of Hb (35,44). The amide I band (1700 –1600 cm^{-1}) is caused by C=O stretching of peptide linkages. The amide II band (1600 –1500 cm^{-1}) is a combination of N-H bending and C-N stretching. Pure Hb shows microscopic FTIR peaks at 1655 and 1537 cm^{-1} (Figure 5a), which are corresponding to the vibrancy of amide bond. The MWNTs-Gel/GC electrode that measured in the 0.5 mM Hb + 0.1 M phosphate buffer solution, as mentioned above, was washed in triply distilled water and dried by purging nitrogen. The MWNTs-Gel film is scraped carefully, and its microscopic FTIR spectrum is recorded. The peaks at 1649 and 1526 cm^{-1} are also observed (Figure 5b). This obviously indicates that Hb has been adsorbed in the MWNTs-Gel film. The position of peaks changes little, which shows the Hb adsorbed in the film possibly keeps the structure similar to its native state.

The effect of pH on the Hb-MWNTs-Gel/GC electrode has also been tested. Both of the cathodic and anodic peak potentials shift negatively with the increasing of pH in the range of 4.4 to 9.2 (Figure 6). The formal potentials $E^{o'}$ obtained by SWV have a linear relationship with the pH in the range of pH 6.1 to 9.2 with a slope of –48 mV/pH (the inset of Figure 6). This value is reasonably close to the theoretical value of –57.6 mV/pH at 18 ^{o}C (46), indicating that one proton transfer is coupled to each electron transfer in the electrochemical redox reaction of Hb. In the plot of $E^{o'}$ vs. pH (the inset of Figure 6), an inflection point is observed at about pH 6.1. In the range of 4.4 to 6.1, $E^{o'}$ varies with the pH with a smaller slope of –23 mV/pH. This suggests that protonation takes place on one group of Hb that is near the active center below pH 6. The similar phenomena have also been demonstrated in the literature (35-37), but the inflection point reported appears at about pH 5.0 (36) or pH 5.5 (37). The difference of the inflection points may be contributed to the changes of the microenvironment of Hb caused by the CNTs. After the measurements in the phosphate solutions of different pH, Hb-MWNTs-Gel/GC electrode remains responses with the unchanged values of peak currents and potentials position when tested again in the pH 7.0 solution. This means Hb-MWNTs-Gel/GC electrode can provide responses repeatedly in the different pH solutions in the range of pH 4.4 to 9.2.

In order to detect low concentration of Hb, the square wave voltammetry (SWV) is employed. SWV voltammograms are recorded from –0.8 V to 0.2 V (vs. SCE) after the MWNTs-Gel/GC electrode immersed in the 20 μM Hb + 0.1 M phosphate solution (pH 7.0) for 2 minutes, and an oxidation peak of Hb at about –0.35 V (vs. SCE) can be obtained (Figure 7). The peak current increases with the addition of Hb, and the MWNTs-Gel/GC electrode has the linear responses from 5 μM up to 0.1 mM with a sensitivity of 0.3 μA/μM when the accumulation time is 2 minutes. When the concentration of Hb is higher than 0.1 mM the current signals are nearly unchanged, which is due to the saturated adsorption of Hb on the MWNTs-Gel/GC electrode (the inset of Figure 7). Therefore, the MWNTs-Gel/GC electrode can be used as a

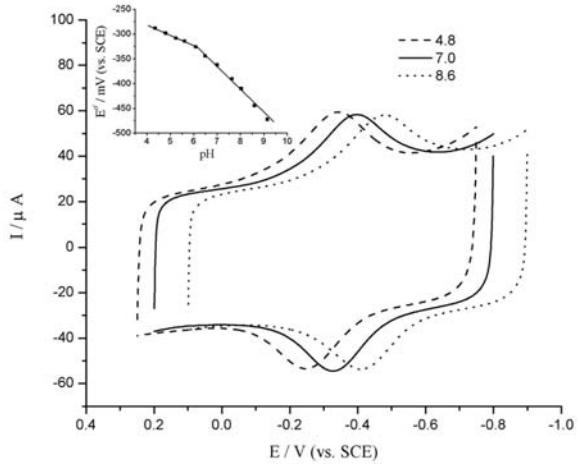

Figure 7. Square wave voltammogram of the MWNTs-Gel/GC electrode in 20 μM Hb + 0.1 M phosphate solution (pH 7.0) after 2 min accumulation. Amplitude: 25 mV. Frequency: 15 Hz. Inset shows the relationship between peak current of SWV and the concentration of Hb.

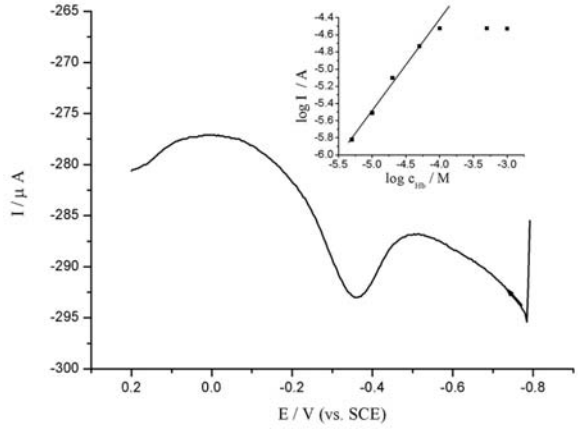

Figure 6. Effects of pH on the CV of Hb-MWNTs-Gel/GC electrode in 0.1 M phosphate solution at 0.1 V/s. Inset shows the influence of pH on the formal potential for Hb-MWNTs-Gel/GC electrode.

sensor to detect Hb, with careful selecting and controlling the experimental conditions.

The response of oxygen on the Hb-MWNTs-Gel/GC electrode is also examined. Figures 8a and 8b shows the cyclic voltammograms of Hb-MWNTs-Gel/GC and MWNTs-Gel/GC electrodes in 0.1 M phosphate buffer solution (pH 7.0) in the absence of oxygen, respectively. When oxygen is present, the reduction current of the Hb-MWNTs-Gel/GC electrode increases with the addition of oxygen while the oxidation current for Hb Fe(II) decreases (Figure 8c). Though the reduction of oxygen is also observed on the MWNTs-Gel/GC electrode (Figure 8d), the increase of reduction current of the Hb-MWNTs-Gel/GC electrode is much larger when oxygen is present. In addition, the reduction potential of oxygen on the Hb-MWNTs-Gel/GC electrode shifts positively about 150 mV, compared with that on the MWNTs-Gel/GC electrode. This shows that the Hb-MWNTs-Gel/GC electrode is electrocatalytic to the reduction of oxygen (37,38).

The electrochemical catalytic reduction of trichloroacetic acid (TCA) by Hb-MWNTs-Gel/GC electrode is also investigated by voltammetry. Figures 9a and 9b shows the cyclic voltammograms of Hb-MWNTs-Gel/GC and MWNTs-Gel/GC electrodes in 0.1 M phosphate buffer solution (pH 7.0) in the absence of TCA, respectively. Figure 9c shows the voltammogram for MWNTs-Gel/GC electrodes in the presence of 24 mM TCA in the solution. For Hb-MWNTs-Gel/GC electrode, with the addition of TCA, the reduction peak current of the Hb increases, accompanied by a decrease of the oxidation peak (Figure 9d), which shows the Hb-MWNTs-Gel/GC electrode is electrocatalytic to the reduction of TCA. These results are consistent with previous report (35,36).

In order to compare the performances of the modified electrodes with and without ILs on the surface, we have tested the ET of Hb at the MWNTs/GC electrode. In the 0.5 mM Hb + 0.1 M phosphate buffer (pH 7.0) solution the MWNTs/GC electrode also gives a pair of peaks at about –0.360V (vs. SCE) (Figure 10a). The similar results have been demonstrated in previous report (47). However, the pair of peaks is not obvious at the beginning. It is different from the result of Hb on the MWNTs-Gel/GC electrode (Figure 4a). The background current of MWNTs/GC electrode is smaller than the MWNTs-Gel/GC electrode, which results from the larger amount of MWNTs on MWNTs-Gel/GC and the porous film structure of the MWNTs-Gel. The peak currents of Hb on MWNTs-Gel/GC electrode are much higher than those on MWNTs/GC electrode. After the MWNTs/GC immersed in the Hb solution for above 24 hours in 4 ^{o}C, washed in the triply distilled water, and measured in the 0.1 M phosphate buffer (pH 7.0). The peak currents of Hb are larger than before and unchanged with time again, which shows the Hb adsorbed on MWNTs/GC with saturation. The result is displayed in the inset of Figure 10. The MWNTs/GC electrode treated with the Hb is called Hb-MWNTs/GC electrode. Experiments show the response time to Hb for MWNTs/GC electrode is slower than the MWNTs-Gel/GC electrode. In addition, compared with the result of Hb-MWNTs-Gel/GC electrode (Figure 10b), the signals of the Hb-MWNTs/GC electrode are much smaller (the inset of Figure 10) at the same sweep rate. The peak currents are only about 10 percent of those obtained on Hb-MWNTs-Gel/GC electrode. One reason is the MWNTs-Gel/GC electrode has much more MWNTs loaded on the film and can adsorb much more Hb. Moreover, due to the easily aggregating of the MWNTs, the MWNTs film on GC prepared by drop coating is not uniform, and only part of the GC electrode surface is covered by MWNTs. The MWNTs-Gel/GC electrode has more ordered structure than the MWNTs/GC electrode, which can be proved by the Figure 2b. Considering the untangling effect of the $BMIPF_6$ on the CNTs (Figure 1), which is resulted from the "cation-π" interaction between imidazolium and nanotubes (28), the MWNTs gel film is more porous than the MWNTs film, thus Hb is easier adsorbed on the MWNTs-Gel/GC electrode, and more Hb can be immobilized on the gel

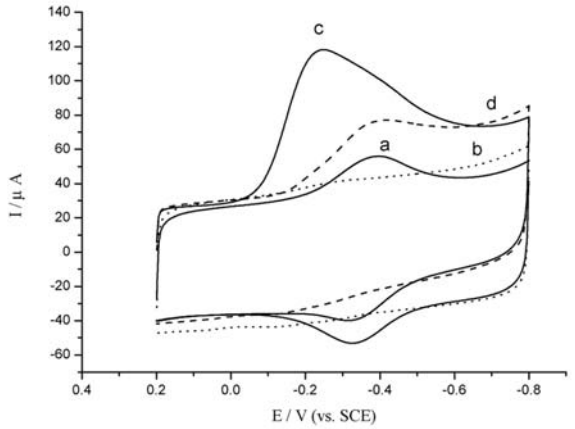

Figure 8. Cyclic voltammograms at 0.1 V/s in 0.1 M phosphate buffer (pH 7.0) for (a) Hb-MWNTs-Gel/GC electrode in the absence of O_2, (b) MWNTs-Gel/GC electrode in the absence of O_2, (c) Hb-MWNTs-Gel/GC electrode in the buffer saturated by air, and (d) MWNTs-Gel/GC electrode in the buffer saturated by air.

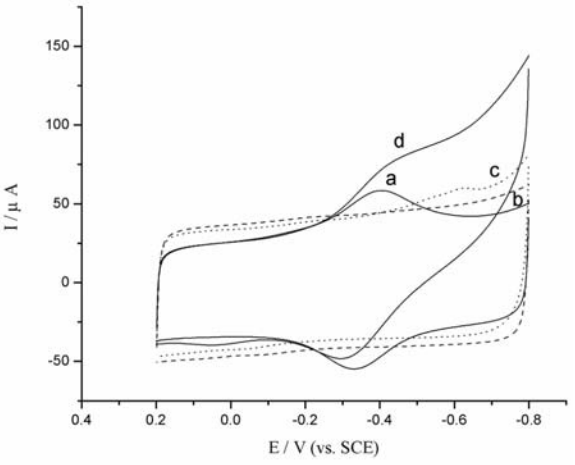

Figure 9. Cyclic voltammograms at 0.1 V/s in 0.1 M phosphate buffer (pH 7.00) for (a) Hb-MWNTs-Gel/GC electrode in the absence of TCA, (b) MWNTs-Gel/GC electrode in the absence of TCA, (c) MWNTs-Gel/GC electrode in the presence of 24 mM TCA and (d) Hb-MWNTs-Gel/GC electrode in the presence of 24 mM TCA.

modified electrode, which may also facilitate the direct electrochemistry of Hb.

The direct ET of Hb is not observed in the 0.5 mM Hb + 0.1 M phosphate buffer solution (pH 7.0) on the bare GC electrode. Even after the bare GC electrode immersed in the Hb solution for above 24 hours at 4 ^{o}C, no obvious electrochemical signals of Hb can be observed. Furthermore, $BMIPF_6$ modified GC electrode that is prepared by dropping 5 μL $BMIPF_6$ on the GC electrode is also measured in the 0.5 mM Hb + 0.1 M phosphate buffer solution (pH 7.0). No redox peaks of Hb are observed on the $BMIPF_6$ modified GC electrode.

The realization of the direct electrochemistry of Hb on the MWNTs-Gel/GC electrode can be attributed to the CNTs. CNTs facilitate the ET due to the specific properties and peculiar structure (3). Nanometer scale size of CNTs also plays an important role. The direct electrochemistry of Hb on other nanomaterials modified electrode such as nano gold (38,39), and other nanoparticles (40-42) have been reported. The nanomaterials can provide a microenvironment to make proteins keep fitted conformation, so that it makes the ET much easier. The main role of $BMIPF_6$ is playing as a tool to make the CNTs form the gel, which facilitates the preparation of the CNTs modified electrode and makes it easy to load large amount of MWNTs on the electrode surface. In addition, the "cation-π" interaction between imidazolium and nanotubes makes the modified film porous. The "cation-π" interaction between imidazolium and nanotubes can also bring positive charge on the surface on the nanotubes, thus the electrostatic interaction may exist between the nanotubes and Hb. The electrostatic interaction and the porous film structure are also favorable for the ET between Hb and electrode.

4.3. Electrochemical behavior of catalase on the MWNTs Gel/GC electrode

The direct electrochemistry of catalase (Cat), a heme enzyme catalyzing the disproportionation of hydrogen peroxide into oxygen and water (48), is also realized on the MWNTs-Gel/GC electrode. In 0.1 mM Cat + 0.1 M phosphate buffer solution (pH 7.0), a couple of redox peaks can be observed on the MWNTs-Gel/GC electrode, and the peak currents increase with the time. It suggests that Cat can be adsorbed on the MWNTs-Gel/GC electrode. The MWNTs-Gel/GC electrode is immersed in the Cat solution overnight at 4 ^{o}C, then washed in triply distilled water, and measured again in the 0.1 M phosphate buffer solution (pH 7.0) containing no Cat. The cyclic voltammogram is displayed in Figure 11a, and a pair of obvious redox peaks of Cat can be obtained. The MWNTs-Gel/GC electrode that adsorbed Cat is called Cat-MWNTs-Gel/GC electrode. The formal potential $E^{o'}$ of Cat on the MWNTs-Gel/GC modified electrode is determined to be –0.472 V, which is close to the previous reported (37,49,50). The formal potential of Cat-MWNTs-Gel/GC electrode shifts negatively with the increase of pH in the range of 4.4 to 9.2, which varies with the pH with a slope of –48 mV/pH. According to the Laviron equation (44,45), the apparent standard rate constant k_s for Cat-MWNTs-Gel/GC electrode can be calculated and is equal to 3.5 s^{-1} at 18 ^{o}C.

After addition of H_2O_2, the reduction current of the Cat-MWNTs-Gel/GC electrode increases greatly, accompanied with the decrease of oxidation current (Figure 11b). Figure 11c displays the voltammogram for MWNTs-Gel/GC electrode in 0.1 M phosphate buffer solution (pH 7.0) in the absence of H_2O_2. The reduction of H_2O_2 on the MWNTs-Gel/GC electrode is shown in Figure 11d. When the H_2O_2 is present, the increase of reduction current on the Cat-MWNTs-Gel/GC electrode is much larger than that on the MWNTs-Gel/GC electrode. This shows the Cat adsorbed on the MWNTs-Gel/GC electrode still keep

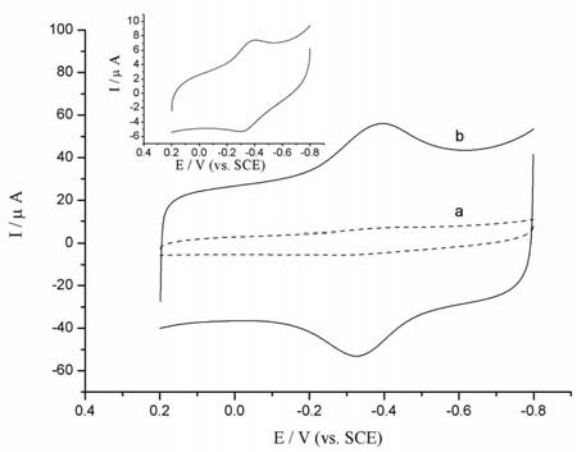

Figure 10. Cyclic voltammograms in 0.5 mM Hb + 0.1 M phosphate buffer (pH 7.0) at 0.1 V/s for (a) MWNTs/GC electrode and (b) MWNTs-Gel/GC electrode. Inset shows cyclic voltammogram in 0.1 M phosphate buffer (pH 7.0) at 0.1 V/s for MWNTs/GC electrode after immersed in 0.5 mM Hb solutions for above 24 hours and washed in triply distilled water.

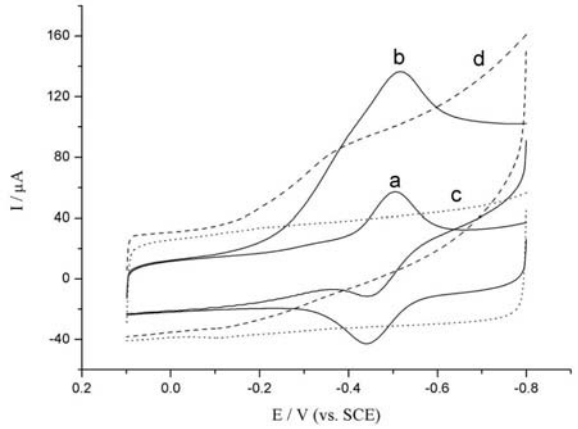

Figure 11. Cyclic voltammograms at 0.1 V/s in 0.1 M phosphate buffer solution (pH 7.0) for (a) Cat-MWNTs-Gel/GC electrode in the absence of H_2O_2, (b) Cat-MWNTs-Gel/GC electrode in the presence of 2 mM H_2O_2, (c) MWNTs-Gel/GC electrode in the absence of H_2O_2, and (d) MWNTs-Gel/GC electrode in the presence of 2 mM H_2O_2.

activity and has good catalysis toward hydrogen peroxide (49,50).

5. CONCLUSIONS

In summary, a gel containing multi-walled carbon nanotubes and ionic liquids is formed by grinding, and has been used to fabricate modified electrodes. The construction of gel modified electrode is simple and easy. The fabricated modified electrode is stable. The direct ETs of hemoglobin and catalase have been realized on such gel modified electrode. Proteins can be adsorbed on the gel modified electrode, and can retain their activities. The facilitation of ETs between proteins and the modified electrode is mainly attributed to the CNTs and the specific film structure resulted from the interaction of CNTs and ionic liquids, but the detailed mechanism is unknown by now. This work provides an easy way to develop CNTs electrode, and will enable the constructions of biosensors based on CNTs and ionic liquids. The further investigation on the new type of modified electrode is under progress in our laboratory.

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Abbreviations: MWNTs: multi-walled carbon nanotubes, $BMIPF_6$: 1-butyl-3-methylimidazolium hexafluorophosphate, FTIR: Fourier transform infrared spectrometry, CNTs: carbon nanotubes, ET: electron transfer, ILs: ionic liquids, GC: glassy carbon, Hb: hemoglobin, Cat: catalase, CCVD: catalytic chemical vapor deposition, CV: cyclic voltammetry, SWV: square wave voltammetry, SCE: saturated calomel electrode, SEM: scanning electron microscope, TCA: trichloroacetic acid

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