

Preparation, Characterization, and Bioelectrocatalytic Properties of Hemoglobin Incorporated Multiwalled Carbon Nanotubes-Poly-L-lysine Composite Film Modified Electrodes Towards Bromate

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Abstract: The present work describes preparation of hemoglobin-incorporated multiwalled carbon nanotubes-poly-L-lysine (MWCNT-PLL)/Hb composite modified electrode film modified glassy carbon electrode (GCE) and its electrocatalytic behavior towards reduction of bromate (BrO_3^-) in 0.1 M acetate buffer (pH 5.6). The modified electrode has been successfully fabricated by immobilizing hemoglobin on MWCNT dispersed in poly-L-lysine. The surface morphologies of MWCNT, PLL and Hb were characterized using atomic force microscopy (AFM). The voltammetric features suggested that the

charge transport through the film was fast and the electrochemical behavior resembles that of surface-confined redox species. Cyclic voltammetry was used to investigate the electrocatalytic behavior of the modified electrode towards bromate and was compared with that of the CNT-modified as well as bare electrode. The analytical determination of bromate has been carried out in stirred solution at an optimized potential with a sensitivity of $7.56 \mu\text{A mM}^{-1}$ and the calibration curve was linear between 1.5×10^{-5} to 6.0×10^{-3} M.

Keywords: Bionanotechnology • Hemoglobin • MWCNT • Poly-L-lysine • Composite electrode • Electrocatalysis • Bromate

1 Introduction

Carbon nanotubes (CNTs) can be described as a sheet of carbon atoms rolled up into a cylinder held together by van der Waals interactions [1–5]. Multiwall carbon nanotubes (MWCNTs) consist of concentric cylindrical shells of grapheme sheets arranged around a central hollow area. Since their discovery [1], they have gained considerable interest due to their unique structural, mechanical and chemical properties [1–5]. The first attempt to use CNTs for developing an electrochemical sensor based on CNTs was performed by Britto et al. [6] who demonstrated the excellent electrocatalytic properties of single wall carbon nanotubes (SWCNTs) dispersed within bromoform. Since then, the interest of using CNTs for developing modified electrodes has largely increased due to their known advantages connected with the high surface area, favorable electronic properties and electrocatalytic effects [7–11]. Compton et al. [12–14] have demonstrated that the interesting electrocatalytic properties of CNTs are mostly due to the presence of defects like the edge planes of pyrolytic graphite located mainly at the end of the tubes.

Recently, Rivas et al [15, 16] have proposed for the first time the design and characterization of electrochemical sensors based on modification of glassy carbon electrode with MWCNT dispersed in poly-L-lysine (PLL). In the present investigation, we propose the use of glassy carbon electrodes (GCEs) modified with MWCNTs dispersed in

PLL as a matrix for the immobilization of hemoglobin and the resulting film, (MWCNT-PLL)/Hb, as sensing layer for the quantification of bromate.

Bromate, a suspected human carcinogen [17–19], can occur in drinking water as a disinfection by-product (DBP) when ozonation is used to purify bromide containing water [20–22]. As this constituent is of general health-care concern [23, 24], maximum tolerable level of bromate in water intended for human consumption is regulated at $10 \mu\text{g L}^{-1}$ concentration [25]. Therefore, the importance of simple analytical methods for trace analysis of bromate has received considerable attention in the recent years.

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In the present work, glassy carbon electrodes (GCE) modified with MWCNTs dispersed in PLL as a matrix for the immobilization of hemoglobin. The electrocatalytic reduction of bromate at hemoglobin incorporated MWCNT-PLL film modified electrodes is investigated. Finally, the possibility of utilizing (MWCNT-PLL)/Hb film electrode as amperometric sensor for bromate determination was examined.

2 Experimental

2.1 Reagents and Chemicals

Multiwalled carbon nanotubes (Aldrich), potassium iodate (Yakuri Chinta), were used as received. Poly-L-lysine hydrobromide (Mol wt = 70 000–150 000), KIO_3 , KIO_4 , H_2O_2 and hemoglobin were obtained from Sigma. All other chemicals used were of analytical grade and used without further purification. 0.1 M acetate buffer was used as supporting electrolyte. Aqueous solutions were prepared using double-distilled deionized water and

then de-aerated by purging with high purity nitrogen gas for approximately 20 min prior to performing the electrochemical experiments. In addition, a continuous flow of nitrogen over the aqueous solution was maintained during the measurements. Indium tin oxide (ITO) ($7 \Omega/\text{cm}^2$) was purchased from Merck Display Technologies (MDT) Ltd (Taiwan).

2.2 Electrochemical Measurements

All electrochemical experiments were carried out with a single compartment cell, in which a BAS (Bioanalytical Systems, West Lafayette, IN) glassy carbon electrode (area = 0.07 cm^2) was used as working electrode and a platinum wire served as counter electrode. An Ag|AgCl (sat KCl) reference electrode was used to monitor the cell potentials. Cyclic voltammetry (CV), Linear Sweep Voltammetry (LSV) and amperometric experiments were performed with CH Instruments 1205A. The amperometric response of working electrode was glassy carbon electrode (geometric area 0.28 cm^2) using CHI-

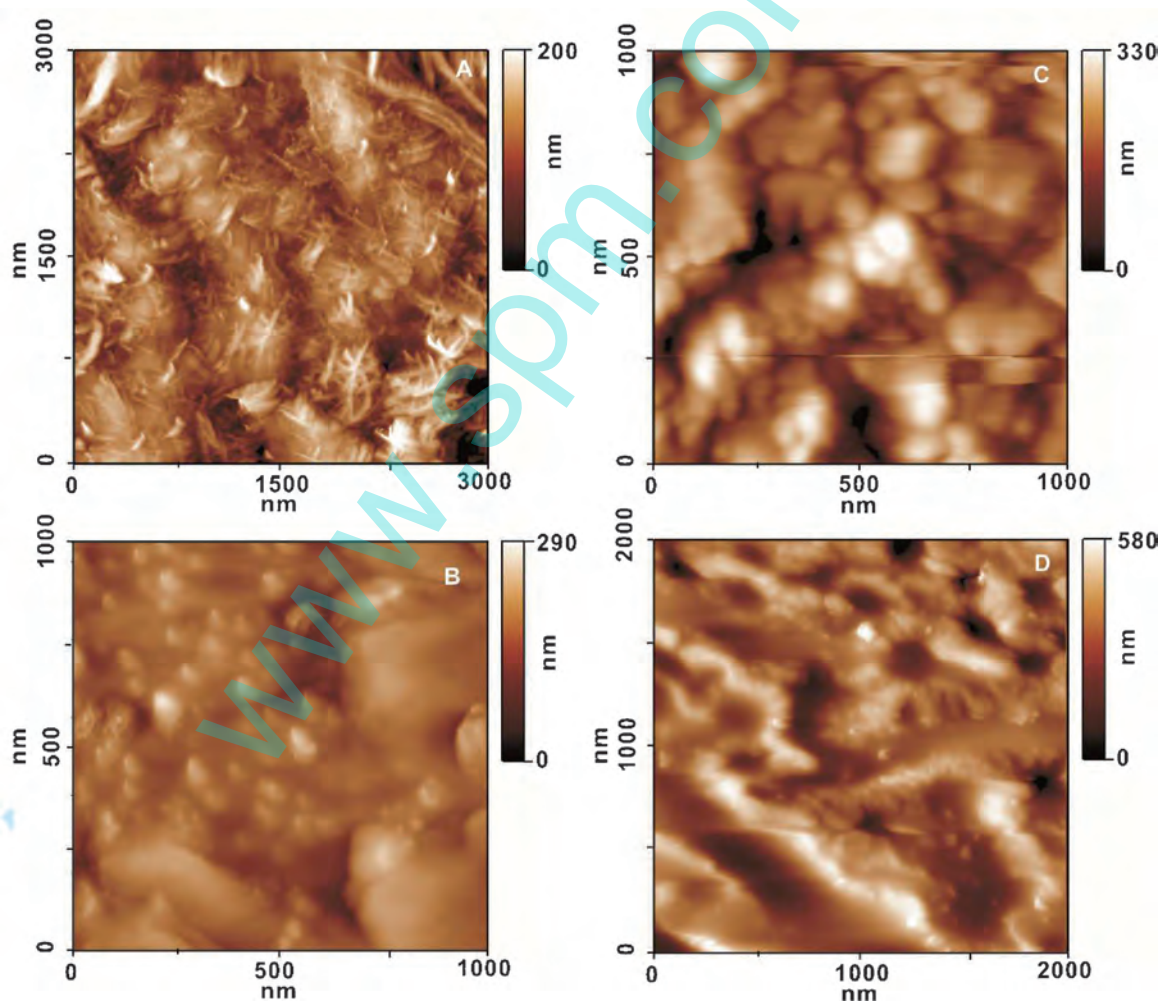


Fig. 1. AFM images of (A) MWCNT, (B) MWCNT-PLL, (C) Hb, and (D) MWCNT-PLL/Hb modified electrode on an ITO electrode.

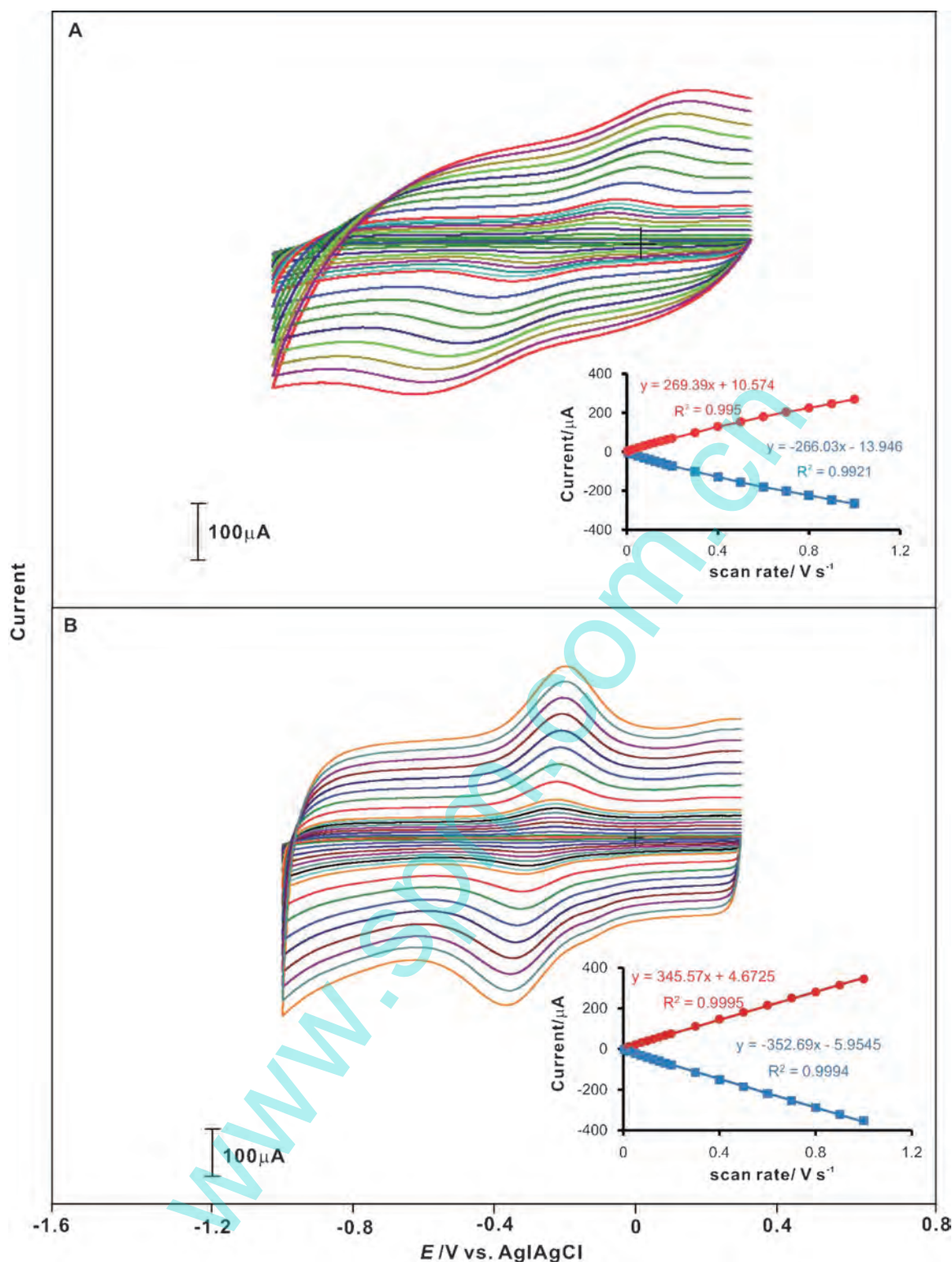


Fig. 2. Cyclic voltammograms of (A) MWCNT/Hb and (B) MWCNT-PLL/Hb in 0.1 M acetate buffer (pH 5.6) at different scan rates (inner to outer voltammograms correspond to 5, 10, 25, 50, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mV s^{-1}). Inset: Variation of anodic (I_{pa}) and cathodic (I_{pc}) peak currents with scan rate.

750 potentiostat. The morphologies of the films was examined using atomic force microscopy (AFM) (Being Nano-Instruments CSPM5000). All the experiments were performed at room temperature ($\approx 25^\circ \text{C}$).

2.3 Preparation of the Modified Electrode

It was obtained by dispersing 1.0 mg of MWCNTs within 1.0 mL of 1.0 mg/mL PLL solution (prepared in 50 : 50 v/v

ethanol/water) followed by sonication for 15 min. Prior to modification, GC electrode was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature. The cleaned GCE was coated with 5 μL of MWCNT-PLL dispersion and the solvent allowed evaporating at room temperature. After evaporating solvent, the inverted electrode was placed in a drying oven maintained at 80 °C for 5 min. The MWCNT-PLL modified electrodes was immersed in 0.1 M acetate buffer (pH 5.6) containing hemoglobin (1 mg hemoglobin/mL) and kept this at 4 °C for at least 12 h. The modified electrode was rinsed with supporting electrolyte and used for further investigation.

Repetitive redox cycling experiments were performed to determine the stability of MWCNT-PLL/Hb films in 0.1 M acetate buffer (pH 5.6). This investigation revealed that after 100 continuous scan cycles with a scan rate of 5 mVs^{-1} , the peak heights of the cyclic voltammograms decreased less than 5%. The MWCNT-PLL/Hb film maintained its initial activity for more than 2 weeks when stored in 0.1 M acetate buffer (pH 5.6). A decrease of 5% was observed in the current response of the electrode after 15 days.

3 Results and Discussion

3.1 Morphological Characterization of the Modified Electrode

The surface morphologies of the electrodes were also examined using AFM. The AFM results provided comprehensive information about the surface morphology of the nanostructures on the ITO surface. Prior to modification, the ITO surfaces were cleaned and ultrasonicated in an acetone–water mixture for 15 min and then dried. Subsequently, MWCNT, MWCNT-PLL, Hb and MWCNT-PLL/Hb films were prepared on the ITO. The AFM images were collected over 3000 \times 3000 nm to 1000 \times 1000 nm surface areas and used to determine the roughness average (s_a), an expression of the surface roughness or texture that is typically used to describe a polished or machined metal surface and the arithmetic average value of the departure (peaks and valleys) of the surface profile from the center-line through the sampling length. From Figure 1(A–D), significant morphological differences among the films can be observed. The MWCNT-modified electrode in (A) exhibited a rough surface with a roughness average (s_a) and root mean square roughness of 15.9 nm and 20.3 nm, respectively. The surface area ratio was 25.3. The MWCNT-PLL-modified electrode in (B) displayed a PLL coating over the entire MWCNT surface. The roughness average (s_a) and root mean square roughness of 6.11 nm and 7.91 nm, respectively. The surface area ratio was 17.6. The Figure 1 (C) displayed Hb-modified electrode on ITO surface. The roughness average (s_a) and root mean square roughness were 6.34 nm and

8.03 nm, respectively. The surface area ratio was 2.8. The Figure 1 (D) displayed MWCNT-PLL/Hb-modified electrode on ITO surface. The roughness average (s_a) and root mean square roughness 80.8 nm and 102 nm, respectively. The surface area ratio was 6.1. The MWCNT displayed excellent surface area ratio.

3.2 Electrochemical Characterization of the Modified Electrode

Figure 2 depicts the cyclic voltammograms of (A) MWCNT/Hb and (B) MWCNT-PLL/Hb composite film modified electrode in 0.1 M acetate buffer (pH 5.6) at different scan rates. It is noteworthy to mention that all voltammograms in the figure exhibit characteristics of hemoglobin in the potential region studied. In (B) can be noticed that a reversible redox couple with $E^{\circ'}$ value of around -265 mV was observed. The redox peaks corresponds to reduction and oxidation of $\text{Fe}^{3+/2+}$ present in Hb. As shown in the Inset, the anodic peak currents are proportional to scan rate plot yields a slope value of nearly unity in the entire scan rate studied. At 100 mVs^{-1} , (A) and (B) the values of peak-to-peak separation (ΔE_p) between anodic and cathodic peak were 202 mV and 76 mV. In addition, the ratio of anodic to cathodic peak current is nearly unity and the formal potentials of two sets of peaks are largely independent of scan rates. Such characteristics imply that the charge transport through the film was fast and the electrochemical behavior resembles that of surface confined redox species [26]. The electron transfer rate constant (K_s) have been calculated from these different scan rate results based on Laviron theory. Where, $K_s = 0.07$ and 0.21 s^{-1} for MWCNTs/Hb and MWCNTs-PLL/Hb composite film, respectively. From these K_s values the increase in the ability of electron transfer between the electrode surface and the Hb in presence of PLL. These results too show that, there is an enhancement in the functional properties of the composite film at lower scan rates in presence of both PLL and MWCNTs.

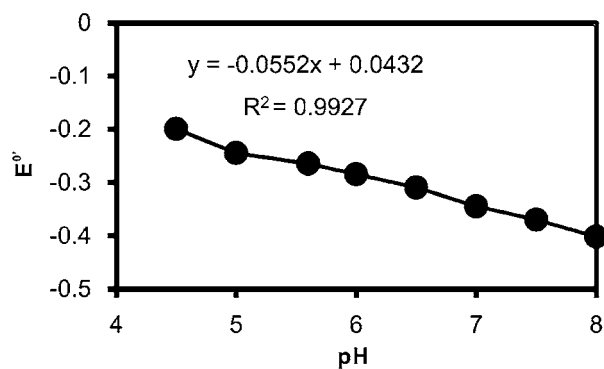


Fig. 3. The MWCNT-PLL/Hb composite film modified electrode in various pH solutions 4.5 to 8.0 of formal $E^{\circ'}$ vs. pH.

Figure 3 showed the MWCNT-PLL/Hb composite film modified electrode in various pH aqueous buffer solutions. This showed that the film is highly stable in the pH range between 4.5 to 8.0. The values of E_{pa} and E_{pc} depends on the pH value of the buffer solution. The re-

sponse shows a slope of -55 mV/pH , which is close to that given by the Nernstian equation for equal number of electrons and protons transfer.

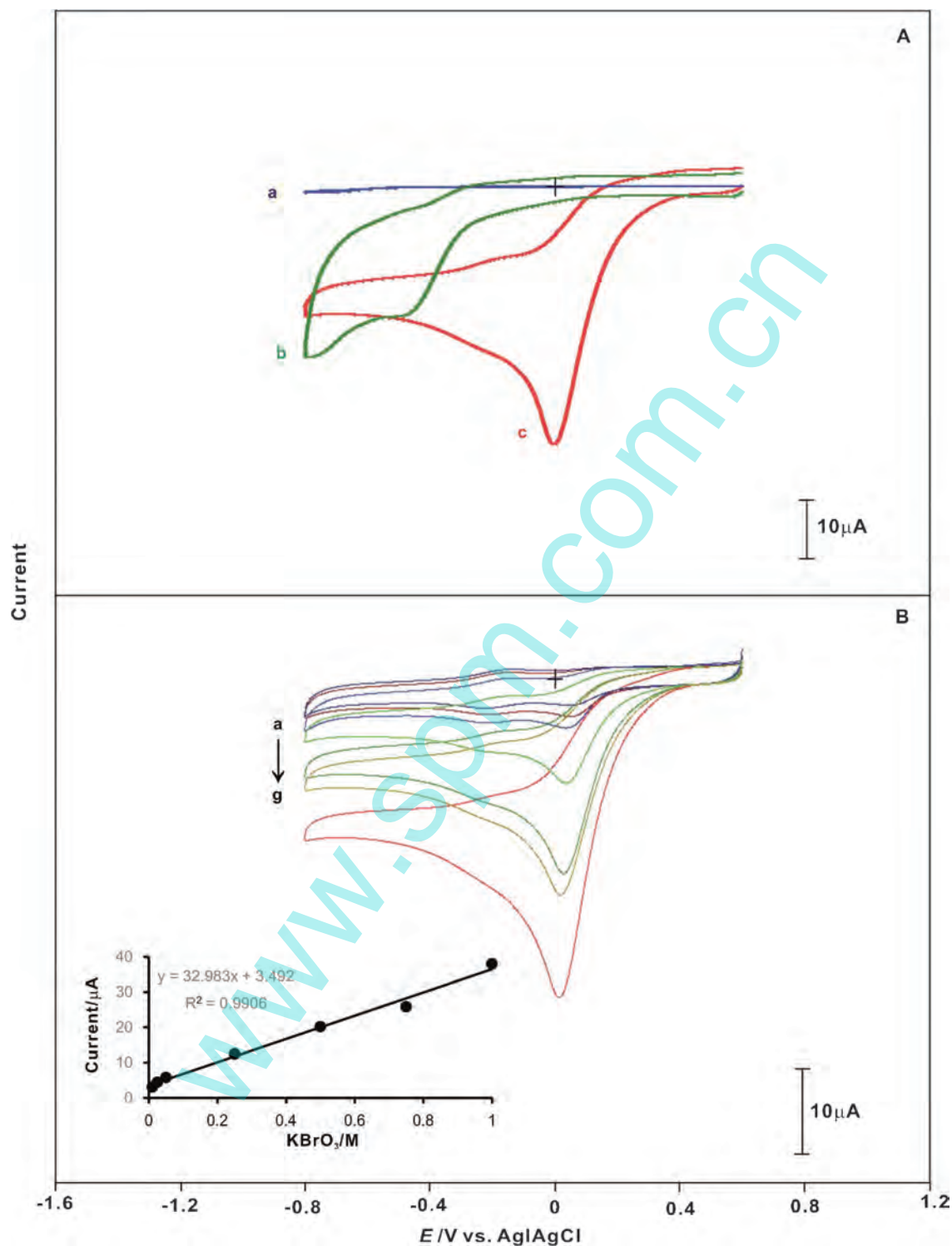


Fig. 4. (A) Cyclic voltammograms in 0.1 M acetate buffer (pH 5.6) containing 2 mM KBrO_3 for different electrodes: (a) bare GCE, (b) MWCNT-PLL, (c) MWCNT-PLL/Hb; scan rate = 10 mV s^{-1} . (B) Cyclic voltammograms of 0.1 M acetate buffer (pH 5.6) containing different concentrations of KBrO_3 at MWCNT-PLL/Hb electrode. Concentrations of KBrO_3 from (a) to (h) are 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75 and 1 mM, respectively; scan rate = 5 mV s^{-1} . Inset: Calibration curve.

3.3 Electrocatalytic Reduction of Bromate at the Modified Electrode

Figure 4A displays cyclic voltammogram of 2.0×10^{-3} M KBrO_3 reduction at different electrodes in 0.1 M acetate buffer (pH 5.6), scan rate 10 mV s^{-1} . Curve (a) in shows cyclic voltammogram of BrO_3^- reduction at unmodified GCE. It is clear that bromate reduction did not take place on bare GCE in the potential region studied. When the electrode was modified with MWCNT-PLL composite film, an ill-defined reduction peak was observed with a peak potential of around -0.5 V. On the other hand, a well-defined reduction peak was observed at MWCNT-PLL/Hb composite film modified electrode at lower negative potential region (0 V), indicating that the MWCNT-PLL/Hb composite film not only accelerate the bromate reduction but also reduced the overpotential of this irreversible reduction reaction. It can be noticed that a reversible redox couple with $E^{\circ'}$ value of around -265 mV was observed. The redox peaks corresponds to reduction and oxidation of $\text{Fe}^{3+/2+}$ present in Hb. Comparison of curve a, b and c reveals that BrO_3^- was electrocatalytically reduced at MWCNT-PLL/Hb composite film electrode. The electrocatalytic activity of modified electrode was further evaluated by investigating the effect of concentration. In order to confirm the electrocatalytic behavior of MWCNT-PLL/Hb composite modified electrode, current response of modified electrode with different concentration of bromate was recorded by cyclic voltammogram. As shown in Figure 4 (B), reduction current increases with increase in bromate concentration in 0.1 M acetate buffer (pH 5.6), scan rate 5 mV s^{-1} . The current response of linear range was 0.1 to 1 mM and sensitivity was $32.983 \mu\text{A mM}^{-1}$ ($R=0.99$) in inset. The limits of detec-

tion was 8.3×10^{-5} M. The results of concentration dependence study clearly showed that MWCNT-PLL/Hb composite modified electrode can effectively catalyze bromate reduction, and can be conveniently used for quantitative applications.

3.4 Optimization of Applied Potential for Bromate Determination

As amperometric experiment under stirred condition has higher sensitivity compared to cyclic voltammetry, this technique has been chosen to explore the application of MWCNT-PLL/Hb composite modified electrode for quantitative determination of bromate. In order to determine the optimum working potential for bromate determination, amperometric measurements were carried out at different potential (curve a to g) MWCNT-PLL/Hb composite film electrode by injecting $100 \mu\text{L}$ of 0.5 M KBrO_3 and the results are illustrated in Figure 5. It can be observed that the steady-state current increase with applied potential near 0 V. The potential of $+25$ mV was selected as the optimum working potential for amperometric detection of bromate.

3.5 Amperometric Determination of Bromate

Figure 6 shows a typical current-time plot for MWCNT-PLL/Hb composite film electrode at $+25$ mV on successive injection of different concentrations (2.4×10^{-6} to 6.5×10^{-3} M) of KBrO_3 into 0.1 M acetate buffer. When an aliquot of KBrO_3 is injected into buffer solution, the reduction current rises steeply to reach a stable value. The sensor achieves 95% of steady-state current in less than 5 s. Such a short response time indicates fast mass

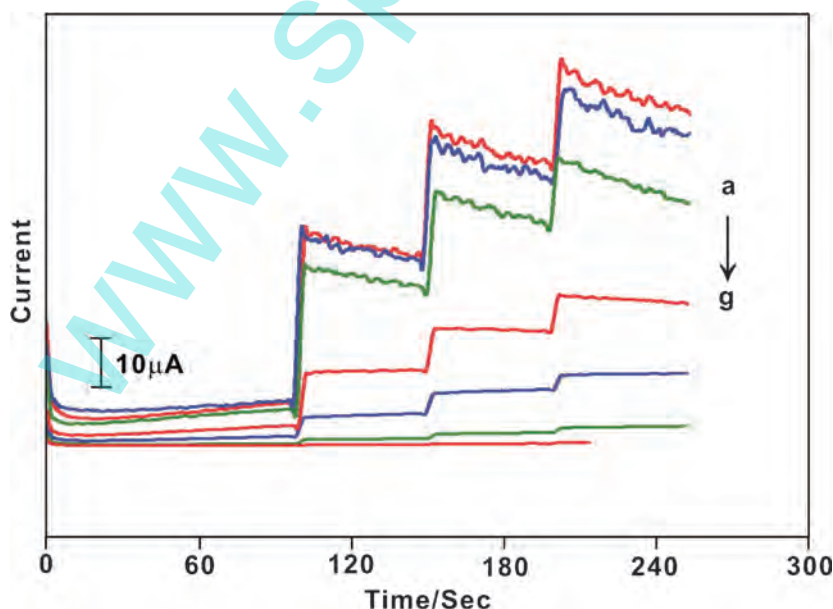


Fig. 5. Dependence of current response of MWCNT-PLL/Hb modified electrode upon successive injections of $100 \mu\text{L}$ of 0.05 M KBrO_3 to 10 mL 0.1 M acetate buffer (pH 5.6) on different applied potentials: a) -600 ; b) -400 ; c) -200 ; d) -50 ; e) 0; f) $+50$ and (g) $+100$ mV.

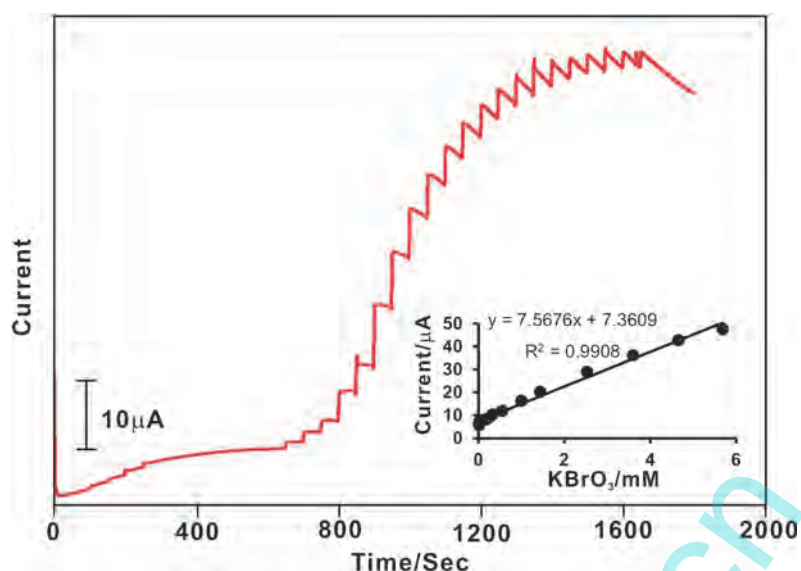


Fig. 6. Amperometric responses of MWCNT-PLL/Hb modified electrode upon successive injections of different concentrations (2.4×10^{-6} to 6.5×10^{-3} M) of KBrO_3 in 0.1 M acetate buffer (pH 5.6) under stirred condition; applied Potential = +25 mV. Inset: Calibration curve.

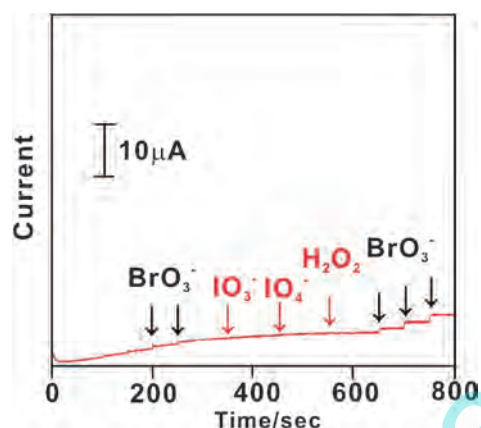


Fig. 7. The interferential experiment of the amperometric response of 2.5 μM bromate in 0.1 M pH 5.6 acetate buffer and after adding 10-fold KIO_3 , KIO_4 and H_2O_2 into buffer.

transfer across the film and also fast electron exchange between mediator and analyte. As demonstrated by Inset, the steady-state reduction current varies linearly with KBrO_3 concentrations in the lower concentration range and level-off at higher concentrations. The calibration curve was linear between 1.5×10^{-5} to 6.0×10^{-3} M. The sensitivity of MWCNT-PLL/Hb composite film electrode for bromate determination was $7.5679 \mu\text{A mM}^{-1}$ with a correlation coefficient of 0.99. The limits of detection was 9.6×10^{-7} M. The linear calibration range and sensitivity of MWCNT-PLL/Hb composite film electrode are comparable with those of other modified electrodes [27–31].

Figure 7 showed the interferential experiment of the fabricated bromate sensor was performed by comparing the amperometric response of 2.5 μM bromate before and

after adding some possible interferences into 0.1 M pH 5.6 acetate buffer. The 10-fold KIO_3 , KIO_4 and H_2O_2 not interfere with the determination of bromate.

3.6 Real Samples Analysis

Linear Sweep Voltammetry (LSV) measurement was used to test the practical applicability of proposed sensor for the determination of bromate from real samples. Figure 8 shows the experimental verification of the method on real samples. MWCNT-PLL/Hb composite film electrode at different concentrations (add (a) 1.0×10^{-5} to (g) 2.0×10^{-3} M) of KBrO_3 in mineral water (Ca 0.1–15.0 mg/L, Mg 0.1–15.0 mg/L, Na 0.1–10 mg/L, Fe < 0.2 mg/L, F < 0.5 mg/L, HCO_3^- 5–50 mg/L). The sensitivity of MWCNT-PLL/Hb composite film electrode for bromate determination was $43.85 \mu\text{A M}^{-1}$ with a correlation coefficient of 0.98.

4 Conclusions

We have demonstrated application of hemoglobin-incorporated multiwalled carbon nanotubes-poly-L-lysine composite modified electrode film modified glassy carbon electrode (MWCNT-PLL)/Hb for the amperometric determination of bromate. The charge transport through the film was fast and the voltammetric features of the modified electrode resemble that of surface-electrochemistry. The MWCNT-PLL-Hb composite film modified electrode showed excellent electrocatalytic activity towards bromate reduction and used as a simple electrochemical sensor for quantitative determination in amperometric mode. The linear region of the calibration curve spans about two orders between 1.5×10^{-5} to 6.0×10^{-3} M with a sensitivity of $7.5679 \mu\text{A mM}^{-1}$. Easy prepara-

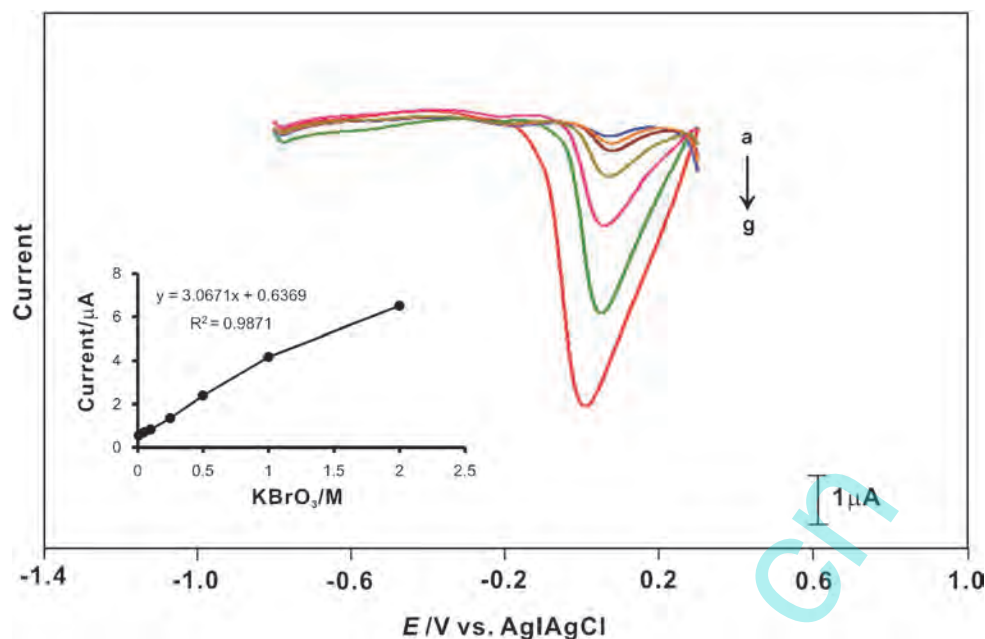


Fig. 8. Linear Sweep Voltammetry (LSV) of different concentrations (add (a) 1.0×10^{-5} to (g) 2.0×10^{-3} M) of KBrO_3 in mineral water (Ca 0.1–15.0 mg/L, Mg 0.1–15.0 mg/L, Na 0.1–10 mg/L, Fe <0.2 mg/L, F <0.5 mg/L, HCO_3^- 5–50 mg/L) at MWCNT-PLL/Hb composite film electrode.

ration of modified electrode makes MWCNT-PLL-Hb composite electrode film electrode as promising candidate for constructing simple electrochemical sensor for bromate.

Acknowledgement

The support of Visiting Professorship to SMC at King Saud University is gratefully acknowledged.

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Received: February 4, 2014
Accepted: February 25, 2014
Published online: April 3, 2014