Electrodeposited Graphene and Silver Nanoparticles Modified Electrode for Direct Electrochemistry and Electrocatalysis of Hemoglobin

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Abstract
By using a 1-butylpyridinium hexafluorophosphate based carbon ionic liquid electrode (CILE) as the working electrode, graphene (GR) nanosheets and silver nanoparticles (Ag NPs) were step by step electrodeposited on the surface of the CILE with potentiostatic method. The fabricated Ag/GR/CILE was used as a new platform for protein electrochemistry and hemoglobin (Hb) was immobilized on its surface with chitosan (CTS) as film forming material. In 0.1 mol/L phosphate buffer solution, a pair of well-defined and quasi-reversible redox peaks appeared on the CTS/Hb/Ag/GR/CILE with a formal peak potential of −0.202 V (vs. SCE) and a peak-to-peak separation (ΔE_p) of 68 mV, which indicated that direct electrochemistry of Hb was realized on the modified electrode. The results could be attributed to the synergistic effects of Ag NPs and GR nanosheets on the electrode surface, which provided a specific three-dimensional structure with high conductivity and good biocompatibility. The Hb modified electrode showed excellent electrocatalysis to the reduction of trichloroacetic acid in the concentration range from 0.8 to 22.0 mmol/L with a detection limit of 0.42 mmol/L (3σ). Moreover, the modified electrode exhibited favorable reproducibility, long term stability and accuracy, with potential applications in the third-generation electrochemical biosensor.

Keywords: Hemoglobin, Silver nanoparticles, Graphene, Carbon ionic liquid electrodes, Direct electrochemistry

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1 Introduction

Hemoglobin (Hb) is a typical multi-cofactor protein that has two heme-containing α- and β-dimmers, which is considered to be an ideal model protein for the investigation of direct electron transfer of heme proteins with advantages like commercial availability, moderate cost and well-documented structure. In general direct electron transfer of redox proteins is difficult to realize on conventional electrodes due to the deep embedment of the redox center of the proteins in polypeptide chain structures and the partly denaturation of proteins adsorbed on the surface of a bare electrode [1]. Thus great efforts have been devoted to design different supporting materials for the acceleration of the electron transfer rate. Various modifiers such as clay [2], ionic liquids [3], hydrogel polymers [4] and nanomaterials [5] have successfully been utilized to immobilize redox proteins and enhance the electron transfer rate effectively.

Different noble metal nanoparticles with high conductivity have been successfully used to construct electrochemical biosensors [6,7]. Among them silver nanoparticles (Ag NPs) that aroused growing interest due to their specific properties such as small granule diameter, large specific surface area, high catalytic activity, good biocompatibility and conductivity [8]. Ag NPs have been used in electrochemical sensors for electrode modification. The presence of Ag NPs exhibited a catalytic activity to the reduction of H_2O_2 with increase of the cathodic current. In protein electrochemistry Ag NPs can provide a microenvironment similar to that of the redox proteins in native systems and accelerate the electron transfer rate. Xu et al. fabricated a Hb-Ag sol modified glassy carbon electrode (GCE) for the electrochemical detection of H_2O_2 [9]. Gan et al. also applied a Hb and Ag NPs comodified pyrolytic graphite electrode for nitric oxide detection [10]. The Ag NPs can be prepared by different methods such as vacuum evaporation [11], self-assembly [12], LB membrane [13] and electrochemical deposition [14].

In recent years a new kind of carbon material, graphene (GR), has aroused great interests in different fields of chemistry due to its properties such as high surface area, excellent electrical conductivity, strong mechanical strength with functionalized groups, which render GR suitable for the fabrication of composite materials, sensors, fuel cells and so on [15]. The feasibility of GR in protein electrochemistry has also been studied recently.

Liu et al. realized the direct electrochemistry of Hb with functionalized GR sheets and ionic liquid (IL) composite films [16]. Wu et al. constructed a kind of GR-based electrochemical biosensors for the detection of nitric oxide [17]. Also nanocomposite films composed of GR with other nanomaterials have been used in protein electrochemistry with the hope of synergizing their exceptional properties. Xu et al. fabricated a GR-ZnO modified gold electrode for the electrochemistry of Hb [18]. Zhou et al. prepared an Au nanoparticles and GR nanocomposite modified GCE for the electrochemistry of horseradish peroxidase and the detection of H_2O_2 [19]. In general GR used for electrode modification is obtained by the chemical reduction of graphene oxide (GO) sheets, with some intrinsic limitations such as lack of control of the film thickness with toxic chemicals involved. Recently electrochemical reduction from GO to GR has drawn great attention with the advantage of a simple, efficient, low-cost and environmental friendly procedure. After depositing GO suspension on the electrode surface, electrochemical reduction can easily be realized with different electrochemical techniques such as cyclic voltammetry or potentiostatic method. Chen et al. [20] prepared an electrode directly from GO dispersions by cyclic voltammetry, which was used for the detection of hydroquinone and catechol. Guo et al. [21] synthesized GR through electrochemical reduction of exfoliated GO at cathodic potentials.

In this paper a carbon ionic liquid electrode (CILE) was prepared by using 1-butylpyridinium hexafluorophosphate ($BPPF_6$) as the binder and the modifier, which was further used as the basal electrode. Due to the specific characteristics of IL such as high chemical and thermal stability, relatively high ionic conductivity, good solubility, negligible vapor pressure and wide electrochemical windows [22], a CILE has been used as the substrate electrode in the electrochemical sensor, acting as a kind of high-performance working electrode like the traditional carbon electrode [23]. CILE displays many good features of different types of carbon electrodes with the advantages such as low cost, low background, simple preparation procedure, easy for surface modification and renewal, high sensitivity and selectivity with good antifouling ability. So it has widely been used in the fields of electrochemical sensors and biosensors [24]. The $BPPF_6$ based CILE has been used as the working electrode for the direct detection of electroactive substances or as the basal electrode for further modification. By using the CILE as the substrate electrode, GR and Ag NPs were further modified on the surface by electrodeposition method step by step to get a specific nanocomposite interface. Then Hb was cast onto the electrode surface with chitosan (CTS) as membrane material. The obtained electrode (denoted as CTS/Hb/Ag/GR/CILE) was used for the study of direct electrochemistry and electrocatalysis of Hb. Due to the biocompatibility and synergistic conductivity of Ag/GR nanocomposite, a favorable microenvironment can be formed for the retaining of the activities of immobilized Hb. Direct electron transfer of Hb was realized with its behaviors investigated carefully and the electrocatalysis of Hb modified electrode to the reduction of trichloroacetic acid (TCA) was further studied.

2 Experimental

2.1 Apparatus

All the electrochemical measurements including the potentiostatic reduction, cyclic voltammetry and electrochemical impedance spectra (EIS) were carried out on a CHI 750B electrochemical workstation (Shanghai Chenhua Instrument, China). A conventional three-electrode system was employed with the Hb modified electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire electrode as the auxiliary electrode. Scanning electron microscopy (SEM) was obtained by a JSM-7500F scanning electron microscope (Japan Electron Company, Japan). Ultraviolet-visible (UV-Vis) absorption spectrum was recorded on a Cary 50 probe spectrophotometer (Varian Company, Australia).

2.2 Reagents

1-Butylpyridinium hexafluorophosphate ($BPPF_6$, $>99\,\%$, Lanzhou Greenchem. ILS. LICP. CAS., China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Co., China), $AgNO_3$ (Sinopharm Chemical Reagent Ltd. Co., China), hemoglobin (Hb, Tianjin Chuanye Biochemicals Ltd. Co., China), chitosan (CTS, Dalian Xindie Limited Co., China) and trichloroacetic acid (TCA, Tianjin Kermel Chemical Reagent Ltd. Co., China) were used as received. Graphene oxide (GO) was synthesized by the modified Hummer's method [25,26]. 0.1 mol/L phosphate buffer solution (PBS) was used as the supporting electrolyte. The stock solution of Hb (10.0 mg/mL) was prepared by dissolving a suitable amount of Hb into 0.1 mol/L PBS and 1.0 mg/mL CTS solution was prepared with 1 % acetic acid. All the reagents were of analytical reagent grade and double distilled water was used throughout the experiments. The solutions in the electrochemical cell were bubbled with high purity nitrogen for 30 min before measurements and a nitrogen environment was maintained over the solution during the measurements.

2.3 Preparation of Modified Electrodes

Based on the reported procedure [27] CILE was fabricated by mixing 3.0 g graphite powder and 1.0 g $BPPF_6$ thoroughly in a mortar and heated at 80 °C to form a homogeneous carbon paste, which was filled into one end of a glass tube ($\varnothing = 4$ mm) with a copper wire inserted through the opposite end to establish an electrical contact. Prior to use a mirror-like surface was obtained by polishing the electrode on a weighing paper.

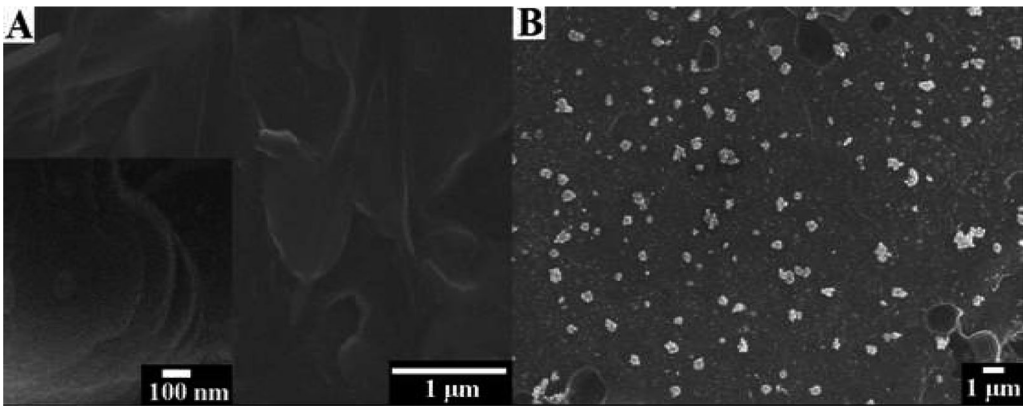

Fig. 1. SEM images of GR/CILE with different amplified multiples (A) and Ag/GR/CILE (B).

Electroreduction of GO had been reported to be an efficient way to fabricate the GR modified electrode, so the GR modified CILE was prepared based on a reported procedure with potentiostatic method [21]. Briefly, a freshly prepared CILE was placed in the 1.0 mg/mL GO dispersion solution with magnetic stirring and N_2 bubbling. After applying a potential of −1.3 V for 300 s, rinsing with doubly distilled water and drying in nitrogen atmosphere, a stable electrochemical reduced GR film formed on the surface of the CILE, and the modified electrode was denoted as GR/CILE. Next, electrodeposition of Ag NPs on the GR/CILE was further performed in a 0.5 mol/L KNO_3 solution containing 5.0 mmol/L $AgNO_3$ and potentiostatically fixed at −0.7 V for 150 s without stirring. The Ag/GR/CILE was thoroughly washed with doubly distilled water and dried in nitrogen atmosphere. Then 8.0 μL of 10.0 mg/mL Hb solution was dropped on the freshly prepared Ag/GR/CILE and dried in the air. Finally, 5.0 μL of 1.0 mg/mL CTS solution was coated onto the electrode surface to acquire the composite film modified CILE (CTS/Hb/Ag/GR/CILE). For comparison other electrodes were also prepared with the same procedure described above and all the modified electrodes were stored at 4 °C in a refrigerator when not in use.

3 Results and Discussion

3.1 Characterization of Modified Electrodes

Scanning electron microscopy (SEM) was used to illustrate the morphologies of GR/CILE and Ag/GR/CILE. It can be seen that crumpled and wrinkled flake-like structures could be observed on the electrode surface, indicating the forming of GR nanosheet on the CILE surface (Figure 1A). The enlarged SEM image further proved that the clearly layered structure appeared (inset of Figure 1A), which are the typical sheets of GR. On the Ag/GR/CILE (Figure 1B) many small nanoparticles appeared with sizes in the range from 50 nm to 100 nm and distributed uniformly on the electrode surface, indicating successfully deposition of the Ag NPs on the GR/CILE surface.

Electrochemical impedance spectroscopic (EIS) measurements are often used to monitor the interface changes of modified electrodes during the modification process. The Nyquist plot contains a semicircular part and a linear part. The semicircular part at higher frequencies corresponds to the electron-transfer-limited process and its diameter is equal to the electron transfer resistance (R_{et}), which controls the electron transfer kinetics of the redox probe at the electrode interface, while the linear part at lower frequencies corresponds to the diffusion-limited process. EIS was performed in a solution containing 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ with a frequency range from 10^4 Hz to 0.1 Hz. Figure 2 showed the Nyquist plots of CTS/CILE, CTS/Ag/CILE, CTS/GR/CILE, CTS/Ag/GR/CILE and CTS/Hb/Ag/GR/CILE, respectively. It can be seen that the R_{et} value of CTS/CILE (curve a) was 150.5 Ω. After electrodeposition of Ag NPs or GR on the surface of CILE, the R_{et} values of CTS/Ag/CILE (curve b) and CTS/GR/CILE (curve c) were 78.05 Ω and 21.69 Ω, respectively. The values were smaller than that of CTS/CILE, indicating that successful deposition of high conductive Ag NPs or GR on the electrode surface. While on the CTS/Ag/GR/CILE the smallest R_{et} value (11.15 Ω) can be obtained, indicating the fastest electron transfer rate of $[Fe(CN)_6]^{3-/4-}$. The result proved that Ag NPs were deposited not only on the surface of GR but also inside the GR nanosheets, which resulted in a most conductive interface. On CTS/Hb/Ag/GR/CILE the R_{et} value increased to 98.8 Ω (curve e), indicating the presence of Hb on the electrode surface hindered the electron transfer rate of the redox probe. The result also suggested that Hb molecules were successfully trapped on the outer layer of the Ag/GR film.

3.2 Direct Electrochemistry of Hb

Cyclic voltammetric results of different modified electrodes in N_2 saturated pH 3.0 PBS were studied with the results shown in Figure 3. No current response appeared in the scanned potential range for CTS/CILE (curve a), in-

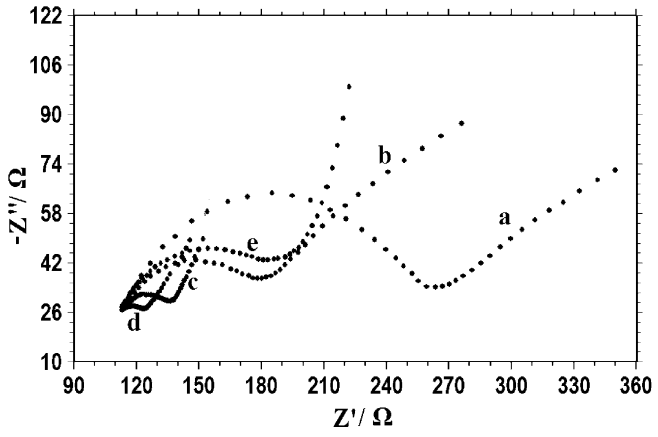

Fig. 2. Electrochemical impedance spectroscopy for (a) CTS/CILE, (b) CTS/Ag/CILE, (c) CTS/GR/CILE, (d) CTS/Ag/GR/CILE and (e) CTS/Hb/Ag/GR/CILE in the presence of 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl with frequencies swept from 10^4 to 0.1 Hz

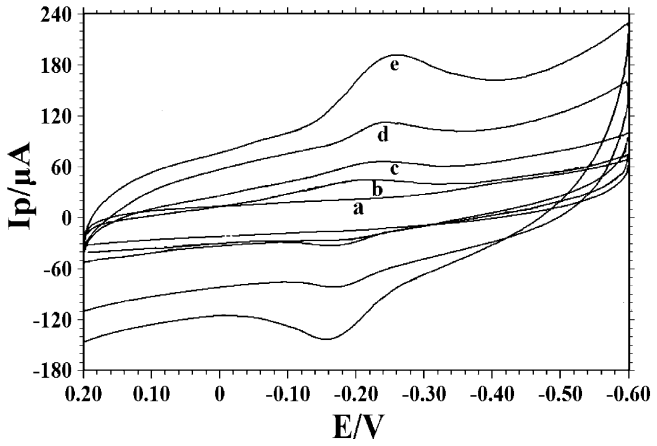

Fig. 3. Cyclic voltammograms of (a) CTS/CILE, (b) CTS/Hb/CILE, (c) CTS/Hb/Ag/CILE, (d) CTS/Hb/GR/CILE and (e) CTS/Hb/Ag/GR/CILE at a scan rate of 100 mV/s in pH 3.0 PBS.

dicating no redox substance present on the electrode surface. With the incorporation of Hb molecules on the electrode, a pair of redox peaks appeared with different responses, indicating that the direct electron transfer of Hb could be realized on the electrode with different electron transfer rates. On the CTS/Hb/CILE (curve b) a pair of small redox peaks appeared, indicating that the direct electron transfer was slow between Hb and the bare CILE. Meanwhile, on the CTS/Hb/Ag/CILE (curve c) and CTS/Hb/GR/CILE (curve d), the redox peak increased gradually, indicating that the presence of electrodeposited Ag NPs or GR nanosheets could accelerate the electron transfer rate between Hb and the underlying electrode. Electrodeposition is an effective method for electrode modification. The Ag NPs or GR can be easily electrodeposited on the CILE surface and resulted in a high conductive interface, which could accelerate the electron transfer rate. On the CTS/Hb/Ag/ GR/CILE (curve e) the largest redox peaks appeared, which implied that it was the best interface suitable for direct electron transfer of Hb. It might be mainly ascribed to the Ag/GR composite film formed after GR and Ag NP were deposited. The synergistic effects of GR and Ag NPs on the electrode surface could greatly increase the interface conductivity and promote direct electron transfer between Hb and the electrode. The cathodic (E_{pc}) and anodic peak potential (E_{pa}) were obtained as −0.236 V and −0.168 V (vs. SCE), respectively. The formal peak potential ($E^{\circ\prime}$) was estimated to be −0.202 V, which was the typical characteristic of Hb heme redox couple [28]. The peak-to-peak separation (ΔE_p) was 68 mV and the ratio of the anodic current and the cathodic one was nearly a unit, implying that the entrapped Hb underwent a nearly reversible electrochemical reaction. These results suggested that the electron transfer between the redox center of Hb and the modified electrode was effectively promoted through the combination effects of Ag NPs and GR nanocomposite, which provided a friendly microenvironment for the Hb molecules to establish a favorable orientation on the electrode surface with fast electron transfer.

3.3 Effect of Scan Rate

The influence of scan rate on the cyclic voltammetric responses of the immobilized Hb was further investigated and the results were shown in Figure 4A. A pair of well-defined redox peaks appeared at different scan rates and the redox peak current increased gradually with scan rate in the range from 50 to 500 mV/s. As shown in Figure 4B two linear regression equations were got as $I_{pa}(\mu A) = -91.29\ v\ (V/s) - 0.426$ ($n = 14$, $\gamma = 0.996$) and $I_{pc}(\mu A) = 176.1\ v\ (V/s) - 1.753$ ($n = 14$, $\gamma = 0.998$). The results implied that the electrode reaction was a surface-confined electrochemical process, in which all the electroactive heme Fe(III) of Hb was reduced to heme Fe(II) on the forward cathodic scan and then converted to heme Fe(III) in the reverse anodic scan. The surface concentration (Γ^*, mol/cm^2) of electroactive Hb on CTS/Hb/Ag/GR/CILE can be estimated according to the Faraday's law of $Q = nFA\Gamma^*$, where Q is the charge involved in the reaction, n is the number of electrons transferred, F is the Faraday's constant, and A (cm^2) is the area of the electrode. By integration of cyclic voltammetric curves, the charge value (Q) was nearly constant in the scan rate range from 50 to 500 mV/s. The average surface concentration (Γ^*) of electroactive Hb was calculated as 1.29×10^{-9} mol/cm^2, which was much higher than that of the theoretical monolayer coverage (2.0×10^{-11} mol/cm^2), indicating that multilayers of Hb immobilized on the surface of Ag/GR/CILE could participate in the electron transfer process. While the total amount of Hb cast on the electrode surface was 9.87×10^{-9} mol/cm^2, so 13.07 % of total Hb on the electrode surface took part in the electrochemical reaction. The result can be attributed to the existence of Ag NPs and GR nanocomposite providing a large sur-

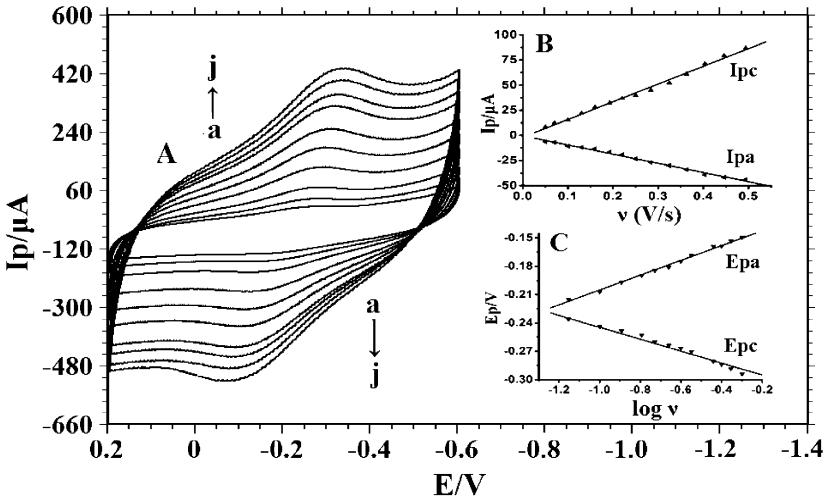

Fig. 4. Cyclic voltammograms on CTS/Hb/Ag/GR/CILE in pH 3.0 PBS at scan rates of 50, 100, 150, 180, 200, 250, 300, 350, 400 and 500 mV/s (from a to j). (B) Linear relationship of the cathodic peak current (I_{pc}) and anodic peak current (I_{pa}) versus scan rate (v); and (C) Linear relationship of the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) versus log v.

face area and three-dimensional architecture for Hb immobilization and promoting the electron transfer of Hb.

Also the redox peak potential varied with increasing scan rate. Two regression equations were plotted as $E_{pc}(V) = -0.0831 \log v - 0.307$ ($n = 13$, $\gamma = 0.998$) and $E_{pa}(V) = 0.0783 \log v - 0.126$ ($n = 13$, $\gamma = 0.997$) (as shown in Figure 4C). According to Laviron's equations:

$$E_{pc} = E^{\circ\prime} - \frac{2.3RT}{\alpha nF}\log v$$

$$E_{pa} = E^{\circ\prime} + \frac{2.3RT}{(1-\alpha)nF}\log v$$

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nFv} - \frac{(1-\alpha)\alpha Fn\Delta Ep}{2.3RT}$$

where v is the scan rate, n is the electron transfer number, α is the charge transfer coefficient, k_s is the electron transfer rate constant and R, F and T have their common meanings. The values of n, α and k_s were estimated to be 1.23, 0.49 and 1.03 s^{-1}, respectively. This k_s value is higher than those previously reported on TiO_2 modified graphite electrode (0.62 s^{-1}) [29], Ag-AgO modified silver electrode (0.239 s^{-1}) [30], Au colloid-cysteamine modified gold electrode (0.49 s^{-1}) [31], CTS-multiwall carbon nanotubes-Au NPs modified gold electrode (0.74 s^{-1}) [32] and similar to that of electrodeposited sol-gel SiO_2 thin film modified GCE (1.2 s^{-1}) [33]. Also under the same conditions, the values of k_s on different electrode such as CTS/Hb/CILE, CTS/Hb/Ag/CILE and CTS/Hb/GR/CILE were calculated as 0.15, 0.33, 0.51 s^{-1}, respectively. These results suggested that the electron transfer process between the Hb molecules and Ag/GR/CILE was very fast. Because the electron transfer rate in protein molecule between the donator and acceptor decreases exponentially with the distance of the electron transfer centers [34]. On the Ag NPs and GR nanosheets decorated CILE the distance between the active site of Hb and the modified electrodes was smaller than the original distance between the protein and the bare electrode surface due to the presence of a nanocomposite with three-dimensional structures [35], so the electron transfer rate was increased significantly. The presence of the Ag/GR composite film was an excellent promoter to establish a fast electron transfer rate between the redox center of immobilized Hb and the electrode interface.

Furthermore, the influence of buffer pH on the redox peak currents of CTS/Hb/Ag/GR/CILE was studied in the pH range from 2.0 to 7.0. The increase of buffer pH led to a negative shift of both reduction and oxidation peak potentials, which indicated that protons are involved in the electrode reaction. The maximum redox peak currents appeared at pH 3.0 buffer solution, which was selected for the further investigation.

3.4 Spectroscopic Result

The UV-Vis absorption spectrum was used for monitoring the possible denaturation on the tertiary structure of Hb. Figure 5 showed the UV-Vis spectra of Hb in double distilled water (curve a) and pH 3.0 PBS (curve b), respectively. It can be seen that the Soret absorption band of Hb appeared at the same wavelength of 406 nm in different solutions, suggesting that the native structure of Hb was not changed in pH 3.0 PBS.

3.5 Electrocatalytic Activity Towards TCA

The electrocatalytic behavior of CTS/Hb/Ag/GR/CILE towards the reduction of TCA was investigated. Figure 6 showed the cyclic voltammograms of the Hb modified

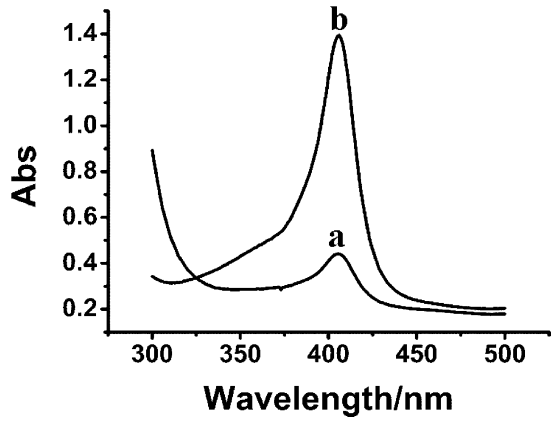

Fig. 5. UV-Vis absorption spectra of 0.03 mg/mL Hb in double distilled water (a) and 0.09 mg/mL Hb in 0.1 mol/L pH 3.0 PBS (b).

electrode in 0.1 mol/L deoxygenated PBS containing different amounts of TCA. With the increase of TCA concentration in the solution a significant increase of the reduction peak current was observed at −0.22 V with the disappearance of the oxidation peak, demonstrating a typical electrocatalytic reduction reaction. According to the Reference [36], the Hb Fe(II) was oxidized to Hb Fe(III) by TCA again in the process of electrode reaction. With increasing TCA concentration in the PBS solution, the emergence of the new reduction peak at a −0.49 V may result from the high reduced Hb Fe(I) causing TCA to set free chlorine again. The electrocatalytic mechanism is proposed as follows:

$$\text{Hb Fe(III)} + e \rightarrow \text{Hb Fe(II)} \quad (1)$$

$$2\text{Hb Fe(II)} + Cl_3CCOOH + H^+ \rightarrow 2\text{Hb Fe(III)} + Cl_2CHCOOH + Cl^- \quad (2)$$

$$\text{Hb Fe(II)} + e \rightarrow \text{Hb Fe(I)} \quad (3)$$

$$2\text{Hb Fe(I)} + Cl_2CHCOOH + H^+ \rightarrow 2\text{Hb Fe(II)} + ClCH_2COOH + Cl^- \quad (4)$$

$$2\text{Hb Fe(I)} + ClCH_2COOH + H^+ \rightarrow 2\text{Hb Fe(II)} + CH_3COOH + Cl^- \quad (5)$$

The catalytic reduction peak currents increased with the TCA concentration in the range from 0.8 to 22.0 mmol/L with a linear regression equation of $I_{pc}(\mu A) = 12.70C$ (mmol/L) + 9.620 ($n = 18$, $\gamma = 0.996$) and a detection limit of 0.42 mmol/L (3σ). The detection limit was smaller than that of Nafion-CdS-Hb modified CILE (10.0 mmol/L) [37] and {poly-diallyldimethylammonium/Hb}$_8$ films modified PGE (1.98 mmol/L) [38], indicating the higher sensitivity of the modified electrode. When the TCA concentration was larger than 22.0 mmol/L, the peak current leveled off to a plateau, indicating a typical Michaelis–Menten kinetic mechanism. So the apparent Michaelis–Menten constant (K_M^{app}) was further calculated from the electrochemical version of the Lineweaver–Burk equation [39], which can be used to evaluate the catalytic activity of the immobilized Hb. Based on the equation of $1/I_{ss} = 1/I_{max} + K_M^{app}/I_{max}C$, where I_{ss} is the steady current after the addition of substrate, C is the bulk concentration of the substrate, and I_{max} is the maximum current measured under saturated substrate condition. The value of K_M^{app} and I_{max} can be obtained from the plot of the reciprocal of the steady-state current and TCA concentration. So the value of K_M^{app} was calculated as 10.2 mmol/L, which was smaller than that on agarose film modified GCE [40].

3.6 Stability and Reproducibility

The stability of CTS/Hb/Ag/GR/CILE was investigated. The modified electrode was evaluated by examining the cyclic voltammetric peak currents after continuous scan-

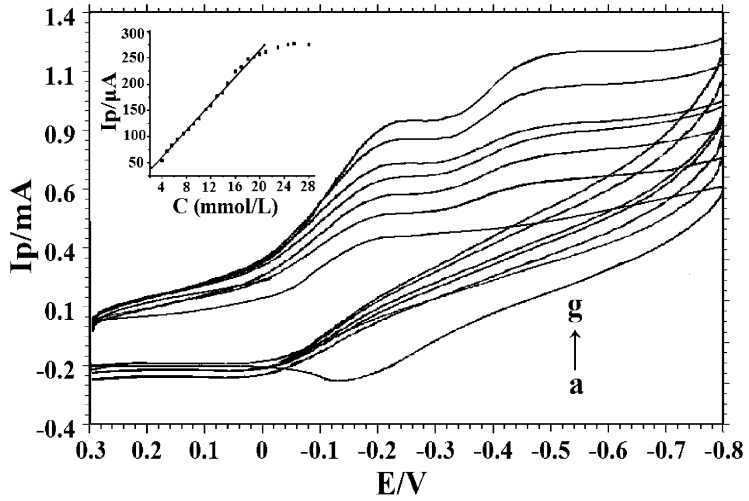

Fig. 6. Cyclic voltammograms obtained at a CTS/Hb/Ag/GR/CILE in pH 3.0 PBS with addition of 0, 1.6, 4.8, 8.6, 12.0, 16.0, 24.0 mmol/L TCA (from a to g), respectively. Scan rate: 100 mV/s. Inset: Plot of catalytic peak currents versus concentrations of TCA.

ning for 100 cycles. There was no obvious decrease of the voltammetric response, indicating that CTS/Hb/Ag/GR/CILE was stable in buffer solution. Also, the electrode was stored at 4 °C when it was not use. The electrode could retain 96.1 % of its initial current response after 1 week storage and 93.6 % after 2 weeks storage, demonstrating a good stability. The modified electrode was applied to the detection of 18.0 mmol/L TCA seven times with the a RSD value of 3.6 %, indicating a good reproducibility.

4 Conclusions

By using electrodeposition technique, a convenient method for the preparation of Ag NP and GR nanosheet modified CILE was established, which was further used as the platform for the construction of a Hb biosensor. The high conductive Ag/GR nanocomposite on the CILE surface can provide a favorable microenvironment for the immobilization of Hb. Direct electrochemistry of Hb on the modified electrode was achieved with fast electron transfer rate, which was attributed to the specific characteristics of GR nanosheets and Ag NPs. The proposed biosensor can be used to determine TCA in the concentration range from 0.8 to 22.0 mmol/L with a detection limit of 0.42 mmol/L. So the fabricated electrode was suitable for the investigation of the direct electrochemistry of redox proteins and acted as the third-generation electrochemical bisensors.

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