Electrochemistry of Hemoglobin-Ionic Liquid-Graphene-SnO$_2$ Nanosheet Composite Modified Electrode and Electrocatalysis

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A composite that consisted of SnO$_2$ nanosheet, graphene (GR), ionic liquid (IL) and hemoglobin (Hb) was modified on carbon ionic liquid electrode (CILE). By using Nafion film for fixing, the modified electrode was named as Nafion/Hb-IL-GR-SnO$_2$/CILE. Spectroscopic data indicated that Hb molecules kept native structure within the composite. Cyclic voltammetric data gave a pair of well-defined redox peak in 0.1 mol L^{-1} phosphate buffer solution, proving that direct electron transfer of Hb in the composite with CILE was realized. The result was due to synergistic effects of the materials used, which fastened the electron transfer rate. Nafion/Hb-IL-GR-SnO$_2$/CILE had excellent electrocatalytic ability to the reduction of trichloroacetic acid in a linear concentration range from 2.0 to 11.0 mmol L^{-1} with a detection limit of 0.615 mmol L^{-1}. The modified electrode was valuable for the preparation of a third-generation electrochemical biosensor.

1. INTRODUCTION

Electrochemistry of redox proteins has attracted many attentions due to the important theoretical and practical significance on understanding the electron transfer mechanism [1]. Also the results can be used for the construction of biosensors and biodiagnostic equipments [2]. In general the electron transfer efficient is low due to the deep burying of the electroactive center inside the biomolecules. Therefore various modified electrodes have been used for the enhancement of the electron transfer rate [3]. Among them nanomaterials based electrodes have been the research focus

due to their unique properties including high electrocatalytic activity, large surface area and good biocompatibility [4].

As a carbon nanosheet with many specific advantages, graphene (GR) has been used in the electrode modification [5, 6]. GR and its related composite exhibit the properties including large surface area, high conductivity and synergistic effects, which can be used in the different fields including electrochemistry and sensors [7, 8]. Nanosized SnO_2 exhibits unique characteristics such as large surface area and special morphology, which has been used in electrochemical sensor, solar cell, smart gas sensor and supercapacitor [9, 10]. By combined GR with SnO_2 together, the hybrid material shows many applications in optoelectronics and electrochemical devices [11]. GR-SnO_2 nanocomposite has also been used in the electrode modification and electrochemical application. Yao et al. applied GR-SnO_2 composite modified electrode for lithium-ion batteries [12]. Li et al. applied GR-SnO_2 nanocomposite for the electrochemical supercapacitors [13]. Sun et al. fabricated GR-SnO_2 modified electrode for sensitive electrochemical detection of dopamine [14].

Ionic liquids (IL) are organic substances with high ionic conductivity, good adhesive ability and wide electrochemical windows, which are often used in electrochemistry [15]. IL can be used as the modifier on electrode, or the electrolyte in electrochemical measurement [16]. The presence of IL in the composite can bind them together with good stability and fast electron transfer rate, which is benefit for the electrochemical applications. Ding et al. applied IL as the electrolyte for the direct electrochemistry of myoglobin [17]. Niu et al. used TiO_2 nanowire and IL modified carbon paste electrode for the voltammetric detection of metol [18]. Lv et al. applied IL-TiO_2-graphene oxide nanocomposite for the dopamine detection [19].

In this paper IL was used for the construction of carbon ionic liquid electrode (CILE), which was used as the substrate electrode for the modification. IL was also added in the composite to adhere the materials together with the enhanced effect. Hemoglobin (Hb) was mixed with SnO_2 nanosheet, GR and IL to obtain a biocomposite, which was used for the construction a Hb modified electrode with direct electrochemistry of Hb realized. This electrochemical Hb sensor exhibited excellent electrocatalytic ability to trichloroacetic acid (TCA) reduction.

2. EXPERIMENTAL

2.1 Apparatus and reagents

Ionic liquid 1-ethyl-3-methyl-imidazolium tetrafluoroborate ([EMIM]BF_4) and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF_4) were purchased from Lanzhou Yulu Fine Chemical. Co. Ltd.. Nafion (5% ethanol solution, Sigma), GR (Nanjing XFNano Materials Tech. Ltd. Co., China) and graphite powder (30 μm, Shanghai Colloid Chem., China) were used as received. 0.1 mol L^{-1} phosphate buffer solutions (PBS) were used as the supporting electrolyte. SnO_2 nanosheet was synthesized based on the reference [20]. Other chemicals were of analytical reagent with doubly distilled water used in the experiments.

Electrochemical investigations were performed on CHI 440A electrochemical workstation (Shanghai Chenhua Instrument, China). A three-electrode model was employed with a Hb based electrode as working electrode. A platinum wire was auxiliary electrode and a saturated calomel electrode (SCE) was reference electrode. FT-IR spectrum and UV-Vis absorption spectrum were got on Tensor 27 FT-IR spectrophotometer (Bruker, Germany) and Cary 50 probe spectrophotometer (Varian Company, Australia). Scanning electron microscopy (SEM) was recorded with a JSM-6700F scanning electron microscope (Japan Electron Company, Japan).

2.2. Preparation of the modified electrode

[EMIM]BF_4 modified carbon paste electrode (CILE) was prepared with the following procedure [21]. In brief 150 μL of EMIMBF_4, 850 mL of liquid paraffin and 3.2 g of graphite powder were hand-mixed in an agate mortar to get a homogenous paste. Then it was inserted into a glass electrode tube (Φ=4 mm) with copper wire as electric contact. The surface of CILE was smoothed on a piece of weighing paper just before use. A homogeneously mixture contained 20 mg mL^{-1} Hb, 1 mg mL^{-1} GR, 1 mg mL^{-1} SnO_2 and 15% [BMIM]BF_4 were modified on CILE. After dried 8 μl 0.5% Nafion solution was drop-coated on the electrode surface. During these steps a bottle was put tightly on the electrode to make sure the slowly evaporation of solvent with a uniform film formed. The modified electrode was named as Nafion/Hb-IL-GR-SnO_2/CILE and stored at 4 °C refrigerator. Other modified electrodes used in the experiments were prepared for comparison.

2.4. Procedure

All the voltammetric measurements were carried out at the ambient temperature (20±2 °C). PBS was purged with N_2 for 30 min to deoxygenate and the experiments were done at N_2 atmosphere. The mixture of Hb-IL-GR-SnO_2 was casted on a glass slide and dried for FT-IR experiments. UV-Vis spectroscopy were recorded with a Hb, [BMIM]BF_4, GR and SnO_2 nansheet mixture colution.

3. RESULTS AND DISCUSSION

3.1 Spectroscopic results

Spectroscopy is a conformational probe for checking the secondary structure of heme proteins. The change of Soret band can give the data about the possible denaturation of the redox proteins [22]. As show in Fig. 1A, the native Hb in water gave a Soret band at 406 nm (Fig. 1Aa), which was the same as that of Hb mixed with IL-GR-SnO_2 (Fig. 1Ab). FT-IR spectroscopy can also be used for structural identification [23]. As shown in Fig. 1B, FT-IR spectra of amide I and II bands of Hb (1648 and 1568 cm^{-1}, curve a) was almost the same as that of Hb with IL-GR-SnO_2 composite (1647 and 1568 cm^{-1}, curve b). The similarities of spectra indicated that Hb kept its native structure within IL-GR-SnO_2 composite, which was due to the biocompatibility of the material used.
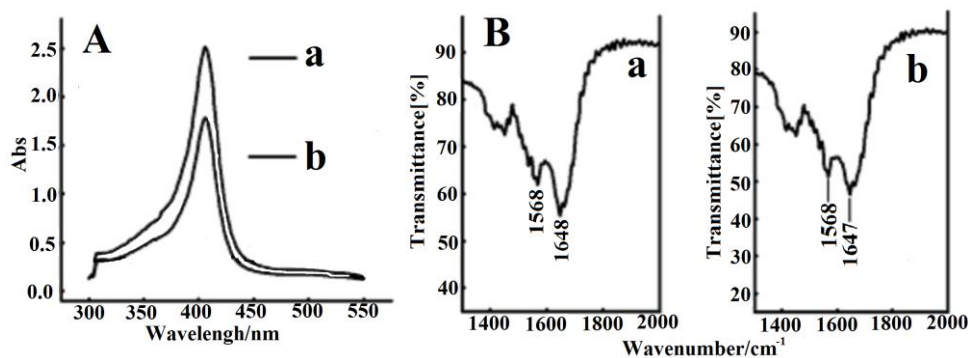
Figure 1. (A) UV-Vis absorption spectra of Hb in water (a) and Hb-IL-GR-SnO$_2$ mixture (b); (B) FT-IR spectra of (a) Hb and (b) Hb-IL-GR-SnO$_2$ film.

3.2 Electrochemical characteristics of the modified electrodes

Cyclic voltammetric investigation of ferricyanide on different electrodes is a convenient tool to record the electrode modification. As shown in Fig. 2, a pair of well-defined redox responses were got on CILE (curve c), which was the typical characteristic of CILE due to its excellent electrochemical performance [24]. On Nafion/Hb/CILE the redox responses decreased greatly (curve d), indicating the presence of Hb molecules on the electrode surface prevented the electron transfer. On Nafion/Hb-GR-SnO$_2$/CILE the redox responses increased (curve b), which was ascribed to the presence of nanosized SnO$_2$ and GR with high conductivity that accelerated the electron transfer. On Nafion/Hb-IL-GR-SnO$_2$/CILE the biggest redox peak currents appeared (curve a), which was due to the addition of high ionic conductive IL within the composite that could improve the whole interfacial conductivity with good adhesive ability.
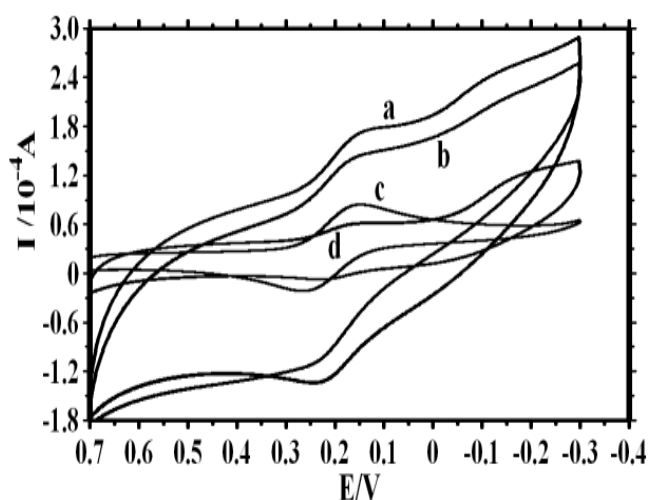

Figure 2. Cyclic voltammograms of (a) Nafion/Hb-IL-GR-SnO$_2$/CILE, (b) Nafion/Hb-GR-SnO$_2$/CILE, (c) CILE and (d) Nafion/Hb/CILE in a mixture solution of 1.0 mmol L^{-1} [Fe(CN)$_6$]$^{3-}$ and 0.1mol L^{-1} KCl with the scan rate as 100 mV s^{-1}.

3.3 Direct electrochemistry of Hb on the modified electrodes

Cyclic voltammograms of different modified electrodes were recorded in pH 7.0 PBS and shown in Fig. 3A. On CILE and Nafion/IL-GR-SnO$_2$/CILE no electrochemical responses appeared (curve e and d), indicating a stable background response. On Nafion/Hb/CILE a pair of small redox peak appeared (curve c), which indicated that direct electron transfer rate between Hb and CILE was slow. On Nafion/Hb-IL/CILE (curve b) the redox peak current increased due to the good ionic conductivity of IL that was benefit for electron transfer. While on Nafion/Hb-IL-GR-SnO$_2$/CILE (curve a) the biggest redox peak appeared, which was due to the presence of SnO$_2$ nanosheet and GR that enhanced the electron transfer rate of Hb. The cathodic (Epc) and the anodic (Epa) peak potential were located at -0.300 V and -0.201 V, respectively. Then the formal peak potential (E$^{0'}$), which was the average of Epa and Epc, was calculated as −0.252 V (vs. SCE). The ratio of Ipc/Ipa was close to 1. All the data indicated that a quasi-reversible electrode reaction of electrotroactive Hb heme Fe(III)/Fe(II) redox couples had taken place on the electrode.
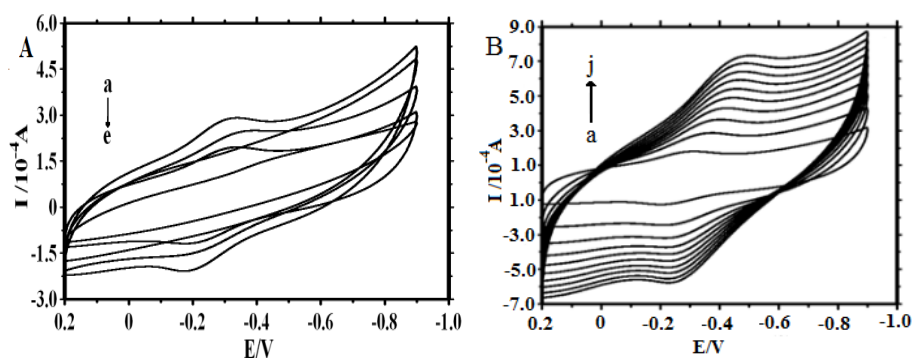

Figure 3. (A) Cyclic voltammograms of (a) Nafion/Hb-IL-GR-SnO$_2$/CILE, (b) Nafion/Hb-IL/CILE, (c) Nafion/Hb/CILE, (d) Nafion/IL-GR-SnO$_2$/CILE and (e) CILE at the scan rate of 100 mV s^{-1}. (B) Influence of scan rate on cyclic voltammetric results of Nafion/Hb-IL-GR-SnO$_2$/CILE in pH 7.0 PBS (from a to j: 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 mV s^{-1}).

Fig. 3B showed the cyclic voltammograms of Nafion/Hb-IL-GR-SnO$_2$/CILE at different scan rates from 50 to 500 mV s^{-1} in pH 7.0 PBS. A pair of well-defined redox peak could be observed with the currents increased gradually. The relationships between the redox currents and scan rate were calculated with linear regression equations as Ipc(μA)=0.36 υ (mV s^{-1}) + 34.11 (γ=0.995) and Ipa(μA) = -0.26 υ (mV s^{-1}) + -33.26 (γ=0.994). So the electroactive Hb heme Fe(III) was electroreduced to Hb heme Fe(II) and then reoxidized to Hb heme Fe(III) in a cyclic scan. For a thin-layer electrochemical behavior, the total charge (Q) involved in the redox reaction of electroactive species can be calculated by integration of cyclic voltammetric peaks. With the following equation of Q=nAFΓ* [25], the average surface concentration (Γ*) was calculated as 6.77×10^{-9} mol cm^{-2}, which accounted for 32.75 % of the total Hb molecules (2.067×10^{-8} mol cm^{-2}) on the electrode. Therefore the large surface area and specific functions of SnO$_2$ nanosheet, GR and IL provided unique microstructure and interface for Hb to transfer the electron with electrode at higher rate. The gradually increase of scan rate also resulted in the enlargement of the peak-to-peak separation and the formal peak potential was not changed, which

indicated a typical quasi-reversible process. By using Laviron's equations the electrochemical parameters could be calculated [26]. Two linear regression equations between Ep and lnυ were constructed with the results as Epc(V)=-0.059lnυ - 0.061 (γ=0.999) and Epa(V)= 0.025 lnυ-0.36 (γ=0.999). Therefore the electron transfer coefficient (α) and the apparent heterogeneous electron transfer rate constant (k_s) were 0.29 and 0.973 s^{-1}. The electron transfer of Hb was facile on the modified electrode due to the synergistic effects of materials used with direct electron transfer of Hb enhanced.

3.4. Electrocatalytic activity

Electrocatalytic activity of this Hb modified electrode to the reduction of TCA was evaluated by cyclic voltammetry with the curves overlapped in Fig. 4. The addition of TCA in PBS resulted in a significant increase of the reduction peak current at -0.295 V and the oxidation peak disappeared, which was ascribed to the catalytic reduction of TCA by Hb molecules [27]. The electrocatalytic reduction of TCA can be expressed with following equations:
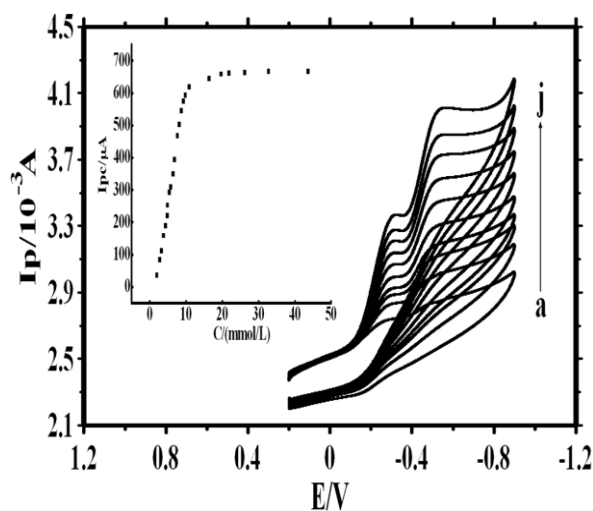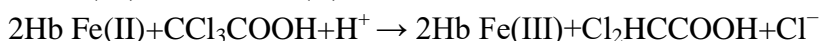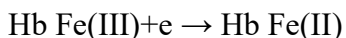
$$Hb\ Fe(III)+e \rightarrow Hb\ Fe(II)$$
$$2Hb\ Fe(II)+CCl_3COOH+H^+ \rightarrow 2Hb\ Fe(III)+Cl_2HCCOOH+Cl^-$$

Figure 4. Cyclic voltammograms of Nafion/Hb-IL-GR-SnO$_2$/CILE in 0.1 mol L^{-1} pH 7.0 PBS with different concentrations of TCA (curve a to j: 2, 3.5, 4.5, 4.8, 5.0, 5.5, 6.5, 7.5, 9.0, 11.0 mmol L^{-1}) at the scan rate of 100 mV s^{-1}. Inset is linear relationship of reduction peak currents and the TCA concentration.

The catalytic peak currents increased with TCA concentration from 2.0 to 11.0 mmol L^{-1} with the linear regression equation as I$_{ss}$(μA)= 72.12 C (mmol L^{-1}) – 111.68 (n=23, γ=0.994) and the detection limit as 0.615 mmol L^{-1} (3σ). When the TCA concentration was more than 11.0 mmol L^{-1}, the current increased slowly, indicating a typical Michaelis-Menten kinetic mechanism [28]. Then the apparent Michaelis-Menten constant ($K_M{}^{app}$) was obtained as 17.87 mmol L^{-1} based on the electrochemical data. The comparison of electrocatalysis to TCA by this Hb based electrochemical

biosensor with previous results was summarized and listed in Table 1. It can be seen that this modified electrode showed a competitive analytical performance for TCA detection.

Table 1. Comparisons of the analytical parameters for TCA detection with other Hb based biosensor.

Modified electrodes	Linear range (mmol L^{-1})	Detection limit (mmol L^{-1})	K_M^{app} (mmol L^{-1})	Refs.
Hb/PDDA/PGE	3.92-58.4	1.98	/	[29]
Hb/Fe$_3$O$_4$/PIGE	5.0-65.4	0.116	/	[30]
Nafion/nano-CdS/Hb/CILE	14.0-46.0	10.0	98.5	[31]
Nafion/Hb-CPE	32.0-148.0	5.6	203	[32]
SA/nano-SiO$_2$/Hb/CILE	6.25-137.5	5.0	0.28	[33]
CTS/ZnWO$_4$-Hb/CILE	2.0-10.0	0.613	5.76	[34]
CTS/Hb-Fe$_3$O$_4$/CILE	2.4-20.0	0.033	14.9	[35]
CTS/ELDH-GR-Hb/CILE	5-135	1.506	7.93	[36]
Nafion/Hb-IL-GR-SnO$_2$/CILE	2.0-11.0	0.615	17.87	This Work

PDDA: Poly(diallyldimethylammonium), PGE: pyrolytic graphite electrodes, PIGE: paraffin impregnated graphite electrode, CPE: carbon paste electrode, SA: sodium alginate, ELDH: exfoliated Co$_2$Al layered double hydroxide.
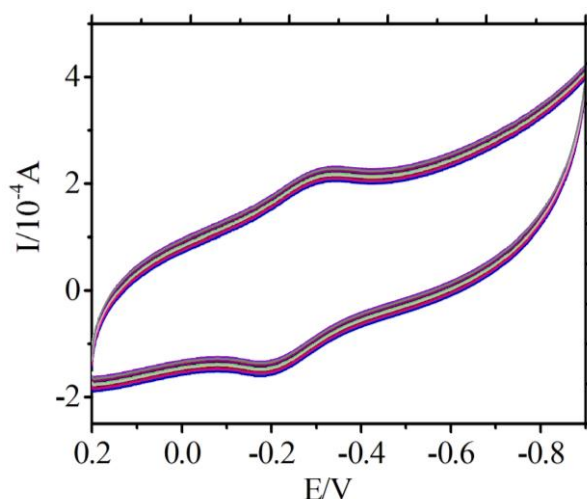
3.5. Stability

Figure 5. Multi-scan cyclic voltammatric curves of Nafion/Hb-IL-GR-SnO$_2$/CILE in pH 7.0 PBS for 100 cycles at the scan rate of 100 mV s^{-1}.

The stability of Nafion/Hb-IL-GR-SnO$_2$/CILE was investigated by multi-scan cyclic voltammetry for 100 cycles in buffer solution and the curves were shown in Fig.5. No obvious changes of the voltammetric response indicated the good stability of the modified electrode in buffer solution. The Hb modified electrode was stored at 4 $^{\circ}$C refrigerator, which retained 96.2% of its initial response

after 14 days and 94.1% after 30 days, implying good long-term storage stability. Eight electrodes prepared by the same steps were used for the detection of 6.0 mmol L^{-1} TCA, which gave the relative standard deviation (RSD) of 1.9% with good repeatability.

4. CONCLUSION

A composite consisted of SnO_2 nanosheet, GR, IL and Hb was used for the preparation of electrochemical sensor. The synergistic effects of the materials were beneficial for the realization and enhancement of direct electrochemistry of Hb on the modified electrode. Electrochemical behaviors of Hb were investigated and electrochemical parameters were calculated. The Hb modified electrode was applied to the electrocatalysis of TCA with wide detection range and low detection limit. Therefore the composite can be used as a promising sensing platform for the electrochemical applications.

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