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Magnesium Oxide/Carbon Nanofiber Nanocomposite Modified Electrode for Electrochemical Hemoglobin Sensor

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MgO decorated carbon nanofiber (MgO@CNFs) nanocomposite was prepared by electrospinning and carbonization method. Then the nanocomposite was casted on carbon ionic liquid electrode (CILE) with the following immobilization of hemoglobin (Hb) by Nafion polymer film. UV-Vis spectroscopic results showed that Hb maintained its original structure without changing after mixing with nanocomposite. The resultant MgO@CNFs modified electrode provided a favorable microenvironment for Hb to realize electrochemistry and the bioelectrochemical properties of Hb were studied in details. The results showed that MgO@CNFs nanocomposite on electrode improved Hb loading amount with its bioactivity maintained and electron transfer rate fasten, which was attributed to big specific interface area, excellent biocompatibility and high conductivity. Nafion/Hb/MgO@CNFs/CILE also displayed high electrocatalytic activity to the electroreduction of trichloroacetic acid. This study provided a novel way for the fabrication of the nanostructured biosurfaces for electrochemical biosensors.

Keywords: MgO decorated carbon nanofiber; Hemoglobin; Biosensor; Direct electrochemistry; Electrocatalysis

1. INTRODUCTION

Among different inorganic metal oxides, magnesium oxide (MgO) has gained much interest due to its specific electrochemical properties, good chemical stability and biocompatibility. Nano/micro-structured MgO has been applied to amperometric biosensors, which can be served as absorbent modifier for enzyme immobilization with improved sensitivity [1, 2]. Carbon nanofiber (CNF) exhibits larger functionalized surface area, attractive physiochemical and mechanical properties, which make it an ideal supporting material for various applications. The edge sites of CNFs can provide a fast electron transfer rate with the usage in electronic devices [3]. Also CNFs can work as the hard template for loading of other nanomaterials such as hydroxyapatite [4], TiO_2 [5] and Co_3O_4 [6] on its surface to fabricate a homogeneous nanocomposite. Various functional groups at the CNFs surface can be used for the selective immobilization and stabilization of biomolecules including proteins, enzymes and DNA. Besides, the high conductivity of CNFs is excellent for the electrochemical response transduction [7], which provides not only more active sites at the electrode surface, but also sufficient surface tunnels for the electrochemical reactions.

Hemoglobin (Hb) is a biological redox protein with long-term stability and high catalytic activity [8, 9]. Electron transfer from Hb to electrode interface has been realized to overcome deep buring of electroactive heme group [10, 11]. Recently, various new nanomaterials or procedures for Hb immobilization on working electrode are devised for realizing direct electrochemical reactions with bioactivities retained.

In this paper, MgO-decoroated polyacrylonitrile (PAN) was prepared by electrospinning, then it was carbonized at suitable temperature to obtain MgO-decoroated CNFs (MgO@CNFs). The morphology of MgO@CNFs nanocomposite was investigated by scanning electron microscopy (SEM). MgO@CNFs and Hb modified carbon ionic liquid electrode (Nafion/Hb/MgO@CNFs/CILE) was selected as the working electrode in the electrochemical biosensor. Direct electrochemistry of Hb on MgO@CNFs based electrode was checked in detail. Due to the electrocatalytic activity to trichloroacetic acid (TCA) by Nafion/Hb/MgO@CNFs/CILE, a new electrochemical sensor was constructed for TCA analysis with excellent performance including wide linear range, low detection limit, good reproducibility and stability.

2. EXPERIMENTAL

2.1 Reagents

Polyacrylonitrile (PAN, J&K Chem. Co., China), MgO nanoparticles (Nanjing XFNANO Materials Tech. Co. China), 1-hexylpyridinium hexafluorophosphate ($HPPF_6$, Lanzhou Yulu Fine Chem. Co., China), graphite powder (average particle size 30 μm, Shanghai Colloid Chem. Co., China), bovine Hb (MW. 64500, Sinopharm Chem. Reagent Co., China), Nafion (5.0%, Sigma, USA) and TCA (Tianjin Kemiou Chem. Co., China) were used as received from the producer. 0.1 $mol \cdot L^{-1}$ phosphate buffer solution (PBS) was selected as the supporting electrolyte, and deoxygenized with N_2 for 30 minutes before experiments. All other chemicals were of A.R. grade and ultrapure water (Milli-Q IQ 7000) was used to the experiments.

2.2 Instruments

Electrochemical experiments were carried out on CHI 604E electrochemical workstation (Shanghai CH Instrument, China) with Nafion/Hb/MgO@CNFs/CILE ($\Phi = 4.0$ mm) as working electrode, a platinum wire auxiliary electrode and a saturated calomel as reference electrode (SCE) SEM was performed on a JSM-7100F scanning electron microscope (JEOL, Japan) and UV-visible spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China) was used for obtaining UV-vis spectroscopy.

2.3 Preparation of MgO@CNFs nanocomposite

MgO@CNFs nanocomposite was electrospinned and synthesized by the following steps. 0.6264 g MgO nanoparticle and 2.75 g PAN were firstly dispersed in 35 ml N'N'-dimethyl formamide (DMF) by ultrasonication for 20 minutes to obtain a homogeneously spinning solution. Electrospinning instruments were laboratory-built and the MgO-PAN-DMF mixed solution was putted into a 5 mL syringe. The tip of the syringe needle was linked to the positive terminal of the high-voltage power supply with the collector clamped to the negative terminal, and the applied voltage was set as 11.22 kV by a direct current supply. The distances between the needle tip and the collector was 12 cm with the mixed solution pumped out at a rate of 11 $\mu L \cdot s^{-1}$ by a syringe pump. Electrospinning was carried out at 25°C with the nanofiber collected and wrapped on aluminum foil. Finally, the resultant MgO@PAN nanofiber was peeled off from the collector and treated at 800°C calcining for 2 h under N_2 atmosphere to obtain MgO@CNFs.

2.4 Working electrode fabrication

CILE was home-made based on the reference with graphite power, $HPPF_6$ and a glass electrode tube [12]. The newly prepared CILE surface was drop-casted with 8.0 μL of MgO@CNFs (1.0 $mg \cdot mL^{-1}$), 8.0 μL of Hb (15.0 $mg \cdot mL^{-1}$) and 6 μL of 0.5% Nafion in sequence by a microsyringe, and dried at room temperature in air. The resultant electrode was denominated as Nafion/Hb/MgO@CNFs/CILE and used as the working electrode.

3. RESULTS AND DISCUSSION

3.1 SEM

SEM is applied to characterize the morphology of the synthesized materials. Fig.1 showed the SEM photographs for CNFs (A, B) and MgO@CNFs nanocomposites (C, D) at different magnification. CNFs were of exclusively uniform sizes with average diameter sizes of 400 nm (Fig. 1 A), and smooth surface characteristics could be observed in Fig. 1 B. As for the nanocomposite, irregular nanoparticles were clearly observed on the surface of CNFs (Fig. 1 C and D) with the interfacial roughness increased and the complexity of CNFs network structure appeared, providing a microstructure for the Hb immobilization.

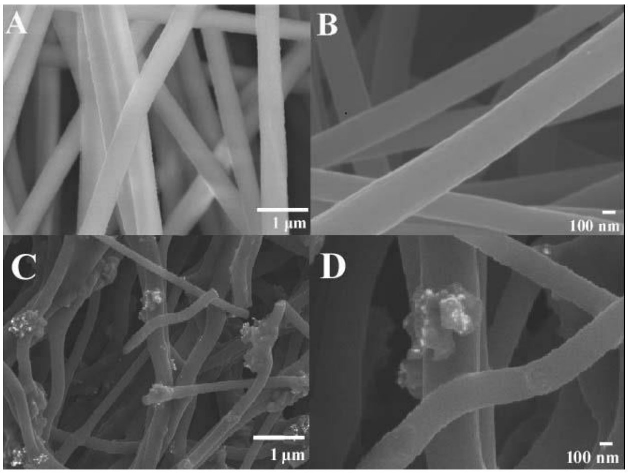

Figure 1. SEM photographs of (A, B) CNFs and (C, D) MgO@CNFs at various magnifications.

3.2 UV-Vis absorption spectroscopic investigation

UV-Vis absorption spectroscopy is often applied to examine the proteins structure with the position of Soret absorption peak provided the state change of heme region [13]. Fig.2 indicated that both UV-Vis spectra of Hb solution (curve a) and the Hb-MgO@CNFs solution (curve b) showed a same absorption peak at 406 nm, which proved that Hb in MgO@CNFs solution was not distorted with the secondary biostructure retained same as the original Hb.

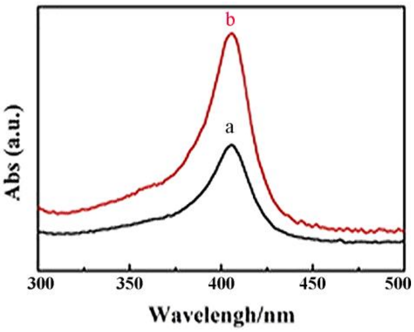

Figure 2. UV-Vis absorption spectra of (a) Hb and (b) Hb-MgO@CNFs mixture in water.

3.3 Electrochemical investigations

As shown in Fig. 3A, electrochemical performances were checked with cyclic voltammetric scan performed in pH 4.0 PBS. It can be clearly noted that no evident redox peaks were shown on Nafion/MgO@CNFs/CILE (curve a) and Nafion/CILE (curve b) in the selected potential range. On Nafion/Hb/CILE (curve c) a couple of redox peaks were found with E_{pc} as -0.266 V and E_{pa} as -0.179 V, which was the typical peaks of Hb heme Fe(III)/Fe(II) [14]. Therefore direct electron transfer (DET) from Hb to electrode was preceded with CILE as the conductive promoter. When Nafion/Hb/MgO@CNFs/CILE (curve d) was applied, redox responses were larger with more symmetrical peak shape (E_{pc}=-0.298 V and E_{pa}=-0.220 V), indicating that DET of Hb on MgO@CNFs modified electrode surface was accelerated. Therefore MgO@CNFs nanocomposite showed important role for increase of electron transfer rate, which provided a better electron transport channel with high conductive network and synergistic functions in electrochemical process.

Influence of scan rates from 0.05 to 1.00 $V·s^{-1}$ on cyclic voltammetric behaviors of Nafion/Hb/MgO@CNFs/CILE were checked in pH 4.0 PBS and the curves were shown in Fig.3 B. The approximately symmetric peaks of Hb molecules appeared at different scan rates. The redox peak potentials shifted towards positive and negative positions with the scan rate along with the increase of ΔE_p. This indicated that the electron transfer of Hb turned to irreversible at higher rate scan zone. Also the responses of redox peak amplified with scan rate, proving a typical interface-controlled process. Two regression equations were got as $I_{pc}\ (mA) = 153.71\upsilon\ (V·s^{-1}) +8.531\ (n=10,\ \gamma= 0.99)$ and $I_{pa}\ (mA) =-181.56\upsilon\ (V·s^{-1}) -0.145\ (n=10,\ \gamma= 0.99)$. Also the relationships of peak potential with *lnυ* at high scan rates zone were got with the regression equations as $E_{pc}\ (V) = -0.046ln\upsilon\ (V·s^{-1}) -0.32\ (n=8,\ \gamma= 0.99)$ and $E_{pa}\ (V) = 0.045ln\upsilon\ (V·s^{-1}) -0.14\ (n=8,\ \gamma= 0.99)$ (Fig. 3 C). Then electron transfer efficient (α) and apparent heterogeneous electron transfer rate constant (k_s) were computed as 0.459 and 0.690 s^{-1}. Therefore MgO@CNFs nanocomposite on the electrode was benefit for faster electron transfer of Hb.

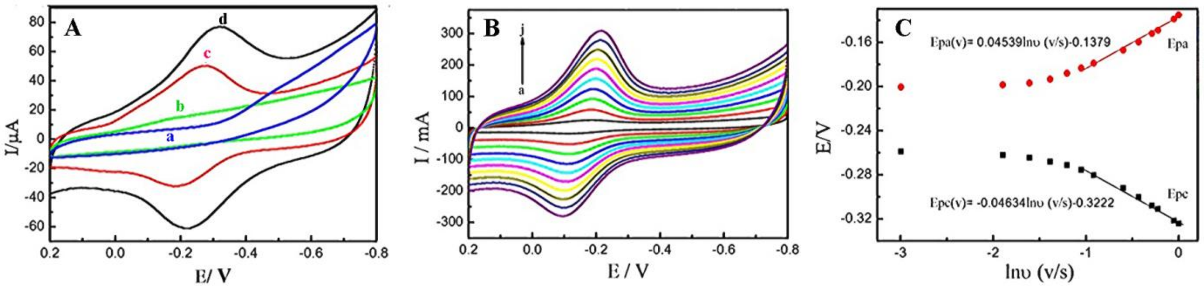

Figure 3. (A) Cyclic voltammograms of (a) Nafion/MgO@CNFs/CILE, (b) Nafion/CILE, (c) Nafion/Hb/CILE, (d) Nafion/Hb/MgO@CNFs/CILE in pH 4.0 PBS at 100 $mV·s^{-1}$; (B) Influence of scan rate on electrochemical responses of Nafion/Hb/MgO@CNFs/CILE in pH 4.0 PBS with scan rate as (a to j) 50, 150, 250, 350, 450, 550, 650, 750, 850, 950 $mV·s^{-1}$; (C) Relationship between the peak potentials and lnυ.

It is known that the increasing pH of buffer solution can reduce the formal potential of heme proteins that are subject to electron transfer along with protons. This shifting of the formal potential as a function of pH is known as Bohr oxidation-reduction effect, which results from a change in the water molecule's prominence at the sixth coordination position in heme iron as well as the product of the trans heme groups with pH changed [15]. As shown in Fig. 4, the formal potential ($E°'$) of Hb presented a negative shift with the increase of the buffer pH from 3.0 to 8.0. A good linear relationship was obtained with linear regression equation as $E°'(V)=0.00431\ pH+0.00159\ (n=5,\ \gamma=0.99)$. The slope (-43.1 mV/pH) was little smaller than the theoretical value (-56.0 mV/pH at 20°C) for reverse reaction of one electron transfer-proton transfer, which may be ascribed to the changes of microenvironment for Hb molecules. The equation of the electrode reaction is presented as following: Hb Fe (III) + H^+ + e^- → Hb

Fe(II) [16], in which Hb Fe (III) was reduced to Hb Fe (II) in cathodic scanning process, and the product was oxidized to Hb Fe (III) at reverse scan, with the reversible redox peaks.

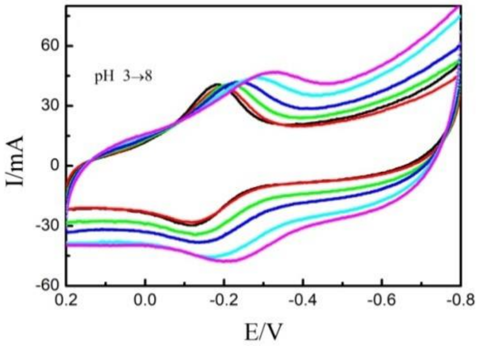

Figure 4. Cyclic voltammograms of Nafion/Hb/MgO@CNFs/CILE in different pH PBS with the scan rate of 100 mV·s^{-1}.

3.4 Electrocatalytic properties

Nafion/Hb/MgO@CNFs/CILE was applied to electrocatalytic reduction of TCA with cyclic voltammetric results present in Fig. 5. After the adding of the increase TCA concentration in pH 4.0 PBS, a clear increase in the reduction current (I_{pc}) was recorded at -0.26 V with a lower oxidation peak current (I_{pa}) of Hb, which was the results of a typical electrical stimulation [17] and good electrocatalytic reduction of Nafion/Hb/MgO@CNFs/CILE to TCA was realized. A linear regression equation of $I\ (mA) = 0.981\ C\ (mM) + 80.322\ (n = 8, \gamma = 0.997)$ was obtained from 0.28 mM to 70.0 mM with a detection limit of 0.067 mM (3σ). According to the Lineweaver-Burk equation [18], the Michaelis-Menten constant (K_M^{app}), which is an important indicator of reaction kinetics around the enzymatic reaction, was calculated as 10.5 mM. Comparison of detection properties for TCA by various Hb modified electrodes showed that Nafion/Hb/MgO@CNFs/CILE had lower detection limit and was suitable for low concentration quantitative analysis (Table 1).

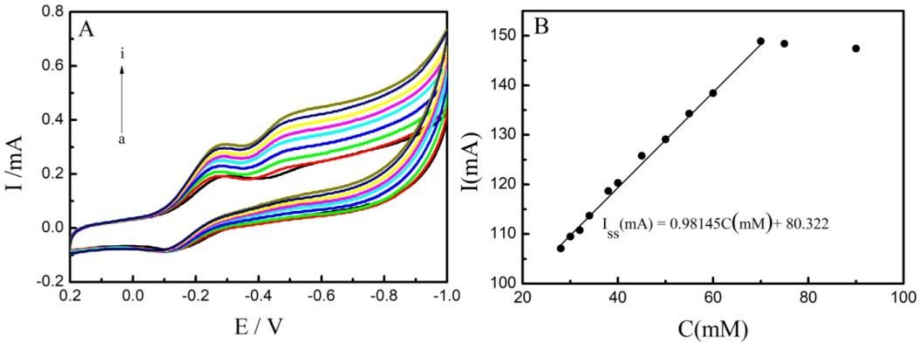

Figure 5. (A) Cyclic voltammograms of Nafion/Hb/MgO@CNFs/CILE with TCA (a to i: 0.1, 2.2, 4.0, 6.0, 8.0, 10.0, 11.0, 12.0, 14.0 mmol L^{-1}) in pH 4.0 PBS at 100 mV s^{-1}; (B) the relationship between I_{pc} and the TCA concentration with a linear portion.

Table 1. Comparison of electrochemical data of different Hb modified electrodes to TCA analysis.

Modified electrodes	Linear range (mmol·L^{-1})	Detection limit (mmol·L^{-1})	K_M^{app} (mmol·L^{-1})	Refs.
Nafion/Hb/Co_3O_4-CNFs/CILE	40.0−260.0	1.33	/	[6]
Nafion/GR-TiO_2-Hb/CILE	0.6–21.0	0.22	3.3	[19]
CTS/CMS-Hb/CILE	2.0−70.0	0.30	1.60	[20]
BPE-PEDOT:PSS-hemin/CILE	2.0-180.0	0.67	4.31	[21]
CTS-HRP-GR-IL/CILE	2.0-16.0	0.58	8.99	[22]
Nafion/Hb/MgO@CNFs/CILE	0.28−70.0	0.067	10.5	This work

Note: graphene (GR); carbon microspheres (CMS); black phosphorene (BPE); poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS); chitosan (CTS); ionic liquid (IL)

4. CONCLUSIONS

In summary, the proposed MgO@CNFs nanocomposites are successfully prepared by using nanosized MgO and carbon fiber precursor, and MgO NPs can be successfully doped within CNFs. Using MgO@CNFs modified electrode, Hb can achieve faster direct electron transfer with electrochemical performance investigated. The Hb modified surface and the strong electrocatalytic activity provided favorable results for the detection of low concentration TCA with a detection limitation of 0.067 mmol·L^{-1}. These special properties extended the applications of MgO@CNFs nanocomposite in the fields of electrochemical biosensor.

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