

Electrodeposited ZnO@three-dimensional Graphene Composite Modified Electrode for Electrochemistry and Electrocatalysis of Myoglobin

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A three-dimensional graphene (3DGR) was electrodeposited on carbon ionic liquid electrode (CILE) and further decorated with electrodeposited zinc oxide (ZnO) to get a ZnO@3DGR hybrid composite. Then myoglobin (Mb) was immobilized on the surface of ZnO@3DGR modified electrode with Nafion to prepare the biosensor named as Nafion/Mb/ZnO@3DGR/CILE. On cyclic voltammogram a pair of well-defined redox responses resulted from the electron transfer of Mb with the working electrode was got, which could be ascribed to the specific characteristics of ZnO@3DGR including porous macrostructure, biocompatibility, high conductivity and big surface area. Electrochemical parameters including the electron transfer coefficient (α) and the apparent heterogeneous electron transfer rate constant (k_s) were got by electrochemical methods with the values as 0.31 and 0.76 s⁻¹. This biosensor showed excellent electrocatalysis to the electro reduction of trichloroacetic acid (TCA). A good linear relationship between the catalytic reduction peak current and TCA concentration was in the range from 0.5 to 30.0 mmol L⁻¹ and the detection limit was 0.167 mmol L⁻¹ (3 σ). The results indicated that a third-generation electrochemical Mb biosensor with ZnO@3DGR/CILE was prepared and exhibited good stability with reproducibility.

Keywords: Three-dimensional graphene; ZnO; Myoglobin; Carbon ionic liquid electrode; Electrochemistry; Electrocatalysis.

1. INTRODUCTION

Investigation on electrochemical behaviors of redox proteins and enzymes has been the research focus. The efficient direct electron transfer from redox enzymes to electrode can understand

the redox mechanism of electron transfer process in biological process. The results can also be used to establish the third-generation biosensors or biomedical designs [1]. In general direct electron transfer is hard to be realized due to the deeply embedded of the electroactive group in the molecule and the unfavorable orientation of enzymes on the interface. Therefore various methods have been made to accelerate the communication from the active centers to the electrode with different mediators, promoters or nanostructured materials [2]. Nanomaterials have specific properties including good biocompatibility, big active surface areas, unique catalytic activity, good stability and high conductivity. Therefore various nanomaterials and related hybrid composites are applied to study the electrochemistry of redox enzymes [3].

As a two-dimensional sp^2 conjugated carbon nanosheets, graphene (GR) has been widely investigated due to its unique electrical, optical and mechanical characteristics [4]. GR has good electrical conductivity and electrocatalytic ability, therefore it has been applied to the fields of electrochemistry and electroanalytical chemistry [5]. However, GR nanosheets tend to aggregate by Van der Waals and π - π interaction, which limit its practical usages. Recently three-dimensional (3D) GR macrostructure had been fabricated by different methods such as template, sol-gel, electrochemistry and so on [6]. 3DGR can provide a network structure with big interface area and high conductivity, which can be used in electrode modification and electrochemical applications [7-10]. ZnO is a kind of semi-conductor that processes many specific properties and is used as the modifier on electrode [11, 12]. The presence of nanosized ZnO is good for the electrochemical investigation with increased surface area and biocompatibility. For example Sun et al. studied hemoglobin electrochemistry on nanosized ZnO film based electrode [13]. Tak et al. applied flower-like ZnO nanostructure for the electrochemical DNA sensor [14].

In this paper 3DGR modified electrode was prepared by electrodeposition and ZnO was electrodeposited on 3DGR to prepare a ZnO@3DGR nanocomposite. Then myoglobin (Mb) was casted on the electrode and Nafion film was used for immobilization. Electrochemistry of Mb was achieved on ZnO@GR/CILE and a pair of well-defined redox peaks appeared. The results proved that ZnO@GR nanocomposite could facilitate the electron transfer of Mb because of its large surface area and increased conductivity. Electrocatalysis of this Mb based biosensor was investigated to trichloroacetic acid (TCA) reduction and the water samples were detected by the proposed method.

2. EXPERIMENTAL

2.1 Reagents and Apparatus

Mb (MW 17800) and Nafion (5% ethanol solution) were purchased from Sigma (USA), 1-hexylpyridinium hexafluorophosphate (HPPF₆, Lanzhou Yulu Fine Chem. Co., China), graphene oxide (GO, Taiyuan Tanmei Ltd. Co., China), lithium perchlorate (LiClO₄, Chengdu Kelong Chem. Co., China), graphite powder (particle size 30 μ m, Shanghai Colloid Chem. Co., China), TCA (Tianjin Kemiou Chem. Co., China) and Zn(NO₃)₂•5H₂O (Tianjin Chem. Reagent, China) were used as received. 0.1 mol L⁻¹ phosphate buffer solutions (PBS) were prepared, deoxygenated and remained in a

nitrogen atmosphere in the experiments. Other chemicals used were of analytical reagent grade and all aqueous solutions were prepared with doubly-distilled water.

Voltammetric experiments were carried out on a CHI 1210A electrochemical workstation. Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660D electrochemical workstation (Shanghai CH Instrument, China). Three-electrode cell was employed with Nafion/Mb/ZnO@3DGR/CILE as working electrode. A platinum wire electrode acted as counter electrode and a saturated calomel electrode (SCE) as reference electrode. A JSM-7100F scanning electron microscope (SEM, Japan Electron Company, Japan) was used for scanning electron microscopic images.

2.2 Construction of Nafion/Mb/ZnO@3DGR/CILE

HPPF₆ based CILE was home-made by the reported method [15], which was polished smoothly for further modification. 3DGR modified CILE was electrodeposited with 3.0 mg mL⁻¹ GO and 0.1 mol L⁻¹ LiClO₄ solution by magnetic stirring and N₂ bubbling [16]. After electrochemical reaction at -1.3 V for 300 s, electroreduced 3DGR could be formed on CILE (3DGR/CILE), which was washed with water and dried in nitrogen atmosphere. ZnO was electrodeposited on the 3DGR/CILE surface with a 0.02 mol L⁻¹ zinc nitrate solution. The reduction potential was -0.6 V for 5 min with electrochemical cell in a 65 °C water bath. After rinsed 8.0 μL Mb solution (15.0 mg mL⁻¹) was directly casted onto ZnO@3DGR/CILE. After dried at room temperature 5.0 μL Nafion solution (0.5%) was put on Mb/ZnO@3DGR/CILE. Finally the modified electrode (Nafion/Mb/ZnO@3DGR/CILE) was got and put at 4 °C refrigerator.

3. RESULT AND DISCUSSION

3.1 SEM images of modified electrode

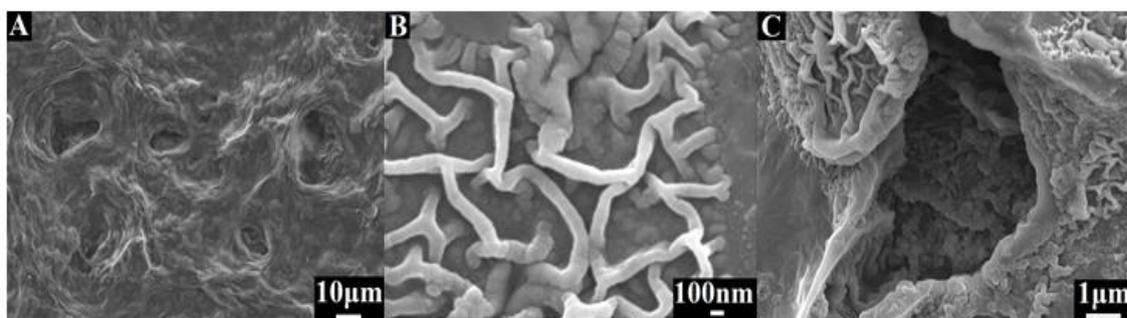


Figure 1. SEM of 3DGR/CILE (A), ZnO@/3DGR/CILE with different magnitude (B) and (C).

SEM image of 3DGR/CILE was recorded and shown in Fig. 1A. An interconnected 3D porous network could be observed, which indicated that GO was electrochemical reduced on the electrode surface [16]. The surface morphologies of ZnO@3DGR composite were shown at different

magnifications (Fig. 1B and 1C), which gave a layer of continuous ZnO film on the surface of 3DGR. Electrodeposition of ZnO had been reported and ZnO can be formed on the surface of 3DGR with the maintenance of the 3D structure, which was helpful for the protein adsorption.

3.2 EIS results

EIS is used for obtaining the electrode interfacial information at different modification process, and the diameter of the semicircle usually equals to the electron transfer resistance (R_{et}). EIS experiments were performed in a $10.0 \text{ mmol L}^{-1} [\text{Fe}(\text{CN})_6]^{3-/4-}$ and $0.1 \text{ mol L}^{-1} \text{ KCl}$ solution, and the frequency swept was from 10^4 to 0.1 Hz . As shown in Fig. 2, the R_{et} value of Nafion/CILE was got as $110.3 \text{ } \Omega$ (curve b), which was smaller than that of Nafion/Mb/CILE ($290.1 \text{ } \Omega$, curve a). Therefore the nonconductive Mb on the electrode prevented the diffusion of ferricyanide and acted as the insert blocking layer. On Nafion/Mb/ZnO/CILE the R_{et} value was decreased to $70.53 \text{ } \Omega$ (curve c) and on Nafion/Mb/ZnO@3DGR/CILE a straight line with the R_{et} value close to $0 \text{ } \Omega$ was got (curve d). Therefore the presence of ZnO and 3DGR on the electrode surface decreased the interfacial resistances due to the high conductivity.

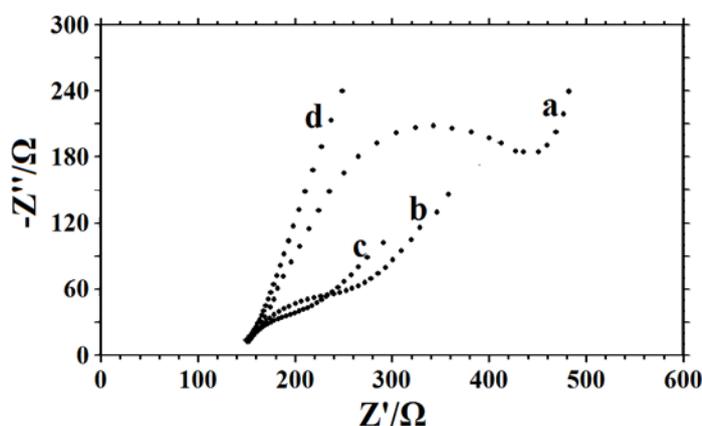


Figure 2. EIS of (a) Nafion/Mb/CILE, (b) Nafion/CILE, (c) Nafion/Mb/ZnO/CILE and (d) Nafion/Mb/ZnO@3DGR/CILE.

3.3 Direct electrochemistry of Mb

Electrochemistry of Mb was checked by cyclic voltammetry (shown in Fig. 3). No electrochemical responses appeared on Nafion/CILE (curve a) and a pair of redox peaks from direct electrochemistry of Mb with CILE appeared on Nafion/Mb/CILE (curve b) [17]. CILE can provide a high conductive and biocompatible interface that facilitates the electron transfer. On Nafion/Mb/ZnO/CILE the redox responses increased (curve c) due to the presence of ZnO that was helpful to transfer electron. On Nafion/Mb/ZnO@3DGR/CILE the redox responses were increased to the maximum data (curve d), which was ascribed to the presence of ZnO@3DGR nanocomposite on the electrode surface. The potentials were got as -0.223 V (E_{pc}) and -0.107 V (E_{pa}), and the formal

peak potential ($E^{0'}$) was -0.165 V. 3DGR has an interconnected structure with fast conductivity and ZnO is biocompatibility. Therefore ZnO@3DGR is benefit for Mb immobilization and the electron transfer rate is fastened. Then a fast electron transfer of Mb was realized on ZnO@3DGR/CILE.

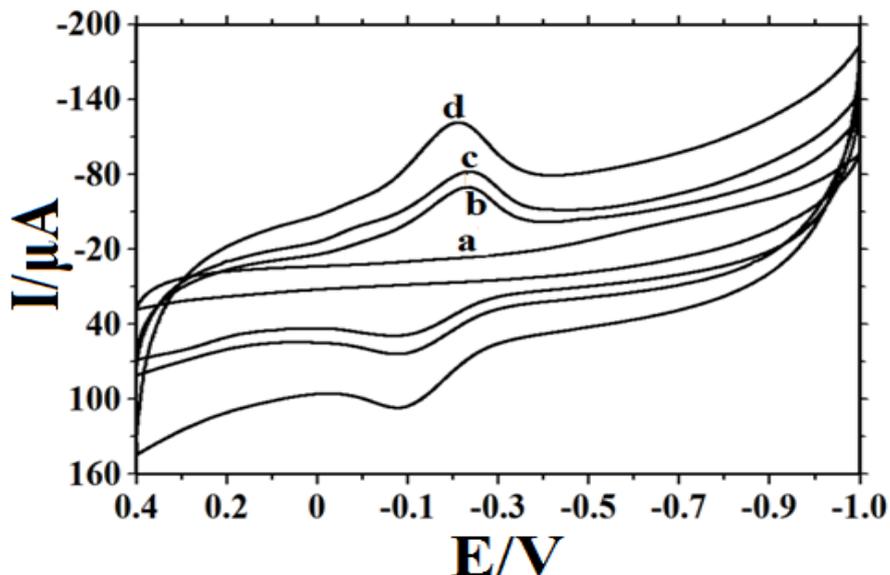


Figure 3. Cyclic voltammograms of (a) Nafion/CILE, (b) Nafion/Mb/CILE, (c) Nafion/Mb/ZnO/CILE and (d) Nafion/Mb/ZnO@3DGR/CILE in pH 3.0 PBS at the scan rate of 100 mV s^{-1} .

3.4 Effect of scan rate

The influence of scan rate on the cyclic voltammetric responses was checked. As shown in Fig. 4, in the scan rate from 100 to 900 mV s^{-1} a pair of symmetric redox peaks could be observed. The currents increased with scan rate and two regression equations were got as $I_{pc}(\mu\text{A})=124.2 v (\text{V s}^{-1})-1.48$ ($n=17$, $\gamma=0.996$) and $I_{pa}(\mu\text{A})=-112.5 v (\text{V s}^{-1})+2.75$ ($n=17$, $\gamma=0.995$), showing a surface-controlled thin-layer process. The increase of scan rate resulted in the increase of the peak-to-peak separation (ΔE_p), indicating a quasi-reversible electrode process. Therefore the electrochemical parameters could be calculated with Laviron's equations [18]. Two regression equations were $E_{pc}(\text{V})=-0.135 \ln v (\text{V s}^{-1})-0.248$ ($n=13$, $\gamma=0.994$) and $E_{pa}(\text{V})=0.109 \ln v (\text{V s}^{-1})-0.116$ ($n=13$, $\gamma=0.994$). Then the electron transfer coefficient (α) and the apparent heterogeneous electron transfer rate constant (k_s) were 0.31 and 0.76 s^{-1} . This k_s value was larger than that of Mb on Ag-CNTs/GCE (0.41 s^{-1}) [19] and Nafion/MWCNTs/CILE (0.33 s^{-1}) [20], indicating that ZnO@3DGR/CILE was a good mediator for the electron transfer with large constant. The surface concentration (Γ^*) of Mb involved in the electrode reaction can be calculated based on $Q=nF\Gamma^*$ [21]. By the integration of the cyclic voltammetric reduction peaks, Γ^* was $9.5 \times 10^{-9} \text{ mol cm}^{-2}$, bigger than the theoretical monolayer coverage ($1.89 \times 10^{-11} \text{ mol cm}^{-2}$) [22], therefore multilayers of Mb molecules on the electrode transferred electrons.

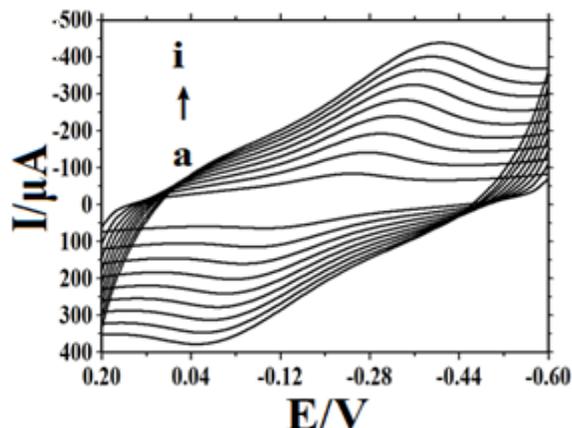


Figure 4. Cyclic voltammograms of Nafion/Mb/ZnO@3DGR/CILE at scan rates from a to i as 100, 200, 300, 400, 500, 600, 700, 800, 900 mV s^{-1} in pH 3.0 PBS.

3.5 Effect of pH

The influence of buffer pH on voltammetric response of Nafion/Mb/ZnO@3DGR/CILE was investigated in PBS (0.1 mol L^{-1}) from pH 2.0 to 7.0. The maximum peak currents appeared at pH 3.0, which was chosen for the following experiment. Also the negative shifts of redox peak potentials indicated that protons involved in the electrochemical reaction. A linear relationship of $E^{0'}$ with buffer pH was $E^{0'} (\text{V}) = 0.055 - 0.040\text{pH}$ ($\gamma = 0.996$). The slope value (-40.0 mV pH^{-1}) was a little smaller than the theoretical value (-59.0 mV pH^{-1}) for a one-electron and one-proton reaction. Therefore Mb took place the following electrode reaction and the equation was $\text{Mb Fe(III)} + \text{H}^+ + \text{e} \rightleftharpoons \text{Mb Fe(II)}$.

3.6 Electrocatalytic investigation

Electrocatalysis of this Mb modified electrode to the electroreduction of TCA was investigated with cyclic voltammetry. As shown in Fig. 5, the increase of TCA concentration resulted in the appearance of a new reduction peak at -0.546 V and the disappearance of the oxidation peak, which was a typical electrocatalytic behavior of Mb to TCA [23]. The catalytic reduction currents had a linear relationship with TCA concentration in the range from 0.5 to 30.0 mmol L^{-1} and the regression equation was $I_{\text{ss}}(\mu\text{A}) = 9.12C(\text{mmol L}^{-1}) + 1.99$ ($n=10$, $\gamma=0.992$) with the detection limit calculated as $0.167 \text{ mmol L}^{-1}$ (3σ). The peak current leveled off to a stable value at TCA concentration more than 30.0 mmol L^{-1} , indicating a Michaelis-Menten kinetic reaction process. Then the apparent Michaelis-Menten constant (K_M^{app}) was calculated with the Lineweaver–Burk equation [24] and the value was 1.78 mmol L^{-1} . A systematic comparison of this Mb modified electrode for the TCA detection with other reported results was listed in table 1. It can be seen that a relatively wider dynamic range and smaller detection limit were achieved with the proposed biosensor, indicating that ZnO@3DGR was benefit for Mb immobilization.

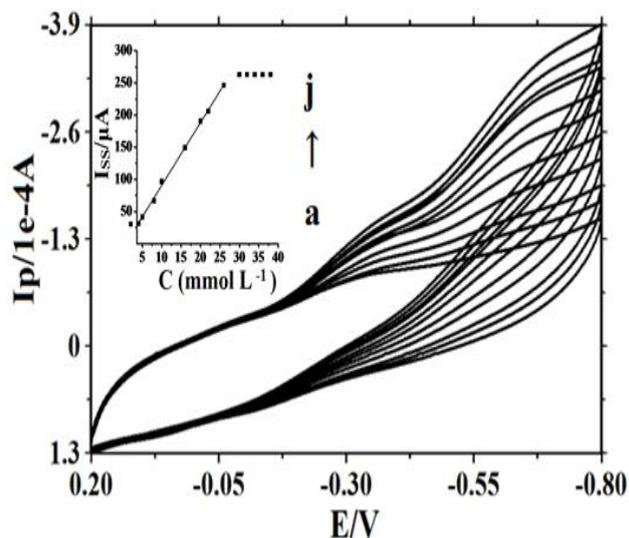


Figure 5. Cyclic voltammograms of Nafion/Mb/ZnO@3DGR/CILE with 2, 4, 8, 10, 12, 16, 20, 24, 26, 30 mmol L⁻¹ TCA (curve a to j), scan rate 100 mV s⁻¹; Inset was the linear relationship of catalytic reduction peak currents and the TCA concentration.

Table 1. Comparisons of the analytical parameters for TCA detection with different redox protein based biosensor.

Modified electrodes	Linear range (mmol L ⁻¹)	Detection limit (mmol L ⁻¹)	K_M^{app} (mmol L ⁻¹)	Refs.
Nafion/Mb/Co/CILE	0.01-1.4	0.006	0.499	[25]
Hb/PDDA/PGE	3.92-58.4	1.98	/	[26]
Nafion/Mb-Co ₃ O ₄ -Au/IL-CPE	2.0-20.0	0.5	4.70	[27]
Nafion-BMIMPF ₆ /Mb/CILE	1.6-19.6	0.2	90.8	[28]
Nafion/Mb-SA-TiO ₂ /CILE	5.3-114.2	0.152	32.3	[29]
Nafion/Mb/NiO/GR/CILE	0.69-30.0	0.23	10.67	[30]
Mb/DNA/CILE	0.5-40.0	83	0.82	[31]
CTS/ZnWO ₄ -Hb/CILE	2.0-10.0	0.613	5.76	[32]
CTS/(GR/Mb) ₂ /CILE	0.6-26.0	0.15	0.459	[33]
CTS/ELDH-GR-Hb/CILE	5-135	1.506	7.93	[34]
Nafion/Mb/ZnO@3DGR/CILE	0.5-30.0	0.167	1.78	This Work

PDDA: Poly(diallyldimethylammonium), SA: sodium alginate, ELDH: exfoliated Co₂Al layered double hydroxide

3.7 Analytical application

The Mb modified electrode was used to detect TCA concentration in water samples and the standard addition method was used for recovery. As shown in table 2, no TCA could be detected in the lab water with the recovery from 91.3 % to 95.0 %. Therefore the modified electrode was able to determinate TCA with satisfactory results.

Table 2. Determination of TCA in the water samples (n=3)

Water Sample	Found (mmol L ⁻¹)	Added (mmol L ⁻¹)	Found (mmol L ⁻¹)	Recovery (%)	RSD (%)
1	0	4.0	3.70	92.5	1.93
2	0	6.0	5.70	95.0	2.56
3	0	8.0	7.30	91.3	3.03

3.8 Stability, reproducibility and repeatability

The storage stability was investigated by storing Nafion/Mb/ZnO@3DGR/CILE at 4°C. After 10 days the peak current retained 96.2% and 30 days later the peak current decreased for 8.5%. The reproducibility was examined in a TCA solution (6.0 mmol L⁻¹) with the relative standard deviation (RSD) as 2.8% for ten independent determinations. Ten Mb modified electrodes were fabricated independently and used for TCA (6.0 mmol L⁻¹) detection with the RSD value as 3.45%. Therefore the good stability, reproducibility and repeatability were got.

4. CONCLUSION

A 3DGR was electrochemically formed on the CILE and further decorated by electrodeposited ZnO. Then Mb molecules were immobilized on its surface with Nafion film. Due to synergistic effects such as high conductivity, big surface area of 3DGR with interconnect structure, the biocompatibility of ZnO, Mb gave a faster electron transfer rate with a pair of well-defined redox peaks appeared. This biosensor had good electrocatalytic ability to TCA reduction. Therefore ZnO@3DGR composite modified electrode could be served as an electrochemical sensing platform for electrochemical applications.

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