

石墨烯-金纳米复合材料:水热合成及在生物传感器中的应用

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摘要: 通过水热法合成了石墨烯-金纳米复合材料。透射电子显微镜直接证明了制备的石墨烯薄而透明的片状结构。X-射线光电子能谱和 X-射线衍射结果也表明了金的存在和氧化石墨烯的还原。作为一个模型,将肌红蛋白固定到该复合材料修饰的玻碳电极上并用于生物传感器。所固定的肌红蛋白显示了一对对称的氧化还原峰并对过氧化氢的还原具有高的催化活性。在信噪比等于 3 的时候该生物传感器的线性范围在 0.1 到 1.5 $\mu\text{mol}\cdot\text{L}^{-1}$,检测限为 0.05 $\mu\text{mol}\cdot\text{L}^{-1}$,并且具有好的选择性、重现性和稳定性。

关键词: 石墨烯; 金; 纳米复合材料; 生物传感器

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Graphene-Au Nanocomposite: Hydrothermal Synthesis and Application to Biosensor

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Abstract: The graphene-Au nanocomposites were synthesized through hydrothermal route. Transmission electron microscopy (TEM) images gave direct evidence of the thin and transparent sheet structure of graphene. X-ray photoelectron spectroscopic (XPS) and X-ray diffraction (XRD) results also showed the presence of Au and the reduction of graphene oxide. As a model, myoglobin (Mb) was entrapped onto the nanocomposite modified glassy carbon electrode (GCE) and applied to construct a sensor. The immobilized Mb showed a pair of well-defined redox peaks and high catalytic activity for the reduction of H_2O_2 . The biosensor with a linear range from 0.1 to 1.5 $\mu\text{mol}\cdot\text{L}^{-1}$ and a detection limit of 0.05 $\mu\text{mol}\cdot\text{L}^{-1}$ (S/N=3) showed good selectivity, reproducibility and stability. The graphene-Au nanocomposites provide a novel matrix for protein immobilization and the construction of biosensors via the direct electron transfer of protein.

Key words: graphene; gold; nanocomposite; biosensor

0 Introduction

Graphene, as an integral part of graphite, is a monolayer of sp^2 -bonded carbon atoms packed into a dense honeycomb crystal structure, attracting tremendous attention from both the experimental and

theoretical scientific communities^[1]. Graphene-based composite materials have shown to improve electronic and thermal conductivity^[2]. The dispersion of metal nanoparticles on graphene sheets potentially provides a new way to develop catalytic, magnetic, and optoelectronic materials.

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Myoglobin (Mb) is a single-chain protein of 153 amino acids containing a heme (iron-containing porphyrin) group in the center which is found in mammalian skeleton and muscle tissues with the functions to store and transport oxygen. Mb with an α -helical polypeptide chain folds into several segments that serve to stabilize the conformation of iron heme through hydrophobic interaction and hydrogen bonding. It is an ideal model molecule for the study of electron transfer reactions of heme proteins and also for biosensing and biocatalysis. However, the direct electron transfer between the redox center of the immobilized enzyme and an electrode is often shielded by the insulating outer protein shell^[3]. Great efforts have been made to enhance its electron transfer by using mediators, promoters, or some special electrode materials^[4]. The exploitation of Mb-based mediator-free H_2O_2 biosensors is a promising area of research. Because of the good biocompatibility, graphene can act as a connector to bind the enzyme and enhance the possibility of direct electron transfer between protein and electrode surface. Au nanomaterials with good biocompatible can provide a mild microenvironment similar to that of redox proteins in native systems and give the protein molecules more freedom in orientation. They also have great potential to immobilize proteins via reducing efficiently the insulating property of the protein shell for direct electron transfer and facilitating the rate of electron transfer through the conducting tunnels of Au^[5]. Therefore, exploitation of this hybrid material for construction of a Mb-based mediator free H_2O_2 biosensor is a promising area of research.

Herein, we report the facile hydrothermal synthesis and application to the biosensor of graphene-Au nanocomposite. The product was characterized by TEM, XRD, and XPS. The obtained graphene nanosheets are almost transparent and Au nanoparticles with an average diameter less than 50 nm are dispersed on the graphene sheets substrate without aggregation. Based on the good biocompatibility and conductivity, the composite was applied to entrap Mb and construct a H_2O_2 biosensor.

Direct electron transfer between Mb and electrode could be easily achieved and the protein exhibits bioactivity in solutions with a wide range of pH value.

1 Experimental

1.1 Materials

Graphite, sulfuric acid, potassium permanganate, hydrogen peroxide (H_2O_2 , 30% (W/V), solution), polyvinylpyrrolidone (PVP), trisodium citrate, HAuCl_4 , Nafion, and Mb were purchased from Shanghai Chemical Reagent Co. All reagents were of analytical grade and used as received.

1.2 Synthesis of graphene-Au nanocomposite

Graphene oxide (GO) was prepared from graphite by the Hummers method^[6-7]. GO (20 mg) was dispersed into 20 mL of deionized water containing 0.4 g of PVP by sonication. Then 1 mL of HAuCl_4 was added under stirring. Stirring was continued for 30 min. And then the mixture was transferred into a Teflon-lined stainless steel autoclave and treated hydrothermally at 150 °C for 24 h. After the autoclave was cooled down to room temperature, the solution was centrifuged and the precipitate was washed with distilled water and ethanol for several times. The final product was dried in vacuum at 40 °C for 24 h.

1.3 Fabrication of the biosensor

The glassy carbon electrode (GCE) was first polished with 1.0-, 0.3-, and 0.05- μm alumina powder successively, followed by rinsing thoroughly with double distilled water. The polished electrode was then sonicated in acetone and double distilled water and finally dried at room temperature. The graphene-Au nanocomposites obtained above were dispersed in water ($2\text{ mg}\cdot\text{mL}^{-1}$). 10 μL of this suspension and 10 μL of the Mb solution ($5\text{ mg}\cdot\text{mL}^{-1}$) were mixed, and then 5 μL of the mixture was deposited onto the surface of the pretreated GCE. It was left to dry at room temperature. 2 μL Nafion was then added for encapsulation. The electrode was left to dry and stored for at least 24 h at 4 °C. The biosensor was stored under the same condition when not in use.

1.4 Characterization

The morphologies of the graphene-Au nanocom-

posites were investigated by transmission electron microscopy (TEM, JEOL JEM-200CX). TEM operated at 100 kV. X-ray diffraction patterns were taken on a Philip-XPert X-ray diffractometer with a Cu $K\alpha$ X-ray source (tube voltage: 40 kV, current: 40 mA, scanning range: $5^\circ \sim 90^\circ$, scanning speed: $0.5^\circ \cdot s^{-1}$, Cu $K\alpha$, $\lambda = 0.154\ 065\ \text{nm}$). X-ray photoelectron spectroscopic (XPS) analysis was performed on an ESCALAB MK II X-ray photoelectron spectrometer. Electrochemical experiments were performed using a CHI660C workstation (Shanghai Chenhua, Shanghai). All experiments were carried out using a conventional three-electrode system, where the GCE modified with graphene-Au as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode as the reference electrode. All solutions were deoxygenated by nitrogen (high purity: 99.999%)

before and during the measurements.

2 Results and discussion

The shapes of the graphene-Au nanocomposites were analyzed by low and high resolution TEM observations, as shown in Fig.1. The graphene nanosheets are almost transparent and exhibit a very stable nature under the electron beam. From Fig.1, we also can see that Au nanoparticles with a diameter between 10 ~ 50 nm are dispersed on the graphene sheets substrate. Since GO and HAuCl₄ could be reduced to graphene and Au by hydrothermal reduction, respectively^[8-11]. When GO and HAuCl₄ were put into hydrothermal environment in the presence of PVP at the same time, graphene-Au nanocomposites could be obtained. Herein, PVP acts as an anchor agent.

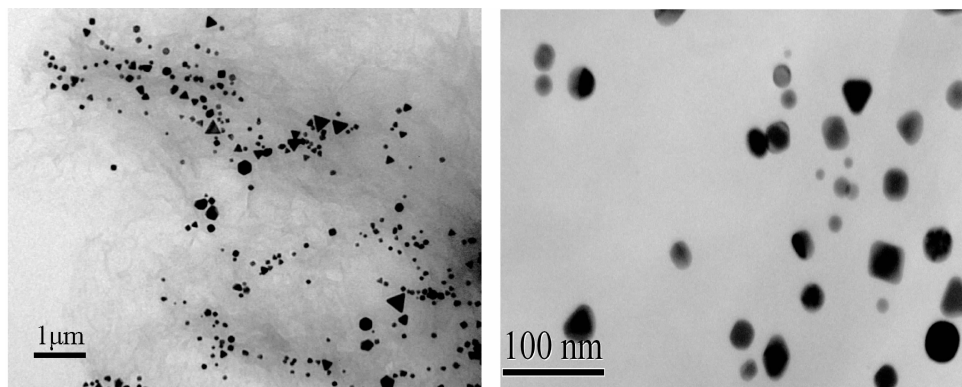


Fig.1 Low and high resolution TEM images of graphene-Au nanocomposites

The XRD pattern confirms the presence of Au nanoparticles in the composites. As shown in Fig.2, the broad peak at 2θ value of 24.5° is ascribed to the graphene nanosheets^[12]. Another five diffraction peaks above 30° correspond to Bragg reflections from (111),

(200), (220), (311), and (222) of the face-centered cubic (fcc) Au crystal structure, and are in good agreement with the reported data^[12], indicating that the Au is formed after being reduced by hydrothermal route.

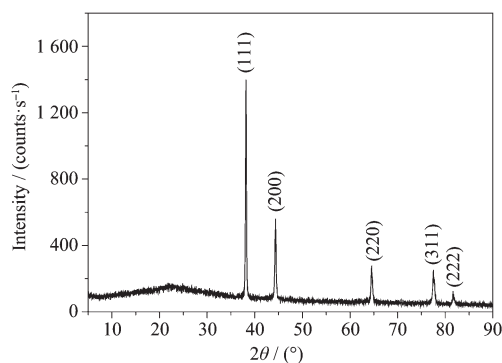


Fig.2 XRD pattern of graphene-Au nanocomposites

The GO and graphene-Au were also characterized by XPS and the corresponding results are shown in Fig.3(a) and 3(b), respectively. The C1s XPS spectrum of graphene-Au nanocomposite shows the same oxygen functionalities with GO. However, the absorbance peaks of graphene-Au at 286.9 (C-O) and 287.2 (C=O and O-C=O) are sharply decreased, which indicates the reduction of GO. In addition, there is an additional component at 285.7 eV corresponding to the C-N bonds that might be caused by the presence of PVP in the resulting product^[13].

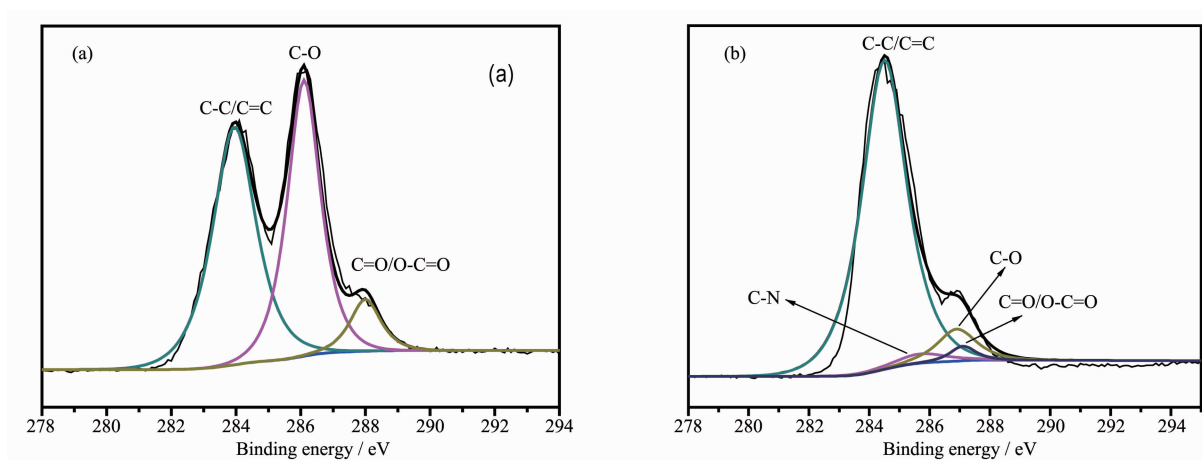
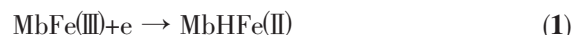


Fig.3 C1s XPS spectra of GO (A) and graphene-Au (b) nanocomposites

In this work, the direct electron transfer between Mb and electrode can be achieved through the immobilization of Mb on a novel graphene-Au nanocomposite. The CVs of Mb/graphene-Au modified GCE without (a) and with $1 \mu\text{mol} \cdot \text{L}^{-1}$ (b) H_2O_2 in nitrogen-saturated $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS solution ($\text{pH}=7.0$) are given in Fig.4(A). A pair of quasi-reversible redox peaks could be observed, as shown in Fig.4a. With the addition of H_2O_2 , an increase in reduction current for the Mb/graphene-Au modified GCE could be

observed, indicating that Mb remains its bioelectrocatalytic activity and could catalyze the reduction of H_2O_2 . The electrocatalytic process can be expressed as follows:



In the presence of H_2O_2 , MbHFe(II) is efficiently converted to its oxidized form, MbFe(III). Consequently, more MbFe(III) molecules are reduced at the electrode surface by the direct electron transfer. Fig.4(B) and 4

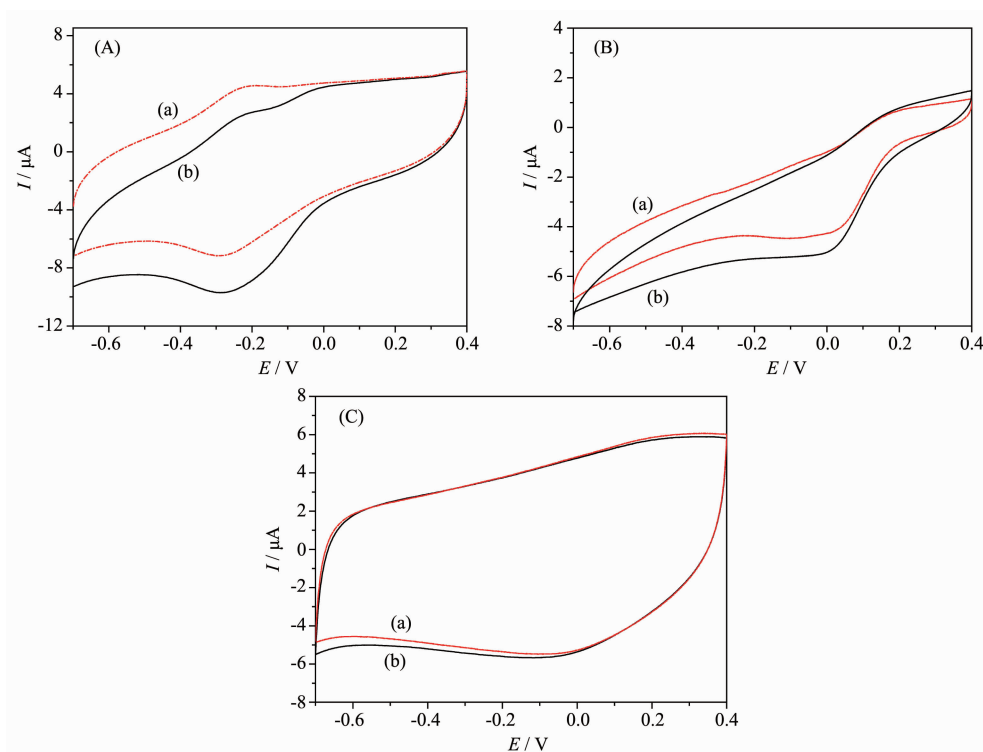


Fig.4 Cyclic voltammograms of the Mb/graphene-Pt (A), Mb (B), and graphene-Pt (C) modified GCE in the absence (a) and in the presence (b) of H_2O_2 ($1 \text{ mol} \cdot \text{L}^{-1}$) in N_2 atmosphere at a scan rate of $100 \text{ mV} \cdot \text{s}^{-1}$

(C) show the CVs of Mb and graphene-Au modified GCE before and after addition of $1 \mu\text{mol} \cdot \text{L}^{-1}$ H_2O_2 in nitrogen-saturated $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS solution ($\text{pH}=7.0$). From the figure, we could see that the catalytic ability of the pure Mb and graphene-Au is lower than that of Mb/graphene-Au modified GCE. Furthermore, Mb can be difficult to realize its direct electrochemistry without the help of graphene-Au. Therefore, a good catalytic effect on the reduction of H_2O_2 of Mb/graphene-Au modified GCE is obtained by the coordinative interaction of Mb and graphene-Au.

The CVs of Mb/graphene-Au modified GCE show a strong dependence on the pH value of the solution (Fig.5). The formal potential shifts negatively with the increase in pH value, and it exhibits a linear relationship over a wide pH value range from 4.0 to 8.0 with a slope of $56.0 \text{ mV} \cdot \text{pH}^{-1}$. This value is very close to the expected value of $58 \text{ mV} \cdot \text{pH}^{-1}$, indicating a one-proton process coupled with single-electron transfer. It can be concluded that Mb exhibits excellent bioactivity in a pH value range from 3.0 to 8.0. And the results also indicate that graphene-Au nanocomposite can provide a suitable microenvironment for Mb.

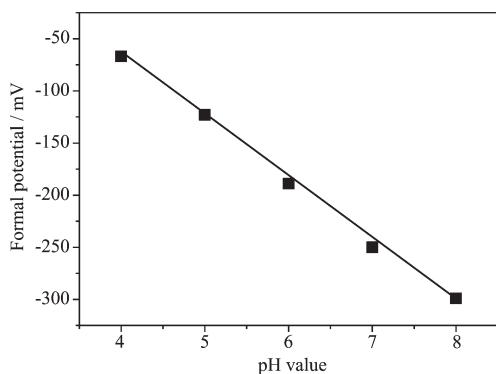


Fig.5 Effect of pH value on the formal potential of the Mb/graphene-Au modified GCE

Fig.6 shows the differential pulse voltammetric (DPV) measurements using Mb/graphene-Au modified GCE in N_2 -saturated $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS solution (pH value of 7.0) upon addition of H_2O_2 with different concentrations. The peak current of Mb/graphene-Au modified GCE increases with the increasing concentration of H_2O_2 . The linear calibration range for

H_2O_2 is $0.1 \sim 1.5 \mu\text{mol} \cdot \text{L}^{-1}$ (inset of Fig.6) with a detection limit of $0.05 \mu\text{mol} \cdot \text{L}^{-1}$ as defined by a signal-to-noise ratio of 3, which is lower than the reported data^[14].

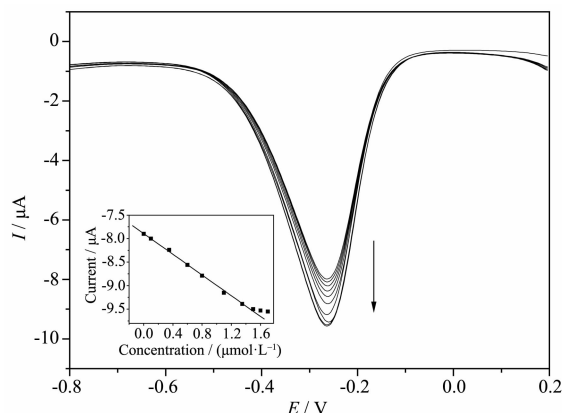


Fig.6 Differential pulse voltammograms obtained at the Mb/graphene-Au modified GCE in $0.1 \text{ mol} \cdot \text{L}^{-1}$ phosphate buffer solution ($\text{pH}=7.0$) containing 0, 0.1, 0.35, 0.6, 0.8, 1.1, 1.35, 1.5, 1.6, and $1.7 \text{ mol} \cdot \text{L}^{-1}$ H_2O_2 (from top to bottom); Inset: relationship between the peak current and the concentration of H_2O_2

The biosensor shows a good selectivity for H_2O_2 . In a N_2 -saturated and stirring PBS solution ($0.1 \text{ mol} \cdot \text{L}^{-1}$, $\text{pH}=7.0$) containing $100 \mu\text{mol} \cdot \text{L}^{-1}$ H_2O_2 , the response of interest from $10 \mu\text{mol} \cdot \text{L}^{-1}$ uric acid (UA) and ascorbic acid (AA) is negligible. The peak current retains 98% of its initial response for Mb/graphene-Au modified GCE after 100 cycles at a H_2O_2 concentration of $1 \mu\text{mol} \cdot \text{L}^{-1}$ (scan rate: $100 \text{ mV} \cdot \text{s}^{-1}$). Moreover, during storage in PBS solution with pH value of 7.0 ($0.1 \text{ mol} \cdot \text{L}^{-1}$) at 4°C , the biosensor still remains about 8% of its initial sensitivity after 3 weeks. This could be due to protein loss from the electrode surface into the PBS solution. The relative standard deviation (RSD) of the peak current in five successive determinations at a H_2O_2 concentration of $1 \mu\text{mol} \cdot \text{L}^{-1}$ is 0.5% for Mb/graphene-Au modified GCE. The corresponding RSD value determined at a H_2O_2 concentration of $1 \mu\text{mol} \cdot \text{L}^{-1}$ is 4.4% for five Mb/graphene-Au modified GCEs fabricated independently.

3 Conclusions

In this work, graphene-Au nanocomposites were

synthesized through facile hydrothermal route. TEM and XRD results show that Au nanoparticles distribute on the single graphene sheet without obvious aggregations. As a model, a third-generation H_2O_2 biosensor was developed based on the direct electrochemistry of Mb immobilized on graphene-Au nanocomposite. The graphene-Au exhibits excellent biocompatibility, high conductivity and good electrocatalytic performance toward the reduction of H_2O_2 and could accelerate the electron transfer between the electroactive sites embedded in protein and the electrode. The proposed biosensor shows sensitive amperometric biosensing for H_2O_2 with a linear range from 0.1 to $1.5 \mu\text{mol} \cdot \text{L}^{-1}$ and a detection limit of $0.05 \mu\text{mol} \cdot \text{L}^{-1}$ (S/N=3), high affinity, excellent selectivity, acceptable reproducibility and stability.

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