Electrochemical Determination of Sulfonamide Based on Glassy Carbon Electrode Modified by Fe$_3$O$_4$/Functionalized Graphene

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A novel strategy for the sensitive determination of sulfonamide using glassy carbon electrode(GCE) modified by Fe$_3$O$_4$/functionalized Graphene (Gr/Fe$_3$O$_4$) was successfully developed in this work. The fabricated Gr/Fe$_3$O$_4$/GCE sensor demonstrated remarkable merits such as higher electrocatalytic activity, higher sensitivity and lower detection limit. Under optimized conditions, the modified electrode achieved a linear range of 5×10^{-7}～1.1×10^{-4} mol/L with a detection limit of 5.0×10^{-8} mol/L. The calibration curve could be expressed by the equation i$_{pa}$(10^{-6}A) =1.356×10^{-4} c (10^{-5} mol/L)+0.898 with a linear coefficient of 0.994. The recovery rate was in the range of 88.5%～104.0%, indicating the enormous potential and prospects of this method.

1. INTRODUCTION

Sulfonamide is a group of synthetic antibiotic drugs which is effectively employed in the prevention and treatment of infection diseases caused by gram-positive, gram-negative bacteria and some protozoa[1-4]. Owing to the merits of low cost and wide antimicrobial spectrum, sulfonamide has been widely used as veterinary drugs in animal husbandry[5-6]. But improper utilization will cause enrichment of sulfonamide in animal body and result in side effect to human body directly or indirectly[7-8]. Consequently, it is imperative to develop valid methods for the determination of sulfonamide quantitatively[9].

Several analytical techniques such as liquid chromatography, high performance liquid chromatography, spectrophotometry, chemiluminescence, and capillary electrophoresis-mass spectrometry have been used for the detection of sulfonamides due to their high sensitivity and great selectivity[10-14]. However, all these methods are complicated, time-consuming and require expensive

equipment. Compared with aforementioned methods, electrochemical method overcomes those drawbacks owing to its low cost, rapid response and application in on-site test[15].

Magnetite Fe_3O_4 nanocomposites with unique properties such as superparamagnetism, high surface activity and high conductivity has caused great interest in the field of electrochemical sensor[16-18]. Graphene, a one-atom-thick sheet of honeycomb carbon lattice, has been universally used for constructing various modified electrodes in virtue of the high surface area, excellent electrical conductivity and strong mechanical strength[19-21]. Nevertheless, graphene tends to form agglomerates, which limits the further application in fabricating sensors[22-23].

Thus, a composite material named Gr/Fe_3O_4 with high electrical conductivity and extended catalytic active sites was constructed in this work since the magnetite Fe_3O_4 nanocomposites could overcome the accumulation of graphene sheets and increasing the distance between the graphene layers[24]. Herein, a novel electrochemical sensor was proposed for sensitive determination of Sulfonamides based on GCE modified with Gr/Fe_3O_4. The proposed method show sufficient simplicity, sensitivity and selectivity in detection of Sulfonamides in real samples.

2. EXPERIMENTAL

2.1. Reagents and Instruments

Sulfonamide was obtained from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin,China). Graphene was purchased from Ningbo Institute of Industrial Technology Co., Ltd. (Shanghai, China). Sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, 99%~101%), sulphuric acid (H_2SO_4, 98%), hydrogen peroxide (H_2O_2, 30%) and sodium hydroxide (NaOH, ≥96%) were donated from Luoyang Chemical Reagent Factory (Luoyang, China). 0.04 mol/L phosphate, 0.004 mol/L acetic acid, 0.04 mol/L boric acid, and 0.04 mol/L sodium hydroxide were used to prepared the BR buffer solution. All other chemicals were analytical grade and all aqueous solutions were prepared by double distilled water.

All electrochemical measurements were performed with a CHI660E electrochemical work station (Shanghai Chen Hua Instrument Co., China) equipped with a three-electrode system which was constructed with a bare GCE or modified GCE as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The transmission electron microscope (TEM) characterization was carried out on HT7700 (Hitachi, japan). And the XRD analysis was performed on Bruker D8 diffractometer with high-intensityCu Kα (λ= 1.54 Å).

2.2. Preparation of Gr/Fe₃O₄ nanocomposites

Graghene oxide was obtained from natural graphene according to the Hummers method[25]. 20 mg of the synthesized graphene oxide was dissolved in 100 mL double distilled water and then under ultrasound for 20 minutes. Next, NaOH was added into the mixture to adjust pH to 11 or 12. The mixture was stirred in ultrasonic bath for 15 minutes after adding 0.8224 g ferrous sulfate and 5 mL hydrazine hydrate. Afterwards, the mixture was dried in the vacuum drying oven at 100℃

temperatures and then washed until pH 7.0. Gr/Fe_3O_4 was finally obtained after filtrating the mixture by a vacuum pump and dried under $60^{\circ}C$ vacuum environment. The Gr/Fe_3O_4 suspension was prepared by dispersing 3 mg Gr/Fe_3O_4 into 2 mL DMF under ultrasonic for 1 hour.

2.3. Fabrication of modified electrode

Prior to modification, the bare GCE was polished with 1.0, 0.3, 0.05 μm graininess Al_2O_3 on a circular polishing cloth, followed by washing ultrasonically with acetone and double distilled water, and then dried at room temperature. 2 μL Gr/Fe_3O_4 suspension was casted on the surface of a bare GCE and dried at room temperature. The Gr/GCE was prepared with the same method.

3. RESULTS AND DISCUSSION

3.1. TEM characterization of Gr/Fe_3O_4 nanocomposites

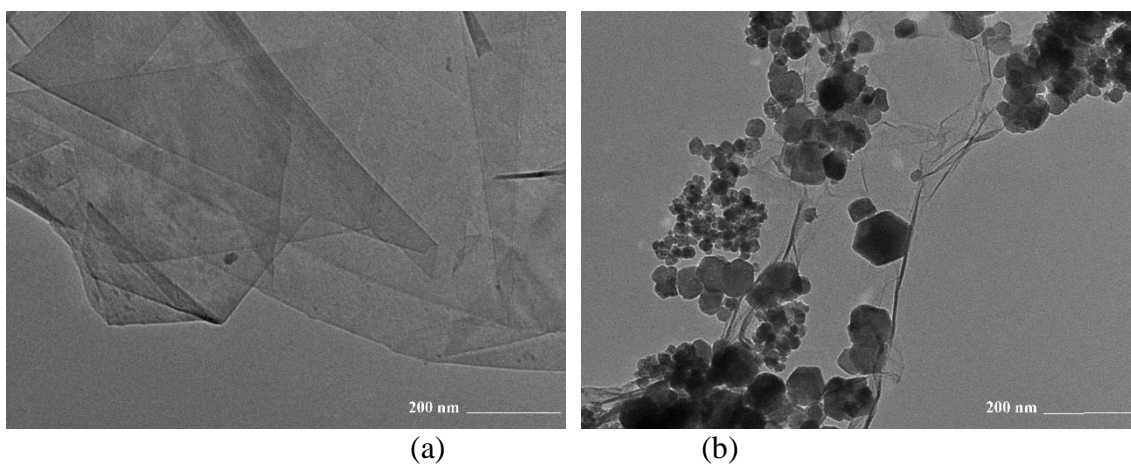The transmission electron microscopy (TEM) was employed to investigate the surface morphology of Gr and Gr/Fe_3O_4.

(a)   (b)

Figure 1. TEM images of Gr (a) and Gr/Fe_3O_4.

As shown in Fig. 1a, the flake structure of graphene was clearly observed. And there were some crumpled areas and folded structures between layers, indicating the high surface area and excellent conductivity of graphene. Spherical shape Fe_3O_4 nanocomposites could be seen on the surface of graphene as shown in Fig. 1b, which enhanced the surface and prevent graphene layers from accumulating. These results were consistent with those in other works[26-27]. However, some Fe_3O_4 nanocomposites agglomerated together instead of distributing uniformly on the surface. Surfactants were introduced to improve the dispersity of Fe_3O_4 nanocomposites in other works.

3.2. XRD characterization of Gr/Fe₃O₄ nanocomposites

The structural property of Gr/Fe$_3$O$_4$ nanocomposites was analyzed by XRD. As shown in figure 2, the diffraction peak of graphene appeared at about 26.35°(curve b).
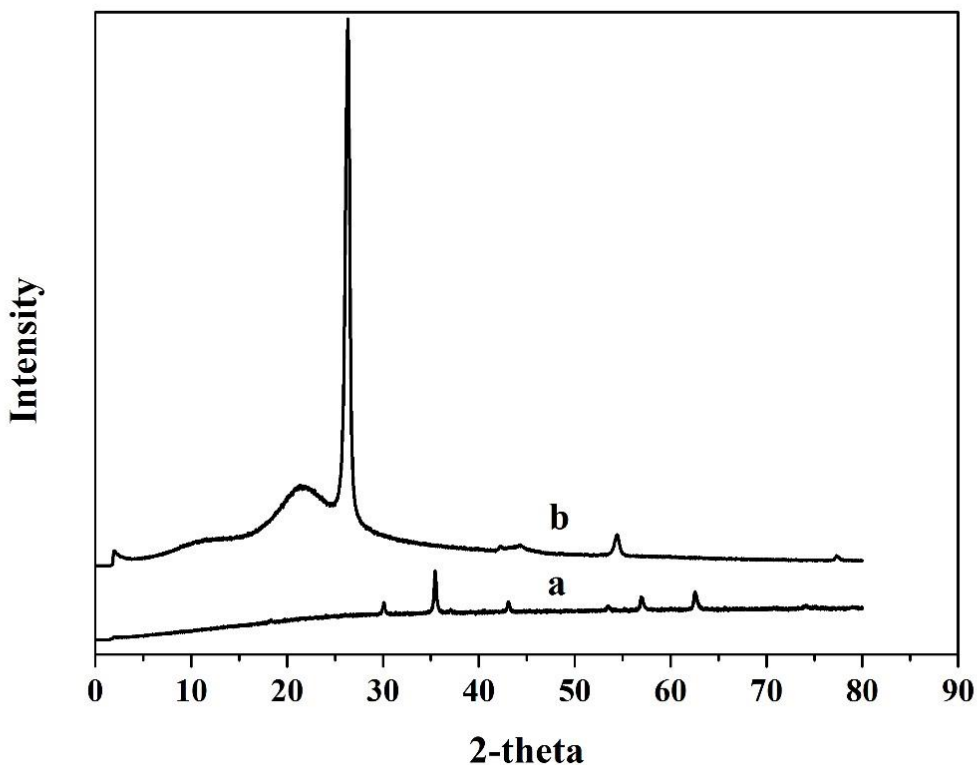

Figure 2. XRD patterns of Gr/Fe$_3$O$_4$ (curve a) and Gr (curve b).

And the diffraction peak positions at 2θ=30.09, 35.39, 43.07, 53.45, 56.95 and 62.53 could be attributed to (220), (311), (400), (422), (511) and (440) planes of cubic Fe$_3$O$_4$ lattice, respectively (curve a). These facts indicated that the Gr/Fe$_3$O$_4$ nanocomposites were successfully prepared and were consistent with those in previous reports[28-29].

3.3. Electrochemical impedance spectroscopy of the electrodes

Eletrochemical impedance spectroscopy (EIS) was performed to calculate the impedance changes of the electrode surface. The comparison of EIS responses of 10 mM K$_3$[Fe(CN)$_6$] at different electrodes was shown in Fig. 3.
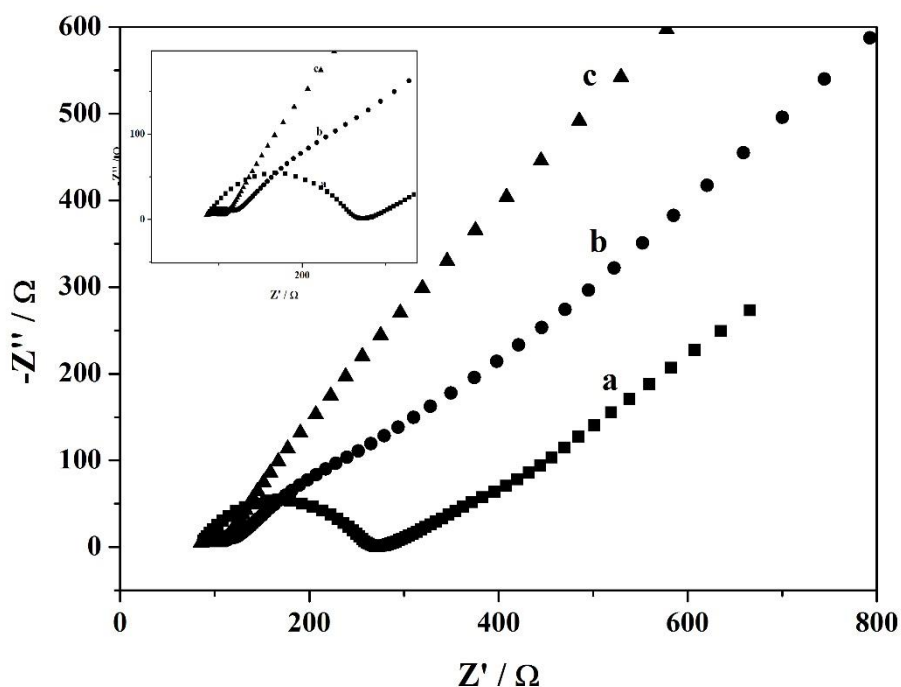
Figure 3. Nyquist diagrams for the bare GCE (a), Gr/GCE (b) and Gr/Fe$_3$O$_4$/GCE (c) in solution of 10 mM [Fe(CN)$_6$]$^{3-/4-}$ and 0.10 M KCl solution.

The electron transfer resistance (Rct) at bare GCE was estimated to be about 170 Ω (a). A smaller semicircle corresponding to a smaller Rct value of 28 Ω was observed at Gr/GCE (b). The decrease of Rct value (17 Ω) at Gr/Fe$_3$O$_4$/GCE (c) indicated the improvement of conductivity, owing to the high surface area and excellent conductivity of Gr/Fe$_3$O$_4$ nanocomposites. Therefore, the Gr/Fe$_3$O$_4$/GCE was used in subsequent experiments.

3.4. Electrochemical Behaviors of Sulfonamides

The electrochemical performance of 1×10^{-3} mol/L sulfonamide in BR buffer solution (pH 2.0) was investigated by cyclic voltammetry at different electrodes surface, which was noted in Fig. 4.

The cyclic voltammetry measurement was performed in the range of 0.4~1.5 V with a scan rate of 100 mV/s. As can be seen in this figure, there was no obvious peak found at bare GCE without the existence of sulfanilamide (curve a). After the addition of sulfanilamide, an anodic peak was observed at about 1.0 V on bare GCE (curve b). For Gr/GCE, there was a slight peak current enhancement (curve c). And a considerable increase in the peak current about two-fold for sulfanilamide detection compared with bare GCE was found at Gr/Fe$_3$O$_4$/GCE (curve d). All of these results indicated that graphene could be used as a suitable modified material because of its high surface area and excellent electrical conductivity. And the electron transfer between sulfanilamide and electrode could be promoted by the composite material consisting of Gr and Fe$_3$O$_4$, leading to improvement of sensitivity for sulfanilamide determination. The results were consistent with the TEM and EIS analysis.
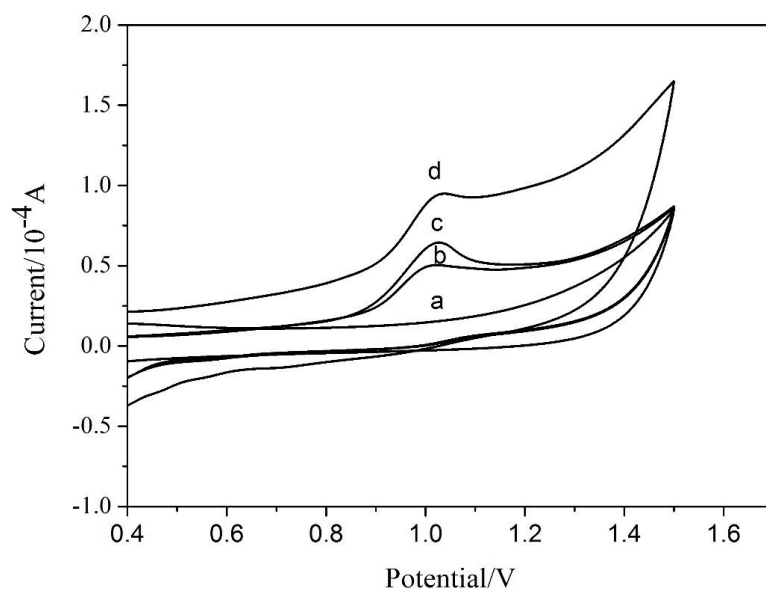
Figure 4. Cyclic voltammograms of BR buffer solution (pH 2.0) with the absence of sulfonamide at bare GCE (a), Cyclic voltammetries of BR buffer solution (pH 2.0) containing 1×10^{-3} mol/L sulfonamide at bare GCE (b), Gr/GCE (c) and Gr/Fe$_3$O$_4$/GCE (d).
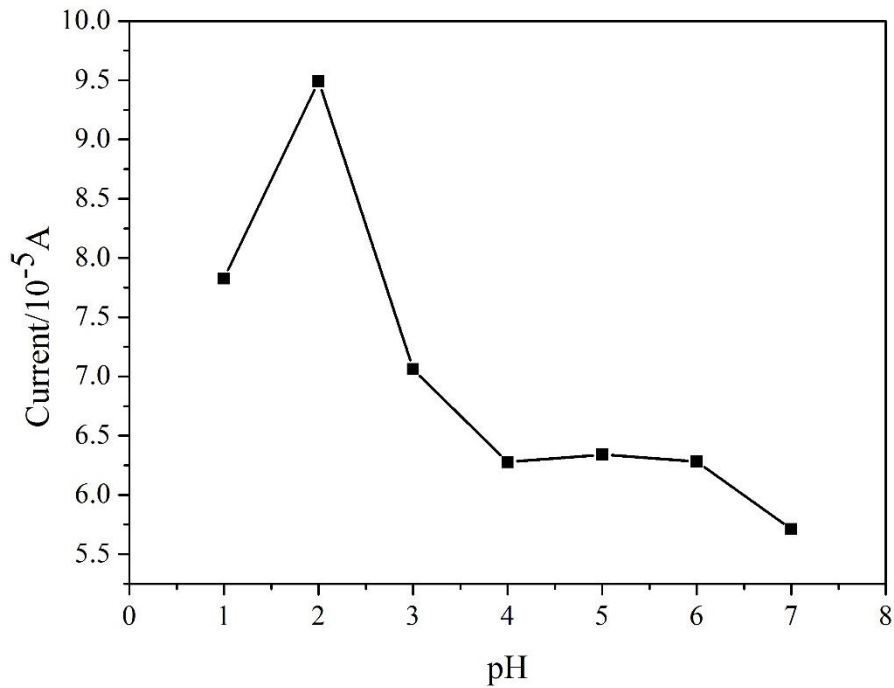
3.5.Effect of pH

Figure 5. The dependences of peak current on solution pH values.

Cyclic voltammograms of 1×10^{-3} mol/L sulfonamide on Gr/Fe$_3$O$_4$/GCE were recorded in BR buffer solution in the pH range of 1.0~7.0. It was found that the peak currents of sulfonamides increased with the changing of pH values from 1.0 to 2.0 (Fig.5).

The maximum peak current was observed at pH 2.0 and then the peak currents decreased as the pH further increased, indicating that H$^+$ might involve in the oxidation process of sulfonamide at the surface of the modified electrode. Consequently, BR solution of pH 2.0 was chose in further determination to obtain the best sensitivity.

3.6. Effect of scan rate

To study the effect of scan rate, cyclic voltammetry of 1×10^{-3} mol/L sulfonamide was performed in BR buffer solution (pH2.0) with various scan rates at Gr/Fe$_3$O$_4$/GCE. As shown in Fig. 6, the oxidation peak current was increased as the scan rates increased from 10 to 140 mV/s.
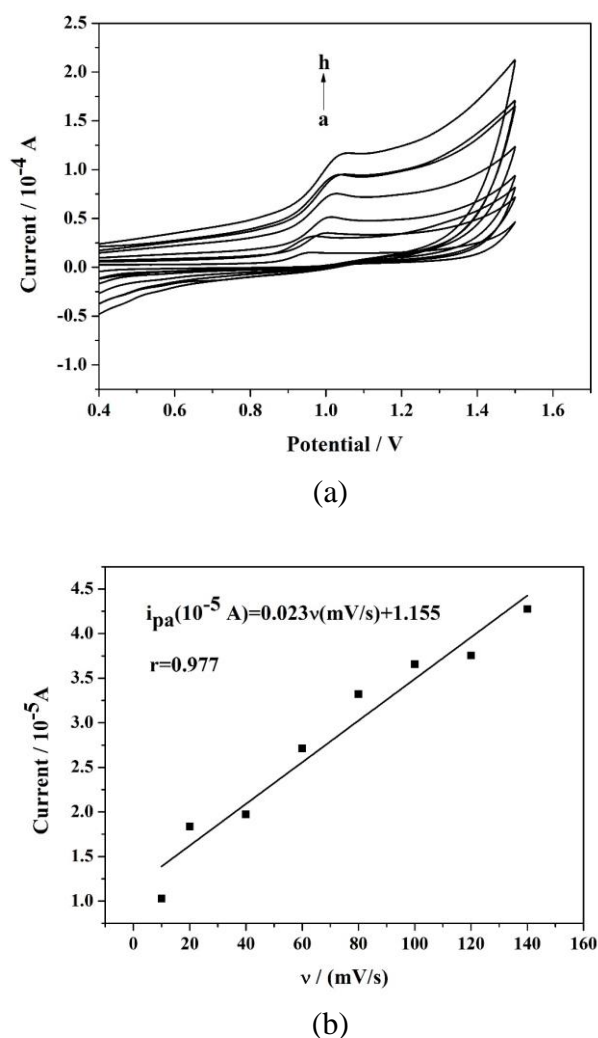

(a)

(b)

Figure 6. Cyclic voltammograms of 1×10^{-3} mol/L sulfonamide at Gr/Fe$_3$O$_4$/GCE in BR buffer solution (pH 2.0) at different scan rates. (a) Curve a to h corresponds to the scan rate: 10 mV/s; 20 mV/s; 40 mV/s; 60 mV/s; 80 mV/s; 100 mV/s; 120 mV/s; 140 mV/s. (b) Plots of peak currents and scan rates.

It was found that the peak current was proportional to the scan rate, with a regressive equation of $i_{pa}(10^{-5}A)=0.023v$ (mV/s)$+1.155(r=0.977)$, indicating that the electrochemical reaction of sulfonamide was controlled by surface adsorption.

3.7. Determination of sulfonamides concentration

(a)

$$i_{pa}(10^{-6}A)=1.356\times10^{-4}c \ (10^{-5}mol/L)+0.898$$
$$r=0.994$$
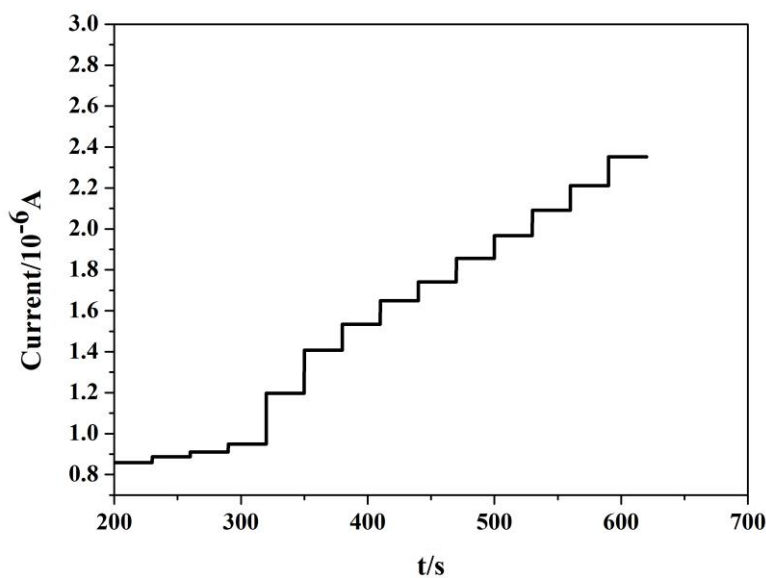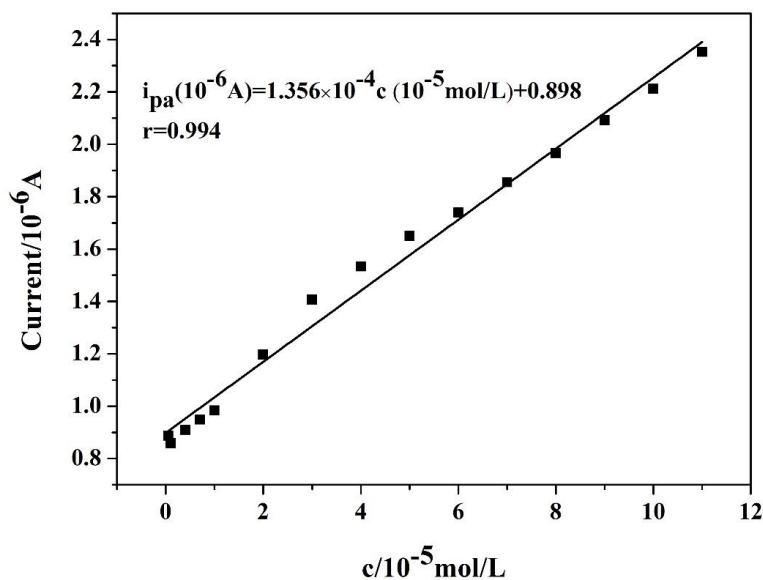
(b)

Figure 7. (a) Typical amperometric signals at Gr/Fe₃O₄/GCE with successive increments of sulfonamides in pH 2.0 BR buffer solution at the potential of 1.0 V. (b) The corresponding linear calibration curve of peak currents versus concentration; scan rate 100 mV/s.

Under optimized conditions, the results of amperometric measurement for various concentrations of sulfonamide on the surface of Gr/Fe$_3$O$_4$/GCE in BR buffer were recorded (Fig. 7a). Calibration curve was obtained in the concentration range of $5\times10^{-7}\sim1.1\times10^{-4}$ mol/L (Fig. 7b). The obtained linear regression equation was $i_{pa}(10^{-6}A)=1.356\times10^{-4}c(10^{-5}$ mol/L$)+0.898(r=0.994)$ with a detection limit (LOD) of 5.0×10^{-8} mol/L(S/N=3). These results illustrated the practical applicability of the modified electrode for the determination of sulfonamides.

The comparison of performance of the proposed sensor and other sensors reported in literatures for the determination of sulfonamide was shown in Table 1. The results in this table showed that Gr/Fe$_3$O$_4$/GCE exhibits lower detection limit and wider linear ranges than other sensors, indicating Gr/Fe$_3$O$_4$/GCE was more suitable for the detection of sulfonamide.

Table 1. Comparison of some characteristics of different sensors for the determination of sulfonamides.

Electrode	Method	Linear range (mol/L)	LOD (mol/L)	Reference
Carboxyl/DMF/MWCNTs/ GCE	CV	1.0×10^{-6}-1.0×10^{-4}	5.0×10^{-7}	30
Nafion-MWCNTs PME	CV	1.0×10^{-5}-1.0×10^{-4}	2.69×10^{-6}	31
MWCNTCOOH/BA-SPCE	DPV	1.0×10^{-6}-7.0×10^{-5}	3.0×10^{-7}	32
SQX-TT/CPE	Potentiometry	5.0×10^{-6}-1.0×10^{-2}	3.0×10^{-6}	33
MIP/CPE	DPV	2.0×10^{-7}-1.0×10^{-4}	1.4×10^{-7}	34
Gr/Fe$_3$O$_4$/GCE	i-t	5×10^{-7}-1.1×10^{-4}	5.0×10^{-8}	This work

3.8. Interference of coexisted substance

The potentially influence of several substances was investigated by cyclic voltammetry in 1×10^{-4} mol/L sulfonamide solution. The variations of peak currents with the presence of 200-fold of K$^+$, Na$^+$, Ca$^+$, Mg^{2+} and Cl$^-$ were less than ±5%, indicating that these ions had no significant influence on sulfonamides determination at Gr/Fe$_3$O$_4$/GCE.

3.9. Detection of sulfonamides in real samples.

In order to examine the applicability of the proposed sensor for real samples determination, analysis of sulfonamide was carried out in pork using standard addition method. Firstly, the mashed pork sample and 1 mL ethylacetate got mixed in a 1.5 mL centrifuge tube. Then, the mixture was sonicated for 10 minutes and centrifuged for 10 minutes at 5000 rpm. 0.5 mL aliquot of supernatant was ten folds diluted with BR buffer solution. Afterward, the resulting solution spiked with various amount of standard sulfonamide was used to evaluate the recovery. As shown in Table 2, the recoveries were in the range of 88.5%～104.0%, indicating that the developed sensor could be successfully applied to detection of sulfonamides in real samples.

Table 2. Recovery test of sulfonamides in real sample.

SAs	Addition (10^{-5} mol/L)	Found (10^{-5} mol/L)	Recovery (%)	RSD (%, n=3)
	1.0	0.945	94.5	3.28
	2.0	1.77	88.5	3.16
	3.0	3.12	104.0	2.79

4. CONCLUSION

In the study, a novel strategy for the determination of sulfanilamide using glassy carbon electrode(GCE) modified by Gr/Fe_3O_4 was successfully developed. The voltammetric response of sulfonamide at $Gr/Fe_3O_4/GCE$ was evidently enhanced compared with that at bare GCE, indicating the remarkable merits such as good conductivity and excellent sensitivity of the proposed method. The modified electrode was used for the determination of sulfonamide in real sample with acceptable recovery results, illustrating the great potential of this method.

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