Voltammetric Determination of Sudan II in Food Samples at Graphene Modified Glassy Carbon Electrode Based on the Enhancement Effect of Sodium Dodecyl Sulfate

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Summary: Herein, a novel electrochemical method was developed for the determination of Sudan II based on the electrochemical catalytic activity of graphene modified glassy carbon electrode (GME) and the enhancement effect of sodium dodecyl sulfate (SDS). In a pH 6.0 phosphate buffer solution, Sudan II exhibited a pair of well-defined quasi-reversible redox peaks at the GME in the presence of 5.0×10^{-5} mol L^{-1} SDS. The oxidation peak current of Sudan II was linearly proportional to its concentration in a range from 4.0×10^{-8} to 4.0×10^{-6} mol L^{-1}, with a linear regression equation of $i_{pa}(A) = 3.35c + 5.96 \times 10^{-6}$, r = 0.9988 and a detection limit of 8.0×10^{-9} mol L^{-1}. The recoveries from the standards fortified blank samples were in the range of 94.7% to 97.5% with RSD lower than 4.0%. The novel method has been successfully used to determine Sudan II in food products with satisfactory results.

Key words**:** Sudan II; Sodium dodecyl sulfate; Graphene; Modified electrode.

Introduction

Sudan dyes (Sudan I, II, III, and IV) are a family of synthetic azo dyes that are extensively used in industries such as car wax, petrol, shoe polish etc [1]. They have been banned for food usage in most countries due to their carcinogenicity [2]. However, illicit use of Sudan dyes can still be found in various food products, especially in ketchup and chili sauce. Therefore, development of specific and convenient analytical methods for these Sudan dyes in food products is of great interests for many researchers.

By now, many methods have been developed for the determination of Sudan II in food products, including HPLC [3, 4], HPLC-DAD [5], HPLC/MS [6, 7], HPLC/UV-vis [8, 9], HPLC-PAD [10], UPLC [11], HPLC-electrochemical detection [12], GC/MS [13], resonance light scattering [14], capillary electrophoresis [15], enzyme-linked immunosorbent assay [16] and voltammetric method [17, 18]. However, almost all these reported methods require expensive equipments, large amount of organic solvents and are time-consuming, and some of them are not very sensitive. Thus, it is still of great significance to develop convenient, cheap and sensitive detection methods for Sudan II.

Graphene is an allotrope of carbon, whose structure comprises a single layer of sp^2-hybridized carbon atoms joined by covalent bonds, forming a flat hexagonal lattice [19]. It has been gaining popularity due to its unique properties such as electronic, structural and mechanical characteristics. Graphene shows outstanding ability such as fast electron transfer kinetics, excellent activity for a variety of electro-active species [20] and has been used to prepare a new generation of electrodes for electrochemical studies [21-24]. Surfactants are amphiphilic compounds that contain both hydrophobic groups and hydrophilic groups. They can adsorb on hydrophobic electrode surface and change the properties of electrode/solution interface, which in turn can heavily influence the electrochemical process of electroactive species. The applications of surfactants in electrochemistry and electroanalytical chemistry have been widely reported [25-28].

In this work, a novel electrochemical method for the determination of Sudan II in food samples based on the electrochemical catalytic activity of graphene modified glassy carbon electrode (GME) and the enhancement effect of sodium dodecyl sulfate (SDS) is described. The electrochemical behavior of Sudan II was investigated in detail, revealing that graphene film can greatly increase the redox peak currents of Sudan II and SDS can remarkably enhance detection sensitivity. The oxidation peak current showed a linear relationship with the concentrations of Sudan II in the range of 4.0×10^{-8} to 4.0×10^{-6} mol L^{-1}, with a detection limit of 8.0×10^{-9} mol L^{-1}. The novel convenient sensing method possesses high sensitivity and good accuracy.

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Its practical application has been demonstrated by the determination of Sudan II in ketchup and chili sauces.

Results and Discussion

Characterization of graphene and the GME

The obtained graphene and GME were fully investigated by IR, Raman spectroscopy, SEM and TEM. The IR spectra of the obtained graphene show the absorption bands of C–OH, C=C, phenyl and C–O–C in the wave range number of $3450cm^{-1}$, $1558cm^{-1}$, $2800-3000cm^{-1}$ and $1110-1200cm^{-1}$ respectively, which demonstrate that graphene has been successfully prepared and the graphene platelets contain abundant C–O–C and C–OH functional groups. The functionalized and defective graphene platelets are more hydrophilic and can be easily dispersed in solvents with long-term stability. Fig. 1 shows the Raman spectrum of the obtained graphene, which shows the D band at 1347 cm^{-1} that occurs at the edges or in defectives of the graphene platelets as well as the G band at 1597 cm^{-1} that shows the in-phase vibration of the graphite lattice. The relative intensity ratio of the D and G lines provides a sensitive measure of the disorder and crystallite size of the graphitic layers. The Raman spectrum is similar to reported one that shows a D band at 1340 $cm-1$ and a G band at 1592 cm^{-1} [20]. Fig. 2 shows the SEM image of the graphene film on the GCE, revealing the crumpled and wrinkled structure of the graphene film on the electrode. In reported literature, similar SEM image was obtained [22]. Fig. 3 shows the TEM image of the nano graphene platelets, revealing a mono- or few-layer planar sheet-like morphology.
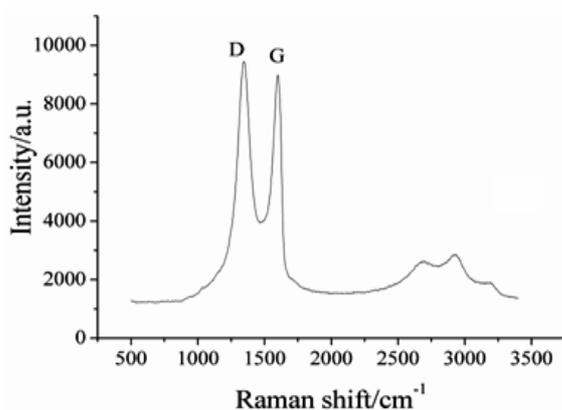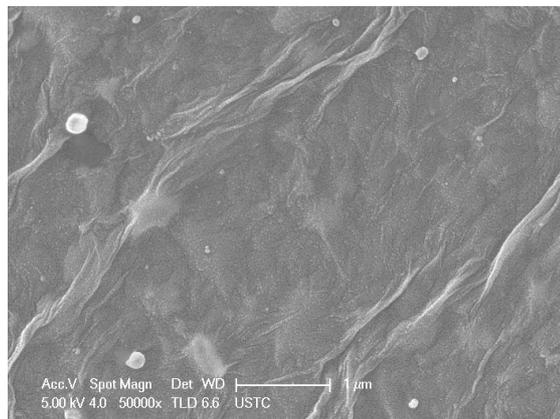

Fig. 1:   Raman spectra of graphene.

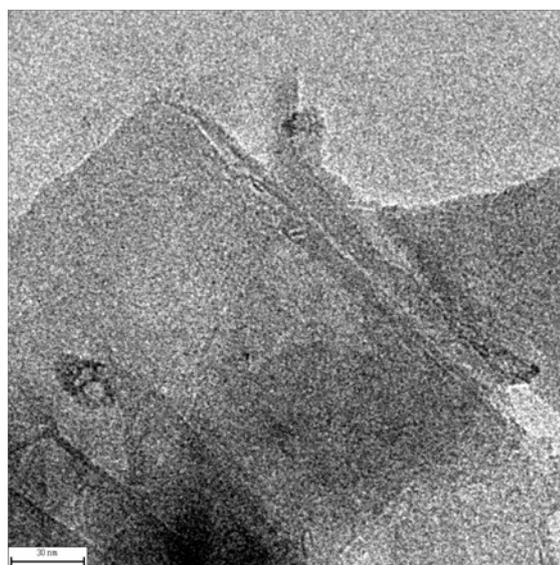Fig. 2:   SEM image of graphene film on the glassy carbon electrode.

Fig. 3:   TEM image of graphene.

Electrochemcial Behavior of Sudan II

The electrochemical behavior of Sudan II at a bare GCE and the GME was studied using cyclic voltammety. Fig. 4 shows that the current response of 2.0×10^{-6} mol L^{-1} Sudan II at the bare GCE (curve a) and the GME (curve c) in pH 6.0 PBS. From the figure we can see that only very weak current response can be observed at the bare GCE. While at the GME, a pair of well defined redox peaks are observed with $E_{pa} = 88$ mV, $E_{pc} = -46$mV and $i_{pa} = -6.15$ μA, $i_{pc} = 8.03$μA. The peak-to-peak separation (134 mV) and the peak currents ratio ($i_{pa}/i_{pc} < 1$) indicate that the reaction process of Sudan II at the

GME is a quasi-reversible redox process. The obvious peak current increase suggests that graphene film can significantly catalyze the oxidation process of Sudan II and the electron transfer rate in the graphene film is much faster. This may be attributed to the special chemical and nano-mesh structure of graphene, which has a large specific surface area and a large number of defects. These defects in graphene are resulted from its oxidation-reduction preparation process and they serve as highly active reaction sites in the electrochemical reactions at the GME. All these unique physical and chemical properties make the reactivity of Sudan II at the modified electrode significantly improved and the response signal greatly increased.
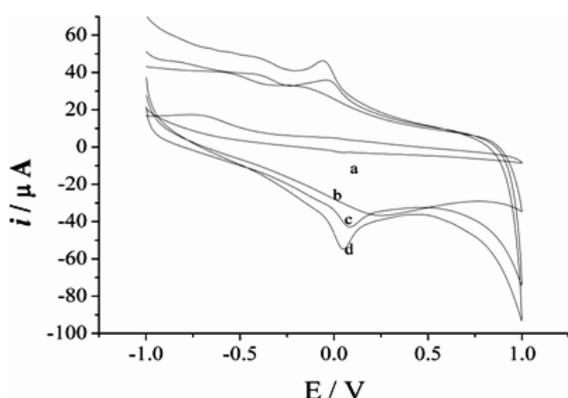

Fig. 4: Cyclic voltammograms of Sudan II (2.0×10^{-6} mol L^{-1}) at the bare GCE (a); Cyclic voltammograms of 5.0×10^{-5} mol L^{-1} SDS (b), 2.0×10^{-6} mol L^{-1} Sudan II (c), and 5.0×10^{-5} mol L^{-1} SDS + 2.0×10^{-6} mol L^{-1} Sudan II (d) at the GME (pH=6.0). Scan rate: 100 mV s^{-1}.

The enhancement effect of SDS to the redox peaks of Sudan II was also investigated by cyclic voltammetry. As shown in Fig. 4, curve b, no obvious redox peaks can be observed for 5.0×10^{-5} mol L^{-1} SDS at the GME. However, from the comparison of curve c and curve d in Fig. 4, we can see that the oxidation peak current of 2.0×10^{-6} mol L^{-1} Sudan II almost doubled and its reduction peak current also increased when tested in the presence of 5.0×10^{-5} mol L^{-1} SDS (Fig. 4, curve d). The oxidation and reduction currents were i_{pa} = –11.90 μA and i_{pc} = 12.23 μA, respectively. The enhancement effect of SDS may be attributed to the amphiphilic properties of SDS, which enable SDS form a layer at the electrode surface via the hydrophobic interaction [29, 30]. The long hydrophobic C–H chain of SDS can efficiently accumulate Sudan II towards the electrode surface, enhancing the electrochemical response of Sudan II. As a result, peak currents of Sudan II are increased and the determining sensitivity is improved.

Effect of Solution pH

The electrochemical behavior of Sudan II at the GME was firstly investigated utilizing different supporting buffers such as pH 2.2 – 8.0 phosphate buffer, pH 3.5 – 5.6 NaAc–Hac buffer, and pH 1.8 – 12.0 BR buffer. Results showed that the redox peak currents were higher and the peak shapes were better in phosphate buffer compared with the other buffers. Then, the influence of the pH value of phosphate buffer on the signal response of Sudan II was examined. As shown in Fig. 5, when the pH value gradually increased from 2.2 to 6.0, the oxidation peak current of Sudan II increased accordingly with the increase of pH value. However, as the pH value continued to increase from 6.0 to 8.0, the oxidation peak current conversely decreased. As a result, pH 6.0 phosphate buffer was chosen as the supporting buffer for the determination of Sudan II. We also observed that the redox peaks of Sudan II shifted negatively with increasing pH value and the oxidation peak potential changed linearly depending on a pH from 2.2 to 8.0, with a linear equation of E_{pa} = 0.54 – 0.074pH, r = –0.9982, indicating that the redox reaction involves protons.
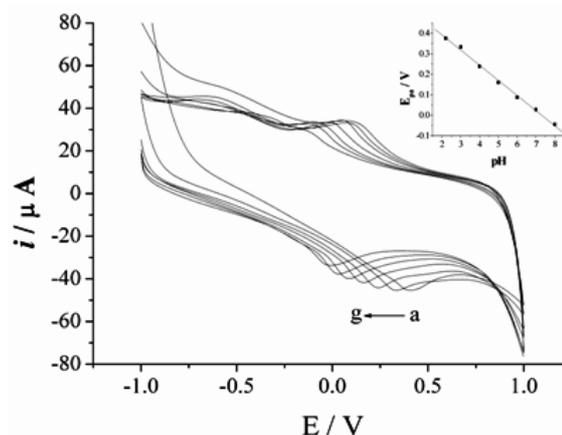

Fig. 5: Cyclic voltammograms of 1.0×10^{-6} mol L^{-1} Sudan II at different pH values. Each of the letters from a to g corresponds to a pH of 2.2, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0, respectively. Inset is the plot of the peak potential of Sudan II versus pH value of buffer solutions. Scan rate: 100 mV s^{-1}.

Effect of Ethanol Amount

Sudan II is insoluble in water whereas soluble in ethanol. In this study, a certain amount of

ethanol was added into the solution to increase the solubility of Sudan II. The amount of ethanol exerts great influence on electrochemical response of Sudan II at the GME. Measurements were made with different amounts of ethanol added into the solution and results are shown in Fig. 6. It was found that the maximum redox peak current was obtained when PBS (pH 6.0), ethanol and water were in the ratio of 10:6:4. Thereby, a supporting electrolyte with a ratio of 10:6:4 among PBS (pH 6.0), ethanol and water was chosen in this study.
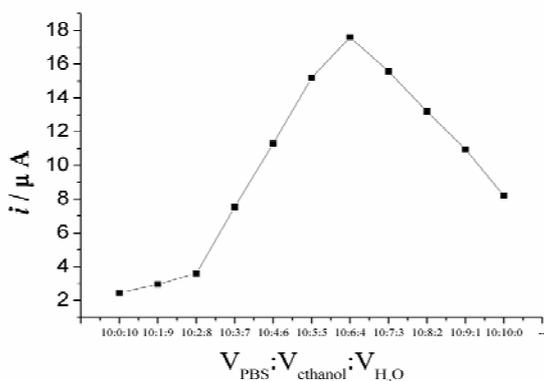

Fig. 6:   Plot of the oxidation peak current of 3.0×10^{-6} mol L^{-1} Sudan II versus the ratio of PBS (pH 6.0), ethanol and water.

Effect of Surfactant

The oxidation behaviors of Sudan II in the absence of surfactant and in the presence of different surfactants were investigated. It was found that the redox peak currents of Sudan II increased in the presence of all investigated surfactants such as sodium dodecyl sulfonate (SDSF), sodium dodecyl sulfate (SDS), sodium dodecyl benzene sulfonate (SDBS), cetyl trimethyl ammonium bromide (CTAB) and cetyl pyridium bromide (CPB). The enhancement effect of SDS was most significant and the oxidation peak current of Sudan II was highest in the presence of SDS. The effect of SDS concentration on the oxidation peak current of Sudan II was further studied. Results showed that, as SDS concentration gradually increased from 0 to 5.0×10^{-5} mol L^{-1}, the oxidation peak current of Sudan II increased accordingly with the increase of SDS concentration and reached maximum at 5.0×10^{-5} mol L^{-1}, When SDS concentration continued to increase, the oxidation peak current conversely decreased. Therefore, 5.0×10^{-5} mol L^{-1} was chosen as the concentration of SDS in this work. This may be

attributed that, when SDS concentration is low, the adsorption of SDS as the monomer and could effectively affect the charge transfer rate instead of the surface properties of the GME. When SDS concentration becomes higher, SDS forms a monolayer on the electrode surface and results in a change of electrode/solution interface.

Effect of Scan Rate

The effect of scan rate on the redoxidation of Sudan II was investigated by cyclic votammetry at a concentration of 2.0×10^{-7} mol L^{-1} in pH 6.0 PBS in a range of from 40 to 600 mV s^{-1}. As shown in Fig. 7, the redox peak currents of Sudan II increased with increasing scan rate. The oxidation peak shifted towards the positive direction and reduction peak shifted towards the negative direction. The oxidation peak current was linearly proportional to the square root of the scan rate with linear equations expressed as $i_{pa}(A) = 3.22 \times 10^{-7} v^{1/2} + 9.96 \times 10^{-7}$ (mV), r = 0.9985, which indicates that the electrode process under these conditions is a diffusion controlled process. Scan rate of 100 mV s^{-1} gave the best redox peaks of Sudan II, therefore, 100 mV s^{-1} was chosen as the best scan rate.
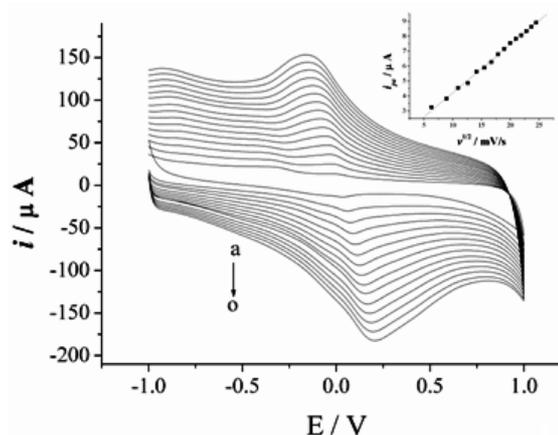

Fig. 7:   Cyclic voltammograms of 2.0×10^{-7} mol L^{-1} Sudan II at the GME. Inset is the plot of oxidation peak currents of Sudan II versus scan rates. Each of the letters from a to o corresponds to a scan rate of 40, 80, 120, 160, 200, 240, 280, 320, 360, 400, 440, 480, 520, 560, 600 mV s^{-1}, respectively.

Effect of Accumulation Time

Accumulation is a simple and effective way to enhance the determining sensitivity. In this work,

open-circuit accumulation was employed to further improve the oxidation peak current of Sudan II in the presence of SDS. When the accumulation time increased from 0 to 120 sec, the oxidation peak current increased with increasing accumulation time. However, the oxidation peak current did not increase as further increasing the accumulation time. Considering both sensitivity and working efficiency, an accumulation time of 120 sec was employed. Further study showed that stirring can eliminate the overpotential caused by concentration polarization.

Linearity Range, Detection Limit and Reproducibility

The variation of oxidation peak current with concentration was studied using linear sweep voltammetry (LSV). Fig. 8 showed the oxidation peak current response of Sudan II at the GME with different concentrations. From which we can see that the oxidation peak current of Sudan II was linearly proportional to its concentration in a concentration range from 4.0×10^{-8} to 4.0×10^{-6} mol L^{-1}, with a linear regression equation of $i_{pa}(A) = 3.35c + 5.96 \times 10^{-6}$, r $= 0.9988$ and a detection limit of 8.0×10^{-9} mol L^{-1}. The relative standard deviation (RSD) for six replicate determinations of 2.0×10^{-6} mol L^{-1} Sudan II at the GME was 3.5%, suggesting that this method possesses excellent reproducibility. After each measurement, the GME was regenerated by several cyclic sweeps in ethanol/pH 6.0 PBS (1:1) until there was no oxidation peak observed.
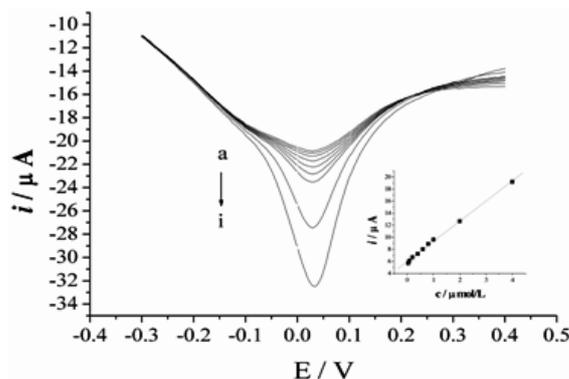

Fig. 8:　Linear sweep voltammograms of Sudan II at the GME at different concentrations. Each of the letters from a to i corresponds to a concentration of 4.0×10^{-8}, 8.0×10^{-8}, 2.0×10^{-7}, 4.0×10^{-7}, 6.0×10^{-7}, 8.0×10^{-7}, 1.0×10^{-6}, 2.0×10^{-6}, 4.0×10^{-6} respectively (in mol L^{-1}). Inset is the plot of the oxidation peak current versus concentration of Sudan II. Scan rate: 100 mV s^{-1}.

Determination of Sudan II in Food Samples

Measurements of Sudan II in chili sauce and ketchup samples were performed by linear sweep voltammetry. 5.0325g of accurately weighed chili sauce was extracted three times with ultrasound assistance, each time with 20 mL of absolute ethanol for 20 min. Extracts were combined, filtered and diluted to 100 mL. The determination conditions were set as discussed above and determinations were conducted in accordance with the analytical procedure. No peak current of Sudan II was observed, which indicates that the chili sauce contained no Sudan II and the determination was not interfered by other substances contained in the chili sauce. Then certain amount of Sudan II standard solution was added into the chili sauce. Extraction and determination were performed as above. The recoveries were calculated and the results are listed in Table-1. Ketchup was similarly tested and the results are listed in Table-1 too.

Table-1: Determination results of Sudan II in chili sauce and ketchup samples (n=6).

Sample	Added ($\times$ mol L^{-1})	Found ($\times$ mol L^{-1})	Recovery (%)	RSD (%)
chili sauce	0	0	-	-
	0.50	0.48	96.0	2.9
	1.00	0.95	95.0	3.3
	1.50	1.42	94.7	3.4
ketchup	0	0	-	-
	1.00	0.97	97.0	2.8
	2.00	1.91	95.5	3.6
	4.00	3.81	95.2	3.8

Experimental

Reagents and Chemicals

Graphite powder (<20μm) was obtained from Qindao Graphite Corporation (Qingdao, China), sodium borohydride was from Tianjin Daofu Chemical New Technique Development Co., Ltd. (China), Sudan II was purchased from Acros (Germany) and 8.0×10^{-4} mol L^{-1} Sudan II stock solution was prepared by dissolving it in absolute ethanol. Sodium dodecyl sulfate was from Shanghai Chemical Factory (China). All other chemical reagents (analytical-reagent grade) were obtained from Beijing Chemical Reagent Company (Beijing China). Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.2 mol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} citric acid. All aqueous solutions were prepared with double distilled water.

Apparatus

Electrochemical measurements were conducted on a CHI 660C Electrochemical

Workstation (Chen-hua, Shanghai, China). Infrared spectra were recorded using a Varian 660-IR spectrometer (Agilent, America). Raman spectra were obtained using a LabRAM-HR Raman Spectrometer (Jobin-Yvon, France). Scanning electron microscope (SEM) image was obtained using a field emission SEM Sirion 200 (FEI, America). TEM image was obtained using a JEM-2010 transmission electron microscope (JEOL, Japan). All electro-chemical experiments were carried out using a three-electrode system consisted of a working electrode (a bare or graphene-modified glassy carbon electrode, 3 mm in diameter) a counter electrode (a platinum wire electrode), and a reference electrode (a Ag/AgCl electrode). Acidity was measured by a PHS-3B Precision pH Meter (Shanghai, China), and all sonication was done using a KQ-100 Ultrasonic Cleaner (Kunshan, China).

Preparation of the Nano Graphene Platelets

Nano graphene platelets were prepared according to a slightly modified literature procedure. Briefly, Graphite powder was oxidized with potassium permanganate in sulfuric acid to give graphite oxide (GO). The obtained GO was then subjected to thermal exfoliation in water, followed by a further flake separation treatment with ultrasonication. The resulting GO platelets then underwent a chemical reduction treatment with sodium borohydride to give nano-graphene platelets [31-35, 20].

Preparation of the Graphene-Modified Electrode (GME)

A glassy carbon electrode (GCE, $\varnothing$ = 3 mm) was polished before each experiment with gold sand paper and 0.05 μm alumina powder, respectively, and rinsed thoroughly with doubly distilled water between each polishing step, then washed successively with 50% nitric acid, ethanol and doubly distilled water in ultrasonic bath, and dried in air. The GME was prepared by casting 4 μL of graphene suspension (0.5 mg mL^{-1} in water) on the GCE and drying under an infrared lamp.

Electrochemical Measurement

All electrochemical measurements were performed with a CHI 660C Electrochemical Workstation. The electrochemical system consisted of a GME as working electrode, a platinum wire as counter electrode and an Ag/AgCl electrode as reference electrode. Unless otherwise stated, a mixture of PBS (pH 6.0), ethanol and water with a ratio of 10:6:4 was chosen as the supporting electrolyte. During measurement, preconcentration was firstly performed at the surface under open-circuit for 120 s with stirring. Then, cyclic voaltammograms (CVs) or linear sweep voltammograms (LSVs) were obtained by scanning in the potential range of –1.0 V to 1.0 V and –0.3 V to 0.4 V, respectively. Upon completion of each scan, the modified electrode was placed in a mixed solution of ethanol and pH 6.0 PBS (ethanol:PBS = 1:1) and cyclic scan was continued until no peak came out, then the electrode was washed with water and dried under an infrared lamp for reuse.

Conclusions

The results of the present work revealed that the graphene modified glassy carbon electrode exhibited excellent electrocatalytic activity towards the electrochemical oxidation of Sudan II and sodium dodecyl sulfate could significantly enhance the sensitivity of the determination. Therefore, the electrochemical responses of Sudan II were greatly increased at the GME in the presence of SDS. The peak currents obtained by linear sweep voltammetry were linearly proportional to Sudan II concentrations in a range from 4.0×10^{-8} to 4.0×10^{-6} mol L^{-1}, with a linear regression equation of $i_{pa}(A) = 3.35c + 5.96 \times 10^{-6}$, r = 0.9988 and a detection limit of 8.0×10^{-9} mol L^{-1}. Due to its high sensitivity, good accuracy, rapid response and simplicity, the method provides a practicable solution for determining Sudan II in food products.

Acknowledgements

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