

## Electrochemical determination of Sudan IV in food samples by using graphene-modified glassy carbon electrodes

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**Abstract:** A simple and sensitive modified electrode was fabricated with graphene via the drop-casting method and applied for the electrochemical detection of Sudan IV. Cyclic voltammetry (CV) was used to investigate the electrochemical behaviors of Sudan IV in phosphate buffer solution (PBS). The experimental conditions such as determining medium, scan rate, and accumulation time were optimized for the determination of Sudan IV. The sensor has excellent performance associated with high sensitivity, a low detection limit ( $6.00 \times 10^{-8}$  M), and a wide linear range of  $2.00 \times 10^{-7}$  M to  $8.00 \times 10^{-5}$  M with a correlation coefficient as follows:  $i_{pc}(A) = 4.37 \times 10^{-6} + 0.35C$ ,  $R = 0.9930$ . Under optimized conditions, the applicability of the method for rapid determination of Sudan IV was corroborated by analyzing food samples with recoveries from 96.8% to 99.2%, and the related RSD values were within the range of 1.51% to 3.78%. A simple extraction procedure using ethanol was applied for the extraction of Sudan IV from samples of chili powder and tomato sauce.

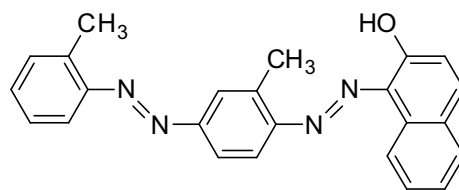
**Key words:** Sudan IV, graphene, food, modified electrode, determination

### 1. Introduction

Sudan IV is a synthetically produced azo dye used for different industrial and scientific applications (coloring of fuel, staining for microscopy, etc). Because of its low cost and wide availability, Sudan IV is also attractive as a food colorant. Sudan IV was classified as a category-3 human carcinogen by the International Agency for Research on Cancer due to its possible mutagenic and carcinogenic effects, and its use in foodstuffs has been banned in many countries to ensure food safety.<sup>1</sup> Nevertheless, according to the European Union Rapid Alert System for Food and Feed reports, there have been a large number of cases where Sudan IV has been found in food. Therefore, it is necessary to adopt a decision on emergency measures to deal with Sudan IV in food. Hence, accurate analysis of low levels of Sudan IV in food is of huge importance.

In recent years, various methods for determination of Sudan IV have been described, including high-performance liquid chromatography (HPLC),<sup>2</sup> high performance liquid chromatography-mass spectrometry (HPLC-MS),<sup>3-5</sup> and others.<sup>6</sup> HPLC and MS are the dominant methods for analysis of Sudan IV dye as they offer more reliable identification possibilities, but these methods suffer from obvious drawbacks; they are expensive and time consuming, require complicated pretreatment, and so on. Therefore, it is necessary to develop a cheaper and simpler method.

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Molecular formula of Sudan IV.

The molecular formula of Sudan IV contains electroactive groups (-N=N- and -OH). In recent years, Lin, Yin, Ming, and so on<sup>7-10</sup> reported an electrochemical determination method for Sudan I. However, very limited electrochemical methods have been proposed for the determination of Sudan IV.<sup>6</sup> As far as we know, there is no report based on using graphene-modified electrodes for the determination of Sudan IV. Graphene is planar sheets of sp<sup>2</sup>-bonded carbon atoms that are densely packed in a honeycomb crystal lattice.<sup>11</sup> The current carrying capability of graphene is orders of magnitude higher than that of metals.<sup>12-14</sup> It has been usually utilized to modify electrodes for electrochemical studies.<sup>15-17</sup>

In this work, we proposed a simple electrochemical sensor based on graphene modified glassy carbon electrodes for trace detection of Sudan IV contamination in chili samples.

## 2. Experimental

### 2.1. Reagents and apparatus

Graphite powder (<20 μm) was obtained from Qindao Graphite Corporation (Qingdao, China). Sodium borohydride was obtained from Tianjin Daofu Chemical New Technique Development Co., Ltd (Tianjin, China). Sudan IV was obtained from Sigma (USA) and dissolved in methanol to prepare a stock solution of  $1.0 \times 10^{-3}$  M. All reagents were at least of analytical grade and used as received without further purification. Double-distilled water was used throughout. The PBS was prepared by mixing 0.2 M disodium hydrogen phosphate solution and 0.1 M citric acid solution.

Electrochemical measurements were performed with a CHI 660C Workstation (CH Instruments, Shanghai, China). A conventional 3-electrode system, consisting of a working electrode, a Ag/AgCl (saturated KCl) reference electrode, and a platinum wire counter electrode, was employed. All the potentials were recorded versus Ag/AgCl. Solution pH was measured using a PHS-3B pH meter (Shanghai Analytical Instruments, Shanghai, China), and all ultrasonic cleaning was performed using an ultrasonic cleaner (KQ-100, Kunshan, China).

### 2.2. Preparation of graphene

Graphene was prepared according to a modified literature procedure.<sup>18-20</sup> Graphite powder (8 g) was added to 40 mL of sulfuric acid and the reaction mixture was stirred at 25 °C for 10 h. Potassium permanganate (6 g) was added, the reaction mixture was stirred for 40 min at 36 °C, and then heated to 80 °C for 45 min, followed by addition of 90 mL of water. The reaction was continued for 30 min at 95 °C. Another 100 mL of water and some amount of H<sub>2</sub>O<sub>2</sub> were added and the hot mixture was filtered. The cake was washed with 5% HCl and deionized water until no SO<sub>4</sub><sup>2-</sup> could be detected in the filtrate (by BaCl<sub>2</sub>) and dried at 85 °C for 5 h to give graphite oxide. The graphite oxide (0.2 g) was added to 200 mL of deionized water; the graphite oxide was then homogeneously dispersed in water by sonication to give a colloidal solution of graphene oxide. The solution was adjusted to pH 10.0 with sodium carbonate solution and heated to 80 °C with a water bath.

Next, reduction of graphite oxide was performed for 1 h by addition of sodium borohydride (0.6 g); it was then washed, filtered, and dried in vacuo to give graphene powder.

### 2.3. Preparation of graphene-modified electrode

Graphene powder (0.3 mg) was dispersed in 10 mL of double-distilled water by ultrasonication for about 30 min to give a stable and homogeneous graphene suspension of  $0.3 \text{ mg mL}^{-1}$ . Prior to modification, a glass carbon electrode (GCE) (3.8 mm diameter) was polished with abrasive paper (grit 2000) and wet alumina powder ( $0.05 \text{ }\mu\text{m}$ ); rinsed ultrasonically with 1:1  $\text{HNO}_3$ , acetone, and distilled water, consecutively; and dried under an infrared lamp. Then  $5 \text{ }\mu\text{L}$  of the graphene suspension was cast on the surface of the GCE and it was left to dry under an infrared lamp.

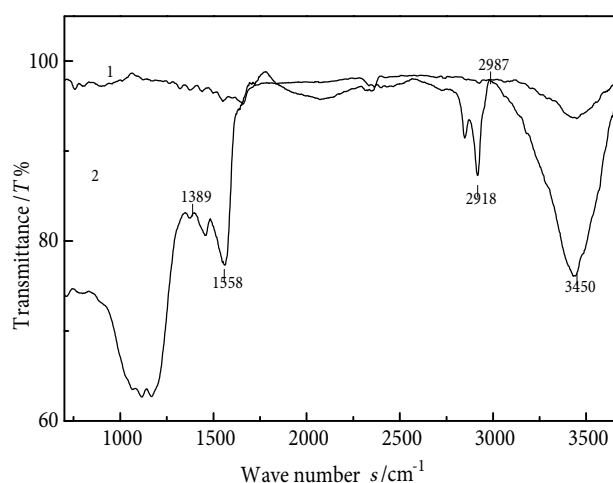
### 2.4. Analytical procedures

Electrochemical measurements were performed with a CHI 660C Workstation using PBS (pH 4.0) as the supporting electrolyte. Cyclic voltammograms (CVs) were obtained by scanning in the potential range from  $-0.6 \text{ V}$  to  $0.8 \text{ V}$  with a certain scan rate. Prior to and after each measurement, the modified electrode was placed in a blank PBS (pH 4.0) and scanned until no peak was seen for reuse.

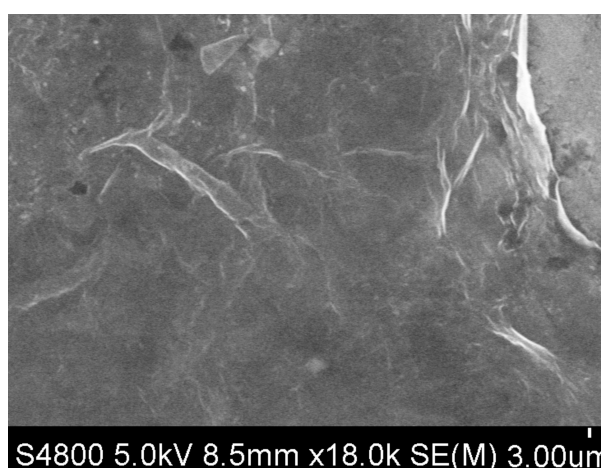
## 3. Results and discussion

### 3.1. Characterization of the graphene and graphene-modified GCE

The graphene and graphene-modified GCE were characterized by IR (Figure 1) and SEM (Figure 2). Figure 1 shows the IR spectra of the graphite and graphene. The IR spectra demonstrate that graphene was successfully prepared. It can be seen from Figure 1 that functional groups of C–O–C and C–OH still exist. It is clear that the GO is partly reduced to sheets by the reduction procedure by removing the oxygen-containing groups with the recovery of a conjugated structure. The functional groups of C–OH and C–O–C cannot be reduced by sodium borohydride.<sup>15,21</sup> The existence of these hydrophilic groups provides a means to disperse rather than dissolve in solvent. Figure 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.<sup>15</sup>



**Figure 1.** IR spectra of (1) graphite and (2) graphene.



**Figure 2.** SEM image of the graphene-modified GCE.

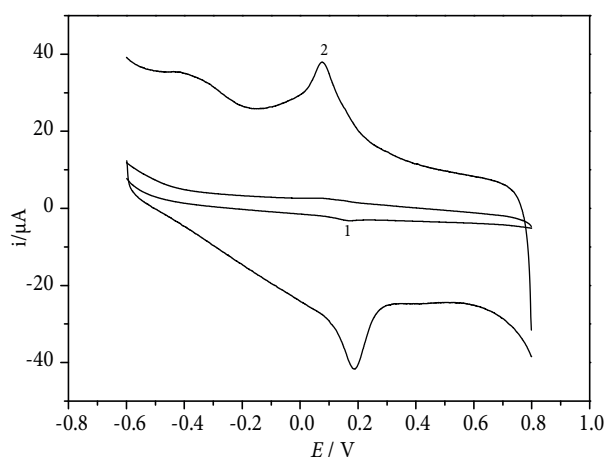
### 3.2. Electrochemical behavior of Sudan IV

The electrochemical behavior of Sudan IV at the graphene-modified GCE was examined using CV within a certain potential window. Figure 3 compares CVs of the GCE (1) and the graphene-modified GCE (2) in 0.1 M PBS (pH 4.0) in the presence of  $2.0 \times 10^{-5}$  M Sudan IV. The peak current intensity at the graphene-modified GCE was sharply increased, and in contrast the peak current was very low at the GCE, which confirms that graphene has excellent electrocatalytic activity to Sudan IV. Such electrocatalytic behavior of graphene is attributed to its unique physical and chemical properties. The relationships between the peak current ( $i_p$ ) of Sudan IV and the volume of graphene on a GCE were investigated by CV. The  $i_p$  clearly increased as the volume of graphene at a GCE from 1  $\mu\text{L}$  to 5  $\mu\text{L}$  increased, and then the  $i_p$  increased slightly from 5  $\mu\text{L}$  to 10  $\mu\text{L}$ . However, the  $i_p$  decreased while the volume of graphene exceeded 10  $\mu\text{L}$ , which may be ascribed to the thicker film of graphene hampering the electrical conductivity. The volume of graphene suspension on the surface of the GCE was kept at 5  $\mu\text{L}$  in this work.

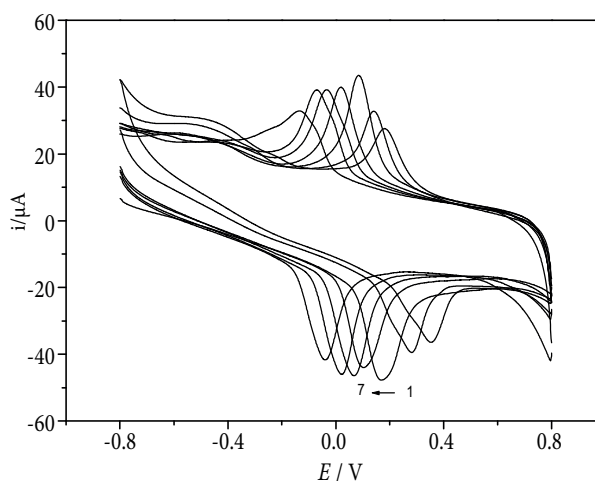
### 3.3. Effect of supporting electrolytes

The effect of the medium's pH including pH 2.2–8.0 PBS, pH 2.0–10.0 Britton-Robinson, and pH 4.0–6.0 HAc–NaAc buffer (0.1 M of each buffer) on the electrochemical signal was analyzed. The best reduction response was obtained in pH 4.0 PBS in that the peak shape was well defined with the highest peak current as compared to that in the other buffer systems. Thus, PBS was chosen as the supporting electrolyte in this work.

With increasing pH value of the solution the redox peak negatively shifted (Figure 4), which indicates that the redox reactions involve the protons. Reduction potential ( $E_c$ ) changed linearly depending on a pH from 2.2 to 8.0, and the equation was  $E_c = 0.30 - 0.057 \text{ pH}$ ,  $R = 0.9983$ . According to the Nernst equation, the slope of  $-57 \text{ mV pH}^{-1}$  reveals that the proportion of the electron and proton involved in the reactions is 1:1. The pH of Sudan IV solutions was changed from pH 2.2 to 8.0, and potential was scanned in the range of  $-0.8 \text{ V} \sim 0.8 \text{ V}$ . Figure 4 shows that the reduction peak current increases with increasing pH and reaches its maximum at pH 4.0. Thus, the buffer solution of pH 4.0 was chosen as the supporting electrolyte in this work.



**Figure 3.** CVs of the bare GCE (1) and the graphene-modified GCE (2) when placed in 0.1 M PBS (pH 4.0) in the presence  $2.0 \times 10^{-5}$  M Sudan IV. Scan rate:  $100 \text{ mV s}^{-1}$ .



**Figure 4.** CVs of  $2.0 \times 10^{-5}$  M Sudan IV at different pH. 1–7: 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0, respectively.

### 3.4. Effect of scan rate and accumulation time

Figure 5 gives the CVs of Sudan IV at different scan rates, which shows the reduction peak potentials are slightly shifted with increased scan rate, and the reduction peak currents are proportional to the scan rates when scan rates are between  $40 \text{ mV s}^{-1}$  and  $600 \text{ mV s}^{-1}$ . The linear equation is  $i_{pc}(\text{A}) = 1.01 \times 10^{-5} + 1.98 \times 10^{-7} v (\text{mV s}^{-1})$ ,  $R = 0.9938$ . This shows that the electrode reaction is controlled by the adsorption process.

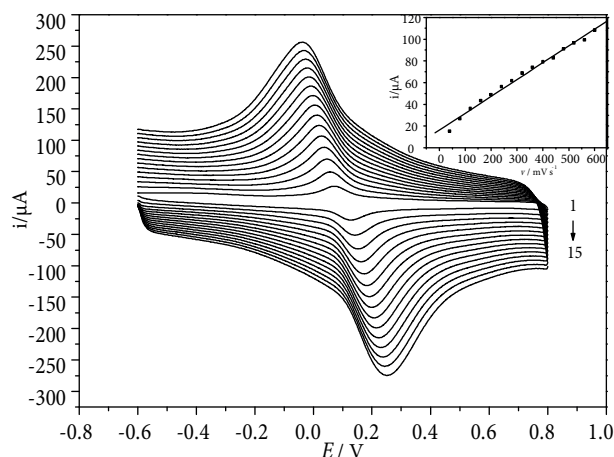
As to the effect of the accumulation time on the reduction peak current, we varied the accumulation time between 20 s and 200 s for  $5.0 \times 10^{-6} \text{ M}$  Sudan IV. CVs of Sudan IV were recorded every 40 min in the potential range from  $-0.6 \text{ V}$  to  $0.8 \text{ V}$ . The  $i_p$  increased greatly with time and reached a maximum at 120 s. Therefore, 120 s was used as the accumulation time, suggesting that the Sudan IV accumulation process very rapidly achieves the saturation adsorption of Sudan IV on the graphene-modified GCE.

### 3.5. Reproducibility and stability

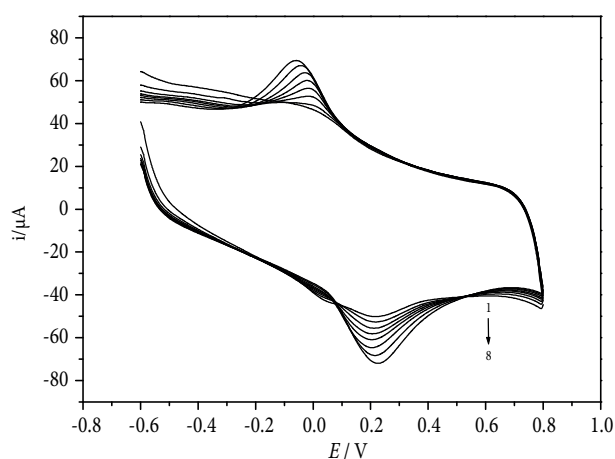
Ten parallel measurements for  $5.0 \times 10^{-6} \text{ M}$  Sudan IV were conducted using a graphene-modified GCE. It was found that the graphene-modified GCE had good reproducibility when the related RSD was less than 5.0%. When the electrode was stored in PBS solution (2.2–8.0) for 14 days at room temperature when not in use, 95.2% of its initial response was kept after storage, indicating that the graphene-modified GCE had good storage stability.

### 3.6. Linearity range, detection limit, and method validation

Figure 6 gives the CVs of Sudan IV at different concentrations. In pH 4.0 PBS, the reduction peak current of Sudan IV at the graphene-modified GCE is linearly proportional to its concentration ( $C$ ) in a range from  $2.00 \times 10^{-7} \text{ M}$  to  $8.00 \times 10^{-5} \text{ M}$ , with a correlation coefficient of 0.9930. The linear regression equation is  $i_{pc}$



**Figure 5.** CVs of  $5.0 \times 10^{-6} \text{ M}$  Sudan IV on the graphene-modified GCE. The numbers from 1 to 15 correspond to scan rates of 40, 80, 120, 160, 200, 240, 280, 320, 360, 400, 440, 480, 520, 560, and  $600 \text{ mV s}^{-1}$ , respectively. Inset is the plot of reduction Sudan IV peak currents versus scan rates.



**Figure 6.** CVs of different concentrations of Sudan IV at graphene-modified GCE in pH 4.0 PBS. The numbers from 1 to 8 correspond to concentrations of 0.20, 0.60, 1.00, 4.00, 8.00, 10.00, 40.00, and  $80.00 \mu\text{M}$ , respectively.

(A) =  $4.37 \times 10^{-6} + 0.35 C$ , R = 0.9930. The limit of detection was estimated by gradually decreasing the concentration levels of Sudan IV<sup>22</sup> and the detection limit of  $6.00 \times 10^{-8}$  M. The comparison of the proposed method with other methods for determination of Sudan IV is shown in Table 1. The results indicate that the sensor for the detection of Sudan IV has lower detection and a wide linear range.

**Table 1.** Comparison of the proposed method with other methods for determination of Sudan IV.

Analyte	Other methods	Linear range (M)	Detection limit (M)	Reference
Sudan IV	GC	$2.6 \times 10^{-7}$ – $3.9 \times 10^{-4}$	$1.3 \times 10^{-7}$	6
Sudan IV	Nanotube modified electrode	$1.3 \times 10^{-7}$ – $6.6 \times 10^{-5}$	$6.6 \times 10^{-8}$	6
Sudan IV	HPLC-DAD	$1.3 \times 10^{-7}$ – $6.6 \times 10^{-6}$	$6.5 \times 10^{-8}$	3
Sudan IV	Graphene-modified electrode	$2.0 \times 10^{-7}$ – $8.0 \times 10^{-5}$	$6.0 \times 10^{-8}$	This work

### 3.7. Analytical application

Ketchup and chili sauce purchased from a local market were accurately weighed (10.0 g) and added to a stoppered flask with absolute methanol (50 mL) under sonication for 30 min. The combined extracts were centrifuged at 12,000 rpm to obtain the supernatant, which was collected followed by appropriate dilution with electrolyte solution to furnish a desired concentration for the sample analysis.

Under the optimized conditions, the prepared test solution was detected at the graphene-modified GCE by CV. Fortunately, no observable peaks appeared and a recovery experiment was carried out by adding a known amount of Sudan IV to the sample. Recovery was calculated with reduction peak current values, and the results are shown in Table 2. The average recoveries (n = 6) varied from 96.8% to 99.2%, and the related RSD values were within the range of 1.51% to 3.78%.

**Table 2.** Recovery of determination of Sudan IV in samples (n = 6).

No.	Content in samples	Sudan IV added (M)	Average found (M)	Recovery (%)	R.S.D. (%)
1	Not detected	$8.00 \times 10^{-7}$	$7.91 \times 10^{-7}$	98.9	3.11
2	Not detected	$1.00 \times 10^{-6}$	$0.97 \times 10^{-6}$	97.0	3.78
3	Not detected	$4.00 \times 10^{-6}$	$3.87 \times 10^{-6}$	96.8	2.74
4	Not detected	$8.00 \times 10^{-6}$	$7.94 \times 10^{-6}$	99.2	1.82
5	Not detected	$2.00 \times 10^{-5}$	$1.97 \times 10^{-6}$	98.5	1.51

### 3.8. Interference

The suitability of the graphene-modified GCE was tested for the determination of Sudan IV in food in the presence of potential interference (such as capsorubin, beta-carotene, zeaxanthin, violaxanthin, neoxanthin, lutein, and metal ion). These species differ greatly from Sudan IV in chemical structure and electrochemical characteristics, and no interference in the current response was observed for 2.0  $\mu$ M Sudan IV in the presence of 1000 times  $K^+$ ,  $Na^+$ ,  $Fe^{3+}$ ,  $Ca^{2+}$ , and  $Mg^{2+}$ ; or 100 times capsorubin, beta-carotene, leaxanthin, violaxanthin, neoxanthin, lutein, glucose, and ascorbic acid, indicating that the graphene-modified GCE is highly selective towards the determination of Sudan IV.

## 4. Conclusions

A graphene-based electrochemical sensor has been demonstrated, and this sensor shows an excellent electrocatalytic activity towards Sudan IV. Owing to the unique properties of graphene, including subtle electronic

characteristics and strong adsorptive ability, the graphene-modified GCE obviously shows excellent sensitivity, selectivity, and stability. The newly established method for determination of Sudan IV has been successfully used in food analysis.

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