



Selective Determination of Sudan IV in Food Using Sulfamic Acid-Modified Electrode

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A simple and sensitive electrochemical method was proposed for the determination of Sudan IV. A glassy carbon electrode was successfully modified with electropolymerized film of sulfamic acid. Cyclic voltammetry was used to study the electrochemical properties of the Sudan IV and the appropriate condition for electropolymerization process. The behaviour and method for the determination of Sudan IV were studied on the sulfamic acid modified electrode. The results showed that the sulfamic acid sensor exhibited excellent performance for detecting Sudan IV. A well-defined redox waves were obtained in phosphate buffer solution of pH = 4. The oxidation peak currents increased linearly with the concentration of Sudan IV in the range of 4.0×10^{-7} to 1.0×10^{-5} mol L⁻¹ with a detection limit of 8×10^{-8} mol L⁻¹. The linear regression equation is $i_{pa} (A) = 3.12 \times 10^{-6} + 1.46c$ ($r = 0.9974$). The sulfamic acid modified electrode showed good sensitivity, selectivity and stability. The method has been successfully applied to food sample analysis with recoveries ranging from 95 to 99.5 %.

Key Words: Sudan IV, Sulfamic acid, Modified electrode.

INTRODUCTION

Staining is an important technique and colour is very important characteristic for food industries. So colourants are often added to food. Sudan IV is used for the staining. Sudan IV was classified as a category 3 carcinogen by the International Agency for Research on Cancer¹ due to their possible mutagenic and carcinogenic effects and its use of Sudan IV in food has been banned in several countries. Nevertheless, there are a large number of cases where Sudan IV has been found in food products, due to its colour fastness, low cost and wide availability. For this reason, it is required to develop a convenient, accurate and reliable method for the determination of Sudan IV.

Many methods have been used for detecting Sudan IV, for example chemiluminescence², liquid chromatography-electrospray-tandem mass spectrometry method^{3,4}, high performance liquid chromatography-diode array detection-electrospray mass spectrometry⁵, HPLC and UPLC⁶⁻¹⁰. But these methods often suffer from the obvious disadvantage of expensive cost, time consuming and/or complicated procedures. So it is necessary to develop a low cost and simple method.

In recent years, electrochemical method has become one of the most interesting methods because it is highly sensitive and selective. As can be seen from Fig. 1 that Sudan IV

contains electroactive groups (-N=N- and -OH), so electrochemical determination method can be used. Amino acids modified electrodes have many merits such as stability, easiness of the preparation and easily available materials. To the best of our knowledge, there is no report based on using sulfamic acid (SA) modified glass carbon electrode (SA/GCE) for the determination of Sudan IV. In this work, a simple electrochemical sensor based on sulfamic acid modified electrode for detection of Sudan IV is proposed. This method was successfully applied to the determination of Sudan IV in food with recoveries ranging from 95 to 99.5 %.

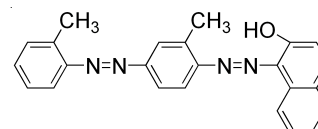


Fig. 1. Chemical structure of Sudan IV

EXPERIMENTAL

Sudan IV was obtained from Tianjin Kermel Chemical Reagent Development Center (Tianjin, China) and dissolved in ethanol to prepare a stock solution of 4.0×10^{-4} mol L⁻¹. The working standard solutions of Sudan IV were obtained by serial dilution with phosphate buffer solution (PBS, pH 4.0) prior to use. Sulfamic acid was purchased from Tianjin

Jinhuitaiya Chemical Reagent Co., Ltd (Tianjin, China). All reagents were used as received without further purification. Redistilled water was used throughout. The PBS was prepared by mixing 0.2 mol L⁻¹ disodium hydrogen phosphate and 0.1 mol L⁻¹ citric acid.

Electrochemical measurements were performed with a CHI 660C Workstation (CH Instruments, Shanghai, China). The electrochemical experience with conventional three-electrode system, which are composed of a bare or modified electrode, a platinum electrode and a Ag/AgCl electrode as working electrode, counter electrode and reference electrode respectively. 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). Scanning electron microscope (SEM) image was obtained using a SEM Sirion 200 (FEI, America).

Electrode preparation and modification: A bare glassy carbon electrode (GCE) was pretreated carefully with gold sand paper and 0.05 μm alumina powder to polish, rinsed thoroughly several times with redistilled water between each polishing step, then washed with 1:1 nitric acid, ethanol and redistilled water in ultrasonic bath and dried in air.

The sulfamic acid modified glass electrode (SA/GCE) was obtained by cyclic voltammetry (CV) in the potential range between -1.5 V and 2.5 V for 15 cycles at a scan rate of 160 mV s⁻¹. The modified solution was made of 8 mL 0.05 mol L⁻¹ sulfamic acid and 20 mL pH 6.8 PBS. Then the SA/GCE was treated in PBS (pH 4.0) by repetitive scanning in the potential range between -0.6 V and 0.8 V at a scan rate of 80 mV s⁻¹ and rinsed with redistilled water to be used.

Analytical procedure: Cyclic voltammogram method was used in the electrochemical measurements using conventional three-electrode system (referred in the Reagents and apparatus section). A certain amount of Sudan IV, pH 4.0 PBS and absolute ethanol was added to the electrolytic cell (20 mL). Cyclic voltammograms (CVs) were recorded between -0.6 V and 0.8 V at a scan rate of 80 mV/s. After each experiment, the modified electrode was restored by repeatedly running the scan in PBS 4.0 until no peak being detected.

RESULTS AND DISCUSSION

Electrochemical polymerization of sulfamic acid on glass carbon electrode: Cyclic voltammograms of polymerization process of sulfamic acid on glass carbon electrode are shown in Fig. 2. Fig. 2 shows that sulfamic acid has one cathodic peak (peak 1) and two anodic peaks (peak 2, 3), which demonstrate that the sulfamic acid film formed in weak acidic media. The optimization of electrochemical polymerization such as potential range, scan rate and accumulation cycles were tested for the determination of Sudan IV. The results showed that the SA/GCE was obtained in the potential range between -1.5 V and 2.5 V for 15 cycles at a scan rate of 160 mV s⁻¹.

Sulfamic acid/glass carbon electrode characterization: The morphology of SA/GCE was characterized by scanning electron micrograph (Fig. 3). It shows that the poly(sulfamic acid) film formed by electrochemically synthesized. Fig. 3 shows sulfamic acid formed of club-shaped granules by cyclic voltammetry on the glass carbon electrode. These club-shaped granules tend to disperse on the glass carbon electrode.

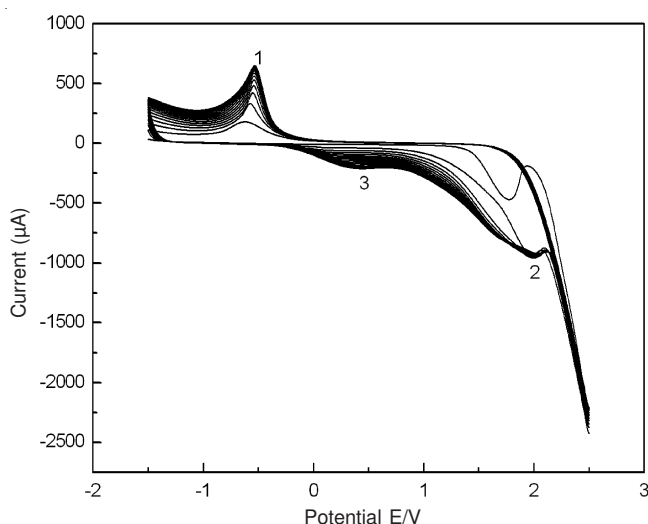


Fig. 2. CVs of sulfamic acid in polymerization process; Scan rate: 160 mV s⁻¹; 1, peak 1; 2, peak 2; 3, peak 3

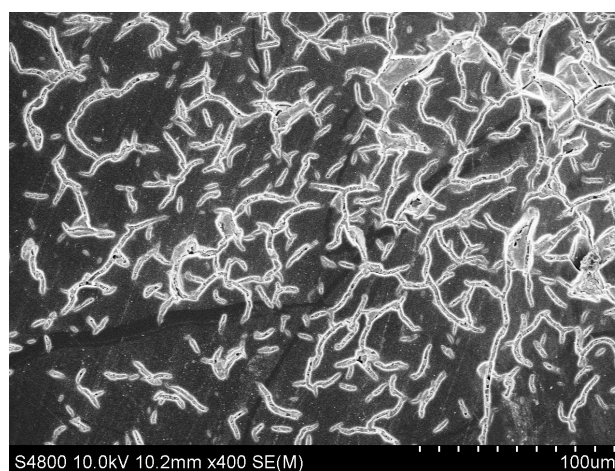


Fig. 3. SEM images of the SA/GCE

Electrochemical behaviours of Sudan IV on the SA/GCE: Electrochemical behaviours of Sudan IV on the SA/GCE were studied by CV and the results are shown in Fig. 4. Sudan IV has a couple of definite redox peaks on the SA/GCE in PBS (pH 4). The peak current intensity on the SA/GCE was sharply increased and in contrast the peak current was near zero on the glass carbon electrode, which confirmed that sulfamic acid has excellent electrocatalytic activity to Sudan IV. The peak currents on the SA/GCE are $i_{pa} = 31.37 \mu\text{A}$ and $i_{pc} = 29.24 \mu\text{A}$, suggesting the sensor of sulfamic acid modified has great promise for detection of Sudan IV. The good electrocatalytic properties of the membrane are ascribed to the formation of hydrogen bond between the hydrogen in hydroxyl groups of Sudan IV and the nitrogen in the system of poly(sulfamic acid). The hydrogen bond could weaken the bond energy of hydroxyl in Sudan IV and enrich Sudan IV on the electrode surface. Therefore, the rate of electron transfer is accelerated and the current is increased^{11,12}.

Effect of solution pH on the determination of Sudan IV: The effect of medium's pH on the electrochemical determination of Sudan IV was analyzed. Fig. 5 shows important influence of pH on the redox reaction of Sudan IV on the

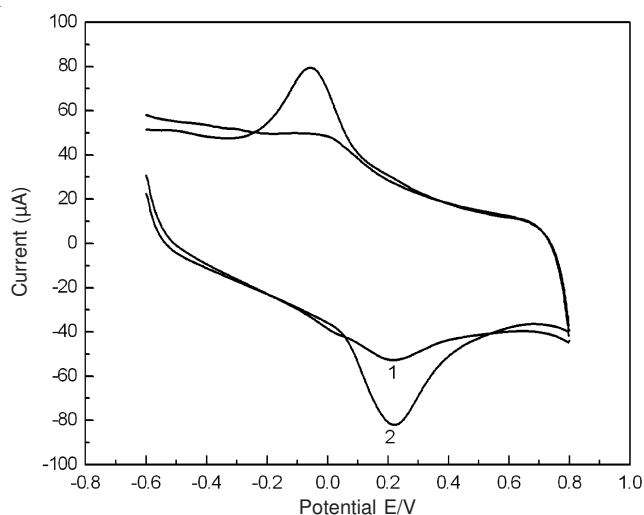


Fig. 4. CVs of 1.0×10^{-5} mol L^{-1} Sudan IV at the GCE and the SA/GCE in pH 4 PBS. Scan rate: 80 mV s^{-1} ; 1, the bare GCE; 2, the SA/GCE

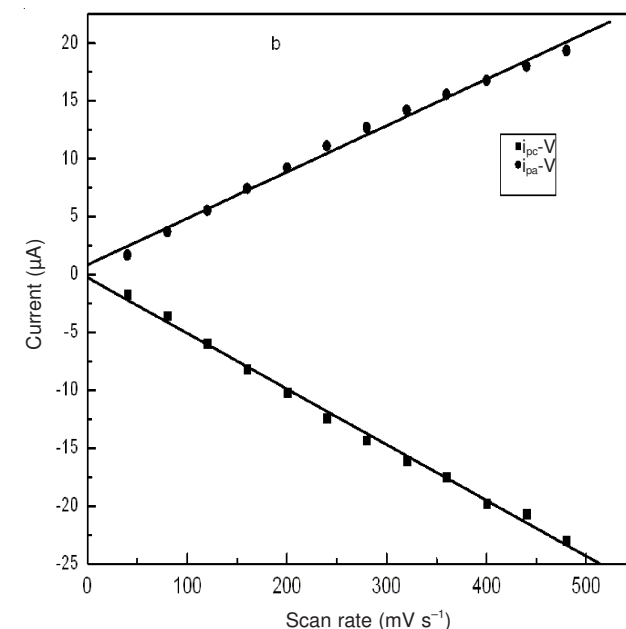
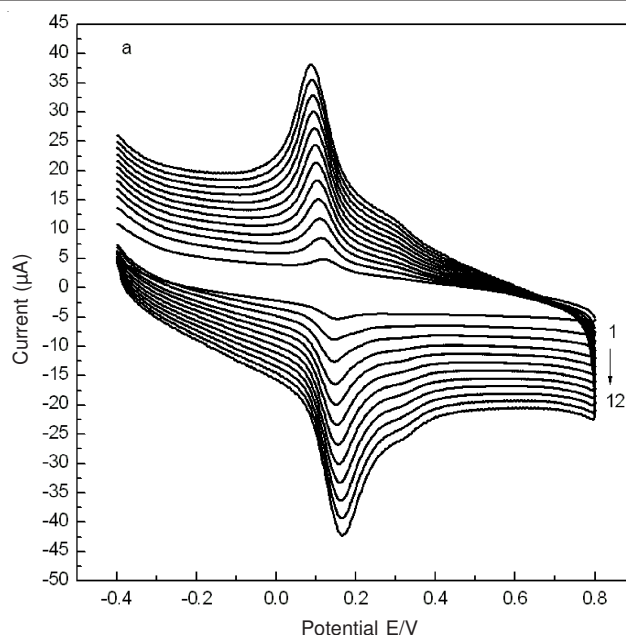


Fig. 6. CVs of 1.0×10^{-5} mol L^{-1} Sudan IV on the SA/GCE at different scan rates from 40 to 480 mV s^{-1} (a). Plots of redox peak currents of Sudan IV vs. scan rates (b); Scanning rate: 1, 40 mV s^{-1} ; 2, 80 mV s^{-1} ; 3, 120 mV s^{-1} ; 4, 160 mV s^{-1} ; 5, 200 mV s^{-1} ; 6, 240 mV s^{-1} ; 7, 280 mV s^{-1} ; 8, 320 mV s^{-1} ; 9, 360 mV s^{-1} ; 10, 400 mV s^{-1} ; 11, 440 mV s^{-1} ; 12, 480 mV s^{-1} ; Plots 1, E_a vs. pH; 2, E_c vs. pH

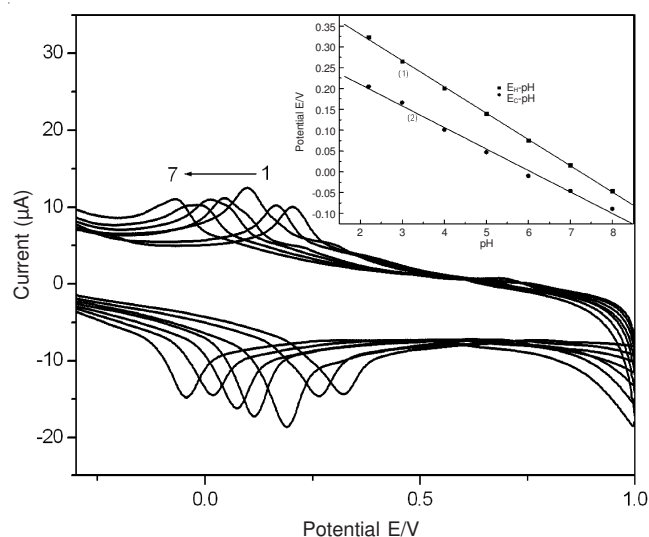


Fig. 5. CVs of 1.0×10^{-5} mol L^{-1} Sudan IV at different pH values. Inset is the shift of the redox potential value with the value of pH. Scan rate: 80 mV s^{-1} ; The pH value of PBS: 1, 2.2; 2, 3.0; 3, 4.0; 4, 5.0; 5, 6.0; 6, 7.0; 7, 8.0; Plots 1, E_a vs. pH; 2, E_c vs. pH

SA/GCE. The redox peak currents increased as the pH changing from 2.2 to 4.0 and then decreased, indicating that the redox reactions involve the protons. The buffer solution of pH 4.0 PBS was chosen as the supporting electrolyte in this work. The redox peak negatively shifted with increasing pH value of the solution, which is demonstrated in insert. The equations were $E_{pa} = 0.45 - 0.062 \text{ pH}$, $r = 0.9996$ and $E_{pc} = 0.31 - 0.057 \text{ pH}$, $r = 0.9961$.

Effect of scan rate on the determination of Sudan IV:

Fig. 6a shows the cyclic voltammograms of Sudan IV at different scan rates, which shows that the redox peak potential are slightly shifted negatively with increased scan rate. Fig. 6b shows that redox peak currents are proportional to scan rates from 40 mV s^{-1} to 480 mV s^{-1} . The linear equations are $i_{pa}(\text{A}) = -2.67 \times 10^{-7} - 4.81 \times 10^{-8} v (\text{mV s}^{-1})$, $r = 0.9977$ and $i_{pc}(\text{A}) = 8.43 \times 10^{-7} + 4.01 \times 10^{-8} v (\text{mV s}^{-1})$, $r = 0.9961$. This showed that the electrode reaction was controlled by the adsorption process.

To study the effect of the accumulation time on the redox peak current, we varied the accumulation time between 20 s and 140 s for 1.0×10^{-5} mol L^{-1} Sudan IV. CVs of Sudan IV were recorded every 20 min. The peak current increased greatly with time and reached a maximum at 60 s (Fig. 7).

Linearity range, detection limit and precision: In pH 4 PBS, the oxidation peak current of Sudan IV on the SA/GCE is linearly proportional to its concentration (c) in a range from 4.0×10^{-7} mol L^{-1} to 1.0×10^{-5} mol L^{-1} , with a correlation coefficient of 0.9964 and a detection limit of 8.0×10^{-8} mol L^{-1} (S/N = 3). The linear regression equation is $i_{pa}(\text{A}) = 3.12 \times 10^{-6} + 1.46 c$ ($r = 0.9965$).

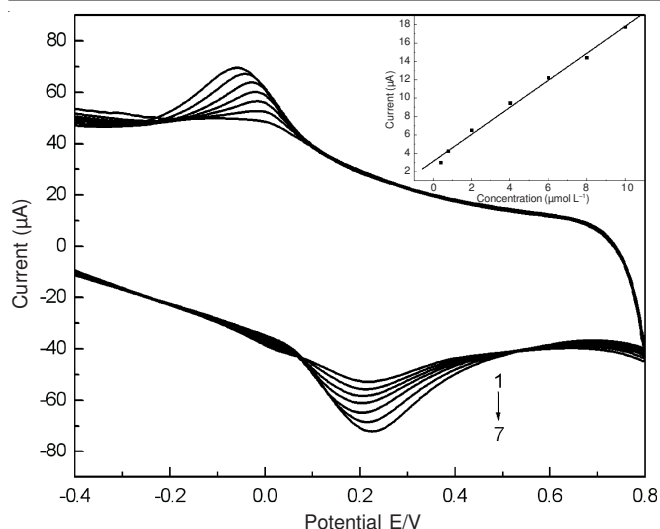


Fig. 7. CVs of different concentrations of Sudan IV at the SA/GCE in pH 4 PBS. Inset is oxidation peak currents of Sudan IV vs. concentrations; Content: 1, 0.40 $\mu\text{mol L}^{-1}$; 2, 0.80 $\mu\text{mol L}^{-1}$; 3, 1.00 $\mu\text{mol L}^{-1}$; 4, 2.00 $\mu\text{mol L}^{-1}$; 5, 4.00 $\mu\text{mol L}^{-1}$; 6, 8.00 $\mu\text{mol L}^{-1}$; 7, 10.00 $\mu\text{mol L}^{-1}$

Analytical application: Following the procedure for determination of Sudan IV, the proposed method was applied to determine Sudan IV in food samples. Ketchup or chilli sauce samples were accurately weighed (10 g) and absolute ethanol (50.00 mL) added to a stoppered flask under sonication for 40 min. The mixture was centrifuged to obtain the supernatant for the sample analysis. Peak identification was carried out by the standard addition method. The recovery was calculated with oxidation peak current value and the results are shown in Table-1. The average recoveries ($n = 6$) varied from 95 to 99.5 % and the related RSD were within the range of 1.68 to 4.11 %.

TABLE-1
RECOVERY OF DETERMINATION OF
SUDAN IV IN SAMPLES ($n = 6$)

No.	Content in sample	Std. Added (mol L^{-1})	Total found (mol L^{-1})	Recovery (%)	R.S.D. (%)
1	n.d.	6.00×10^{-7}	5.91×10^{-7}	98.5	3.59
2	n.d.	1.00×10^{-6}	0.98×10^{-6}	98.0	4.11
3	n.d.	6.00×10^{-6}	5.97×10^{-6}	99.5	2.97
4	n.d.	1.00×10^{-5}	0.95×10^{-5}	95.0	1.68

n.d. = not detected.

Interference: Potential interference (such as ions, capsorubin, β -carotene and ascorbic acid) to the signals of Sudan IV was investigated by cyclic voltammetry. These species differ greatly from Sudan IV in chemical structure and electrochemical characteristics and no interference in the current response was observed for 2 $\mu\text{mol L}^{-1}$ Sudan IV in the presence of 1000 times of ions, such as K^+ , Na^+ , Fe^{3+} , Ca^{2+} and Mg^{2+} , 100 times of other species, such as capsorubin, β -carotene, leaxanthin, indicating that the SA/GCE was highly selective towards the determination of Sudan IV.

Conclusion

The poly(sulfamic acid) membrane on the glass carbon electrode surface has been prepared by cyclic voltammetry. The morphology of SA/GCE and the electropolymerization condition were investigated. The sensor can be prepared and regenerated by a simple procedure with good reproducibility and long-term stability. The newly established method for determination of Sudan IV has been successfully used in food analysis.

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