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Determination of Sudan I in hot chili powder by using an activated glassy carbon electrode

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Abstract

A novel electroanalytical method is proposed for the determination of Sudan I in hot chili powder. Sudan I was firstly pre-concentrated by adsorption and then electroreduced at the electrochemically activated glassy carbon electrode (AGCE). A linear relationship between the reduction current and concentration of Sudan I was obtained over the range from 2.4×10^{-6} mol/L to 1.8×10^{-5} mol/L with a correlation coefficient of 0.9981. The detection limit was estimated to be about 7.1×10^{-7} mol/L.

Keywords: Sudan I; Activated glassy carbon electrode; Cyclic voltammetry

1. Introduction

The lipophilic Sudan I (1-phenylazo-2-naphthol, CAS 842-07-09) (Abraham, Amin, & Zissimos, 2002) is a synthetic red dye widely used as a coloring matter in many industrial and daily used products. Its carcinogenic action was found in 1970s (Marmion, 1979). Recently its potential as a carcinogen to humans was demonstrated (Martinek & Stiborov, 2002; Stiborova, Martinek, Rydlova, Hodek, & Frei, 2002). Therefore, Sudan I used in food products is forbidden worldwide for any purpose at any levels regulated in any national or international food standards.

In April 2003, that Sudan I was detected in hot chili and hot chili products from India engenders fears in the EU. And two years later, in March 2005, similar food panic happened in China as well as in the EU again. Sudan I was found in many food products in China – from the international famous brands, such as the Chili Oil of Heinz–Meiweiyuan (Guangzhou) Food Co. and the New Orleans baking chicken of KFC, and also from the native brands, such as Tantanxiang spicy turnip pickle and Yuxiang Chili powder. So that it is imperative to establish a sensitive, selective and practicable technique for fast detection of Sudan dyes in food products.

Several techniques have been reported in the literatures, the usually proposed methods for the determination of Sudan I in foodstuffs were established on the combination of the separation methods like high performance liquid chromatography (HPLC) with the detection methods like spectrophotometry and mass spectrometry (MS), such as HPLC–UV/Vis (Chen, Mou, Hou, Ni, & Riviello, 1998; Daood & Biacs, 2005; Nagase, Osaki, & Matsueda, 1989; Sproll, Ruge, Strichow, Attig, & Marx, 2005), HPLC-Fluorimetry (Pielesz, Baranowska, Rybak, & Wlochowicz, 2002), and HPLC/APCI–MS/MS (Di Donna, Maiuolo, Mazzotti, De Luca, & Sindona, 2004; Tateo & Bononi, 2004), HPLC/ESI–MS/MS (Calbiani et al., 2004; Zhang, Zhang, Gong, Gopalan, & Lee, 2005). Now HPLC–UV/Vis, as a standard method, is recommended to evaluate Sudan I in food products.

Other available methods are based on the adsorptive nature of Sudan I, combine with spectrophotometry or

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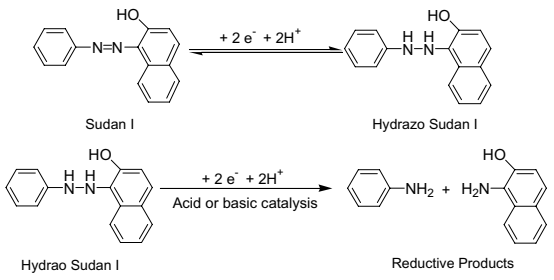
HPLC for detection, such as solid-phase spectrophotometry (Capita, Capitan-Valley, Fernadez, De Orbe, & Avidad, 1996; Capitanvallvey, Fernandez, Deorbe, & Avidad, 1995; Chen et al., 1998; Valencia, Uroz, Tafersiti, & Capitan-Vallvey, 2000), molecularly imprinted solid-phase extraction (MISPE)–HPLC (Puoci et al., 2005), high-performance gel-permeation-chromatography (HPGPC)–HPLC (Mazzetti et al., 2004) etc.

However, the disadvantages in the HPLC methods mentioned above, e.g. multifarious sample pretreatment, expensive cost and time-consuming, extremely restrict its applications in food safety examination. It is well known that electrochemical method is simple and inexpensive as an analytical technique. But rare literatures were reported dealing with measurement of Sudan I by electrochemical method. Yaguez once studied the polarographic behavior of Sudan II (1-(2,4-dimethylphenylazo)-2-naphthol) in hydroalcoholic medium at drop mercury electrodes (De La Cruz Yaguez, Pingarron Carrazon, & Polo Diez, 1986). Despite of the toxicity of mercury, this gave a good choice of detection methods to Sudan dyes: electrochemistry other than spectrophotometry or mass spectrometry.

In this study, we discuss about a new method to detect Sudan I at an activated glassy carbon electrode (AGCE) using cyclic voltammetry and linear sweep voltammetry. The approaches to activate glassy carbon electrode are various. S.B. Khoo obtained an AGCE by applying a high positive oxidation potential at GCE in phosphate buffer solution (pH 7.0) to determine biological molecules (Premkumar & Khoo, 2005), and S. Abraham John obtained in 0.05 M H_2SO_4 to determine uric acid and ascorbic acid (John, 2005). The activation strategies of GCE with electrochemical pretreatment have been previously reviewed by Engstrom and Xiaobing et al., respectively (Engstrom, 1982; Hanchang, Xiaobing, & Zhaorong, 1997).

Florence considered azo-dye compounds be classified in four kinetic groups depending on the behavior of their corresponding hydrazo intermediates and the acid or basic catalytic effect on the corresponding electrode reaction (Florence, 1974). According to our experimental results, we proposed an electrochemical reduction mechanism of Sudan I involving four electrons and four protons at AGCE, as can be presented by



and also an electrochemical analytical method was established based on the electrode reaction mechanism to determine Sudan I in hot chili powder.

2. Experimental

2.1. Materials

2.1.1. Reagents

Sudan dyes (I–IV) from Aldrich were used as received. Hot chili powder was purchased from a supermarket. Acetonitrile, absolute alcohol and carbinol were provided by Shanghai Chemicals Company (Shanghai, China). A series of BR buffer solutions of different pH values were obtained by adjusting mixed acid solution (phosphoric acid + glacial acetic acid + borax) with 1.0 mol/L aqueous sodium hydroxide. All chemicals used were of analytical grade and the water used was doubly distilled.

2.1.2. Preparation of BR buffer stock solution

Mix 17 mL 85% phosphoric acid, 15 mL 95.5% acetic acid and 24.84 mL borax, then dilute with doubly distilled water to 1 L (pH 1.69, total conc. = 0.25 mol/L). Before used, the solution is adjusted to a required pH value with 1 mol/L aqueous sodium hydroxide.

2.1.3. Pretreatment of hot chili powder

Dry 5.0 g hot chili powder in a vacuum drying oven, followed grinding and extracting ultrasonically three times by methanol each for 20 min, and then the resulting extraction is gathered and diluted with methanol to 100 mL.

2.2. Apparatus

Electrochemical measurements were performed on a LK98BII Electrochemical Analysis System (LANLIKE, Tianjin, China), in a three-electrode arrangement, equipped with the AGCE as working electrode, a platinum counter electrode and a KCl-saturated Ag/AgCl reference electrode. The other equipments, a vacuum drying oven, a pH-acidimeter and a magnetic stirrer, were also used in this study.

2.3. Procedure

2.3.1. Preparation of AGCE

The glassy carbon electrode (GCE) of 3.0 mm in diameter was firstly polished with 1, 0.3 and 0.05 μm alumina slurry, respectively, rinsed thoroughly with doubly distilled water, then ultra-sonicated in nitric acid (1 + 1, v/v), ethanol (1 + 1, v/v) and doubly distilled water successively, each for 3 min. The activation treatment of the GCE was carried out by cycling the potential between 0 and −1.2 V until stabilization in a spiriting solution at a scan rate of 50 mV/s. After that, the electrode was rinsed carefully with doubly distilled water, and dried on a clean tissue.

2.3.2. Determination of samples

10.0 mL stock BR buffer, 10.0 mL absolute alcohol and Sudan I sample were added into a 50 mL volumetric flask, diluted with doubly distilled water to the mark line and

shaken to uniformity. Before each experiment, the solution was deoxygenated by bubbling high-pure nitrogen gas (99.99%) for 5 min. Then cyclic voltammograms or linear sweep voltammograms were recorded.

3. Results and discussion

3.1. Selection of spiriting media

To understand the effect of different AGCEs on the voltammetric responses of Sudan I, several spiriting solutions were used to activate the glassy carbon electrode: 0.25 mol/L BR buffer, 0.2 mol/L phosphate and 1.0 mol/L sodium hydroxide solutions. Fig. 1 gives the cyclic voltammograms of Sudan I at these AGCEs in BR buffer solution containing 9.41×10^{-6} mol/L Sudan I, and also at bare GCE (Curve 3 in Fig. 1).

As can be seen in Fig. 1, the AGCE activated in BR buffer solution shows the best sensitivity and well-defined current peak than that activated in sodium hydroxide and phosphate. Therefore, the BR buffer solution was chosen as the spiriting medium for GCE activation.

3.2. Selection of co-solvents

The effect of co-solvents on reductive current of Sudan I was investigated. Due to the comparatively weak solubility of Sudan I in water, three organic solvents, acetonitrile, alcohol and carbinol, in which Sudan I can be well dissolved (Sproll et al., 2005; Zhang et al., 2005), were chosen as the co-solvent, respectively. Experimental results indicate that Sudan I has a comparatively high sensitivity in alcohol, and additionally, alcohol is inexpensive and nontoxic, thus alcohol was selected as a co-solvent to dissolve Sudan I. Furthermore, the influence of alcohol in different ratio in working solution on the voltammetric response of Sudan I was investigated. Different quantity of alcohol, 10 mL BR buffer solution and 0.2 mL Sudan I solution were added into a volumetric flask, diluted with doubly distilled water to 40 mL (the concentration of Sudan I was 4.76×10^{-6} mol/L). Table 1 illustrates the influence of alcohol volume on the voltammetric response of Sudan I. The highest sensitivity and well-defined peak shape were obtained in the solution containing 20% of alcohol (8.0 mL alcohol was added).

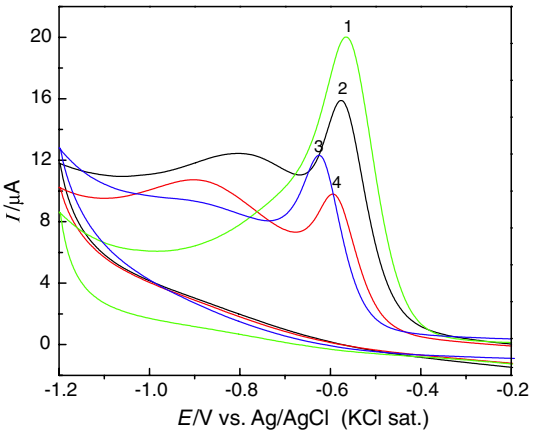


Fig. 1. Cyclic voltammograms of Sudan I at bare (3) and various activated GCEs (1-BR buffer; 2-NaOH solution; 4-KH_2PO_4 solution) The concentration of Sudan I: 9.41×10^{-6} mol/L in BR buffer. Scan rate: 100 mV/s.

3.3. Selection of working solution and acidity

3.3.1. Selection of working solution

In order to compare the effect of different electrolytes on the electrochemical response of Sudan I, three working solutions, BR buffer, acetic acid–sodium acetate and phosphate buffer solutions, were examined respectively. Sudan I shows a strong reductive peak both in BR and phosphate buffer solutions, however, the peak shape in BR buffer is more well-defined, so that BR buffer was chosen as the working solution for determination of Sudan I. Furthermore, the influence of BR buffer in different ratio in working solution on the voltammetric response of Sudan I was investigated. Different quantity of BR buffer, 8 mL alcohol and 0.3 mL Sudan I solution were mixed in a volumetric flask, and diluted with doubly distilled water to 40 mL (the concentration of Sudan I was 7.101×10^{-6} M). The highest sensitivity and well-defined peak shape were obtained in working solution containing 25% of BR buffer (10.0 mL BR buffer was added).

3.3.2. Selection of solution acidity

The influence of the solution acidity on the electrochemical behavior of Sudan I at AGCE was examined in the mixture of 10 mL absolute alcohol and 10 mL BR buffer of different pH, as illustrated in Fig. 2. It is demonstrated that the potential of reductive peak moves negatively with the increase of pH, and that the peak current has a positive correlation with pH over the range between 5.0 and 9.0 (slope, 2.3 μA/pH; correlation coefficient, 0.996). Contrarily, the peak current decreases when pH reaches beyond 9.0. This is caused by the change of reaction mechanism from acid catalysis to basic catalysis (De La Cruz Yaguez

Table 1
The influence of the amount of alcohol on the peak currents of Sudan I at AGCE

	Volume of alcohol (mL)							
	2.0	4.0	6.0	7.0	8.0	9.0	10.0	12.0
Peak potentials (V)	−0.639	−0.628	−0.614	−0.607	−0.607	−0.606	−0.605	−0.598
Peak currents (μA)	1.665	2.353	5.861	6.208	7.598	7.052	6.451	4.471

Potential scan range: 0.00 to −1.20 V; scan rate: 50 mV/s.

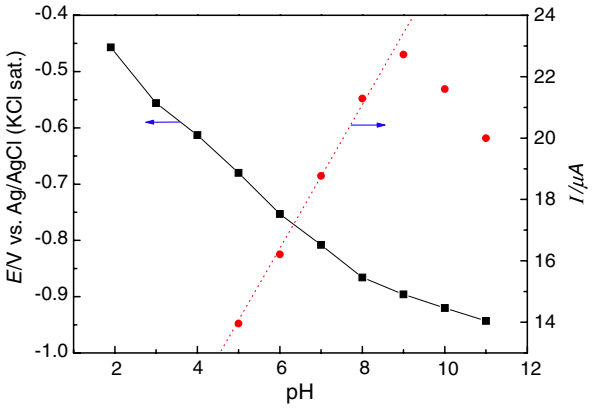


Fig. 2. Dependence of reductive peak potentials and currents on pH.

et al., 1986). In order to obtain a high enough sensitivity, the pH of working solution was taken 8–9.

3.4. Selection of potential scan rate

The influence of potential scan rate was also taken into account. It was observed that the peak current raises, and also that the reductive peak potential moves negatively with the increase of potential scan rate. This phenomena indicates the typical electrochemical process controlled by surface absorption, which can be used to pre-concentrate micro-quantity of Sudan I onto the surface of AGCE for quantitative analysis.

3.5. Selection of pre-concentration time

Sudan I is pre-concentrated onto the surface under stirring before the voltammogram was recorded. As is known that the pre-concentration time has a distinct influence on analytical sensitivity. It was observed that the peak current increases sharply with the pre-concentration time when it is less than 3 min, and then reaches a almost invariable limiting value. This demonstrates the adsorption of Sudan I on the surface of AGCE is fast and easy to reach saturated. So the pre-concentration time for Sudan I was taken 3 min in the analytical procedure.

3.6. Determination of Sudan I in real samples

3.6.1. Analysis procedures

The determination of Sudan I was carried out in different amount of extract, which was mixed with 10.0 mL BR buffer, 8.0 mL ethanol and diluted with doubly distilled water to 40 mL. No Sudan I was detected in the three kinds of hot chili powder purchased in a supermarket by linear sweep voltammetry (LSV). Fig. 3 demonstrates the voltammograms obtained for the mixture of extract and different amount of Sudan I standard solutions.

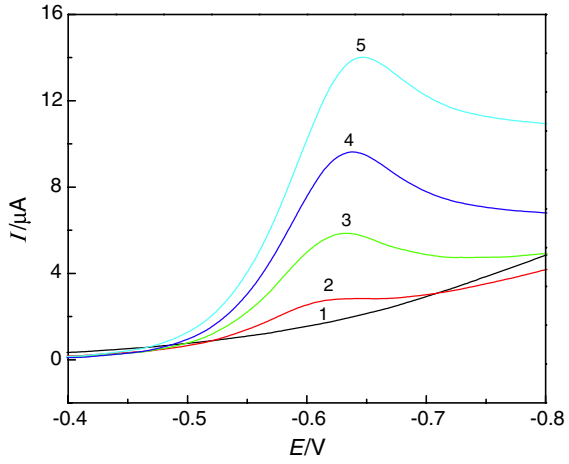


Fig. 3. Voltammograms of Sudan I in the samples of hot chili powder (1 – extract of hot chili powder, 2,3,4,5 – the extract mixed with 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL standard solution of Sudan I, respectively, scan rate: 100 mV/s).

3.6.2. Linear range and detection limit

The linear range of calibration curve is related to the amount of co-solvent in working solution. The more the amount of co-solvent was added, the wider the linear range and the lower the sensitivity was obtained. Inversely, the less the amount of co-solvent was added, the narrower the linear range and the higher the sensitivity was obtained. Under the selected conditions (20% alcohol, v/v), peak currents showed a well linear relationship with the concentration of Sudan I in the range between 2.4×10^{-6} and 1.8×10^{-5} mol/L, with a slope of 10.9 A/mol/L and a correlation of 0.9981, and the detection limit of about 7.1×10^{-7} mol/L can be estimated from the signal-to-noise characteristics of these data ($S/N = 3$). Six parallel determinations of Sudan I samples (containing 9.41×10^{-6} mol/L Sudan I) were carried out and the RSD was 4.8%.

3.6.3. Recovery

Table 2 gives the recovery of determination of Sudan I in hot chili powder. Mixed 5.0 g hot chili powder, which has been dried and grinded, with 0.0224 g Sudan I, and extracted with methanol ultrasonically three times (each time for 20 min). Admix the resulting extracts, dilute with methanol to 100 mL, then measurement is performed. Parallel six measurements gave the average recovery of 101.3%.

Table 2
Recovery of determination of Sudan I in hot chili powder

Analyte	Added (mol/L)	Measured (mol/L)	Recovery (%)	Average (%)
Sudan I	9.88×10^{-6}	1.02×10^{-6}	103.0	101.3
	1.18×10^{-5}	1.17×10^{-5}	99.2	
	1.38×10^{-5}	1.45×10^{-5}	105.5	
	1.57×10^{-5}	1.58×10^{-5}	100.8	
	1.76×10^{-5}	1.75×10^{-5}	99.2	
	1.95×10^{-5}	1.95×10^{-5}	100.0	

Table 3
The voltammetric responses of Sudan dyes at AGCE

Analytes	Sudan I	Sudan II	Sudan III	Sudan IV
Peak potentials (V)	−0.580	−0.606	−0.648	−0.683
Peak currents (μA)	28.22	27.80	39.21	40.08

Potential scan range: 0.00 to −1.20 V; scan rate: 50 mV/s.

3.6.4. Interferences

The natural pigments in chili are capsorubin (50–60%), one kind of tetraterpene compound, beta-carotene, cryptoxanthin, capsanthin (all three belong to ketenes compound), and capsaicin, one derivative of *ortho*-methoxy phenol. Consider the marked difference of electrochemical characteristics between pigments mentioned above and Sudan I, there is no interference during the detection procedure just as indicated in Fig. 3. The analogues of Sudan I are Sudan II, Sudan III and Sudan IV that might be added into food products as red coloring matters. Their voltammetric responses are listed in Table 3. It is shown that all the azocompounds possess a similar electrochemical behavior and well-defined reductive peak in the same working solution, i.e. 20% alcohol, 25% BR buffer and 2.35×10^{-5} mol/L Sudan dyes. Therefore, they might be detected quantitatively as they exist alone. Obviously they interference each other as they coexist due to their overlapped voltammetric peaks. It is suggested that the total amount of all Sudan dyes in the hot chili sample might be determined quantitatively by the area of overlapped peaks.

4. Conclusions

The present study has established a novel method to detect Sudan I in the samples of hot chili powder using AGCE. The glassy carbon electrode was activated in BR buffer solution, that enhances enormously the selectivity and sensitivity to determine Sudan dyes. The voltammetric technique, which is simple, convenient, fast and inexpensive, enriches the analytical means for Sudan dyes in food checking up besides the expensive and time-cosuming HPLC–MS or HPLC-spectrophotometry. Without doubt, HPLC or capillary electrophoresis (CE) combines with a amperometric detector employing an AGCE might give a well separated and more efficient tactics for detecting Sudan dyes in complex matrixes. And also, since the electrochemical system can be made into a small sensor, it is conceivable that the proposed method is more easy than others to be used in micro-total analysis system (μ-TAS).

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