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Electrochemical Behavior of Sudan II and Its Determination in Food Using Activated Glassy Carbon Electrode

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A glassy carbon electrode activated in sulfuric acid was applied in the determination of Sudan II in food samples. The experimental parameters, such as supporting electrolyte, scanning rate and accumulation time, were tested. It was very clear that the redox peak currents of Sudan II obviously increased. In pH 4.0 citric acid-phosphate buffer solution, the oxidation peak current of Sudan II at the activated glassy carbon electrode was linearly proportional to its concentration in a range from 4.00×10^{-7} mol L^{-1} to 1.00×10^{-5} mol L^{-1}. The limit of detection was estimated by gradually decreasing the concentration levels of Sudan II and the detection limit was found to be 8.00×10^{-8} mol L^{-1}. The electrochemical parameters was Calculated and mechanism related to the electrode reaction of Sudan II was proposed. The sensor showed great promise for the simple, sensitive, and quantitative detection of Sudan II and was used for the determination of Sudan II in real food samples with satisfactory results.

Keywords: Activation, Determination, Food, Sudan II, Cyclic voltammetry

1. INTRODUCTION

Colour is an important attribute of a food. Therefore, many colorants have been added to food product by food manufacturers. It is necessary that food businesses are responsible for ensuring their food safety. Food additives must comply with specified legislation. Unfortunately, some colorants are poisonous chemicals, which have been added to food, such as four Sudan dyes and Para Red. Sudan dyes have been used illegally in spices, sauces, chutneys, and vinegars. Sudan II (1-(2, 4-dimethylphenylazo) - 2-naphthol) (see Fig. 1) is one of Sudan dyes that is extensively used because of its colorfastness and low cost [1]. Sudan II is not permitted for use in food, as it is carcinogenic [2-4]. A study about its toxicity that can cause tumors in the liver or urinary bladder in rats was reported [5]. But it was still added in food supplies because of its good colour, colorfastness, and low cost.

Therefore, it is very important to develop a method for the fast detection and quantification of Sudan II.

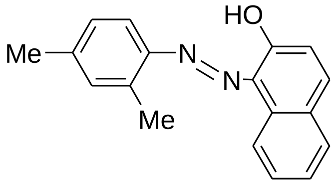

Figure 1. Chemical structures of Sudan II.

Some methods have been employed to detect Sudan II including high performance liquid chromatography (HPLC) or (HPLC-MS) [1, 6-9], capillary electrophoresis[10], and electrochemistry detection[11]. It can be seen that the HPLC or HPLC-MS methods are the major methods which require complicated procedure, time-consuming and costly apparatus. Electrochemistry detection for Sudan II has been studied at drop mercury electrodes. Despite of the toxicity of mercury, this gave a good choice of detection methods to Sudan II by electrochemistry method. It was well known that electrochemical methods are the most widely used methods in direct determination chemical molecules as they are not only rapid and sensitive but also more feasible for microanalysis. Glass carbon electrode (GCE) has been widely used in electrochemical direct determination. Its can be easily enhanced by various electrochemical pretreatments. The simplest pretreatment among them is the electrochemical activation. The activation of GCE (AGCE) with electrochemical pretreatment has been previously reported in references [12-15]. It is also well known that pretreatment resulted in the increase in oxygen content and the activated surfaces showed unusual adsorption ability [16]. Electrochemically activated glassy carbon electrodes have been applied to detect organic molecules [17, 18], but, to the best of our knowledge, this activated glassy carbon electrode has not been applied to detect Sudan II so far.

This report describes the electrochemical behavior of Sudan II and its determination in food using AGCE. A new method for determination of Sudan II in food was established. The sensor shows great promise for the simple, sensitive, and quantitative detection of Sudan II. Finally, this method was used for the determination of Sudan II in real food samples with satisfactory results.

2. EXPERIMENTAL

2.1 Chemicals and materials

Sudan II was purchased from Sigma-Aldrich and 2.0×10^{-4} mol L^{-1} Sudan II stock solution was prepared by dissolving it in absolute ethanol. All other chemical reagents were analytical-reagent grade. Citric acid-phosphate buffer solution was prepared by mixing the stock solutions of 0.1 mol L^{-1}

citric acid and 0.2 mol L^{-1} disodium hydrogen phosphate. All aqueous solutions were prepared in double distilled water.

2.2 Apparatus

Voltammetric measurements were performed by using a CHI 660C Electrochemical Workstation (Chen-hua, Shanghai, China) in a three-electrode arrangement, equipped with a glassy carbon working electrode, a platinum counter electrode and a Ag/AgCl reference electrode. PHS-3B Precision pH Meter (Shanghai, China) was used to measure acidity and all sonication was done using a KQ-100 Ultrasonic Cleaner (Kunshan, China).

2,3 Preparation of the AGGCE

Glassy carbon of electrode (GCE) 3.0 mm in diameter were polished thoroughly with gold sand paper and alumina powder (0.05 μm) and cleaned successively with 50% nitric acid, ethanol and doubly distilled water in an ultrasonicating bath for 1 min before being used, respectively. The AGCE was prepared by cycling the potential between -1.3 V and 2.2 V in 0.5 mol L^{-1} H_2SO_4 solution at a scan rate of 160 mV s^{-1} for nine cycles. After that, the electrode was taken out, and then rinsed carefully with doubly distilled water, and dried in the air to be used.

2.4 Electrochemical measurement

The electrochemical determination of Sudan II is performed in a certain amount of citric acid-phosphate buffer solution (pH 4.0) at the scan rate of 100 mV s^{-1}. Cyclic voltammograms (CVs) and differential pulse voltammetry (DPVs) were used for the determination of analytes. Upon completion of each determination, the electrode was continued scanned in a mixed solution of 10 mL ethanol and 10 mL pH 4.0 citric acid-phosphate buffer solution until no peak comes out for reuse.

3. RESULTS AND DISCUSSION

3.1 Electrochemical activation treatment of GCE

The AGCE was prepared by cycling the potential between -1.3 V and 2.2 V in 0.5 mol $L^{-1}$$H_2SO_4$ solution at a scan rate of 160 mV s^{-1} for nine cycles. The CVs are shown in Figure 2. According to the literatures [12, 14], the peaks of redox came from the electrode oxidation process on the surface of electrode. Oxygen-containing functional groups (such as carboxyl, carbonyl, hydroxyl, etc.) were generated on the carbon surface. In addition, the surface of the electrode became very fluffy and had a porous structure after treatment, so that the effective surface area increased [12]. It is beneficial to adsorption of the analyte.

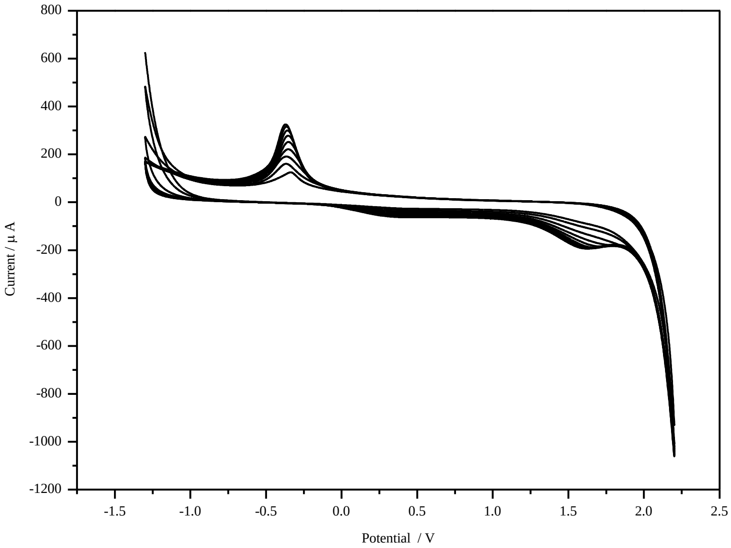

Figure 2. CVs of electrochemical activation of the bare GCE. Scan rate: 160 mV s^{-1}.

3.2 Electrochemical behaviors of Sudan II on AGCE

Figure 3 shows the CVs of 5.0 × 10^{-6} mol L^{-1} Sudan II in citric acid-phosphate buffer solution (pH 4.0) at a GCE and AGCE. The peak-current intensity at the AGCE greatly enhances in contrast to the GCE. Such electrocatalytic behavior of Sudan II attributed to functional groups and enlarged surface of the AGCE. In general, the AGCE has an excellent conductivity.

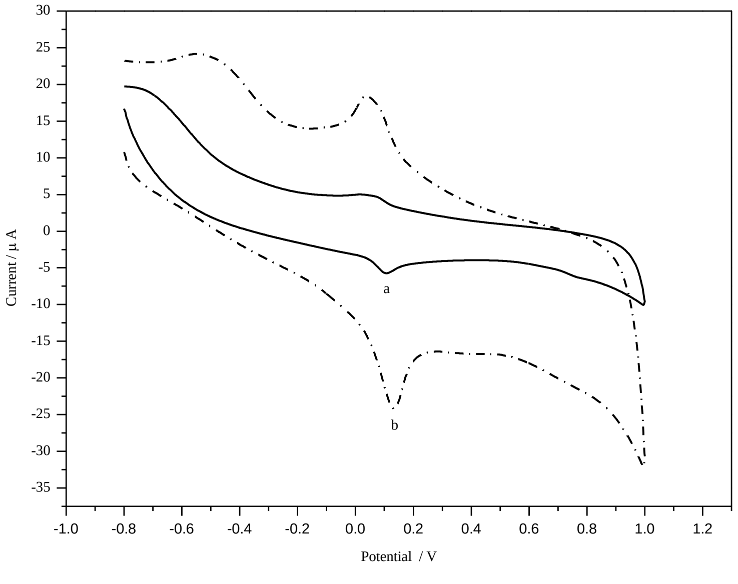

Figure 3. CVs of Sudan II at bare (a) and activated GCE (b). The concentration of Sudan II: 5.0 × 10^{-6} mol L^{-1} in citric acid-phosphate buffer solution (0.1 mol L^{-1}, pH 4.0). Scan rate: 100 mV s^{-1}.

3.3 Effect of the solution pH

The effect of the solution pH on the electrochemical signal of Sudan II was evaluated (Figure 4). Figure 4 shows the influence of the pH on the redox reaction of Sudan II at the AGCE. It can be seen that both the cathodic and anodic peaks potential of Sudan II negatively shifted with the increase of pH values, which shows that the protons took part in the electrode reaction of Sudan II. The peak potential of Sudan II changes linearly depending on a pH from 2.2 to 7.0, and the equations are $E_{pa} = 0.50 - 0.07$ pH and $E_{pc} = 0.26 - 0.048$ pH, the corrections are 0.9966 and 0.9950, respectively. The peak current reaches the biggest value at pH 4.0. So the pH 4.0 values were chosen to carry out the electroanalytical study in this work.

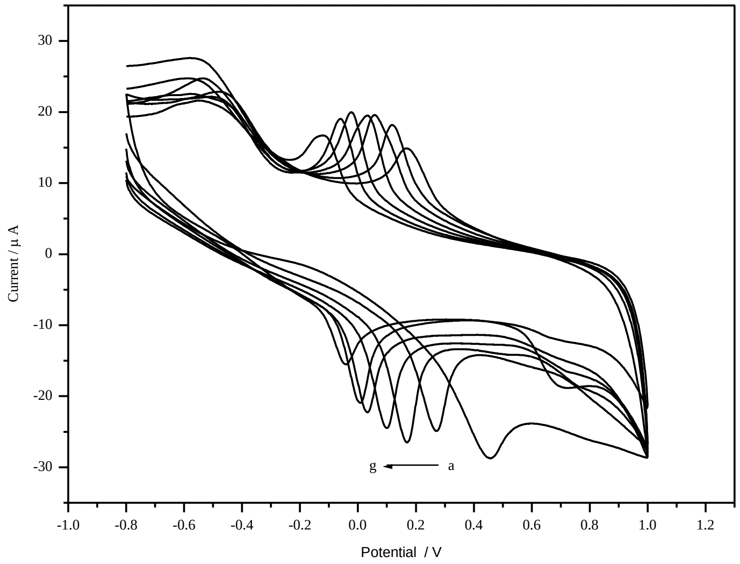

Figure 4 CVs of 5.0×10^{-6} mol L^{-1} Sudan II at different pH values. The pH of curve a-g was 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0, respectively. Scan rate: 100 mV s^{-1}.

3.4 Effect of the scan rate

The redox peak currents of Sudan II increased, with the scan rates ranging from 60 to 300 mV s^{-1} (Figure 5). The linear-regression equations of i_{pa} and i_{pc} of the scan rates are expressed as i_{pa} (A) = $-6.69 \times 10^{-7} - 1.45 \times 10^{-8} v$ (in millivolts per second), $R = 0.9923$; i_{pc} (A) = $5.24 \times 10^{-7} + 1.56 \times 10^{-8} v$ (in millivolts per second), $R = 0.9922$, respectively, indicating that the electrochemical behaviors of Sudan II on AGCE is an adsorption process. However, the symmetry of the potential becomes worse with the scan rates, ranging after 100 mV s^{-1}. Therefore, 100 mV s^{-1} was used as the scan rate in this work.

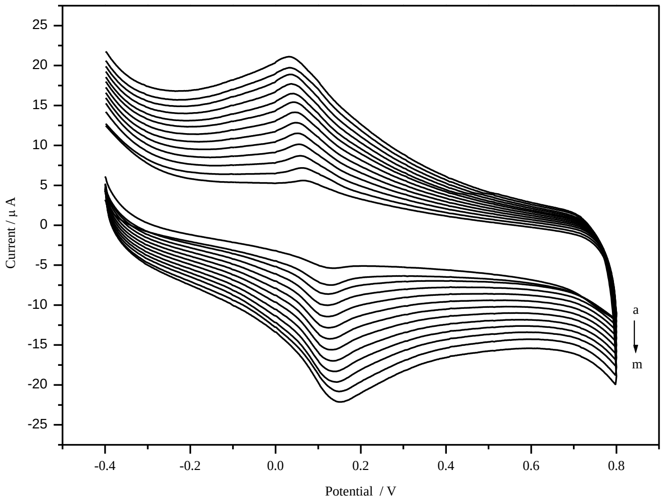

Figure 5 CVs of Sudan II on AGCE in the buffer of citric acid-phosphate buffer solution (0.1 mol L^{-1}, pH 4.0) at different scan rates from 60 to 300 mV s^{-1}.

3.5 Calculation of the electrochemical parameters and proposed mechanism related to the electrode reaction of Sudan II

The relationships between E_p and ln*v* in the cyclic voltammetry could be expressed as: E_{pa}= 0.20 - 0.02 ln*v*; E_{pc}= 0.38 - 0.039 ln*v*. According to the following Laviron's equation (1)[19]:

$E_{pa}=A+RT/[(1-\alpha)nF]\ \ln\nu$; $E_{pc}=B-RT/(\alpha nF)\ \ln\nu$ (1)

Where α (generally, $0.3< \alpha <0.7$) was the electron transfer coefficient, F the Faraday constant (96487 coulombs), R the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the Kelvin temperature (K) (T = 298 K in this work), and n the number of electron-transferred.

So α is calculated to be 0.35 and the number of electron (n) involved in the reaction is calculated to be $1.87 \approx 2$. According to the relationships between E_p and pH and the Nernst equation, the slope of -64 mV pH^{-1} reveals that the proportion of the electron and proton involved in the reactions is 1: 1. Consideration of above results and the literatures [20], the oxidation mechanism of Sudan II on the AGCE could be deduced as figure 6.

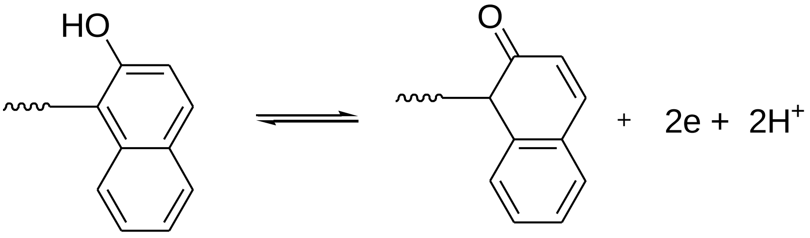

Figure 6. Proposed mechanism of oxidation of Sudan II on the AGCE.

3.6 Effect of the accumulation time

The accumulation time enabled us to ascertain the level of Sudan II adsorption on the electrode surface. To accomplish this, we varied the accumulation time and recorded the CVs of 20, 40, 60, 80, 100, 120, and 140 s for 5.0×10^{-6} mol L^{-1} Sudan II. The peak currents of Sudan II increased significantly with the increase of the accumulation time, and reached a maximum at 100 s, suggesting that AGCE can effectively accumulate Sudan II. Therefore, 100 s was used as the accumulation time.

3.7 Effect of the concentration and the detection limit

To quantitively detect Sudan II, the concentrations of Sudan II were varied from 4.00×10^{-7} mol L^{-1} to 1.00×10^{-5} mol L^{-1} and DPVs were recored. In pH 4.0 citric acid-phosphate buffer solution, the oxidation peak current of Sudan II on AGCE is linearly proportional to its concentration (c) in a range from 4.00×10^{-7} mol L^{-1} to 1.00×10^{-5} mol L^{-1}, with a correlation coefficient of 0.9933. The linear regression equation is i_{pa} (A) = $1.87 \times 10^{-6} + 0.68\, c$ (in moles per liter), $R = 0.9933$. The limit of detection was estimated by gradually decreasing the concentration levels of Sudan II with the detection limit of 8.00×10^{-8} mol L^{-1} ($S / N = 3$).

3.8 Interference

Potential interference (such as capsorubin, beta-carotene, leaxanthin, violaxanthin, neoxanthin, et al.) on the selective determination of Sudan II was investigated. These species differ greatly from Sudan II in chemical structure and electrochemical characteristics, and no interference in the current response was observed for 5.0×10^{-6} mol L^{-1} Sudan II. Furthermore, experiments show that no interferences in the redox peak current of Sudan II in the presence of 1000 times K^{+}, Na^{+}, Fe^{3+}, Ca^{2+} and Mg^{2+}, indicating that the AGCE is highly selective towards the determination of Sudan II.

3.9 Sample analysis

In order to ascertain the applications in food sample analysis, this newly method was used to detect Sudan II in chilli powder, chilli sauce, strawberry sauce, and tomato sauce. The 10.00 g of sample was accurately weighed and added to a stoppered flask with absolute methanol (50 mL) under sonication for 40 min, respectively. After filtration, the filtrate was just filtrated and the filtrate was collected for measurement. Under the optimized conditions, the prepared sample solution was detected at the AGCE. Fortunately, there was no observable peaks appeared and a recovery experiment was carried out by the standard addition method. Recoveries were calculated with oxidation peak current value and results are shown in Table 1. Six parallel measurements were carried out for each sample, and the RSD were within the range of 3.41% to 4.76%, indicating that this method has good reproducibility. The average recoveries varied from 95.0% to 101.7%, indicating that this method has good accuracy.

Table 1. Recovery of determination of Sudan II in chilli samples (n=6)

Samples	Electrooxidation				HPLC		
Samples	Sudan II Added / (μmol L^{-1})	Average Found / (μmol L^{-1})	Recovery / %	R.S.D / %	Average Found / (μmol L^{-1})	Recovery / %	R.S.D / %
Chilli powder	0.60 6.00 1.00	0.58 5.91 0.97	96.7 98.5 97.0	4.26 3.41 3.56	0.57 5.96 0.96	95.0 99.3 96.0	4.41 4.11 3.96
Strawberry sauce	0.60 6.00 1.00	0.57 5.96 0.96	95.0 99.3 96.0	4.65 3.71 3.96	0.59 5.80 0.97	98.3 96.7 97.0	3.65 4.71 4.16
Tomato sauce	0.60 6.00 1.00	0.61 5.94 0.99	101.7 99.0 99.0	4.06 4.41 4.76	0.59 5.97 0.96	98.3 99.5 96.0	3.86 4.01 2.96

4. CONCLUSIONS

In this paper, a glassy carbon electrode in sulfuric acid was activated by a rapid and simple procedure for the determination of Sudan II. The electrochemical behavior of Sudan II was greatly promoted with an increased oxidation peak current, which provided a sensitive method for Sudan II. The electrochemical parameters was Calculated and mechanism related to the electrode reaction of Sudan II was proposed. The sensor shows great promise for the simple, sensitive, and quantitative detection of Sudan II. The ability of the modified electrode for the determination of Sudan II was evaluated and demonstrated in food samples.

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