Voltammetric Determination of Sudan 1 in Food Samples Using Its Cu(II) Compound

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SUMMARY

In this work, we developed a sensitive, simple and convenient electrochemical method to determine Sudan 1 in food samples using its Cu(II) coordination compound. Using phosphate buffer solution at pH=5.0 as supporting electrolyte (75 % methanol), differential pulse voltammetry and 6-fold concentration of Cu(II), the electrochemical oxidation signal of Sudan 1–Cu(II) coordination compound at glassy carbon electrode significantly increased when compared to the one without the added Cu(II). The experimental conditions such as the amount of methanol, pH, the concentration of Cu(II) and the instrumental parameter were optimized for the determination of Sudan 1. Under the optimal experimental conditions, the oxidation peak current of Sudan 1 was proportional to its concentration in two ranges: 0.04–0.09 to 0.09–5.3 µM with a detection limit of 0.71 nM (S/N=3). The interference effects of Sudan 2-4 with the determination of Sudan 1 was also evaluated. The developed method was successfully applied to tomato, chilli sauces, ketchup and chilli powder. The analysis results of Sudan 1 in food samples obtained by the proposed method were in a good agreement with the reference values detected by HPLC.

Key words: Sudan 1, voltammetric determination, Cu(II), food analysis

INTRODUCTION

For food industries, colour is the first indicator of the quality of the food. Organic colourants are often used to enhance both the sales and visual aesthetics of foods. Food colourants are usually classified into pigments from natural sources and artificially synthesized dyes (1,2). Azo compounds, a kind of synthetic organic colourants, are among the widely used food colourants. The azo dye molecules with the help of biocatalyst are transformed into respective aromatic amines, which are more toxic to the environment than the intact dye molecule (3). Sudan dyes, mainly Sudan 1, Sudan 2, Sudan 3 and Sudan 4, are non-ionic fat-soluble dyes. These phenyl-azoic derivatives are classified by the International Agency for Research on Cancer (IARC), Lyon, France, as category 3 carcinogens. They can induce some forms of liver and bladder cancer in animals (4) so their use in food is banned in most countries, including the European Union (5). However, Sudan 1 (1-phenylazo-2-naphthol), which is a carcinogenic compound containing an azo group, is still used in foodstuffs as additive due to its low price, bright and stable colour (6–8). Therefore, developing a sensitive, suitable and rapid method for the determination of Sudan 1 is very important and essential.

Until now, several analytical methods have been developed for the analysis of Sudan 1, such as capillary liquid chromatography (9), Raman spectroscopy (10), UFLC-MS/MS (11), HPLC (12–14) and fluorescence spectroscopy (15), among which HPLC is preferred for determination of Sudan 1 (16). These techniques are sensitive but they require expensive instruments, time for pretreatment, experienced operators and large amount of organic solvents (17). Apart from that, these instruments are not suitable for fieldwork because they are cumbersome. On the other hand, electroanalytical techniques are cost-effective, simple and use portable instruments, which are easy to handle, and they require short analysis time (18,19). For the electrochemical determination of Sudan 1, some methods...
using multi-wall carbon nanotube (MWNT) thin film-modified glassy carbon electrode (20), self-assembly ionic liquid-functionalized reduced graphene oxide–gold nanoparticle composites (6), or multi-wall carbon nanotube modified glassy carbon electrode (27) have been reported.

Sudan dyes and their Cu(II) complexes were investigated both experimentally and theoretically and characterized by analytical and spectroscopic X-ray diffraction methods (22,23).

In this paper, we developed a simple, stable and sensitive electrochemical method for the determination of Sudan 1 in food samples. For the voltammetric determination of Sudan 1, Sudan 1-Cu(II) coordination compound and non-modified glassy carbon electrode were used. Besides, interference effects of Sudan 2-4 were also evaluated for the electrochemical determination of Sudan 1. It was discovered that the proposed method has advantages such as high sensitivity, low detection limit and low cost. The suggested new method was used for the determination of Sudan 1 in tomato, chilli sauces, ketchup and chilli powder.

MATERIALS AND METHODS

Chemicals

Phosphate-buffered saline (PBS) tablets were purchased from Oxoid Ltd. (Thermo Fisher Scientific, Basingstoke, UK) and Cu²⁺, Ca²⁺, Mg²⁺, Al³⁺, Na⁺, Fe³⁺ and Zn²⁺ solutions (inductively coupled plasma (ICP) standard, in 2% HNO₃) were purchased from Merck (Darmstadt, Germany). Sudan 1 (≥95%), Sudan 2 (90%), Sudan 3 (technical grade), Sudan 4 (≥80%), glucose (ACS reagent (analytical reagents of the American Chemical Society)), fructose (≥99%), sucrose (≥99.5%) and methanol were all purchased from Sigma-Aldrich (Merck, St. Louis, MO, USA). All of them were used without further purification. The tomato, chilli sauces, ketchup and chilli powder were purchased from a local market (Çankaya, Ankara, Turkey). The supporting electrolyte, phosphate buffer (pH=5.0), was prepared using PBS tablets in methanol (75%) and pH was adjusted using 0.1 M H₃PO₄ (Sigma-Aldrich, Merck) and 0.1 M NaOH (Sigma-Aldrich, Merck). All chemicals were of analytical grade. Double distilled water (18.2 MΩcm) was used to prepare the solution.

Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were carried out with a PalmSens3 potentiostat/galvanostat (PalmSens BV, Houten, The Netherlands) and CHI 660B (CH Instruments, Inc., Austin, TX, USA). A conventional three-electrode system, consisting of glassy carbon working electrode (MF-2012, 3.0 mm diameter; BASi, West Lafayette, IN, USA), Ag/AgCl reference electrode (MF-2052; BASi) and platinum wire auxiliary electrode (MW-1032; BASi), was used. Moreover, Agilent 1200 series HPLC system (Agilent Technologies, Inc., Santa Clara, CA, USA) was used for Sudan 1 chromatographic analysis on the ACE C18 (250 mmx4.6 mm) column (Advanced Chromatography Technologies Ltd, Aberdeen, UK), with a mobile phase consisting of methanol/acetoniitrile (40:60, by volume) and a constant flow rate of 0.5 mL/min. The injection volume was 10 µL and the column temperature was set at 25 °C. Finally, the wavelength was set at 254 nm for quantitative analysis.

Analytical procedure

Phosphate buffer solution (pH=5.0) as supporting electrolyte (75% methanol) was used for the determination of Sudan 1. Differential pulse voltammograms were recorded from 0.4 to 1.2 V with the parameters of pulse amplitude of 0.05 V, pulse time of 4 s and pulse width of 0.025 s. The oxidation peak current values at 0.96 V were used for the determination of Sudan 1 in the presence of Cu(II).

Sample preparation

A mass of 1.0 g of tomato and chilli sauces, ketchup and chilli powder was weighed exactly and then 40 mL methanol were added. After 30 min, ultrasonication, the mixtures were filtered through 0.22-µm syringe filter (Sartorius, Goettingen, Germany) in a 50-mL volumetric flask and then diluted to 50 mL with methanol.

RESULTS AND DISCUSSION

Voltammetric behaviour of Sudan 1 and Sudan 1-Cu(II)

The electrochemical behaviour of 1.96 µM Sudan 1 and Sudan 1-Cu(II) coordination compound was investigated on glassy carbon electrode using cyclic voltammetry (CV) in PBS at pH=5.0 (75% methanol). Fig. 1a shows the cyclic voltammogram of supporting electrolyte (curve A), and the cyclic voltammogram of 1.96 µM Sudan 1 in which an oxidation peak was observed at 0.86 V during the anodic scanning from 0.4 to 1.2 V (curve B). There is no peak on the reverse scan. Curve C shows the influence of Cu(II) on electrochemical oxidation of Sudan 1.

The oxidation peak potential of Sudan 1 shifted from 0.86 to 0.96 V in the presence of 11.76 µM Cu(II) and the oxidation peak current increased greatly; on the reverse scan, a slightly visible reduction peak appeared at 0.87 V. In addition, the oxidation peak current of Sudan 1-Cu(II) increased remarkably; however, the reduction peak current remained almost unchanged. This may be attributed to the special structure of Sudan 1-Cu(II) complex because it exhibits oxidation waves at more positive potentials as a result of the destabilizing effect of the electron-withdrawing group on the aryl-azo moiety (24). Therefore, with the addition of Cu(II), Sudan 1 oxidation peak current increased and the sensitivity was enhanced. Besides, the oxidation of Sudan 1 is an irreversible reaction in the presence of Cu(II), too. Finally, the electrochemical behaviour of Cu(II) solution (1.96 µM) was evaluated in the same medium, but any oxidation or reduction peak was not observed (Fig. 1b, curve B).
The effect of the amount of cosolvent

Sudan dyes are fat-soluble dyes. While Sudan 1 is insoluble in water, it is soluble in various organic solvents (in mol/L: trichloromethane 1.49, dichloromethane 0.57, toluene 0.30, benzene 0.17, acetonitrile 0.04, ethanol 0.02 and methanol 0.017) (26). Solubility of Sudan 1 is similar in methanol and ethanol, so in this study, methanol was chosen as a co-solvent because of its solubility in water and its low toxicity (27). The influence of methanol ratio to Sudan 1-Cu(II) oxidation peak current was studied and changes of peak currents were evaluated in PBS (pH=5.0) supporting electrolyte (Fig. 3).

The effect of pH

The pH is always involved in the electrochemical reaction of organic compounds and it exerts significant effect on the reaction speed (18). The influence of pH on the electrochemical oxidation signal of Sudan 1 was studied at different pH values (from 2.0 to 12.0) with different supporting electrolytes (75 % methanol) with CV technique and 117.6 μM Cu(II). Fig. 2a shows the effect of pH value on the oxidation peak current of 19.6 μM Sudan 1. Sudan 1 oxidation peak potential shifted negatively with the increase of pH and changed linearly depending on the change in the pH ranging from 2.0 to 12.0, which was determined using the following equation:

\[ E_{pa} = -0.0455 \text{pH} + 1.0934 \quad (R^2=0.924) \]

indicating that the oxidation of Sudan 1 includes the protons. This result is consistent with literature (25).

When pH value increased gradually from 2.0 to 12.0, the oxidation peak current of Sudan 1 at glassy carbon electrode obviously decreased (Fig. 2b). Sudan 1 oxidation peak current was the highest at pH=2.0 (1.95 μA), but since this is an extremely acidic medium, phosphate buffer solution at pH=5.0 was used for the determination of Sudan 1. In this medium, Sudan 1 oxidation peak current was 1.90 μA. As a result, PBS at pH=5.0 was chosen as the supporting electrolyte.

**Fig. 1.** Cyclic voltammograms recorded on glassy carbon electrode with scan rate 125 mV/s of: a) supporting electrolyte (phosphate-buffered saline (PBS) at pH=5.0 in 75 % methanol) (curve A), Sudan 1 (c=1.96 μM) in PBS (curve B), and Sudan 1-Cu(II) complex (curve C); and b) PBS (curve A), and solution of Cu(II) (c=11.76 μM) in PBS (curve B)

**Fig. 2.** Cyclic voltammograms of c(Sudan 1)=19.6 μM recorded on glassy carbon electrode in supporting electrolyte (phosphate-buffered saline (PBS) at pH=5.0 in 75 % methanol): a) at scan rate 100 mV/s for different pH values, and b) plot of peak currents of Sudan 1 versus pH value

**Fig. 3.** Influence of methanol volume fractions on stability of Sudan 1-Cu(II) complex with time. \( I_p \)=peak current
voltammogram (DPV) peak current obviously decreased with time. At the end of 35 min, Sudan 1-Cu(II) oxidation peak disappeared in 25 % methanol solution (PBS at pH=5.0) and the decrease of peak current was around 85 % in the 50 % methanol solution. Due to the stability of the oxidation peak currents in 75 % methanol aqueous solution, this volume fraction of methanol was chosen for further experiments. For electrochemical studies 5 mL of supporting electrolyte were used.

Effect of scan rate

The scan rate is linearly proportional to the peak current according to Randles-Sevcik equation in diffusion-controlled systems:

\[ I_p = k \nu^{1/2} \]

where \( I_p \) is the peak current, and \( \nu \) is the scan rate.

The logarithmic equation between the scan rate and the peak current is given as:

\[ \log I_p = \log k + x \log \nu \]

In diffusion-controlled systems the \( x \) value is 0.50 (28). Firstly, the effect of scan rate (from 2.5 to 150 mV/s) on oxidation of c(Sudan 1)=2.92 µM in supporting electrolyte PBS in 75 % methanol (pH=5) at the glassy carbon electrode was investigated (Fig. 4a).

From the experimental data, the slope was calculated to be 0.36 (Fig. 4b). This value shows that although this is the adsorption-controlled reaction, there is no significant diffusion effect on it. Then, the effect of scan rate (from 2.5 to 200 mV/s) on oxidation of Sudan 1-Cu(II) complex (c(Sudan 1)=2.92 µM, c(Cu(II))=17.52 µM) was investigated (Fig. 4c). The slope was calculated to be 0.46 (Fig. 4d). This value shows that although this is a diffusion-controlled reaction, there is no significant adsorption effect on it. The results suggest that the adsorption-controlled electrochemical process changes into diffusion-controlled process with the addition of Cu(II).

Besides, when the scan rate was studied between 2.5 and 200 mV/s, it was observed that the peak current increased to 125 mV/s, scan rate above 125 mV/s did not improve the electrochemical current response significantly. Therefore, 125 mV/s was used as the optimal scan rate for Sudan 1-Cu(II) studies.

Effect of Cu(II) concentration

Azo dyes can act as chelating reagents for metal ions. Sudan 1 can react with Cu(II) to form metal-Sudan complex (15,16), where alkaline solution was preferred for the investigation of interaction between Sudan 1 and copper. In our study, oxidation peak potentials of Sudan 1 at pH=5.0 and 10 (75 % methanol) were 0.879 and 0.489 V, respectively. The oxidation peak potentials of the Sudan 1-Cu(II) complex at pH=5.0 and 10 (75 % methanol) were 0.969 and 0.934 V, respectively. Our study at both pH values showed that the peak potentials for the oxidation of Sudan 1 and Sudan 1-Cu(II) complex shifted towards negative direction with an increase in Cu(II) concentration.

\[ y=0.36x+1.79 \]

\[ R^2=0.9989 \]

\[ y=0.4537x+1.1656 \]

\[ R^2=0.9995 \]

Fig. 4. Differential pulse voltammograms of c(Sudan 1)=2.92 µM recorded on glassy carbon electrode in supporting electrolyte phosphate-buffered saline in 75 % methanol (pH=5.0): a) different scan rates: 2.5, 5.0, 10, 50, 125, 150 mV/s (from A to G), b) influence of scan rate (\( \nu \)) on peak current (\( I_p \)) of Sudan 1, c) Sudan 1-Cu(II) complex: c(Cu(II))=17.52 µM at different scan rates (from A to H), and d) influence of scan rate (\( \nu \)) on peak current (\( I_p \)) of Sudan 1-Cu(II) complex.
in the pH and the oxidation peak currents of Sudan 1-Cu(II) at pH=5.0 and 10 (75 % methanol) were 1.53·10^{-6} and 0.88·10^{-6} A. As the peak current at pH=5.0 was twofold higher than at pH=10.0, we used pH=5.0 for the determination of Sudan 1.

The influence of Cu(II) concentration on oxidation peak current value of 19.6 µM Sudan 1 was investigated in PBS at pH=5.0 (75 % methanol). When Cu(II) concentration gradually increased from 19.6 to 130.7 µM, the oxidation peak currents of Sudan 1-Cu(II) increased. The oxidation peak currents and oxidation potential of Sudan 1-Cu(II) increased gradually and reached the maximum value at 120.06 µM Cu(II) (Fig. 5).

Calibration curves for the determination of Sudan 1

DPV had a higher sensitivity than CV for analytical determination of Sudan 1 (29). Fig. 6a shows the differential pulse voltammograms of Sudan 1-Cu(II) complex recorded under optimized conditions. The DPV oxidation peak current of Sudan 1-Cu(II) complex increased with the increasing concentration of Sudan 1. In the concentration range from 0.04 to 5.32 µM, two linear ranges can be seen in the insert of Fig. 6b. These ranges can be obtained in a range between 0.04–0.09 and 0.09–5.3 µM. The corresponding regression equations can be expressed as:

\[ I_p = 1.441c + 0.0085 \quad (R^2=0.9972) \quad /4/ \]

and

\[ I_p = 0.227c + 0.0969 \quad (R^2=0.9979) \quad /5/ \]

Two slopes were observed with two different regions. This is because higher concentrations of Sudan 1 molecules are attached at the interface, and thus, the saturation occurs in the system, resulting in lower signal in the current value (30). The limit of detection (LOD) was estimated to be 0.71 nM (S/N=3).

For the evaluation of the prevalence of this work, Table 1 compares our results with the literature findings, and shows that the detection limit of Sudan 1 is lower than those of previously reported works.

Firstly, the quantitative analysis of Sudan 1 was carried out by the DPV under the optimum conditions such as PBS at pH=5.0 (75 % methanol), scan rate 125 mV/s, and 6-fold concentration of Cu(II). The analysis of the known concentrations of samples was repeated five times using the glassy carbon electrode. The data in Table 2 show that the quantitative determination of Sudan 1 involves a very small error and standard deviation.

In real samples, standard addition method was carried out and each sample was determined five times under the optimized conditions (Table 2). For comparison, the Sudan 1 concentrations in food samples were also determined by HPLC (34). The electrochemical results show good agreement with HPLC results. The percentage recoveries ranged from 97.71 to 102.21, which indicates that the developed method may have useful applications in food samples.

**Table 1**

| c(Cu(II))/µM | I_p/µA |
|-------------|--------|
| 0.04        | 0.06   |
| 0.08        | 0.09   |
| 1.8         | 2.7    |
| 3.6         | 4.5    |
| 5.3         | 19.6   |

**Table 2**

| c(Sudan 1)/µM | Recovery (%) |
|---------------|--------------|
| 0.04          | 97.71        |
| 0.06          | 98.21        |
| 0.08          | 97.71        |
| 1.8           | 97.71        |
| 3.6           | 97.71        |
| 5.3           | 97.71        |
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**Table 1.** The comparison of the determination of Sudan 1 in this study with previously reported ones

| Electrode | Method | LOD/nM | Linear range/M | Reference |
|-----------|--------|--------|----------------|-----------|
| Ag nanoparticles on graphene oxide-modified glassy carbon | Amperometry | 1140.0 | (3.90–31.90)·10⁻⁵ | (19) |
| Multi-wall carbon nanotube | SWV | 20.10 | 4.03·10⁻⁶–4.03·10⁻⁴ | (20) |
| Graphene-modified glassy carbon | CV | 40.0 | 7.50·10⁻⁶–7.50·10⁻⁵ | (25) |
| Expanded graphite paste | DPV | 0.90 | 5.0·10⁻⁶–7.0·10⁻⁶ | (31) |
| Platinum nanoparticles attached to graphene-β-cyclodextrin | DPV | 1.60 | 5.0·10⁻⁹–68.68·10⁻⁴ | (32) |
| Graphene-ZnSe quantum dots hybrid | SW-ASV | 1.20 | 4.02·10⁻⁶–2.01·10⁻⁴ | (33) |
| Non-modified glassy carbon | DPV | 0.71 | (0.38–0.93)·10⁻⁷ to (0.93–53.2)·10⁻⁷ | This work |

LOD=limit of detection

**Table 2.** The determination of Sudan 1 in synthetic and food samples

| Sample | c(Sudan 1)c(Sudan 1) | c(Sudan 1) | Recovery/% | RSD/% | Reliability range**c(Sudan 1) | Determination by HPLC c(Sudan 1)10⁻⁷ M |
|--------|-------------------|--------------|-------------|-------|-----------------------------|----------------------------------|
| Synthetic sample 1 | 0.66 | 0.66±0.03 | 100.51 | 4.17 | 0.66±0.03 | 0.66±0.03 |
| Synthetic sample 2 | 2.38 | 2.37±0.02 | 99.87 | 0.80 | 2.37±0.02 | 2.38±0.02 |
| Chilli sauce | 2.76 | 2.73±0.02 | 98.76 | 0.62 | 2.73±0.02 | 2.75±0.02 |
| Tomato sauce | 4.42 | 4.46±0.01 | 98.60 | 0.27 | 4.46±0.02 | 4.46±0.02 |
| Chilli power | 2.76 | 2.78±0.02 | 100.60 | 0.68 | 2.78±0.02 | 2.77±0.01 |
| Ketchup | 4.42 | 4.48±0.03 | 99.19 | 0.58 | 4.48±0.03 | 4.50±0.03 |

*Result=x±sx; **95 % reliability level, N=5; RSD=relative standard deviation

**Interference studies**

The influence of some potential interfering species, such as Sudan 2-4, glucose, fructose and sucrose, on the determination of 2.82 µM Sudan 1 was studied by DPV. The results indicate that 25 times higher concentrations of glucose, fructose and sucrose would not affect the determination of Sudan 1. There was a 5 % decrease in peak current because of increasing supporting electrolyte volume; nevertheless, there was no deformation in the shape of the peak or any shift in Sudan 1 oxidation potential. Under the same conditions, the effects of Sudan 2-4 were investigated and it was detected that 0.04 times higher concentration of Sudan 2, and 0.03 times higher concentrations of Sudan 3 and Sudan 4 had an influence on the signal of Sudan 1. There was a deformation in the shape of the peak and shift in Sudan 1 oxidation potential. The total approximate content of all Sudan compounds such as Sudan 1-4 might be detected using this method in food samples.

Metal ions Ca²⁺, Mg²⁺, Al³⁺, Na⁺, Fe³⁺ and Zn²⁺ can be found in food samples. Previous studies show that about 1000-fold concentration of these metal ions has almost no influence on the electrochemical determination of Sudan 1 (35,36), so the interference effects of these metals were not investigated in this study.

**CONCLUSIONS**

We developed a simple, sensitive, inexpensive and selective electrochemical method for the determination of Sudan 1 in foods by differential pulse voltammetry based on the addition of methanol and Cu(II). The electrochemical response of Sudan 1 remarkably increased in the presence Cu(II). In all previous studies, Sudan 1 was determined by a modified electrode; however, this method needs no modification and the detection limit was much lower (0.71 nM) than previously reported values. The interference effects of Sudan 2-4 were demonstrated in this study.

**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

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