

Oxidation and Complexation-Based Spectrophotometric Methods for Sensitive Determination of Sudan II Dye in Commercial Food Samples

Elham Y. Hashem^{1,*}, Magda M.S. Saleh¹ and Najat O. A. Al-Salahi²

¹Chemistry Department, Faculty of Science, Assiut University, 71516 Assiut, Egypt.

²Chemistry Department, Aden University, Yemen.

Received: 2 Jun. 2018, Revised: 11 Aug. 2018, Accepted: 18 Aug. 2018.

Published online: 1 Sep. 2018.

Abstract: A simple solvent extraction procedure for separation – enrichment of Sudan II (1-[(2,4-dimethylphenyl)azo]-2-naphthol from commercial food products had been presented. Two highly simple and sensitive spectrophotometric methods were developed to quantitate Sudan II in pure and in some commercial food samples. The two methods are based on redox reaction with copper followed by copper complex formation (method I) as well as oxidation–reduction reaction with alkaline KMnO₄ (method II). These reactions were monitored spectrophotometrically at two maximum absorbance's 364 and 610 nm for methods I and II respectively. Variables affecting these reactions were carefully studied and the conditions were optimized. The stoichiometry of each reaction was determined. The equilibrium and stability constants (K, B) of Sudan II – copper complexes were calculated. Under optimized experimental conditions, Beer's law is obeyed in the concentration range 5.53–44.21, 2.21–15.48 µg.ml⁻¹ for copper and KMnO₄ reagents respectively. The apparent molar absorptivity, Sandell's sensitivity, detection and quantification limit were calculated. Matrix effects were also investigated. The procedures were successfully applied for the determination of trace levels of Sudan II in the selected commercially food samples by using multiple points standard addition method. Statistical comparison of the results is in good agreement with the reported methods.

Keywords: Sudan II, Complexation, Solvent extraction, Commercial food products, Spectrophotometric analysis.

1 Introduction

Synthetic dyes used in various industries are potential pollutants for food and environmental samples. Sudan dyes (I–IV) are a class of azo dyes. They have been illegally used as additives in food products to maintain their red–orange color and thus stimulate sales of these products in the market (Qiao et al. 2011; Rebane et al. 2010; Sun et al. 2007; Soylak et al. 2011). Sudan dyes are thought to be carcinogenic yet have been determined as a contaminant in chili powder (Calbani et al. 2004; Liu et al. 2007). The EU has set the detection limit at 0.5–1 mg.kg⁻¹ for Sudan dyes, and any food material containing more than those limits showed be withdrawn from the market (Chailapakul et al. 2008). Sudan dyes are of interest because of their chromophoric nature and the bidentate character of their

orthophenolichydroxy group. These properties have made azo dyes useful for metal complexation (Gürkan and Altunay 2013).

A variety of analytical methods have been proposed to detect the presence of Sudan dyes in food stuffs as recently reviewed (Rebane et al. 2010). The most popular analytical methods are based on high–performance liquid chromatographic method associated with different detectors (Qiao et al. 2011; López-Jiménez et al. 2010; Qi et al. 2011; Enríquez-Gabeiras et al. 2012; Li et al. 2013). Electrochromatography has been used to analyze Sudan dyes in foods (Fukuji et al. 2011; Mejia et al. 2007; Zhang et al. 2012). In addition many other analytical methods such as fluorescence (Huang et al. 2013), electro–analytical technique (He et al. 2007; Ensafi et al. 2012) and Raman spectroscopy (Di Anibal et al. 2012) have been available for their individual and simultaneous analysis. Most of these methods are expensive and time consuming, and thus restrict their application in food safety examination.

* Corresponding author E-mail: elham_hashem@yahoo.com

Although UV and Vis. spectrophotometric methods are simple and inexpensive, available literature is very scanty dealing with measurement of Sudan II (Di Anibal et al. 2009; Mustafa et al. 2013; Mustafa et al. 2013). In besides that there has been no report of spectrophotometric determination of Sudan II depending on complexation reaction with copper or oxidation with alkaline KMnO_4 . Two important limitations in UV-Vis. spectrophotometric determination of food colorants arise when levels of analyte are lower than the quantitative limits of UV-Vis. spectrometry and where there are influence of the concomitants on the signal of the colorants (Pourreza et al. 2011). So various separation–enrichment procedures have been used to solve these problems (Pourreza et al. 2011; Soylak et al. 1996; Soylak et al. 1997; Ertaş et al. 2007). In our research work, Sudan II were extracted from samples of chili powder, catsup and tomato sauces by ethyl alcohol (Ertaş et al. 2007) and were estimated by two simple and sensitive spectrophotometric methods. The first is based on redox reaction followed by complexation reaction of Sudan II with copper ions and the second is based on oxidation of Sudan II with alkaline KMnO_4 under optimum conditions.

2 Experimental

2.1 Apparatus

An evolution 300 UV-Vis. Spectrophotometer with 1.0 cm matched cells fitted with vision pro software of Thermo Electron Corporation (Cambridge, U.K) was used for electronic spectral measurements. pH measurements were made with Jenway 3040 ion analyzer – pH meter, equipped with Jenway 924005 combined glass electrode. All measurements were made in presence of ethanol–water (50% v/v) at 25°C. pH values of partially aqueous were corrected as described by Douheret (Douheret 1967).

2.2 Chemicals and Solutions

All reagents and solvents used (ethanol, methanol, acetone, DMSO and DMF) were of analytical reagent grade. Deionized water was used to prepare all solutions. A $1.0 \times 10^{-3} \text{ mol L}^{-1}$ stock solution of copper nitrate was prepared by dissolving the required amount of the Analar product in deionized water and standardized complex imetrically with EDTA (Vogel 1973). The ionic strength of solutions was maintained at a constant value of $I = 0.1 \text{ mol L}^{-1}$ (NaClO_4). A borate buffer solution ($\text{pH} = 9.0$) was prepared in 100 ml volumetric flask. All measurements were made in 50% (v/v) ethanol–water medium (method I) and in $1.0 \text{ mol L}^{-1} \text{NaOH}$ (method II). Potassium permanganate (Merck, Germany) $1.0 \times 10^{-2} \text{ mol L}^{-1}$ was prepared in deionized water.

2.3 Standard Sudan II Solutions

A $2 \times 10^{-3} \text{ mol L}^{-1}$ standard solution of Sudan II (Sigma, USA) was prepared by dissolving a suitable amount of solid in ethanol (method I) and in $1.0 \text{ mol L}^{-1} \text{NaOH}$ (method II). Working, standard solutions were obtained by stepwise dilution of the stock standard solution just prior to use.

2.4 Preparation of Real Samples

Chili powder catsup and tomato sauce samples were obtained from local markets of Assiut city, Egypt. According to the procedures of (Paleček et al. 1993), 2.0 g of sample was accurately weighed and added into 20 ml ethanol in 50 ml calibrated centrifuge tube. The resultant solution was kept in a thermostatic water bath at 50°C with stirring for 30 min separation of the solid and Sudan II rich phase (supernatant solution) was accomplished by centrifugation for 20 min at 2500 rpm. After filtration with a filter membrane, the extraction process for sediment phase was performed three times using three separate additions (10 mL each) of solvent. All the filtrate were collected in 50 ml volumetric flask and diluted to the mark with ethanol.

For determination of Sudan II in real samples, 0.2 mL extracted sample solution was used and analyzed according to our methods. Finally the Sudan II content of real food samples was determined using the regression equation and standard addition calibration procedure.

2.5 General Procedure

2.5.1 Method I (Complexation Reaction with Cu(II))

Into a 10 mL volumetric flasks, transfer a suitable aliquot of standard solution (in ethanol) containing up to 55.68 μg of Sudan II and 3.0 mL of $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{Cu(II)}$ solution. After mixing with appropriate amount of ethanol, the mixture was buffered to pH 9.0 with 1.0 mL of 0.05 mol L^{-1} borate buffer. Dilute the resulting solution to volume with deionized water and measure the absorbance at 364 nm by using 1.0 cm quartz cell against a similarly prepared blank of the same pH. The calibration graph was prepared by plotting absorbance vs. Sudan II concentration.

2.5.2 Method II (Oxidation with KMnO_4)

Solutions containing 1.65–27.634 $\mu\text{g mL}^{-1}$ Sudan II (in $1.0 \text{ mol L}^{-1} \text{NaOH}$) were transferred into individual 10 mL calibrated flasks. 2.5 mL of 1.0 mol L^{-1} sodium hydroxide solution was added followed by 3.0 mL of $5.0 \times 10^{-3} \text{ mol L}^{-1} \text{KMnO}_4$ solution and it was diluted to the final volume with deionized water. After 40 min, the absorbance was measured at 610 nm against reagent blank treated similarly.

2.6 Interference from Matrix

Samples were prepared by mixing 0.267 mg of Sudan II (method I) or 0.055 mg (in case of method II) with various amounts of common matrix cations, anions and dyes such as Sunset Yellow, Sudan Orange G or Sudan III. The procedure was continued as described under general procedures.

3 Results and Discussion

3.1 Acid–Base Equilibria of Sudan II

The solution of Sudan II in water/ EtOH, 50% (v/v) at $I = 0.1 \text{ mol L}^{-1}$ (NaClO_4) show four main absorption bands within the pH range 1.0–13.8. At pH 1.0–2.5 the absorption spectra of Sudan II exhibit a symmetrical intense band at 525 nm corresponding to absorption by cationic form (H_2^+L) of the compound. The double headed band appearing at 500 and 525 nm in the pH range 2.5–10.5 is presumably due to tautomericequilibria of the neutral molecule (Gilani et al. 2012). Accordingly lower band at 500 nm is corresponding to an intramolecular CT within the hydrazone form, whereas that at 525 nm is due to the CT transition within the azo form. The double headed band acquires a symmetrical nature with decreasing acidity of the medium at $\text{pH} > 10.5$. The symmetrical band at 500 nm with shoulder at 420 nm is due to absorption by monoanionic form of the compound. According to the results obtained in this work, the protonation process of Sudan II can be represented in Scheme I.

The absorbance versus pH graphs at selected wavelengths were interpreted (Kuban and Havel 1973) assuming that a particular equilibrium is established under selected conditions. Under our experimental conditions the calculated dissociation constants are $\text{pK}_{a1} [\text{H}_2^+\text{L} / \text{HL}] = 2.40$, $\text{pK}_{a2}, [\text{HL} / \text{L}^- (\text{OH})] = 11.40$ in 50% (v/v) ethanol / water at 25°C . Also, acidic constant (K_{a2}) of Sudan II was calculated using H-point standard addition method (HPSAM) (Kubista et al. 1993; Reig and Falcó 1988) along pH 10.0–13.0. The super acidic and basic spectra of Sudan II titration was used as HL and L^- species respectively. The best wavelength pair that gives the higher value in differences of slopes of calibration lines for pure standard ethanol / aqueous solution of Sudan II was selected. So, the wavelength pair 500 and 530 nm that gives greatest slope increment was selected for obtaining the highest accuracy. Concentration of L^- was obtained by using HPSAM equation (Kubista et al. 1993; Reig and Falcó 1988) and the calculated $\text{pK}_{a2} [\text{HL} / \text{L}^- (\text{OH})]$ is 11.68.

3.2 Absorption Spectra of Sudan II Reaction Products

Sudan dyes possess two reducible groups a nitrogen–nitrogen double bond and a phenol group. The latter is transformed into a phenate ion at a sufficiently high

pH and becomes reducible and thus could be oxidized by Ag^+ ions (Wu et al. 2006). A similar reduction process, which corresponds to a spontaneous–auto reduction of the Cu^{2+} into Cu^+ was also observed in the cases of complexation of Cu^{2+} with Sudan I (Huo et al. 2010) or Sudan II (Gürkan and Altunaye 2013).

On the basis of redox sensitivity in the Cu(II) – Sudan II interaction, the solution spectrum in water–ethanol (50% v/v) at $\text{pH} = 9.0$ shows two absorption bands with maximum at 364 and 576 nm corresponding to Cu(I)–L (MLCT) and Cu(II)–L (LMCT) complexes respectively (Fig. 2a). Under the same conditions the metal ion has no absorbance over the 250–700 nm, all measurements were performed against a reagent blank. Sudan II does not absorb at the given pH and wavelength range. Fig. 2b shows the spectrum obtained for KMnO_4 solution (maximum at 530 nm). With the addition of Sudan II dissolved in NaOH solution, absorption curve with two maxima at 430 and 610 nm was obtained. The latter band is attributed to the formation of manganate ions as a result of the oxidation of Sudan II with alkaline KMnO_4 (Hassan and Belal 2002; Saleh et al. 2015; Saleh et al. 2016).

3.3 Complexation Equilibria of Sudan II – Copper(II)

The complexation equilibria of Sudan II with Cu(II) was studied in water–ethanol (50% v/v) solution in the pH range 4.5–11 at $I = 0.1 \text{ mol L}^{-1}$ (NaClO_4), 25°C . The visible absorption spectra of the solution were recorded in equimolar solutions and in presence of excess metal ion. The absorption spectra of equimolar and of solutions with excess concentration of Cu(II) (Fig. 3a,b) at various pH values are analogous and exhibit two absorption bands with λ_{max} at 364 and 576 nm (minor in excess Cu(II)). The absorption band at 364 nm belongs to Cu(I) complexation with the oxidized hydrazone form while the other absorption band (576 nm) is resulted from complexation reaction of Cu(II) with the high conjugated azo form (Scheme II). Maximum color development is attained at pH 8.5–10 and pH 9.5–10.5 for Cu^+L and Cu^{2+}L^- complexes respectively. At higher pH values a decrease of absorbance is observed due to hydrolysis effect. The absorbance versus pH graphs at λ_{max} 364 and 576 nm were interpreted using the relationships reported by (Voznica et al. 1980), (Idriss et al. 1988; Idriss et al. 2000; Idriss and Saleh 1993) and (Saleh et al. 2015; Saleh et al. 2016).

The following equations (1,2) are valid for equimolar solutions and solutions with an excess of metal ion respectively.

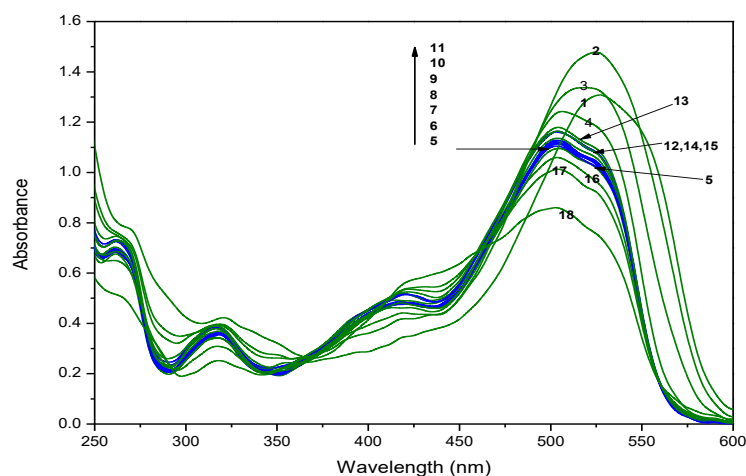
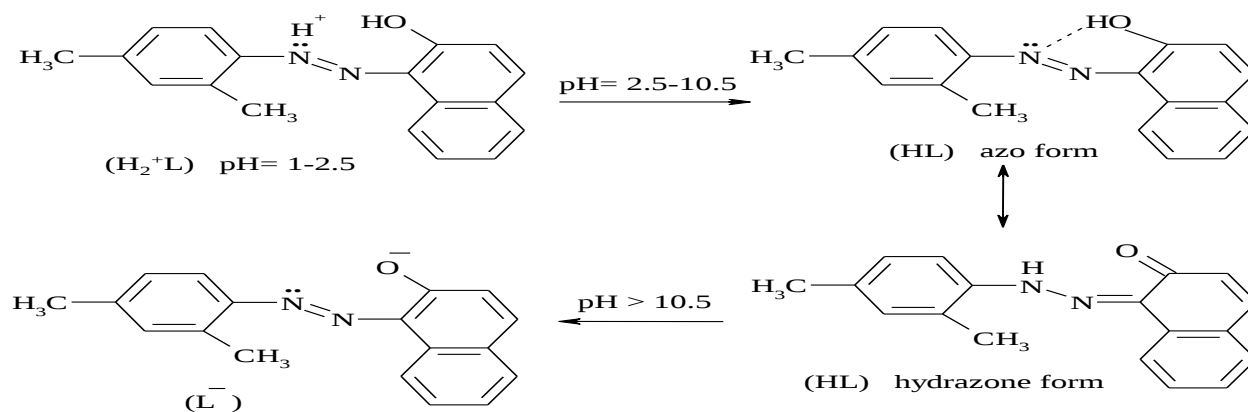


Fig.1: Absorption spectra of $5 \times 10^{-5} \text{ mol.L}^{-1}$ Sudan II in ethanol–water (50% v/v) at different pH values: 1, pH= 1.1; 2, pH= 1.38; 3, pH=1.46; 4, pH=1.57; 5, pH=2.76; 6, pH=3.9; 7, pH=5.6; 8, pH=7.27; 9, pH=8.58; 10, pH=9.50; 11, pH=10.23; 12, pH=11.0; 13, pH=11.73; 14, pH=12.80; 15, pH=13.20; 16, pH=13.75; 17, pH=13.85; 18, pH=13.95 .I=0.1mol.L⁻¹ NaClO₄ at 25°C.

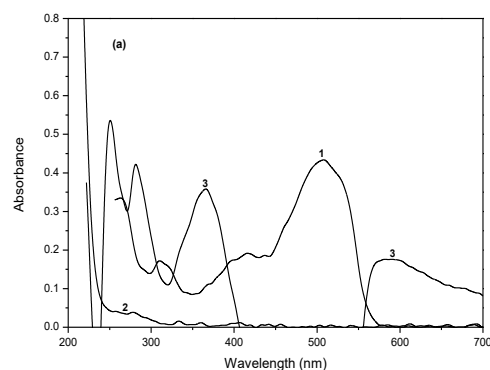


Fig.2-a) Absorption spectra of Sudan II-Copper in 50% (v/v ethanol-water, pH=8.57, I= 0.1mol.L⁻¹ NaClO₄). (1) Sudan II $2 \times 10^{-5} \text{ mol.L}^{-1}$; (2) Cu(II) $1 \times 10^{-4} \text{ mol.L}^{-1}$; (3) 1:1 Sudan II-Copper complex; [Sudan II]= [Cu(II)]= $1 \times 10^{-4} \text{ mol.L}^{-1}$.

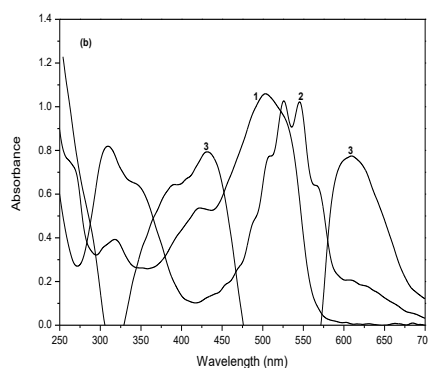
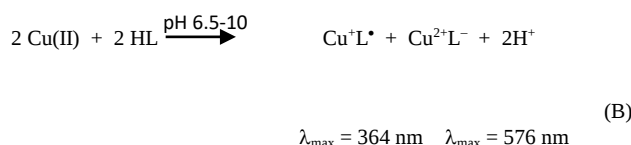
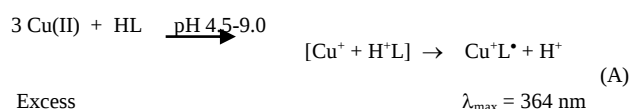


Fig.2-b) Absorption spectra of Sudan II-KMnO₄ reaction (in alkaline medium). (1) Sudan II= $5 \times 10^{-5} \text{ mol.L}^{-1}$ (pH= 13.75); (2) alkaline KMnO₄ ($5 \times 10^{-5} \text{ mol.L}^{-1}$); (3) Sudan II-KMnO₄ reaction products, [Sudan II]= $5 \times 10^{-5} \text{ mol.L}^{-1}$, [KMnO₄]= $1.5 \times 10^{-3} \text{ mol.L}^{-1}$, [NaOH]= 0.25 mol.L^{-1} .

$$\log \left[\frac{\Delta A}{(\varepsilon_1 C_L - \Delta A)^2} \right] = q \text{ pH} + \log \varepsilon + \log K_1^* \quad (1)$$

$$\log \left[\frac{\Delta A}{(\varepsilon_1 C_L - \Delta A)} \right] = q \text{ pH} + \log C_M + \log K_1^* \quad (2)$$

All symbols have their usual meanings. The logarithmic transformation are linear with a slope of $q=1$, indicating the release of one proton during complication and an intercept including K_{eq} . By considering the value of the dissociation constant of Sudan II under our experimental conditions, one can assume the following complication equilibrium with Cu(II) in the pH range 4.5–9.0 and 6.5–10 respectively.



The equilibrium constants $K_{1\text{Cu}^+ \text{L}^*}^*$ and $K_{1\text{Cu}^{2+} \text{L}^-}^*$ were determined by considering equilibrium A and B. The stability constant of Cu(I) or Cu(II) complexes is related to the equilibrium constant K^* by the expression $B = K_1^* / K_{a2}$. The calculated values of $\log K_1^*$ and $\log B$ for Cu(I) and Cu(II) complexes are (-1.5, 10.04) and (-3.68, 7.86) respectively. It is appeared that Cu(I)–complex is more stable than Cu(II)–complex at the same temperature (25°C). This order reflects the changes in the heat of complex formation and arises from a combination of the influence of both additional stability of d^{10} metal ion [Cu (I)] and expected back donation ability.

The proposed binding models of Sudan II–copper complexes are shown in Scheme II.

3.4 Optimization of Variables

3.4.1 Complexation Method

In order to optimize the conditions we have investigated a number of parameters such as pH, solvent, reagent concentration and time.

3.4.1.i Effect of Solvent

Since Sudan II is insoluble in water, different solvents such as ethanol, methanol, acetone, DMSO and DMF mixed with water (50% v/v) have been tried but the best results

were obtained with ethanol for copper–Sudan II complex.

3.4.1.ii Effect of pH

With other conditions fixed the effect of pH on absorbance of Cu(I)-L complex at $\lambda_{\text{max}} = 364 \text{ nm}$ was evaluated at pH values varying from 5.5–13.0. As can be seen from Fig. 3a, the quantitative determination of Sudan II was achieved at pH 9.0. Hence, pH 9.0 was selected as the optimal working value for further studies.

In order to determine the best buffer solution at pH 9.0, several buffer systems including $\text{HCO}_3^-/\text{CO}_3^{2-}$, $\text{NH}_3/\text{NH}_4\text{Cl}$ and boric acid, KCl, NaOH at isomolar concentrations of 0.05 mol L^{-1} were studied. The best analytical sensitivity was obtained in presence of borate buffer. Next, the effect of buffer volume on analytical sensitivity was also studied in the range 0.5–2.5 ml, and the maximum absorbance was obtained at a buffer volume of 1.0 mL. The results are shown in Fig. 4a.

3.4.1.iii Effect of Copper(II) Concentration

At optimum other conditions, effect of different volumes of Cu(II) ($2 \times 10^{-3} \text{ mol L}^{-1}$) on absorbance of Sudan II–copper complex was studied in the range (0.25–3 mL) at 25°C (Fig 4a). The maximum absorbance was observed when 1.0 mL of Cu(II) was used. To ensure the complete complexation for determination of Sudan II, 1.5 mL of Cu(II) was used.

3.4.2 Oxidation with KMnO_4 Method

Potassium permanganate, as a strong oxidizing agent, has been used in the oxidation–based analytical methods for the determination of many compounds (Hassan and Belal 2002). Sudan II was found to be susceptible for oxidation with alkaline KMnO_4 producing a green color peaking at 610 nm (Fig. 2b). At this wavelength, the various experimental parameters affecting the development and stability of the reaction product were carefully studied and optimized.

3.4.2.1 Effect of Time

During the current study the produced color intensity gradually increased with time to reach maximum after 40 min and was stable for at least 24 h.

3.4.2.2 Effect of Solvents

NaOH (1.0 mol L^{-1}) solution was used to dissolve Sudan II since KMnO_4 oxidizes other solvents with the production of green manganate.

3.4.2.3 The Effect of Potassium Permanganate

Concentration

Under optimum conditions, the reaction increases substantially with increasing the concentration of KMnO_4 in Sudan II solution (Fig. 4b). Maximum absorbance was obtained when 2.0 mL of $5.0 \times 10^{-3} \text{ mol L}^{-1}$ KMnO_4 was used. Thus, the adoption of 2.5 ml of potassium permanganate in the final solution proved to be adequate for the maximum concentration of Sudan II used in determination process.

3.4.2.4 Effect of Sodium Hydroxide Concentration

With other conditions fixed, maximum absorption was obtained when 2.0 mL of 1.0 mol L^{-1} NaOH was used. Over this volume no change in absorbance could be detected and 2.5 ml of 1.0 mol L^{-1} NaOH was used as an optimum value.

3.5 Stoichiometry of Sudan II Reactions

The stoichiometry of Sudan II reaction with Cu(II) or KMnO_4 was determined using Job's continuous variation (Saleh et al. 2015; Saleh et al. 2016; Job 1928). In solutions have $C_0 = C_R + C_{\text{Sudan II}} = 3.0 \times 10^{-4} \text{ mol L}^{-1}$ at pH = 9.0 or > 12.0. The plot of absorbance at λ_{max} 364 or 610 nm versus mole fraction of reagent Cu(II) or KMnO_4 respectively shows a maximum at 0.5 for copper suggesting the formation of 1:1 (Sudan : copper) complex, and a maximum at 0.66 revealing the interaction ratio 1:2 (Sudan II : KMnO_4).

3.6 Quantification

3.6.1 Validation of the Proposed Methods (I, II)

Standard calibration curves for Sudan II were determined under optimized conditions using Cu(II) (I) and KMnO_4 (II) methods. Beer's law for I and II methods was obeyed over the concentration ranges of 5.53–44.21 and 2.21–15.48 $\mu\text{g mL}^{-1}$ at λ_{max} 364 and 610 nm respectively. For both methods Sandell's sensitivity, regression equations and molar absorptivity are calculated. To validate the analytical procedures both the detection limit (DL) and the quantification limit (QL) were determined (Narade et al 2010). The parameters of the proposed methods are summarized in Table 1.

3.6.2 Accuracy and Precision

The accuracy and precision of the proposed spectrophotometric methods (I and II) were determined at three different concentration levels of Sudan II by analyzing five replicate samples of each concentration. The relative standard deviation (R.S.D) obtained for the analytical results did not exceed 2% (Table 2) which

proved a high reproducibility of the results and precision of the methods.

3.6.3 Intraday and Interday Precision and Accuracy

Under optimum conditions the intraday precision was carried out for our procedures through replicate analysis ($n = 5$) of Sudan II corresponding to 19.34, 23.49, 27.63 $\mu\text{g mL}^{-1}$ (2.76, 3.32, 3.87 $\mu\text{g mL}^{-1}$ for KMnO_4 method). The interday precision was also evaluated through replicate analysis of the pure sample for three consecutive days at the same concentration levels as in within day precision. The results of these assays are reported in Table 2. For intraday and interday precision, the recovery and RSD values were in the range of 81.07–102.17% and 0.61 to 1.99% respectively. The green manganate produced from Sudan II – KMnO_4 reaction was stable only for 24 hours.

3.6.4 Analytical Recovery and Matrix Effects

The effect of the matrix components of the real commercial food samples are a critical parameter in extraction studies. Therefore, under our proposed methods, the influence of various cations and anions on the recoveries was investigated. Tolerance limits were defined by the concentration of dyes, cations and anions which caused an < 5% error in the preconcentration and determination of Sudan II. The obtained mean recoveries and standard deviation ranged between 95.18–106.90 and ± 0.8 –2.5 respectively are shown in Table 3. These results proved the accuracy of the proposed methods and absence of interference from common matrix. The accuracy of our methods was also checked by studying the influence of various dyes on the recovery of Sudan II. Sudan II determination was possible in presence of few mg L^{-1} of Sunset Yellow, Sudan Orange G and Sudan III (Table 3). This was attributed to the great sensitivity of the methods that necessitated dilution for the food sample and consequently the matrix beyond their interference capability.

3.7 Applications

The complexation method was successfully applied for the determination of Sudan II in extracted chili powder, catsup and tomato sauce by applying standard addition method. The results are given in Tables 4 and 5. The recovery values for Sudan II were in the range 96.233–99.378%. The results were compared with reported methods (Qiao et al. 2011; López-Jiménez et al. 2010; Long et al. 2011) using student's t-test (to determine the accuracy) and F-test (to determine the precision). Tables 4 and 5 list the calculated t- and F-values at the 95% confidence level. The results for Sudan II contents in the investigated commercial food samples are given in Table 6.

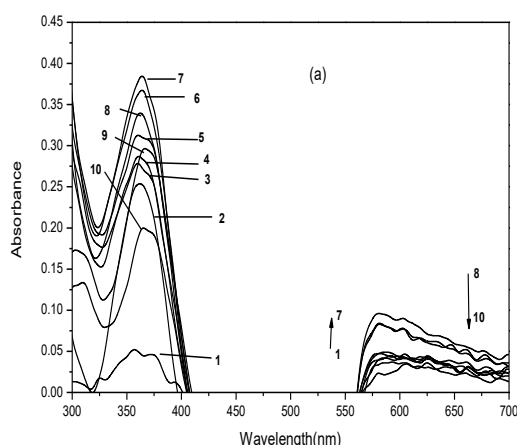


Fig.3-a: Absorption spectra of Sudan II-Copper complexes in 50% (v/v) ethanol, $[Cu^{+2}]=3 \times 10^{-4} \text{ mol.L}^{-1}$, $[Sudan II] = 1 \times 10^{-4} \text{ mol.L}^{-1}$ at different pH values, 1, pH=5.53; 2, pH=6.2; 3, pH=6.35; 4, pH=6.74; 5, pH=8.34; 6, pH=9.11; 7, pH=10.36; 8, pH=11.08; 9, pH=11.8; 10, pH=12.5

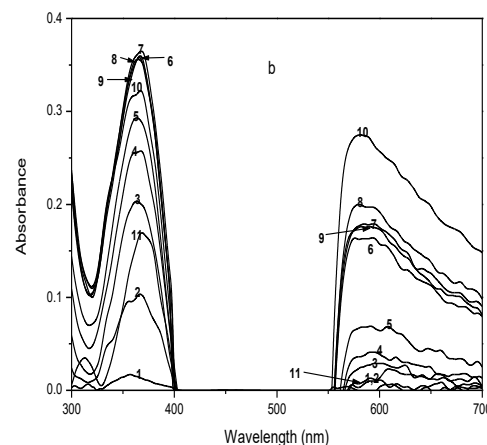


Fig.3-b: Absorption spectra of Sudan II-Copper complexes in 50% (v/v) ethanol - water, $[Cu^{+2}] = [Sudan II] = 1 \times 10^{-4} \text{ mol.L}^{-1}$ at different pH values, 1, pH=4.68; 2, pH=5.94; 3, pH=6.3; 4, pH=6.6; 5, pH=6.9; 6, pH=8.57; 7, pH=9.9; 8, pH=10.9; 9, pH=11.6; 10, pH=11.9; 11, pH=13.21

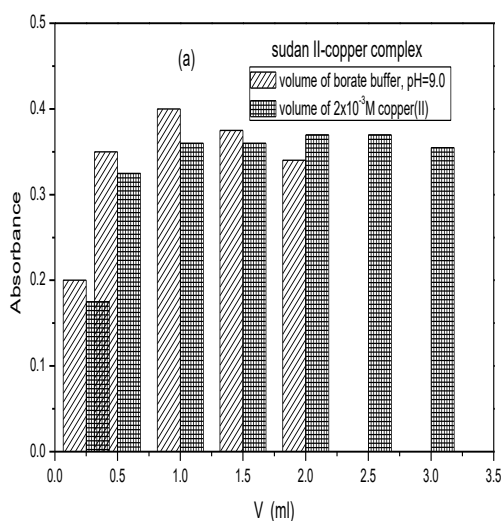


Fig.4-a (I) Effect of borate buffer volume (pH= 9.0) on absorbance of Sudan II-copper complex, $[Sudan II]=1 \times 10^{-4} \text{ mol.L}^{-1}$, $[Cu^{+2}]=3 \times 10^{-4} \text{ mol.L}^{-1}$, 50% (v/v) ethanol at 25°C. (II) Effect of copper(II) volume ($2 \times 10^{-3} \text{ mol.L}^{-1}$) on absorbance of Sudan II-copper complex, $[Sudan II]=1 \times 10^{-4} \text{ mol.L}^{-1}$, 50% (v/v) ethanol - water, pH= 9.0 (borate buffer) at 25°C.

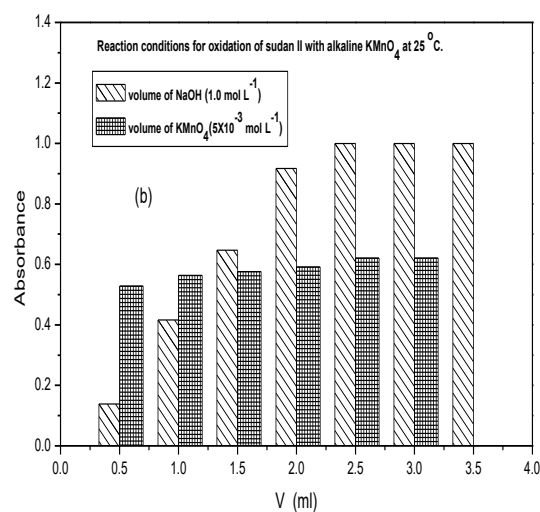


Fig.4-b (I) Effect of NaOH (1.0 mol.L^{-1}) (by volume) on absorbance of MnO_4^{2-} , $[Sudan II]=8 \times 10^{-5} \text{ mol.L}^{-1}$, $[KMnO_4]=1 \times 10^{-3} \text{ mol.L}^{-1}$, $\lambda_{max}=610 \text{ nm}$, $t=40 \text{ min}$. (II) Effect of $KMnO_4$ ($5 \times 10^{-3} \text{ mol.L}^{-1}$) by volume, $[Sudan II]=8 \times 10^{-5} \text{ mol.L}^{-1}$, $[NaOH] = 0.25 \text{ mol.L}^{-1}$, $\lambda_{max}=610 \text{ nm}$, $t=40 \text{ min}$.

Table 1: The analytical parameters of the proposed methods for determination of Sudan II dye.

Method / Reagent Parameter	Copper(II)	KMnO ₄
Colour	Yellow	Green
$\lambda_{\max}$ (nm)	364	610
Beer's law limits ($\mu\text{g.mL}^{-1}$)	5.53-44.21	2.21-15.48
Ringbom limits ($\mu\text{g.mL}^{-1}$)	6.31-39.81	3.98-13.49
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	3.75×10^3	1.17×10^4
Sandell's sensitivity ($\mu\text{g.cm}^{-2}$)	0.074	0.0236
Regression equation $A = a + bc$	$A = 0.01243c + 6.613 \times 10^{-3}$	$A = 0.0401c + 7.3096 \times 10^{-3}$
Slope (b)	0.01243	0.0401
Intercept (a)	6.613×10^{-3}	7.3096×10^{-3}
Correlation coefficient	0.9928	0.9985
Limit of detection (LOD) ($\mu\text{g.ml}^{-1}$)	0.4194	0.1300
Limit of quantification (LOQ) ($\mu\text{g.ml}^{-1}$)	1.2711	0.3940

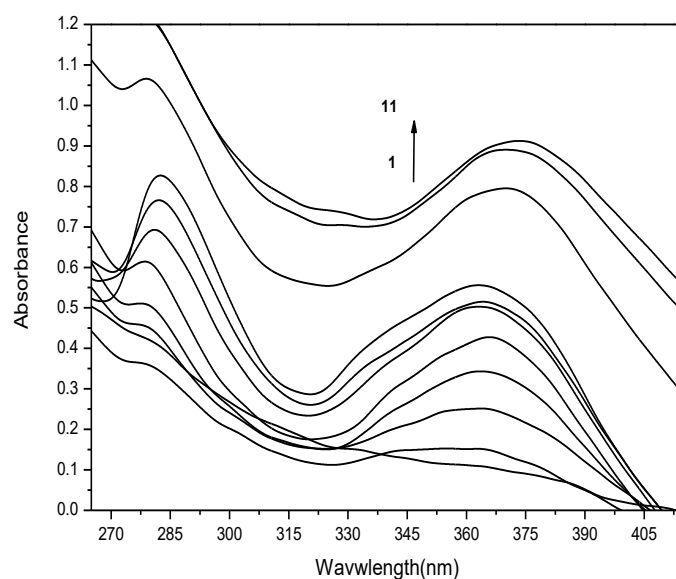
**Fig.5:** Absorption spectra of Sudan II-Copper complex in 50% (v/v) ethanol-water, pH=9.0 (borate buffer), Sudan II concentration range $3.0 \times 10^{-5} \text{ mol.L}^{-1}$ (I) to $2.0 \times 10^{-4} \text{ mol.L}^{-1}$ (II) with regular successive additions in presence of $3 \times 10^{-4} \text{ mol.L}^{-1}$ Cu(II) at 25°C.

Table 2: Inter- and Intra-day accuracy and precision of the proposed methods for determination of Sudan II in pure form.

Method / Reagent	Amount $\mu\text{g.ml}^{-1}$		RSD %	Recovery %	C.L ^b
	Taken	Found $\pm\text{SD}^a$			
Cu(II) Intraday assay	19.34	19.66 ± 0.12	0.610	101.65	± 0.149
	23.49	21.35 ± 0.17	0.796	90.89	± 0.211
	27.63	23.04 ± 0.21	0.911	83.39	± 0.261
Interday assay	19.34	19.42 ± 0.19	0.978	100.41	± 0.236
	23.49	20.91 ± 0.24	1.148	89.02	± 0.298
	27.63	22.40 ± 0.22	0.982	81.07	± 0.273
KMnO ₄ Intraday assay	2.76	2.82 ± 0.05	1.773	102.17	± 0.062
	3.32	3.19 ± 0.06	1.881	96.08	± 0.074
	3.87	3.51 ± 0.07	1.990	90.70	± 0.087
Interday assay	2.76	2.64 ± 0.03	1.136	95.65	± 0.037
	3.32	3.09 ± 0.06	1.807	93.07	± 0.074
	3.87	3.54 ± 0.05	1.412	91.47	± 0.062

(a) Mean for 3 independent analysis.

(b) C.L., confidence limit at 95% confidence level and 4 degree of freedom ($t = 2.776$).**Table 3:** Matrix effects on the recovery of Sudan II.

Matrix components	Complexation method		Oxidation method	
	Concentration mg.L^{-1}	Recovery %	Concentration mg.L^{-1}	Recovery %
1) Na ⁺ , K ⁺ , Mg ²⁺ , Ca ²⁺ , Cl ⁻ , SO ₄ ²⁺ , PO ₄ ³⁻	2500	99.1 ± 1.0	2300	98.2 ± 1.5
2) Fe ³⁺ , Zn ²⁺ , Ni ²⁺ , Cr ³⁺ , Cd ²⁺	150	96.16 ± 2.4	100	95.18 ± 2.5
3) Sudan orange G	5.3	105.5 ± 1.2	0.4	106.7 ± 1.6
4) Sudan III	5.0	101.85 ± 1.0	0.44	102.45 ± 1.7
5) SunsetYellow	4.52	104.69 ± 0.8	0.34	106.9 ± 1.0

Table 4: Statistical analysis of results obtained by the complexation method for Sudan II in chili powder samples.

Comercial simple ^a	Present $\mu\text{g.ml}^{-1}$	Added $\mu\text{g.ml}^{-1}$	Found $\mu\text{g.ml}^{-1}$	Recovery %	Mean		t-value ^b	F-value ^b	Reportedmethod(López-Jiménez et al. 2010)	
					Recovery %	$\pm\text{RSD}$ %			Recovery %	$\pm\text{RSD}$ %
Chili powder	0.552	5.526	5.941	97.745	99.378	± 3.2	1.802	1.485	96.8	3.9
		8.290	8.981	101.561						
		11.052	11.468	98.83						
Red peper powder	0.414	5.526	5.803	97.694	98.96	± 2.9	1.669	1.81		
		8.290	8.843	101.59						
		11.052	11.192	97.61						
Páprika powder ^c	0.552	5.526	5.94	97.75	97.843	± 2.4	0.973	2.641		
		8.290	9.119	96.96						
		11.052	11.468	98.82						

(a) From local markets of Assiut city, Egypt.

(b) Theoretical t-value ($\nu = 4$) and F-value ($\nu = 4, 4$) at 95% confidence level are 2.78 and 6.39.

(c) Made in Lebanon.

Table 5: Statistical analysis of results obtained by the complexation method for Sudan II in catsup and tomato sauce samples.

Comercial sample ^a	Present $\mu\text{g.ml}^{-1}$	Added $\mu\text{g.ml}^{-1}$	Found $\mu\text{g.ml}^{-1}$	Recovery %	Mean		t-value ^b	F-value ^b	Reportedmethod(Qiao et al. 2011; Long et al. 2011)	
					Recovery %	$\pm\text{RSD}$ %			Recovery %	$\pm\text{RSD}$ %
Hot cátsup	0.414	2.763	3.04	95.688	96.996	± 2.8	1.437	1.94	95.2	3.9
		5.526	5.803	97.693						
		11.052	11.192	97.61						
Hot sauce	0.552	2.763	3.178	95.587	98.139	± 3.6	1.829	1.174		
		5.526	6.079	100.00						
		11.052	11.468	98.83						
Extra hotsauce ^c	0.552	2.763	3.178	95.587	96.233	± 2.3	1.006	2.87		
		5.526	5.803	95.475						
		11.052	11.33	97.638						
Tomito cátsup	0.552	2.763	3.316	100.00	98.85	± 3.7	2.21	1.111		
		5.526	5.94	97.73						
		11.052	11.468	98.83						
Tomito paste (1)	0.4698	2.763	3.178	98.298	98.57	± 3.9	1.476	1.78	96.0	5.2
		5.526	5.941	99.08						
		11.052	11.33	98.33						
Tomito paste (2)	0.552	2.763	3.178	95.87	97.08	± 2.7	0.896	3.7		
		5.526	5.941	97.746						
		11.052	11.33	97.638						

(a) From local markets of Assiut city, Egypt.

(b) Theoretical t-value ($\nu = 4$) and F-value ($\nu = 4, 4$) at 95% confidence level are 2.78 and 6.39.

(c) Made in Saudi Arabia.

Table 6: Sudan II content in chili powder, catsup and tomato sauce samples.

Samples	Chili powder	Red pepper powder	Paprika powder	Hot catsup	Hot sauce	Extra hot sauce	Tomito catsup	Tomito paste (1)	Tomato paste (2)
Sudan II $\mu\text{g.ml}^{-1}$	690 $\pm 15^a$	517.5 ± 9	690 ± 14	517.5 ± 8	690 ± 15	690 ± 16	690 ± 14	690 ± 14	587.25 ± 12

(a) Mean $\pm$ standard deviation.

4 Conclusions

The proposed methods are accurate, time saving and have the advantages of simplicity, sensitivity and reproducibility. Method I is based on Cu(II)–Sudan II redox reaction followed by complexation to form Cu(I)-L complex. While method II is based on oxidation of Sudan II with alkaline KMnO_4 to produce green manganate species under optimum conditions. From method I, it is found that the stability constant of Cu(I) complex formation is higher than that of Cu(II). This order reflects the changes in the heat of complex formation from a combination of the influence of both additional stability of d^{10} copper ion and expected back donation ability. Student t- and F-values gave lower values than theoretical ones indicating high accuracy and precision compared to the reported one. Complexation method is sensitive enough to determine trace amounts of Sudan II in extracted chili powder catsup and tomato sauce using standard addition method.

Compliance with Ethical Standards

Conflict of Interest. Elham Y. Hashem has no conflict of interest. Najat O. A. Al-Salahi has no conflict of interest. This article does not contain any studies with human or animal subjects.

Informed Consent. Informed consent was obtained from all individual participants included in the study .

References

- [1] Calbiani, F, Careri, M, Elviri, L, Mangia, A, Zagnoni, I (2004) Accurate mass measurements for the confirmation of Sudan azo-dyes in hot chili products by capillary liquid chromatography–electrospray tandem quadrupole orthogonal- acceleration time of flight mass spectrometry. *Journal of Chromatography A.*, **1058**(1), 127-135(2004).
- [2] Chailapakul, O, Wonsawat, W, Siangproh, W, Grudpan, K, Zhao, Y, Zhu, Z (2008) Analysis of Sudan I, Sudan II, Sudan III, and Sudan IV in food by HPLC with electrochemical detection: Comparison of glassy gel modified electrode. *Food Chemistry.*, **109**(4), 876- 882(2008).
- [3] Di Anibal, C V, Marsal, L F, Callao, M P, Ruisánchez, I (2012) Surface Enhanced Raman Spectroscopy (SERS) and multivariate analysis as a screening tool for detecting Sudan I dye in culinary spices. *SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy.*, **87**, 135-141.
- [4] Di Anibal, C V, Odena, M, Ruisánchez, I, Callao, M P (2009) Determining the adulteration of spices with Sudan I-II-III-IV dyes by UV–visible spectroscopy and multivariate classification techniques. *Talanta.*, **79**(3), 887-892(2009).
- [5] Douheret, G (1967) Comparison between glass electrode with hydroorganic and aqueous fillings. *Bull. Soc. Chim. Fr.*, **1412**, 1419(1967).
- [6] Enríquez-Gabeiras, L, Gallego, A, Garcinuño, R M, Fernández-Hernando, P, Durand, J S (2012). Interference-free determination of illegal dyes in sauces and condiments by matrix solid phase dispersion (MSPD) and liquid chromatography (HPLC–DAD). *Food Chemistry*, **135**(1), 193-198(2012).
- [7] Ensafi, A A, Rezaei, B, Amini, M, Heydari-Bafrooei, E (2012) A novel sensitive DNA–biosensor for detection of a carcinogen, Sudan II, using electrochemically treated pencil graphite electrode by voltammetric methods. *Talanta.*, **88**, 244-251(2012).
- [8] Ertaş, E, Özer, H, Alasalvar, C (2007) A rapid HPLC method for determination of Sudan dyes and Para Red in red chili pepper. *Food Chemistry.*, **105**(2), 756-760(2007).
- [9] Fukuji, T S, Castro-Puyana, M, Tavares, M F, Cifuentes, A (2011) Fast determination of Sudan dyes in chili tomato sauces using partial filling micellarelectrokinetic chromatography. *Journal of agricultural and food chemistry.*, **59**(22), 11903-11909(2011)
- [10] Gilani, A G , Moradi, E, Binay, S, Moghadam, M (2012) Tautomeric behavior of some azoquinoline dyes

- in liquid and liquid crystalline media. *SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy.*, **87**, 112-118(2012).
- [11] Gürkan, R, Altunay, N (2013) A reliable method of quantification of trace copper in beverages with and without alcohol by spectrophotometry after cloud point extraction. *Química Nova.*, **36(8)**, 1146-1154(2013).
- [12] Hassan, E M, Belal, F (2002) Kinetic spectrophotometric determination of nizatidine and ranitidine in pharmaceutical preparations. *Journal of pharmaceutical and biomedical analysis.*, **27(1)**, 31-38(2002).
- [13] He, L, Su, Y, Fang, B, Shen, X, Zeng, Z, Liu, Y (2007) Determination of Sudan dye residues in eggs by liquid chromatography and gas chromatography–mass spectrometry. *AnalyticaChimicaActa.*, **594(1)**, 139-146(2007).
- [14] Huang, S T, Yang, L F, Li, N B, Luo, H Q (2013) An ultrasensitive and selective fluorescence assay for Sudan I and III against the influence of Sudan II and IV. *Biosensors and Bioelectronics.*, **42**, 136-140(2013).
- [15] Huo, J, Guo, Y, Meng, S, Wang, M, Wang, Y (2010) Complex Formation of Sudan I with Cu (II) and Its Identification from Chili Species. In *Bioinformatics and Biomedical Engineering (iCBBE)*, 2010 4th International Conference on (pp. 1-4). IEEE.
- [16] Idriss, K A, Hashem, E Y, Abdel-Aziz, M S, Ahmed, H M (2000) Direct spectrophotometric determination of aluminium oxide in Portland cement and cement clinker. An insight into the solution equilibria and analytical aspects of the aluminium–quinizarin system. *Analys.t*, **125(1)**, 221-225(2000).
- [17] Idriss, K A, Seleim, M M, Saleh, M S, Abu-Bakr, M S, Sedaira, H (1988) Spectrophotometric study of the complexationequilibria of zirconium (IV) with 1-amino-4-hydroxyanthraquinone and the determination of zirconium. *Analyst.*, **113(11)**, 1643-1647(1988).
- [18] Idriss, K A, Saleh, M M (1993) Acid–base characteristics of naphthazarin and solution equilibria of yttrium (III) chelates. *Analyst.*, **118(2)**, 223-227(1993).
- [19] Job, P (1928) Formation and stability of inorganic complexes in solution. *Ann. Chem. (Paris).*, **9**, 113-203(1928).
- [20] Kuban, V, Havel, J (1973) Some 2-(2-Thiazolylazo)-4-Methoxyphenol (Tamp) Complex Equilibria. 1. Acid-Base Properties of Tamp In Water And In Various Mixed Solvents. *ActaChemicaScandinavica.*, **27(2)**, 528-540(1973).
- [21] Kubista, M, Sjoeback, R, Albinsson, B (1993) Determination of equilibrium constants by chemometric analysis of spectroscopic data. *Analytical Chemistry.*, **65(8)**, 994-998(1993).
- [22] Li, J., Ding, X M, Liu, D D, Guo, F, Chen, Y, Zhang, Y B, Liu, H M (2013) Simultaneous determination of eight illegal dyes in chili products by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B.*, **942**, 46-52(2013).
- [23] Liu, W, Zhao, W J, Chen, J B, Yang, M M (2007) A cloud point extraction approach using Triton X-100 for the separation and preconcentration of Sudan dyes in chili powder. *Analyticachimicaacta.*, **605(1)**, 41-45(2007).
- [24] Long, C, Mai, Z, Yang, X, Zhu, B, Xu, X, Huang, X, Zou, X (2011) A new liquid–liquid extraction method for determination of 6 azo-dyes in chili products by high-performance liquid chromatography. *Food Chemistry.*, **126(3)**, 1324-1329(2011).
- [25] López-Jiménez, F J, Rubio, S, Pérez-Bendito, D (2010) Supramolecular solvent-based microextraction of Sudan dyes in chili-containing foodstuffs prior to their liquid chromatography-photodiode array determination. *Food chemistry.*, **121(3)**, 763-769(2010).
- [26] Mejia, E, Ding, Y, Mora, M F, Garcia, C D (2007) Determination of banned Sudan dyes in chili powder by capillary electrophoresis. *Food Chemistry.*, **102(4)**, 1027-1033(2007).
- [27] Mustafa, S O, Khan, R A, Sultana, I, Nasir, N, Tariq, M (2013) Estimation of Para Red Dye in Chili Powder and Tomato Sauces by a Simple Spectrophotometric Method followed by Thin layer Chromatography. *Journal of Applied Sciences and Environmental Management.*, **17(2)**, 177-184(2013).
- [28] Mustafa, S, Nasir, N, Mahmood, T, Mahmood, I, Khan, R A, Khaliq, S (2013) Spectrophotometric Determination of Sudan I-IV Dyes in Selected Chili Samples: A Survey of Karachi City, Pakistan. *J. European Academic Research.*, **1(6)**, 1202-1215(2013).
- [29] Narade, S, Patil, S, Surve, S, Shete, D, Pore, Y (2010) Simultaneous UV spectrophotometric method for the determination of diacerein and aceclofenac in tablets. *Journal of Pharmaceutical Science and Research.*, **2**, 137-142(2010).
- [30] Paleček, E, Jelen, F, Teijeiro, C, Fučík, V, Jovin, T M (1993) Biopolymer-modified electrodes in the voltammetric determination of nucleic acids and proteins at the submicrogram level. *Analyticachimicaacta.*, **273(1)**, 175-186(1993).
- [31] Pourreza, N, Rastegarzadeh, S, Larki, A (2011) Determination of Allura red in food samples after cloud point extraction using mixed micelles. *Food chemistry.*, **126(3)**, 1465-1469(2011).
- [32] Qi, P, Zeng, T, Wen, Z, Liang, X, Zhang, X (2011)

- Interference-free simultaneous determination of Sudan dyes in chili foods using solid phase extraction coupled with HPLC–DAD. *Food Chemistry.*, **125(4)**, 1462-1467(2011).
- [33] Qiao, F, Geng, Y, He, C, Wu, Y, Pan, P (2011) Molecularly imprinted microspheres as SPE sorbent for selective extraction of four Sudan dyes in catsup products. *Journal of Chromatography B.*, **879(27)**, 2891-2896(2011).
- [34] Qiao, J, Yan, H, Wang, H, Wu, Y, Pan, P, Geng, Y (2011) Simultaneous determination of four Sudan dyes in egg yolks by molecularly imprinted SPE coupled with LC-UV detection. *Chromatographia.*, **73(3-4)**, 227-233(2011).
- [35] Rebane, R, Leito, I, Yurchenko, S, Herodes, K (2010) A review of analytical techniques for determination of Sudan I–IV dyes in food matrixes. *Journal of Chromatography A.*, **1217(17)**, 2747-2757(2010).
- [36] Reig, F B, Falcó, P C (1988) H-point standard additions method. Part 1. Fundamentals and application to analytical spectroscopy. *Analyst.*, **113(7)**, 1011-1016(1988)
- [37] Saleh, M M, Hashem, E Y, Al-Salahi, N O (2016) Oxidation and Complexation-Based Spectrophotometric Methods for Sensitive Determination of Tartrazine E102 in Some Commercial Food Samples. *Computational Chemistry.*, **4(02)**, 51-64(2016).
- [38] Saleh, M M, Hashem, E Y, Youssef, A K, Abdel-Kader, D A (2015) UV-Visible Spectrophotometric Methods for Direct Determination of Sulfasalazine Antibiotic Drug in Its Pharmaceutical Formulations. *World Journal of Pharmacy and Pharmaceutical Sciences.*, **4**, 205-226(2015).
- [39] Soylak, M, Elci, L, Dogan, M (1997) Determination of trace amounts of cobalt in natural water samples as 4-(2-thiazolylazo) resorcinol complex after adsorptive preconcentration. *Anal. Lett.*, **30(3)**, 623-631(1997)
- [40] Soylak, M, Şahin, U, Elçi, L (1996) Spectrophotometric determination of molybdenum in steel samples utilizing selective sorbent extraction on Amberlite XAD-8 resin. *Analytica Chimica Acta.*, **322(1)**, 111-115(1996).
- [41] Soylak, M, Unsal, Y E, Yilmaz, E, Tuzen, M (2011) Determination of rhodamine B in soft drink, waste water and lipstick samples after solid phase extraction. *Food and chemical toxicology.*, **49(8)**, 1796-1799(2011).
- [42] Sun, H W, Wang, F C, Ai, L F (2007) Determination of banned 10 azo-dyes in hot chili products by gel permeation chromatography–liquid chromatography–electrospray ionization–tandem mass spectrometry. *Journal of Chromatography A.*, **1164(1)**, 120-128(2007).
- [43] Vogel, A I (1973) Text-book of quantitative inorganic analysis, including elementary instrumental analysis. Third Ed. Longman, London
- [44] Voznica, P, Havel, J, Sommer, L (1980) The reactions of gallium, indium and thallium with 2-(2-pyridylazo)-1-naphthol-4-sulphonic acid and their spectrophotometric determination. *Collection of Czechoslovak Chemical Communications.*, **45(1)**, 54-79(1980).
- [45] Wu, L P, Li, Y F, Huang, C Z, Zhang, Q (2006) Visual detection of Sudan dyes based on the plasmon resonance light scattering signals of silver nanoparticles. *Analytical chemistry.*, **78(15)**, 5570-5577(2006).
- [46] Zhang, Z, Xu, S, Li, J, Xiong, H, Peng, H, Chen, L (2012) Selective solid-phase extraction of Sudan I in chili sauce by single-hole hollow molecularly imprinted polymers. *Journal of agricultural and food chemistry.*, **60(1)**, 180-187(2012).