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Utility of Spectrophotometry for Novel Quantitation of Sudan Orange G in some Commercial Food Products

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Abstract: A simple solvent extraction procedure for separation enrichment of Sudan Orange G 1-phenylazobenzene 2,4-ol from commercial food samples (chili powder, red pepper powder, paprika powder, hot catsup, hot sauce, extra hot sauce, tomato catsup, tomato paste and pasta sauce) is presented. Two highly simple and sensitive spectrophotometric methods were developed to quantitate Sudan Orange G in pure and in some commercial food samples. The two methods are based on Sudan Orange G redox reaction with copper followed by complex formation (method I) as well as oxidation – reduction reaction with alkaline $KMnO_4$ (method II). These reactions were monitored spectrophotometrically at maximum absorbance's 346 and 610 nm for I and II methods 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^*, β) of Sudan Orange G copper complexes formation are calculated. Under optimize experimental conditions, Beer's law is obeyed in the concentration range 2.14 – 34.27, 0.64 – 7.07 μg ml^{-1} for copper and $KMnO_4$ reagents respectively. The apparent molar absorptivity, Sandell`s sensitivity, detection and quantification limits are calculated. Matrix effects were also investigated. The procedures were successfully applied for the determination of trace levels of Sudan Orange G in the selected commercially food samples by using multiple points standard addition method. Statistical comparison of the results are in good agreement with the reported methods.

Keywords: Sudan Orange G, Complexation, $KMnO_4$, Solvent extraction, Commercial food products, Spectrophotometric analysis.

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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 [1-4]. Sudan dyes are thought to be carcinogenic yet have been determined as a contaminant in chili powder [5,6]. 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 [7]. Sudan dyes are of interest because of their chromophoric nature and the bidentate character of their orthophenolic hydroxy group. These properties have made azo dyes useful for metal complexation [8]. A variety of analytical methods have been proposed to detect the presence of Sudan dyes in food stuffs as recently reviewed **[2]**. The most popular analytical methods are based on high–performance liquid chromatographic method associated with different detectors [9-13]. Electrochromatography has been used to analyze Sudan dyes in foods [14-16]. In addition, many other analytical methods such as fluorescence [17], electro–analytical technique[18,19] and Raman spectroscopy [20] 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. Although UV and Vis. spectrophotometric methods are simple and inexpensive, available literature is very scanty dealing with measurement of Sudan dyes. In besides that there has been no report of spectrophotometric determination of Sudan Orange G depending on complexation reaction with copper or oxidation with

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alkaline $KMnO_4$. In Our research work, Sudan Orange G were extracted from samples commercial food products by ethyl alcohol [21] and were estimated by two simple and sensitive spectrophotometric methods. The first method is based on Sudan Orange G redox reaction with copper (II) followed by complex formation at pH = 6.1. The second method is based on oxidation of Sudan Orange G 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 (40% v/v) at 25°C. pH values of partially aqueous were corrected as described by Douheret [22].

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 $1.0x10^{-3}$ mol l^{-1} stock solution of copper nitrate was prepared by dissolving the required amount of the Analar product in deionized water and standardized compleximetrically with EDTA [23]. The ionic strength of solutions was maintained at a constant value of I= 0.1 mol l^{-1} ($NaClO_4$). A borate buffer solution (pH= 6.1) was prepared in 100 ml volumetric flask. All measurements were made in 50% (v/v) ethanol–water medium (method I) and in 1.0 mol l^{-1} NaOH (method II). Potassium permanganate (Merck, Germany) $1.0x10^{-2}$ mol l^{-1} was prepared in deionized water.

2.3 Standard Sudan Orange G Solutions

A $1x10^{-3}$ mol l^{-1} standard solution of Sudan Orange G (Sigma, USA) was prepared by dissolving a suitable amount of solid in ethanol (method I) and in 1.0 mol l^{-1} 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

The real samples (chili powder, red pepper powder, paprika powder, hot catsup, hot sauce, extra hot sauce, tomato catsup, tomato paste and pasta sauce) were bought from local supermarket in Assiut City (Egypt). Each sample were weighed exactly 2.0 gm 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 at 2500 rpm. After filtration with a filter membrane, the extraction process for sediment phase was performed three times using three separate addition (10 ml each) of solvent. All the filtrate was collected in 50 ml volumetric flask and diluted to the mark with ethanol.

For determination of Sudan Orange G in real samples, 0.2 ml extracted sample solution was used and analyzed according to our methods. Finally, the Sudan Orange G content of real food samples were 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 49.271 µg of Sudan Orange G and 3.0 ml of $1.0x10^{-3}$ mol l^{-1} Cu (II) solution. After mixing with appropriate amount of ethanol, the mixture was buffered to pH 6.1 with 0.5 ml of borate buffer. Dilute the resulting solution to volume with deionized water and measure the absorbance at 346 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. SOG concentration.

2.5.2 Method II (Oxidation with KMnO4)

Solutions containing 0.643−19.280 µg ml^{-1} Sudan Orange G (in 1.0 mol l^{-1} NaOH) were transferred into individual 10 ml calibrated flasks. 3 ml of 1.0 mol l^{-1} sodium hydroxide solution was added followed by 3.0 ml of $5.0x10^{-3}$ mol l^{-1} $KMnO_4$ solution and it was diluted to the final volume with deionized water. After 30 min, the absorbance was measured at 610 nm against reagent blank treated similarly.

2.6 Interference from Matrix

Samples were prepared by mixing 0.21 mg of Sudan Orange G (method I) or 0.045 mg (in case of method II) with various amounts of common matrix cations, anions and Sunset Yellow, Tartrazine, Tropaeolin 000, Sudan III dyes. The procedure was continued as described under general procedure.

3 Results and Discussion

3.1 Acid–Base Equilibria of Sudan Orange G`

The solution of Sudan Orange G dye has been studied as a function of pH. The main objectives have been to study the pH-dependence of the spectral changes and to obtain a desirable level of control over the various acid-base equilibria existing in solution. The spectral changes are explained in terms of shifts equilibria amongst different molecular and ionic species existing in solution. The pK_a values corresponding to the different ionization steps have been calculated by treating the non-linear dependences A=f (pH*). The absorption spectra of 2.5×10^{-5} mol l^{-1} of the Sudan Orange G dye in 40% ethanol, I= 0.1 mol l^{-1} ($NaClO_4$) at various concentrations of $HClO_4$ or NaOH are recorded as the dependence A=f (λ) for various pH Fig. 1.

The UV - Visible spectra of this dye (Sudan Orange G) in all mixtures investigated display four absorption bands within the pH range 0.93-13.17. The maximum absorption of these bands is located at 382, 420, 432 and 474 nm. The band at 382 nm corresponds to absorption by the cationic

molecule [24] Scheme I. The band at 432 and 474 nm are apparently due to absorption by the form mono-anionic (HL^-) species and the di-anionic (L^{2-}) species respectively for SOG dye.

According to our results, the acid-base equilibria existing in Sudan Orange G solution within the pH range (0.93 – 13.17) can be represented as:

$$H_3L^+ \rightleftharpoons H_2L \rightleftharpoons HL^- \rightleftharpoons L^{-2}$$

H_3L^+	H_2L	HL^-	L^{-2}
Momocationic from	Neutral form	Monocationic from	Dianionic form
pH (0.93-3.63)	pH (4.04-9.01)	pH (9.06-11.35)	pH (13.63-13.17)

The absorbance versus pH graphs at selected wavelengths were interpreted [25] assuming that a particular equilibrium is established under selected conditions. Under our experimental conditions the calculated dissociation constants are pK_b [H_3^+L / H_2L] = 3.1, pK_{a1}[H_2L /HL^-] = 7.2

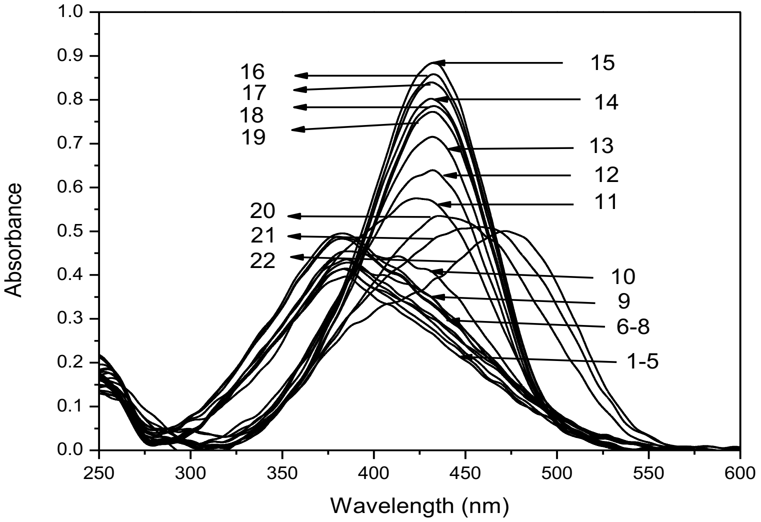


Fig. 1: Absorption spectra of 2.5×10^{-5}mol l^{-1} Sudan Orang G in 40% (v/v) ethanol-water, I= 0.1 mol l^1 $NaClO_4$, 25°C at different pH values; 1) pH= 0.93; 2) pH= 1.12; 3) pH= 1.53; 4) pH= 2.34; 5) pH=3.13; 6) pH=3.37; 7) pH= 3.63; 8) pH= 4.04; 9) pH= 5.20; 10) pH= 6.09; 11) pH= 6.68; 12) pH= 7.23; 13) pH= 7.63; 14) pH= 8.42; 15) pH= 9.01; 16) pH= 9.06; 17) pH= 10.43; 18) pH= 10.86; 19) pH=11.35; 20) pH= 12.63; 21) pH= 13.05; 22) pH= 13.17.

2.3 Absorption Spectra of Sudan Orange G Reaction Products

Sudan dyes possess two reducible groups a –N=N–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[26]. A similar reduction process, which corresponds to a spontaneous–auto reduction of the Cu^{2+} into Cu^+ was

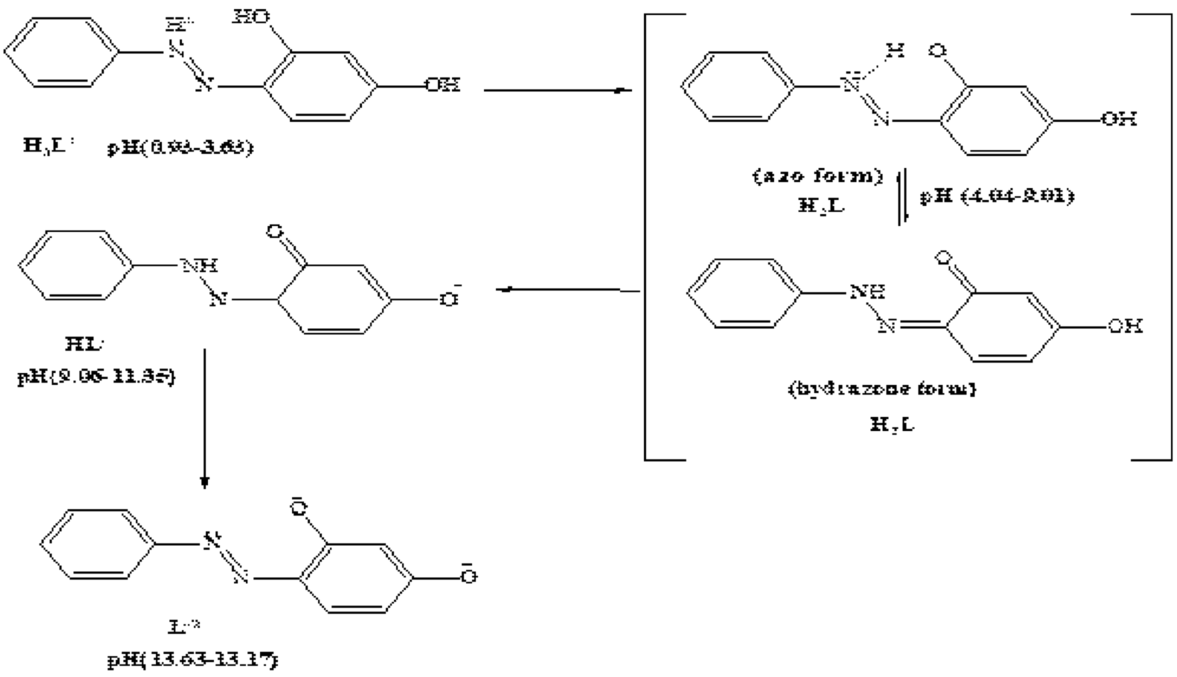
from (H_3L^+) of the compound and disappears at pH > 3.63. The symmetrical bands at 420-432 nm corresponds to the absorption the neutral from (H_2L) of the reagent, and also its presumably due to tautomeric equilibria of the neutral

and pK_{a2} [HL^- /L^{2-}] = 11.40 in 40% (v/v) ethanol / water at 25°C.

also observed in the cases of complexation of Cu^{2+} with Sudan I [27] or Sudan Orange G [8].

The complexation reaction between Cu(II) – SOG dye was studied in 40% (v/v) ethanol / water medium at I = 0.1 mol l^{-1} ($NaClO_4$) , pH = 6.1at 25°C.

On the basis of redox sensitivity in the Cu(II)–SOG interaction, the solution spectrum shows two absorption bands with maximum at 346 and 506 nm corresponding to Cu(I)–L (MLCT) and Cu(II)–L (LMCT) complexes



Scheme I: Different Species of Sudan Orange G in 40% (v/v) Ethanol.

respectively Fig. 2-a. Under the same conditions the metal ion has no absorbance over the 200–600 nm, all measurements were performed against a reagent blank. Sudan Orange G does not absorb at the given pH and wavelength range. Fig. 2-b 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 Orange G with alkaline $KMnO_4$ [28-30].

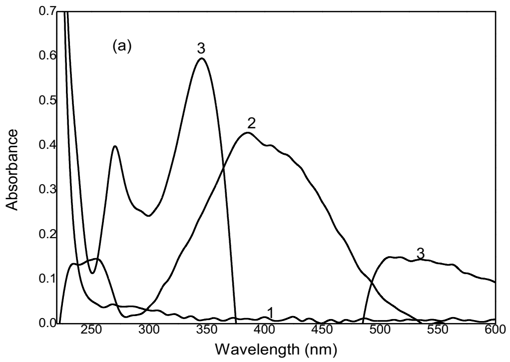


Fig. 2-a: Absorption spectra of SOG-Cu(II) system in 40% (v/v) ethanol - water, pH= 6.1, I = 0.1 mol l^{-1} $NaClO_4$ at 25 ºC. (1) 2ml Cu(II) $1x10^{-3}$ mol l^{-1} (2) 0.5 ml (Sudan Orange G) $5x10^{-4}$mol l^{-1} (3) 1:1 SOG – Cu(II) complex; [SOG] = [Cu(II)] =$1x10^{-4}$ mol l^{-1}

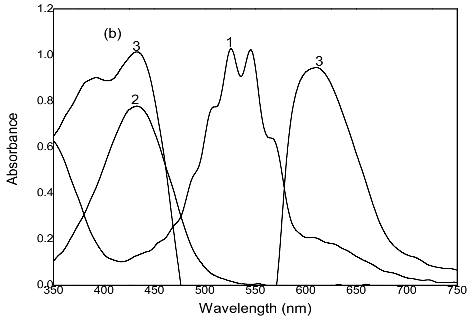


Fig. 2-b: Absorption spectra of SOG-$KMnO_4$ reaction (in alkaline medium) (1) alkaline $KMnO_4$ ($1x10^{-2}$ mol l^{-1}) (2)0.5 ml Sudan Orang G (in 1.0 mol l^{-1} NaOH) ($0.5x10^{-3}$ mol l^{-1} (3) SOG-$KMnO_4$ reaction products [SOG] = $3.9x10^{-5}$mol l^{-1}, [$KMnO_4$] = $1.5x10^{-3}$mol l^{-1}, [NaOH] = 0.3 mol l^{-1}

2.2 Complexation Equilibria of Sudan Orange G–

Copper(II)

The complexation equilibria of Cu(II) with Sudan Orange G are studied in 40% (v/v) ethanol-water medium I = 0.1 mol l^{-1} ($NaClO_4$), 25°C, in the pH range 3.51 – 7.97. The visible absorption spectra of solutions are recorded in the presence of an excess of metal ion, and equimolar solution.

The solution spectra reflect the formation of one complex species with λ_{max} at 346 and 506 nm and the existence of a single chelate equilibrium in the pH range 3.51 – 7.97 **Figs. 3-a, 3-b and Scheme II**. The absorbance versus pH graphs at λ_{max} 346 nm for solutions having different C_M/C_L molar ration, show the range of formation of complexed ligand. All the absorbance versus pH graphs exhibit a similarly shaped descending branch above pH 6.8 due to the hydrolysis of complexed ligand.

By considering the values of the dissociation constants of Sudan Orange G under our experimental conditions, we can assume that the neutral form (H_2L) of the dye is the prevalent ligand species in the pH range of complexation, and the complex forming equilibrium which exists at pH 6.1 probably represents interaction of Cu(II) with SOG dye according to the general equation.

$$\mathbf{m\,Cu^{2+} + nH_2L \rightleftharpoons Cu_mL_nH_z + qH^+} \qquad (1)$$

The hydrolysis of the complex formed is carried out according to the equation,

$$\mathbf{Cu_mL_nH_z + yH_2O \rightleftharpoons Cu_mL_nH_c\,(OH)_y + qH^+} \qquad (2)$$

The absorbance versus pH graphs for the Cu(II) – SOG dye system are interpreted by direct graphical and logarithmic analysis using transformations derived by earlier by **Sommer et al. [31], Hashem et al. [32-34]**

The following equilibria are considered during graphical and logarithmic analysis of the absorbance verses pH-graphs

$$\mathbf{Cu^{2+} + H_2L \rightleftharpoons [\overset{+}{C}uH\overset{-}{L}]^+ + H^+} \qquad \textbf{(A)}$$

$$\mathbf{Cu^{2+} + H_2L \rightleftharpoons [CuHL]^+ + H^+} \qquad \textbf{(A)}$$

$$\mathbf{[CuHL]^+ + 2H_2O \rightleftharpoons [CuHL\,(OH)_2]^- + 2H^+} \qquad \textbf{(B)}$$

The absorption band at 346 nm belongs to Cu(I) complexation with the oxidized hydrazone form while the other absorption band 506 nm is resulted from complexation reaction of Cu(II) with the high conjugated azo form **(Scheme I)**. Maximum color development is attained at pH 4.40–7.17 and pH 3.50–7.97. At higher pH values a decrease of absorbance is observed due to hydrolysis effect. The absorbance versus pH graphs at λ_{max} 346 and 506 nm were interpreted using the relationships reported by **Sommer et al. [31], Hashem et al. [32-34]**.

The following equations (3), (4) are valid for equimolar solutions and solutions with an excess of metal ion respectively.

$$log\left[\frac{\Delta A}{(\varepsilon_1 C_L - \Delta A)^2}\right] = qpH + log\varepsilon + logK_1^* \qquad (3)$$

$$log\left[\frac{\Delta A}{(\varepsilon_1 C_L - \Delta A)}\right] = qpH + logC_M + logK_1^* \qquad (4)$$

The logarithmic transformation (3) and (4) were found to be linear with a slope q=1, indicating the release of one proton during complexation of Cu(II) with Sudan Orange G dye in the pH range of study. The calculated values of equilibrium (log K^*) constant and stability constants (log β) and molar absorptivity of [Cu(II)-SOG] complexes are given in **Table 1**.

Table 1: Mean values of equilibrium (log K_{eq}^*), stability constant (log β) and molar absorptivity (Ɛ) of Cu (II) Sudan Orange G, I = 0.1 mol l^{-1} $NaClO_4$, 25°C.

Equilibrium( )	**Constant**	**Log Constant**	**molar absorptivity (Ɛ) L mol^{-1} Cm^{-1}**
$[CuLH][H^+] / [H_2L][Cu^{2+}]$	K_{eq}^*	**$(-2.11)^a$ $(-2.18)^b$**	**$Ɛ_1 = 0.389\times 10^4$**
$[CuLH] / [LH][Cu^{2+}]$	**β**	**$(8.41)^c$**	
$[CuHL(OH)_2]^-[H^+]^2/[CuHL]^+[H_2O]^2$	**$K_{(OH)}$**	**$(-1.20\pm0.02)^a$ $(-1.13\pm0.03)^b$**	
$[CuHL(OH)_2]^- / [Cu][HL][OH]^2$	**$\beta_{(OH)}$**	**$(46.02)^d$**	

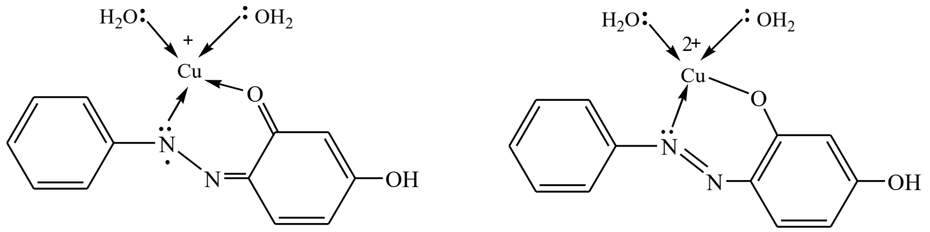
( ) Change are omitted

[a] From the absorbance versus pH graphs for solution of equimolar solution

[b] From the absorbance versus pH graphs for solution of excess metal

[c] log β= log K_{eq}^* / K_{a_2}

[d] log $\beta_{(OH)}$ = log $K_{(OH)}^*$ + pK_{a_2} + 2 pK_w



(a) λ_{max} = 346 nm

(b) λ_{max} = 506 nm

Scheme II: The proposed binding models of Cu(I) and Cu(II) complexes with Sudan Orange G

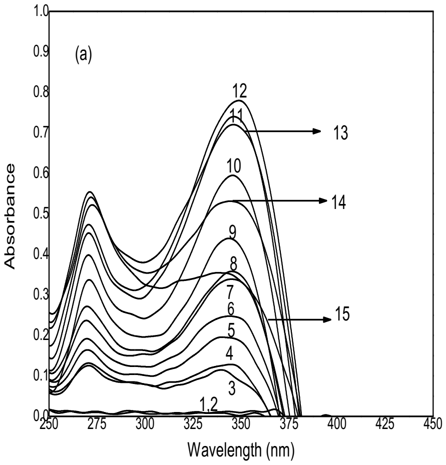


Fig. 3-a: Absorption spectra of SOG-Cu(II) system in 40% (v/v) ethanol-water, $[Cu^{+2}]$ = [Sudan II] = $1x10^{-4}$ mol L^{-1} at different pH values, 1) pH= 3.51; 2) pH=3.80; 3) pH=4.47; 4) pH=4.98; 5) pH=5.22; 6) pH=5.36; 7) pH=5.44; 8) pH=5.55; 9) pH=5.68 10) pH=5.93; 11) pH=6.11; 12) pH=6.28; 13) pH=6.56; 14) pH=6.96; 15) pH=7.47; 16) pH=7.96

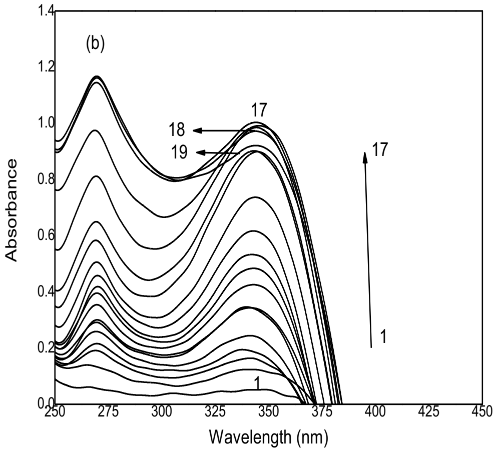


Fig. 3-b: Absorption spectra of SOG-Cu(II) system in 40% (v/v) ethanol-water, [Cu+2] = 3x10-4 mol L-1, [SOG] = 1x10-4 mol L-1 at different pH values, 1) pH=4.35; 2) pH=4.73; 3) pH=4.97; 4) pH=5.10; 5) pH=5.23; 6) pH=5.32; 7) pH=5.38; 8) pH=5.50; 9) pH=5.67; 10) pH=5.95; 11) pH=6.06; 12) pH=6.10; 13) pH=6.12; 14) pH=6.18; 15) pH=6.27; 16) pH=6.40; 17) pH=6.67; 18) pH=6.94; 19) pH=7.17

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.

3.4.1.1 Effect of Solvents

Since Sudan Orange G is insoluble in water, different solvents such as ethanol, methanol, acetone , DMSO and DMF mixed with water (40% v/v) have been tried but the best results were obtained with ethanol for copper (II)–Sudan Orange G complex.

3.4.1.2 Effect of pH

With other conditions fixed the effect of pH on absorbance of Cu(I)-SOG complex at λ_{max} = 346 nm was evaluated at pH values varying from 3.5-7.96. As can be seen from the quantitative determination of Sudan Orange G was achieved at pH 6.1. Hence, pH 6.1 was selected as the optimal working value for further studies.

3.4.1.3 Effect of Buffer

In order to determine the best buffer solution at pH 6.1, several buffer systems including HCO_3^-/CO_3^{2-}, NH_3/NH_4Cl 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 0.1–3.0 ml, and the maximum absorbance was obtained at a buffer volume of 0.5 ml. The results are shown in **Fig. 4-a**.

3.4.1.4 Effect of Copper (II) Concentration

At optimum conditions, effect of different volumes of Cu (II) ($1x10^{-3}$ mol l^{-1}) on absorbance of Sudan Orange G–copper complex was studied in the range (0.25–3.0 ml) at 25ºC **Fig. 4-b**. The maximum absorbance was observed when 1.0 ml of Cu(II) was used. To ensure the complete complexation for determination of Sudan Orange G, 3.0 ml of Cu (II) was used.

3.4.1.5 Effect of Time

Under optimum condition, the reaction time was determined by following the absorbance of the complex at different time intervals. Complete complex formation was attained after 3.0 min.

3.4.2 Oxidation with KMnO4 Method

Potassium permanganate, as a strong oxidizing agent, has been used in the oxidimetric–based analytical methods for the determination of many compounds [28]. SOG was found to be susceptible for oxidation with alkaline $KMnO_4$ producing a green color peaking at 610 nm **Fig. 2-b**. 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 30 min and was stable for at least 24 hr.

3.4.2.2 Effect of Potassium Permanganate Concentration

Under optimum conditions, the reaction increases substantially with increasing the concentration of $KMnO_4$ in SOG solution **Figs. 5-a**. Maximum absorbance was obtained when 2.0 ml of $5.0x10^{-3}$ mol l^{-1} $KMnO_4$ was used. Thus, the adoption of 3.0 ml of potassium permanganate in the final solution proved to be adequate for the maximum concentration of SOG used in determination process.

3.4.2.3Effect of Sodium Hydroxide Concentration

Fig. 5-b shows the effect of varying NaOH concentration upon the reaction of this Sudan Orange G dye with $KMnO_4$. The 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 3.0 ml of 1.0 mol l^{-1} NaOH was used as an optimum value.

3.5 Stoichiometry of Sudan Orange G Reactions

The stoichiometry of Sudan Orange G reaction with Cu (II) or $KMnO_4$ was determined using Job's continuous variation [35,28]. In solutions have $C_o= C_R + C_{SOG} = 3x10^{-4}$ mol l^{-1} at pH = 6.1 or pH > 12 in case of $KMnO_4$. The plot of absorbance at λ_{max} 346 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 (SOG: Cu(II)) complex, and a maximum at 0.66 revealing the interaction ratio 1:2 (SOG: $KMnO_4$).

3.6 Quantification

3.6.1 Validation of the Proposed Methods (I, II)

Standard calibration curves for Sudan Orange G 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 2.14 – 34.27and 0.64 – 7.07µg ml^{-1} at λ_{max} 346 nm and 610 nm respectively **Figs. 6-a, 6-b**, for both methods Sandell sensitivity, regression equations and molar absorptivity are calculated. To validate the analytical procedures both the detection limit (LOD) and the quantification limit (LOQ) were determined [36]. The parameters of the proposed methods are summarized in **Table 2**.

3.6.2 Accuracy and Precision (Intraday and Interday)

The accuracy and precision of the proposed spectrophotometric methods with (Cu (II) and $KMnO_4$) were determined at three concentration levels of Sudan Orange G dye by analyzing five replicate samples of each concentrations. The relative standard deviation (RSD %) obtained for the analytical results did not exceed 2% **Table 3** which proved a high reproducibility of the results and precision of the methods. The good level of precision was suitable for quality control analysis of Sudan Orange G in food samples. Under optimum conditions the intraday precision assay were carried out for our procedures used through replicate analysis (n = 5) for Sudan Orange G corresponding to 10.71, 14.99, 19.28, 25.70, 32.13 µg ml^{-1} (2.57, 3.21, 3.86, 4.50,5.14 µg 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 used in within day precision. The results of these assays are reported in **Table 3**. The (RSD %) and recovery values for intraday and interday

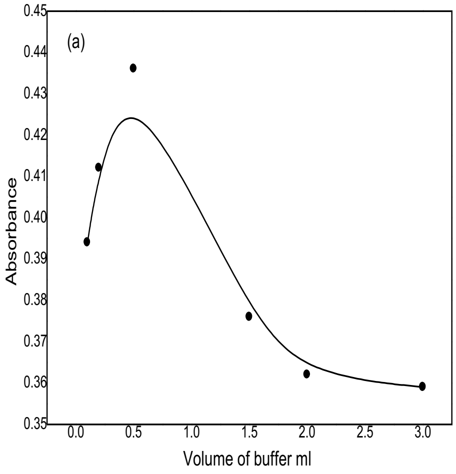


Fig. 4-a: Absorbance –vs acetate buffer volume for Cu(II)-SOG system in 40% (v/v) ethanol-water, [Cu(II)] = $3X10^{-4}$ mol l^{-1}, [SOG] = $1x10^{-4}$ mol l^{-1}, λ_{max} = 346 nm, pH=6.1

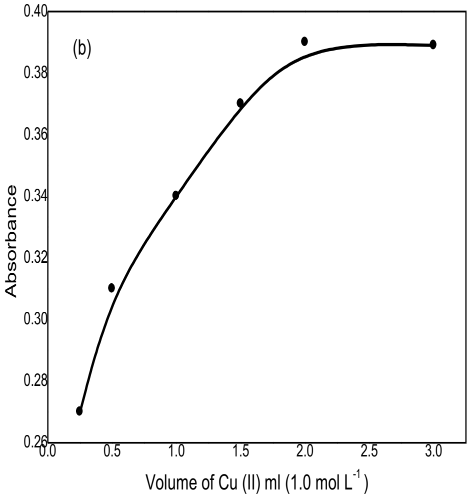


Fig. 4-b: Absorbance –vs volumes of Cu(II) ($1X10^{-3}$ mol l^{-1}) for system in 40% (v/v) ethanol-water, [SOG] =$1X10^{-4}$ mol l^{-1}, λ_{max} =346 nm, pH=6.1, acetate buffer volume = 0.5 ml

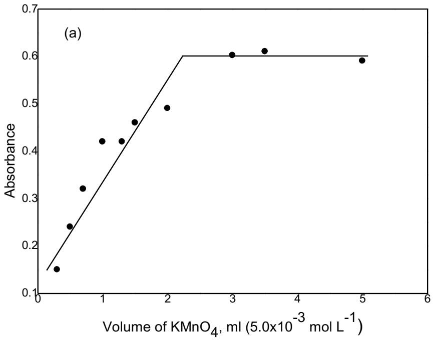


Fig. 5-a: Absorbance – vs volume of $KMnO_4$ ($5.0X10^{-3}$ mol l^{-1}) of SOG-$KMnO_4$ system in aqueous medium, [SOG] = $5.0X10^{-5}$ mol l^{-1}, [NaOH] = 0. 3 mol l^{-1}, t = 30 min, λ_{max} = 610 nm

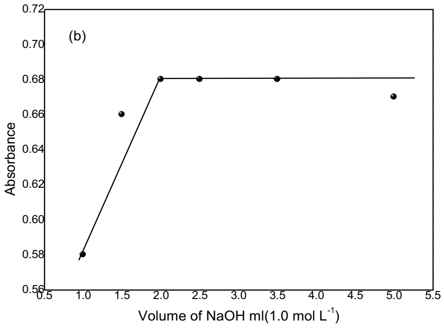


Fig. 5-b: Absorbance –vs volume of NaOH (1.0 mol l^{-1}) of SOG-$KMnO_4$ system in aqueous medium, [SOG] = $5X10^{-5}$ mol l^{-1}, [$KMnO_4$] =$2X10^{-3}$ mol l^{-1}, t = 30 min, λ_{max} =610 nm

precision were in the range 0.30 to 0.96% and 91.40–101.56% respectively. The green manganite produced SOG – $KMnO_4$ reaction in alkaline medium was stable for about 24 hr.

3.6.3 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 were 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 4. 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 Orange G. Sudan Orange G determination was possible in presence of few mg L^{-1} of Sunset Yellow, Sudan II, Tartrazine **Table 4**.

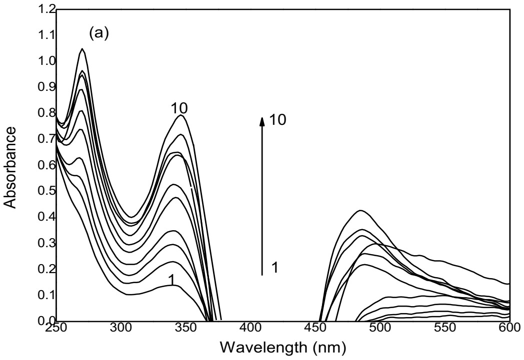


Fig. 6-a: Absorption spectra of SOG-Cu(II) system in 40% (v/v) ethanol-water, pH=6.1 (acetate buffer), Sudan Orange G concentration range $3.0x10^{-5}$ mol L^{-1} - $2.3x10^{-4}$mol L^{-1} with regular successive additions in presence of $3x10^{-4}$mol L^{-1} Cu(II), λ_{max} = 346 nm at 25ºC.

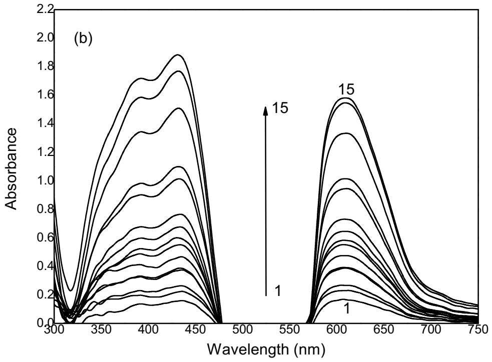


Fig. 6-b: Absorption spectra of SOG-$KMnO_4$ system in aqueous medium, Sudan Orange G concentration range $3x10^{-6}$ mol L^{-1} - $9x10^{-5}$mol L^{-1} with regular successive additions [$KMnO_4$] = 1.5 ml mol L^{-1}, [NaOH] = 0.3 mol L^{-1}, λ_{max} = 610 nm at 25ºC

Table 2: Summary of optical and regression characteristic of the proposed methods for determination of Sudan range G.

Method / Reagent **Parameter**	**Copper(II)**	**$KMnO_4$**
Color	**Colorless**	**Green**
λ_{max} (nm)	**346**	**610**
Beer's law limits (µgml^{-1})	**2.14 – 34.27**	**0.64 – 7.07**
Ringbornlimts (µgml^{-1})	**3.16 – 33.11**	**1.02 – 6.31**
Molar absorptivity (L mol^{-1}cm^{-1})	**0.389×10^4**	**2.6×10^4**
Sandell's sensitivity (µg cm^{-2})	**0.0551**	**0.0082**
Regression equation A = a+bc	**$A= 0.0177C + 4.21{\times}10^{-4}$**	**$A= 0.1177C - 7.8 \times 10^{-3}$**
Slope (b)	**0.0177**	**0.1177**
Intercept (a)	**$4.21{\times}10^{-3}$**	**$- 7.8 \times 10^{-3}$**
Correlation coefficient	**0.9998**	**0.9830**
Limit of detection (LOD) (µgml^{-1})	**0.2954**	**0.4429**
Limit of quantification (LOQ) (µgml^{-1})	**0.8952**	**1.3423**

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.

4.2.5.4 Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of detection (LOD) and limit of Quantification (LOQ) define the sensitivity of the method.

LOD and LOQ were calculated [37], according to $ICHQ_2$ recommendation [36] from the following equations:

$$LOD = 3.3\ \delta / \text{slope}$$

$$LOQ = 10\ \delta / \text{slope}$$

Where δ is the standard deviation of the intercept of regression line and the slope of calibration. LOD was found to be 0.2954, 0.4429µg ml^{-1}, while LOQ was found to be 0.8952, 1.3423µg ml^{-1} for the two methods respectively. The small values of LOD and LOQ indicated high sensitivity **Table 2**.

Table 3: Summary of accuracy and precision of the proposed methods for determination of Sudan Orange G in pure form.

Method / Reagent	**Amount μg ml^{-1}**		**RSD %**	**Recovery %**
	Taken	**Found ±SD[a]**		
Complexation method **Intraday assay**	**10.71**	**10.50 ± 0.05**	**0.48**	**98.04**
	14.99	**14.45 ± 0.08**	**0.55**	**96.40**
	19.28	**18.41 ± 0.07**	**0.38**	**95.49**
	25.70	**25.41 ± 0.11**	**0.43**	**98.87**
	32.13	**31.17 ± 0.13**	**0.42**	**97.01**
Interday assay	**10.71**	**10.16 ± 0.03**	**0.30**	**94.86**
	14.99	**13.89 ± 0.07**	**0.50**	**92.66**
	19.28	**17.84 ± 0.10**	**0.56**	**92.54**
	25.70	**23.49 ± 0.08**	**0.34**	**91.40**
	32.13	**29.71 ± 0.15**	**0.5**	**92.47**
Oxidation method **Intraday**	**2.57**	**2.61 ± 0.02**	**0.77**	**101.56**
	3.21	**3.12 ± 0.03**	**0.96**	**97.20**
	3.86	**3.72 ± 0.02**	**0.54**	**96.37**
	4.50	**4.48 ± 0.04**	**0.89**	**99.37**
	5.14	**5.16 ± 0.03**	**0.58**	**100.39**

[a] Mean for 5 independed analysis

Table 4: Matrix effects on the recovery of Sudan Orange G.

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**	**98.2 ± 1.0**	**2300**	**96.2 ± 1.2**
2) Fe^{3+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, Cd^{2+}	**150**	**97.16 ± 2.3**	**100**	**94.15 ± 2.5**
3) Sudan II	**0.28**	**97.06 ± 1.1**	**0.55**	**95.24± 1.5**

3.7 Applications to Commercial Food Samples

To confirm the usefuless of the proposed two methods, Sudan Orange G dye has been determined in nine different kinds of commercial food samples. The two proposed spectrophotometric methods were successfully tested without any interference from different matrix in commercial food samples. The complexation method was successfully applied for the determination of Sudan Orange G in extracted chili powder, ground paprika, ground red pepper, tomato paste, tomato ketchup, hot ketchup, hot sauce, pasta sauce and extra hot sauce by applying standard addition method. The results are given in **Table 5**. The recovery values for Sudan Orange G were in the range 91.96–101.24%.

Table 5: Statistical analysis of results obtained by the complexation method for Sudan Orange G in commercial food samples applying the standard addition technique.

Commercial Samples[(a)]	Present µg ml^{-1}	Added µg ml^{-1}	Found µg ml^{-1}	Recovery %	Mean	SD	RSD %
Chili Powder	**1.928**	**14.995** **21.422** **25.706**	**16.067** **22.065** **25.921**	**94.94** **91.96** **93.08**	**93.33**	**2.79**	**2.99**
Red Pepper Powder	**0.428**	**14.995** **21.422** **25.706**	**14.995** **20.779** **25.278**	**97.22** **95.10** **96.72**	**96.35**	**2.62**	**2.72**
Paprika Powder	**1.285**	**14.995** **21.422** **25.706**	**16.067** **22.065** **26.349**	**98.69** **97.17** **97.62**	**97.83**	**2.3**	**2.35**
Hot Catsup	**0.321**	**14.995** **21.422** **25.706**	**15.210** **21.422** **26.349**	**99.31** **98.52** **101.24**	**99.69**	**2.6**	**2.61**
Hot Sauce	**0.857**	**14.995** **21.422** **25.706**	**15.424** **22.493** **26.349**	**97.30** **100.96** **99.19**	**99.15**	**3.3**	**3.33**
Extra Hot Sauce	**1.607**	**14.995** **21.422** **25.706**	**15.210** **21.422** **25.278**	**91.62** **93.02** **92.55**	**92.40**	**2.4**	**2.60**
Tomato Catsup	**1.071**	**14.995** **21.422** **25.706**	**15.423** **22.493** **26.563**	**96.00** **100.00** **99.20**	**98.40**	**2.99**	**3.04**
Tomato Paste	**2.142**	**14.995** **21.422** **25.706**	**16.281** **22.493** **26.563**	**95.00** **95.45** **95.39**	**95.28**	**3.14**	**3.30**
Pasta Sauce	**1.500**	**14.995** **21.422** **25.706**	**16.067** **22.493** **26.778**	**97.41** **98.13** **98.43**	**97.99**	**2.5**	**2.55**

[(a)] from local supermarket in Assiut city, (Egypt).

4 Conclusion

The proposed methods are accurate, time saving and have the advantages of simplicity, sensitivity and reproducibility. Method I is based on Cu (II)–Sudan Orange G redox reaction followed by complexation to form Cu(I)-L complex. While method II is based on oxidation of Sudan Orange G 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. Complexation method is sensitive enough to determine trace amounts of Sudan Orange G extracted from Commercial Food Samples using standard addition method

Compliance with Ethical Standards:

Funding Assuit University, Faculty of Science Conflict Interest Elham Y. Hashem declares that she has no conflict of interest, Najat O. A. Al-Salahi declares that she has no conflict of interest.

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