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Oxidation and Complexation-Based Spectrophotometric Methods for Sensitive Determination of Tartrazine E102 in Some Commercial Food Samples

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Abstract

Two new sensitive spectrophotometric methods are reported for determination of tartrazine (Tz) (E102) in some commercial food samples. The first method involves two coupled reactions, the reduction of Cu(II) to Cu(I) by the analyte in acetate buffer medium (pH = 5.9) at 30°C and the complexation reaction between Cu(I) and Tz oxidized form to yield Cu-Tz complex (method I). The other method is based on oxidation of Tz by alkaline $KMnO_4$. These reactions are monitored spectrophotometrically at maximum absorbances 332 and 610 nm for methods (I and II) respectively. Variables affecting these reactions are carefully studied and the conditions are optimized. The stability constants are calculated at 293, 303, 313 and 323 K. The thermodynamic parameters, Gibb's free energy change (ΔG), entropy change (ΔS), and enthalpy change (ΔH) associated with the complexation reaction are calculated and discussed. Under optimized conditions the proposed methods (I, II) obey Beer's law 10.69 - 85.50, 5.34 - 34.12 μg·ml^{-1} of Tz respectively. The molar absorptivity, sandel sensitivity, detection and quantification limits are calculated. Matrix effects are also investigated. The methods are successfully applied to the quantification of Tz in different commercially food samples. The results obtained are in good agreement with those obtained by the reported methods at the 95% confidence level.

Keywords

Tartrazine E102, Food Colorants, Complexation, $KMnO_4$, Spectrophotometric Analysis

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1. Introduction

Food dyes are often added to foodstuffs and drinks in order to supply, intensify or restore their colour to create the desired coloured appearance [1]. Synthetic dyes are widely used as they show several advantages compared with natural dyes such as high stability to light, oxygen and pH, colour uniformity, low microbiological contamination and relatively lower production costs. Tartrazine is a synthetic organic food dye that can be found in common food products such as bakery products, dairy products, candies, and beverages. According to the limitations of European Union [EU] and Federal Food, Drug and Cosmetic Act [2], the presence and content of Tz dye must be controlled in food products due to their potential harmfulness to human beings [3]. Also it appears to cause the most allergic and intolerance reaction of all the azo dyes, particularly among asthmatics and those with an aspirin intolerance [4] [5]. Therefore the determination of tartrazine in commercial food products is very important. Until now, different methods such as chromatography [6]-[12], electroanalytical methods [13]-[15] and spectrophotometry [16]-[24], have been reported for the determination of tartrazine. However, some of these methods are not suitable for routine monitoring as they are time consuming, complicated and have poor sensitivity and selectivity. To the best of our knowledge, no spectrophotometer methods based on complexation with copper have been reported for the quantification of Tz. This paper describes the development of a simple, and rapid two spectrophotometric methods for the assay of Tz in food samples. The first method is based on oxidation of tartrazine by Cu(II) in acetate-acetic acid medium (pH = 5.9) at 30°C followed by complex formation. The other method is based on oxidation of Tz 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 Electronic Corporation (Cambridge, U.K.) was used for electronic spectral measurements. pH measurements were made with Jenway 3040 ion analyzer-pH meter, equipped with Jenway 924,005 combined glass electrode.

2.2. Chemicals

All chemicals were of analytical reagent grade. Deionized water was used to prepare all solutions. The tartrazine dye (E102) was supplied from Alfa Aesar (Germany). Stock standard solution containing 2.672 g·L^{-1} of the dye was prepared in deionized water. The working solutions were prepared daily by appropriate dilution. A 5×10^{-3} mol·L^{-1} of copper nitrate was prepared by dissolving the required amount in deionized water and standardized complexmetrically with EDTA [25]. The ionic strength of the solutions was maintained at a constant value of I = 0.1 mol·L^{-1} ($NaClO_4$). Acetic acid (0.2 mol·L^{-1}) sodium acetate (0.2 mol·L^{-1}) buffer (pH = 5.9) was prepared in 100 ml volumetric flask. Potassium permanganate (Merck, Germany) 1×10^{-2} mol·L^{-1} was prepared in deionized water.

2.3. Preparation of Real Samples

Powdered gelatin samples (Lemon, pineapple, green apple, apricot, banana and peach) and powdered drinks (apple, orange and tamarind) were bought from local supermarket in Assiut city (Egypt). These samples contained sugars, fumaric and citric acids, beef gelatin, artificial flavour, colour (E102), tricalcium phosphate and aspartame. Each gelatin or drink samples were weighed exactly (about 1 g) and dissolved in 50 ml of deionized water. Then each solution was centrifuged (10 min, 3500 rpm) to remove the insoluble particles. The filtrate was collected in 100 ml volumetric flask and diluted to the mark with deionized water. For determination of Tz in the above real samples, 0.2 ml of sample solution was used and analyzed according to method I. Finally, the Tz content in food samples were determined using the calibration equation and standard addition calibration curves procedure.

2.4. General Procedures

2.4.1. Method I (Complexation Reaction)
Into a 10 ml volumetric flasks, transfer a suitable aliquot of standard solution in deionized water containing up

to 181.6 µg of tartrazine and 4 ml of 2×10^{-3} mol·L^{-1} of Cu(II) solution. After mixing, the mixture was buffered to pH 5.9 with acetic acid-acetate buffer. Dilute the resulting solution to volume with deionized water and measure the absorbance at 332 nm, 303 K by using 1.0 cm quartz cell against a similarly prepared blank of the same pH. The calibration graph was constructed by plotting absorbance vs. Tz concentration.

2.4.2. Method II (Oxidation with KMnO₄)

Standard solutions in deionized water containing 16.031 - 187.03 µg·ml^{-1} Tz were transferred into individual 10 ml calibrated flasks, 4.0 ml of 1.0 mol·L^{-1} sodium hydroxide solution was added followed by 2.0 ml of 1×10^{-2} mol·L^{-1} KMnO₄ solution and it was diluted to the final volume with deionized water. After 60 min, the absorbance was measured at 610 nm against reagent blank treated similarly.

2.5. Interference from Matrix

Samples were prepared by mixing 0.5344 mg of Tz (method I) or 0.267 mg (in case of method II) with various amounts of common matrix cations, anions, sugar, gelatin, aspartame and dyes such as sunset yellow, and allura red. The procedure was continued as described under general procedures.

2.6. Determination of the Thermodynamic Parameters

The decomposition of stability constant into its enthalpy change (ΔH), Gibbs free energy change (ΔG) and entropic (ΔS) contributions is of fundamental importance to understand the various factors that may influence coordination [26] [27]. Enthalpy changes due to coordination reactions can always be obtained from the determination of stability constants at different temperatures according to the Van't Hoff [28] relationship (1, 2):

$$\triangle G = -2.303RT \log K_i = \triangle H - T\triangle S \tag{1}$$

$$\log K_i = \left(\frac{-\Delta H}{2.303\,R}\right)\frac{1}{T} + \frac{\Delta S}{2.303\,R} \tag{2}$$

where R is ideal gas constant (8.314 JK^{-1}·mol^{-1}), K_i is the stability constant of the complex and T is the absolute temperature in Kelvin (K).

3. Results and Discussion

3.1. Acid-Base Equilibria of Tartrazine

The absorption spectra of tartrazine salt (HL^{3-}) solution (5×10^{-5} mo·L^{-1}) in aqueous medium at $I = 0.1$ mol·L^{-1} (NaClO₄), 25°C were recorded at various pH values (**Figure 1**). The food additive tartrazine salt (HL^{3-}) yields four acid—base forms in solution of pH 0.86 - 12.4, H₅L$^+$, H₂L^{2-}, HL^{3-} and L^{4-} exhibiting the absorption maxima at 440, 430, 420 and 400 nm. The protonated acid form H₅L$^+$ at azo-nitrogen (λ_{max} = 440 nm) predominates in strongly acidic medium (pH < 3). The solution spectra of food colorant display a symmetrical and highly intense band (λ_{max} = 430 nm) at pH 3.2 - 4.3 corresponding to the protonated form of the dye salt (H₂L^{2-}). On decreasing, the acidity of the medium (4.2 - 6.5), a blue shift of the latter band at 420 nm is obtained resulting from dissociation of protonated nitrogen azo group (HL^{3-}). The electronic spectra of Tz in aqueous medium within pH 7.2 - 10.4 exhibit a broad band at 400 nm with a shoulder at 425 nm corresponding to tautomeric equilibria of monoanionic form of tartrazine salt (L^{4-}), **Scheme 1**. The absorbance versus pH graph were interpreted [29] assuming that a particular equilibrium established under selected conditions. Under our experimental condition pKa₁ [H₂L^{-2}/HL^{-3} (–HN=N–)] = 5.15 ± 0.10, pKa₂ [HL^{-3}/L^{-4} (OH)] = 9.25 ± 0.16.

3.2. Absorption Spectra of Tartrazine Reaction Products

Tartrazine likes Sudan dyes possess two reducible groups, a nitrogen-nitrogen double bond and a phenol group [30]. A reduction process, which corresponds to a spontaneous auto–reduction of the Cu^{2+} into Cu$^+$, was observed in cases of complexation of Cu^{2+} ions with sudan(I) [31] and sudan(II) [32]. Because of these features of sudan dyes, tartrazine is considered as chromogenic chelating and redox sensitive agent reacting with copper(II) to form Cu$^+$-Tz complex under optimum conditions. On the basis of redox sensitivity in the Cu(II)-tartrazine
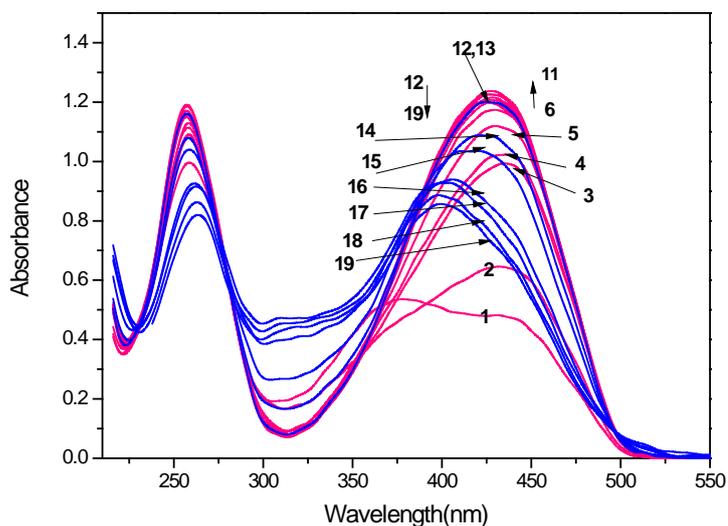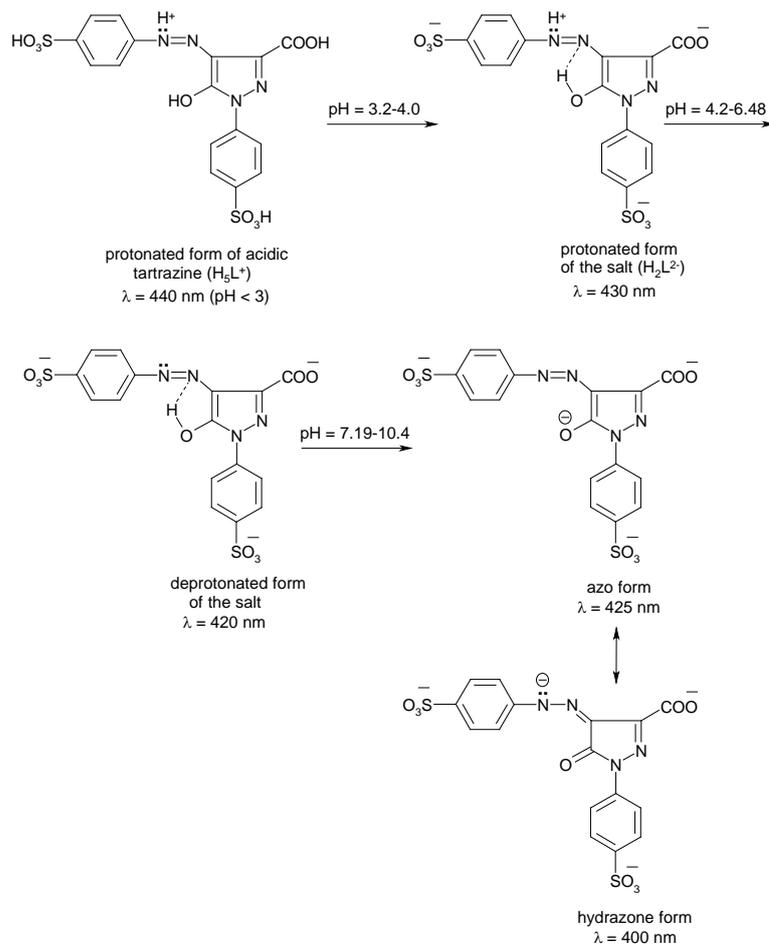
Figure 1. Absorption spectra of 5×10^{-5} mol·L^{-1} tartrazine in aqueous medium, I = 0.1 mol·L^{-1} (NaClO$_4$), 25°C at different pH values; 1, pH = 0.86; 2, pH = 0.9; 3, pH = 1.0; 4, pH 1.17; 5, pH = 1.6; 6, pH = 2.26; 7, pH = 2.73; 8, pH = 3.29; 9, pH = 3.73; 10, pH = 4.21; 11, pH = 5.7; 12, pH = 6.48; 13, pH = 7.19; 14, pH = 8.7; 15, pH = 9.2; 16, pH = 9.97; 17, pH = 10.58; 18, pH = 11.12; 19, pH = 12.83.

Scheme 1. Protonation process of tartrazine.

interaction in aqueous medium at I = 0.1 mol·L⁻¹ (NaClO₄), pH = 5.9, 30°C, solution spectrum shows an absorption band with a maximum at 332 nm corresponding to Cu(I)-Tz complex (MLCT) (**Figure 2(a)**).

Figure 2(b) shows the absorption spectrum obtained for alkaline KMnO₄ solution (λ_{max} = 530 nm). With addition of tartrazine solution, an absorption spectrum with two absorption bands at 390, 410 (double headed) and 610 nm was obtained. The latter band is attributed to the formation of manganate ion as a result of the oxidation of tartrazine with KMnO₄ in alkaline medium [33] [34] and the highly intense double headed band is related to Tz oxidized form.

3.3. Complexation Equilibria of Cu(II) with Tartrazine at Different Temperatures

The complexation equilibria of Cu^{2+} with Tz were investigated at I = 0.1 mol·L⁻¹ (NaClO₄) in aqueous medium over the pH range 3.5 - 10 at 293, 303, 313 and 323 K. The solution spectra were recorded in presence of an excess of the metal ion and in equimolar solutions. The absorption spectra for both metal/Tz ratios at various pH are analogous at the same temperature and exhibit an absorption band at 332 nm.

The absorbance versus pH graphs for the above solutions at different temperatures show the range of complex
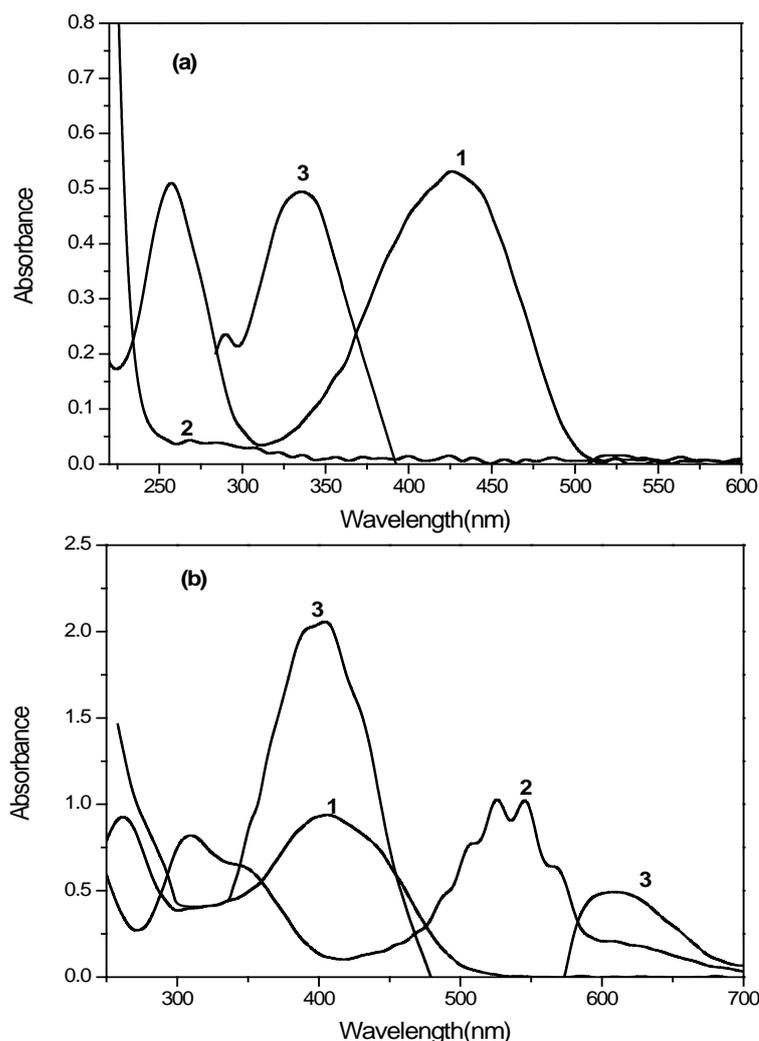
Figure 2. (a) Absorption spectra of Tz-Cu(II) (pH = 5.9), I = 0.1 mol·L⁻¹ (1) Tz 2 × 10⁻⁵ mol·L⁻¹; (2) Cu(II) 2 × 10⁻⁴ mol·L⁻¹; (3) 1:1 Tz-Cu(II); [Cu²⁺] = [Tz] = 2 × 10⁻⁴ mol·L⁻¹. (b) Absorption spectra of Tz-KMnO₄ reaction (in alkaline medium), (1) Tz 5 × 10⁻⁵ mol·L⁻¹; (2) alkaline KMnO₄ (5 × 10⁻⁴ mol·L⁻¹); (3) Tz-KMnO₄ reaction product [Tz] = 7 × 10⁻⁵ mol·L⁻¹, [KMnO₄] = 2 × 10⁻³ mol·L⁻¹, [NaOH] = 0.4 mol·L⁻¹.

formation (3.0 - 6.5) and existence of one complex equilibria within the pH range studied (**Figure 3(a)**). All graphs exhibit a similar descending branch above pH 6.5 which is due to the hydrolysis of the complexed Tz. The absorbance versus pH graphs for copper-Tz system at different temperatures were interpreted using relations derived earlier by Sommer *et al.* [35], Idriss *et al.* [36] [37] and Saleh *et al.* [34]. The following Equations (3, 4) were valid for equimolar and solutions with an excess of metal ion respectively:

$$\log\left[\Delta A / \left(\varepsilon_1 C_L - \Delta A\right)^2\right] = q \, \text{pH} + \log \varepsilon_1 + \log K^* \tag{3}$$

$$\log\left[\Delta A / \left(\varepsilon_1 C_L - \Delta A\right)\right] = q \, \text{pH} + \log C_M + \log K^* \tag{4}$$

All symbols have their usual meanings. The logarithmic transformation are linear with a slope of $q = 2$, indicating the release of two protons during complexation and an intercept including K^*. By considering the values of the dissociation constants of Tz under our experimental conditions, one can assume the following complexation equilibria with Cu(II) in the pH range 3.0 - 6.5.

$$\text{Cu}\left(\text{II}\right) + \text{H}_2\text{L}^{-2} \xrightarrow[\text{pH=3.0 - 6.5}]{} \left[\text{Cu}^+ + \text{H}_2\text{L}^-\right] \xrightarrow[\text{Intermediate state}]{} \left[\text{Cu}^+\text{L}^{3-}\right] + 2\text{H}^+, K^* \tag{A}$$
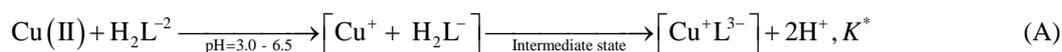
The mean equilibrium constant K^* was determined at different temperatures by considering equilibrium A. the stability constant of Cu(I) complex is related to the equilibrium constant K^* by the expression $K_1 = K^* / K_{a1} \cdot K_{a2}$. The calculated values of log K_1 for the complex at 293, 303, 313 and 323 K are given in **Table 1**. The proposed binding model of Tz-copper complex is shown below.
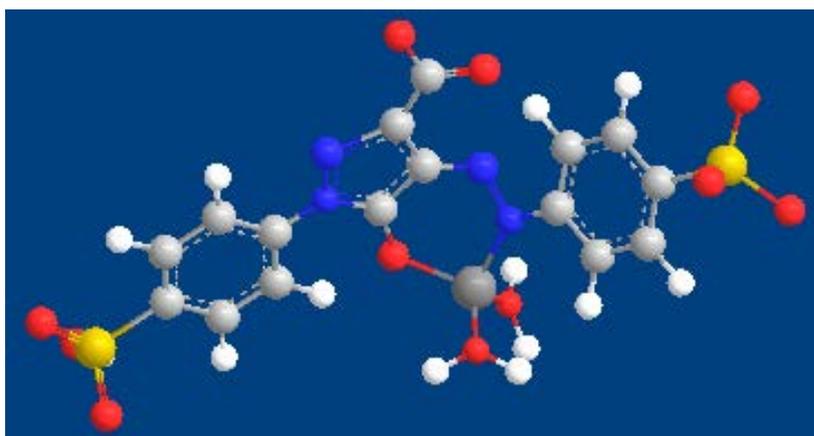
The proposed binding model of Tz with copper(I).

3.4. Thermodynamic Functions of Tartrazine-Copper(II) Reaction

According to Van't Hoff equation the values of overall thermodynamic parameters ΔG, ΔH and ΔS accompanying complex formation have been determined (**Figure 3(b)**). The ΔH and ΔS values can then be considered as a sum of three contributions, Cu(II)-Tz redox reaction, release of H_2O molecules from octahedral copper(II) ions and metal-ligand bond formation. From **Table 1**, the −ve value of ΔG for the complexation process Cu(I)-Tz suggests a spontaneous nature of such process [38]. The +ve value of ΔH means that this reaction is endothermic favourable at high temperature. The +ve value of ΔS for the complexation process confirm that the Cu(I)-Tz complex formation is entropically favourable [39].

Table 1. Stability constants at different temperatures and thermodynamic parameters ΔG, ΔH and ΔS of copper(I)-Tz complex.

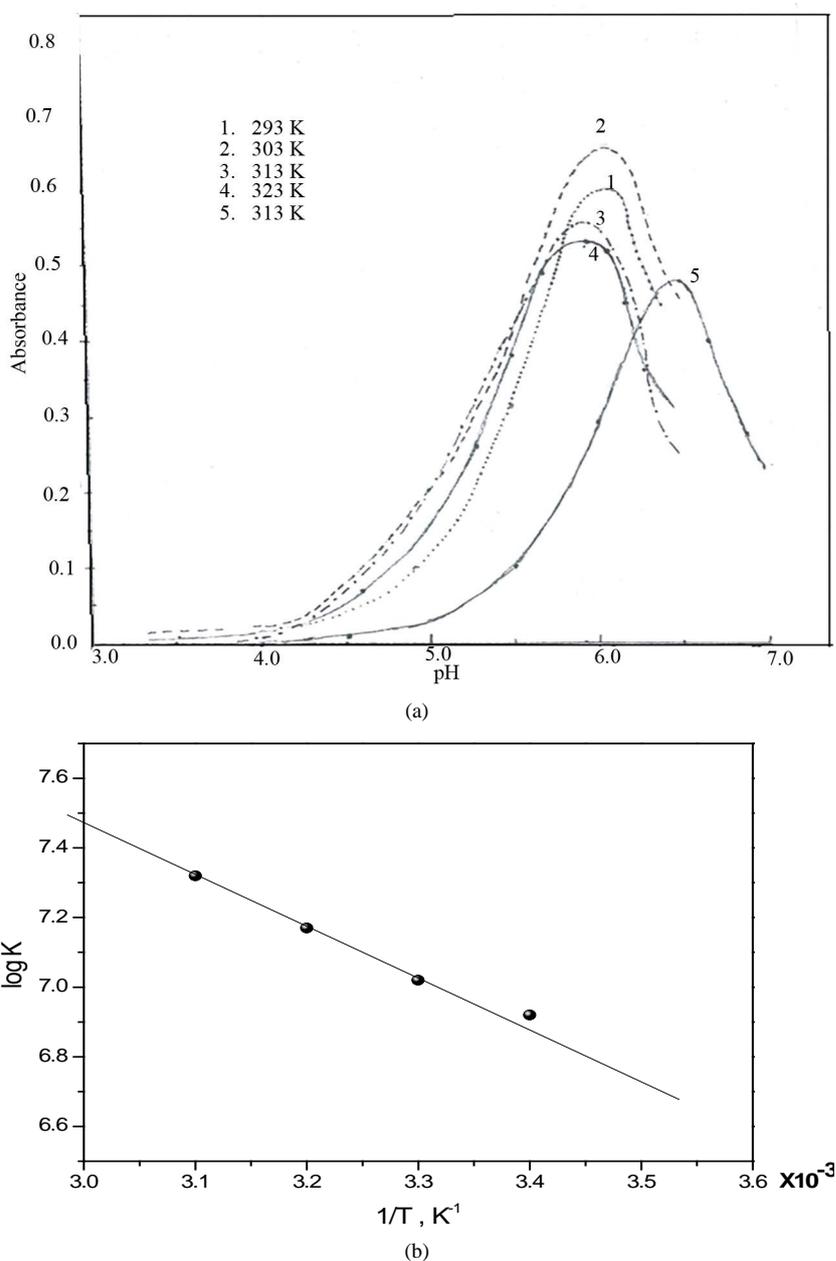| Complex species	Log K_1 at temperature, K				$-\Delta G$ $Kj \cdot mol^{-1}$	ΔH $Kj \cdot mol^{-1}$	ΔS $J \cdot mol^{-1} \cdot K^{-1}$
	293	303	313	323			
$[\text{Cu}^+\text{L}^{3-}]^{2-}$	6.922	7.02	7.172	7.322	41.319	27.354	142.65

(a)

(b)

Figure 3. (a) Absorbance vs pH graph for Tz-Cu(I) complex in aqueous medium, I = 0.1 mol·L^{-1} (NaClO$_4$), λ_{max} = 332 nm at various temperature, (1 - 4) [Tz] = 2 × 10^{-4} mol·L^{-1}, [Cu^{2+}] = 6 × 10^{-4} mol·L^{-1} at 293, 303, 313, and 323 K respectively; (5) [Tz] = [Cu^{2+}] = 2 × 10^{-4} mol·L^{-1} at 313 K. (b) Van't Hoff plot of log K_1 of Tz-Cu(I) complex against 1/T.

3.5. Optimization of Variables

3.5.1. Complexation Method

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

Effect of pH

With other conditions fixed the effect of pH on absorbance of Cu(I)-Tz complex at λ_{max} = 332 nm was investigated from pH 3.5 - 10.0. From **Figure 3(a)**, the quantitative determination of Tz was achieved at pH = 5.9.

In order to determine the best buffer solution at pH = 5.9, several buffer systems at isomolar concentration of 0.2 mol·L^{-1} were studied. The best analytical sensitivity was obtained in presence of acetate-acetic acid buffer. Next, the effect of buffer volume on analytical sensitivity was also studied in the range of 0.2 - 2.5 ml, and the maximum absorbance was obtained at a buffer volume of 0.5 ml. the results are shown in **Figure 4(a)**.

Effect of Copper Concentration

The effect of copper(II) concentration on the absorbance of the complex was investigated by varying the reagent concentration under the optimum conditions ([Tz] = 2×10^{-4} mol·L^{-1}), pH = 5.9, T = 293 K. It is clear from **Figure 4(a)** that the maximum absorbance was attained with 3.0 ml; above this volume, the absorbance remained unchanged. To ensure the complete complexation for determination of tartrazine, 4.0 ml Cu(II) was used.

Effect of Temperature and Time

The effect of temperature on the absorbance of the formed complex was studied in aqueous medium at pH = 5.9 in the range 393 - 323 K, keeping constant concentration of Tz (2.0×10^{-4} mol·L^{-1}) and copper (8.0×10^{-4} mol·L^{-1}). The maximum absorbance was obtained at 303 K (**Figure 4(b)**). Under optimum conditions, the reaction time was determined by following the absorbance of the complex at different time intervals. Complete complex formation was attained after 5 min. at 303 K.
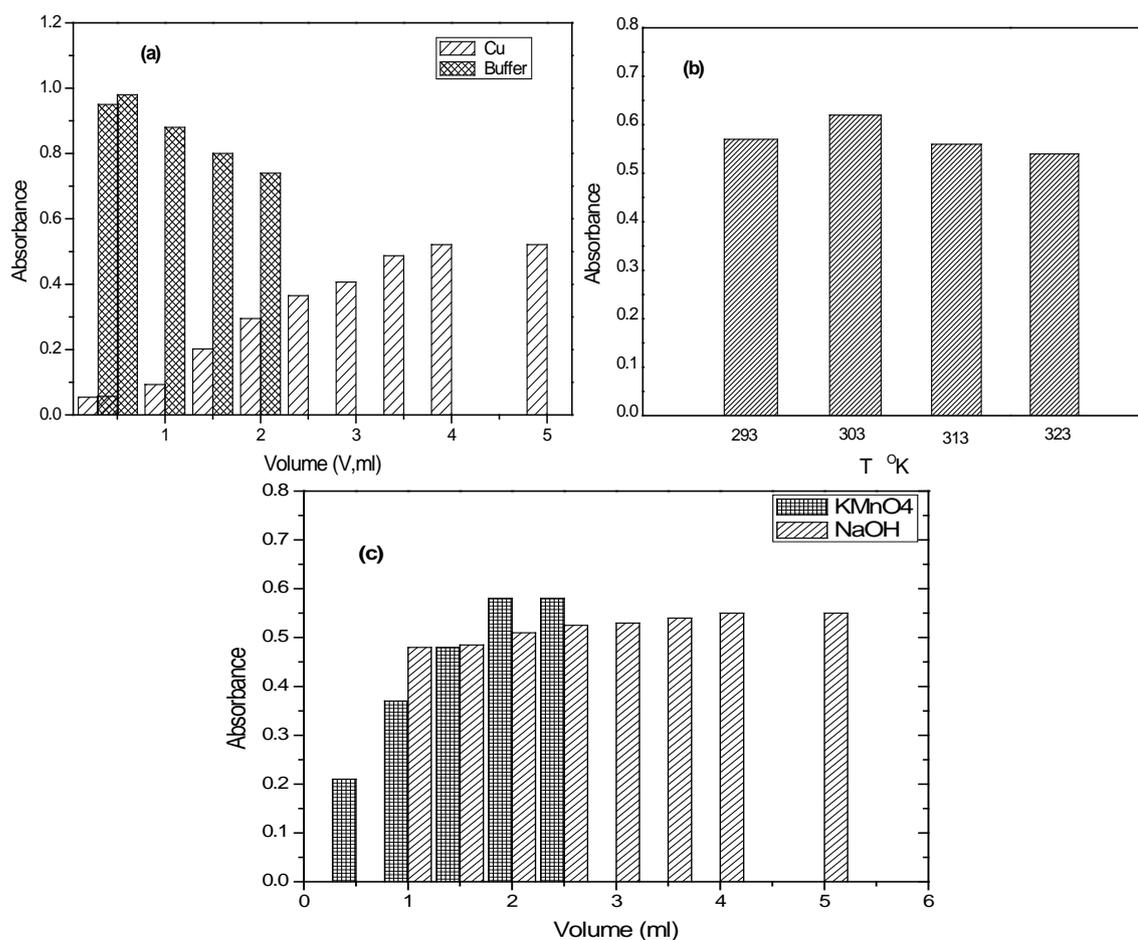
Figure 4. (a) Reaction conditions for complexation reaction at 293 K, [Tz] = 2×10^{-4} mol·L^{-1}, λ_{max} = 332 nm, pH = 5.9. (1) Effect of different volumes of 2×10^{-3} mol·L^{-1} Cu^{2+} on absorbance of Cu$^+$-Tz^{3-} complex, acetate buffer volume = 1 ml. (2) Effect of different acetate buffer volume on absorbance of Cu-Tz complex, [Cu^{2+}] = 8×10^{-4} mol·L^{-1}. (b) Effect of absolute temperature on absorbance of Tz complex, [Tz] = 2×10^{-4} mol·L^{-1}, [Cu^{2+}] = 8×10^{-4} mol·L^{-1}; buffer volume = 0.5 ml. (c) Reaction conditions for oxidation reaction of Tz with alkaline KMnO$_4$ at 293 K, [Tz = 1×10^{-4} mol·L^{-1}]. 1) Effect of KMnO$_4$ (1×10^{-2} mol·L^{-1}) (by volume), [NaOH] = 0.4 mol·L^{-1}, t = 60 min. 2) Effect of NaOH (1.0 mol·L^{-1}) (by volume), [KMnO$_4$] = 2×10^{-3} mol·L^{-1}, t = 60 min.

3.5.2. KMnO₄ Oxidation Method

Redox reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many compounds [33] [40]. Tartrazine reacts with $KMnO_4$ in strongly alkaline medium producing green manganate ($\lambda_{max} = 610$ nm) (**Figure 2(b)**). During the current study the produced colour intensity increased gradually with time to reach maximum after 60 min and was stable for at least 24 h. The factors affecting the formation of manganate ions were further studied and optimized.

Effect of NaOH and KMnO₄

The dependence of redox reaction on the volume of NaOH (1.0 mol·L^{-1}) was investigated in the range 1.0 - 5.0 ml. The results show that maximum absorbance was obtained using 4.0 ml of 1.0 mol·L^{-1} NaOH with no significant changes indicated as the volume was increased (**Figure 4(c)**). Under optimum conditions, the dependence of redox reaction on the volume of $KMnO_4$ was investigated over the range from 0.5 - 3.0 ml (1×10^{-2} mol·L^{-1}). The results show that the maximum absorbance was obtained using 2.0 ml of 1×10^{-2} mol·L^{-1} $KMnO_4$ (**Figure 4(c)**).

3.5.3. Stoichiometry of Tartrazine Reactions

The stoichiometryt of tartrazine reaction with Cu(II) or $KMnO_4$ was established by the continuous variation method [34] [41]. The solutions having $C_o = C_R + C_{Tz} = 6.0 \times 10^{-4}$ mol·L^{-1} at pH 5.9 or > 11.0. The plot of absorbance versus mole fraction of reagent at λ_{max} 332 or 610 nm, reveals the formation of 1:1 (Tz:copper) complex and 1:2 (Tz:KMnO₄).

3.6. Quantification

3.6.1. Validation of the Proposed Methods

For our two methods (I, II) the ranges of linearity of absorbance as a function of food additive Tz concentration provide a satisfactory measure of the sensitivity of the method. Under the optimum conditions the absorbance of the complex (**Figure 5**) or manganate obeys Beer's law in Tz concentration range of 10.69 - 85.50 or 5.34 - 34.12 µg·ml^{-1} respectively. For the two proposed methods (I, II), the molar absorptivity and Sandell sensitivity [42] values are 0.361×10^4, 0.80×10^4 L·mol^{-1}·cm^{-1} and 0.1480, 0.0668 µg·cm^{-2} respectively. The regression equations, correlation coefficient, limit of detection, and limit of quantification [43] were also calculated and summarized in **Table 2**.

3.6.2. Accuracy and Precision

The accuracy and precision of the proposed spectrophotometric methods were determined at three different concentration levels of additive food colour-Tz 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 3**) which proved a high reproducibility of the results and precision of the methods.
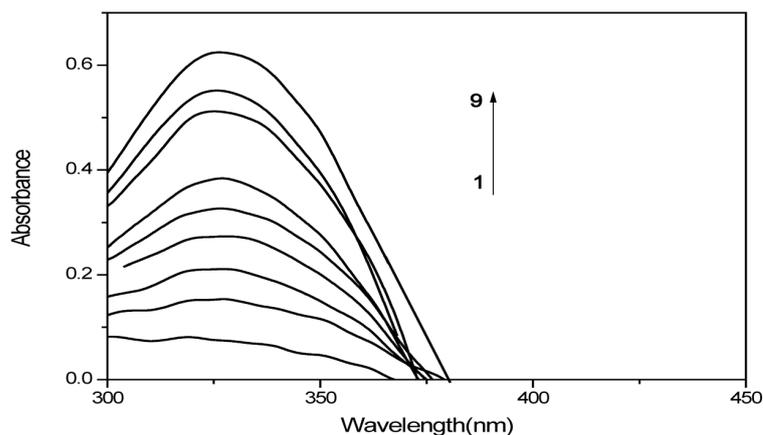

Figure 5. Absorption spectra of tartrazine–copper complex, pH = 5.9 (acetate buffer), tartrazine concentration range 2×10^{-5} (1) to 2.0×10^{-4} mol·L^{-1} (9) with regular successive additions in presence of 8.0×10^{-4} mol·L^{-1}Cu(II) at 303 K.

Table 2. Summary of optical and regression characteristic of the proposed methods for determination of tartrazine.

Parameter / Method/reagent	Copper(II)	KMnO$_4$
Colour	Colourless	Green
λ_{max} (nm)	332	610
Beer's law limits ($\mu g \cdot ml^{-1}$)	10.69 - 85.50	5.34 - 34.12
Ringborn limts ($\mu g \cdot ml^{-1}$)	12.59 - 81.28	7.08 - 31.62
Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	0.361×10^4	0.80×10^4
Sandel's sensitivity ($\mu g \cdot cm^{-2}$)	0.1480	0.0668
Regression equation $A = a + bc$	$A = 6.55 \times 10^{-3}C - 8.62 \times 10^{-4}$	$A = 0.0147C + 6.79 \times 10^{-3}$
Slope (b)	6.55×10^{-3}	0.0147
Intercept (a)	8.62×10^{-4}	6.79×10^{-3}
Correlation coefficient	0.9999	0.9969
Limit of detection (LOD) ($\mu g \cdot ml^{-1}$)	0.101	0.355
Limit of quantification (LOQ) ($\mu g \cdot ml^{-1}$)	0.305	1.075

Table 3. Summary of accuracy and precision of the proposed methods for determination of tartrazine in pure form.

Method/Reagent	Amount $\mu g \cdot ml^{-1}$ Taken	Found ± SD[a]	RSD %	Recovery %	C.L[b]
Cu(II) Intraday assay	32.062	31.929 ± 0.11	0.34	99.585	±0.137
	53.437	51.777 ± 0.13	0.25	96.894	±0.162
	64.124	63.532 ± 0.20	0.31	99.076	±0.249
Intreday assay	32.062	31.166 ± 0.12	0.39	97.205	±0.149
	53.437	52.54 ± 0.15	0.29	98.321	±0.186
	64.124	63.838 ± 0.19	0.30	99.554	±0.236
KMnO$_4$ Intraday	16.031	16.001 ± 0.05	0.31	99.813	±0.062
	26.719	26.205 ± 0.07	0.27	98.076	±0.087
	32.062	32.395 ± 0.11	0.34	101.039	±0.137

a) Mean for 5 independed analysis. b) C.L., confidence limit at 95% confidence level at 4 degree of freedom (t = 2.776).

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) for Tz corresponding to 32.062, 53.437, 64.124 $\mu g \cdot ml^{-1}$ (method I) and 16.031, 26.719, 32.062 $\mu g \cdot ml^{-1}$ (method II). The interday precision was also evaluated through replicate analysis of the standard Tz for three consecutive days at the same concentration levels as in within day precision. The results of these assays are reported in **Table 3**. For intraday and interday precision, the recovery and RSD values were in the range of 96.89% - 101.04% and 0.25% to 0.39% respectively. The green manganate ions produced from Tz-KMnO$_4$ reaction in alkaline medium was stable only for 24 hours.

3.6.4. Analytical Recovery and Matrix Effects

To investigate the selectivity of the proposed methods, the effect of various substances on the determination of Tz (53.437 and 26.719 $mg \cdot L^{-1}$) for method I and II respectively was tested under optimum conditions. Several representative potential interferences such as inorganic cations, anions, molecular species and dyes were investigated individually for their effect on Tz recovery. Tolerance limits were defined by the concentration of interferents which caused on <5% error in the determination of Tz. The obtained mean recoveries and standard deviation ranged between 99.0% - 96.0% and ± 1.0 - 2.0 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 method was also checked by studying the influence of various dyes on the recovery of Tz. Tartrazine determination

Table 4. Matrix effects on the recovery of tartrazine (E = 102).

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$_4^{3-}$	3000	99.0 ± 1.0	2500	98.0 ± 1.5
2) Citrate, stearat, fumarate, aspartame, gelatin, sugar, L-ascopric acid.	1000	98.0 ± 1.0	8.0	98.5 ± 1.0
3) Sunset yellow	4.5	97.5 ± 1.5	3.5	97.0 ± 1.5
4) Tropaeolin ooo	6.1	96.5 ± 2.0	4.5	96.0 ± 2.0
5) Allura red Ac	5.0	97.0 ± 2.0	4.0	96.8 ± 1.0

Table 5. Statistical analysis of results obtained by the complexation method for tartrazine in powdered drink samples.

Drink[a] sample	Present µg·ml^{-1}	Added µg·ml^{-1}	Found µg·ml^{-1}	±RSD %	Recovery %	Mean Recovery %	±RSD %	t-value[b]	F-value[b]	Reported method[41] Recovery %	±RSD %
Apple	3.741	10.690	13.893	0.68	96.48	98.121	0.70	1.98	1.15	97.5	0.75
		21.375	24.578	0.69	97.86						
		32.070	35.800	0.73	100.02						
Orange	1.603	10.690	11.756	0.55	95.632	98.75	0.60	-	-	-	-
		21.375	23.510	0.62	102.320						
		32.070	33.130	0.63	98.390						
Tamarind	2.1375	10.690	12.825	0.60	99.97	98.46	0.65	-	-	-	-
		21.375	22.440	0.63	95.44						
		42.760	34.199	0.72	99.96						

a) From local markets of Assiut city, Egypt. b) Theoretical t-value ($\upsilon = 4$) and F-value ($\upsilon = 4.4$) at 95% confidence level are 2.78 and 6.39.

Table 6. Statistical analysis of results obtained by the complexation method for tartrazine in powdered gelatin.

Gelatin[a] sample	Present µg·ml^{-1}	Added µg·ml^{-1}	Found µg·ml^{-1}	±RSD %	Recovery %	Mean Recovery %	±RSD %	t-value[b]	F-value[b]	Reported method[21] Recovery %	±RSD %
Lemon	2.137	10.690	12.82	0.54	99.92	97.40	0.60	1.49		97.0	–
		21.375	22.44	0.62	95.44						
		32.070	33.13	0.64	96.84						
Apricot	3.206	10.690	12.820	0.66	92.25	95.93	0.71	–	–	–	–
		21.375	23.512	0.72	95.57						
		32.070	35.270	0.75	99.98						
Banana	2.1375	10.690	12.820	0.59	99.94	98.45	0.64	–	–	–	–
		21.375	22.440	0.70	95.45						
		32.070	34.199	0.63	99.96						
Pineapple	3.206	10.690	13.894	0.66	99.95	97.52	0.68	–	–	–	–
		21.375	23.512	0.69	95.66						
		32.070	34.199	0.69	96.94						
Green apple	1.068	10.690	11.75	0.67	99.93	97.29	0.70	–	–	–	–
		21.375	21.37	0.69	95.23						
		32.070	32.06	0.74	96.67						
Peach	1.068	10.690	10.687	0.66	90.89	96.95	0.69	–	–	–	–
		21.375	22.440	0.69	99.98						
		32.070	33.130	0.72	99.97						

a) From local markets of Assiut city, Egypt. b) Theoretical t-value ($\upsilon = 4$) and F-value ($\upsilon = 4.4$) at 95% confidence level are 2.78 and 6.39.

was possible in presence of 0.045 mg/10 ml sunset yellow (0.035 mg in case of method II), 0.05 mg Allura red (0.04 mg in case of method II) and 0.061 mg Tropaeolin 000 (0.045 mg in case of method II). 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 to the determination of Tz in two different commercial food products (powered drink and powdered gelatin samples). To investigate the applicability of the proposed method, recovery experiments were performed using multiple points standard addition method. For this purpose, a known amount of Tz was spiked to the formulated preparations and the total amount of the dye was estimated. The results are summarized in **Table 5** and **Table 6**. The t- and F-tests indicated no significant differences between the calculated and theoretical values of both the proposed and the reported methods [24] [44] at 95% confidence level, which indicate good precision and accuracy in the analysis of investigated Tz in commercial food products.

4. Conclusion

In this paper, two spectrophotometric methods for the determination of Tz with Cu(II) and $KMnO_4$ were proposed and successfully validated. The methods were based on the redox reaction with Cu(II) followed by complex formation and oxidation with strongly alkaline $KMnO_4$. The positive value of change in enthalpy (ΔH) for complexation reaction suggested that the reaction was endothermic favourable at high temperature. The negative value of free energy change indicated that copper-Tz redox reaction, dissociation of the ligand and complexation process were spontaneous. It was found that the complexation method was selective and sensitive enough to enable the determination of lower amounts of Tz and could be applied to the food quality control.

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