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Spectrophotometric Estimation of Trimethoprim By Charge Transfer Complex Formation Reaction

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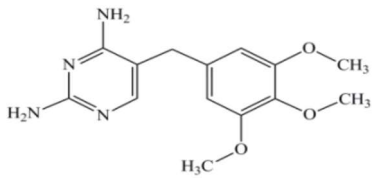
Abstract

A new simple method is proposed for the spectrophotometric estimation of trimethoprim drug (TMP), depending on the formation of an n-π charge transfer complex between TMP acting as n-donor and o-chloranil acting as π-acceptor in ethanolic medium. The complex has a maximum absorbance at 379 nm. Beer's law is applied within the limits of concentration of 5–60 µg/mL. The method is accurate, precise, and sensitive, as the average recovery was 101.57%. The relative standard deviation (RSD) was less than 3.03%, and the molar absorptivity was 1.11×10^4 L/mol.cm. The limit of the quantitation (LOQ) was 0.2323 µg/mL, and the limit of detection (LOD) was 0.0697 µg/mL. The complex was formed at a ratio of 1:1. TMP is usually found mixed with sulfamethoxazole in its pharmaceutical preparations, so the uniqueness of this method over others is that TMP can be estimated alone in tablets containing sulfamethoxazole and other excipients after the simple extraction. The method was contrasted favorably with the British Pharmacopoeia by computing the t-exp and F-test and other literature methods.

Keywords: Spectrophotometry, o-Chloranil, Trimethoprim, Charge transfer complex.

Introduction

Trimethoprim ($C_{14}H_{18}N_4O_3$) is chemically known as [2,4-diamine-5-(3,4,5-trimethoxy-benzyl) pyrimidine; TMP]. It is one of the drugs that best exemplifies the category of pharmaceutical and personal care products (PPCPs) (Scheme 1), in combination with sulfamethoxazole (SMX), has a synergetic effect, for instance, in the treatment of urinary tract infections [1].



Scheme 1: **Structure formula of Trimethoprim**

TMP possesses bacteriostatic activity against a wide variety of Gram-positive and Gram-negative bacteria [2].

MWt=390.3 g/mol

Most of the analytical methods and the techniques have been used for the determination of TMP and SMX in their mixtures. Few analytical methods were described for the determination of TMP in the presence of SMX. The methods included HILIC-HPLC [3], TLC-densitometry [4], ion-selective electrode [5-7], LC [8], RP-LC [9], RP-HPLC [10], fluorometry [11], voltammetry [12, 13], and cyclic voltammetry [14].

Various reagents have been used for the spectrophotometric methods for the determination of TMP, including 2,3-dichloro-5,6-dicyano-p-benzoquinine (DDQ) and p-chloranilic acid [15, 16], 2,4-dinitro-1-fluorobenzene [17], persulfate as an oxidizing agent [18], bromocresol green, alizarin red S with bromophenol blue [19], diazotized (Z)-1-(4 - ((1 - hydroxynaphthalen - 2 - yl) diazenyl) phenyl) ethan-1-one and (E)-1-(4-((2,3-dihydroxyphenyl)diazenyl)phenyl)ethan-1-one [20]. However, all mentioned methods suffered from the SMX interference, which required additional procedures to overcome this interference by the separation processes using complex devices that require training and are expensive or complex chemical calculations.

In the current work, a low-cost, simple, fast, and reliable method has been proposed for the determination of TMP spectrophotometrically, depending on the charge transfer complex (CTC) formation in ethanol medium where the amine group reacts, as an n-donor, with the o-chloranil (*o-CA*) reagent as a π-acceptor. This suggested method was applied to pharmaceutical formulation.

Materials and Methods

Materials and Reagents

In this research work, all the chemicals used were purchased from Fluka, Molekula, and SDI, suppliers. They were of analytical grade and used without any further purification, including o-chloranil (*o-CA*), potassium hydroxide, and sodium hydroxide (Fluka, Germany); absolute ethanol and chloroform (Molekula, United Kingdom); and Trimethoprim supplied from SDI-Iraq.

Instruments

The measurements were conducted by using a Shimadzu model UV–1650PC UV-Visible spectrophotometer. Cells of silica with a width of 1 cm were used. Heating processes were carried out using a Lab-Companion Bs-11 water bath, and pH was measured using the pH meter (9421) Philips PW connected to a CEIO-12 electrode and a GR-200 sensitive balance was used to weigh the samples.

Preparation of Standard Solutions

The TMP of 100 µg/mL stock solution was prepared by dissolving 0.01 g in 100 mL of distilled water. An *o-CA* solution was prepared at a concentration of $5x10^{-3}$ M by dissolfcaEHNYving 0.123 g of o-chloranil in 100 mL of absolute ethanol, and ~ 0.01 M potassium hydroxide solution was prepared by dissolving 0.14 g in 250 mL of distilled water.

General Procedure

Accurate volumes of TMP (100 µg/mL) were added to 5-mL volumetric flasks to obtain 5-60 µg/mL in final volume, followed by the addition of 0.8 mL of *o-CA* reagent (5×10^{-3} M) and 0.75 mL of potassium hydroxide (0.01 M), and the volume was diluted with absolute ethanol to the mark. The solutions were then allowed to stand for 5 min at ambient temperature before being measured at 379 nm against a reagent blank.

Analysis of Tablet

Each tablet contains 400 mg SMX and 80 mg TMP. Both compounds contain an amino group that reacts with the *o-CA* reagent, causing interference in the estimation process. Therefore, TMP was extracted from the tablet before determination by the following procedure [16]. After exactly 10 tablets had been powdered and well mixed, a quantity equal to one tablet was added to a separating funnel that contained 30 mL of 0.1 M sodium hydroxide. Chloroform was used to extract

TMP (4×25 mL). After the organic layer was completely evaporated, the residue was dissolved in 5 mL of absolute ethanol, then transferred to a 100 mL volumetric flask, and the volume was completed with distilled water. A working solution with an appropriate dilution was prepared, and a solution containing an amount within the calibration graph was treated in accordance with the general procedure.

Results and Discussion

TMP, as an n-donor, reacted with *o-CA* reagent in ethanolic medium to form a charge transfer complex which produced a colored solution with a maximum absorption wavelength of 379 nm. Fig. 1 shows that the blank displayed an absorption band at 312 nm.

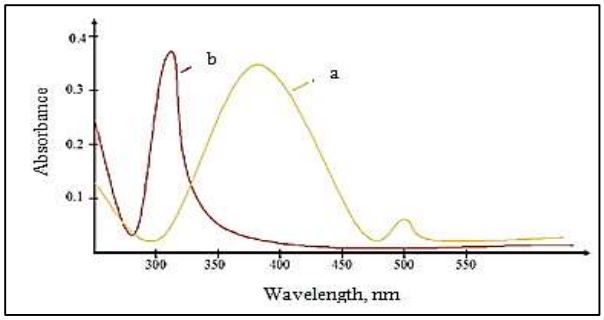


Figure 1. **The absorption spectra of TMP-*o-CA* complex against blank solution (a) and blank solution against ethanol (b).**

Optimization of the Experimental Conditions

Various factors influencing the intensity of the formed complex's absorption were studied in order to reach the optimum conditions for the interaction between the *o-CA* reagent and TMP medicine.

Effect of Solvents

To find out the best solvent that gives the highest sensitivity to the interaction between TMP and the *o-CA* reagent, different solvents were used, which included ethanol, methanol, acetone, acetonitrile, and water. The results recorded in Table 1 show that the complex is formed with the highest sensitivity when dissolving TMP with distilled water, and the *o-CA* with absolute ethanol, and final dilution has been done with absolute ethanol. This gave the highest absorption at 378 nm, and this solvent system has been adopted in subsequent experiments.

Table 1. **Effect of solvent on the absorbance of 10 µg/mL TMP.**

TMP dissolved in	*o-CA* dissolved in	Dilution solvent	λ max (nm)	Absorbance
Water	Ethanol	Ethanol	379	0.312
Water	Ethanol	Water	-	-
Ethanol	Ethanol	Ethanol	354	0.245
Ethanol	Ethanol	Water	361	0.213
Methanol	Methanol	Methanol	371	0.291
Methanol	Methanol	Water	-	-
Water	Methanol	Methanol	373	0.302
Water	Methanol	Water	-	-
Water	Acetone	Acetone	364	0.213
Water	Acetone	Water	409	0.115
Water	Acetonitrile	Acetonitrile	411	0.123
Water	Acetonitrile	Water	418	0.082

pH and Buffer Solutions Effects

HCl and NaOH at a concentration of 0.01 M have been used to study the effect of pH on the formation of the n-π charge transfer complex at various pH levels ranging from 2 to 12. As seen in Fig. 2, this complex was formed at pH 7.81 in the presence of 0.75 mL NaOH (Fig. 2). The addition of HCl revealed a significant decrease in absorbance, which may be related to the hydrogen chloride liberation. As a result, several types of buffer solutions, including borates, bicarbonates, and carbonates with a pH of 7.81, were studied on the absorbance of the complex. It was found that the buffer solutions lead to a decrease in absorption, so they were excluded.

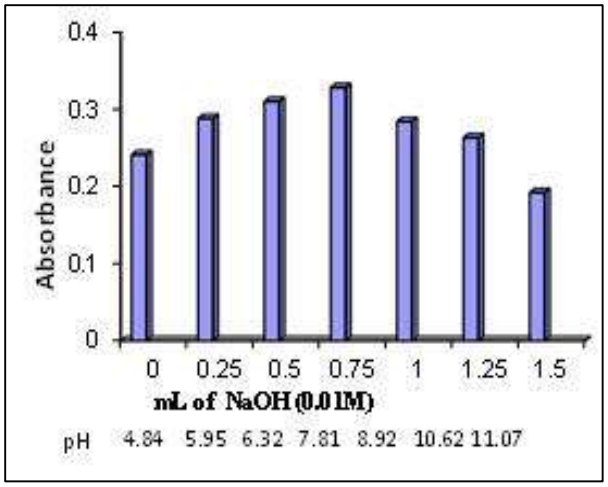


Figure 2. **Effect of pH on CTC absorbance using 10 µg/ mL TMP is present.**

In order to achieve the complex with high sensitivity, various bases, such as sodium hydroxide, sodium carbonate, sodium bicarbonate, and potassium hydroxide, were investigated in the presence of a fixed amount of TMP. These bases have a fixed volume of 0.75ml and a concentration of 0.01 M. The results showed that potassium hydroxide gave the best color intensity, and the optimum amount of this base was at 0.75 mL.

Effect of o-CA amount

Increasing volumes of 5×10^{-3} M *o-CA* solution, ranging from 0.1 to 1.4 mL, were added to equal amounts of TMP (10 µg/mL) in the presence of potassium hydroxide (0.75 mL) and added into volumetric flasks of 5 mL. Then the volumes were completed to the mark with absolute ethanol, left for 10 min at the laboratory temperature, and the absorbance was measured versus the blank solutions at 378 nm. The volume of 0.8 mL was the best amount to obtain the highest sensitivity.

Effect of Temperature, Reaction time, and Stability

In order to find out the optimal temperature at which the TMP-*o-CA* complex is formed, the formation of the complex has been studied at different temperatures ranging from the laboratory temperature 25 °C to 60 °C. The results showed that the highest sensitivity of the complex was at the laboratory temperature and the highest absorption was obtained after 5 min, with a stability time of 60 min (Fig. 3).

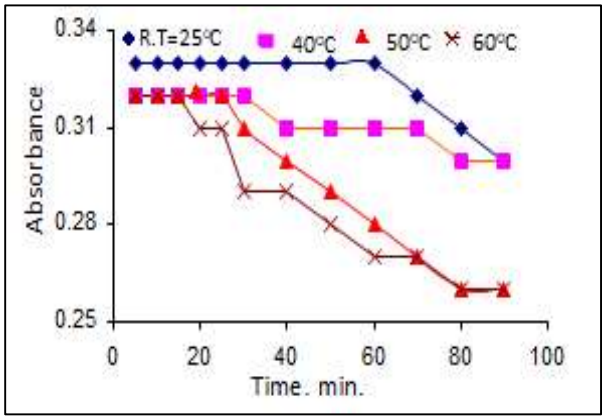


Figure 3. **Temperature and time effect on the absorption of 10 µg/mL TMP.**

Addition Sequence Effect

Three sequences of reagent additions were studied to achieve the highest sensitivity. The sequence TMP+*o-CA*+KOH was found to be the best.

Optical Properties and Method Statistics

A calibration graph of the colored TMP-*o-CA* complex was made by plotting absorbance against concentration under the optimum experimental conditions outlined in the suggested procedure. A good correlation coefficient was obtained. Beer's law was followed in the 5-60 µg/mL range, and the method's high sensitivity was indicated by its molar absorptivity value. The detection and quantitation limits were calculated according to the following equations:

$$LOD = 3\sigma/S$$
$$LOQ = 10\sigma/S$$

S is the slope of the calibration graph, and σ is the response's standard deviation.

Table 2 provides an overview of the proposed method's optical characteristics and statistics.

Table 2. **The optical characteristics and statistical data of the suggested method.**

Parameters	Value
Linearity conc. range (µg/mL)	5-60
Molar absorptivity (L/mol.cm)	1.12×10^4
Sandell sensitivity (µg/cm^2)	0.261
LOD (µg/mL)	0.0697
LOQ (µg/mL)	0.2323
Average recovery (%)*	101.57
RSD*	≤ 3.037
Coefficient of determination (R^2)	0.9982
Regression equation (Y)	y=0383X
Slope, *a*	0.0383
Intercept, *b*	0.0

*Average of four determinations

The Effect of Excipients

The effect of some drug excipients was studied in the determination of a solution containing 10 µg/mL of TMP. The results recorded in Table 3 showed that the excipients do not interfere even when they were present in high quantities (more than 50-fold excess), except for sodium chloride, which showed significant interference at high concentrations, and the reason may be due to the formation of a complex between the *o-CA* reagent and sodium ion as follows:

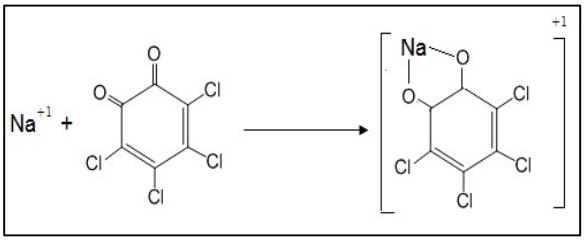


Table 3. **Excipient effects on TMP assay.**

Excipient	Recovery % of 10 µg/mL TMP per µg/mL excipient added					
	50	100	150	200	250	500
Glucose	96.8	100.8	96	98.4	99.2	101.6
Starch	97.6	96	98.4	99.2	100.8	102.4
Lactose	96.8	96	97.6	100.8	98.4	103.2
Acacia	96	97.6	99.2	100.8	104	103.2
NaCl	97.6	99.2	100.8	85.6	112	228.8

Validity of the Method

The suggested method was successfully applied for the estimation of TMP in a commercially available pharmaceutical (tablet). The results, cited in Table 4, indicated that the method is accurate, precise and showed no interferences with excipients. In order to know the reliability of the proposed method, it was compared with the standard method mentioned in the British Pharmacopoeia [21], which is based on the potentiometric titration of TMP in pure form by perchloric acid, through the t-test, which is utilized for comparing two groups' means to see if there is a significant difference between them, and the F-test to determine whether there are substantial differences between two or more groups by comparing their variances, by applying the following equations:

$$T_{exp} = \frac{|\mu - \bar{X}| \times \sqrt{n}}{s} \quad \text{F-test} = S^2{}_1 / S^2{}_2$$

where X, s, and n represent the average quantity found and standard deviation for replicates (n), respectively, µ is the drug's certified value, s_1^2 and s_2^2 are the variances of the first and second samples. It can be concluded from the results in Table (4) that the experimental t value is less than the tabular t value (2.571) and the experimental F value is less than its tabular value (9.28) at a 95% confidence level and for six degrees of freedom, which indicates that there is no significant difference between the two methods and that the proposed method is reliable and valid in application to pharmaceutical preparations.

Table 4. **Application of the proposed method for estimation of TMP in tablet formulation.**

Pharmaceutical preparation	TMP Concentration prepared (µg/mL)	Recovery* (%)	Average recovery (%)	TMP recovery per tablet (mg)	RSD	t-test	F-test
Bactrim tablet **	10	104.60					
(400 mg SMX/80mg TMP)	30	101.82	102.38	81.90	2.28	0.552	2.6
	50	100.73					

*Average of six determinations
** Manufactured by EUMEDICA Pharmaceuticals GmbH, Germany

Composition and Stability Constant (K_{st}) of the Complex

The molar ratio of the produced complex between TMP and the o-*CA* reagent was investigated using both continuous variations and mole ratio approaches [22]. The results are consistent with the idea that only one location (the more sterically free terminal basic primary amino group with *o-CA*) is involved in the interaction between the investigated drug and the reagent. The outcomes demonstrated that the complex was formed in a 1:1 ratio (Fig. 4).

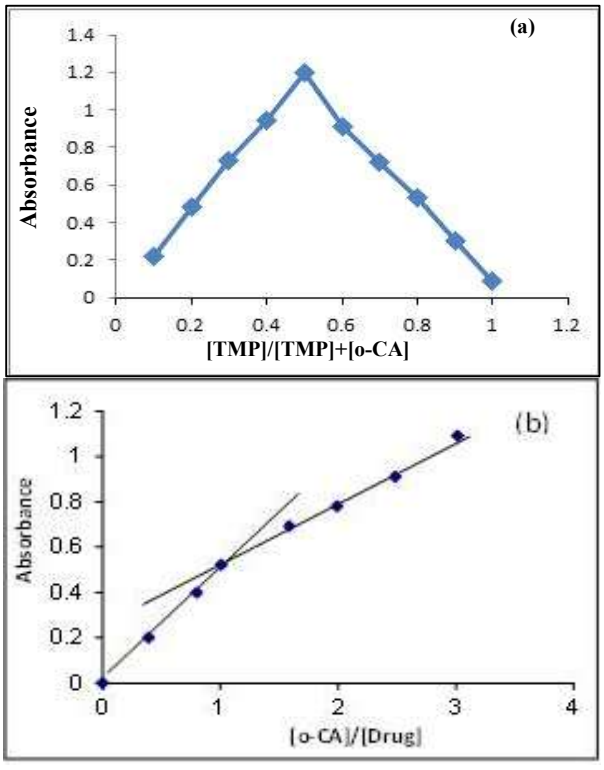


Figure 4. **Continuous variations (a) and mole ratio (b) plots of *o-CA* - TMP complex**

The apparent stability constant was calculated in accordance with the aforementioned findings by contrasting the absorbance of a solution having stoichiometric amounts of *o-CA* and TMP (As) to that of a solution containing an optimum amount of *o-CA* reagent (Am). By using the following equations, the complex's average conditional stability constants were determined:

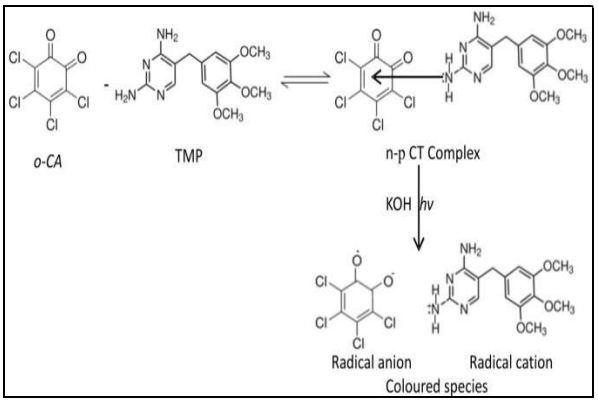
$$Kc = \frac{1-\alpha}{\alpha^2 C} \qquad \alpha = \frac{Am-As}{Am}$$

Where Kc is the apparent stability constant, α is the degree of dissociation, and C is the complex's concentration, which is the same as TMP's concentration.

It was found that the average stability constant was 2.425×10^3 L/mol, which indicates that the complex has good stability.

Suggested Mechanism

Charge transfer complexes have been formed when basic nitrogenous compounds that act as n-donors interact with π-acceptors. A radical anion is formed as a result of an electronic transition to an excited state that involves a partial transfer of electronic charge from the donor to the acceptor moiety [23]. The proposed mechanism is presented in Scheme 2.



Scheme 2: **Proposed mechanism of the formation of charge transfer complex in the assay of TMP by *o-CA***

Comparison of the Proposed Method

The suggested method was compared to other spectrophotometric methods described in the literature. As shown in Table 5, the proposed method is more delicate than the other mentioned alternative methods and is characterized by simplicity, rapidity, larger estimated ranges, and being more sensitive than other methods. It does not require heating, the complex is formed quickly, and it has good stability.

Table 5. **A comparison of the current method with other spectrophotometric methods in the determination of TMP.**

Analytical parameters	Present method *o-CA*	Literature methods			
		CA[a] [15]	DDQ[b] [15]	DNFB[c] [17]	Alizarin [24]
λ_{max} (nm)	379	523	585	538	390
solvent	Ethanol	1,4-dioxane	1,4-dioxane	Acetone	HCl
pH	7.81	-	-	7.4	<1
Temp. (°C)	R.T	R.T	R.T	60	30
Development time (min.)	5	30	30	40	10
Stability period (min.)	>70	-	-	40	-
Beer's law (µg/mL)	0-60	0.2-0.4	0.02-0.1	10-75	2-45
Molar absorptivity (L/mol.cm)	1.11×10^4	0.83×10^3	1.11×10^2	1.91×10^3	1.33×10^3
Recovery (%)	101.57	100.09	100.09	100.43	101.03
RSD (%)	≤ 3.037	0.740	1.890	1.067	≤ 2.97
Application	Tablet	Tablet	Tablet	Tablet	Tablet

[a] Chloranilic acid
[b] 2,4-Dinitro-1-fluorobenzene
[c] 2,3-Dichloro-5,6-dicyano-p-benzoquinine

Conclusion

The *o-CA* reagent was used in the direct spectrophotometric determination of TMP in the presence of a base, depending on the formation of an n-π charge transfer complex. The results indicated that the method is sensitive (molar absorptivity = 1.11×10^4 L/mol.cm), accurate (average recovery % = 101.57), precise (RSD= ≤ 3.037), and inexpensive (using a simple device and few reagents). The applicability of the method was also examined in comparison with the standard method by calculating t and F-tests, which confirmed the obtained results and also proved that the method has a good applicability and was successfully applied in the determination of TMP in the presence of some pharmaceutical additives. However, the limitation of the method is the need to eliminate SMX present in trimethoprim formulation, which causes significant interference with the estimation process.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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