



Determination of Trimethoprim Based on Charge-Transfer Complexes Formation

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(Received 25 Mar. 2013; Final version received 02 Jul. 2013)

Abstract

A spectrophotometric study concerning the interaction between Trimethoprim (TMP), Sulfamethoxazole (SFMx), as n-donor and 2,3-dichloro-5,6-dicyano-P-benzoquinone (DDQ) and chloranilic acid (CA) as π -acceptor were performed at 25°C. The results of interaction of CA and DDQ with TMP indicate the formation of a 1:1, 1:2, charge transfer complexes through non equilibrium reactions. In the case of SFMx, the formation of 1:2 (SFMx/DDQ, SFMx/CA) charge-transfer complexes is confirmed. The formation constants of the equilibrium step were evaluated from the computer fitting of the absorbance-mole ratio data. Also, this study was carried out to simultaneously determine quantitatively TMP in Cotrimoxazole. The complexes of TMP with DDQ and chloranilic acid absorbed maximally in 512 and 585, respectively.

Keywords: Trimethoprim, Sulfamethoxazole, DDQ, Chloranilic acid, Donor/acceptor complexation, KINFIT program, determination.

Introduction

The study of the charge-transfer complexes formed in the reaction of aromatic electron acceptors (π -acceptors) with various electron donors have attracted considerable interests and growing importance owing to their significant physical and chemical properties [1–6].

Charge-transfer complexes using organic species are intensively studied because

of their special type of interaction, which is accompanied by transfer of an electron from the donor to the acceptor [7, 8]. Also, protonation of the donor from acidic acceptors are generally route for the formation of ion pair adducts [9].

Recently, the charge transfer (CT) reactions had been studied spectrophotometrically in the determination of drugs that are easy

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to be determined based on CT complex formation with some electron acceptors. 7,7,8,8-Tetracyanoquinodimethane (TCNQ) and tetracyanoethylene (TCNE), DDQ and chloranilic acid are strong electron acceptors and are applied in the determination of several electron donor drugs.

The study of charge-transfer interactions is important as it may help to explain intramolecular interaction across space that

may be involved in the maintenance of structure of some biological macromolecules, or they may explain drug–drug and drug–receptor interactions. The combination of TMP-SFMx is known as co-trimoxazole, results in an *in vitro* synergistic antibacterial effect by inhibiting successive steps in folate synthesis [10, 11]. As a consequence, the use of co-trimoxazole was restricted in 1995 [12].

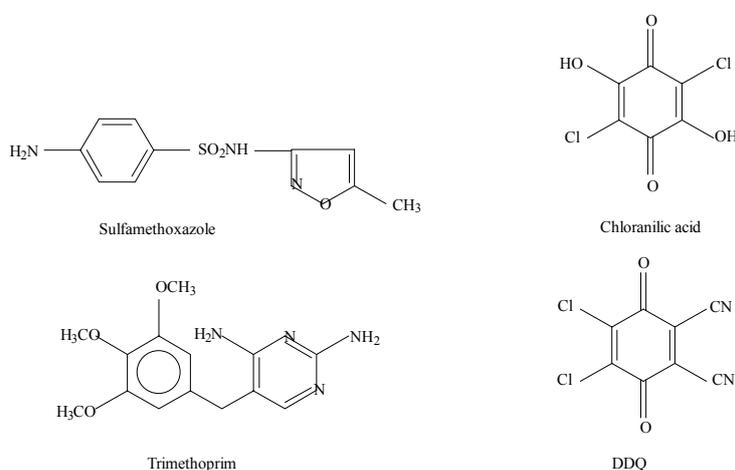


Figure 1. Used compounds structure.

Various techniques for the simultaneous determination of SFMx and TMP in pharmaceuticals have been developed [13-19]. The most widely used technique for simultaneous determination is HPLC [19, 20]. The present research aims chiefly to study the reaction of both DDQ and chloranilic acid, reagents (electron acceptors) at first time with TMP and SFMx (electron donors) and to use these reagents in spectrophotometric determination of the given TMP in pure form and in some of their pharmaceutical preparation and evaluation of equilibrium

constants of complexation of these drugs with chloranilic acid and DDQ. The used compounds structures are shown in Figure 1.

Experimental

Materials

TMP and SFMx were kindly provided from Alborz Pharmacy company, Iran. Chromatropic acid was purchased from BDH Chemicals. All of chemical compounds were obtained from MERCK chemicals. All other reagents used were of analytical grade and were freshly prepared each time.

Apparatus

AJASCO model V-530 UV-Vis spectrophotometer with 1 cm matched cell was used for electronic spectral measurements.

Determination of absorption maxima

DDQ (1×10^{-2} M) & chloranilic acid (2×10^{-2} M) solutions were prepared in 1,4-dioxan and its absorption maximum determined using spectrophotometer. Solutions of SFMx (4×10^{-2} M) and TMP (2×10^{-2} M) were also prepared in 1,4-dioxan. One milliliter each of SFMx and TMP solutions was transferred into 10- ml volumetric flasks and 1.0 ml of chloranilic acid or DDQ added. The volumes were brought up to mark with more 1,4-dioxan solvent. The solutions were scanned on a UV-visible spectrophotometer (Figures 2 and 3). Higher concentrations of the reagent did not affect the color intensity.

Spectrophotometric titrations

Titration of TMP (2.0×10^{-3} M) or SFMx (4×10^{-4} M) were carried out by the addition of microliter amounts of a concentrated standard solution of the CA or DDQ in using a pre-calibrated micropipette, followed by absorbance intensity reading at 25.0°C at the related λ_{max} . The complete color development was obtained by raising the temperature to $50 \pm 2^\circ\text{C}$ on a water-bath for 15 min. But, the color reaction should be carried out at room temperature (25°C) after 30 min.

Construction of calibration curves

Standard solutions of TMP equivalent to 2.0 mg/ml were prepared in 1,4-dioxan. Serial volumes of the TMP stock solution, i.e. 1.0, 2.0 to 5.0 ml were accurately transferred to 10-ml volumetric flasks and 1.0 ml of chloranilic acid or DDQ each added. Triplicate preparations for each volume of TMP were measured. The solutions were left to stand for 30 min before their absorbance was measured.

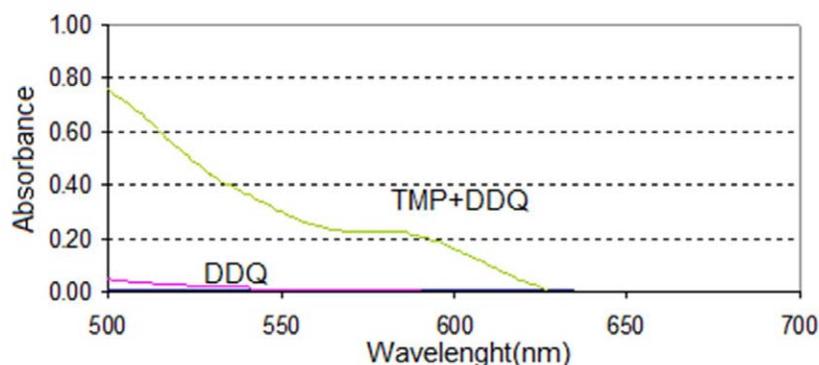


Figure 2. Absorption spectra of TMP, charge transfer complex of TMP and DDQ in the range of 500-700 nm.

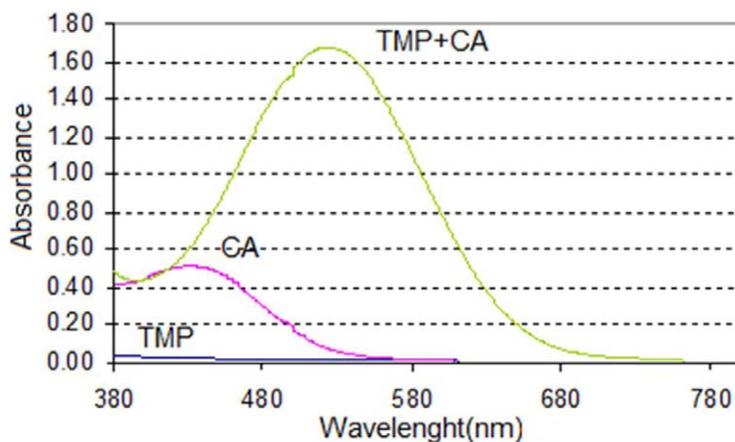


Figure 3. Absorption spectra of TMP , charge transfer complex of TMP and CA in the range of 380–780 nm.

Simultaneous assay of TMP in their dosage forms

For assay of TMP in their dosage forms, brand of pharmaceutical tablets containing SFMx and TMP were extracted into 100-ml volumetric flasks using 1,4- dioxan as

described previously. Serial volumes were transferred into 10-ml volumetric flasks and 1.0 ml chloranilic acid or DDQ added to each flask. Their absorbance was read at their absorption maxima.

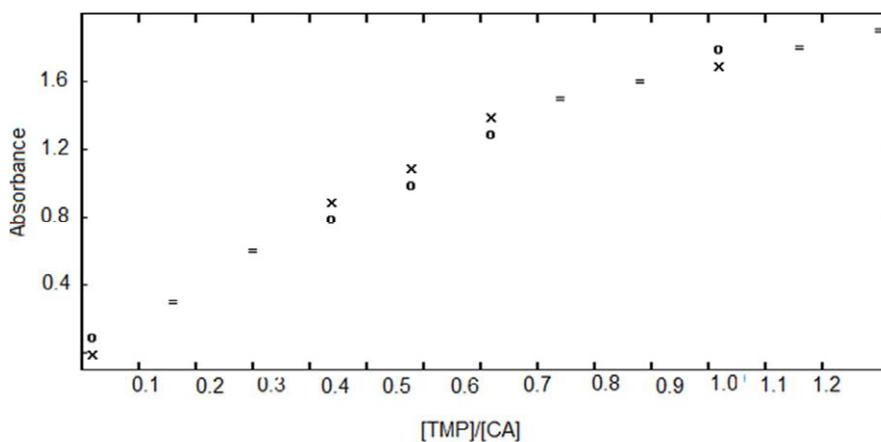


Figure 4. Computer fits of the plots of absorbance vs. $[TMP]/[CA]$ mole ratios at 25 °C: (x) experimental point, (o) calculated point, (=) experimental and calculated points are the same within the resolution of the plots.

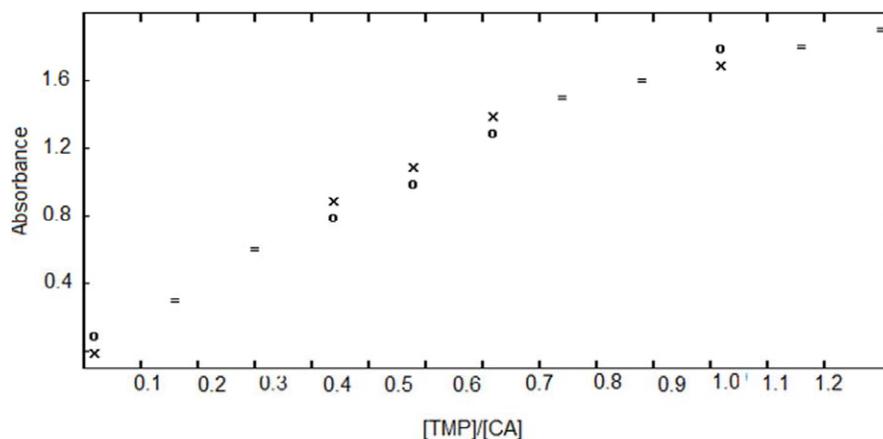


Figure 4. Computer fits of the plots of absorbance vs. [TMP]/[CA] mole ratios at 25 °C: (x) experimental point, (o) calculated point, (=) experimental and calculated points are the same within the resolution of the plots.

Results and discussion

The composition of the complexes and stability constants

The electronic absorption spectra of SFMx and TMP and their DDQ or chloranilic acid complexes are shown in Figures 2 and 3. The stoichiometric ratio of SFMx and TMP to DDQ or chloranilic acid in the complexes were determined by the Job method of equimolar solutions. Also, the stoichiometries of the Charge-Transfer complexes were examined by the mole ratio method at λ_{\max} of their complexes.

The formation constants of the resulting complexes were obtained at 25°C by absorbance measurements of solutions in which varying concentrations of CA or DDQ were added to fixed amounts of TMP or SFMx solutions, at λ_{\max} of complexes.

For evaluation of the formation constants of the resulting 1:1 TMP:CA or 1:2 drugs:DDQ

and SFMx:CA complexes, K_f , from the absorbance-mole ratio data, the nonlinear least-squares curve fitting program *KINFIT* was used [21]. The program is based on the iterative adjustment of calculated to observed absorbance values by using either the Wentworth matrix technique [22] or the Powell procedure [23]. Refinement of the parameters was continued until the sum-of-squares of the residuals between calculated and observed values of the absorbance for all experimental points was minimized. The mass balance equation of AD type (Eq. (1) used in computer program *KINFIT* should be solved in order to obtain Eq. (2) for the free donor concentration [D].



$$K = \frac{[AD]}{[A][D]}$$

$$C_A = [A] + [AD], \quad C_D = [D] + [AD] \quad (1)$$

$$K[D]^2 + (1 + K(C_A - C_D))[D] - C_D = 0 \quad (2)$$

The observed absorbance of solution is also given by the following equation:

$$A_{obs} = \varepsilon_D[D] + \varepsilon_{AD}[AD] \quad (3)$$

Where ε values are the molar absorptivities of the species denoted. For evaluation of the formation constants of the resulting 1:2. The mass balance equations can be written as Eqs.

$$(2K_f - 1)[D(A)_2]^2 - K_f(C_A + 2C_D)[D(A)_2] + K_f C_A C_D = 0 \quad (7)$$

The complex concentrations, $[AD_2]$, were calculated from Eq. 7 by means of a Newton-Raphson procedure. Once the value of $[AD_2]$ had been obtained, the concentrations of all other species involved were calculated from the corresponding mass balance equations by using the estimated value of K_f at the current iteration step of the program. Refinement

4 and 5, and the formation constant of the complex as in Eq. 6:

$$C_A = [A] + 2[D(A)_2] \quad (4)$$

$$C_D = [D] + [D(A)_2] \quad (5)$$

$$K_f = [D(A)_2]/[D][A]^2 \quad (6)$$

Substitution of Eqs. 4 and 5 into 6 and rearrangement yields Eq. 7:

of the parameters was continued until the sum of squares of the residuals between calculated and observed absorbance values for all experimental points was minimized. The output of the program *KINFIT* comprises the refined parameters, the sum of squares and the standard deviation of the data.

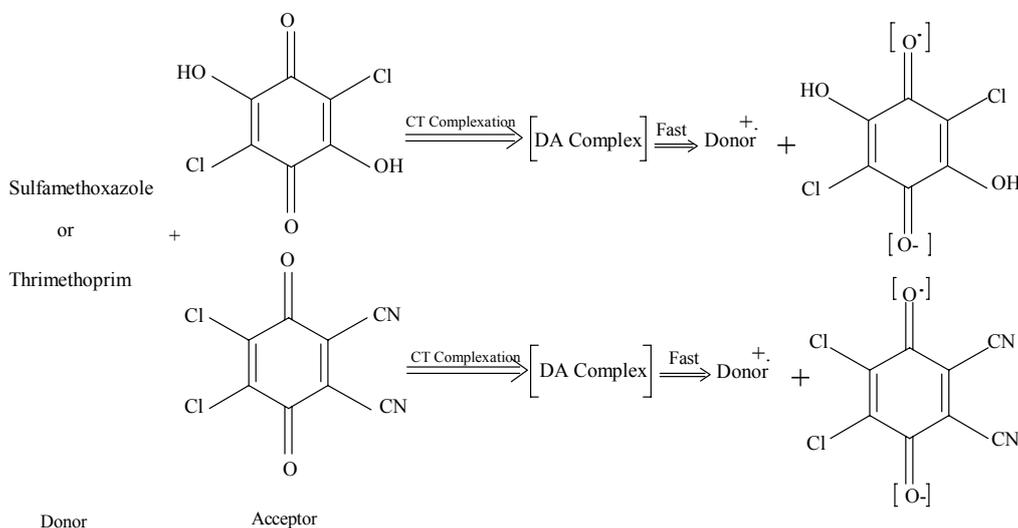


Figure 6. Reaction possible mechanism.

Figures 4 and 5 show the resulting computer fit of the absorbance mole ratio data. (two sample of the resulting plots). As seen, the fair agreement between the calculated and

observed absorbances further supports the existence of a 1;1 or 1:2 complexation between donor and acceptor.

Determination of TMP in Co-trimoxazole 400/80

The complexes formed between DDQ, chloranilic acid and TMP, appeared amber and violet with absorption maxima at 512 and 585 nm, respectively (Figures 2,3). SFMx didn't appear peak in this region. The observed colour change suggests complex formation between DDQ, chloranilic acid and drug. This observation is associated most with donor and acceptor interaction as is the case in this investigation. Suggestion mechanism is represented in Fig. 6. The relationship between the absorbance and concentration was quite linear in the concentration ranges given in (Table 1). The intercept (a), slope (b), correlation coefficient (r), molar absorptivities (ϵ), and sandell sensitivity values are summarized in (Table 1). The detection limit

(LOD) and the limits of quantization LOQ is defined as the minimum level at which the analyte can be reliably detected for the 3 drugs was calculated and listed in Table 1.

The accuracy and precision of the methods were evaluated by performing six replicate analyses on pure drug solution at four different concentration levels (within the working range). Percentage relative standard deviation (RSD %) of the proposed spectrophotometric methods were calculated. The relative standard deviation (RSD) indicates good repeatability of the suggested methods. The following equations satisfied the mathematical model developed for the linearity of the range of concentrations employed.

$$A_1 = 0.1937 X_1 + 0.1276 \text{ (for TMP+ DDQ)}$$

$$A_2 = 0.4216 X_2 + 0.3114 \text{ (for TMP + chloranilic acid)}$$

Table 1. Optical characteristics and statistical data of the regression equations for the complexes of CA, DDQ and TMP and SFMx.

Parameters	SFM/ddq	TMP /CA	TMP/ddq	SFMx /CA
$\lambda_{\max} (nm)$	270	523	585	296
Molar absorbitivity ($l \text{ mol}^{-1} \text{ cm}^{-1}$)	6717	836.6	111.41	5838
Beer s law rang (μgml^{-1})	8-80	0.2-0.4	0.02-0.1	8-80
Sandel s sensitivity (μgcm^{-2})	0.076	0.1607	0.001	0.1279
Limit of detection (μgml^{-1})	0.2127	0.0104	0.039	0.3039
Limit of quantitation (μgml^{-1})	0.644	0.0316	0.1184	0.9209
Confidence limits for intercept	± 0.032	± 0.0013	± 0.002	± 0.033
Confidence limits for slope	± 0.0013	± 0.0481	± 0.216	± 0.053
Log k	6.72	6.54	6.69	7.21
Regression equation(Y)				
Slope(b)	0.028	5.149	4.033	0.0254
Intercept(a)	0.5959	0.4697	0.1625	0.4106
Correlation coefficient(r)	0.9931	0.9946	0.9911	0.9932
R.S.D %	0.911	0.7407	1.8909	0.0473
Range of error (95%)Confidence limits	0.1118	0.0908	0.0913	0.0976

Conclusions

Spectrophotometric analysis by charge-transfer technique is constrained by the nature of interactions that take place between the donor and acceptor molecules, influence of solvent and stability of the complex formed. Molecules that absorb in the visible region, usually absorb very strongly so that absorbances can readily and accurately be determined. From the calculation of stability constants of the complexes, the later were found to be stable. The TMP complexes formed under the above mentioned conditions offer a sensitive, simple, reproducible and accurate procedure for the determination of TMP in bulk, tablets dosage forms. The low values of RSDs are an indication of the precision and accuracy of the measurements.

The spectrophotometric methods use an inexpensive instrument compared to many reported procedures. The methods are simple and rapid taking not more than 20–25 min for the assay. In terms of the linear range of applicability, the spectrophotometric methods are even more sensitive than the chromatographic [19, 20] and are free from such experimental variables as heating or extraction step. The methods should therefore find ready application in pharmaceutical industrial quality control.

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