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Physico-chemical studies on the charge-transfer complex formed between sulfadoxine and pyrimethamine with chloranilic acid

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Abstract

Thermodynamic studies on the charge-transfer complexes of sulfadoxine and pyrimethamine with chloranilic acid in non-aqueous 1,4-dioxan were investigated by spectrophotometric method. The absorption maxima for sulfadoxine and pyrimethamine were observed at 500 and 520 nm, respectively. We observed a bathochromic shift in the transitions of these complexes with respect to chloranilic acid, which absorb at 420 nm. The enthalpy of complexation was found to be −3.044 kJ/mole for pyrimethamine and −5.934 kJ/mole for sulfadoxine. Equilibrium constant values were generally high, that is 261.202 l/mole and 344.29 l/mole for pyrimethamine and sulfadoxine, respectively, at 303 K. Free energy values were negative, of the order of −14.420 kJ/mole for pyrimethamine and −15.384 kJ/mole for sulfadoxine, indicating that complex formations were exothermic. These data suggest that the complexes are very stable. Arguments are presented to support the application of charge-transfer interactions in the quantitative analysis of these drugs. © 2002 Published by Elsevier Science B.V.

Keywords: Sulfadoxine; Pyrimethamine; Charge-transfer complexation; Spectrophotometric analysis

1. Introduction

All molecules when in contact with each other somehow interact creating some kind of intermolecular force. These forces may be small when compared with intramolecular forces within the molecule [1]. Compounds with unshared pairs of electrons may interact with other compounds through the donation of such electrons in a manner different from the traditional dative bond formation. Those interactions giving rise to intermolecular forces may be sufficiently strong or show features that do not exactly fit the definition of the classical dipole–dipole, dipole-induced dipole and/or van der Waals interactions.

Depending upon the orbital that accepts these electrons, this form of interaction may be called π- or δ-donor. Of course acceptors may also be described as π- or δ-acceptors. π-Acceptors are the most common type of organic acceptors. They generally include unsaturated systems like aromatic or hetero-aromatic nuclei containing electron-withdrawing groups such as nitro, cyano, halo, quinones and acid anhydrides [1,2].

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Although these interactions are divided into electron donors or acceptors, it must be recognized that this division is entirely relative. For example, one species may be a donor in one instance and an acceptor in another, e.g. iodine. Chemists recognized very early that these donor–acceptor interactions are a form of addition reactions that do not fit normal rules of valency [2]. Many compounds that engage in donor–acceptor interactions produce highly coloured compounds. Very often these products exist only in solution formed in equilibrium with the component molecules.

Solutions of donors and acceptors, particularly of the π-donors and π-acceptors give little evidence of association other than the observed spectra, suggesting that the lifetime of the complex which is evidently present, must be extremely short, probably of the order of a few vibrational periods of the molecules [1,2].

It is the understanding of this phenomenon that has been utilized in the spectrophotometric assay or analysis of pharmaceutical products [3–10]. Similarly, studies have been carried out in which π-acceptors were utilized as locating (spray) reagents on thin layer chromatographic plates for detection of mixtures containing electron-donating molecules [11,12]. Charge-transfer interactions have also been employed to the quantitative determination of carbonyl compounds after derivatization [13].

No report has appeared in the literature involving the interaction between a π-acceptor like chloranilic acid with sulfadoxine or N^1-(5,6-dimethoxypyrimidin-4-yl) sulfanilamide and pyrimethamine or [2,4-diamino-5 (*p*-chlorophenyl)-6-ethylpyrimidine]. They are very important anti-malarial agents with amino functional groups (potential π-donors).

The objective of the present study is to investigate the thermodynamics of complexation in terms of equilibrium constants (K_e), free energy changes (ΔG), enthalpy (ΔH) and entropy (ΔS), in relation to the stability of the complexes. The information gained on the stability of the complex will guide the subsequent adaptation of the technique to the quantitative determination of these drugs.

2. Experimental

2.1. Materials

Fansidar® tablets were purchased from the Pharmacy Department of the Jos University Teaching Hospital Jos, Nigeria. Methanol (AnalaR grade: BDH Chemicals, England), chloroform (AnalaR BDH Chemicals, England), chloranilic acid (Riedel de Haem, Germany) was recrystallized from acetone, 1,4-dioxan (May and Baker, England) was used without further purification. All the other reagents used were of analytical grade.

2.2. Isolation of sulfadoxine and pyrimethamine from pharmaceutical formulation

Fansidar® tablets containing 500 mg each of sulfadoxine and 25 mg each of pyrimethamine equivalent to 5.0 g of sulfadoxine was powdered and transferred into a beaker. 100 ml of aqueous methanol (4:1) was added with constant stirring for 15 min. The mixture was filtered and the residue returned to the beaker. Further extraction was carried out until all the sulfadoxine was isolated from the excipients. The aqueous methanol was filtered, evaporated to dryness under reduced pressure and the crystals of sulfadoxine recrystallized from water. The sulfadoxine isolated was subjected to identity tests using the methods described in the British Pharmacopoeia [14]. Pyrimethamine is practically insoluble in water and so could not contaminate sulfadoxine so obtained. The purity of the isolates was further confirmed by thin layer chromatography.

Similarly, pyrimethamine was extracted from it tablet formulation (Daraprim®) by continuous extraction with chloroform. Again the chloroform extract was evaporated and the pyrimethamine recrystallized from 96% ethanol. The pyrimethamine thus isolated was also subjected to identity and TLC tests by the method described in the British Pharmacopoeia [14]. The other components of the formulations were insoluble in 1,4-dioxan and therefore the presence of interfering substances were eliminated from the isolates.

2.3. Determination of absorption maxima for chloranilic acid and its complexes with sulfadoxine and pyrimethamine

Solutions (1% each) of chloranilic acid, sulfadoxine and pyrimethamine were prepared in 1,4-dioxan and their absorption maxima determined. 1.0 ml volume of chloranilic acid was added individually to 1.0 ml each of sulfadoxine and pyrimethamine contained in a volumetric flask. The volumes were made up to mark with dioxan. The solutions were allowed to stand for 30 min for the colour to be fully developed before scanning on a UV/visible spectrophotometer (Pye-Unicam SP 800, England).

2.4. Determination of extinction coefficient

Stock solutions of sulfadoxine and pyrimethamine equivalent to 5 mg/ml was prepared in 1,4-dioxan. Serial volumes of 1.0, 2.0 to 5.0 ml were transferred to 10 ml volumetric flasks and 1.0 ml chloranilic acid added to each flask. The solutions were brought up to mark with more 1,4-dioxan. Triplicate preparations were made, the contents were thoroughly mixed and their absorbances measured after 30 min. The absorbances were further monitored for another 5 h at regular intervals of 1 h.

The means of the absorbances were calculated and plotted against the concentrations. The curve was regressed by the method of least squares [15]. The slope gives the measure of the extinction coefficient.

2.5. Determination of stoichiometric relationships

Equimolar concentrations of sulfadoxine and pyrimethamine, 5.0×10^{-2} M each were prepared. A series of mixtures of chloranilic acid with sulfadoxine and pyrimethamine were prepared in the following ratios: 1:9, 2:8, 3:7, to 9:1. The complexes were allowed to stand for 30 min before their absorbances were measured at the respective absorption maximum. The continuous variation plots were constructed for each complex.

2.6. Determination of equilibrium constant and free energy change

The equilibrium constant and the free energy change of the interaction of chloranilic acid with sulfadoxine and pyrimethamine were, respectively, determined essentially by the method reported earlier [16]. Serial volumes of the solution of sulfadoxine (5.0×10^{-2} M), i.e. 1.0, 2.0, to 5.0 ml were transferred to different test-tubes and 1.0 ml of chloranilic acid (5.0×10^{-2} M added to each test-tube. The contents were mixed thoroughly and the volumes brought up to 10 ml by adding 1,4-dioxan. The mixtures were analyzed at the absorption maximum. The procedure was repeated for pyrimethamine.

Further analyses of the solutions were carried out by repeating the procedure at 30, 40, 50 and 60 °C in a thermostated water bath.

3. Result and discussion

Sulfadoxine and pyrimethamine isolated from the formulation were very pure since the determined melting point of 198–201 °C for sulfadoxine and 240–243 °C for pyrimethamine are consistent with the literature values of 197–200 °C and 239–242 °C, respectively. The purity was further confirmed on TLC plates. The isolated drugs were found to be stable for over 1 month.

Solutions of sulfadoxine and pyrimethamine in the presence of chloranilic acid spontaneously formed purple complex with absorption maxima at 500 and 520 nm, respectively. This represents a bathochromic shift with respect to chloranilic acid that absorbs at 420 nm. The purple colour was stable over a period 24 h, therefore we reasonably believed that the analyte in solution was also stable during the actual period of the analysis which was 90 min. The procedure was optimized by the choice of 1,4-dioxan which has a lower dielectric constant that other organic solvents like chloroform and acetonitrile, therefore is likely to dissolve less of the excipients contained in the formulation. The limits of detection of these complexes were 5.0 ppm for pyrimethamine and 10.0

ppm for sulfadoxine. Quantitative analyses (LOQ) were reproducible at 15.0 and 20.0 ppm for pyrimethamine and sulfadoxine, respectively. Curves generated in these optimization procedures (linearity test) satisfy the following equations: $y = 0.25x + 0.00619$ for pyrimethamine and $y = 0.193x + 0.00952$ for sulfadoxine. The relative standard deviations were 0.895 and 1.005%, respectively.

Pyrimethamine and sulfadoxine both contain amino groups that are powerful electron donors so in this investigation we believe that the complexation is specific between chloranilic acid and the drug molecules. Excipients did not give any colour reaction with chloranilic acid. We therefore assume that there were no interferences for other constituents of the formulation. The absorption maxima for chloranilic acid, sulfadoxine and pyrimethamine are illustrated in Fig. 1.

The spontaneous formation of purple colour from yellow appearance of chloranilic acid upon reaction with these drugs is sufficient evidence that charge-transfer complex was formed since sulfadoxine and pyrimethamine used were in their pure state. We attribute these bands (Fig. 1) to charge-transfer transitions from the π-donors to π-acceptor i.e. chloranilic acid. The molar extinction coefficients for the complexes are 105.04 for sulfadoxine and 161.55 for pyrimethamine. These figures were obtained from the regressed plots, which satisfied the equations generated earlier. The continuous variation plots for both drugs shows the stoichiometric ratios to be 1:1 each (Fig. 2). Similar stoichiometry has been reported in the literature for nearly all investigations involving drug molecules [4,5,17–20].

Based on the stoichiometry of 1:1, the equilibrium constant for the interactions was investigated [20]. The interaction between a π-donor (D) and an π-acceptor (A) may be formulated as follows [1]:

$$D + A \leftrightarrow D{:}A \quad (1)$$

Benesi–Hildebrand model equation [21] was adapted to evaluate the equilibrium constant, K_e, for this form of interaction as follows

$$[A]_0/A = 1/K_e^A\ \varepsilon_\lambda^{AD}[D]_0 + 1/\varepsilon_\lambda^{AD} \quad (2)$$

where $[D]_0$ and $[A]_0$ are the initial concentrations of the donor and acceptor species; A is the ab-

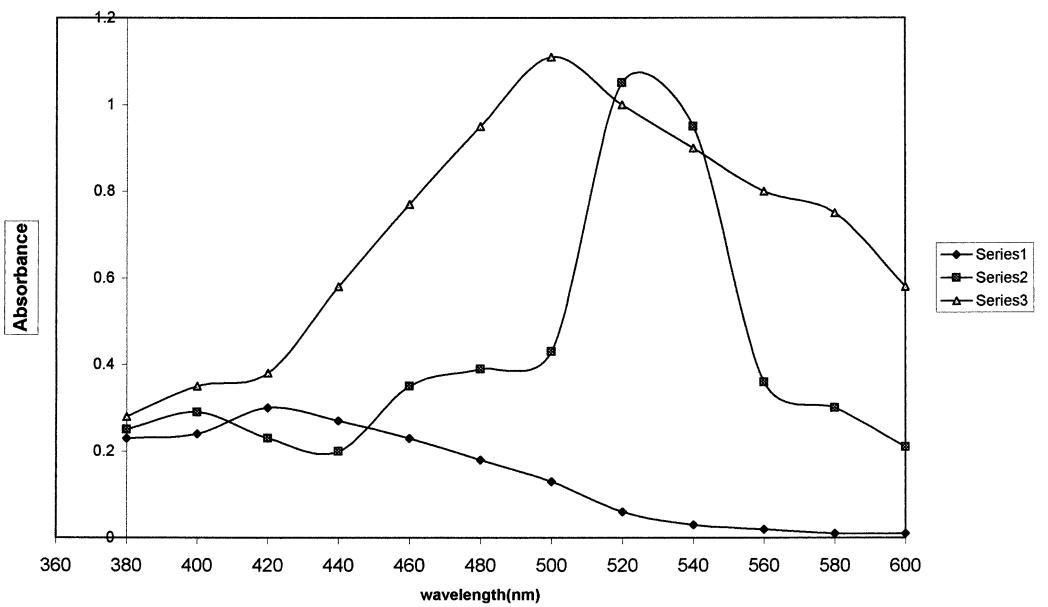

Fig. 1. Absorption maxima for chloroanilic acid (1), chloroanilic acid–pyrimethamine (2) and chloroanilic acid–sulfadoxine complexes (3).

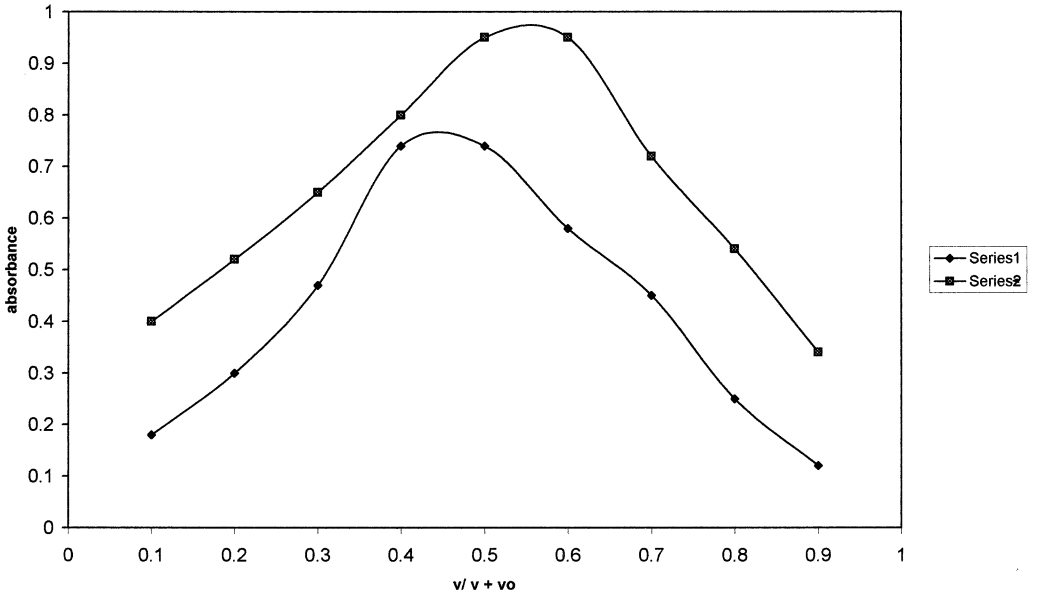

Fig. 2. The continuous variation plot of the complex between chloroanilic acid with pyrimethamine (1) and sulfadoxine (2).

sorbance of the complex at λ_m; ε_λ^{AD} is the molar absorptivity and K_e^{AD} is the equilibrium constant.

The plots of $[A]_0/A$ versus $1/[D]_0$ gave a linear graph from which the values of the K_e^{AD} and ε_λ^{AD} were obtained, respectively, from the slopes and intercepts. The curves were regressed by the method of least squares [15]. The fundamental requirement for the application of Benesi–Hildebrand equation is that $[A]_0$ must be kept constant and low and $[D]_0 \gg [A]_0$ so that K_e^{AD} can be extrapolated to infinite dilution. Graphical representations of the interaction between chloranilic acid, sulfadoxine and pyrimethamine at different temperatures employing Eq. (2) are shown in Figs. 3 and 4. These plots are satisfied by the following equations: $y = 0.0157x + 0.185$ and regression coefficient of 0.98 for Fig. 3 and $y = 0.0356x + 0.133$ and regression coefficient of 0.96 for Fig. 4. The calculated intercepts varied slightly with temperature. The curves appear packed closely because of the slight differences in K_e^{AD} values with temperature.

The experimental point of each curve has been omitted because of the closeness of the curves to each other.

The Gibbs free energy, enthalpy and entropy changes were calculated from the following well-established equations.

$$\Delta G = -2.303RT \log K_e \quad (3)$$

$$\log K_e^{AD} = -\Delta H/2.303RT + \text{constant} \quad (4)$$

$$\Delta G = -\Delta H - T\Delta S \quad (5)$$

The enthalpy of the reaction was obtained by plotting $\log K_e^{AD}$ against the reciprocal of temperature (Eq. (4)), from which the slope yielded ΔH (see Fig. 5). Again the plots are described by the regression equations $159x + 0.00419$ for pyrimethamine and $310x + 0.00419$ for sulfadoxine. The entropies were then calculated from Eq. (5). These parameters are represented in Table 1.

Although the molar absorptivity of the absorbing species is supposed to be independent of temperature, empirically this is not always true, hence the slight variations in this study. The figures represented in Table 1 are averages calculated from the different curves.

Generally, the equilibrium constants derived from the Benesi–Hildebrand equations are high and the figures presented here are consistent with earlier reports [16]. The equilibrium constants il-

lustrated in Table 1 show slight decrease in value with temperature indicating that the complex formation was an exothermic interaction. The high values of the equilibrium constants, free energy changes and entropy explain the spontaneity of the complexation. Even though the enthalpy of complexation is relatively low, it is significant to the spontaneous formation of the complex and its stability.

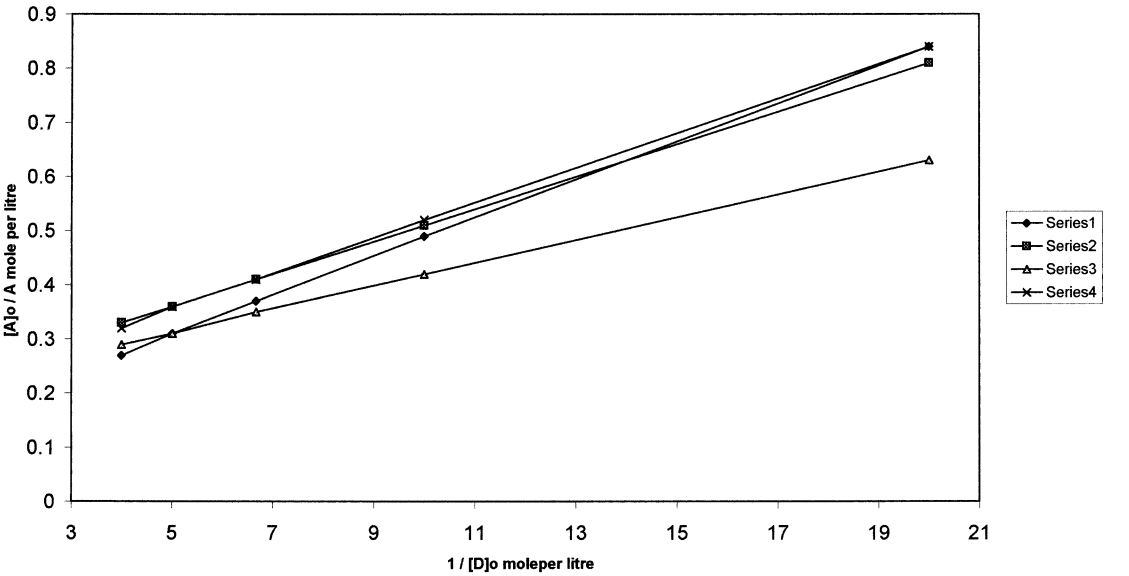

Fig. 3. Plot of $[A]_0/A$ against $1/[D]_0$ for pyrimethamine–chloroanilic acid complex at different temperatures (S1 = 30 °C, S2 = 40 °C, S3 = 50 °C, S4 = 60 °C). $[D]_0 = 5.02 \times 10^{-2}$ M and pyrimethamine $[D]_0 = 0.05$–0.25 M.

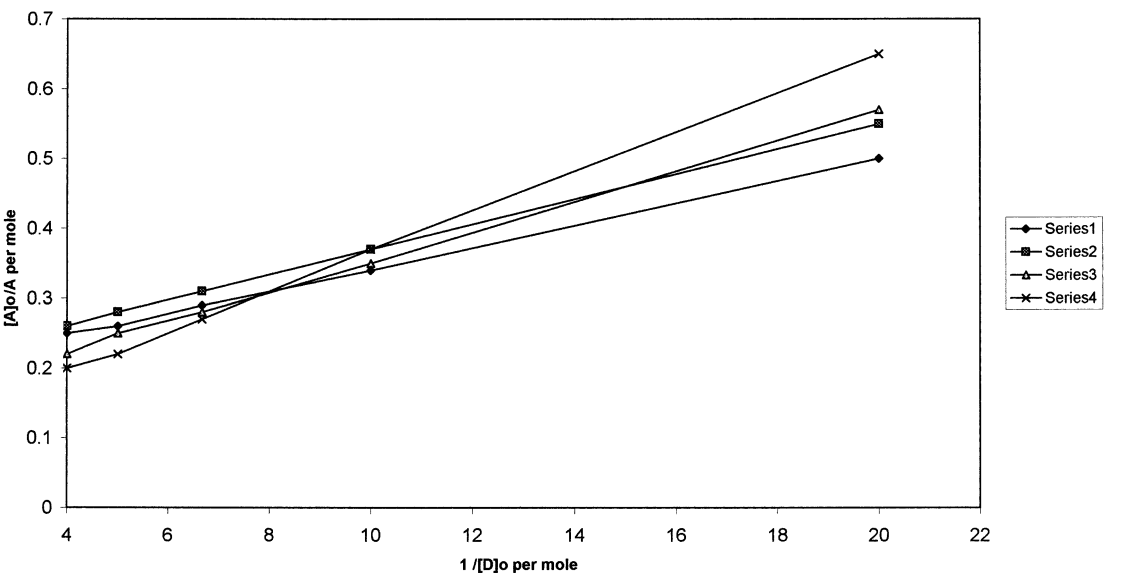

Fig. 4. Plots of $[A]_0/A$ against $1/[D]_0$ for sulfadoxine–chloroanilic acid complex at different temperatures (S1 = 30 °C, S2 = 40 °C, S3 = 50 °C, S4 = 60 °C). $[A]_0 = 0.05$ M and sulfadoxine $[D]_0 = 0.05$–0.25 M.

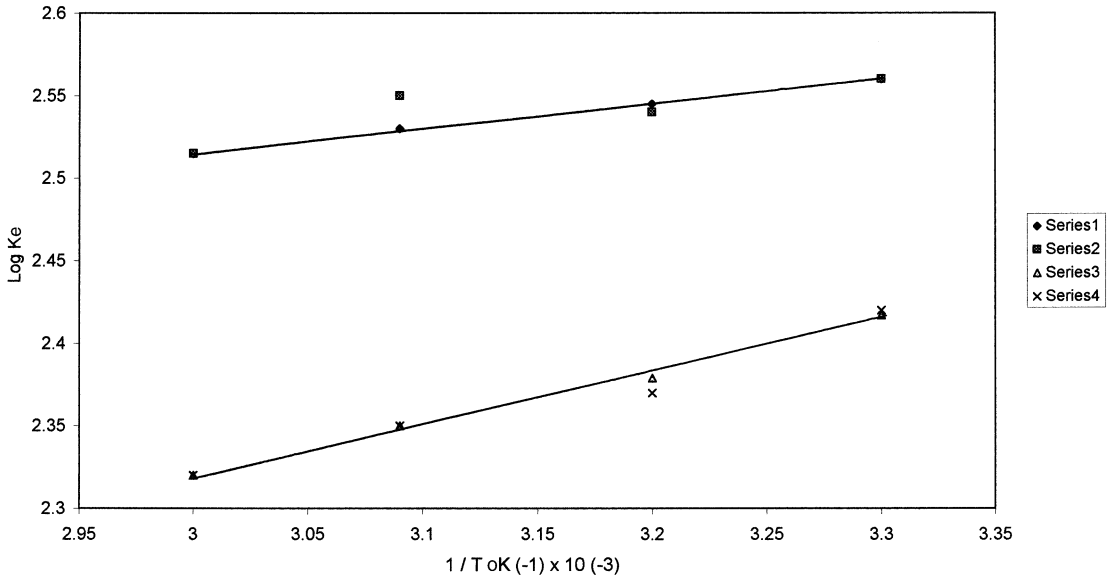

Fig. 5. Plot of log K of sulfadoxine (1) and pyrimethamine (3) complexes with chloranilic acid against $1/TK$ (-1). Serial 2 and 4 are experimental points.

Table 1
Thermodynamic changes following the complexation reaction between chloranilic acid and pyrimethamine and sulfadoxine at different temperatures

Temperature (K)	K_e (mole)	ΔG (kJ/mole)	ΔH (kJ/mole)	ΔS (kJ/mole)
Pyrimethamine–chloranilica acid complex ($\varepsilon_{520} = 161.55$)				
303	261.202	−14.022		−0.0362
313	239.436	−14.259	−3.044	−0.0358
323	229.190	−14.597		−0.0358
333	208.604	−14.801		−0.0353
Sulfadoxine–chloranilic acid complex ($\varepsilon_{500} = 105.04$)				
303	344.29	−14.718		−0.029
313	340.6	−15.176	−5.934	−0.0295
323	338.62	−15.745		−0.0301
333	322.66	−15.996		−0.0302

K_e is the equilibrium constant, ΔG is the free energy change, ΔH is the enthalpy change and ΔS is the entropy change.

The thermodynamic parameters presented in this study may be affected by several variables like the formation of termolecular and isomeric complexes, solvation of the component molecules, interference due either to the donor or acceptor on the absorption maximum of the complex and temperature. The preliminary observation that Beer–Lambert Law was satisfied at both low and high concentrations of donor and acceptor molecules and the stoichiometry of 1:1 strongly suggest that isomeric or termolecular complexes were not formed. Virtually all the reports earlier presented on donor–acceptor interactions in the analysis of pharmaceutical products showed a 1:1 stoichiometry [5–10,20,22]. Similarly, the low solvating power of 1,4-dioxan and the relatively large sizes of the drug molecules mean that solvation effects were absolutely minimal. Also the

donor did not absorb in the visible region of the spectrum and so could not have interfered with the absorption of the complexes.

Temperature effects equilibrium constant negatively if the reaction is already exothermic and if it leads to changes in the structure of the solvent. The equilibrium constants in this study decrease with increase in temperature, thus supporting the exothermic nature of the interaction. The working temperature of 30–60 °C reported here had no effect on the structure of 1,4-dioxan because the boiling point of dioxan is much higher (b.p. 101 °C). Since the limitations enumerated above have been minimized, the thermodynamic values obtained approximate closely with the true or absolute values. From general principles, when K_c, ΔH and ΔS are large and negative, the products formed are stable.

The existing methods of determining thermodynamic and kinetic parameters include direct heat measurements and determination of activities of the reacting species. These procedures are suitable for reactions that evolve large quantities of heat energy. As would be expected, the dynamic changes associated with π-donor/π-acceptor complexations would be extremely small; so that adapting the classical methods would fail to measure the heat changes accurately. Spectrophotometric technique, despite its limitations, represents a more sensitive and accurate method of investigating the energy changes associated with the complexation. This is because each mole of the complex formed absorbs strongly in the visible spectrum and can be accurately determined. The procedure for adapting this technique for analytical purposes should involve the construction of fully validated Beer–Lambert curves and using such curves to conduct recovery experiments.

This study has addressed the fundamental problems of the donor–acceptor interactions, i.e. free energy change, enthalpy and entropy and the equilibrium constant as a direct measure of the stability of the complex. Therefore the result of this investigation has demonstrated that the charge-transfer complexes between sulfadoxine and pyrimethamine are sufficiently stable and can be used as a basis for the quantitative analysis of these drugs. The real life application of this technique has been presented in a separate communication. This charge-transfer technique of drug analysis is currently being investigated for use as a stability-indicating method in the analysis of these drugs.

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