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THERMODYNAMIC AND ANALYTICAL STUDIES ON THE CHARGE-TRANSFER COMPLEX FORMED BETWEEN CHLORANICIC ACID AND AMINO DERIVATIVE OF METRODINAZOLE

JOHNSON OGODA ONAH.

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ABSTRACT

The nitro group of metronidazole was converted to the amine derivative before complexation with chloranilic acid; the charge-transfer complex was analyzed spectrophotometrically. There was a bathochromic shift in the absorption of chloranilic acid from 420 to 440 nm on the formation of the complex. The stoichiometry of complexation was found to be 1:1. The equilibrium constant for the interaction at different temperatures was determined according to Benesi-Hildebrand model. The equilibrium constant and molar absorptivities varied only slightly with temperature. The thermodynamic parameters like free energy change (ΔG), enthalpy change (ΔH) and entropy change (ΔS) showed that the interaction was exothermic. Quantitative analysis of four brand of metronidazole showed mean percentage recoveries of 98.78 ± SD 0.045 for charge-transfer technique and 95.93 ± SD 0.31 for the Standard method. The limits of detection (LOD) and quantitation (LOQ) were 25 ng/ml and 70 ng/ml respectively. Comparison of these techniques shows that there is no significant difference ($p > 0.05$) between the two methods. Charge-transfer technique is suitable for the quantitative analysis of metronidazole because it is simple, sensitive and accurate.

KEY WORDS: metronidazole derivative, charge-transfer complex, chloranilic acid.

INTRODUCTION

Metronidazole (2-methyl-5-nitro-1-imidazoleethanol) is probably unique as single direct-acting amebicide that is very effective in the bowl and liver without any significant toxicity associated with neuropsychiaric disturbance or electrocardiographic changes (Massarat et al., 1998; Raymond et al., 1998). In humans, it is the drug of choice for the treatment of acute amebic dysentery and amebic liver abscess (British National Formulary; Braun and Frayha, 1998). The other clinical uses of metronidazole are also reported in the literature (Gobeil, 1997; Wolff, 1998; Anon, 1998; Hicks et al., 1999).

Pharmaceutical analysis of metronidazole have been carried out by bivariate spectrophotometry (Lopez de Alba et al., 1997) and absorbance subtraction methods (Du, 1997). Differential spectrophotometry have also been used (Issa et al., 1990) which provides results comparable with the British Pharmacopoeia (BP, 1993) method of non-aqueous titration.

The phenomenon of π-π^* interaction (Mulliken, 1964; Foster, 1969) has been applied to the analysis of pharmaceutical formulations (Muralikrishna et al., 1984; Ibrahim et al, 1986; Rizk et al., 1981). This molecular interaction is generally associated with the formation of intensely coloured complex that absorbs radiation in the visible region (Issa et al, 1990). Some workers have adapted vibrational spectral method (Taha et al., 1980) for the study of the complexes involving tertiary amines. Amines and other organic compounds containing hydroxy, methoxy, mercapto and anhydrides that possess nonbonding electrons are suitable for charge-transfer interactions. Molecules like iodine (Ruosteuo et al. 1988), chloranilic acid (Belal et al. 1986; Onunkwo and Adikwu, 1995) and tetracyanoethylene (Ibrahim et al. 1986; Ayad et al., 1984) are the π-acceptor molecules. Depending upon the source of electrons and the orbital into which they are accepted, these interactions may be classified into π-donors or π-acceptors.

For example spectrophotometric studies on charge-transfer complexes of benzanilides with π-acceptors have been reported (Mourad, 1988). Substituted benzanilides acts as electron donor and the nature of electronic interaction is either π-π^* or n-π^* depending upon the substituent group present.

Although charge-transfer (CT) interactions have been applied extensively to pharmaceutical

JOHNSON OGODA ONAH, Department of Pharmaceutical Chemistry, University of Jos, Jos, Nigeria.

analysis (Murlikrishna et al.,1984; Ibrahim et al. 1986; Rizk et al., 1981) the technique is not without some limitations. For example the empirically determined value of absorption maximum (λ_m) for the complex may be affected by the absorption of the individual molecules and the possible formation of termolecular and isomeric complexes. Solvation of the donor or acceptor molecules may also pose serious limitations to the interpretation of data. The application of Benesi-Hildebrand equation and its various modifications to the determination of the association constant makes several fundamental assumptions that affect reliability of data.

Despite these limitations, the technique gives reliable approximation of the theoretical situation as its application has demonstrated (Murlikrishna et al.,1984; Ibrahim et al. 1986; Rizk et al., 1981). No report has been cited in the literature for the analysis of metronidazole and its derivatives using chloranilic acid (CA) or the thermodynamic parameters of such interactions.

The objective of the present study is to report the thermodynamics parameters of the complexation between chloranilic acid and the derivative of metronidazole as a basis for its application in the real analysis of its pharmaceutical formulation.

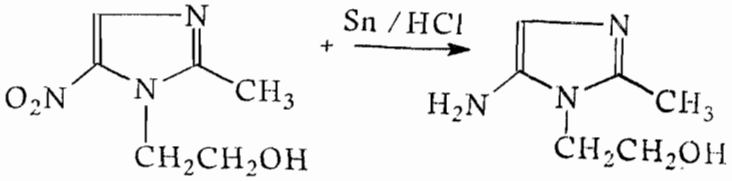
EXPERIMENTAL

Materials

Chloranilic acid (Riedel de Haem,) was recrystallized from acetone (May & Baker,). Chloroform (AnalaR: BDH) and 1,4- dioxan (May & Baker) were used without further purification. Granulated tin (May & Baker) was used as supplied. Department of Pharmaceutics and Pharmaceutical Technology, University of Jos, kindly donated pure metronidazole powder. All other reagents used were of analytical grade and were prepared fresh whenever required.

Reduction of nitro-group of metronidazole

Metronidazole was subjected to nitro group reduction by reacting 1.0 g of metronidazole with 1.0 g of tin in 100 ml of 4M concentrated hydrochloric acid under reflux. When the vigorous reaction has subsided, it was further refluxed on a water bath until the nitro compound completely reacted. The reaction mixture was allowed to cool, 40 % solution of sodium hydroxide was added until the precipitate was re-dissolved. The derivative formed was 2-methyl-5-amino-1-imidazoleethanol (RM). It was recovered by exhaustive extraction with chloroform followed by evaporation to recover the product. The product was re-crystallized from ethanol.



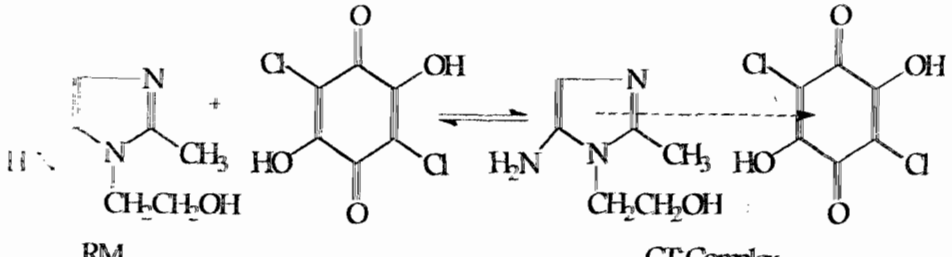
metronidazole RM

Spectral characteristic of chloranilic acid and the CT. complex

Stock solutions of chloranilic acid (4.79 x 10^{-3} M l^{-1}) and RM (7.14 x 10^{-2} M l^{-1}) were prepared in 1,4- dioxan and their absorption maxima individually determined using Pye-Unicam SP 800. Equal volumes of these solutions were mixed and again the absorption maximum determined. Serial volumes of the RM solution i.e. 1.0 ml, 2.0 ml to 5.0 ml were transferred to 10 ml calibrated flasks and 1.0 ml of chloranilic acid was added; the solutions were brought up to mark with 1,4-dioxan. Quintuplets preparations were made for each volume. The contents were thoroughly mixed and their absorbances measured after 30 minutes when equilibrium should have been established. The stability of the purple colour was further monitored for 72 h. The mean values of the absorbances were calculated and plotted against the concentrations. The curve was regressed by the method of least squares (Bauer, 1971); the slope gave the measure of the extinction coefficient.

Stoichiometric relationship

Mixtures of chloranilic acid and RM in the ratios of 1:9, 2:8, 3:7 to 9:1 were transferred to calibrated flasks, the complex formed was



RM CT Complex

Table 1: Thermodynamic changes associated with the complexation between chloranilic acid and metronidazole derivative at different temperatures.

Temp. (°K)	K_c l mol^{-1} ± SD	ΔG KJ mol^{-1}	ΔH KJ mol^{-1}	ΔS KJ mol^{-1}
303	858.40 ±17.2			
313	567.22 ± 8.6	16.83 ± SD 0.52	15.24± SD 0.05	0.05± SD 0.02
323	501.45 ± 10.1			
333	389.33 ± 7.8			

$\varepsilon_\lambda^{max}$ = 10559 l mol^{-1} cm^{-1}

n=5 for each parameter.

allowed to stand for 30 minutes before their absorbances were measured at the absorption maximum.

Determination of equilibrium constant and free energy change

The equilibrium constant and the free energy change of the interaction was determined by the method already described (Adikwu et al., 1998). Serial volumes of the stock solution of RM, i.e. 1.0ml; 2.0ml; to 6.0ml were transferred to different 10 ml volumetric flasks and 1.0 ml of chloranilic acid added to each flask. The contents were analyzed at the absorption maximum. Similar experiments were carried out at 30°, 40° 50° and 60°C in a thermostated water bath.

Calibration curve

From the stock solutions of RM (7.14 x 10^{-2} M) measured volumes of 1.0 ml to 5.0 ml were transferred to 10 ml calibrated flasks and 1.0 ml of chloranilic acid (4.79 x 10^{-3} M) added to each flask. The mixture was made up to mark and absorbances read after 15 minute; the absorbance was monitored for another 72 h. Quintuplet preparations were made for each volume of RM. Mean values of absorbances and standard deviations were calculated. The graphs

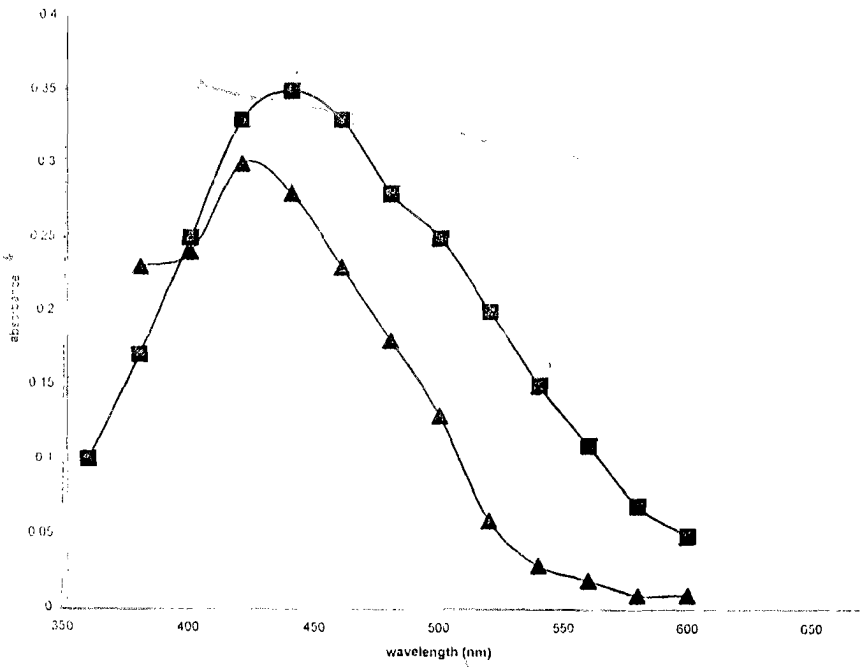


Fig. 1 Absorption spectra (λ_{max}) of (a) chloranilic acid (■) and (b) chloranilic acid-metronidazole complex (▲)

Table 2: Summary of recovery experiments. The label claim on each tablet was 400 mg.

Brands	Charge-transfer Method		Non-aqueous method	
	Recovery (%) n=5	SD %	Recovery N = 5	SD %
A	99.85	0.055	96.84	0.27
B	98.80	0.040	95.50	0.29
C	99.04	0.038	96.10	0.37
D	98.74	0.052	95.30	0.31

were generated and regressed as described previously. The curve was validated using different concentrations of RM but employing the same procedure. The mean values of absorbances of the fresh preparations were converted to the corresponding concentrations using the calibration curve. Comparison of this concentration with the actual concentration spiked gave the recovery.

Assay of metronidazole in tablets

Four different brands of metronidazole tablets equivalent to 400 mg each were individually powdered and extracted with excess chloroform. The chloroform was evaporated off and the residue quantitatively transferred into conical flasks. The reduction of nitro group was carried out as described previously. The percentage yield after reduction of each brand was A, 99.0 % (326.7 mg); B. 100 % (330.05 mg); C. 99.5 % (327.76 mg) and D 99.7 % (329.01 mg). Stock solutions of each brand was prepared (5.0 mg/ml) from which serial volumes of 1.0, 2.0 to 5.0 ml were transferred in triplicates into test - tubes and 1.0 ml of chloranilic acid (4.79 x 10^{-3}M) was added to each test-tube. The contents were

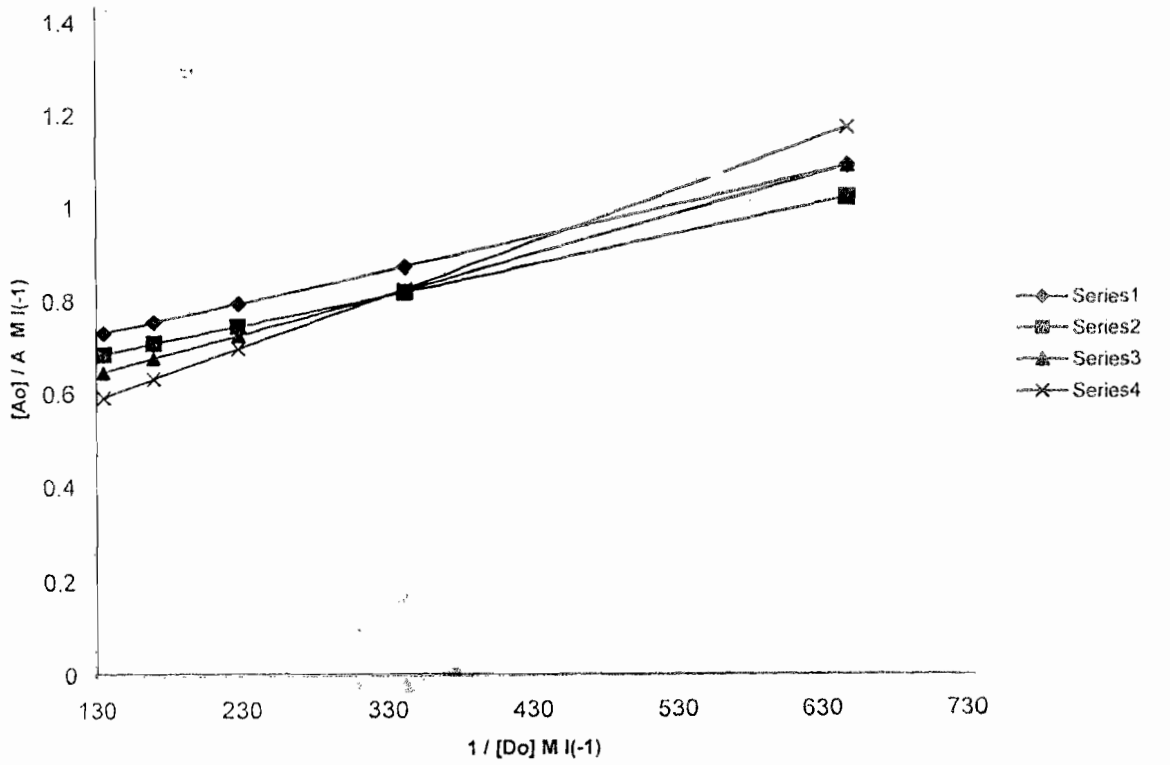


Fig.2: Plots of $[A_o] / A$ against $1 / [D_o]$ at different temperatures: (♦) 30° ; (■) 40°; (▲) 50° and (✕) 60 °C . $[A_o] = 5.2 \times 10^{-3}$ M and $[Do] = 1.04 \times 10^{-2}$ to 5.8×10^{-2} M

brought up to 10 ml mark after mixing thoroughly and their absorbances were determined. Using the calibration curve the percent recoveries were calculated. The results were found to be comparable to the values obtained with Pharmacopoeia method (BP, 1993).

RESULT AND DISCUSSION

Thermodynamic studies

The absorption maximum for chloranilic acid solution in 1,4-dioxan was found at 420 nm, but turned purple in the presence of 2-methyl-5-amino-1-imidazoleethanol (RM). Evidence for the formation of amino derivative was confirmed when the IR spectrum of it was compared with IR spectrum of pure metronidazole. The amino derivative or RM showed absorption at 3350 cm^{-1} supporting the presence of NH_2 group (Kemp. 1991). The absorption maximum of the complex occurred at 440 nm, indicates a bathochromic shift (Fig. 1). The molar absorptivity, ε, was calculated to be 10511 $l\ mol^{-1}\ cm^{-1}$. The stoichiometric ratio of the interaction was 1:1 This is consistent with the earlier reports on π-π^* electron transfers (Muralikrishna et al.,1984; Ayad et al., 1984).

The equilibrium constant for the interaction was investigated assuming 1:1 stoichiometry using the Benesi-Hildebrand model derived originally from the following equation:

$$D + A \rightarrow D{:}A \qquad (1)$$

and summarized as

$$[A_o]/A = 1/K_e^{AD} \cdot \varepsilon^{AD} \cdot 1/[D_o] + 1/\varepsilon^{AD} \qquad (2)$$

where $[A_o]$ and $[D_o]$ are the initial concentrations of the acceptor and donor species,
A = absorbance of the complex D:A at λ_m, ε^{AD} is molar absorptivity and K_e^{AD} is equilibrium constant.

The plots of $[A_o]$ / A against 1 / $[D_o]$ gave linear plots from which the values of K_e^{AD} and ε^{AD} were calculated from the slope and intercept. The graphs were regressed by the method of least squares. Graphical representations of the interactions between chloranilic acid and RM at different temperatures employing equation 2 are shown in Fig.2 The close packing of the curves demonstrates the slight differences in their equilibrium constants with temperature; the calculated intercepts varied slightly with temperature. Figure 2 shows only the regressed points as the experimental points were omitted because of the closeness of the curves. The fundamental requirement for the application of this equation is that $[A_o]$ must be kept constant while $[D_o] >> [A_o]$.

The Gibb's free energy change, ΔG, of the reaction was calculated from the following established equation

$$\Delta G = -RT\ln K_e^{AD} \qquad (3)$$

since $-\Delta G = \Delta H - T\Delta S$
enthalpy and entropy of the reaction was also calculated from the following equation

$$\ln K_e^{AD} = \Delta H / RT + \Delta S / R \qquad (4)$$

i.e a plot of $\ln K_e^{AD}$ versus the reciprocal of T gives a linear graph (Fig. 3), the slope of which yield ΔH, and ΔS from the intercept. The thermodynamic parameters calculated from these equations are shown in Table 1.

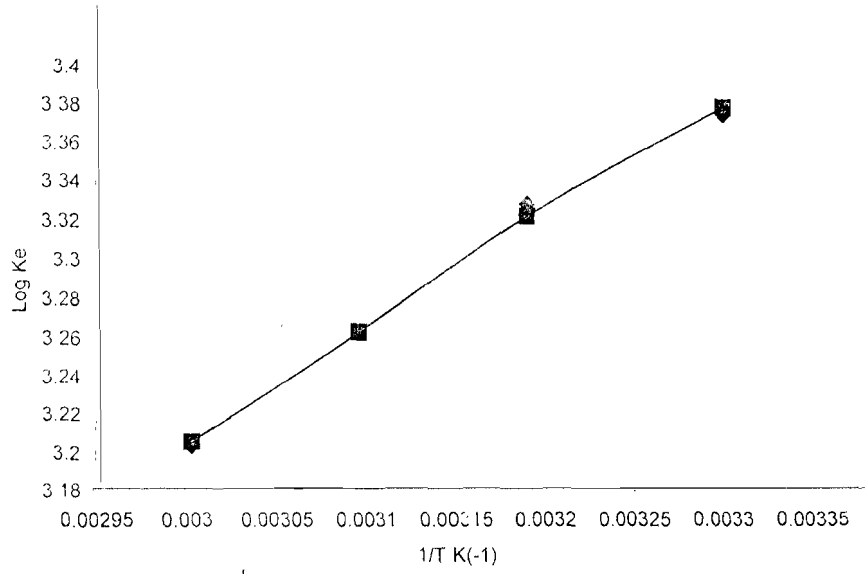


Fig .3 Plot of Log K_e against 1/ T K^{-1}. The data points are represented by (◆) and the regressed points by (□)

From theoretical considerations the molar absorptivity is supposed to be independent of temperature, but in this investigation, slight variations were observed (Fig.2), therefore the value quoted in Table 1.0 was a calculated average. The equilibrium constants and the entropy changes in this investigation are high and consistent with figures reported in the literature (Foster, 1969; Adikwu et al. 1998). The equilibrium constant in Table 1 shows a decrease with temperature. This decrease suggests that the interaction was exothermic (negative ΔG).

The equilibrium constant determined by the Benesi-Hildebrand equation may be affected by several factors like the formation of termolecular and isomeric complexes, solvation of the donor and acceptor molecules, absorption due either to the donor and or acceptor on the absorption maximum of the complex and temperature.

Preliminary observation that Beer-Lambert law was obeyed at the working concentrations and the stoichiometry of 1:1, suggest the absence of these termolecular, isomeric and solvation effects. Similarly, the size of the donor molecule (drug) and the extremely low solvating power of 1,4-dioxan suggest that solvation effect was absolutely minimal. Literature reports have similarly indicated the absence of these interactions (Ibrahim et al., 1986; Muralikrishna et al. 1984; Wolff, 1998; Hicks et al., 1999;) at low concentrations. Also neither the donor nor the acceptor absorbed near λ_m of the complex, so no interference was observed due to these possible absorptions.

Temperature affected equilibrium constant since the reaction was originally exothermic. Similarly, temperature affects K_e^{AD} if it leads to changes in the structure of the solvent (Foster, 1969). The temperature of 60 °C reported here had no effect on the structure of 1,4-dioxan since its temperature was well below the boiling point.

The highly negative K_e^{AD} and ΔH was an indication of high stability of the charge-transfer complex formed and this has been demonstrated by the stable colour over 72 h.

Quantitative studies

Based on the results of the thermodynamic studies, real application of this principle was adapted in the quantitative determination of the pharmaceutical formulation. The CT interaction between CA and RM obeys Beer-Lambert law over a wide range of concentrations. The analytical usefulness of this technique is dependent upon the quantitative reduction of metronidazole. In this study, the mean percent recovery of the reduced product was 99.55 and so it is reasonable to conclude that nitro-reduction was quantitative. The quantitative interaction between RM and CA was justifiably investigated spectrophotometrically using the Benesi-Hildebrand model equation. The result was intrapolated to a situation in which 100 % reduction of the drug took place (Table 2). Non-aqueous titration was similarly carried out on the pure molecule.

The precision of this method gave an average relative standard deviation (RSD) of 0.92 % (SD 0.046), suggesting good reproducibility. The limit of detection (LOD) was 25 ng/ml and that of quantitation (LOQ) was 70 ng/ml. Various graphs of absorbance versus the concentration of RM were generated and regressed (n = 5) by the method of least squares. The following equation generally satisfies the mathematical model developed to test linearity:

$$A_{440} = 6.95 \times 10^{-3} x + 6.34 \times 10^{-2}$$

where A_{440} is absorbance, x is the concentration of the drug in the final assay mixture in M l^{-1} i.e. 7.14×10^{-3} M to 7.14×10^{-2} M. The mean regression coefficient was 0.9964 and the RSD of 1.05% (SD 0.053) and 1.5 % (SD 0.075) for slope and intercept respectively. The molar absorptivity was calculated to be 10511 l mol^{-1} cm^{-1}

Quantitative recovery experiment was carried out on four brands of metronidazole out of several brands that are commercially available (Table 2). The Pharmacopoeia method (British Pharmacopoeia, 1993) of non-aqueous titration was similarly carried out for comparative purposes. The precision and reproducibility of non-aqueous titration depends upon the strength of the base, and the sharpness of the colour change at the equivalence point. The apparently lower recovery values for non-aqueous titration can be ascribed to the precision at equivalence point. Statistical comparison of the two methods by Student t-test ($p > 0.05$) did not however show any significant difference in their sensitivity and accuracy.

CONCLUSION

Nitro-reduction of metronidazole is a quantitative reaction, hence the technique was used for analytical purposes. This investigation has revealed that π-donor/π-acceptor interaction can produce very stable complexes that can be adapted in the quantitative determination of basic drugs containing amino functionality. Many drug molecules are weak bases and so their analysis by non-aqueous titration may not produce the desired degree of precision. The single most important limitation of CT complexation is that its

presence cannot be demonstrated beyond the colour formation and the stability of the complex determines its suitability for analytical purposes.

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