Research Article

Spectrophotometric Determination of Charge Transfer Complexation between Cimetidine and Chloranilic Acid

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Abstract A simple and sensitive spectrophotometric method is described for the assay of cimetidine. Modern analytical technique for cimetidine in various dosage forms are limited by cost and absence of high technological equipment especially in the third World countries including Nigeria. The research investigates the use of charge transfer complexation in the spectrophotometric assay of cimetidine as an alternative method to conventional methods. Spectrophotometer was employed to determine charge transfer complex formation between cimetidine (n-donor) and chloranilic acid (π-aceptor) in 1,4- dioxan. The developed method was employed in the assay of commercial cimetidine in solid dosage forms. The wavelength of maximum absorption (λ_{max}) of the complex was at 254 nm compared to 315 nm for π-acceptor alone. Chloranilic acid was found to form a charge-transfer complex in a 1:1 stoichiometry. Conformity with Beer's law was evident over the concentration range 1.260×10^{-3} to 12.60 mg %. A detailed investigation of the complex formed was made with respect to its composition, association constant and molar absorptivity. This method has been applied successfully to the analysis of commercially available cimetidine tablet with good recovery (99.90 ± 1.20 %) and reproducibility.

Keyword: Spectrophotometer; Chloranilic acid; Cimetidine; Stoichiometry; Maximum absorption

Introduction

Charge-transfer complex or electron-donor-acceptor complex is an association of two or more molecules, such that a fraction of electronic charge is transferred between the molecular entities. Molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensely colored charge-transfer complexes, which absorb radiation in the visible region [1].Charge transfer complexes are formed between electron donors having sufficiently low ionization potential and electron acceptors having sufficiently high electron affinity. Variety of electron-accepting compound including chloranilic acid have been reported to yield charge-transfer complexes leading to their utility in the development of simple and convenient spectrophotometric methods for the analysis of many drugs [2,3 and 4] .This type of analysis is usually simple, reliable and reproducible. Cimetidines are H_2 blocker and are reversible, competitive antagonist of the actions of histamine on H_2 receptors. They are highly selective in their action. Advanced chromatographical technique that make use of Ultraviolet detectors, Gas Chromatography (GC) as well as infra-red (IR) spectrophotometry have been employed in the determination of cimetidine [5] but these analytical techniques are very expensive and require high level of skill. Hence the need for a very cost effective assay method for cimetidine an important drug of choice in the management of ulcer which is a tropical disease condition [6]. Cimetidine [7] has the structural formular presented in fig 1a while the structure of chloranilic acid is shown in fig 1b.
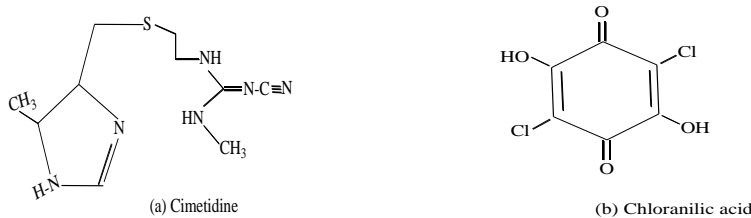
Figure: 1a (Cimetidine) & 1b (Chloranilic acid)

Materials and Methods

The following materials were procured respectively, commercial cimetidine (400 mg) tablet (unbranded) from Zim Lab Ltd India and cimetidine (400 mg) tablets product of (Juhel Pharmaceutical, Enugu. The pure sample of cimetidine was a gift from Glaxo-welcome pharmaceuticals through the federal medical center, Owerri, Nigeria. Other materials used were 1,4-dioxan (BDH, UK), methanol (Scharlan chemic, S.A, Spain). All other reagents and solvents were of analytical grade and were used as received.

Preparation of Standard Solutions

Chloranilic acid

A stock solution of chloranilic acid (5.0×10^{-3} M) was prepared by dissolving 0.26 g of chloranilic acid power, accurately weighed, in 1,4-dioxan and making up to 250 ml with the same solvent. Further dilutions were carried out using 1,4-dioxan to obtain lower concentrations.

Cimetidine

A standard stock solution of cimetidine in methanol was prepared by dissolving 0.315g of cimetidine powder in methanol in a 250ml volumetric flask and made up to volume with same solvent to provide a 5.0×10^{-3} M solution. Further dilutions were carried out using methanol to obtain lower concentrations.

Absorption spectrum of chloranilic acid

Chloranilic acid (4ml) stock solution was scanned in a (Perkin Elmer Lambda 35 UV-Vis spectrophotometer in the wavelength range of 400-700 nm to determine its wavelength of maximum absorption.

Absorption spectrum of cimetidine-chloranilic acid complex

Chloranilic acid (4 ml) stock solution (5.0×10^{-3} M) was mixed with (4 ml) cimetidine (5.0×10^{-3} M) solution. The content was left at room temperature for 1h. A colour was developed and the complex formed was scanned spectrophotometrically against the blank of dioxan and methanol in the wavelength range of 400-700nm.

Beers calibration plot for the cimetidine-chloranilic acid complex

Serial volumes (ranging from 0.4 to 2.8 ml) of the stock cimetidine solution (5.0×10^{-3} M) were transferred to different test tubes. Sufficient volumes of methanol were added to bring the volume in each test tube to 3 ml. A 2ml of chloranilic acid (5.0×10^{-3} M) was added to each of the test tubes to afford a final concentration range of 1.26 $\times 10^{-3}$ mg% - 12.6 mg%. The contents were mixed and left at room temperature for 1h. The absorbance of each sample was determined at the wavelength of maximum absorption (λmax 525nm) against blank of 1,4-dioxan (2 ml) and methanol (3 ml).

Time-absorbance relationship

A time-absorbance relationship was established for the complex to determine the time it takes the complex to develop fully. Equal volumes (4 ml) equimolar concentrations (5.0×10^{-3} M) of the drug and chloranilic acid were mixed and absorbance taken at various time intervals from 10 to 120min at 525nm.

Determination of stoichiometry of the complex

Job's method of continuous variation was employed [8]. Equimolar concentration ($5.0x10^{-3}$ M) of solutions of cimetidine and chloranilic acid were used for the experiment. A series of 5ml volumes of mixtures of the above solutions comprising complimentary proportions of the two solutions in various ratios ranging from 0.5:4.5 to 4.5:0.5 (chloranilic acid: cimetidine solution) were transferred into different test tubes and the complex formed for each reaction mixture was allowed to stand for 1h at room temperature before analysis at 525 nm. A blank solution consisting of 4 ml Chloranilic acid and Methanol respectively were used.

Determination of association constant (K_A) and molar absorptivity (E_λ) of the complex

Serial volumes of $5.0x10^{-3}$ M solution of cimetidine ranging from 0.4 ml to 2.8 ml were transferred to different test tubes. The solutions were diluted to 3.0 ml with methanol and 2 ml of $5.0x10^{-3}$ M

Table 1: Table of values for Benesi-Hildebrand`s plot

Volume of Cimetidine ($5.0x10^{-3}$ M)	Molar concentration of P-Chloranilic acid ($5.0x10^{-3}$ M)	Molar core of Do Cimetidine ($5.0x10^{-3}$ M)	Abs	Ao/Ax10^2	1/Dox10^2
1.5 ml	5.0	7.5	0.0030	1.67	13.3
2.0 "	5.0	10.0	0.0036	1.40	10.0
2.5 "	5.0	12.5	0.0040	1.20	8.0
3.0 "	5.0	15.0	0.0045	1.10	6.7
3.5"	5.0	17.5	0.0050	1.00	5.7
4.0"	5.0	22.5	0.0056	0.90	4.4
4.5"	5.0	25.0	0.0060	0.80	4.0

Solution of chloranilic acid in 1,4-dioxan was added to each test tube. The contents were capped and mixed by gentle shaking. The test tubes were allowed to stand for 1h at room temperature (25°c) and absorbance measurements were taken spectrophotometrically at 525nm against a blank of methanol and dioxan. Further analysis of the reaction mixture were carried out at temperatures of 20-50°c, respectively.

The molar absorptivity and association constant for the cimetidine-chloranilic acid complex were evaluated using the Benesi-Hildebrand equation (Eq1) [9-11].and the data is shown in table 1.

$$\frac{[Do][Ao]}{A_\lambda [A:D]} = \frac{1}{E_\lambda [A:D]} + \frac{1}{E_\lambda [A:D] . K_c [A:D]} \text{ x } \frac{1}{[D_o]} \qquad\qquad ....\text{(Equation 1)}$$

where [Do] and [Ao] are initial concentrations of the reactions Ao$^{[A:D]}$ is the absorbance of the complex at 525nm, E$\lambda^{[A:D]}$ is the molar absorptivity of the complex at 525nm, and Kc$^{[A:D]}$ is the association (stability) constant. The intercepts and slopes of the regression lines from a plot of [Ao]/A$^{[A:D]}$ against 1/[Ao] were used to calculate the values of Eλ and Kc [A:D] respectively.

Assay procedure for cimetidine tablet

Twenty cimetidine tablet from each brand respectively (Juhel Pharma) and cimetidine tablet (India) were emptied, thoroughly mixed by trituration in a motar and an amount equivalent to 400mg active drug was accurately weighed. This was mixed with methanol in a 250ml flask to extract the active drug. The mixture was filtered to remove the excipients after shaking for 45min. The filtrate was thereafter made up to 250ml with methanol to provide a theoretical concentration of $5.0x10^{-3}$ M of cimetidine solution. Gradient volumes (0.5, 1.5, 2.5, 3.0 and 3.5 ml) of the solution were taken into different test tubes to afford concentrations of 10, 15, 20, 25, and 30 mg respectively. Sufficient methanol was added in each case to bring the volume to 4ml. chloranilic acid solution $5.0x10^{-3}$ M, was then added in each case to make up the final volume of 5.0ml each. The content were mixed, left for 1h at room temperate and their absorbance determined at 525nm against a blank of methanol and dioxan. The procedure was repeated for five times. Percentage recovery of cimetidine from dosage form was calculated by reference to its beer's plot. Same step was taken for other brands.

Statistical Analysis

The data so obtained were analyzed by SPSS version 11 and recorded as mean standard deviation (SD, a=5). Statically significant difference between brands of cimetidine assayed by this method was evaluated using the student's t-test and one-way analysis of variance (ANOVA; Fishcher LSD post-hoc test). Significant differences between means were considered at $P < 0.05$.

Results and Discussion

A golden yellow colour was produced by the solution of chloranilic acid in 1,4-dioxan with maximum wavelength of absorpotion at 315 nm, and instantaneous purple colour was obtained on reaction chloranilic acid with solution of cimetidine in methanol [Fig: 2], (λ-$_{max}$ of 525nm).
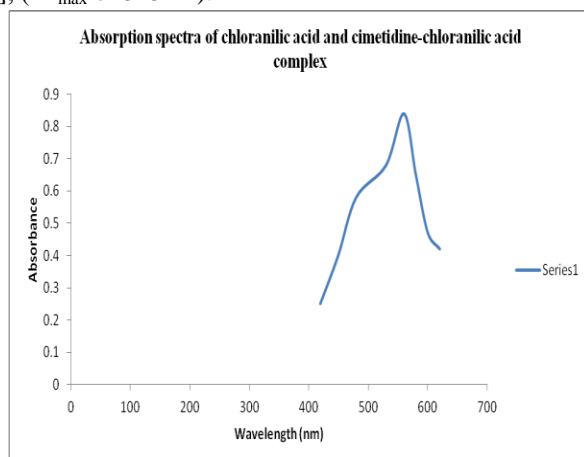

Figure 2: Absorption spectra of Cimetidine-Chloranilic acid complex

Absorbance of the complex was high at zero time and increased slowly over the 150min period of the analysis (fig 5, abs/time curve). The colour of the complex also remained stable after 24h. Beer's Law was obeyed by the complex at 525 nm, a fairly linear relationship was obtained between absorbance and the concentration over the entire range studied (1.26×10^{-3} mg% - 12.6mg% of cimetidine). From the regression equation of the line, it was found that the correlation is fairly high. The stoichiometry of the cimetidine-chloranilic acid complex was studied using job's method of continuous variation. The job's plot for continuous variation is presented in fig 3.
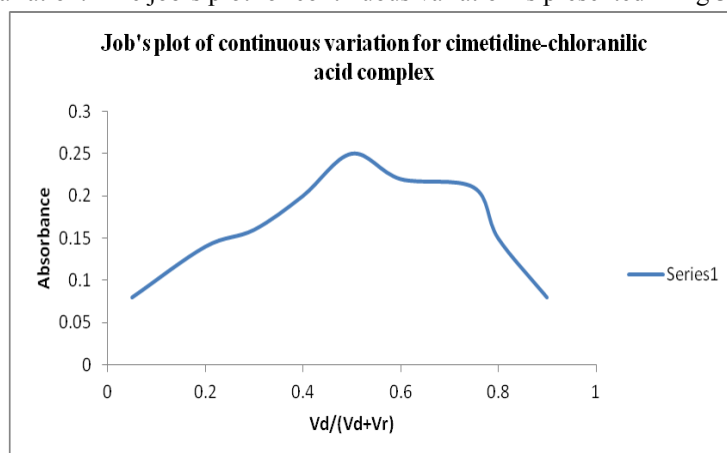

Figure 3: Jobs plot of variation for Cimetidine-Chloranilic acid complex

The molar absorptivity and association constant for cimetidine-chloranilic acid complex were evaluated using the Benesi-Hildebrand equation [12-14], deduction from the graph plot shows the molar absorptivity to be 2.09×10^{2} while the association constant was found to be 0.053 Litre/Mol.
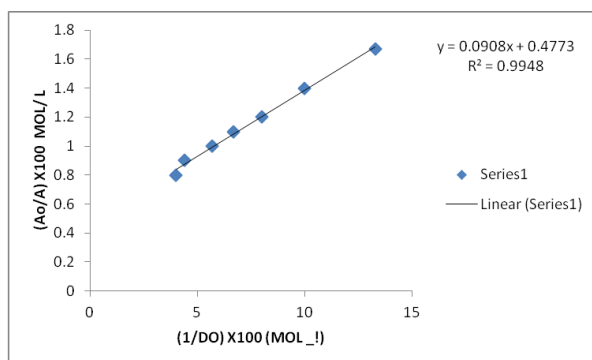
Figure 4: Benesi-Hildebrand`s plot for the complex where A_O is the initial acceptor concentration ($5x10^{-3}$ M). A is the absorbance and Do is the initial drug concentration

Recovery results of cimetidine from the dosage form using the proposed method showed that recovery was 97% for cimetidine from the unbranded commercial products. This indicates a high accuracy method of analysis and reproducibility of the method. The spontaneous color change from yellow for the chloranic acid alone to purple for the complex with a bathochromic shift of absorption maxima 315nm to 525nm) suggests a charge transfer complex formation (see fig 2). The time-absorbance relationship of the complex suggests that the formation of the cimetidine-chloranic acid complex was instantaneous and the complex was stable for over 24h with the purple colour still obvious.
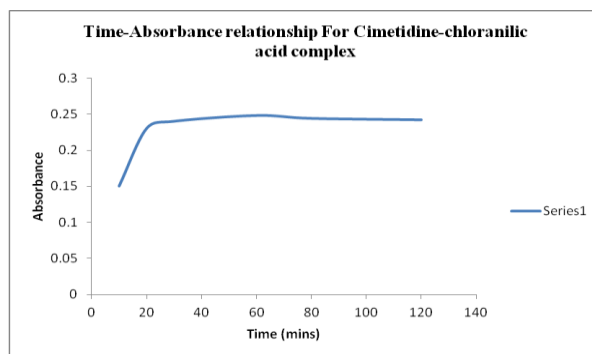

Figure 5: Time-Absorbance relationship for Cimetidine-Chloranilic acid complex

Conclusion

The cimetidine-chloranic acid complex obeyed Beer's law in the concentration range $1.26x10^{-3}$ mg% to 12.60 mg% with fair correlation. This relationship with Beer's Law indicates that spectrophotometirc analysis of electron donor-aceptor formation can be used for the quantitative analysis of cimetidine. The correlation was fairly high indicating reproducibility and reliability. The stoichiometry, which was 1:1 (cimetidine:chloranilic acid) for the complex, indicate good site for electronic association. The association constants presented were high while the molar absorptivity values was found to increase with time and temperature. The complex was quite stable showing that the bond formed between cimetidine and chloranilic acid was fairly strong. From the recovery experiment, there was no statistical difference ($P < 0.05$) between the brands. The recovery was up to 97% amongst the brands. It was found that this proposed method gives precise values validating the claims from the manufacturers. It is easy and reproducible, cheap as well as affordable analytical procedure for the assay of cimetidine. This proposed assay method was successful for cimetidine in solid dosage form. Precision among other analytical techniques should still be investigated.

Conflict of Interest

The authors declare no conflict of interest.

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