Spectrophotometric and Thermodynamic Studies of the Charge-Transfer Interaction between Diethylcarbamazine Citrate and Chloranilic Acid

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The charge-transfer complex formed by the interaction between the electron acceptor, *p*-chloranilic acid and an electron donor diethylcarbamazine was adopted for the assay of the drug in pure powder and in tablets. In dioxane–chloroform medium, chloranilic acid was found to form a 1 : 1 molecular complex with diethylcarbamazine with maximum absorption band at 540 nm. At 540 nm, the absorbance was linear ($r=0.9999$) over the concentration range of 10—400 μg/ml. The pertinent equilibrium constants and molar absorptivities were calculated at different temperatures using the Scott equation and were found to decrease with increase in temperature. The free energy change ($\Delta G°$) and the enthalpy of formation ($\Delta H°$) as well as the entropy ($\Delta S°$) were determined for the various interactions. The proposed method has been conveniently applied in the analysis of commercially available diethylcarbamazine citrate tablets with good accuracy and precision.

Key words charge-transfer complexation; chloranilic acid; diethylcarbamazine citrate; spectrophotometric determination; thermodynamic studies

Diethylcarbamazine citrate (DEC), [*N*, *N*-diethyl-4-methyl-1-piperazinecarboxamide citrate] is the drug of choice in the treatment of filariasis infections caused by a host of organisms commonly found in the tropics.[1)] It is also the alternative drug of choice in *Onchocerca volvulus* infections and tropical eosinophilia.[2)] A thin layer chromatographic procedure for the detection of DEC has been described[3)] and the method was used for the rapid quality assessment of the drug in tablets and in pure powder. Only one direct UV spectrophotometric method is available for the determination of DEC.[4)] A method of non-aqueous titration for the assay of DEC has been described.[5)]

Charge-transfer complexes result from a donor-acceptor mechanism of Lewis acid–base reaction between two or more different chemical constituents. The formation of electron-donor acceptor (EDA) complexes can be rapidly assessed for its validity as a simple quantitative analytical method for many drug substances which can act as electron donors. Chloranilic acid (CA) and other π-acceptors as well as sigma (σ) acceptors have been successfully utilized in the determination of a variety of electron-donating basic compounds.[6—17)]

Rizk *et al.*[18)] assayed DEC spectrophotometrically *via* charge transfer complexation with chloranilic acid. Similarly Wahbi and co-workers[19)] assayed DEC tablets *via* a charge-transfer complex with iodine. High recovery rates were obtained with the above methods. However, the workers did not report on the thermodynamic parameters for the charge transfer complexes. Similarly, the work by Rizk *et al.*[18)] using chloranilic acid as the acceptor was carried out on the salt of diethylcarbamazine. In the present work, pure salt as well as various brands of the drug are evaluated.

This work is a preliminary report on charge-transfer complex of chloranilic acid with DEC and its utilization in a sensitive assay of the drug. The determination of the association constants, molar absorptivities, free energy changes, enthalpies of formation and entropies of the complex formed at different temperature is also described.

Experimental

Materials DEC pure powder (Merck), Banocide® tablets (Wellcome), unbranded DEC tablets (Vadhman Exports and IDA), chloranilic acid (Riedel de Haen), 1,4-dioxane and chloroform (May and Baker). Other reagents and solvents were analytical grade and used as such. All laboratory reagents were freshly prepared.

Standard Solutions An amount of the salt equivalent to 25 mg DEC base was accurately weighed in an analytical balance (Sauter) and dissolved in about 10 ml of water in a 250-ml separating funnel. The solution was made alkaline with 3 ml of 2 M sodium hydroxide and shaken successively for 2 min with 15 and three 10-ml portions of chloroform, each extract being washed with the same 15 ml of water in another separating funnel. The washed extracts were pooled and passed through 2 g of anhydrous sodium sulphate supported on a filter paper in a small funnel into a 50-ml volumetric flask and completed to volume with chloroform to provide a standard DEC base equivalent to 0.1 mg ml^{-1}.

Absorption Spectra A solution of chloranilic acid (3×10^{-3} M) was made in 1,4-dioxane and its wavelength of maximum absorption determined using a Pye Unican double beam UV-Vis spectrophotometer (SP8-100). A colour was developed by mixing 2 ml of chloranilic acid with 2 ml of 2.56×10^{-3} M DEC and made up to 5 ml with dioxane. The wavelength of maximum absorption for the resulting solution was then determined.

Standard Curve For calibration, a 25 mg portion of DEC base in 50 ml chloroform as described under standard solutions was used. Serial concentrations of 0.05 to 0.6 mg in 0.05 mg steps of standard base solution were transferred to different test tubes. Sufficient chloroform was added to bring the volumes to 3 ml, and 2 ml of 3×10^{-3} M chloranilic acid in dioxane added to bring the volumes to 5 ml. The contents were mixed and left at room temperature (30 °C) for 60 min after which their absorbance was measured at 540 nm against a blank, prepared simultaneously without DEC solution.

Assay Procedure for Tablets Ten Banocide® tablets were finely powdered and an amount equivalent to 50 mg of the salt accurately weighed. This was dissolved in about 10 ml of water in a beaker and filtered to remove the excipients. The resulting filtrate was quantitatively transferred to a 250 ml separating funnel and handled exactly as mentioned in the preparation of standard solutions. This procedure was repeated for two other commercial sources of unbranded DEC.

Molecular Ratio of Reactants in DEC–Chloranilic Acid Complex Job's method of continuous variation[20)] was employed. Master solutions of chloranilic acid (5.01×10^{-3} M) and diethylcarbamazine (5.01×10^{-3} M) were prepared. A series of 5-ml quantities of mixtures containing a total of 5 ml of the master solution of DEC base and chloranilic acid in different complementary proportions (0 : 5, 0.5 : 4.5, ...4.5 : 0.5, 5 : 0) were transferred to different test tubes. The complex formed for each reaction mixture was allowed to stand for 60 min before analysis at 540 nm.

Association Constant, Molar Absorptivity and Free Energy Change Serial volumes of 0.1 to 0.6 ml of 3.83×10^{-2} M DEC solution in 0.1 ml steps were transferred to different test tubes. The solutions were diluted with chlo-

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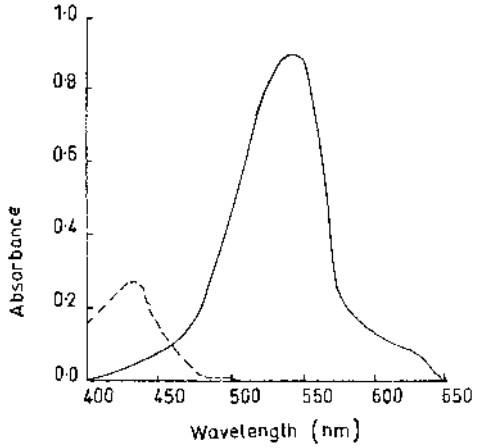

Fig. 1. Absorption Spectra of CA (---) and DEC–Chloranilic Acid Complex (--)
DEC: 2.55×10^{-3} M; CA: 5.0×10^{-3} M.

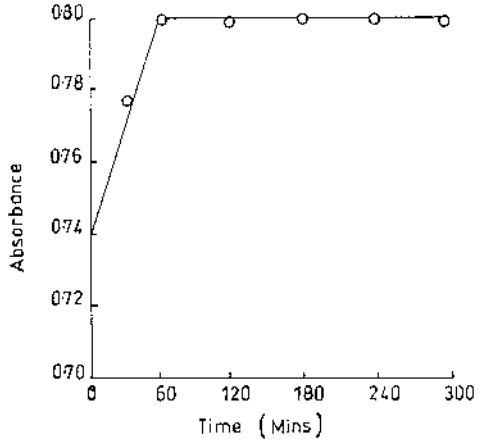

Fig. 2. Time–Absorbance Relationship for DEC–CA Complex at 540 nm

roform to 3 ml, and 2 ml of 3.0×10^{-3} M solution of chloranilic acid in dioxane was added. The procedure was continued as described for the calibration of DEC and was further analysed at temperatures of 50, 70 and 90 °C.

Results and Discussion

Chloranilic acid in a solution of dioxane displayed an absorption peak at 430 nm while the DEC showed a negligible absorption in the 400—700 nm region. Mixing the chloroformic extract of the DEC with the solution of chloranilic acid in dioxane resulted in a change of the yellowish-pink colour of the chloranilic acid to purple. As a consequence, the absorption band of chloranilic acid showed a bathochromic shift (shifted to a longer wavelength). This was indicative of charge-transfer complex formation. The charge-transfer complex between DEC and chloranilic acid exhibited an absorption band at 540 nm (Fig. 1). In other words, the interaction between DEC and CA is a charge-transfer complexation reaction between the n-donor DEC and the π-acceptor (CA) followed by the formation of a radical anion according to the following scheme:

$$R_3\ddot{N}+CA \overset{\text{fast}}{\rightleftharpoons} [R_3\ddot{N}-CA] \quad \text{(outer complex)}$$

$$[R_3\ddot{N}-CA] \longrightarrow R_3\dot{N}^{+}+CA^{-} \quad \text{(inner complex)}$$

Although the complex was formed instantaneously, constant absorbance readings were obtained after no less than 60 min of standing at room temperature (30 °C) and remained constant for at least 24 h (Fig. 2). At 540 nm, linear relationships were obtained ($r=0.9999$) between the absorbance and the concentrations over the 10—400 μg ml^{-1} range. The regression equation of the line as derived using the method of least squares[21] is:

$$A_{540}=1.53\times10^{-2}+4.38\times10^{-2}C \qquad (1)$$

where A_{540} is the absorbance at 540 nm, C is the concentration of DEC in mg per 100 ml of the final dilution and r is the correlation coefficient. The slope of the calibration curve reflects the sensitivity of the procedure.

The determination of the mole ratio of reactants in the complexes is based on the method of continuous variation as proposed by Job.[20] The plot (Fig. 3) indicates a 1 : 1 EDA interaction. This finding was anticipated because of the presence of a single strong electron-donating centre in the molecular structure of DEC. The absorbance of the complex obtained experimentally was used to calculate the molar absorptivities and association constants using the Scott equation[22] which, like the Benesi–Hildebrand equation,[23] depends on the experimental condition that one of the two components species should be present in large excess, so that its concentration is virtually unaltered on formation of a complex. The molar absorptivity and association constant can be obtained from the slope and intercept of the Scott equation plotting $[A_0][D_0]/A$ *versus* $[D_0]$ while keeping $[A_0]$ constant. Representative plots are shown in Fig. 4. The regression equation describing the line obtained from the Scott plot is:

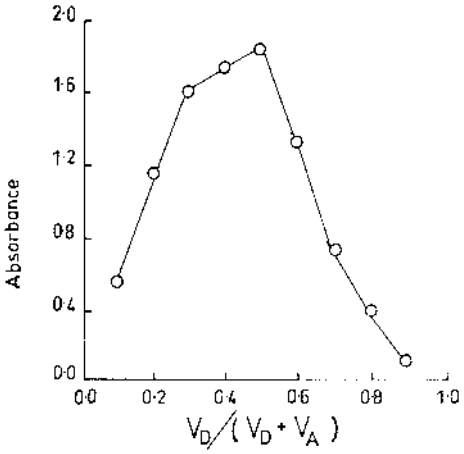

Fig. 3. Continuous Variation Plot for DEC–CA Complex (5.01×10^{-3} M)

$$\frac{[D_0][A_0]}{A_\lambda^{AD}}=1.371\times10^{-6}+7.43\times10^{2}[D_0] \qquad (2)$$

The standard enthalpy change, $\Delta H°$, of an interaction can be obtained graphically by plotting log K_C^{AD} *versus* $1/T$ of the following well-known equation:

$$\log K_C^{AD}=\frac{\Delta H°}{2.303RT}+\text{constant} \qquad (3)$$

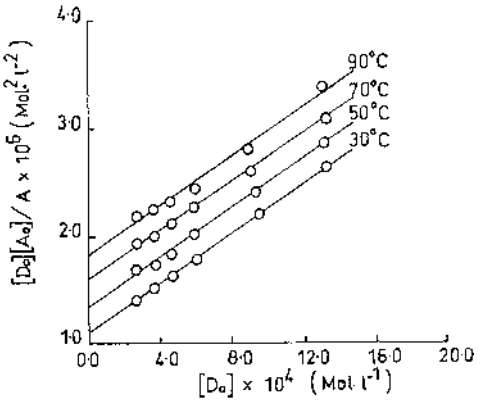

Fig. 4. Plots of $[D_0][A_0]/A$ *versus* $[D_0]$ for DEC–CA Complex at Different Temperatures

DEC: 3.83×10^{-2} M; CA: 3.0×10^{-3} M. The curves have about the same intercepts as those at 30 °C; they were separated to avoid crowding.

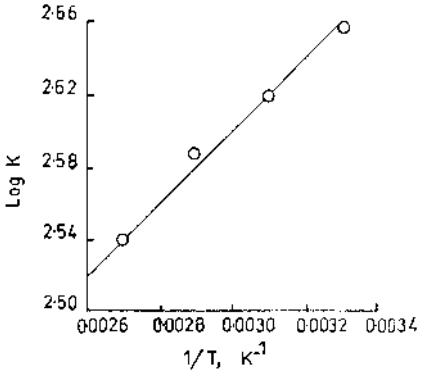

Fig. 5. Log K of the DEC–CA Interaction *versus* $1/T$

A plot of Eq. 3 for DEC–chloranilic acid complex is shown in Fig. 5. The standard free energy, $\Delta G°$, is calculated from:

$$\Delta G° = -RT \ln K_C^{AD} \quad (4)$$

The standard entropy change, $\Delta S°$, is calculated from:

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

The thermodynamic parameters together with the molar absorptivities of the studied interaction are presented in Table 1. All the slopes and intercepts used to calculated the quantities in Table 1 and the figures were determined statistically by the method of least squares.

The molar absorptivities were discovered to be temperature independent. At the same time, the association constants were found to decrease with increase in temperature. This finding is in agreement with earlier studies.[24,25)]

A negative free energy change ($-\Delta G°$) and a positive entropy change ($+\Delta S°$) are indicative of a spontaneous reaction.[26)] The interaction between DEC and chloranilic acid occurs spontaneously and a negative ΔH as can be seen from Table 1, is itself proof of a spontaneous reaction. Andrews and Keefer[27)] had earlier demonstrated that $\Delta H°$ and $\Delta S°$ generally become more negative as the stability constant for molecular complexation increases. As the binding between donor and acceptor becomes stronger, $\Delta H°$ would be expected to have higher negative values. The high negative value of $\Delta H°$ and the high values of association constants as

Table 1. Association Constants (K), Standard Free Energy ($\Delta G°$), Standard Enthalpy ($\Delta H°$), and Standard Entropy ($\Delta S°$) Changes Associated with the Interaction of DEC with CA in Non-aqueous Media at Different Temperatures

Temperature (K)	K (M^{-1})	ε (l^{-1})	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/deg/mol)
303	462.74	924.38	−3.70	—	8.83
323	421.14	939.73	−3.88	−1.022	8.85
343	376.21	879.37	−4.04	—	8.81
363	337.09	891.95	−4.20	—	8.75

Table 2. Assay Results for DEC in Pure Sample and in Tablets (Charge Transfer Complexation)

Parameter	Pure sample	Tablets[a)]		
		A	B	C
Mean recovery (%)[b)]	100.55	100.79	99.86	100.07
Standard deviation (%)	1.30	1.16	0.61	1.08
Calculated value of t[c)]	1.23	0.24	0.69	0.20
Regression equation (Y)[d)]				
Slope (n)	4.38×10^{-2}	4.42×10^{-2}	4.34×10^{-2}	3.73×10^{-2}
Intercept (m)	1.53×10^{-2}	-1.06×10^{-2}	1.85×10^{-3}	2.69×10^{-3}
Correlation coefficient (r)	0.9991	0.9999	0.9999	0.9998

a) DEC tablets manufactured by Wellcome, Nigeria (A), by IDA, Holland (B), and by Vadhman Exports, India (C). *b*) Mean for ten determinations; percentage recovery from the label claim amount (50 mg DEC) *c*) The value for t-theoretical at $\alpha=0.025$ is 1.96 *d*) $Y=m+nc$, where c is the concentration.

are evident in Table 1 are a pointer to the strength of the binding between DEC and CA and the high stability of the resultant charge-transfer complex.

The validity of the regression equation (earlier stated) for the calibration of DEC was assessed in its determination in pure sample and in tablets. Table 2 shows the accuracy of the proposed method. Recovery experiments carried out on DEC in pure sample and in tablets show high quantitative recoveries with low standard deviations.

The performance of the proposed method was judged through calculation of the Student's t-value. At the 95% level of probability, the calculated values of t do not exceed the theoretical values. This is an indication that the proposed method gives results not significantly different from the true values according to label claims and lends further confirmation to the high accuracy of the method. Auxiliary substances that are likely to be present as the preparation base, *e.g.*, starch, lactose, talc and magnesium stearate in tablets exhibited no interference during the assay procedure, as the proposed method is based on the extraction of the free base prior to complexation with chloranilic acid.

Compared to the official method, the proposed method is simpler, faster, and more sensitive and is of high accuracy and precision. These advantages encourage its application in the quality control and analysis of drugs. The data obtained for the new method compares with the official (non-aqueous titration) method (Table 3). It should, however, be noted that there was a higher correlation in all the batches tested when the official method was employed. In the works of Rizk *et al.*[18)] and Wahbi *et al.*,[19)] they compared their results to the official methods and the standard deviations for the official method were smaller than those for the spectrophotometric method *via* charge-transfer complexation. This is not the case

Table 3. Assay Results for DEC in Pure Sample and in Tablets (Official Method[5])

Parameter	Pure sample	Tablets[a]		
		A	B	C
Mean recovery (%)[b]	99.99	100.10	99.30	99.98
Standard Deviation (%)	1.38	1.22	0.82	1.17
Calculated value of t[c]	1.35	0.41	0.92	0.22
Regression equation (Y)[d]				
Slope (n)	4.22×10^{-2}	3.95×10^{-2}	4.42×10^{-2}	3.35×10^{-2}
Intercept (m)	1.47×10^{-3}	-1.12×10^{-2}	1.90×10^{-2}	2.55×10^{-3}
Correlation coefficient (r)	0.9998	0.9999	0.9999	0.9999

a—*d*): same as in Table 2.

with our own studies, in which the standard deviations compare well with those of the official method.

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