



## Studies on the Charge-Transfer Interaction Between Tamoxifen Citrate and Chloranilic Acid

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### Abstract

The complex formed as a consequence of the interaction between the electron-acceptor P-chloranilic acid and an electron donor tamoxifen citrate was employed in the assay of the drug in pure powder and tablets. Chloranilic acid was found to form a charge-transfer complex in a 1:1 stoichiometric ratio, with tamoxifen citrate. The wavelength of maximum absorption for the complex was found to be 550 nm while the absorbance was linear over the concentration range of 2-100 g/ml. Evaluations of the various thermodynamic parameters by means of the Scott equation was carried out and were found to decrease with increase in temperature. The free energy change ( $\Delta G^\circ$ ) and the enthalpy of formation ( $\Delta H^\circ$ ) as well as the entropy ( $\Delta S^\circ$ ) were determined for various interactions. Results obtained suggest that the proposed method may be conveniently applied in the analysis of commercially available tamoxifen citrate tablets with a high degree of accuracy and reproducibility.

**Keywords:** Charge-transfer complexation; Chloranilic acid; Tamoxifen citrate; Thermodynamic studies.

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### 1. Introduction

Tamoxifen citrate (TC), (Z)-2-(P-(1, 2-diphenylbut-1-enyl) phenoxy) ethyl dimethylamine citrate is an anti-oestrogen, which has been shown to inhibit or modify the action of estrogens, and it is the drug of choice for the management of breast cancer [1-3].

Charge-transfer complexes arise from a donor-acceptor interaction of a reacting pair

of Lewis acid-base reagents. The formation of electron-donor acceptor (EDA) complexes can be rapidly evaluated for its usefulness as a simple quantitative analytical method for many drug substances, which can act as electron donors. Chloranilic acid (CA) and other  $\pi$ -acceptors as well as sigma ( $\sigma$ ) acceptors have been successfully utilized in the quantitative determination of a large-number of electron donating basic compounds [4- 13].

This work describes some physical /chemical studies carried out on the charge transfer complex of chloranilic acid with TC.

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Further studies aimed at determining the association constants molar absorptivities, free energy changes as well as the enthalpies of formation and entropies of the complex at different temperatures, were also undertaken.

## 2. Materials and methods

### 2.1. Materials

TC from Generics Ltd, UK, TC (TEVA), TC (NOSTON), and CA (Riedel de Haen) were used. 1,4-Dioxane and chloroform were from May and Baker. Other reagents and solvents were of analytical grade.

### 2.2. Standard solutions

A sufficient quantity of the salt equivalent to 20 mg of the base was dissolved in 10 ml of distilled water. The resulting solution was then made alkaline by the addition of 3 ml of 1.0 M sodium hydroxide. The resulting alkaline solution was then shaken successively for 2 min. with various 15 ml portions of chloroform. The free tamoxifen base was then extracted with 15 ml of chloroform on three different occasions. The combined chloroform extracts were filtered through anhydrous sodium sulphate to remove any trace of water in the solution. The filtered extract was then diluted to 100 ml with chloroform to give a standard solution of 0.4 mg/ml.

### 2.3. Absorption spectra

A solution of CA ( $1.08 \times 10^{-3}$  M) was made in dioxane after which its wavelength of maximum absorption was determined using a Pye Unicam double beam UV-Vis spectrophotometer (SP8-100). Purple colour developed on the mixing of 2 ml volumes of TC and CA. The volume was then made up

to 5 ml with dioxane. The wavelength of maximum absorption for the resulting solution was then ascertained.

### 2.4. Standard curve

To obtain the standard calibration curve, serial volumes (0.1 ml, 0.2... ml) of  $1.08 \times 10^{-3}$  M tamoxifen solution (0.40 % w/v) were reacted with 3 ml of each of  $1.08 \times 10^{-3}$  M CA (0.20% w/v). The contents of the test tubes were then made up to 4 ml with chloroform. The test tubes were allowed to stand for a few min. after which their absorbance was taken at 550 nm.

### 2.5. Stoichiometric determination of complex composition

Job's method of continuous variation was used [14]. This involved the preparation of master solutions of CA ( $1.08 \times 10^{-3}$  M) and tamoxifen ( $1.08 \times 10^{-3}$  M). A series of 5 ml quantities of mixtures containing the drug and chloranilic acid in different complimentary proportions (0:0.5, 0.5:4.6, 4.5:0.5, ... 5:0) were transferred to previously labeled test tubes. The complex formed for each reaction mixture was left to stand for 60 min. before the spectrophotometric absorbance evaluation.

### 2.6. Assay procedure for tablets

Batches of ten tablets from the three sources investigated were crushed in a glass mortar after which an amount equivalent to 25 mg of the salt was accurately weighed out. The amount weighed out was then dissolved in sufficient volume of water to give a 0.4% w/v solution. This procedure was repeated for the two other commercial sources of TC.

### 2.7. Association constant, molar absorptivity and free energy change

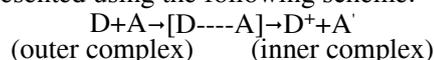
**Table 1.** Association constant (k), standard free energy ( $\Delta G^\circ$ ), standard enthalpy ( $\Delta H^\circ$ ), and standard entropy ( $\Delta S^\circ$ ) changes associated with the interaction of TC with CA.

k (M <sup>-1</sup> )	$\epsilon$ (l <sup>-1</sup> )	$\Delta G^\circ$ Kcal/mol	$\Delta H^\circ$ Kcal/mol	$\Delta S^\circ$ cal/log/min.
$2.389 \times 10^2$	$1.667 \times 10^3$	-3.298	-	-

These thermodynamic parameters were determined by the following procedure: serial volumes of between 0.2 to 1.6 ml of  $1.08 \times 10^{-3}$  M of TC solution in 0.2 ml steps were measured out and transferred to different appropriately labeled test tubes. The solutions were then diluted with chloroform to 4 ml. One ml of chloranilic acid was then added. Next, the resulting solution was then analyzed spectrophotometrically at the temperatures of 50, 70 and 90 °C in a thermostated water bath.

### 3. Results and discussion

The formation of a complex between the electron donor (TC) and the electron acceptor (CA) was indicated by the detection of a shift in the visible spectrum of the mixture towards a longer wavelength (bathochromic shift; Figure 1). This change in the wavelength of maximum absorption owing to the complexation reaction was made manifest by a colour change as chloranilic acid in dioxane changed from the typical yellowish pink colour to purple. This chromogenic transformation, which is instantaneous upon the addition of a solution of TC to chloranilic acid, is clear proof for the validation of a charge-transfer complexation reaction. Chloranilic acid in dioxane normally exhibits a peak absorption band at 430 nm. The charge transfer complex between TC and CA shows a peak at 550 nm (Figure 1). We may now hypothesize that the interaction between the n-donor TC and the  $\pi$ -acceptor CA can be represented using the following scheme:



It has been proposed that on the basis of a simple general quantum mechanical theory, the stoichiometric ratio for the electron donor

and electron acceptor should be 1:1 [15]. In the ground state, the reactants are held together when in close proximity by intermolecular forces such as van der Waals forces and hydrogen bonding [16]. When a complex absorbs light of a required energy, the electrons in its structure are raised from the ground state to the excited state and in the case of the charge transfer complex, the electron that is donated by the TC molecule is almost completely transferred to the electron acceptor. This transfer of electron is responsible for the bathochromic shift observed upon the addition of the TC solution to that of the electron acceptor CA.

The complex formed instantaneously and completely as indicated by the constant maximum absorption band attained. It was found to be stable since the absorption peak remained constant over a period of 24 h and even when the experimental set up was placed in the dark for 12 h.

The association constant of the TC-CA complex was determined by the application of the following equation:

$$A + D = AD \quad (\text{Equation 1})$$

The Scott equation [17], a modified form of the Benesi-Hildebrand equation [18] was also employed:

$$\frac{[Do][Ao]}{A_{\lambda}^{AD}} = \frac{[Do]}{\epsilon_{\lambda}^{AD}} + \frac{1}{K_c^{AD} \epsilon_{\lambda}^{AD}} \quad (\text{Equation 2})$$

where [Do] and [Ao] represent the initial concentrations of the reactant species,  $A_{\lambda}^{AD}$  is the absorbance of the complex at the wavelength,  $\lambda$ ,  $\epsilon_{\lambda}^{AD}$  is the molar absorptivity, and  $K_c^{AD}$  is the association constant.

**Table 2.** Measurement of TC in the pure sample and tablets.

Parameter	Pure sample	Tablet (samples <sup>a</sup> )		
		A	B	C
Mean recovery (%) <sup>b</sup>	100.25	100.36	99.98	100.32
Standard deviation (%)	1.36	1.14	0.78	1.02

<sup>a</sup>TC tablets manufactured by (Generics Ltd, UK), (Teva) an (Noston).

<sup>b</sup>Mean for ten determinations; percentage recovery from the label claim.

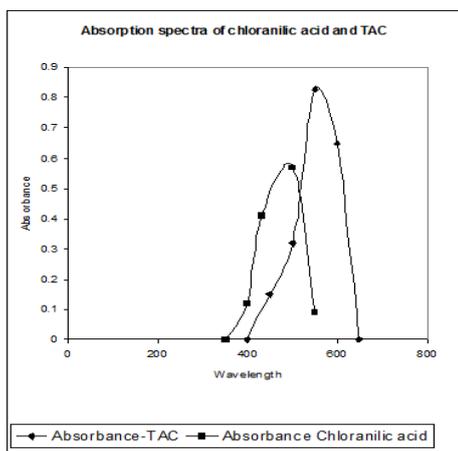


Figure 1. Absorption spectra of chloranilic acid and tamoxifen.

The scrutiny of the graphical representation of the relationship between  $[Do][Ao]/A_{\lambda}^{AD}$  versus  $[Do]$  with  $[Ao]$  being constant, yields the values of  $E_{\lambda}^{AD}$  and  $K_c^{AD}$ , which are represented by the slope and the intercept, respectively. With a view towards the minimization of experimental error, the method of least squares was employed in the determinations [19].

The plot of Equation 2 is shown in Figure 3. The calculated value of the intercept varied slightly with temperature though it cannot be said that this variation is very significant.

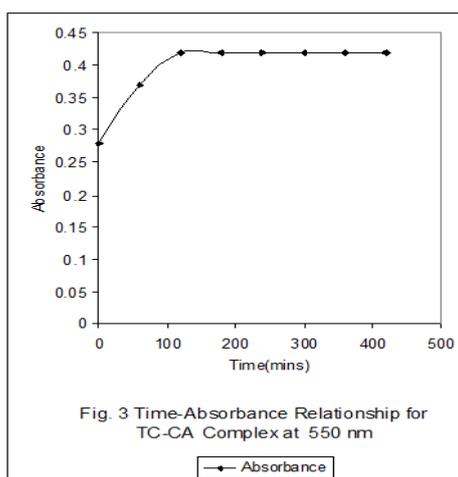


Figure 3. Time-absorbance relationship for tamoxifen citrate-chloranilic acid complex at 550 nm.

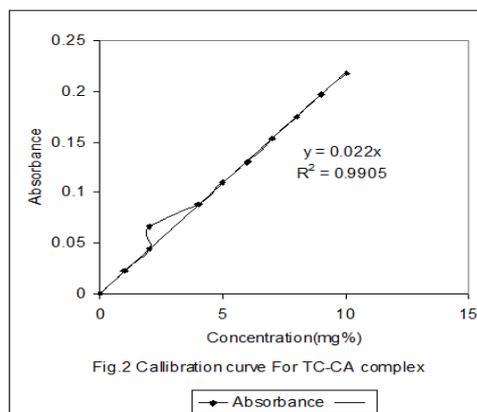


Figure 2. Calibration curve of tamoxifen citrate-chloranilic acid complex

Next, thermodynamic values were calculated by means of the mathematical relationship between the standard enthalpy change,  $\Delta H^{\circ}$ , and the changes in temperature. The thermodynamic equation is shown below:

$$\text{Log } K_c^{AD} = \frac{\Delta H^{\circ}}{2.303RT} + \text{Constant} \quad (\text{Equation 3})$$

A plot of Equation 3 for TC-CA complex is represented in Figure 3. The standard free energy,  $\Delta G^{\circ}$  is determined by the application of the following relation:

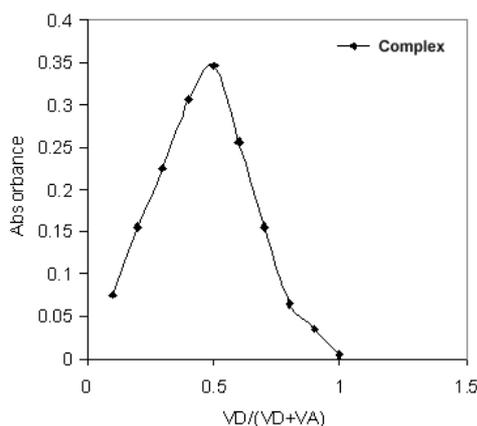


Figure 4. Continuous variation plot for tamoxifen citrate-chloranilic acid complex ( $1.08 \times 10^{-3}$  M).

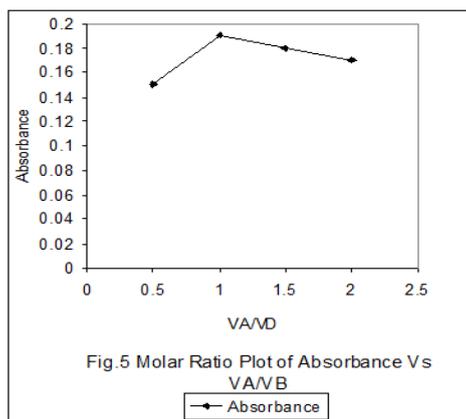


Figure 5. Molar ratio plot of absorbance vs VA/VD.

$$\Delta G^\circ = -RT/K_c^{AD} \quad (\text{Equation 4})$$

The standard entropy change  $\Delta S^\circ$  is obtained using equation 5:

$$\Delta G = \Delta H^\circ - T\Delta S^\circ \quad (\text{Equation 5})$$

The results of this thermodynamic evaluation as well as the molar absorptivity are shown in Table 1. The molar absorptivity was found to be unaffected by temperature while the association constant was found to decrease with increase in temperature.

The negative free energy change ( $-\Delta G^\circ$ ) and the positive entropy change ( $+\Delta S^\circ$ ) both point to a spontaneous reaction [20]. This finding corroborates what was experimentally observed. Some researchers have demonstrated that  $\Delta H^\circ$  and  $\Delta S^\circ$  generally become more negative as the value of the stability constant increases [21]. The high negative value of  $\Delta H^\circ$  and the association constant as can be discerned from Table 1 point to the high strength of the complex formed between TC and CA as well as its high stability.

Details of the assay of the pure sample and tablets of TC are shown in Table 2. Recovery of the active principle is quite high with very low standard deviations.

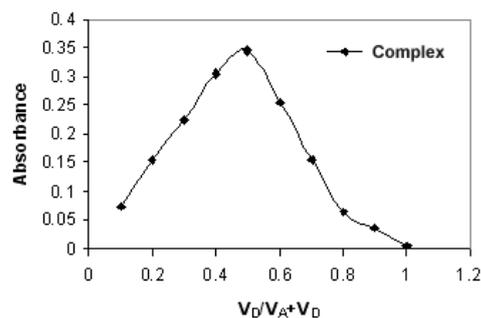


Figure 6. Job's continuous variation plot for TCA complex measured at 550 nm.

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