◆ Scientific
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Spectrophotometric Determination of Lamivudine Using Chloranilic Acid and 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)

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Abstract

A spectrophotometric method for the assay of lamivudine in pure form and in dosage form was developed in this study. The method was based on charge-transfer complex formation between the drug, which acted as n-donor while chloranilic acid and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) acted as a π-acceptor in a non-aqueous solvent in each case. Chloranilic acid was found to form a charge-transfer complex in a 1:1 stoichiometry with lamuvidine (lamivudine-chloranilic acid) with a maximum absorption band at 521 nm. Also, DDQ was found to form a charge-transfer complex in a 1:1 stoichiometry with lamivudine (lamivudine-DDQ) with a maximum absorption band at 530 nm. The pH was obeyed at acid range. The complexes obeyed Beer's law at a concentration range of 0.04 - 0.28 mg/ml. The thermodynamic parameters calculated at different temperatures included the molar absorptivity, association constant, free energy change, enthalpy and entropy. The proposed method has been conveniently applied in the analysis of commercially available lamivudine tablet with good accuracy and precision.

1. Introduction

Lamivudine is chemically 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] primidin-2-(1H)-one. It is an antiretroviral drug belonging to the class called nucleoside reverse transcriptase inhibitors (NRTIs)[1]. It exhibits potent antiretroviral activity [2]. The adult does is 150 mg three times daily. It was indicated that combination therapy of lamivudine with zidovudine is associated with substantial persistent increase in CD_4^+ cell counts and decreases in HIV RNA as measured by polymerase chain reactions [3]. Chloranilic acid, DDQ and other π-acceptors have been variously utilized in the spectrophotometric assay and analysis of many drugs by charge-transfer complexation [4-9]. Uv-visible Spectrophotometric methods are the instrumental methods of choice which are commonly used in industrial labouratories because of their simplicity, accuracy, precision and low cost [10-12]. In the available literatures, the method has not been adopted in the analysis of this drug both in pure sample and in pharmaceutical formulations.

2. Aims and Objectives of the Study

Our contemporary drug market is frequently eroded with fake and substandard drugs. Efforts are therefore directed in this work to the development of simple, accurate and sensitive analytical methods for screening lamiduvine which occupy a strategic position in clinical practice. The aim of the present work is to develop a simple, sensitive and less expensive spectrophotometric method of analysis for the determination of lamivudine using 2,3-dichloro-5,6 dicyano-1,4-benzoquinone and chloranilic acid with methanol as the solvent.

3. Experimental

3.1. Materials

The following materials were procured from their local suppliers and used without further purification: Lamivudine pure powder (Fidson Healthcare Ltd, Lagos Nigeria), chloranilic acid (Sigma-Aldrich Chemie, Germany), DDQ 98% (Sigma-Aldrich Chemie, Germany), methanol (Analytical grade, BDH, UK). All other reagents and solvents were of analytical grade and were used as such. All laboratory reagents were freshly prepared.

3.2. Preparation of Standard Solutions

Lamivudine standard solution (0.00372 M): This was prepared by weighing 0.00853 g accurately on an electronic weighing balance and dissolving in enough methanol in 100 ml standard flask and making up to 100 mL with methanol.

Chloranic acid standard solution (0.00372 M): A 0.07 78 g quantity of chloranilic acid was accurately weighed on an electronic weighing balance and dissolved in methanol in 100 mL standard flask and the volume made up to 100 mL with methanol.

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ 98%) standard solution (0.00372 M): A 0.0844 g quantity of DDQ was accurately weighed on an electronic weighing balance and dissolved in methanol in 100 mL standard flask and the volume made up to 100 mL with methanol.

3.3. Absorption Spectra

A 4 mL volume of the chloranilic acid standard solution was scanned in a double-beam Uv-vis spectrophotometer from –nm to –nm to determine its wavelength of maximum absorption. Similarly, the wavelength of maximum absorption of a coloured solution developed by mixing 4 ml volume of DDQ standard solution and 2 mL of lamivudine standard solution was determined, together with a coloured solution developed on mixing 2 mL of DDQ standard solution and 2 ml of lamivudine standard solution.

3.4. Stoichiometric Determination of the Complex of Lamivudine-Chloranilic Acid

The slope ratio method was employed. Lamivudine solution (3.72×10^{-3} M) was kept constant in each case while chloranilic acid solution (3.72×10^{-3} M) was varied according to the following ratios: 0.25:5, 0.25:7.5, ⋯,

0.25:15 mL of lamivudine:chloranilic acid. They were transferred into different test tubes from the beaker. The mixtures were allowed to stand for 1 h before determining the absorbance at 521 nm against the blank of methanol and the chloronilic acid. Also, the chloronilic acid was kept constant while the lamivudine was varied as mentioned above. They were also transferred into different test tubes from the beaker for colour development and kept for 1 h before determining the absorbance at 521 nm against the blank of methanol and chloranilic acid.

3.5. Stoichiometric Determination of the Complex of Lamivudine-DDQ

The same method described immediately above was adopted but in this case, the absorbance was determined at 430 nm.

3.6. Effect of Time on the Formation of Lamivudine-Chloranilic Acid Complex

The absorbance of a mixture of 2 mL of 3.72×10^{-3} M lamivudine solution in methanol and 2 mL of 3.72×10^{-3} M chloranilic acid solution in methanol was determined at various time intervals from 5 s to 120 min at 53l nm (λ_{max}) at room temperature against methanol blank and the reagent blank.

3.7. Effect of Time on the Formation of Lamivudine-DDQ Complex

The same method described immediatelyabove was adopted but in this case, the absorbance was determined at 430 nm.

3.8. pH Study of Lamivudine-Chloranilic Acid Complex

A 3.72×10^{-3} M solution of lamivudine was mixed with a 3.72×10^{-3} M solution of chloranilic acid at the ratio of 2:2 and 6 mL of buffer solution was added in each case to make up the volume to 10 mL. The same treatment was done with buffer 1 - 13 in different test tubes for colour development and kept for 1 h before determining the absorbance at 521 nm against the blank of methanol, buffer and the reagent blank.

3.9. pH Study of Lamivudine-DDQ Complex

The same method described immediately above was adopted but in this case, the absorbance was determined at 430 nm.

3.10. Determination of Association Constant, Molar Absorptivity and Thermodynamic Parameters of the Complexes

Lamivudine–chloranilic acid complex: Serial volumes (0.4, 0.8, ···, 2.4 mL) of lamivudine solution (3.72×10^{-3} M) were transferred into different test tubes. The solutions were diluted to 3 mL with methanol and serial volumes (0.4, 0.8, ···, 2.4 mL) of chloranilic acid solution in methanol (3.72×10^{-3} M) were added to various test tubes. The content were mixed and left at room temperature for 1 h after which their absorbance was determined at 521 nm against a blank of methanol and chloranilic acid at temperature of 30°C (room temperature). Further analysis of the reaction mixtures were done by subjecting them to temperatures of 40°C, 50°C and 60°C in a thermostated water bath.

3.11. For Lamivudine-DDQ

The same method described immediately was adopted but in this case, the absorbance was determined at 430 nm.

3.12. Beer's Calibration Plot for Lamivudine-Chloranilic Acid Complex

Serial concentrations (0.4, 0.8, ···, 2.8 mL) of the standard solution (3.72×10^{-3} M) were transferred to different test tubes. Sufficient volumes of chloranilic acid in methanol were added to each of the test tubes according to the stoichiometry determined. Sufficient volumes of methanol were also added to bring the volumes to 6 mL in each of the test tubes. The contents were mixed and left at room temperature for 1 h after which the absorbance of each of the samples was determined at a wavelength of 521 nm against a methanol and chloranilic acid blank. The absorbance values were plotted against the concentration to obtain the Beer's calibration curve for lamivudine.

3.13. For Lamivudine-DDQ Complex

The procedure immediately above was adopted but the absorbance was determined at 430 nm.

3.14. Assay Procedure for the Complexes

3.14.1. Lamivudine-Chloranilic Acid Complex
One tablet formulation of lamivudine equivalent to 150 mg of lamivudine was crushed in a crucible and dissolved in a 50 mL standard flask with methanol to extract the active pharmaceutical ingredient (API). The solution was filtered to remove the excipients after shak-

ing for 3 min. The filtrate was made up to 50 mL mark with methanol. The solution was diluted further by taking 1 mL of lamivudine and adding 20 mL of methanol. Serial volumes of 0.4, 1.6 and 2.8 mL of the solution were transferred into different test tubes. These volumes give the corresponding concentration 0.06 mg, 0.24 mg and 0.42 mg respectively as was used in the Beer's plot and sufficient amount of methanol added to bring the volumes to 5 mL each. The constituents were mixed with sufficient volumes of chloranilic acid and left at room temperature for 1 h after which their absorbance was determined at 420 nm against a blank of methanol and chloranilic acid blank.

3.14.2. Lamivudine-DDQ Complex
The same method described immediately above was adopted but their absorbance was determined at 430 nm.

## 4. Results and Discussion

4.1. Absorption Spectra

A solution of chloranilic acid in methanol had a golden yellow colour with maximum wavelength at 434 nm (**Figure 1**). On reacting the colourless drug solution of lamivudine with chloranilic acid solution, a purple colour was obtained. This suggested a charge-transfer complex formation resulting in the scanning of the complex in the visible range of 350 - 600 nm, which showed a maximium peak at 521 nm (**Figure 1**). The interaction between lamivudine and chloranilic acid is as shown in **Scheme 1**. The complex was formed instantaneously. The complex was stable for over 24 h as indicated by the colour of the complex. The plot of absorbance against time is shown in **Figure 2**. The absorbance band of chloranilic acid showed a bathochromic shift (shift to a longer wavelength).
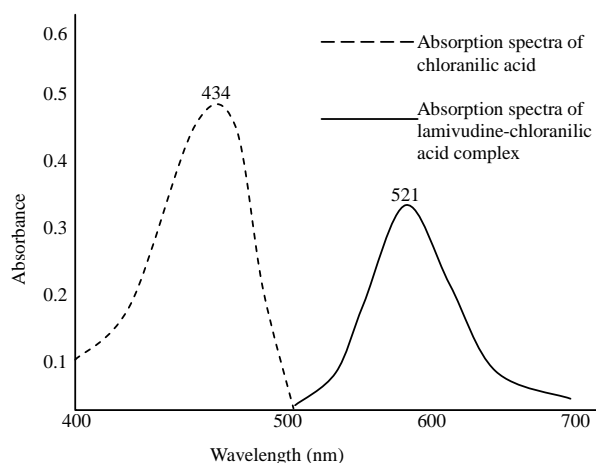
Figure 1.
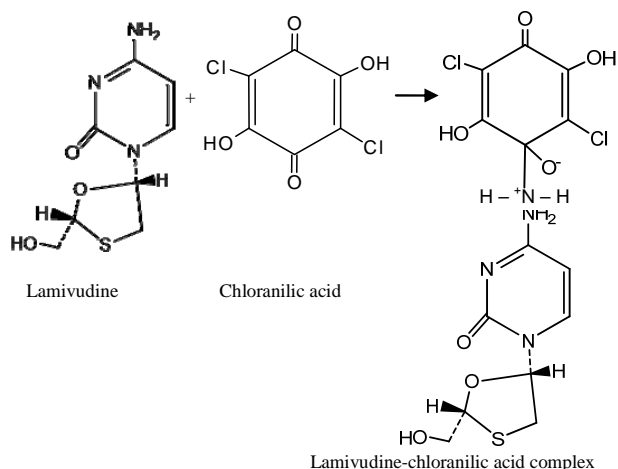
Scheme 1. Proposed structure of lamivudine-chloranilic acid charge transfer complex.
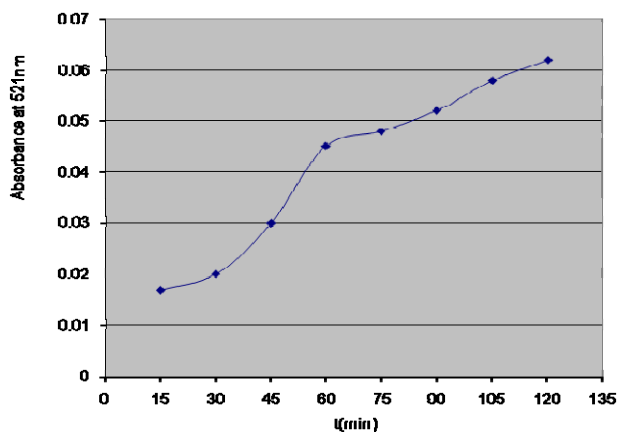

Figure 2. Lamivudine-chloranilic acid complex with respect to absorbance and time.

Also, a solution of DDQ in methanol had a golden colour with maximum wavelength at 351 nm (**Figure 3**). On reacting the colourless drug solution of lamivudine with DDQ solution, a deep golden colour was obtained. This suggested a charge-transfer complex formation resulting in the scanning of the complex in the visible range of 400 - 600 nm, which showed a maximum peak at 430 nm (**Figure 3**). The interaction between lamivudine and DDQ is as shown in **Scheme 2**. The complex was also formed instantaneously. The complex was stable for over 24 h as indicated by the colour of the complex. The plot of absorbance against time is shown in **Figure 4**. The absorption band of DDQ also showed a bathochromic shift.
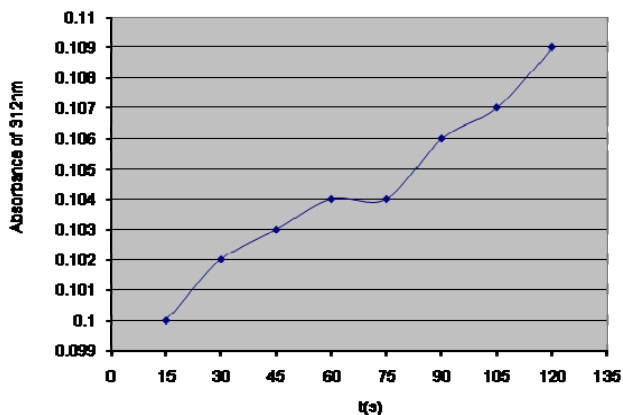
4.2. Stoichiometric Determination of the Complexes

Lamivudine-chloranilic acid: The stoichiometric ratio of
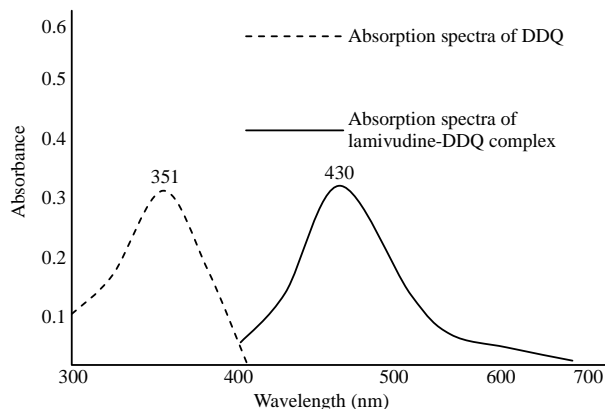
Figure 3.
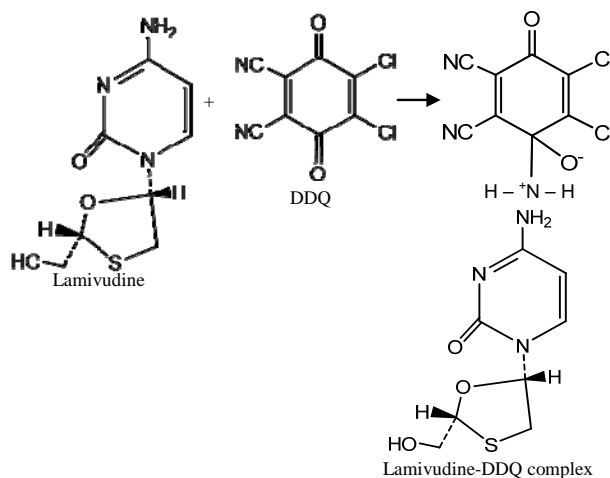
Scheme 2. Proposed structure of lamivudine-DDQ charge transfer complex.


Figure 4. Lamivudine-benzoquinone complex with respect to absorbance and time.

the reactant was determined using slope ratio method. A 1:1 ratio of charge-transfer complex was indicated for the lamivudine-chloranilic acid interaction (**Figures 5(a)** and (**b**)).

Lamivudine-DDQ complex: The stoichiometric ratio of the reactant was determined using slope ratio method. A 1:1 ratio of charge-transfer complex was indicated for the lamivudine-DDQ interaction (**Figures 6(a)** and **(b)**).
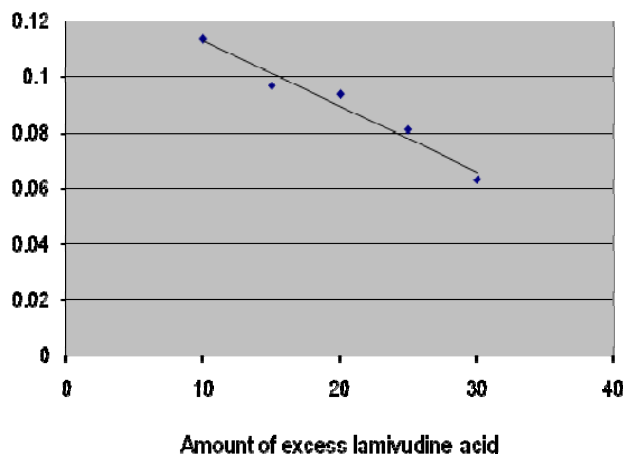
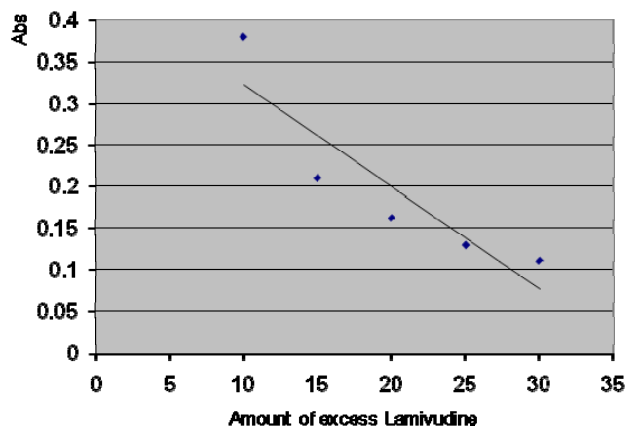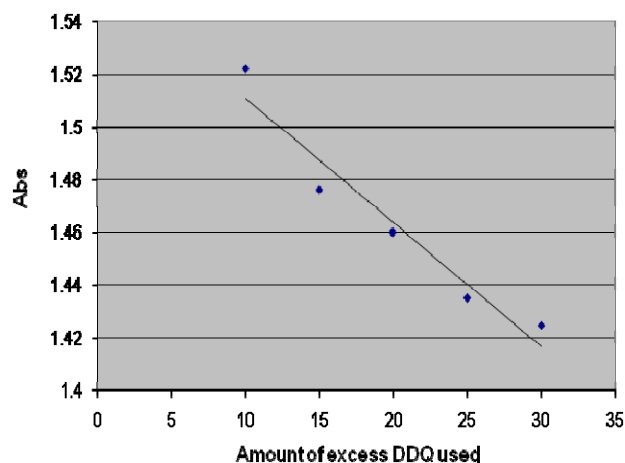
(a)
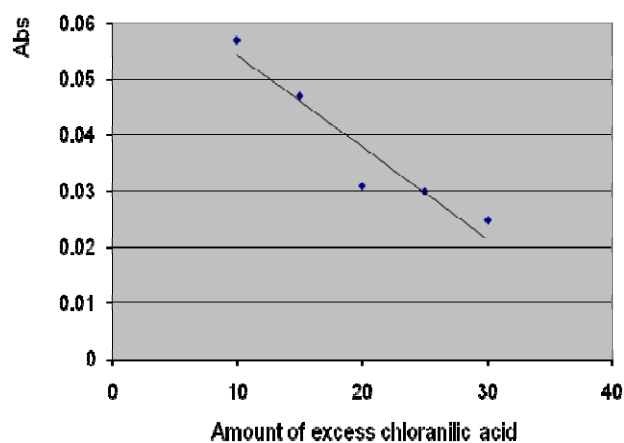
(b)

Figure 5. (a) lamivudine-chloranilic acid complex with excess lamivudine; (b) lamivudine-chloranilic acid complex with excess chloranilic.

(a)

(b)

Figure 6. (a) lamivudine-DDQ complex with excess lamivudine; (b) Lamivudine-DDQ complex with DDQ in excess.

4.3. Association Constant, Molar Absorptivity and Thermodymamic Parameters of the Complexes

Lamivudine-chloranilic acid complex: They were evaluated using modification of the Benesi-Hildebrand Equation [13].

$$\frac{\left[A_o\right]^2}{A_\lambda^{[D:A_2]}} = \frac{1}{E_\lambda^{[D:A_2]}} + \frac{1}{K_C^{(D:A_2)}E_\lambda^{[D:A_2]}}\left(\frac{1}{\left[D_o\right]}\right) \qquad (1)$$

where $[D_o]$ and $[A_o]$ are the initial concentrations of the reactants. $A_\lambda^{[D:A_2]}$ is the absorbance of the complex at 521 nm, $E_\lambda^{[D:A_2]}$ is the molar absorptivity of the complex at 521 nm and $K_C^{[DA_2]}$ is the stability constant. The plot of $\left[A_o\right]^2/A_\lambda^{(D:A_2)}$ against $1/[D_o]$ is shown in **Figure 7**. The intercepts and the slopes of the regression lines were used to obtain the values of $E_\lambda^{[D:A_2]}$ and $K_C^{[DA_2]}$ respectively, at constant [A$_o$]. The molar absorptivities calculated were almost constant at the different temperatures. This was expected since ideally, it should not vary. Increase in temperature may have led to the dissociation of the formed complexes. These are presented in **Table 1**. The standard enthalpy change, ΔH^o, of the lamivudine-chloranilic acid interaction was obtained from this equation:

$$LogK_C^{(D:A_2)} = \frac{\Delta H^o}{2.303RT} + \text{constant} \qquad (2)$$

by plotting $\log K_C^{[D:A_2]}$ against the reciprocal of absolute temperature T, when it was calculated from the slope of the regression line. The plot is shown in **Figure 8** and the result is presented in **Table 1**, where R is the gas constant.
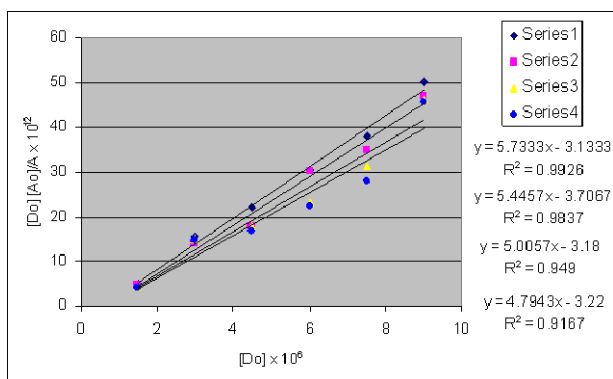
Figure 7. Benesi-Hildebrand plots for lamivudine-chloranilic acid.
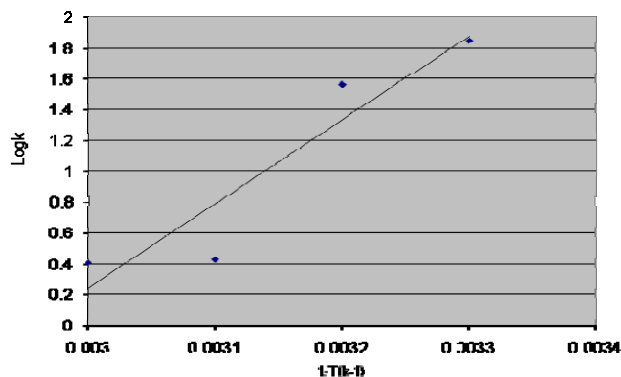

Figure 8. Logk of Lamivudine-chloranilic acid verses Thermodynamic temperature.

Table 1. Logk of Lamivudine-chloranilic acid verses thermodynamic temperature.

K (M^{-1})	ε	Log k	$\frac{1}{T}$ (K^{-1})	Temp (k)	ΔG^0 cal/deg/mol	ΔH^0 cal/deg/mol	ΔS^0 cal/deg/mol
6.9.93	0.286	1.85	0.003	303	−10701.315	−67.015	28.92
35.97	0.278	1.56	0.0032	313	−9323.98	−68.93	24.41
2.67	0.250	1.43	0.0031	323	−2637.35	−76.59	6.60
2.55	0.196	1.41	0.0130	333	−2591.65	−97.65	6.018

Similarly, Gibb's free energy (ΔG^0) and the entropy (ΔS^0) were calculated respectively from the equations 3 and 4 and the results are presented in **Table 1**.

$$\Delta G = -RT \ln K_C^{(D:A_2)} \tag{3}$$

$$\Delta G^o = \Delta H^o - T\Delta S^o \tag{4}$$

Lamivudine-DDQ complex: The same method as in lamivudine-chloranilic acid complex was adopted but the absorbances were read at 430 nm. The plot of

$$\left[A_o\right]^2 \big/ A_\lambda^{[D:A_2]}$$

against $1/D_o$ is shown in **Figure 9**. The results were similar to that of lamivudine-chloranilic acid complex but different plots and tables were generated for lamivudine-DDQ complex (**Figures 9**-**10** and **Table 2**).

4.4. pH Studies of the Complexes

Lamivudine-chloranilic acid complex, buffer solutions of pH 1 - 13 were used. It was observed that the highest peak was at pH of 2.0, which showed that the complex was favoured under high acidic medium which means that the complex dissociates appreciably. The plot of absorbance against pH is as shown in **Figure 11**.

Lamivudine-DDQ complex: Buffer solutions of pH 1 - 13 were also used. It was observed that the highest peak is at the pH of 6.0 which showed that the complex was
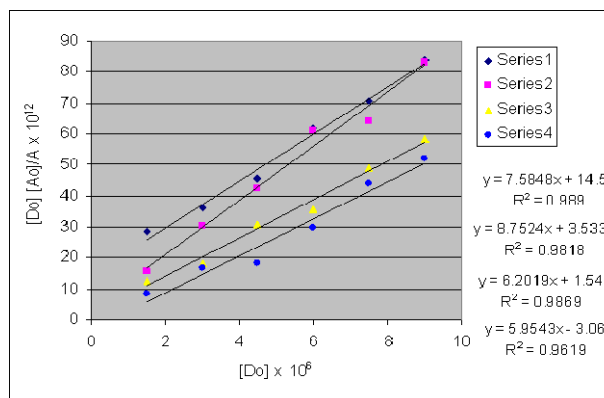

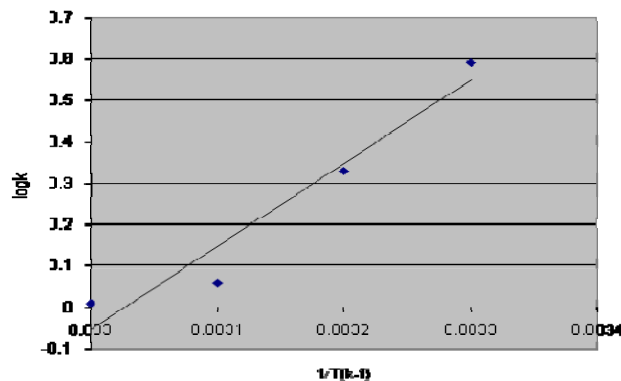**Figure 9. Benesi-Hildebrand plots for Lamivudine-DDQ.**

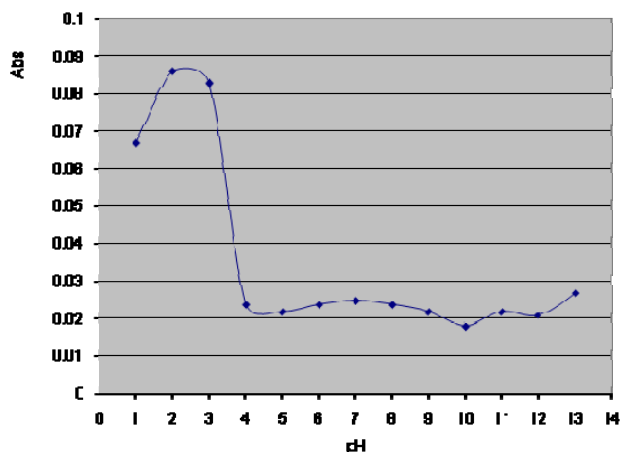**Figure 10. Logk of lamivudine-DDQ verses thermodynamic temperature.**

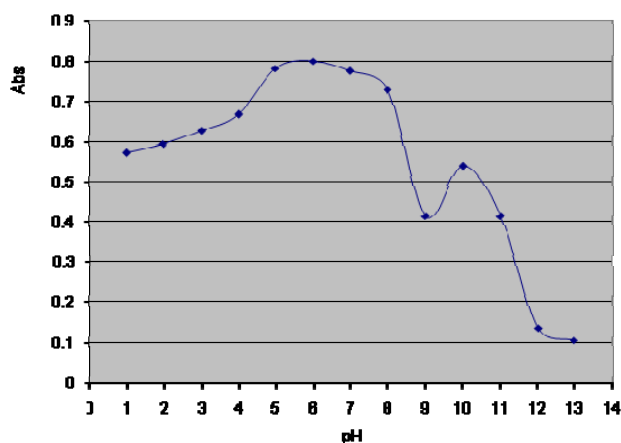**Figure 11. pH study of Lamivudine-chloranilic acid complex.**

Figure 12. pH study of lamivudine-DDQ complex.

Figure 13. Beer's calibration curve for lamivudine-chloranilic acid complex.
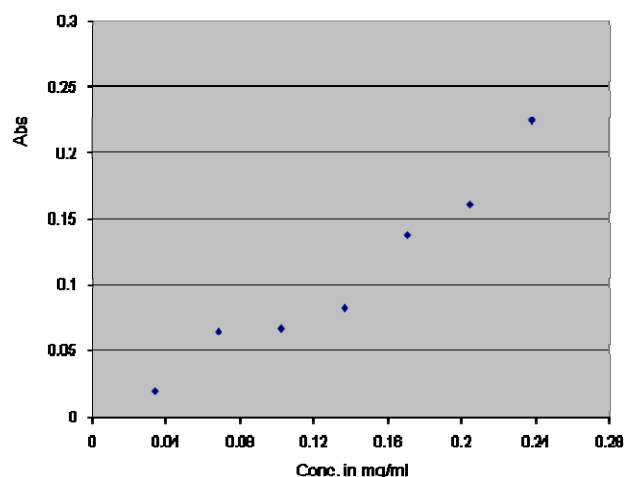
Figure 14. Beer's calibration curve for lamivudine-DDQ complex.

favoured under a very weak acidic medium which means that the complex did not dissociate appreciably. The plot of absorbance against pH is as shown in **Figure 12**.

4.5. Beer's Calibration Plots for the Complexes

Lamivudine-chloranilic acid complex: A standard calibration plot for lamivudine was constructed by plotting absorbance verses concentration of the drug in mg/mL of the lamivudine standard solution. A straight line passing through the origin was obtained for the complexed drug, indicating that spectrophotometric analysis of electron donor-acceptor complex formation can be used for quantitative analysis of the drug (**Figure 13**). Conformity with Beer's law was evident in the concentration rage of 0.04 - 0.28 mg/mL of lamivudine.

Lamivudine-DDQ complex: The same procedure for lamivudine-chloranilic acid complex was adopted but different plot that passed through the origin was obtained (**Figure 14**).
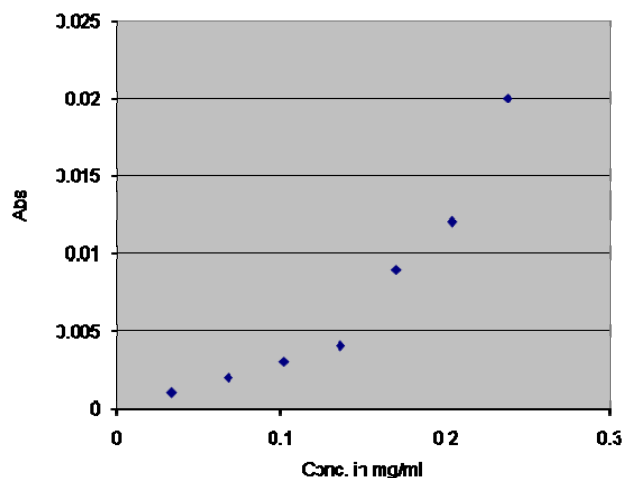
From the assay of the drug, it was discovered that the recovery experiments carried out on lamivudine in tablet dosage form showed high quantitative recoveries with low standard deviations. For lamivudine-chloranilic acid complex, the mean percentage recovery of the drug was found to be $85.1\% \pm 12.3\%$, and that of lamivudine-DDQ, the percentage recovery was $85.1\% \pm 12.1\%$ also. These indicated a high accuracy of the method of analysis.

5. Conclusions

A charge-transfer complexation between lamivudine with different reagents (chloranilic acid and DDQ) occurred with a 1:1 stoichiometry in each case, with maximum wavelength of absorption of 521 nm for lamivudine-chloranilic acid complex and 430 nm for lamivudine-DDQ complex. The reactions were favoured at acidic

medium according to the pH determinations. Thermodynamically, the complexes were found to be very stable at room temperature. The methods were used to assay the drugs in pure form and in dosage form with good precision and accuracy and can therefore be used in rapid qualitative and quantitative determination of lamivudine both in pure form and in dosage form.

6. References

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