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Equilibrium, Thermodynamic and Kinetic Studies for the Color Reaction of Metronidazole with Acridone Using Spectrophotometric Technique

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Abstract

Simple and sensitive spectrophotometric method is described to estimate Metronidazole drug in pure form. The procedure for spectrophotometric determination of Metronidazole based on the reaction of Metronidazole drug with Acridone to forms a highly stable reddish-orange colored complex. As well as their physical thermodynamic functions, chemical kinetic and binding modes were determined. The wavelength of the maximum absorption λ_{max} of the complex against reagent blank was 502nm compared to 313nm for Metronidazole and 295nm for Acridone alone. The complex formed was still stable for one week. Under the optimum reaction conditions, the complex obeyed Beer's law in the concentration range of (1×10^{-3}–6×10^{-3}M) with fair correlation. The composition of the complex was determined using the job's method of continuous variation, results showed that stoichiometric ratio of Metronidazole-Acridone was 1:1.The binding of Metronidazole to Acridone is quite strong as indicated by its equilibrium binding constant K_{eq} and showed a reduction with increase of temperature. Thermodynamic parameter including ΔG°, $\Delta H°$ and $\Delta S°$ were determined at various temperatures (298-313), the negative values of the standard free energy$\Delta G°$ indicate that the reaction of drug with Acridone is spontaneous. The van't Hoff equation of 1/T versus $\ln K_{eq}$ suggests that the Metronidazole drug binds exothermically to Acridone which is characterized by high negative value of the standard enthalpy change $\Delta H°$ as well as the negative values of $\Delta S°$ indicate reduce the freedom of motion in the transition state and relatively slow reaction. A chemical kinetics result shows that the reaction was pseudo-first order reaction. Moreover atomic force microscopy to explain other properties of precipitate formed is topographical information of nanostructure between Metronidazole and phosphotungstic acid.

Keyword: Spectrophotometry, Metronidazole, Acridone, Thermodynamic function. Chemical kinetics, Atomic force microscopy

1. INTRODUCTION

Metronidazole is the drug of choice for human infections caused by various anaerobic and micro-aerophilic bacteria. Metronidazole is chemically named as [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole]. It is a 5-nitroimidazole derivative used as antiamoebic, antiprotozoal and antibacterial drug [1]. It was first synthesized in 1950$_s$under the brand name Flagyl [2]. Metronidazole is a member of the imidazole class of antibacterial agents and is classified therapeutically as an antiprotozoal and antibacterial agent. The nitroimidazoles share a heterocyclic structure consisting of an imidazole-based nucleus with a nitro group (Fig.1A). It appears as a white to brownish cream crystalline substance with a molecular weight of 171.16 and melting point of 160°C [3]. Metronidazole is active against a wide range of pathogenic microorganisms, notably species of ***Bacteroides*** *,Eubacteria,Clostridia anaerobic cocci and Gardnerella vaginalis.*it is also active against *Trichomonas vaginalis, Entamoeba histolytica, Giardia lamblia, Balantidium coli and Helicobacter pylori* [4]. Metronidazole works by disrupts DNA, Inhibits nucleic acid synthesis susceptible organisms, the mechanism of action involves reduction of the nitro group to form radicals. This eads to bacterial DNA damage and subsequent cell death [5].Reduction of the nitro group has been also used as a basis for determination of metronidazole of these drugs [6].

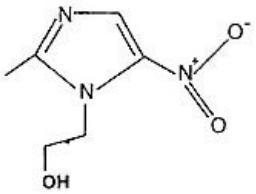


Figure1A.Chemical structure of metronidazole

Acridones are organic compound based on the acridine skeleton; It is oxidized product of acridine Acridone constitutes the scaffold of some synthetic compounds with various pharmacological activities. Acridone is defined as tricyclic ring having nitrogen at 10th and carbonyl group at 9th position (Fig1B), it is also known by the name of 9 (10H)-acridinone, acridine,-9-one, 9-acridanone, acridinone.it is oxidized product of acridine.it may be synthesized by the self-condensation of N-phenylanthranilic acid [7].

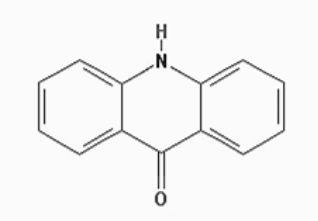


Figure1B.Chemical structure of Acridone

The tricyclic acridone ring system has become a focus of major research by medicinal chemists due to the biological significance of this moiety in drug design and discovery. Acridone has substantial bio-potential signification of since it performs crucial functions, including anticancer, antiviral, antimicrobial, antimalarial and anti-inflammatory activities [8]. Acridone melts at 354°C; it is a pure yellow solid; it is almost insoluble in water, chloroform, ether, ethanol and benzene. Acridone dissolved in alcoholic potassium to give a yellow brown solution of its potassium salt which completely decomposed in water. Acridone is highly associated indicates by the high melting point and low solubility of it in many solvent [9].

Atomic force microscopy or AFM is part of microscopy group called scanning probe microscopy. It is a well-suited and an amazing technique to analyze topographical features and mechanics at the nanoscale. It allows us to see the shape of a surface in three-dimension (3D) detail down to the nanometer scale and measure surface structure with accuracy and unprecedented resolution. AFM has come to be used in all fields of science, such as, biology, physics, chemistry, materials science, nanotechnology, astronomy, and medicine [10, 11].

The objective of this studies it to quantitative estimation of Metronidazole in pure form using spectrophotometric technique. The method based on the formation of colored complexation of Metronidazole with Acridone, as well as, a study was performed to describe the complexation chemistry by determination of the equilibrium constant, physical thermodynamic functions and chemical kinetic of the reaction were carried out at various temperatures.

2. EXPERIMENTAL

2.1. Instrument

Shimadzu UV-VIS double beam spectrophotometer 1800 (Germany) was used for all spectral measurements. Water bath Electro thermal-England was used.

2.2. Chemicals and Reagents

All chemical used were of spectroscopic grade and the solutions were prepared with double distill water**.**

2.3. Preparation of the Standard Stock Solution

A stock solution of Metronidazole (Aldrich-Sigma, Germany, purity: 99.00 %) at 5×10^{-2}M concentration was prepared by dissolving an accurately a weight amount of 2.139g of metronidazole in 20 ml 0.3M H_2SO_4 then the volume was completed to 250 ml with distilled water. A stock solution of Acridone (Hopkins &William LTD, England) 5×10^{-2}M was prepared by dissolving an accurately weight amount of 0.1299g of Acridone in 10% dimethyl sulfoxide DMSO then the volume was completed to 250 ml with distilled water.10% dimethyl sulfoxide DMSO (BDH Co, England) was prepared by transferring quantitatively10mL of DMSO to 100 ml volumetric flask and completed with distal water. 0.3×10^{-3}M H_2SO_4 (Merck Co., Germany), M.wt 98.08 g/mol, sp.gr 1.84 g/mol and purity was 99.00 was preparing by transferred quantitatively 5.4 ml to 25 ml volumetric flask and completed with distilled water. Phosphotungstic acid (PTA) Anhydrous (Hopkins &William LTD, England) $H_3PW_{12}O_{40}$, M.wt 2880.2 prepared by dissolved 28.802 g of PTA in few drops of phosphoric acid, followed by heating until complete dissolution then the volume was completed to 100mL with distilled water and kept overnight for use.

2.4. General analytical procedures

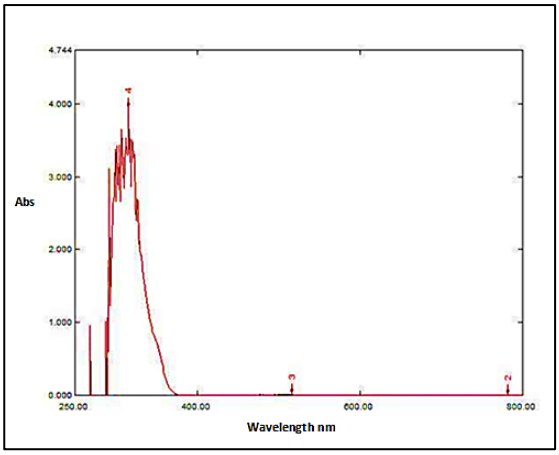
After fixing the optimum conditions, A series of working standard solutions of Metronidazole ranging from (1×10^{-3}–6×10^{-3}M) were prepared into a series of 10mL volumetric flasks by dilution from stock solutions 5×10^{-2}M in 0.3×10^{-3}M H_2SO_4. A volume of 0.5 ml 5×10^{-2}M of Acridone in10% dimethyl sulfoxide DMSO was added and the contents of the flasks were diluted to the mark with distilled water. At (502nm) the absorbance was measured at room temperature against a blank containing (H_2SO_4: DMSO). A calibration graph was drawn and the linear equation was calculated.

Job's method of continuous variation was employed. Equimolar concentrations 5×10^{-2} M of solution of Metronidazole drug and Acridone were used. A series of 1.0 ml volumes of mixture Metronidazole drug and Acridone comprising complementary proportions in various ratios ranging from 0.1:0.9 to 0.9:0.1) Metronidazole: Acridone were transferred into test tubes than the contents of the flasks were diluted to the mark with distilled water, the complex formed for each reaction mixture was allowed for 1h before analysis at (502nm). A solution consisting of (H_2SO_4: DMSO) was used as a blank. The rate constant and order of reaction of Metronidazole with Acridone was done for the stoichiometry ratio by measuring the absorbance of the complex of a concentration 5×10^{-3}M of with time.

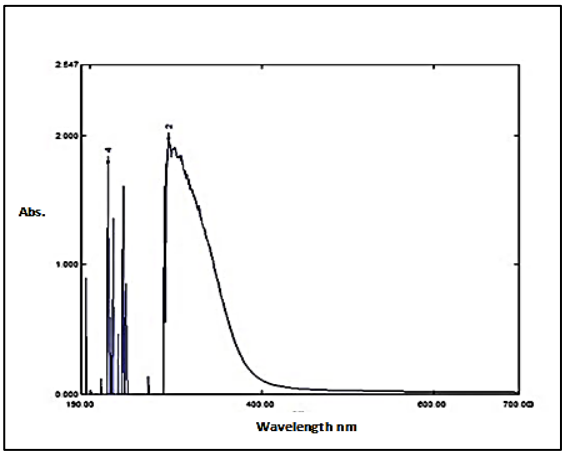
3. RESULT AND DISCUSSION

3.1. Absorption Spectrum of Metronidazole and Acridone

Absorption spectra of Metronidazole 3.5×10^{-3}M versus the blank solution 0.3×10^{-3}M H_2SO_4 and Acridone 2.5×10^{-3}M versus the blank solution 10% dimethyl sulfoxide DMSO. The UV-Vis spectrum of Metronidazole shows a maximum absorbance occurring around 313nm and Acridone at (295nm).The full spectrum is shown in Fig. 2.



A



B

Figure 2: UV-VIS absorption of A: Metronidazole in 0.3×10^{-3} M H_2SO_4 B: Acridone in10%DMSO Using quartze cell of 1cm path length (200-800) nm.

3.2. Optimization of Reaction between Metronidazole with Acridone conditions

3.2.1. Effect of variable Concentration of H_2SO_4 on Absorbance of Drug.

A series of H_2SO_4 solutions ranging from ($0.1\text{-}0.5\times10^{-3}$M) were prepared, and at fixed concentration of Metronidazole 3.5×10^{-3}M and the absorbance measurement were taken for them at λ_{max}313nm of MTZ. All results obtained were summarized in Table1. It was found that the absorbance of Metronidazole increase with increase of acid up to 0.3×10^{-3}M, more than the absorbance was decreased.

Table 1: Absorbance of Metronidazole 3.5×10^{-3}M in variable concentration of H_2SO_4 at λ_{max} 313nm

$[H_2SO_4]\times10^{-3}$M	Absorbance
0.1	0.325
0.2	0.562
0.3	0.812
0.41	0.456
0.5	0.213

3.2.2. Stability of Metronidazole Solutions in the Presence of Solvent

The interaction of Metronidazole with the solvent of H_2SO_4 was studies by the measurements of absorbance at variable selected of time ranged from ((1-120 min.)

It was found that there is no interaction between Metronidazole and the solvent at the optimum concentration of H_2SO_4 0.3×10^{-3}M.This was observed from the constant of absorbance of Metronidazole solution 3.5×10^{-3}M as shown in Table 2.

Table 2: The absorbance of Metronidazole 3.5×10^{-3}M in 0.3×10^{-3}M concentration of H_2SO_4 with variable selected of time at λ_{max} 313 nm.

Time(mints)	Absorbance of MTZ
1	0.813
5	0.824
10	0.832
20	0.814
30	0.829
60	0.831
90	0.822
120	0.814

3.2.3. Effect of DMSO as a Solvent on the Absorbance of Acridone at λ_{max} =295 nm

A series of the variable ratio of DMSO (V_{DMSO}/V_{H2O}) solutions ranging (0.5% -20%) in addition to H_2O only were prepared at a constant concentration of Acridone 2.5×10^{-3}M to prevent the Acridone solution from precipitate. Table 3 summarizes the obtained results, in which it can be seen that the optimum ratio is 10% to obtain the maximum absorbance of Acridone, following this ratio, there was a constant absorbance. So 10% ratio for DMSO to H_2O was chosen for a completely soluble of Acridone and maxima absorbance and the stability of Acridone in this solvent at 10% ratio was studied at variable time at λ_{max} 295 nm of Acridone ranged from (1min.) to (120min.), the results were listed in Table 3.

Table 3: The absorbance of Acridone at variable ratio of DMSO and time with constant concentration of chelate 2.5×10^{-3}M

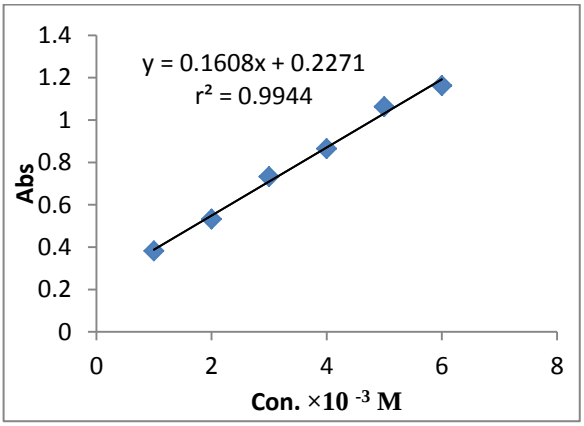
Ratio DMSO/H_2O (mL/mL)	H_2O only	0.5 %	1%	3%	7%	10 %	15 %	20 %
Absorbance	0.102	0.312	0.542	0.683	0.742	0.932	0.920	0.931
Time (min)	1	5	10	20	30	60	90	120
Absorbance	0.931	0.934	0.929	0.928	0.929	0.932	0.933	0.930

3.4. Applicability of Beer's Law and Calibration Graphs Preparation

A series of solutions at variable concentration of Metronidazole ranging from 1×10^{-3}-6×10^{-3}M were prepared in 0.3×10^{-3}M concentration of H_2SO_4 as a solvent atλ_{max}= 313nm and Acridone solutions at variable concentration ranging from 1×10^{-3}-6×10^{-3}M were prepared in 10% DMSO as a best medium at λ_{max}= 295nm. This study lead to test the applicability of Beer'law, the absorbance are plotted against the concentration to obtain a straight line passing through the origin point which shows that the correlation of calibration curve obey the Beer'law, and the slope equal to the molar absorptivity (ε).A result for calibration curve of Metronidazole and Acridone listed in Table 4 and a plot of absorbance vs. concentration of Metronidazole and Acridone in Fig. 3A &B.

Table 4:Summary of results, for absorbance vs. concentration of Metronidazole and Acridone

NO	Conc./M	Absorbance	
		MTZ	Acridone
1	1×10^{-3}	0.380	0.356
2	2×10^{-3}	0.533	0.734
3	3×10^{-3}	0.733	1.136
4	4×10^{-3}	0.888	1.212
5	5×10^{-3}	1.063	1.473
6	6×10^{-3}	1.163	1.562



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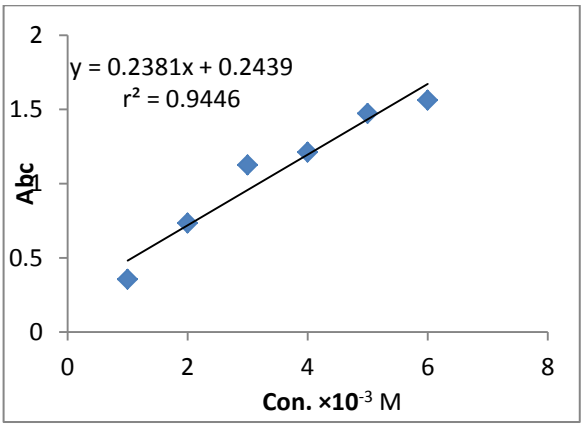


B

Figure 3: The calibration curve of (A) Metronidazole (B) Acridone

As a result, a straight line in Fig 3 shows that metronidazole and Acridone obey Beer's law and their slope equal to the molar absorptivity (ε) .The analytical values of statistical data treatment for calibration curves are summarized in Table 5.

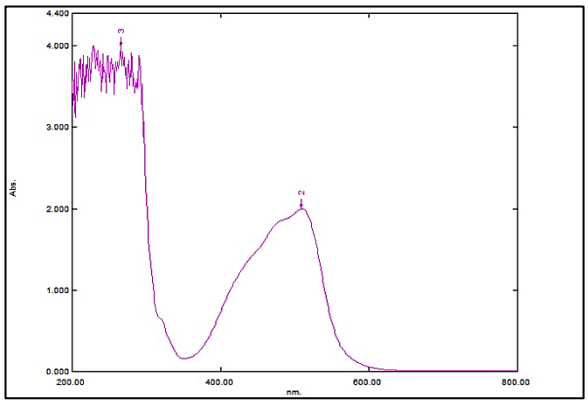
Table5: Statistical data of the regression equations for the determination of Metronidazole and Acridone

Parameter	Metronidazole	Acridone
λ_{max} nm	313	295
Beer's law range (mg ML^{-1})	1×10^{-3}- 6×10^{-3}	1×10^{-3}- 6×10^{-3}
Molar absorptivity ε (L mol^{-1} cm^{-1})	160.8	238.1
Regression equation: Slop(m)	y = 0.1608x + 0.2271	y = 0.2381x + 0.2439
Intercept (a)	0.2271	0.2439
Coefficient of determination (r^2)	0.9944	0.9446
Linearity %	99.44%	94.46%

3.5. Spectral Characteristics of the Reaction for Metronidazole–Acridone Complex

The reaction of Metronidazole drug with Acridone yields a highly stable reddish-orange-colored complex which remained stable for further one day, the formation of Metronidazole-Acridone complex absorbs lights in the visible region with a maximum absorbance occurring around (502 nm) as show in Figure 4.The equilibrium reaction may be written:

Metronidazole +Acridone ⇋ [Metronidazole–Acridone] Complex



(Reddish–orange)

Figure 4: Absorption spectra of the reaction products of Metronidazole with Acridone

The shift in absorbance compared with the free (λ_{max}) of Metronidazole and Acridone attributed to the formation of reddish orange complex according to the following figure as shown below :

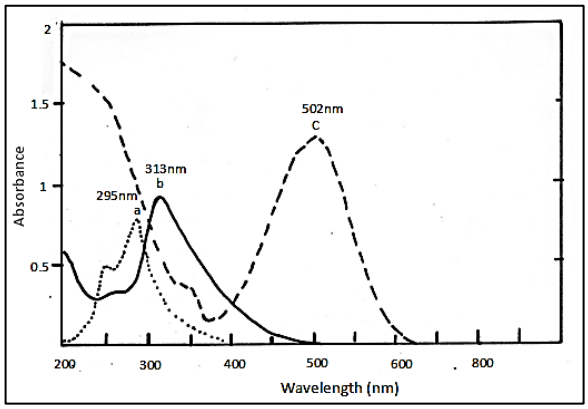


Figure 5: Spectrum at the range of 200-800 showing absorbance maximum peaks for

a: Acridone 2.5×10^{-3}M against 10% DMSO as a blank (λ_{max} = 295nm) (….)

b: Metronidazole 3.5×10^{-3}M against H_2SO_4 (0.3×10^{-3}M as a blank (λ_{max}= 313 nm) (—)

c: Complex for metronidazole with Acridone against (H_2SO_4: DMSO) as a blank (λ_{max} =502 nm) (----)

The molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensity colored complexes which absorb radiation in the visible region [12].

3.6. Stoichiometric Ratio of the Complex by Job's Method of Continuous Variation

The stoichiometry of the Metronidazole–Acridone complex has been determined spectrophotometrically by applying the method of continuous variation known as Job's method [13].

A series of solutions have a mole fraction is varied between 0.1-0.9mlwere prepared by mixing variable volumes of Metronidazole and Acridone as the same concentration 5×10^{-2}M.The absorbance of the resulting solutions were measured at (502 nm) versus the blank. The results summarized in Table 6. The absorbance was plotted against mole fraction as show in (Fig.6).

Table 6: Absorbance of complex (Metronidazole-Acridone) of λ_{max}= (502nm) using 5×10^{-2}M concentration of each solution.

No of solution	X_{MTZ}	$X_{Acridone}$	Abs.
1	0.1	0.9	0.389
2	0.2	0.8	0.765
3	0.3	0.7	1.162
4	0.4	0.6	1.592
5	0.5	0.5	1.992
6	0.6	0.4	2.072
7	0.7	0.3	1.588
8	0.8	0.2	1.051
9	0.9	0.1	0.524

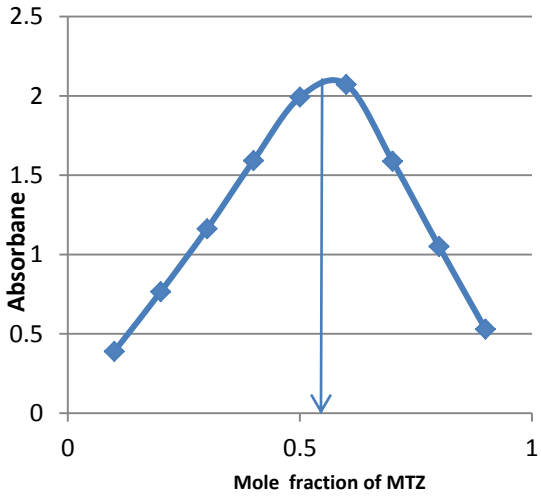
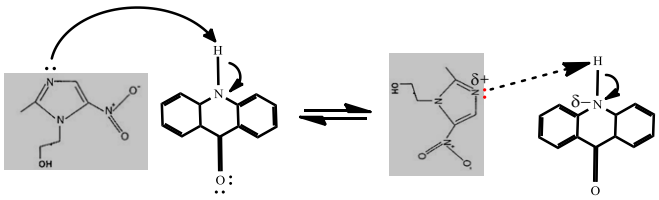


Figure 6: Job's plot for composition Metronidazole-Acridone complex at 502 nm

Job's plot showing a 1:1 binding stoichiometry for a Metronidazole–Acridone complex this Indicated that only one sits is responsible for the formation of the complex [14].

3.7. Reaction of Metronidazole with Acridone

In general, the complexation and the proposed reaction between Metronidazole with Acridone may be expressed as follows in sketch



Sketch 1: proposal mechanism for the reaction between Metronidazole and Acridone

3.8. Beer's Calibration Plot for the Metronidazole-Acridone Complex

A series of solutions with concentration 1×10^{-3}-6×10^{-3}M were prepared from mixing (1:1) Metronidazole 3.5×10^{-3}M with fix concentration of Acridone 2.5×10^{-3}M for the formation the color complex of Metronidazole-Acridone followed by the absorbance

of solutions were measured versus against the blank(H_2SO_4: DMSO). Beer'low was obeyed by the complex. At(502 nm), a fairly linear relationship was obtained with best correlation coeffecient between the absorbance and the concentration ranges of (1×10^{-3}- 6×10^{-3}M) at (25 C°).A result for calibration curve of the complex listed in Table7 and a plot of absorbance versus concentration of complex in Fig 7.

Table7: Summary of result, for absorbance versus concentration of Metronidazole-Acridone Complex.

Conc. MTZ-Arc Complex / M	Abs.
1×10^{-3}	0.386
2×10^{-3}	0.665
3×10^{-3}	0.913
4×10^{-3}	1.181
5×10^{-3}	1.432
6×10^{-3}	1.708

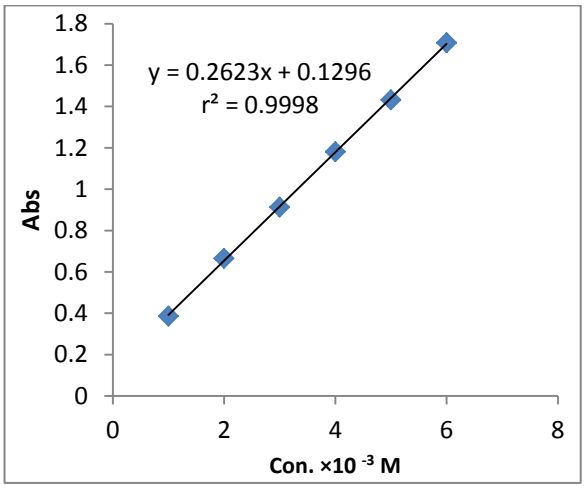


Figure 7: The Calibration Curve of (Metronidazol-Acridone) Complex at 298 K

The analytical values of statistical data treatment for calibration curve are summarized in Table 8.

Table 8: Summary of results for calibration graph using MTZ-Acridone Complex

Parameter	MTZ-Acridone complex
λ_{max} nm	502
Beer's law range (mg ML^{-1})	1×10^{-3}- 6×10^{-3}
Molar absorptivity ε (L mol^{-1} cm^{-1})	262.3
Regression Equation:slop(m)	y = 0.2623x + 0.1296
Intercept (a)	0.1296
Determination of coefficient	r^2 = 0.9998

This conformity with Beer'low indicates that spectrophotometric analysis of electron donor-acceptor by H-Acridone to form a color complex can be used for the quantitative analysis of Metronidazole. The fairly high indicating reliability.

3.9. Calculation of Equilibrium Constant K_{eq}

The system studied here is the reaction of Metronidazole with Acridone to form a (Metronidazole-Acridone) complex

Metronidazole +Acridone ⇋ [Metronidazole –Acridone]

Complex …… (Eqn.1)

(Reddish-orange)

When studying the equilibrium of chemical system, one of the most important quantities to determine is the equilibrium constant, $K_{eq.}$ The equilibrium reaction for the reaction in Equation 1 is given as:

$$Keq = \frac{[(\text{Metronidazole –Acridone})\ complex]eq}{[\text{Metronidazole}]eq\ [\text{Acridone}]eq} \quad \ldots\ldots\ldots (\text{Eqn. } 2)$$

Where the eq subscript denotes equilibrium concentrations.

The value of the equilibrium constant may be determined from experimental data. All equilibrium concentrations can be calculated if a single equilibrium concentration is known along with all other "initial" concentrations.

It may be recalled that in spectrophotometric studies, the Beer's Law, can be used to determine the concentration of highly colored species. Mathematically, Beer's Law can be stated as:

$A=\varepsilon lc$ …… (Eqn.3)

Where "A" is the absorbance, "Ɛ"is the Molar absorptivity, is a proportionality constant that has a specific value for each absorbing species at a given wavelength. "l" is the pathlength, is the distance across the solution in centimeters. In this case, the pathlength will be kept constant at 1.00 cm "c"is the concentration of the absorbing specific is in moles of solute per liter of solution.

The complex absorbs radiation at 502nm.So at this wavelength; Beer's Law can be rewritten as:

$$A=\varepsilon l\left[(\text{Metronidazole} - \text{Acridanon})\ complex\right]eq \quad \ldots\ldots\ldots (\text{Eqn.4})$$

$$\left[(\text{Metronidazole} - \text{Acridanon})\ complex\right]eq = \frac{A}{\varepsilon l} \quad \ldots\ldots\ldots (\text{Eqn.5})$$

Equilibrium concentrations of the reactants can be calculated by subtracting the equilibrium concentration of the product from the initial concentrations of reactants.

$[\text{Metronidazole}]eq = [MTZ]_0 - [(\text{Metronidazole} - \text{Acridone})_{complex}]eq$

$[\text{Acridone}]\ eq = [Acr]_0 - [(\text{Metronidazole} - \text{Acridone})_{complex}]\ eq$

$[MTZ]_0$, $[Arc]_0$ are the original concentration of both Metronidazole and Acridone respectively

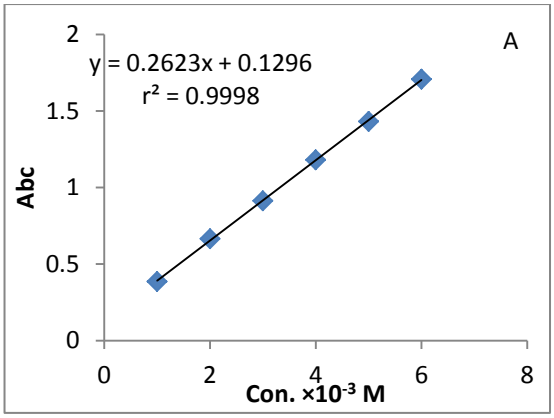
The Equilibrium constant K_{eq} for equation (2) may be formulated as shown in the following expression

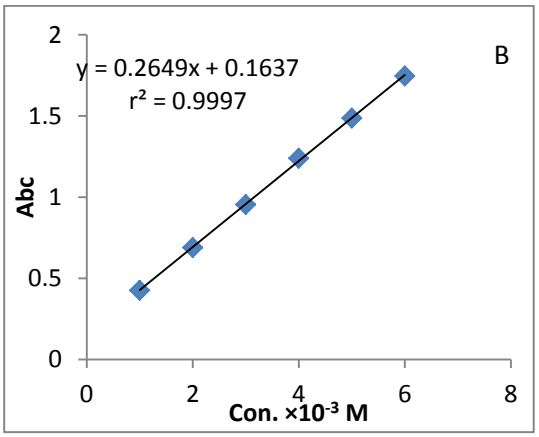
$$Keq = \frac{\left[\frac{A}{\varepsilon l}\right]eq}{([MTZ]0 - \left[\frac{A}{\varepsilon l}\right])eq\ \ ([Acr]0 - \left[\frac{A}{\varepsilon l}\right])eq} \ldots\ldots\ldots (\text{Eqn.6})$$

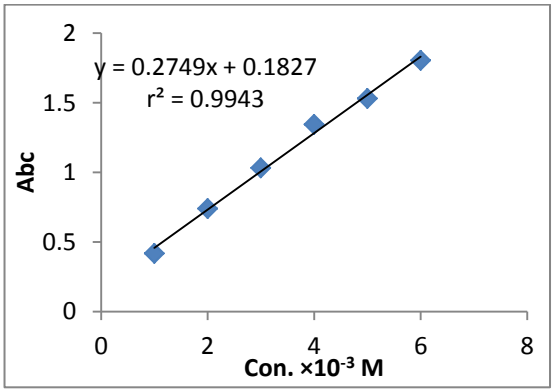
The molar absorptivity of complex was calculated by recording the absorbance A of a various concentration of the (1:1) complex at various temperatures (298,303,308 and 313K) at 502nm and plotting of the absorbance of the complex against concentration given a straight line with the slope equal to ($\varepsilon_{complex}$) Fig.8 and the results were listed in Table 9.

Table 9: Absorbance at variable temperature and the concentrations of 1:1 (Metronidazole – Acridone) complex at 502 nm

Conc. Mtz-Acr complex	Abs.			
	298K	303K	308K	313K
1×10^{-3}	0.386	0.427	0.417	0.411
2×10^{-3}	0.665	0.690	0.740	0.751
3×10^{-3}	0.913	0.955	1.032	1.084
4×10^{-3}	1.181	1.240	1.344	1.442
5×10^{-3}	1.432	1.487	1.531	1.611
6×10^{-3}	1.708	1.746	1.804	1.960







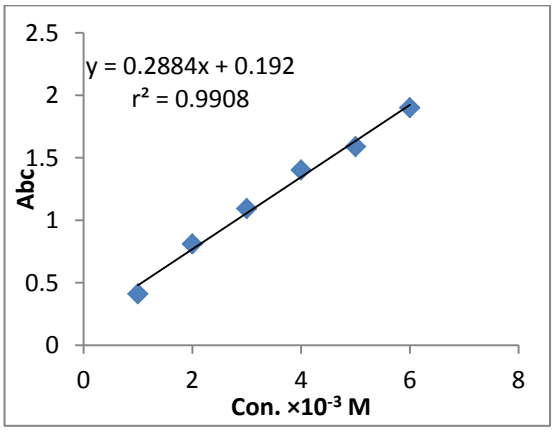


Figure 9: Absorbance vs. concentrations of (Metronidazole - Acridone) complex at various temperatures A/ 298 K B/ 303K C/ K308 D/ 313K.

From the calibration data, molar absorptivity was determined from the linear equation for the straight line which is given at top of the plot and listed in Table 10.

Table 10: Molar absorptivity for Metronidazole and Acridone at four different temperatures.

T(K)	$\varepsilon_{complex}$ /L.Mol^{-1}.cm^{-1}
298	262.3
303	264.9
308	274.9
313	288.4

The molar absorptivity values increases with the increase in temperatures; an interesting observation which suggests that the complex was fairly strong enough to withstand elevated temperature, thus, with increase in temperature; the dissociation of the complex was gradually activated leading to lower concentration of the complex in the reaction medium.

The equilibrium constant K_{eq} were then calculated by the application of equation 6 at various of temperatures (298, 303, 308 and 313K) and the data are shown in Table 11.

Table11: Equilibrium constant for the reaction between Metronidazole and Acridone at various of temperatures.

T(K)	K_{eq} /L.mol^{-1}(Mtz-Acr) $_{Complex}$
298	18×10^3
303	12.7×10^3
308	7.5×10^3
313	5×10^3

The values of the equilibrium constants K_{eq} were high and showed a reduction with increase in temperature, this is probably due to the dissociation of the complexes at higher temperature.it follows that the complex can be regarded as thermostable; it however, showed higher stability at lower temperature [15].

3.10. Determination of Thermodynamic Constants of Complex MTZ-Acridone

Thermodynamic parameters; the standard free energy ΔG° change the standard enthalpy change, ΔH° and standard entropy ΔS° were calculated. The Gibbs free energy of a reaction is determined by the enthalpy ΔH°, and entropy ΔS°:

$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ …….. (Eqn.7)

The thermodynamic of a chemical reaction are completely characterized by measuring the equilibrium constant K_{eq} as a function of temperature. The Gibb's free energy ΔG° and the equilibrium constants of a chemical reaction are related by:

$\Delta G° = -RT\ln K_{eq}$ ……. (Eqn.8)

$\ln K_{eq} = \Delta G°/RT$ Where T is the absolute temperature in kelvins. R is the gas constant, Substitution of this last relationship into (Eq.4) and dividing by RT gives:

$\ln K_{eq} = \left(\frac{\Delta H}{R}\right)\frac{1}{T} + \left(\frac{\Delta S}{R}\right)$…… (Eqn.9)

The enthalpy ΔH° of communication was calculated by van't Hoff equation [16], over moderate temperature ranges a plot of $\ln K_{eq}$ as a function of (1/T) gives a straight line with Slop $= -\Delta H^\circ/R$ Fig10, The entropy change ΔS° for the system can be calculated from the intercept $\Delta S^\circ/R$. or from Equation 7. The thermodynamic parameter are shown in Table 12

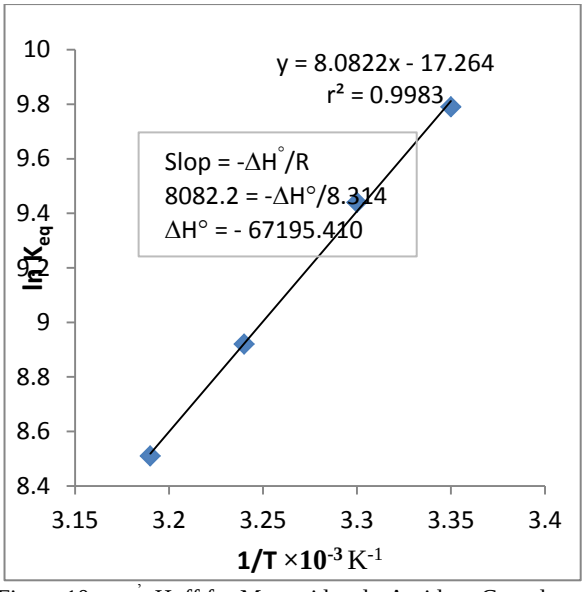


Figure 10: van't Hoff for Metronidazole-Acridone Complex at (502 nm).

Table12: Thermodynamic parameters for (Metronidazole-Acridone) complex at (502 nm).

T(K)	K_{eq}	ln K_{eq}	ΔG°(J.mole^{-1})	ΔH°(J.mole^{-1})	ΔS°(J.mole^{-1}K^{-1})
298	18×10^3	9.79	-24255.42	- 67195.410	-144.093
303	12.7×10^3	9.44	23780.700-		-143.282
308	7.5×10^3	8.92	-22841.55		-144.006
313	5×10^3	8.51	-22145.41		-143.929

The Gibbs free energy is very important when deciding the direction of processes and positions of equilibrium in systems. The negative values of the standard free energy change ΔG° indicate that the reaction of drug with Acridone is spontaneous. The negative values of ΔS° indicate reduce the freedom of motion in the transition state and relatively slow reaction that can be followed spectrophotometrically.

The standard enthalpy change ΔH° of reaction has a high negative value that means the process is exothermic. High negative value of the standard enthalpy change ΔH° together with association constant values indicate strong bonding between Metronidazole and Acridone as well as high stability of the resultant complex [15].

3.11. Kinetics of the Reaction

The best way to obtain the magnitude of the rate constant (k) for reaction is to plot the logarithm of the concentration or the logarithm of any related property versus time. The rate of the reaction may be calculated by variable time method measurement [17] as ΔA/Δt, A is the absorbance and t is the time in minutes. The plot of ln (A) as a function of time produced straight line with slop equal to –k/2.303, from which the rate constant is obtained. The equation that gave the best fit for the experimental data corresponding to first order [18] and the slope represents the rate constant. The kinetic studies of the reaction between Metronidazole and Acridone were carried out at a certain, temperature, wavelength and its stoichiometric ratio (Table 13). Graph of ln (A) versus time for the complex in the concentration of 5×10^{-3}M was plotted and it appeared to be rectilinear (Fig.11) pseudo-first order rate constant (k) was calculated from the slope multiplied by-2.303,It is equal to 3.5×10^{-3}min^{-1}

Table 13: absorbance at time for (Metronidazole-Acridon) complex.

Abs	lnAbs	Time
1.744	0.5561	5
1.732	0.5492	10
1.717	0.5388	15
1.699	0.53	20
1.679	0.5181	25
1.658	0.5065	30
1.636	0.4946	35
1.602	0.4712	40
1.563	0.4485	45
1.520	0.4187	50
1.476	0.3893	55
1.431	0.3583	60

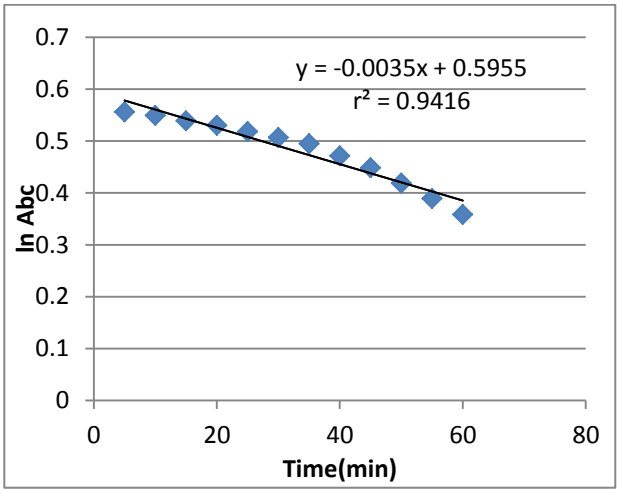


Figure 11: A plot of ln (absorbance) versus time for complex of Metronidazole Acridone at λ_{max} 502 nm

4. Use of Atomic Force Microscopy AFM to Explain the Proposed Precipitate Formed

From Granularity Cumulation Distribution it can be noticed at high concentration more particles of small diameter on an average diameter of 83.49 nm which is within the definition of nanoparticle its less than 100nm while at low concentration of reactant a higher diameter with less number of particles is formed baised to one sided plot (reference is made to Fig 12-A. At high concentration the surface skewness is (7.45×10^{-5}nm) while at low concentration it (0.091nm), this indicate that at high concentration Fig 12-B no. or minimum skewness compered to low concentration. The Roughness coverage indicate that a non-colloidal aspect is available at high concentration (22.1nm) compared to(4.06 nm) at low concentration using functional parameter there is the possibility of reaction of fluid at high concentration (76, 7 nm compared to 13.3nm) for low concentration. concentration

Core Roughness depth indicate that a buildup of grow is possible with or at high concentration 76.7 compared to(13.3nm) at low concentration.

Surface area ratio equal to 1.14 for low concentration compared to (67.2 nm) indicate larger area obtained. Surface Kurtosis indicates that the property granule_formed will show the same property whether low concentration or high concentration.

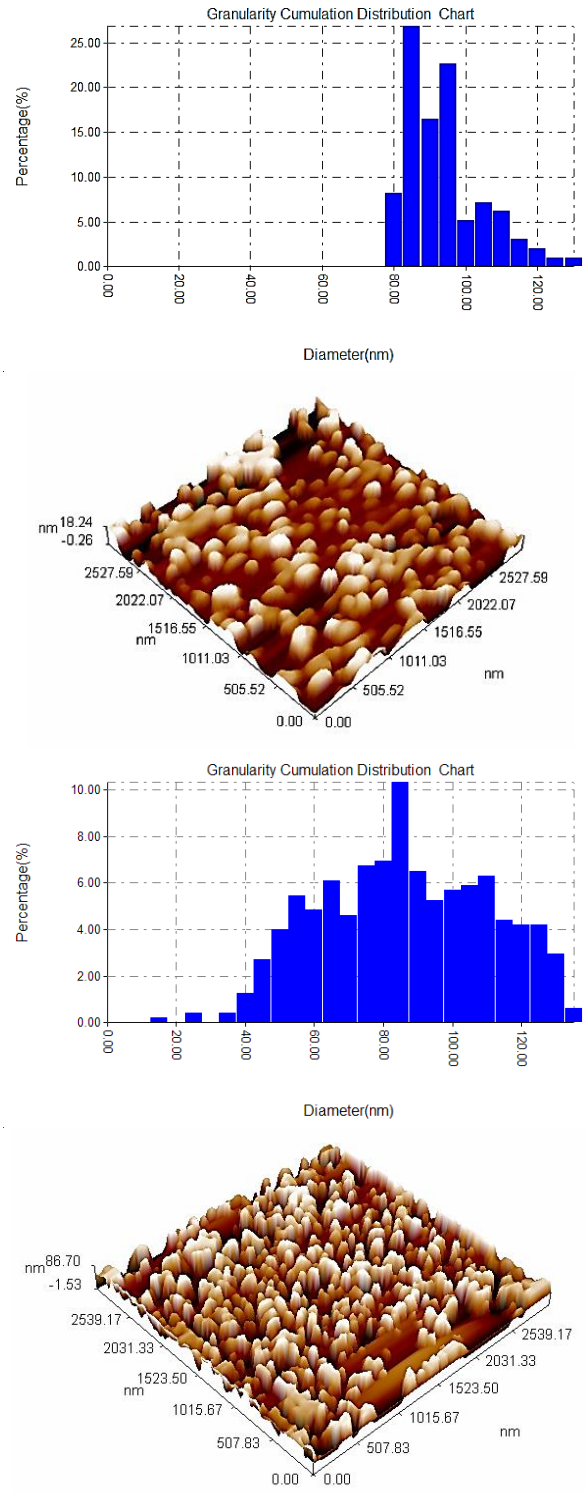


Figure 12 A: Shows distribution of different particles for MTZ-PTA system at low concentration
B: Shows distribution of granule particles for MTZ-PTA system at high concentration

CONCLUSIONS:

The proposed method has been efficiently applied to the estimation of Metronidazole in pure form.

Complexation between Metronidazole and Acridone occurred with a 1:1stochiometry, with maximum absorption at (502nm).The reaction between Metronidazole and Acridone was affected by various parameters such as temperature and equilibrium constant. The kinetic study of the reaction of the drug with Acridone revealed pseudo-first order reaction. Thermodynamically, the complex possessed high stability constant, and was still stable after one week and no significant changes occur in the internal structure therefore the reaction was spontaneous and the negative values of ΔS° indicate reduce the freedom of motion in the transition state and relatively slow reaction in addition to; the negative value of ΔH° leads to an exothermic through the reaction.

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