

Spectrophotometric determination and kinetic study of Sulfamethoxazole using 9-chloroacridine reagent

Abstract

A spectrophotometric method has been developed for analysis of Sulfamethoxazole (SMX) in pure and dosage forms. The method is based on the reaction of the Sulfamethoxazole with 9-chloroacridine (9-CA) reagent in organic and acidic medium, to produce a yellow product having maximum absorption at 448nm. Beer's law was obeyed in the concentration range 1-30 $\mu\text{g mL}^{-1}$ with molar absorptivity of 1.63×10^4 L/mol.cm with good detection and quantification limits. Accuracy (Average recovery%) is 98.43 and precision (relative standard deviation) is 0.651. The reaction mechanism has been proposed and the stability constant of the product was determined. The proposed method was applied successfully for determination of Sulfamethoxazole in its commercial dosage form as tablet and agree well with the official method.

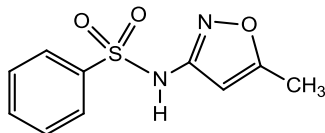
Thermodynamic study is also carried out in this work. The equilibrium constant and the thermodynamic functions (ΔH° , ΔG° and ΔS°) of the complex formation were estimated. The study revealed that the complex formation could occur spontaneously, the type of interaction forces between SMX and 9-CA are physical in nature and association increases the order of the studies system.

Kinetic study is performed. The results indicated that, the reaction is pseudo first order with respect to SMX. The rate constant is determined at various temperatures. The thermodynamic functions of activation were evaluated. Theoretical parameters were calculated by applying the semi-empirical Austin method (AM1). These parameters are helped to suggest reaction mechanism in addition to other results.

Keywords : Spectrophotometric ; Sulfamethoxazole (SMX) ; 9-chloroacridine, Thermodynamic study, kinetic study

Introduction

Sulfamethoxazole is also called Septra, Spetrin and Bactrim. Synonym is 4-amino-N-(5-methyl-3-isoxazole) benzene sulfonamide [1]. It is a medicinal compound in the form of a white powder that partially dissolves in water and has a high solubility in acetone and ethanol. Its melting point ranges from 169-172°C. It is one of the sulfonamides (sulfa drugs), which is considered one of the most important anti-bacterial drugs. Sulfamethoxazole in drugs is often associated with Trimethoprim to form a compound called Co-Trimoxazole, which is used in the treatment of respiratory infections, sinusitis, and middle ear infections, as well as for urinary and reproductive system infections, gastrointestinal infections, and is also used to treat feline disease and skin infections [3,6].



$C_{10}H_{11}N_3O_3S$: Mwt = 253.2 g/mole

Various analytical techniques have been described for the determination of SMX, such as immunoassay (1,2), Electrochemical(3,4,5), high performance liquid chromatography (HPLC) (6,7) and fluorometry (8,9). These techniques are expensive and need special training. Also; spectrophotometric methods have been reported for determination of SMX using different reagents such as, vanillin(10), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, p-chloranil and picric acid (11), phenoxazine (12), 2,4,6-trihydroxybenzoic (13), 1-naphthol (14), 2-naphthol (15), phloroglucinol (16), Salbutamol sulphate (17), pyrocatechol (18), thymol (19), 2,4,6-trihydroxybenzoic acid (20) and Diphenylamine (21). Some of these methods are suffer from disadvantages, such as low sensitive, need extraction step or heating. The present study is developing a new sensitive and simple spectrophotometric method for determination of SMX in pure and dosage forms, depending on formation of a new product by the reaction of SMX with 9-chloroacridine (9-CA).

Experimental

Devices and tools

T92+ UV-Visible spectrophotometer PG instrument supplied with a 1.0-cm path length silica cell used for absorbance measurements, a combined glass electrode type pH meter JEN WAY 3510 used for pH measurements. Heating of solutions was carried out on a water bath type Julabo, a balance type of RN ABS used for weighing and Microsoft Excel for Windows was used for all calculations.

Chemicals

SMX and its pharmaceutical formulations (tablet) were kindly provided by state company for Drug Industries and Medical Appliance-(SDI) Sammara-Iraq. 9-CA was obtained from Eastman chemical co., and other chemicals were obtained from Fluka and BDH companies. All solvents were analytical reagent grade and water was distilled.

Solutions

SMZ standard solution of $100 \mu\text{g}\cdot\text{mL}^{-1}$ was prepared by dissolving 0.01 g of sulfamethoxazole in pure form in absolute ethanol and completed the volume to 100 ml

in volumetric flask. This stock solution was used for the preparation of working solutions.

9-CA Reagent of 1×10^{-2} M solution was prepared by dissolving 0.0213g of 9-chloroacridine in absolute ethanol and then the volume was completed to 100 ml in a volumetric flask. This solution was prepared daily and used immediately [22]

Hydrochloric acid of 0.2M solution was prepared by appropriate dilution of the concentrated solution with distilled water.

Recommended procedure

Increasing volumes of the working *SMX* solution ($100 \mu\text{g mL}^{-1}$) were transferred to cover the concentration range $1\text{-}30 \mu\text{g mL}^{-1}$ to a series of 10 ml calibrated flasks containing 2.5 mL 9-CA (0.01M) and 1 mL HCl (0.2M). The solutions were diluted to the mark with methanol. The solutions were diluted to the mark with ethanol and kept in a water bath at 10°C for 50 min, and the absorbance was measured at 448 nm against the respective blank reagent.

Procedure for *SMX* tablet

Weighed and finely powdered 6 tablets (each containing 400 mg *SMX* and 80 mg Trimethoprim), an accurately weighed amount of powder equivalent to one tablet and dissolved in 50 ml ethanol, shaken to increase the solubility and filtered into 100 ml calibrated flask, then the solution was made to the volume with the ethanol (the solution was equivalent to $4000 \mu\text{g mL}^{-1}$ for *SMX*). A suitable volume was diluted with ethanol and followed the recommended procedure.

RESULTS AND DISCUSSION

In the preliminary investigation work, it was found that 9-CA reagent reacted *SMX* in alcoholic medium of ethanol and produced yellow colored solution after standing for 20 min at room temperature with maximum absorption at 448 nm. Whereas the reagent blank which shows no absorbance at this wavelength but have a maximum absorption at 360 nm and the drug shows maximum absorbance at 250 nm (Fig. 1). However, the wavelength of maximum absorption 448 nm was used in all subsequent experiments.

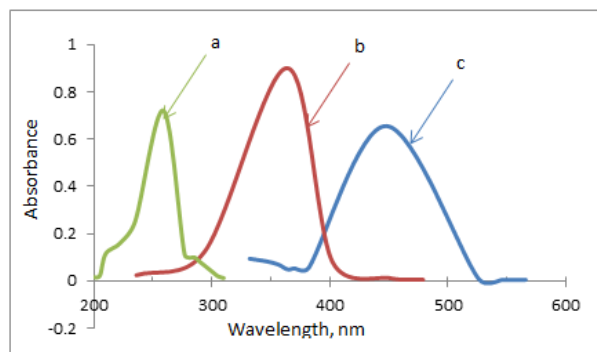


Figure 1, Absorption spectra of (a) SMX ($10 \mu\text{g mL}^{-1}$) Vs ethanol, (b) blank reagent Vs ethanol and (c) SMX ($10 \mu\text{g mL}^{-1}$) - 9-CA product Vs blank in the presence of HCl under the optimum conditions

Optimization of the method conditions

The conditions of the suggested method was optimized by studying the effect of different variables including acids, buffer solutions, concentration of 9-CA, solvents, temperature and reaction time.

Effect of acid

Due to the reaction of 9-CA with SMX was carried out in acidic medium, therefore; the effect of different acids was studied using 1 ml of 0.1 M of each tested acid. It was found that maximum absorbance was in the presence of hydrochloric acid with a final pH value of 1.75. Two buffer solutions including(boric acid + H_3PO_4 + CH_3COOH) and (KCl+HCl) at this pH were also tested and showing decreasing in absorbance. However; the effect of concentration and volume of HCl were examined. It was found that 1ml of 0.2 M gave maximum absorbance, which are recommended in subsequent experiments.

Effect of 9-CA concentration and volume

Different concentrations in the range 10^{-4} - 10^{-1} M of 9-CA reagent were tested using the same amount of SMX ($10 \mu\text{g mL}^{-1}$) and HCl (1ml of 0.2M). The absorbance increases when 9-CA concentration increasing (Fig. 2), and reaches its maximum absorbance when using 10^{-2} M. Effect of volume of this concentration was tested and 2.5 ml gave maximum absorption (Fig. 3). These concentration and volume were recommended in

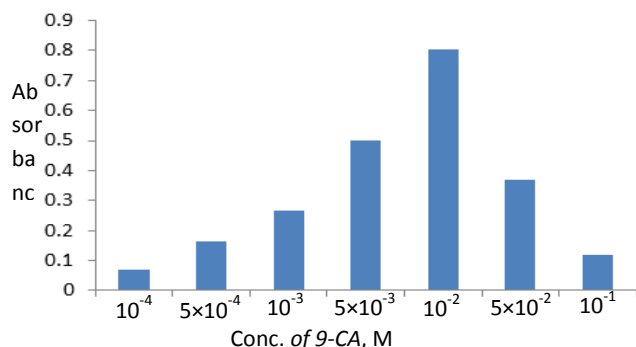


Figure 2, Effect of 9-CA conc.on the reaction with SMX

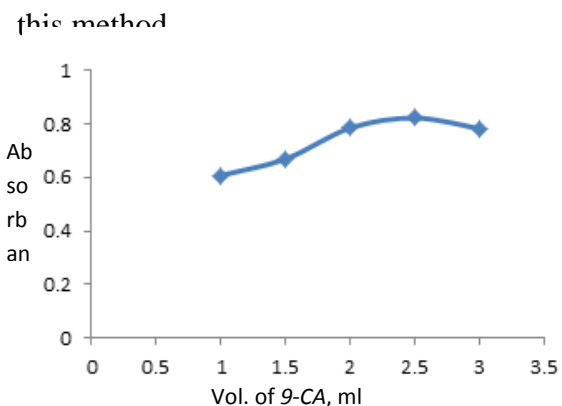


Figure 3, Effect of volume of 10^{-2} M 9-CA on its reaction with SMX

Effect of solvent

9-CA was insoluble in water but free soluble in organic solvents, Therefore effect of the solvent on the absorption of *SMX* -9-CA product was investigated. Different solvents namely methanol, ethanol, propanol and tetrahydrofuran (THF) were tested. The procedure was based on the mixing of $10 \mu\text{g}\cdot\text{mL}^{-1}$ *SMZ* with 2.5 mL of 10^{-2} M 9-CA dissolved in each above solvents in the presence of 1 mL of 0.2 M HCl and diluted with the same solvent of 9-CA in a final volume of 10 mL. Measuring the absorbance after 10 min at room temperature. Ethanol as a solvent gave maximum absorbance 448 nm which was recommended in this method.

Effect of temperature and time

To obtain high color intensity, the time of the reaction was studied by measuring the absorbance at different temperatures ranging from 10°C to 50°C (using thermostatic water bath) after each 5 min intervals. The results showed high color intensity after 40 min with maximum absorbance at 10°C with stability for more than 30 min (Fig. 4). While the absorbance was continuously decreased at higher temperatures, which can be interpreted for the decomposition of the *SMX* -9-CA product. Therefore, the absorbance was measured after 40 min at 10°C was selected.

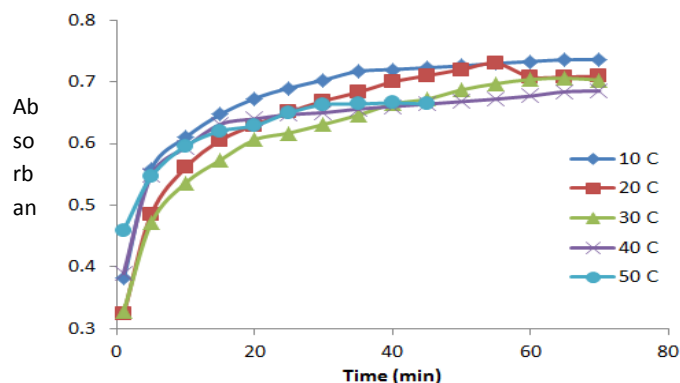


Figure 4, Effect of temperature on the absorbance of *SMX*-9-CA product at various time

Sequence of addition

The high absorption intensity of the product was obtained when 9-CA and HCl were added before adding *SMX*. Otherwise, a loss in color intensity was observed (Fig. 10).

Quantification

Under the optimum conditions obtained above, concentration of *SMX* was found to be proportional to the absorbance with excellent linearity (Fig.5), As seen in Table 1, the method have good accuracy (recovery %) and high molar absorptivity. The results of limit of detection (LOD) and limit of quantitation (LOQ) revealed that the proposed method is sensitive. The value of relative standard deviation (RSD) indicate the method is precise. The low value of intercept indicates that the blank reading at 448 nm was low. The good correlation coefficient indicates that the method is suitable for quantitative analysis of *SMZ*, (Table 1).

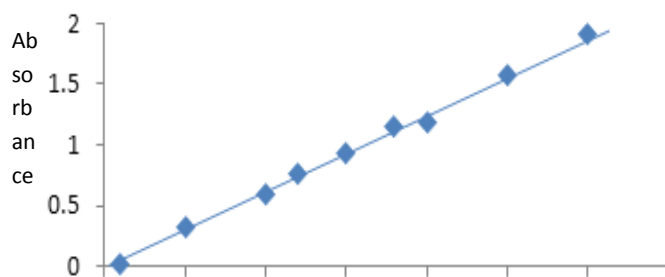


Figure 5, Effect of Conc. of Smx on the reaction with 9CA

Table 1. Summary of the optimum Parameters of the studied reaction

Parameters	Value
Linearity range ($\mu\text{g mL}^{-1}$)	1-30
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	1.63×10^4
Recovery % *	98.43
RSD*	0.651
Intercept	0.0337
Slope	0.0644
Correlation coefficient (R^2)	0.9969
LOD ($\mu\text{g mL}^{-1}$)	0.255
LOQ ($\mu\text{g mL}^{-1}$)	0.849

* Average of four determinations

Selectivity

The selectivity of the method was investigated by applying the standard addition procedure and observing any interference encountered from the excipients containing in pharmaceutical formulations. This was carried on measuring the absorbance of solutions containing a fixed amounts of drug in dosage form (10 and $15 \mu\text{g mL}^{-1}$). The good recovery % (99.9 and 102.5) indicated the proposed method have good selectivity, (Fig.6).

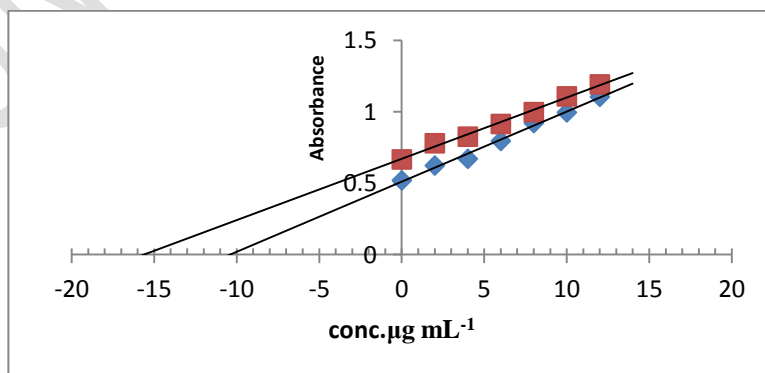


Figure 6,standerd addition method

Analysis of pharmaceutical formulations

The suggested method was successfully applied to determine the *SMX* drug in its commercial form as tablet. The results given in Table 2 indicated that the method is a reproducible and accurate.

Table 2. Assay of *SMX* in pharmaceutical formulation by the proposed method

Pharmaceutical preparation	Amount present (μgmL^{-1})	Average Recovery* (%)	Drug content found (mg)	Certified value (mg)
<i>Trimox Tablet</i>	10	101.7	406.8	400
	15			
	20			

*Average of four determinations

Validity of the method

The proposed method was compared statistically by a Student's t-test for accuracy and a F-test for precision with the official method for pure drug (23) at the 95% confidence level with six degrees of freedom. The results showed in Table 3 that the experimental t-test and F-test were less than the theoretical value ($t=2.45$, $F=6.39$), indicating that there was no significant difference between the proposed method and official method.

Table 3. comparison of the present method with the official method

Pharmaceutical preparation	Recovery %		t-exp.	F-test
	Present method	Official method		
<i>SMX</i> tablet	98.43	101.0	2.12	3.39

Stoichiometry, stability constant and reaction mechanism

The molar ratio of *9-CA* and *SMX* was determined by Job's continuous variation [24] and it was found that the *9-CA*: *SMX* ratio was 1:1 (Fig. 7). According to the result of molar ratio, the apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of each *SMX* and *9-CA* (A_s) to one containing an optimum amount of *9-CA* reagent (A_m). The average conditional stability constant of the product was calculated by applying the following equations:

$$K_c = 1 - \alpha / \alpha^2 C \quad \alpha = A_m - A_s / A_m$$

where K_c is the stability constant, α the dissociation degree and C is the concentration of the product which is equal to the concentration of *SMX* drug. The K_c value is 2.36×10^3 L/mole which indicate that the product is relatively stable.

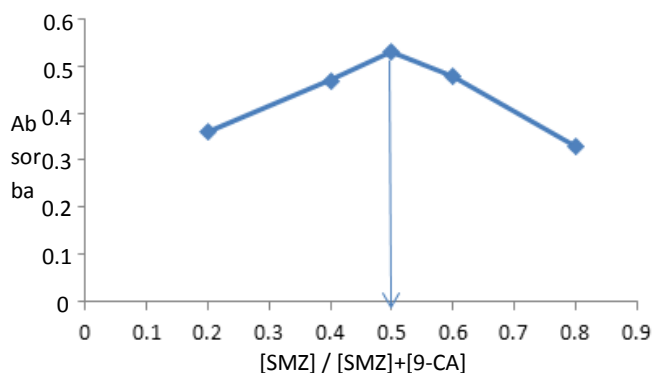


Figure 7, Job's method for SMX -9-CA product

Comparison of the present method with other literature spectrophotometric methods

The present method has been compared with other spectrophotometric methods. As seen in Table 4, the proposed method has some advantages. It is simple, sensitive, selective for determination of SMX in the presence of Trimethoprim as well as more economic .

Table 4: Comparison of the proposed method with other spectrophotometric methods in the determination of SMX

Analytical parameters	Present method	Literature method		
		chloranilic acid ⁽²⁵⁾	<i>N,N</i> -diethyl- <i>p</i> -phenylene-diamine ^(b)	Vanillin ^(c)
Reagent	9-CA			
λ_{max} (nm)	448	520	550	396
Solvent	Ethanol	acetone/water	Water	Water
Temp.(°C)	10	30	60	R.T
Development (min)	40	30	10	5
Beer's law ($\mu\text{g.mL}^{-1}$)	1-30	7.5-60	5-25	5 – 80
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	1.63×10^4	2.98×10^3	8.31×10^3	1.1×10^3
RSD (%)	≤ 0.651	≤ 0.075	≤ 0.600	≤ 6.360
Type of reaction	Substitution reaction	Charge transfer complex	Oxidative coupling	Schiff's base

Kinetic study of the resulting product

The reaction of SMX and 9-CA reagent gives a yellow product with a wave length at 448 nm. This reaction is kinetically studied by applying the pseudo first order equation at optimal conditions obtained in this study.

$$\ln \frac{A_0}{A_0 - At} = kt \dots\dots\dots(1)$$

$$\ln(A_0 - At) = \ln A_0 - kt \dots\dots\dots(2)$$

Where (A_0) is the absorbance of the initial concentration, (At) is the absorbance of the product at time (t), and k is the rate constant. The results obtained from this study are

listed in Table (4). The results showed good fitting of the pseudo first order equation on the experimental data of the reaction between *SMX* and the reagent 9-CA. This is indicated by the values of correlation coefficient (R^2) close to unity and calculated initial concentration (a) matches the experimental value. Figures 8 (A-E)

Figure (A)

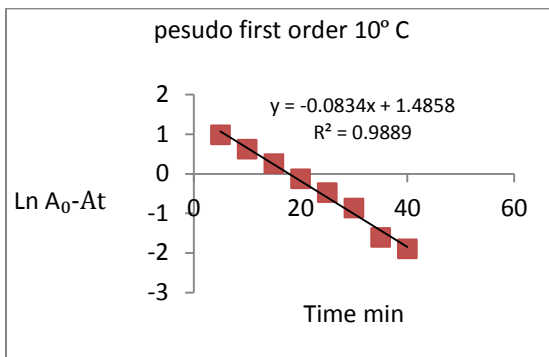


Figure (B)

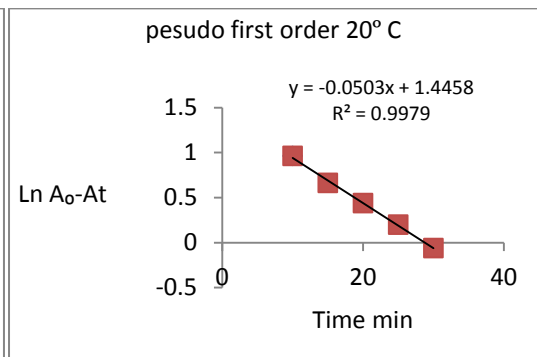


Figure (C)

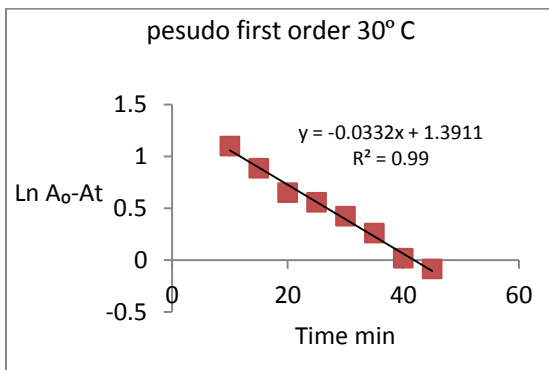


Figure (D)

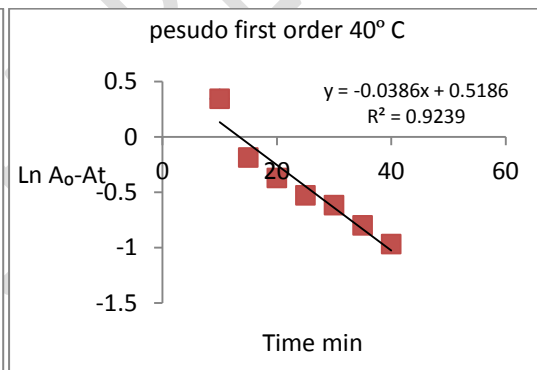


Figure (E)

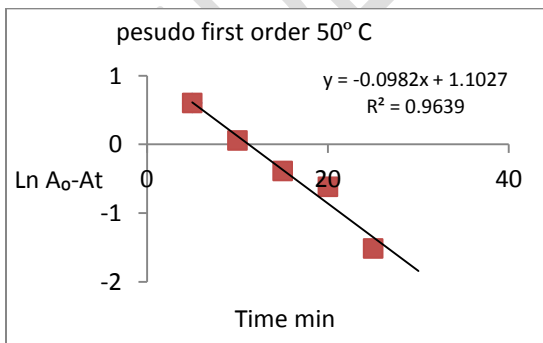


Figure 8: Fitting of the pseudo first order equation on the experimental data of the reaction between *SMX* and the reagent 9-CA at various temperatures.

Arrhenius equation for the reaction of *SMX* with the 9-CA was applied to estimate the activation energy and the values of thermodynamic functions of activation using the rate constants at various temperatures and plotting the logarithm of the rate constant versus the reciprocal of the absolute temperature figure (9) using the relations given below (equation (3)).

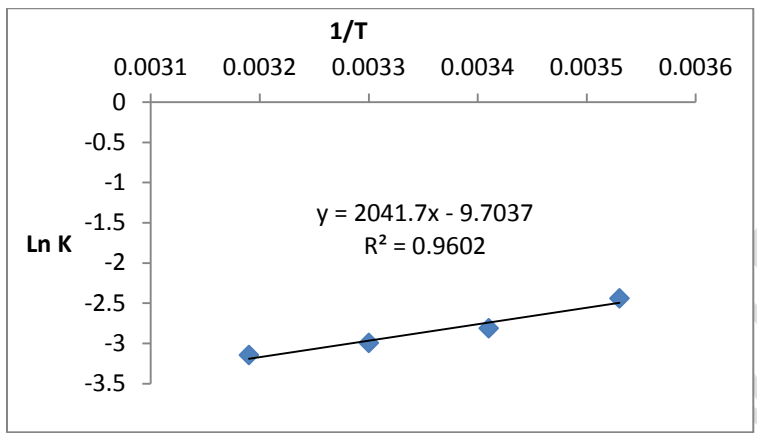


Figure 9: application of Arrhenius equation to calculate the activation energy

Kinetic study of the reaction between *SMX* and 9-CA reagent at optimum conditions obtained so far depending on the formation of a new product using ethanol as a solvent in acidic medium and by employing spectrophotometric technique giving maximum absorbance band at 448nm. The color of the product was found to develop with time indicating to the formation of the product. The investigation revealed that the reaction follow the pseudo first order equation as show in figures (A-B) which gave a straight line with (R^2) close to one and calculated values of (a) consistent with the experimental values. In order to estimate the values of the thermodynamic function of activation, the

experiment is repeated at the same conditions of the pseudo first order reaction at various temperatures (20,30,40 and 50 °C). This work is aimed to calculate the rate constant (k) at different temperatures so the values of the thermodynamic functions of activations (ΔH^* , ΔG^* and ΔS^*) can be estimated^[18].

$$\ln K = \ln A - \frac{E}{RT} \dots\dots\dots(3)$$

Where A is the frequency factor and is used to calculate value of ΔS^* by using equation(4).

$$\Delta S^* = R \left[\ln A - \ln \frac{kt}{h} - 1 \right] \dots\dots\dots(4)$$

Where K is Boltzmann constant (0.38×10^{-23}), h is Plank constant ($6.62 \times 10^{-34} \text{ J}\cdot\text{sec}^{-1}$) and R is the gas constant ($8.314 \text{ J}\cdot\text{mol}^{-1} \cdot\text{K}^{-1}$).

$$\Delta H^* = E - RT \dots\dots\dots(5)$$

$$\Delta G^* = \Delta H^* - T\Delta S^* \dots\dots\dots(6)$$

The values of rate constant at various temperatures, half life time ($t_{1/2}$) activation energy (E), and thermodynamic functions of activations are listed in Table (5).

Table 5. rate constants , half life time ($t_{1/2}$) ,activation energy (E) and thermodynamic parameters of activation

Temp.	K	T _{1/2}	E (kJ.mole ⁻¹)	ΔH^* (kJ.mole ⁻¹)	ΔS^* (J.mole ⁻¹ .K ⁻¹)	ΔG^* (kJ.mole ⁻¹)
283	0.083	8.35	42.409	40.056	- 254.59	112.1
293	0.050	13.86		39.972	- 254.88	114.6
303	0.033	21.0		39.889	- 255.16	117.2
313	0.038	18.24		39.806	- 255.43	119.4
323	0.098	7.07		39.723	- 255.69	122.3

The effect of temperature

The effect of temperature and stability time of the colored product was investigated for the reaction of 10 µg/ml of SMX and 9-CA reagent, in acidic (HCl) medium. It was found that the best absorption of the complex was at 10 °C and the product was stable for 55 minutes as shown in Table (6).

Table 6. The effect of temperature and stability time

Time (min)	Absorbance of the product at temperature				
	10°C	20° C	30° C	40° C	50°C
1	0.381	0.324	0.328	0.390	0.459
5	0.558	0.485	0.471	0.548	0.546
10	0.610	0.561	0.536	0.594	0.596
15	0.647	0.605	0.573	0.631	0.620
20	0.673	0.630	0.606	0.640	0.629
25	0.690	0.651	0.617	0.647	0.650
30	0.703	0.669	0.631	0.650	0.663
35	0.717	0.683	0.646	0.656	0.664
40	0.720	0.700	0.664	0.660	0.666
45	0.723	0.710	0.672	0.664	0.664
50	0.726	0.720	0.687	0.668	

55	0.730	0.730	0.697	0.672	
60	0.733	0.708	0.704	0.677	
65	0.736	0.708	0.706	0.684	
70	0.736	0.709	0.703	0.685	

Thermodynamic study

The thermodynamic functions are very important parameters they could be used to identify the direction of the reaction, its spontaneity, nature of the complex and the order of the studied system. The values the of thermodynamic functions were calculated by employing the Vant Hoff equation ^[19], the values of ΔH were estimated by plotting the logarithm of the equilibrium constant versus the reciprocal of the absolute temperature as illustrated in figure (10).

$$\ln K = \frac{-\Delta H}{RT} + \frac{\Delta S^\circ}{R} \dots\dots\dots(7)$$

The value of ΔS° was calculated, representing the intercept of the obtained straight line of figure (10). While ΔH represent the slope value, as the value of ΔG° was determined by using equation(9) [20].

$$\Delta G^\circ = - nRT \ln k \dots\dots\dots(8) \quad \text{Where T is the absolute temperature}$$

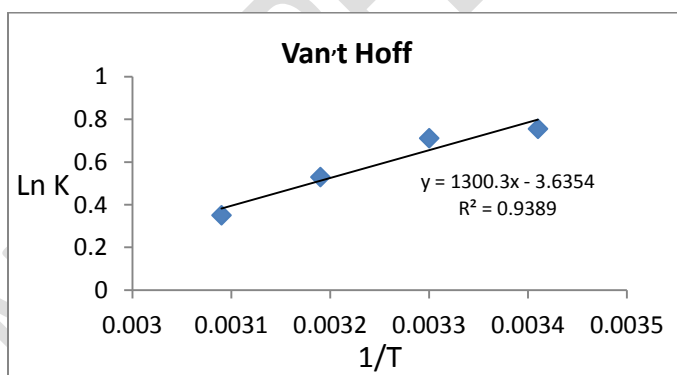


Figure 10: application of Vant Hoff equation to calculate enthalpy of reaction between *SMX* and *9-CA*

The calculated values of the thermodynamic functions of the resulted product are listed in Table (7).

Table 7. equilibrium constants and thermodynamic parameters

Temp.	K	ΔH° (kJ.mole ⁻¹)	ΔG° (kJ.mole ⁻¹)	ΔS° (J.mole ⁻¹ .K ⁻¹)
283	1.93	-10.810	-1.547	- 32.7
293	2.13		-1.841	- 30.6
303	2.04		-1.796	- 29.7
313	1.70		-1.380	- 30.1
323	1.42		-0.941	- 30.55

The values of stability constants (K) of the complex formed from the reaction between SMX and 9-CA indicating increasing the stability with increasing the temperature from 10 to 30°C. This could be improved visually by increasing the intensity of the yellow color of the complex. This was followed by gradual decline with elevating the temperature from 30 – 50 °C which is accompanied by decrease in the color intensity suggesting the occurrence of dissociation process of complex .

The values of thermodynamic functions resulted from the reaction of the complex formation (SMX and 9-CA) listed in Table (6) indicating the following. The negative value of ΔG° refers to that, the complexation reaction could occur spontaneously.

The negative sign of ΔH° indicating that, the complex formation is exothermic reaction, its values pointing into the physical nature of forces controlling the complex formation. These values are supported by the rising temperature which favor the backward reaction (dissociation reaction) (since it is an exothermic reaction).

The negative value of ΔS° refers to the increasing in the order of the studied system. i.e. decreasing the degree of freedom of reacting molecules during the reaction of complex formation. The thermodynamic results conclude that, the complex is less stable at temperature higher than 30°C, supporting the physical natural of forces controlling the interaction between SMX and 9-CA indicated by the value of ΔH . This interaction occurs spontaneously with increasing the order of the studied system. The results could also help in giving an indication for suggesting a proper mechanism for the complex formation according to the kinetic study.

Theoretical study

One of the essential point of studying the kinetic of a reaction is to help the researchers suggest a suitable reaction mechanism. In the considered reaction, the first step is achieved for determining which of the two molecules (SMX or 9-CA) attack and behaves as a nucleophile. Several parameters were selected for performing this job. The Chem. Office program (V.10, 2012) is employed for doing this calculations. These calculations are carried out at 25°C and using the ethanol as solvent (in simulation to the experimental conditions) and by applying the semi-empirical method (Austin method, AM1). The factors used for this comparison are the energy of the molecular orbitals (HOMO and LUMO), the energy gap ($E_{LUMO} - E_{HOMO}$) (L – H), hardness of molecule (η), electronic chemical potential (μ), Global electrophilic (ω), total energy (TE) and atomic charge on the hetro atoms in the molecules the calculated values are listed in Tables (8 and 9) .

Table (8): values of hardness, electrophilicity and nucleophilicity index, molecular orbitals and energy gap for the studied compounds.

compound	η	μ	ω	HOMO	LUMO	L-H
9-CA	2.336	-7.302	11.267	-9.669	-4.936	4.733
SMX	3.967	-5.072	3.245	-9.036	-1.105	7.934

The results obtained indicate the following

- 1- According to the TE values, SMX is more stable (less TE) than 9-CA compound.
- 2- The gap energy (L- H) refers to that, SMX is more stable than 9-CA (larger energy gap).
- 3- The value of hardness (η) of SMX (more stable) is higher than that of 9-CA.
- 4- Comparing the values of energy of molecular orbital, it was noticed that, the SMX has higher E_{HOMO} and lower E_{LUMO} than 9-CA, therefore the SMX behaves as a nucleophile while 9-CA acting as an electrophile

$$\eta = \frac{1}{2} (E_{LUMO} - E_{HOMO}) \dots\dots\dots(9)$$

$$\mu = \frac{1}{2} (E_{HOMO} + E_{LUMO}) \dots\dots\dots(10)$$

$$\omega = \frac{\mu^2}{2\eta} \dots\dots\dots(11)$$

- 5- Finally, the values of μ and ω in compound SMX are lower than those of compound 9-CA, which indicate that SMX is better behaves as a nucleophile than

9-CA. Accordingly, the SMX as a nucleophile will attack the 9-CA (as electrophile) in the first step of the mechanism and as follow.

The reaction of the SMX and 9-CA could occur in the basic medium as in figure (11)

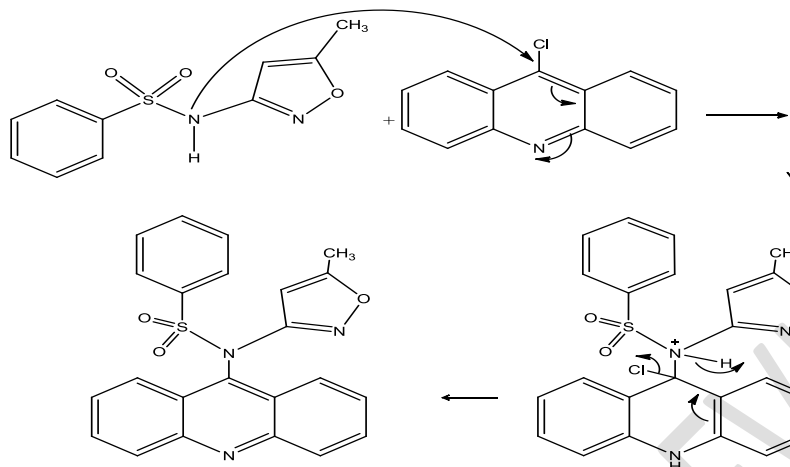


Figure (11) : Suggested mechanism in the basic medium

and in acidic medium, the nitrogen atom of 9-CA is susceptible for protonation and the carbon atom in position 9 (attached to Cl) will be more electronegative. The nitrogen atom in SMX will attack the nitrogen atom in 9-CA instead of the carbon atom in position 9 of 9-CA. The results in the acidic medium pointing out to the formation of complex by physical attraction forces. This result is supported by value of the change in enthalpy obtained from the thermodynamic study ($\Delta H=10.8$ kJ/mole) and the effect of temperature which showed increasing the stability constant with rising temperature from 10-30°C and then decrease with elevating temperature. This reaction could occur as follow (Figure 12)

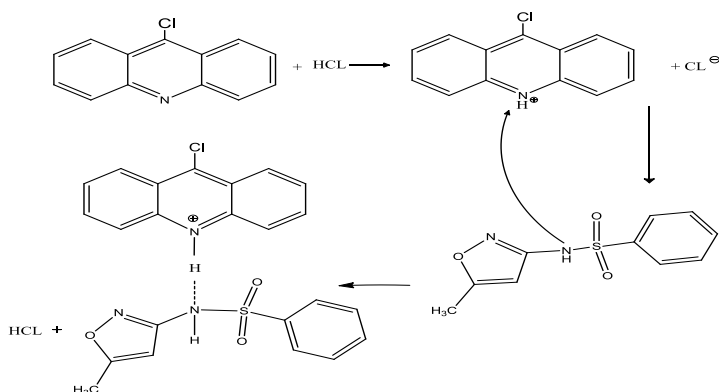


Figure (12) : Suggested mechanism in the acidic medium

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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