

# A Novel Spectrophotometric determination and kinetic study of Sulfamethoxazole in Pure and Tablet formulation 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 SMX with 9-chloroacridine (9-CA) reagent in organic and acidic medium, to produce a yellow product having maximum absorption at 448 nm. Beer's law was obeyed in the concentration range 1-30  $\mu\text{g.ml}^{-1}$  with molar absorptivity of  $1.63 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$  with good detection and quantification limits. Accuracy Average recovery and precision are 98.43% and 0.651, respectively. The proposed method was applied successfully for determination of Sulfamethoxazole in its commercial dosage form as tablet and agree well with the official method.

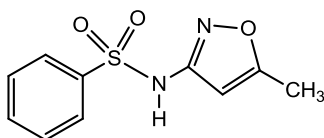
The equilibrium constant and the thermodynamic functions ( $\Delta H^\circ$ ,  $\Delta G^\circ$  and  $\Delta S^\circ$ ) of the complex formation were estimated. The study revealed that the complex formation could occur spontaneously, the type of interacting forces between SMX and 9-CA are physical in nature and association increases the order of the studied systems.

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

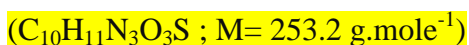
Keywords: Spectrophotometric; Sulfamethoxazole (SMX); 9-chloroacridine; Thermodynamic study; kinetic study

## Introduction

Sulfamethoxazole, 4- amino-N- (5- methyl-3-isoxazole) benzene sulfonamide [1]. It is a white powder medicinal compound, highly soluble in acetone, ethanol, and partially soluble in water. 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].



Molecular structure of Sulfamethoxazole



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 disadvantageous, in terms of sensitivity, and require extraction or heating steps.**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. **The reagent 9-CA** was obtained from Eastman chemical co., and other chemicals were obtained from Fluka and BDH companies. All solvents were analytical reagent grade.

### **Solutions**

**SMX standard solution of  $100 \mu\text{g}\cdot\text{mL}^{-1}$**  was prepared by dissolving 0.01g of SMX 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.

**The 9-CA Reagent of  $10^{-2}\text{M}$  solution was** prepared by dissolving 0.0213g of 9-CA 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 used.**

### **Recommended procedure**

Increasing volumes of the working *SMX* solution ( $100 \mu\text{g}\cdot\text{mL}^{-1}$ ) were transferred to cover the concentration range 1-30  $\mu\text{g}\cdot\text{mL}^{-1}$  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 composition of blank.

### Procedure for SMX tablet

Six Trimox tablets (400 mg SMX and 80 mg Trimethoprim) were taken and ground into fine powder, then accurate weight equivalent to one tablet was dissolved in 50 ml ethanol. Then filtered by wattman No.4 and the solution was made up to 100 ml in calibrated flask. Suitable aliquots were analysed by using the described procedure above.

## RESULTS AND DISCUSSION

Preliminary work has shown that 9-CA reacts with SMX in ethanolic medium, producing a yellow coloration after 20 minutes at room temperature, with maximum absorption observed at 448 nm. Whereas the reagent blank which shows no absorbance at this wavelength but has 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.

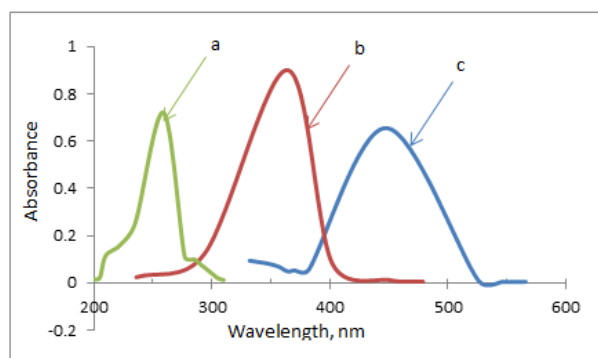


Figure 1, Absorption spectra of (a) SMX ( $10 \mu\text{g}\cdot\text{ml}^{-1}$ ) Vs ethanol, (b) blank reagent Vs ethanol and (c) SMX ( $10 \mu\text{g}\cdot\text{ml}^{-1}$ ) - 9-CA product Vs blank in the presence of HCl under the optimum conditions

### 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 acidic condition medium reaction, different acids were studied using 1 ml of 0.1 M of each tested acid. Maximum absorbance was found in the presence of hydrochloric acid at pH value of 1.75, while the two buffer solutions (boric acid/ $\text{H}_3\text{PO}_4$ / $\text{CH}_3\text{COOH}$ ) and (KCl/HCl) showing decreasing in absorbance at the same pH. However, It was found that

1ml of 0.2 M of HCl 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 ( $10 \mu\text{g}\cdot\text{ml}^{-1}$ ) 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 this method.

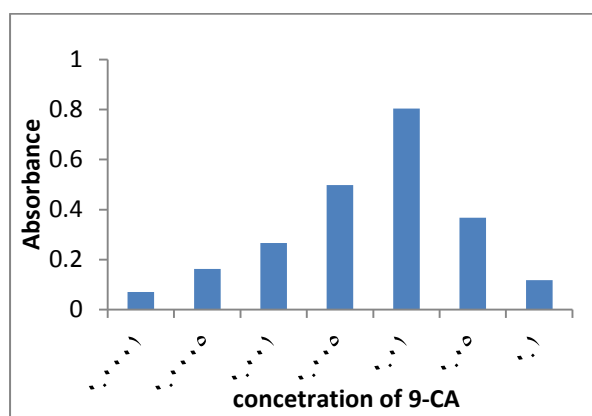


Figure 2, Effect of 9-CA conc.on the reaction with SMX

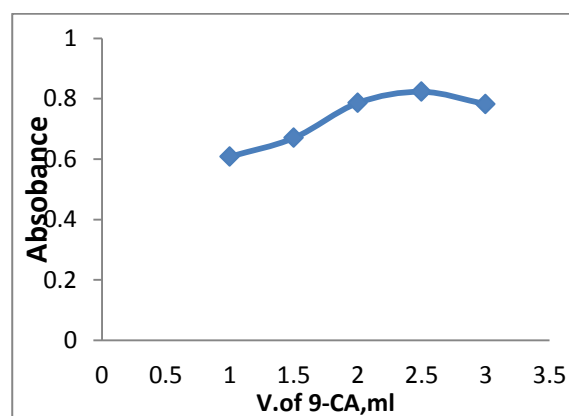


Figure 3, Effect of volume of  $10^{-2}$  M 9-CA on its reaction with SMX

### Effect of solvent

Different solvents namely methanol, ethanol, propanol and tetrahydrofuran (THF) were tested. The procedure consist to mixed  $10 \mu\text{g}\cdot\text{mL}^{-1}$  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, then diluted with the same solvent of 9-CA in a final volume of 10 mL, and absorbances are measured 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  $10^{\circ}\text{C}$  to  $50^{\circ}\text{C}$  (using thermostatic water bath) after each 5 min intervals. The results showed high color intensity after 40 min with maximum absorbance at  $10^{\circ}\text{C}$  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  $10^{\circ}\text{C}$  was selected.

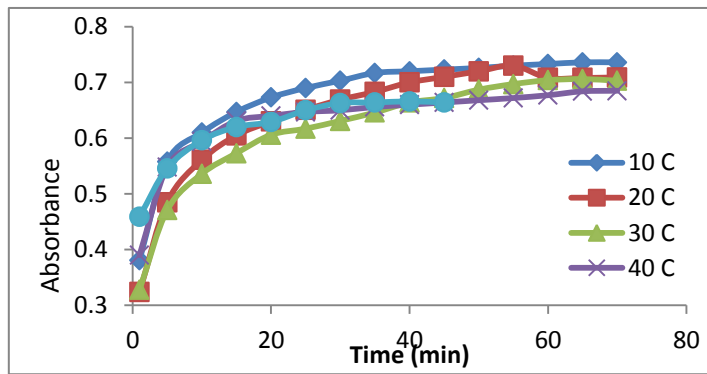


Figure 4, Effect of temperature on the absorbance of SMX-9-CA product at various time

### 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 SMX, (Table 1).

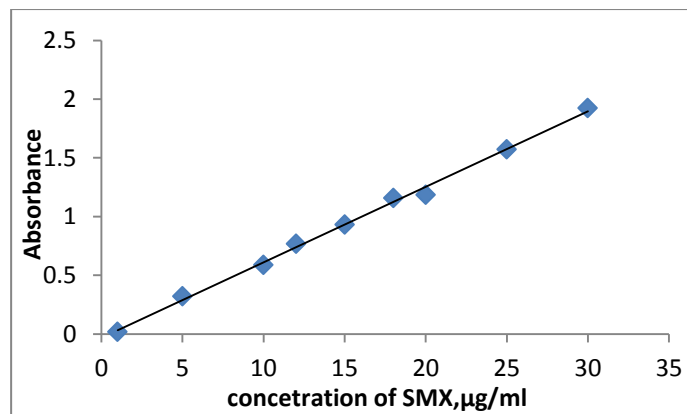


Figure 5, Absorbance of concentration of SMX on the reaction with 9-CA

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

Parameters	Value
Linearity range ( $\mu\text{g.ml}^{-1}$ )	1-30
Molar absorptivity ( $\text{L.mol}^{-1}.\text{cm}^{-1}$ )	$1.63 \times 10^4$
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 amount 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).

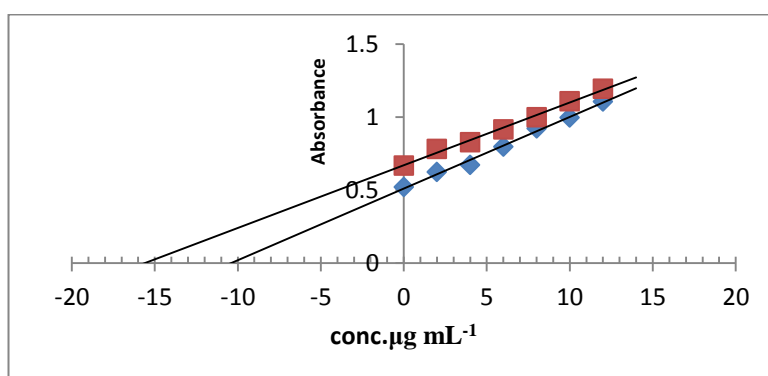


Figure 6, standard addition method

### 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 ( $\mu\text{g.ml}^{-1}$ )	Average Recovery* (%)	Drug content found(mg)	Certified value (mg)
Trimox Tablet	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 an 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		
SMX 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,  $\alpha$  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  $2.36 \times 10^3 \text{ L.mole}^{-1}$  which indicate that the product is relatively stable.

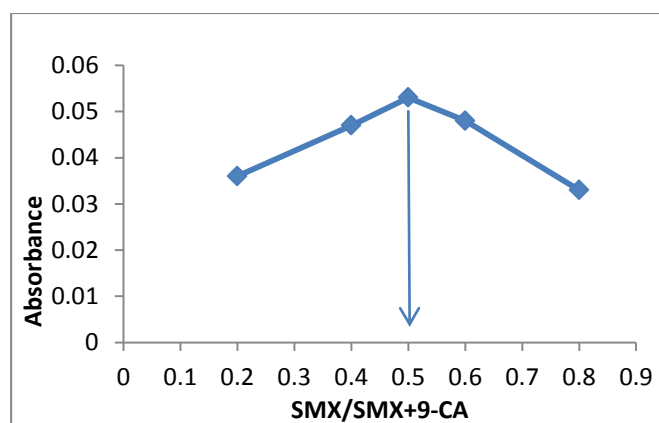


Figure 7, Job's method for SMX -9-CA product

### 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 <sup>(25)</sup>	<i>N,N</i> -diethyl- <i>p</i> -phenylene-diamine <sup>(b)</sup>	Vanillin <sup>(c)</sup>
Reagent	9-CA	chloranilic acid <sup>(25)</sup>	<i>N,N</i> -diethyl- <i>p</i> -phenylene-diamine <sup>(b)</sup>	Vanillin <sup>(c)</sup>
$\lambda_{\max}$ (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 ( $\text{L.mol}^{-1}.\text{cm}^{-1}$ )	$1.63 \times 10^4$	$2.98 \times 10^3$	$8.31 \times 10^3$	$1.1 \times 10^3$
RSD (%)	$\leq 0.651$	$\leq 0.075$	$\leq 0.600$	$\leq 6.360$
Type of reaction	Substitution reaction	Charge transfer complex	Oxidative coupling	Schiff's base

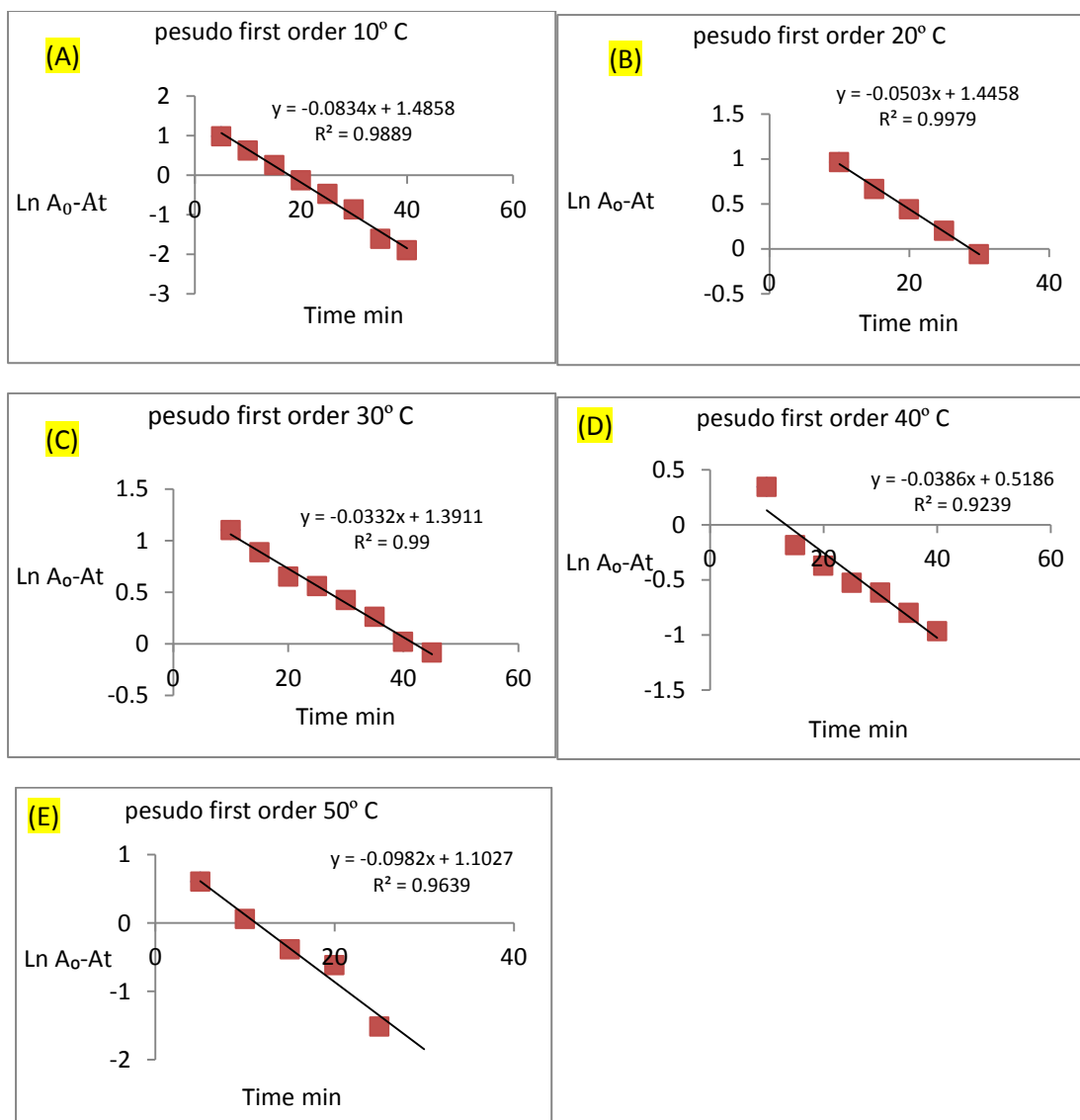
### Kinetic study of the resulting product

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.

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

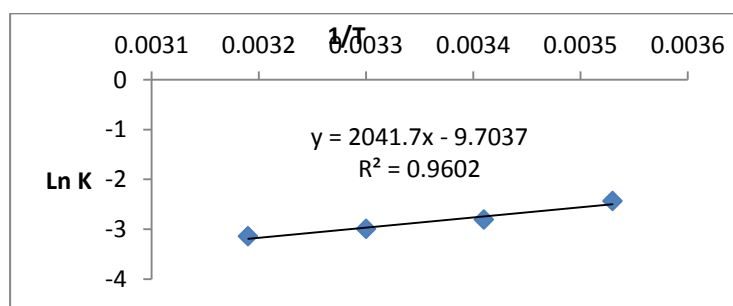
$$\ln(A_0 - At) = \ln A_0 - kt \dots\dots\dots(2)$$

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 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 (Fig. 8A to 8E).



**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 (Fig.9) using the relations given below (equation (3)).



**Figure 9.** Application of Arrhenius equation to calculate the activation energy

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 ( $\Delta H^*$ ,  $\Delta G^*$  and  $\Delta S^*$ ) can be estimated<sup>[18]</sup> .

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

Where A is the frequency factor and is used to calculate value of  $\Delta S^*$  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 ( $0.38 \times 10^{-23}$ ), 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 <sub>1/2</sub>	E (kJ.mole <sup>-1</sup> )	$\Delta H^*$ (kJ.mole <sup>-1</sup> )	$\Delta S^*$ (J.mole <sup>-1</sup> .K <sup>-1</sup> )	$\Delta G^*$ (kJ.mole <sup>-1</sup> )
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  $10 \mu\text{g}\cdot\text{ml}^{-1}$  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, the 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  $\Delta H$  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  $\Delta S^\circ$  was calculated, representing the intercept of the obtained straight line of figure (10). While  $\Delta H$  represent the slope value, as the value of  $\Delta G^\circ$  was determined by using equation(9) [20].

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

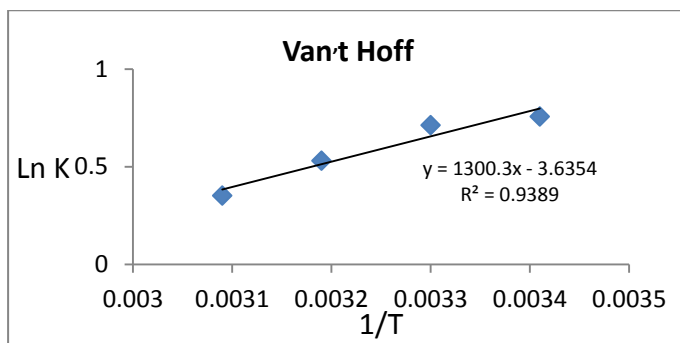


Figure 10, Application of Vant Hoff equation to calculate enthalpy of reaction between SMX and 9-CA

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	$\Delta H^\circ$ (kJ.mole <sup>-1</sup> )	$\Delta G^\circ$ (kJ.mole <sup>-1</sup> )	$\Delta S^\circ$ (J.mole <sup>-1</sup> .K <sup>-1</sup> )
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  $\Delta G^\circ$  refers to that, the complexation reaction could occur spontaneously.

The negative sign of  $\Delta H^\circ$  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**).

The negative value of  $\Delta S^\circ$  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  $\Delta H$ . 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 **points** 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 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 ( $\eta$ ), electronic chemical potential ( $\mu$ ), Global electrophilic ( $\omega$ ), total energy (TE) and atomic charge on the **hetroatoms** 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 gab for the studied compounds.

compound	$\eta$	M	$\omega$	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 gab energy (L- H) refers to that, SMX is more stable than 9-CA (larger energy gab).
- 3- The value of hardness ( $\eta$ ) 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  $\mu$  and  $\omega$  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)

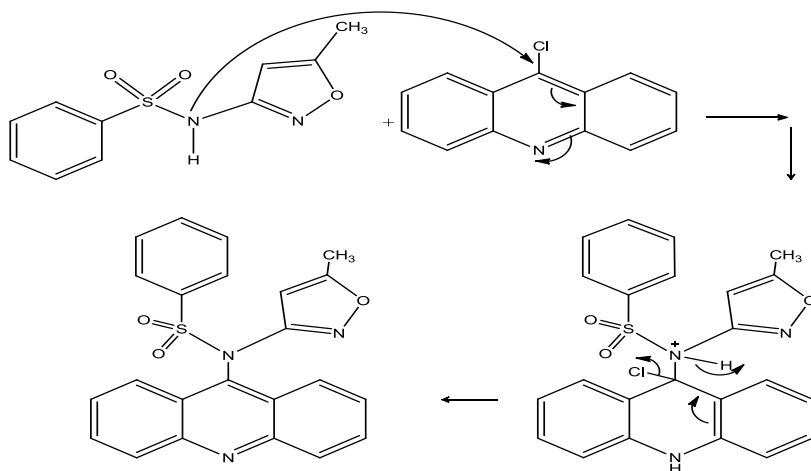


Figure (11), Suggested mechanism in the basic medium

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).

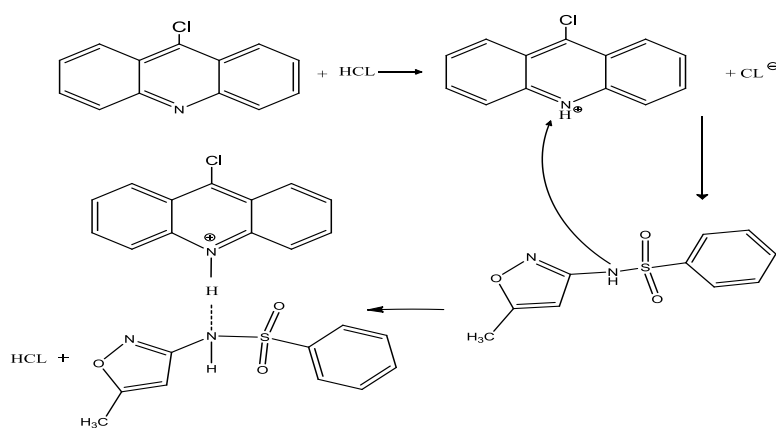


Figure (12), Suggested mechanism in the acidic medium

## Conclusion

A spectrophotometric method for the determination of the SMX and kinetic study based on the formation of a colored product from its reaction with 9-CA reagent in acidic ethanol medium has been developed. The coloured product has maximum absorption at 448 nm and obeyed Beer's law in the range 1-30  $\mu\text{g.ml}^{-1}$  with molar absorptivity of  $1.63 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$  and good accuracy. The method was compared favorably with the official method. The kinetic study of the interaction of sulfamethoxazole with the reagent 9-chloroacridine showed that the reaction is of the pseudo-first-order and estimation of the rate constant of the reaction at different temperatures helped to calculate the activation energy (E) and thermodynamic functions of activation. Thermodynamic study were also conducted. The results indicated that the complex formation is exothermic, its values pointing into the physical nature of forces controlling the complex formation. The reaction could take place spontaneously with an increase in the order of the studied system. Theoretical parameters were calculated by applying the semi-empirical Austin method (AM1). These parameters are helped to suggest reaction mechanism and supporting other results.

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