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Thermodynamic and kinetic studies for the interaction of cephalexin with albumin

دراسات ثرموديناميكية وحركية لارتباط ا دراسات ثرموديناميكية وحركية لارتباط الالبومين مع السفالكسين

Dr.Elham M.Al-Rufaie*(1), Alaa Abd Al-Zahra*(1) and Alaa j.khadim*(2)

Department of Chemistry, College of science, Baghdad University [1]

Department of Chemistry, College of education for pure sciences, Karabla University[2]

E-mail:elhammageed54@yahoo.com

البحث مستل

Abstract:

This work aim to get information about the binding of cephalexin(antibiotics) with albumin, and the influence of the solvent polarity and ionic strength on it by using UV-visb spectrophotometric measurements in phosphate buffer solution pH (7.4), and at three different temperatures, (290, 300, 310)K .The UV absorption shows a change and a shift in the absorbency and a shift in albumin and cephalexin peaks, the two changes are indicative of complex formation. The stoichiometry of the interaction were calculated by the method of continuous variations which was1:1at pH 7.4.The equilibrium constant was calculated at three different temperature (290,300,310)K and the thermodynamic parameter such as ΔG, ΔH and ΔS also calculated. The kinetic studies for this interaction follows first order equation with a rat constant value of 13.8×10^{-4} min^{-1}.

Key words: Albumin, cephalexin, Drug interaction, kinetics, thermodynamic.

الخلاصة :

يهدف البحث الى دراسة ارتباط السفالكسين (مضاد حيوي) مع الالبومين و تأثير المذيب والقوة الايونية على هذا الارتباط، باستخدام الطرق الطيفية (مطيافية الاشعة فوق البنفسجية والمرئية) في المحلول المنظم الفوسفاتي ذو الدالة الحامضية (7.4)عند ثلاث درجات حرارية(290 و300 و310) كلفن .من خلال الامتصاصية لاشعة uv أظهرت ان هنالك ازاحة في قمة الامتصاص للدواء والالبومين و تشيرالنتائج الى حدوث تغيرين ويعزا الى تكوين المعقد بينهما, كما حسبت نسبة الاتحاد بطريقة التغاير المستمر وتبين ان نسبة الدواء الى الالبومين في هذا المعقد هو 1:1.كما تم حساب ثابت الاستقرار equilibrium constant لهذا المعقد عند ثلاث درجات حرارية 290 و300 و310 كلفن، حسبت الدوال الثرموديناميكية(ΔGوالالنثالبيH ΔوالانتروبيΔS)) أظهرت الدراسة الحركية لتأثر الالبومين مع هذه الادوية انها تخضع الى حركيات المرتبة الاولى بثابت سرعة يساوي 13,8 × 10^{-4} دقيقة $^{-1}$

Introduction:

Albumin is the most abundant of the plasma proteins; it is classic protein, which has been the object of extensive researches for many years, especially in the field of protein chemistry [1]. The amino acid sequence of the human serum albumin shows a single chain of 585 residues is cross-linked by 17 cysteine bridges to form 8 double loops and one single loop [2]. The amount of human serum albumin is about 60% of the total protein in blood serum. In the serum of human adults, the concentration of albumin is approximately 40 mg/ by simple (condensation reaction) between aliphatic or aromatic aldehydes or ketones with aliphatic or m [3-7]. The β-lactams antibiotics are an important type of vital antibiotics used to treat infectious disease including tetra cycle β-lactam atom [8].The β-lactam antibiotics comprise two groups of therapeutic agents of considerable

clinical importance -the penicillin and cephalosporin have in their chemical structures a 4-membered lactam[9] The β-lactam antibiotics inhibit bacteria, exhibiting activities that differ in pattern and intensity [10] Cephalexin, 5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-[(amino phenyl acetyl) amino]-3-methyl-8-oxo, monohydrate, ($C_{16}H_{17}N_3O_4S.H_2O$), is a multi-dentate ligand [11] .Figure (1) shows the structural of cephalexin. Cephalexin is a white crystalline powder; Cephalexin is relatively broad-spectrum antibiotics with activity against both Gram-positive and Gram-negative bacteria [12, 13]. Cephalexin is Schiff base compound. Schiff bases are organic compound which contain azo methane group ($-\overset{|}{C}=\ddot{N}-$) which are prepared aromatic primary amines [14, 15].

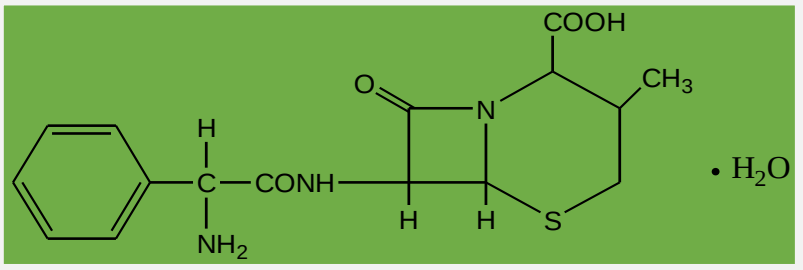

Figure -1: Structural formula of cephalexin

Experimental:

Serum albumin was purchased from Merck chemical company. Cephalexin was perused from the Arab company for antibiotics industries (ACAI).the solution of albumin, cephalexin were prepared by the use of Michal is phosphate buffer of pH (7.4) as solvent. Sodium chloride was purchased from BDHchemicals LTD pool England, absolute ethanol was purchased from fluke/switzer land.

Absorption spectroscopy:

The UV-Vis measurement of serum albumin was recorded on **(Cary-Varian) EL04103410**, using a quartz cell of 1 cm path length. The absorbance value of albumin in the presence and absence of each cephalexin, sodium chloride, ethanol were made in a wavelength (200-800 nm) .albumin concentration was fixed at (4×10^{-5}M), while the concentration of cephalexin were varied from (($1-5)\times10^{-5}$M).

Stoichiometric Analysis:

The stoichiometry of interaction of the drug (cephalexin) with albumin were calculated by the method of continuous variation, this methods sometimes known as Job's method [16]. The stoichiometry of the complex between cephalexin and albumin were obtained by preparing a series of ten solutions of cephalexin and albumin with a total concentration of (4×10^{-5}M) in phosphate buffer of pH=7.4, at the maximum wavelength of cephalexin.

Result and discussion:

Absorption spectroscopy:

The UV –VIS absorption studies were performed to ascertain the complexion of albumin with cephalexin. The mixture of albumin and cephalexin shows a shift in λ_{max} and a change in the absorbance due to a complex formation between the drugs and albumin, Figure (2a, 2b) show the absorbance spectrum of albumin and drug .The existence of the equilibrium is:

Drugs + Albumin $\rightleftharpoons$ Albumin-Drug complex … (1)

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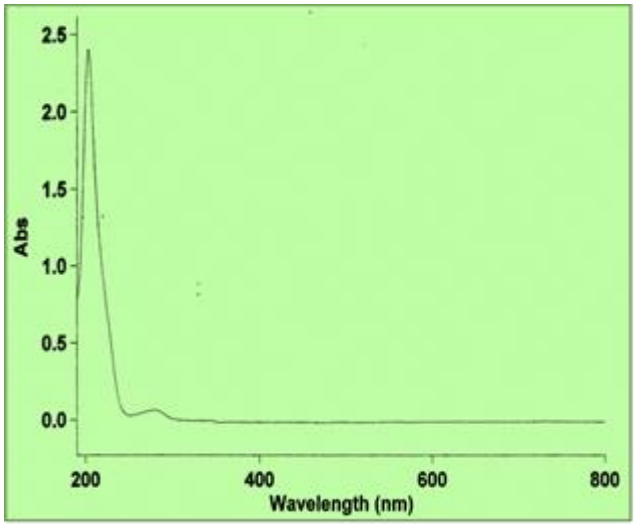
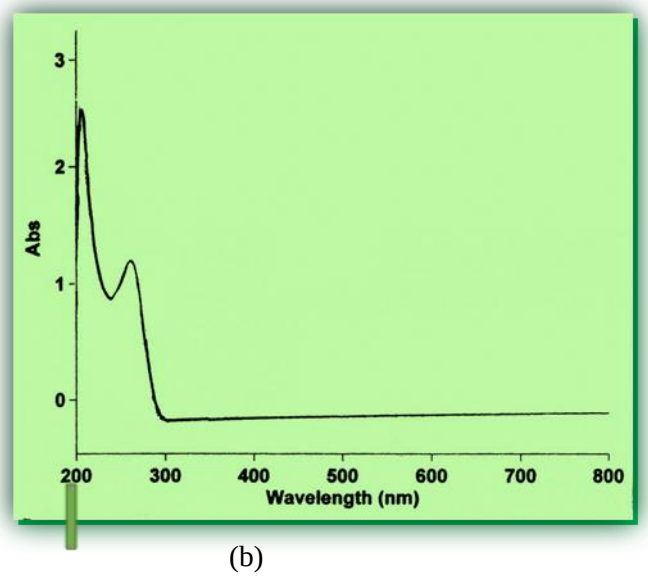


(a)



(b)

Figure -2a: absorption spectra of albumin (4×10^{-5}M) in a phosphate buffer of pH7.4.

Figure -2b: absorption spectra of cephalexin ($4^{\times}$10-5M) in a phosphate buffer of pH7.4

Stoichiometric Analysis:

The stoichiometry of interaction of the drug (cephalexin) with albumin was calculated by the method of continuous variation, this method is sometimes known as Job's method. The coordination number n could be calculated from the plot of absorbance against the mole fraction of drug. As it evident from the figure (3) the job, s plot implies that the stoichiometric ratio n of albumin -drug at 290 k and pH7.4 is 1:1.

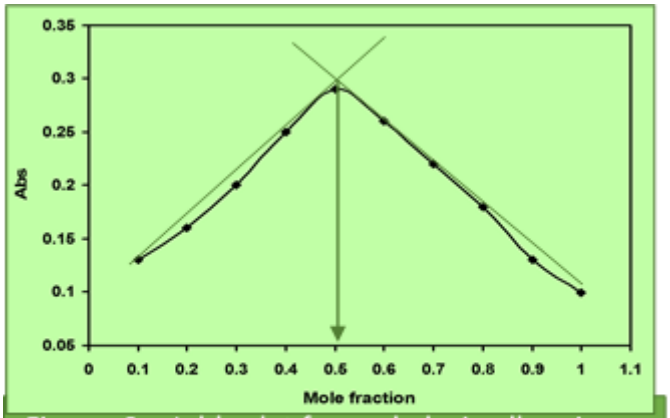


Figure -3a: Job's plot for cephalexin-albumin complex in phosphate buffer of PH 7.4

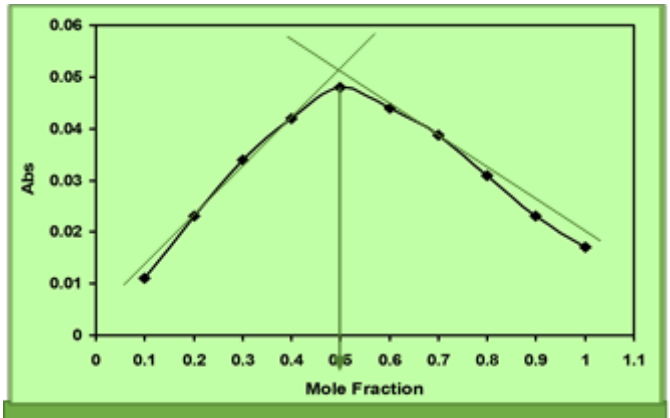


Figure -3b: Job's plot for cephalexin-albumin complex at pH 7.4 and 0.1M NaCl

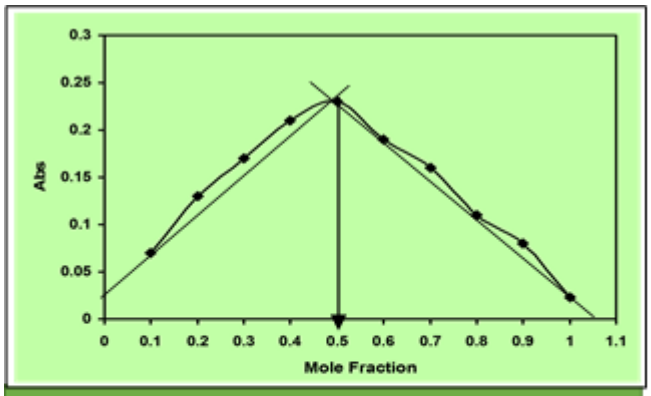


Figure -3c: Job's plot for cephalexin-albumin complex at 290K and 5% ethanol

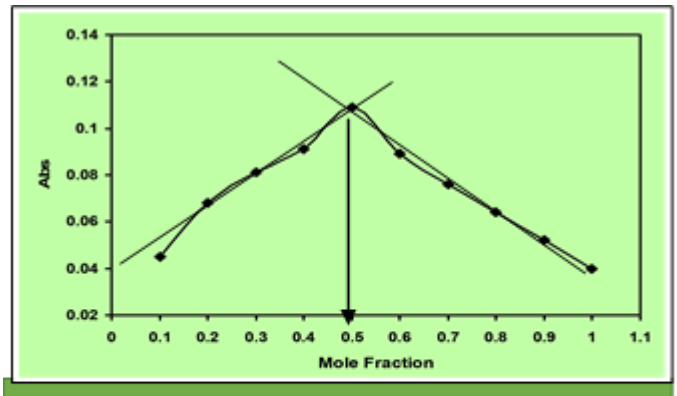


Figure -3d: Job's plot for cephalexin-albumin complex in pH 7.4 and 10% ethanol

The equilibrium constant was calculated using the continuous variation method [17-20].

Drug + Albumin $\longleftrightarrow$ [AD_n complex] … (2)

$$K_{eq} = \frac{[AD_n\ complex]}{[D]_{eq}\,[A]_{eq}} \quad \ldots \quad (3)$$

Knowing the formula of the complex between albumin and the drug (, cephalexin) which was (1:1) it is possible to determine the equilibrium constant of this complex.

The concentration of the complex formed at equilibrium was calculated as [21, 22]

$[AD_n]_{eq}$ = Absorbance $_{(max)}$ / $\varepsilon\, l$ … (4)

Plotting of the absorbance of the complex against concentration given a straight line with the slope equal to ε complex, the obtained data were listed in table (1)

Table -1: Molar absorptivity of the complex:

Complex name	Molar absorptivity ε ($cm^{-1}.L.mol^{-1}$)
cephalexin-albumin in phosphate buffer of pH 7.4	**31510**
cephalexin-albumin in pH 7.4 and (0.1)N NaCl	**190600**
cephalexin-albumin in pH7.4 and 5%ethanol	**39040**
cephalexin-albumin in pH7.4 and 10% ethanol	**36610**

The equilibrium constant for the interaction of albumin with the drug (cephalexin) was then calculated at three different temperature (290, 300 and 310) K; the results were shown in table (2).

Table -2: The equilibrium constant of drug-albumin complexes at different temperatures

Temp. (K)	Keq ($L.mol^{-1}$) in phosphate buffer of pH 7.4	Keq ($L.mol^{-1}$) in phosphate buffer of pH7.4 and 0.1N NaCl	Keq ($L.mol^{-1}$) In phosphate buffer of pH7.4 and 5% ethanol	Keq ($L.mol^{-1}$) in phosphate buffer of pH 7.410% ethanol
290	3.5×10^5	1×10^5	1.4×10^5	1.1×10^5
300	7.5×10^5	2.1×10^5	2.3×10^5	1.83×10^5
310	11.7×10^5	3×10^5	3.6×10^5	2.8×10^5

Table (2) shows the dependence of the equilibrium constant on temperature, the K_{eq} values increase with the increase in temperature for the complex between this drug and albumin and with increase in k_{eq} values the stability of the complex will increase.

Thermodynamic Parameters:

Thermodynamics is concerned with heat and energy transformations, such transformations are considered to take place in a "Universe" that is composed of a system and its surrounding [23, 24]. K_{eq} were determined from the concentrations of all components at equilibrium, which then allows us to calculate ΔG [25, 26].

$$\Delta G^\circ = - RT \ln K_{eq} \quad \dots \quad (5)$$

The enthalpy of a reaction can be determined by measuring the equilibrium constant for a system at different temperature [27]The enthalpy changes were calculated by substituting the value of the slope of the plot (*lin Keq* vs. *1/T*) in the vant Hoff equation (5), the result shown in figure(4). Entropy change for the system can then be calculated from:

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

Table (3a, 3b, 3c, 3d) show the thermodynamic parameter of complex

.

Table -3a: Thermodynamic parameters for cephalexin-albumin complex in phosphate buffer pH 7.4

T(K)	ΔG°($kJ.mol^{-1}$)	ΔH°($kJ.mol^{-1}$)	ΔS°($kJ.mol^{-1}$)
290	-36.3105	+50.2997	+0.04
300	-39.4083	+50.2997	+0.036
310	-42.9333	+50.2997	+0.026

Table -3b: Thermodynamic parameters for cephalexin-albumin complex in phosphate buffer pH 7.4 and 0.1M NaCl

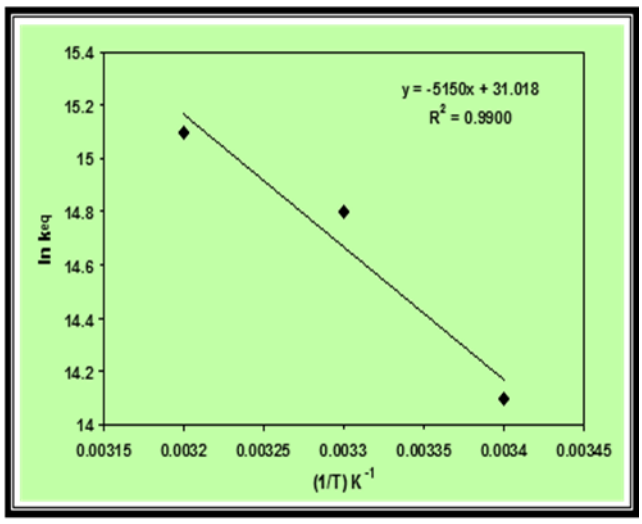
T(K)	ΔG°(kJ.mol^{-1})	ΔH°(kJ.mol^{-1})	ΔS°(kJ.mol^{-1})
290	-33.2726	+45.727	+0.043
300	-36.1659	+45.727	+0.032
310	-38.4023	+45.727	+0.0235

Table -3c: Thermodynamic parameters for cephalexin-albumin complex in phosphate buffer pH 7.4 and 5% ethanol

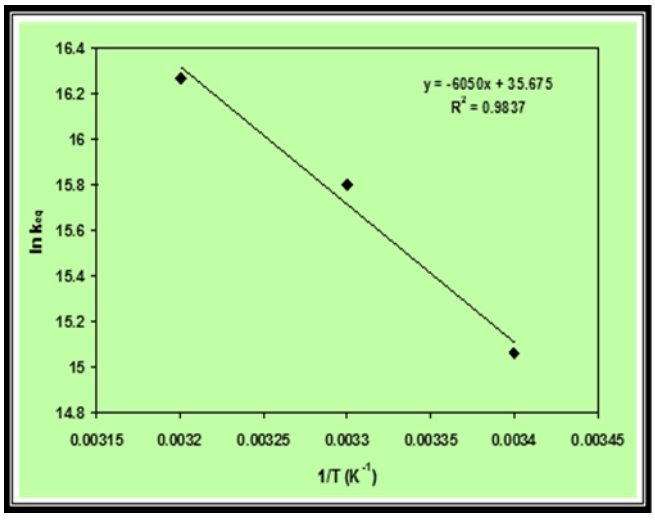
T(K)	ΔG°(kJ.mol^{-1})	ΔH°(kJ.mol^{-1})	ΔS°(kJ.mol^{-1})
290	-33.9959	+42.8171	+0.03
300	-36.9141	+42.8171	+0.019
310	-38.917	+42.8171	+0.012

Table -3d: Thermodynamic parameters for cephalexin-albumin complex in phosphate buffer pH 7.4 and 10% ethanol

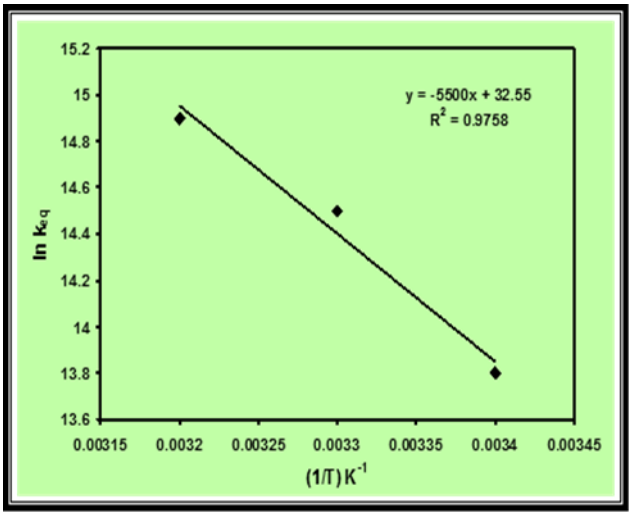
T(K)	ΔG°(kJ.mol^{-1})	ΔH°(kJ.mol^{-1})	ΔS°(kJ.mol^{-1})
290	-33.5137	+41.570	+0.027
300	-36.9	+41.570	+0.018
310	-38.1446	+41.570	+0.0109

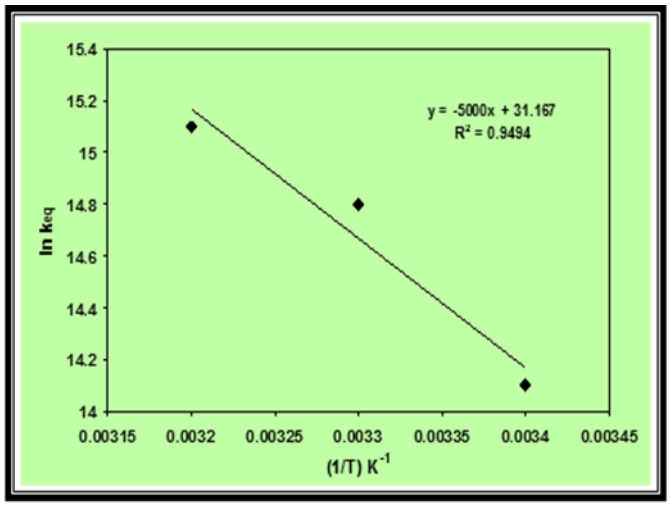


(a)



(b)





(c) (d)

Figure -4a: Van't Hoff plot for cephalexin-albumin complex.
Figure -4b: Van't Hoff plot for cephalexin-albumin in pH 7.4 and 0.1N NaCl.
Figure -4c: Van't Hoff plot for cephalexin-albumin complex in pH 7.4 and 5% ethanol
Figure -4d: Van't Hoff plot for cephalexin-albumin complex in in pH 7.4 and 10% ethanol.

The negative values of Gibbs free energy for cephalexin-albumin) means that process is spontaneous the enthalpy of interaction has a positive value that means the process is endothermic and the system requires input of energy. The positive enthalpy and entropy change also refer to the type of interaction between albumin molecules and cephalexin) which are hydrophobic association and electrostatic interaction [28, 29]

Interaction Kinetics:

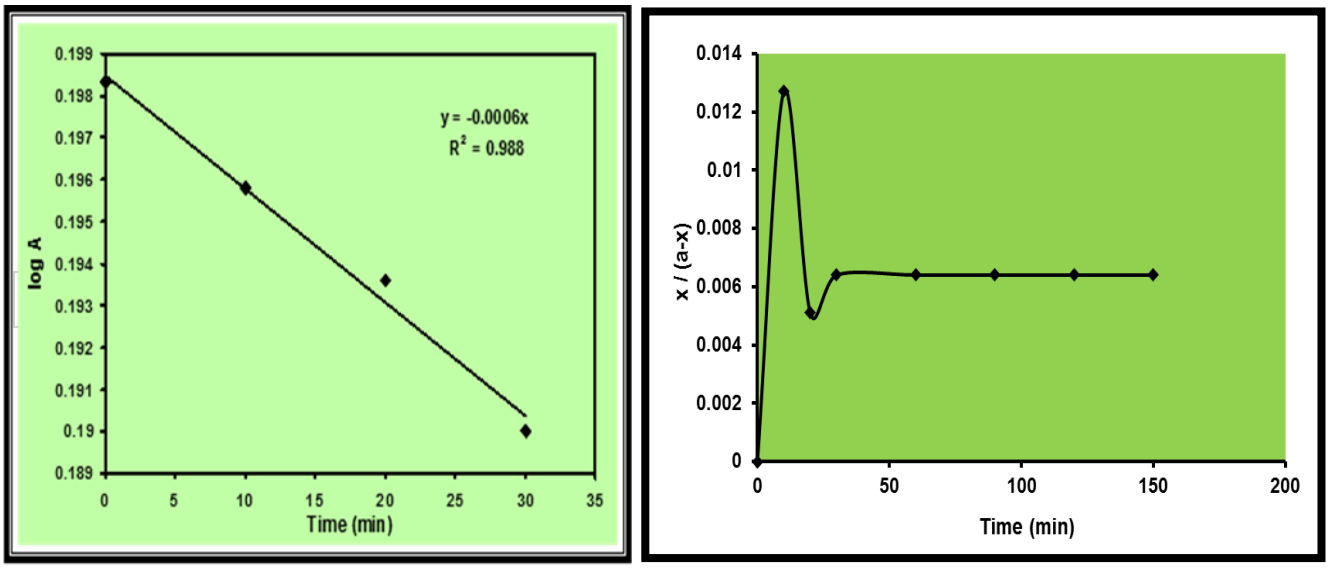
Interaction kinetics of the albumin with the studied drug (cephalexin) was determined by the following of the absorbance of albumin-cephalexin complex with time at a known wavelength. The first order rate equation (7) and the second order rate equation (8) were applied.

$lnA - lnA_{\circ} = -kt$ first order equation … (7)

k: rate constant for the reaction, which is independent of the concentration but depends on the temperature.

$1/[A] - 1/[A]_{\circ} = kt$ Second order equation … (8)

The complex will be stable in about (30-60) minutes, which demonstrated from the constant absorbance. The application of the first and second order of the reaction illustrate in figure 5(a, b).Table (4) illustrate First order Rate constant for the complex of cephalexin-albumin at different ionic strength and polarity.



(a) (b)

Figure (5)
a: The application of the first order reaction equation for complex of albumin-cephalexin at 290K,
b: The application of the second order reaction equation for complex of albumin-cephalexin.

The interaction of this drug was first order with a rate constant presented in table (4).

Table -4: The first order rate constant for albumin drug complex

NO.	**Complex and medium**	**First order Rat constant (min^{-1})at 290(K)**	**First order Rat constant(min^{1})at 300(K)**	**First order Rat constant (min^{1-})at 310(K)**
1	Amoxicillin-albumin in phosphate buffer of pH7.4	13.8×10^{-4}	15.1×10^{-4}	17.6×10^{-4}
2	Amoxicillin-albumin inpH7.4and (0.1N)NaCl	4.6×10^{-4}	5.3×10^{-4}	7.1×10^{-4}
3	Amoxicillin-albumin in pH 7.4 and 5%ethanol at 290K	9.2×10^{-4}	12.03×10^{-4}	$14.6\times10^{-4}4$
4	Amoxicillin-albumin in pH7.4 and10% ethanol at 290K	6.9×10-4	9.30×10^{-4}	12.1×10^{-4}

Conclusions

The interaction of cephalexin (antibiotic) has been investigated in vitro under simulated physiological conditions (phosphate buffer of pH 7.4) using UV-Vis. Spectrophotometric method. Experimental results showed that the interaction is of a first order, with a stoichiometric complex ratio of 1:1.The thermodynamic analysis suggested that cephalexin could bind human serum albumin (HAS) through the hydrophobic forces and ionic interaction, and the extend of the binding influenced by the polarity of solvent the change in ionic strength. The equilibrium constants of the drug and (HAS) complexes here a fundamental role in determining the free drug concentration in the plasma which in turn, induces the pharmacological activities of these drugs. Similarly, the knowledge of the varieties in the enthalpy and entropy value associated the complex formation reactions enable one to predict the nature of the chemical interactions.

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