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Physical Properties and Chemical Kinetics for the Interaction of Albumin with Amoxicillin

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Abstract

Albumin is the most abundant protein in blood plasma, serves as a circulating depot for endogenous and exogenous (e.g. drugs) compounds due to its ligand binding properties, this work aim to get information about the binding of Amoxicillin (antibiotics) with albumin, and the influence of the solvent polarity and ionic strength on it by using UV -vis spectrophotometric measurements in phosphate buffer of pH7.4 and three different temperature (290, 300, 310) K. The UV absorption shows a change and a shift in the absorbency and a shift in albumin and amoxicillin 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 and ΔG°, ΔH° and ΔS° also calculated. The kinetic studies for this interaction follows first order equation with rate constant value of 16×10^{-4} min^{-1}.

Keywords: Albumin, Amoxicillin, Drug interaction, kinetics, thermodynamic.

الخواص الفيزيائية والحركيات الكيميائية لارتباط الالبومين مع الاموكسيسيلين

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الخلاصة

يهدف البحث الى دراسة ارتباط الاموكسسلين (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 قوة هيدروجينية 7.4 عند ثلاثة درجات حرارية (290 و300 و310) كلفن . ان قيمة الازاحة في قمة الامتصاص للدواء والالبومين والنقصان في الامتصاصية، تشير 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 .كما تم حساب كلا من الدوال الثرموديناميكية ((ΔG°), والالنثالبي (ΔH°) والانتروبي(ΔS°) والدراسة الحركية لتأثر الالبومين مع هذه الادوية حيث كانت تخضع الى حركيات المرتبة الاولى بثابت سرعة يساوي16×10^{-4} دقيقة$^{-1}$.

Introduction:

Albumin is one of the smallest known plasma proteins with a molecular mass of approximately 69 KDa. Albumin is one of a small number of plasma proteins that is devoid of a carbohydrate moiety. A major function of albumin is to provide most of the natural osmotic pressure of plasma. It's versatility in binding a great variety of ligands such as fatty acids bilirubin, drugs and many other organic anions, is perhaps its most important property related to its physiological function and it is certainly

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it's most unusual and intriguing property to be explained by its molecular stature [1,2]. Antibiotics are chemical substances produced by various species of microorganisms and other living systems that are capable in small concentration inhibiting the growth of or killing bacteria and other microorganism [3, 4]. Amoxicillin is a p-hydroxyl derivative of ampicillin [5]. The chemical name of amoxicillin is 4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylicacid, six [aminohydroxypheny) acetyl] amino3, 3dimethyl-7-oxo, trihydrate ($C_{10}H_{19}O_5S$), figure-1 shows the structural formula of amoxicillin trihydrate. It is a yellow crystalline powder, soluble in water and alcohol (ethanol, methanol) [6, 7].Amoxicillin is broad-spectrum penicillin. It is active against a wide range of Gram-negative and Gram-positive bacteria; amoxicillin is particularly effective in typhoid fever caused by salmonella typh [8].

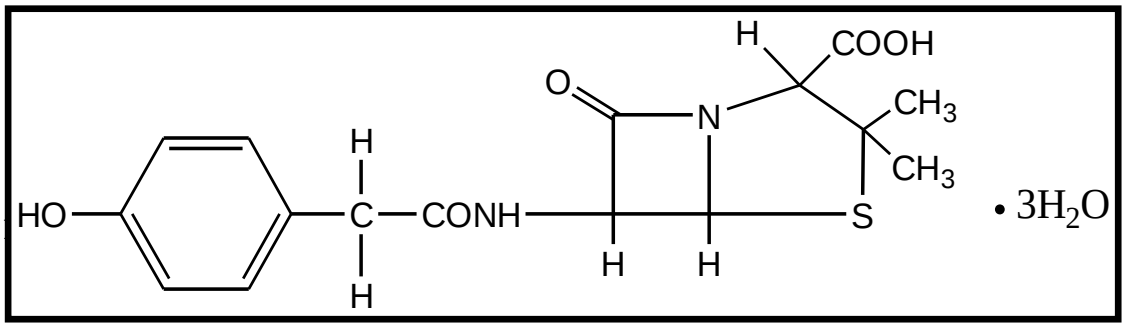

Figure 1- structural formula of amoxicillin trihydrate

On an importance of protein as a structural material, and its involvement in metabolic regulation, transport, defense and catalysis, we originate this research as a completion to our foregoing work in this field. Abd Al-Kareem calculates the energies of hydrogen bonding which was formed between some drugs with specific amino acid, theoretically and practically by the application of IR spectroscopy [9]. Zahra studied the interaction between anticoagulant drugs (warfarin, coumarin, heparin and aspirin) with albumin by the application of different spectroscopic techniques, the equilibrium constants were calculated at different temperatures and it was found that k_{eq} increases with the increase in temperature [10]. Charles F.F. Karney and Joson E. Ferrara (2004), apply a Monte Carlo method to compute the binding affinity of a ligand to a protein. The method involves extending configuration space by a discrete variable [11].

Aim of the work to study the interaction of albumin with amoxicillin by the application of UV-Visible spectroscopic method, the following calculation were done:

a) Confirmation of Albumin-drugs complex.
b) The stoichiometric ratio of the complexes.
c) The rate constant and the order of interaction.
d) Equilibrium constant and thermodynamic parameters ($\Delta H°$, $\Delta S°$, $\Delta G°$) for the interaction. These studies were done in solvents differ in its composition and ionic strength.

Experimetal:

Reagent and chemicals: Michal's phosphate buffer of pH 7.4

Michal is phosphate buffer of physiological pH 7.4 was prepared by mixing certain ratio of each of the two stock solutions, potassium hydrogen phosphate $K_2HPO_4.3H_2O$ (0.067 M) and potassium hydrogen phosphate KH_2PO_4 (0.067 M).Albumin stock solution of (7×10^{-5}M) concentration were prepared by dissolving (0.4634 g) of albumin in 100ml phosphate buffer as a solvent. Serum albumin was purchased from Merck chemical company. Amoxicillin stock solution of (6×10^{-5}M) concentration were prepared by dissolving (0.025 g) of amoxicillin in 100ml phosphate buffer as a solvent. Amoxicillin tri hydrate was purchased from the Arab company for antibiotics industries (ACAI).Absorption spectra were taken with the UV-Vis spectrophotometer (Cary Varian) EL04103410, using a quartz cell of 1 cm path length. The absorbance of albumin and drug were calculated in a wavelength (200-800 nm) by the use of Michal's phosphate buffer of pH 7.4 as a blank.
Stoichiometric analysis: The stoichiometry of the complexion of albumin with drug was determined by continuous variation method (Jobs method): Job method was applied by a series of solutions have a mole fraction in between (0.1 to 0.9) [12], were prepared by mixing different volumes of albumin and drug stock solutions of a concentration (4×10^{-4}M) for each.

Result and Discussion:

Absorption spectroscopy:

The UV –VIS absorption studies were performed to ascertain the complexion of albumin with amoxicillin.Albumin is donors and amoxicillin is acceptor the Donor-acceptor complexes, the compositions of which can be represented by integral mole ratios of the components, are in many instances so unstable that they cannot be isolated in the pure state at ordinary temperatures but exist only in solutions in equilibrium with their components. They can, however, usually be detected readily because of differences in their physical properties (e.g., absorption spectra) from those of the pure [13]. The UV-VI absorbance showed an increase with the increase in drug concentration also a shift in λ max and aching in the absorbance due to complex formation between drug and albumin,Figure-2(a, b,c)show the absorption spectra of albumin and drug.[14,15]

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

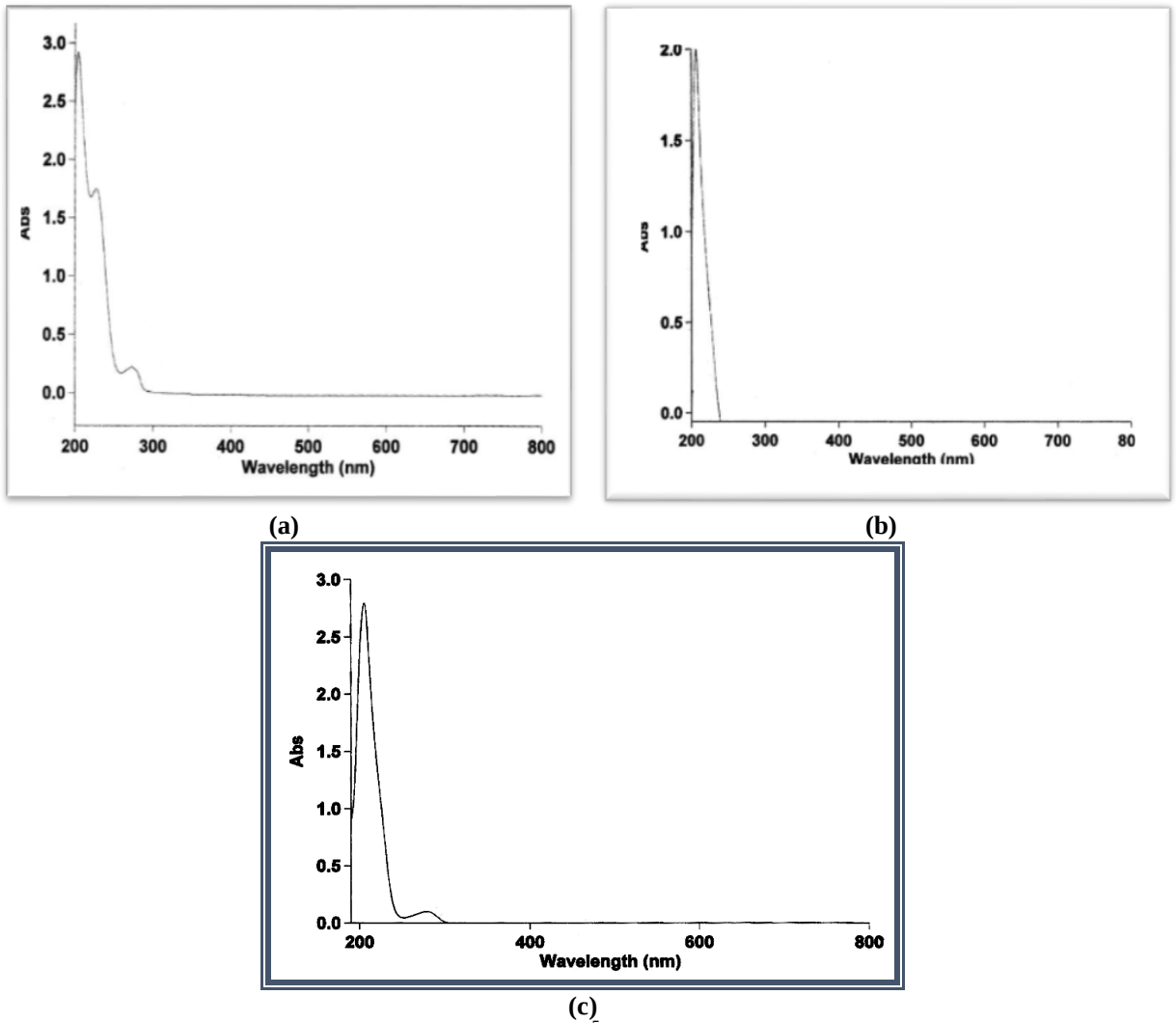

Figure 2- (a) Absorption spectra of amoxicillin (4×10^{-5}M) in a phosphate buffer of pH 7.4 **(b):** Absorption spectra of albumin (4×10^{-5}M) in a phosphate buffer of pH 7.4 **(c)**: Absorption spectra of amoxicillin-albumin complex in a phosphate buffer of pH 7.4

Stoichiometric analysis:

The stoichiometry of the complex of albumin with drug was calculated by the method of continuous variation [16]. The coordination number n could be calculated from the plot of absorbance of amoxicillin-albumin at λ max (279nm) against the mole fraction of drug.as it evident from the Figure-3(a, b, c, d) the Job's plot, implies that the stoichiometric ratio n of albumin -drug at 290 K and PH7.4 is 1:1.

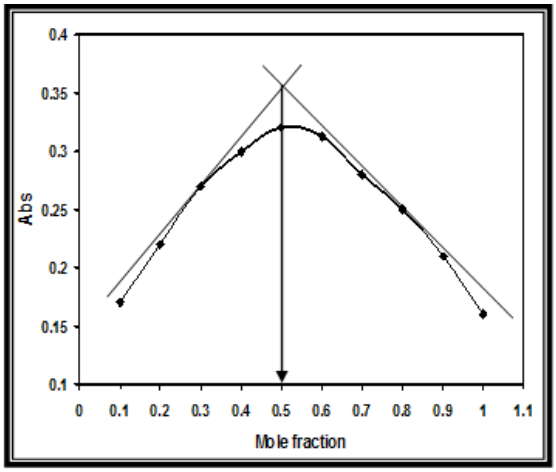

Figure 3-(a) :Job's plot for amoxicillin-albumin complex at pH7.4

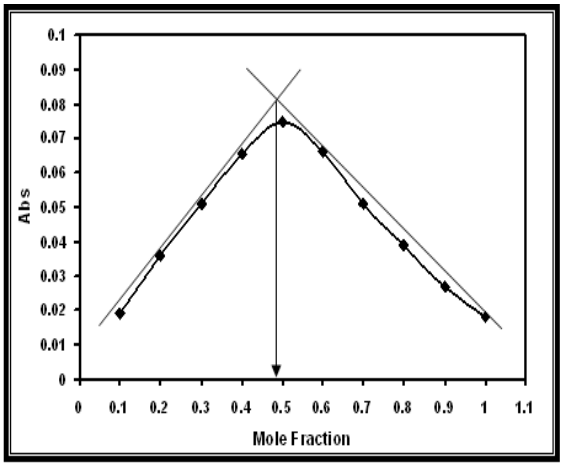

Figure 3-(b): Job's plot for amoxicillin-albumin complex at pH 7.4 and 0.1M NaCl

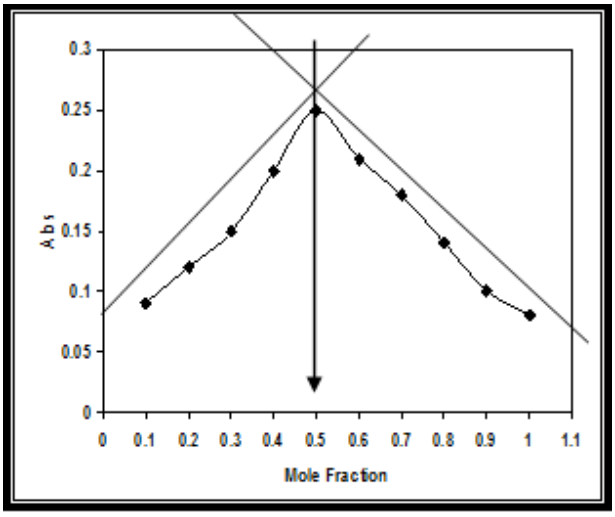

Figure 3- (c) Job's plot for amoxicillin-albumin complex at 290K and 5% ethanol

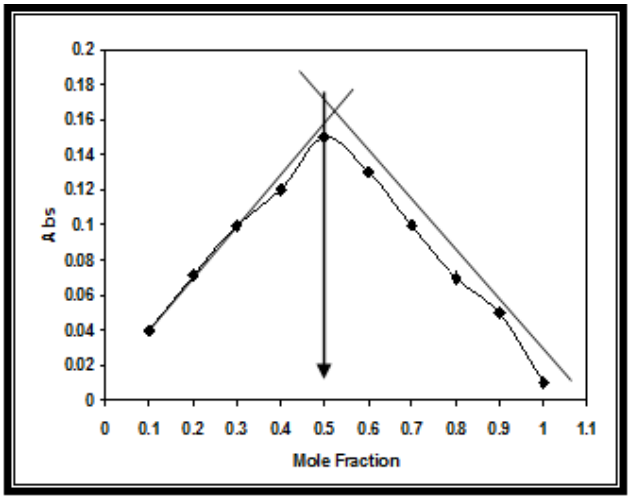

Figure 3- (d) Job's plot for amoxicillin-albumin complex at pH 7.4 and 10% ethanol

Stability constant (Keq): The equilibrium constant can be calculated using the continuous variation method [17, 18]

(A)eq+(D)eq= (AD)eq (2)

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

$$K_{eq} = \frac{[A_{max}/\varepsilon l]}{[C_A - A_{max}/\varepsilon l]\,[C_D - A_{max}/\varepsilon l]} \quad (4)$$

A_{max} = the maximum absorbance of the complex

ε = molar absorptivity of the complex (L. mole^{-1}. cm^{-1})

l = path length. cm.

C_D = Initial concentration of the drug

C_A =Initial concentration of albumin.

The molar absorptivity of the complex was calculated by recording the absorbance of a series concentration of 1:1complex and plotting the absorbance against concentration which given a straight line with a slope equals to ε for this complex was illustrated in Table-1: [19, 20].

Table 1-Molar absorptivity of complexes at different ionic strength and polarity

Complex name	Molar absorptivity ε (L . .mol^{-1}cm^{-1})
Amoxicillin-albumin in phosphate buffer of pH 7.4	291400
Amoxicillin-albumin in pH 7.4 and (0.1)M NaCl	223100
Amoxicillin-albumin in pH7.4 and 5%ethanol	110000
Amoxicillin-albumin inpH7.4 and 10% ethanol	103800

The equilibrium constant calculated by this method were determined in three different temperatures (290,300,310) K as shown in Table-2.

Table 2- The equilibrium constant of drug-albumin complex at different temperatures in pH 7.4.

Temp. (K)	Keq (L.mol^{-1}) in phosphate buffer of pH 7.4	Keq (L.mol^{-1}) in phosphate buffer of pH7.4 and 0.1M NaCl	Keq (L.mol^{-1})in phosphate buffer of pH7.4 and 5% ethanol	Keq (L.mol^{-1})in phosphate buffer of pH 7.4 and 10% ethanol
290	4.2×10^5	1.2×10^5	2.5×10^5	1.6×10^5
300	8.4×10^5	2.5×10^5	4.5×10^5	2.3×10^5
310	13.2×10^5	3.4×10^5	7.3×10^5	3.1×10^5

Table-2 shows the dependence of equilibrium constant with temperature, it increase with increase in temperature for (albumin –drug) complex. That mean the stability of complex increase with temperature that means the bond between them becomes stronger [21, 22].

Thermodynamic Parameters: the enthalpy changes ΔH^0, the entropy changes ΔS^0 and the free energy changes ΔG^0, have been reported of the complexions (albumin-amoxicillin complex) in Table-(3a, 3b, 3c, 3d).

The enthalpy changes were calculated by substituting the value of the slope of the plot (*log Keq* vs. *1/T*) in the vant Hoff equation (5), , the result as shown in Figure-4 and Table-(3a,3b,3c,3d) [23-25].

$$\ln Keq = -{}^{\Delta H^\circ}/_{RT} + {}^{\Delta S^\circ}/_{R} \quad (5)$$

$$Slope = -{}^{\Delta H^\circ}/_{R}$$

R = gas constant.

The change in Gibbs free energy can be determined from equation (6), the relation between K_{eq} and $\Delta G^o$and the entropy changes from equation (7).

$$\Delta G^\circ = -RT \ln K \quad (6)$$

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (7)$$

Table 3-(a) Thermodynamic parameters for amoxicillin-albumin complex in phosphate buffer pH 7.4at different temperature

T(k)	ΔG°(kJ.mol^{-1})	ΔH°(kJ.mol^{-1})	ΔS°(kJ.mol^{-1} K^{-1})
290	-31.218	+49.468	+0.0278
300	-34.024	+49.468	+0.0278
310	-36.323	+49.468	+0.077

Table 3- (b) Thermodynamic parameters for amoxicillin-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} .K^{-1})
290	-28.198	+46.974	+0.259
300	-31.001	+46.974	+0.260
310	-32.827	+46.974	+0.257

Table 3- (c) Thermodynamic parameters for amoxicillin-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} K^{-1})
290	-29.968	+45.727	+0.261
300	-32.467	+45.727	+0.261
310	-34.796	+45.727	+0.260

Table 3- (d) Thermodynamic parameters for amoxicillin-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} K^{-1})
290	-28.892	+41.570	+0.247
300	-30.793	+41.570	+0.241
310	-32.589	+41.570	+0.239

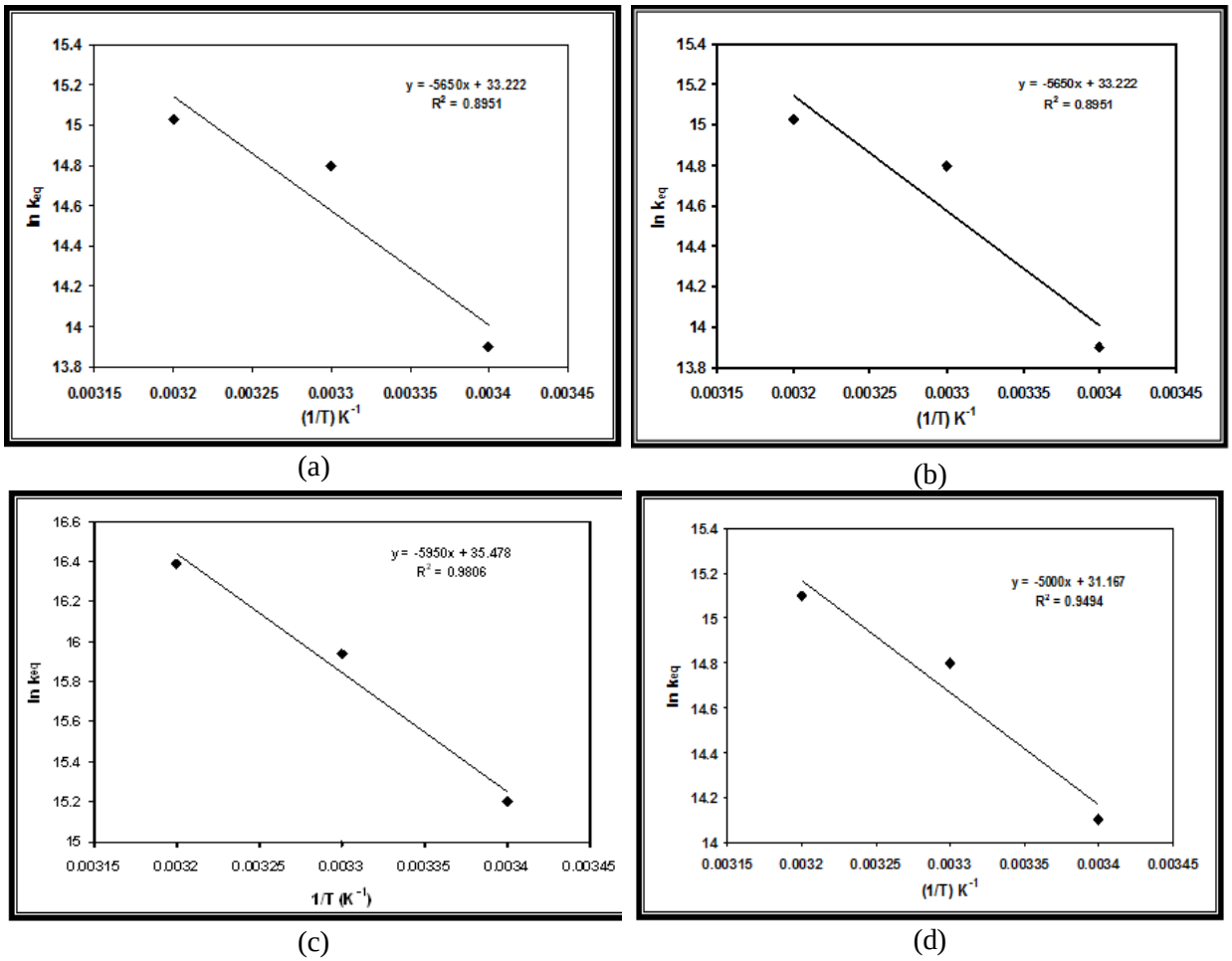

Figure 4- van't hoff plot for(a) amoxicillin-albumin in pH7.4 (b)amoxicillin albumin in pH 7.4and (0.1) M NaCl (c)amoxicillin albumin in pH7.4 and 5%ethanol (d)amoxicillin albumin in pH7.4 and 10%ethanol.

The negative values of Gibbs free energy refer to spontaneous interaction between albumin and amoxicillin, in direction of equilibrium and increase with increase in temperature. The positive values of entropy occur because water molecules that arranged around the albumin and ligand became more random because of hydrophobic interaction also the entropy decrees with increase in temperature. The positive enthalpy, entropy change refer to the hydrophobic interaction between albumin and amoxicillin [26].

Interaction Kinetics: Interaction kinetics of the albumin with the studied drug (amoxicillin) was determined by the following of the absorbance of albumin-amoxicillin complex with time at a known wavelength (279nm). The first order rate equation (8) and the second order rate e-quation (9) were applied.

$\log A = -kt / 2.303 + \log A_o$ first order equation (8)

$t = x / k\,a(a-x)$ second order equation (9)

A = absorbance at time t.

A_o= absorbance at time zero.

k = rate constant.

$x = A_o - A$

$a - x = A$

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

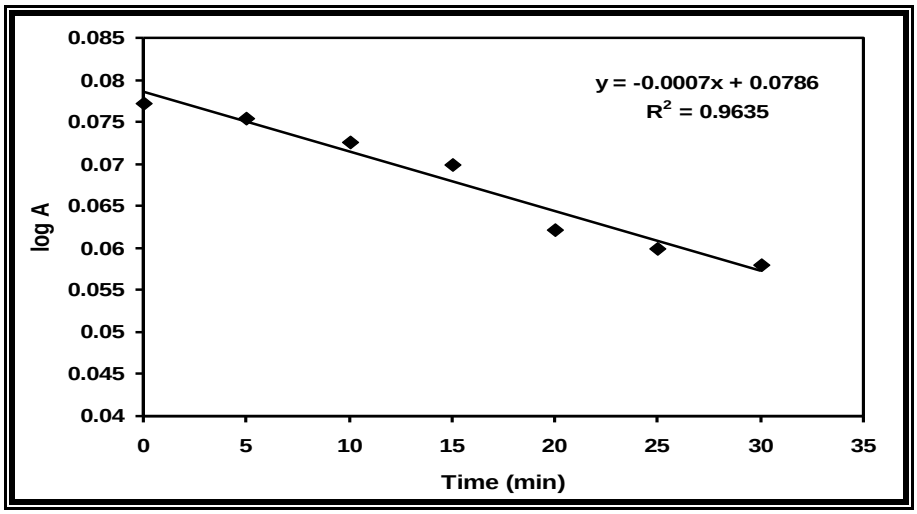

Figure 5- (a): The application of the first order reaction equation for complex albumin – amoxicillin at 290K

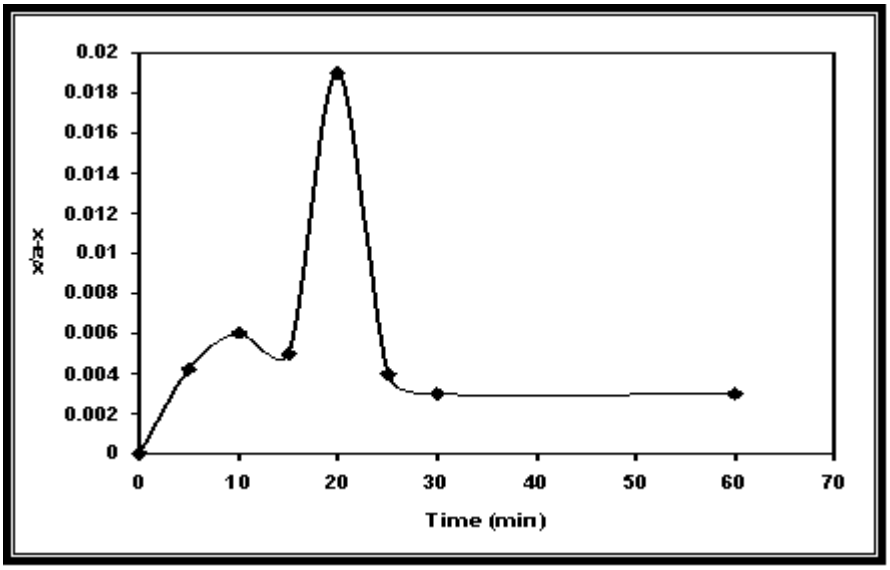

Figure 5- (b) The application of the second order reaction equation for complex of albumin-Amoxicillin

Figure- (5a) illustrate a straight line and Figure-(5b) illustrate a non-linear relation, this indicates that the interaction between albumin and Amoxicillin is a first order with a rate constant $k=16\times10^{-4}min^{-1}$

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	16×10^{-4}	18.2×10^{-4}	20.1×10^{-4}
2	Amoxicillin-albumin inpH7.4and (0.1M)NaCl	3.2×10^{-4}	3.64×10^{-4}	4.02×10^{-4}
3	Amoxicillin-albumin in pH 7.4 and 5%ethanol	11.51×10^{-4}	13.09×10^{-4}	$14.4\times10^{-4}4$
4	Amoxicillin-albumin in pH7.4 and10% ethanol	9.2×10-4	10.47×10^{-4}	11.56×10^{-4}

Table-4 show that the increasing of temperature due to increase in rate constant.

Determination the Activation Energy of Albumin-Drug Comple

Activation energy is the minimum kinetic energy that reactants must have in order to form products. It is calculated from the relation Arrhenius equation:

$k=Ae^{-Ea/RT}$

$\log k=\log A-Ea/2.303RT$ Arrhenius equation (10)

A: Frequency factor, Ea. Activation energy, A and Ea. is called Arrhenius parameters

The relation between log k and 1/T was explaining in Figure-6, the activation energy was calculated and list in Table-5.

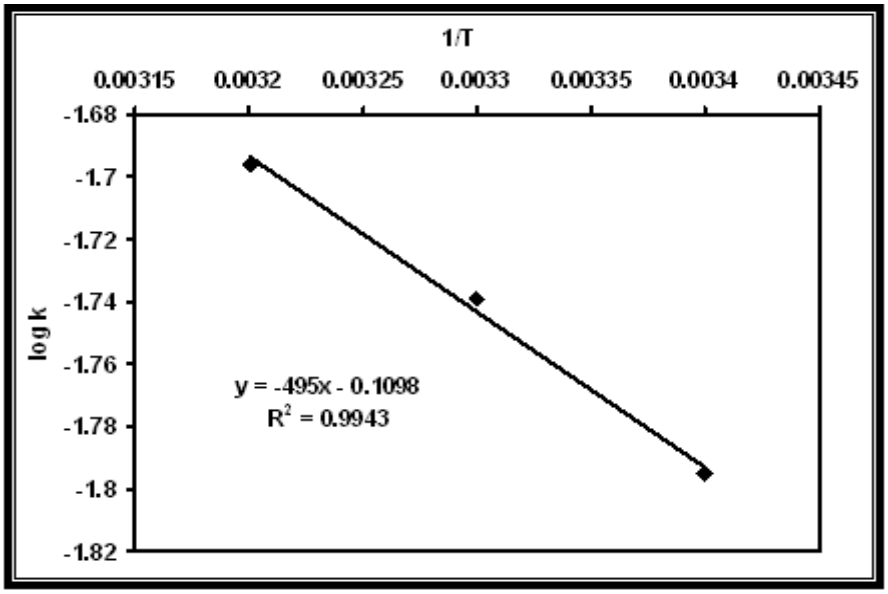

Figure 6- show the relation between log k and 1/T for albumin-amoxicillin complex

Table 5- The activation energy of albumin-drug complex

Drug-albumin complex	**Activation energy J.mol^{-1}**	**A**
Amoxicillin-albumin in phosphate buffer of pH7.4	126.77	0.0166
Amoxicillin-albumin inpH7.4and (0.1M)NaCl	28.8115	0.033
Amoxicillin-albumin in pH 7.4 and 5%ethanol at 290K	174.77	0.012
Amoxicillin-albumin in pH7.4 and10% ethanol at 290K	587.7	0.034

Conclusion:

The interaction of amoxicillin (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 amoxicillin 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 drugs 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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