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Voltammetric Trace Determination of Theophylline in Human Serum-Studies The Interaction with Albumin

Mohammed M.A. Al-Imam[1], Saddalah T. Sulaiman[2]
[1]Chemistry Department, College of Education, University of Mosul, IRAQ
[2]Chemistry Department, College of Science, University of Mosul, IRAQ

ABSTRACT :The study of Voltammetric properties of pure (theophylline) in a direct method in the aqueous solution. The substance has revealed a clear and major reduction peak at potential (-0.315) volt against the reference electrode (Ag/AgCl/Sat.KCl). The calibration curve of the theophylline in the phosphate buffer (pH=7.0) has been studied. The relationship has been lineared at the range of concentration [(0.793×10^{-6})-(3.66×10^{-6})] M with a correlation coefficient is (0.9965). The calibration curve with human serum has been studied also. The way of standard addition is used successfully to determine the drug in serum of the patients. The same way has been a cheered successfully to determine the drug in tablets and syrups. The molecular binding is also studied of theophylline with albumin and the constant binding (k) is calculate, through vant hoff equation to calculate thermodynamic variables. (ΔG, ΔS, ΔH), we conclude the binding is (Ion-Ion) type.

INTRODUCTION

Theophylline is the chemical compound with the formula $[C_7H_8N_4O_2]$[(1)] . Theophylline is a purine alkaloil and belongs to a glass of drugs known as xanthenes [(2)]. Theophylline comes as a tablets, capsule, solution, and syrup to take by mouth[(3,4,5)]. Theophylline is found naturally in plants, but most theophylline used in medicine is synthesized on industrial scale , theophylline is mainly found in black and green tea, but also in green coffee, cocoa and mate[(1)]. Theophylline has many medicinal uses. Theophylline acts as a phosphodiesterase in hibitor and relaxes smooth muscle of the airways of the lungs. Theophylline may also help the improve contraction of the diaphragm, other actions of theophylline are lowering of blood pressure, anti-inflammatory effect and chronoscopic effect[(1)]. This medication is used to treat and prevent wheezing and trouble breathing caused by ongoing lung disease such as asthma, emphysema, chronic bronchitis. This medication does not work immediately and should not be used for sudden attacks of breathing trouble [(2,3)] .

The serum theophylline concentration, varied 24 fold from (2 to 49 µg/ml). The relationship between daily dosage and serum concentration was unpredictable in an individual patient. Measurement of serum theophylline concentration disclosed subtherapeutic concentration theophylline determination appear to be important clinically in guiding effective and safe usage of theophylline[(5)]. Several methods have been described for its determination, chemiluminescent immunoassay (CLIA) is used for quantitative determination of theophylline concentration in human serum (n = 122) and evaluate the assay. The linear range of the (CLIA) method was [0.51 – 40 mg/L]. The average recovery rate was 102.3%[(6)]. Adams etal determined the theophylline in 50 samples of serum . The drug is extracted into a small value of solve at that contains an interval standard, 8-chlorotheophylline. The extract is analyzed by isocratic reversed phase chromatography, with measurement of eluted theophylline at 273 nm[(7)]. George etal use a gas – chromatographic procedure for determination of the bronchodilator, theophylline (1,3 – dimethyl xanthine), in serum and saliva. Substances that interfere in the classical determination of theophylline, such as theobromine (3,7- dimethyl xanthine) and Phenobarbital, are well separated from theophylline by the gas-chromatographic procedure and can be quantitated if desired. It is possible to determine 1 Mg of theophylline in 1 ml of serum or saliva[(8)]. Boron-dopeddiamond (BDD) electrodes were used to examine the electro chemical oxidation of Xanthire and its naturally occurring N-methyl derivatives, theophylline , theobromine and caffeine.

The effect of pH, concentration and potential sweep rate on the voltammetric response were thoroughly investigated, and it was found that BDD exhibits excellent behavior, interms of very well – defined, reproducible oxidation peaks , for Xanthine , theophylline , theobromine and caffeine determination. The results enabled the measurement of the oxidation peak current to be used as the basis for a simple, accurate and rapid method for determining the investigated compounds, within a concentration range of 1 – 400 µ M for theophylline, theobromine and caffeine, and 1-100 µ M for xanthine[(9)]. Reversed – phase , high – pressure liquid chromatography technique is used for determination of

theophylline in body fluids . The chromatography system include a mubonda pack C18 column and acetonitril , 70 m/Liter of sodium acetate buffer (10 mmd/liter , pH = 4.0) as the mobile phase. Test serum or plasma, 30 mul, is mixed with an equal quantity as a solution containing the internal standard, beta-hydroxy ethyl theophylline in acetonitrile / sodium acetate buffer (20 mmol / liter, pH = 4.0) 7/43 by vol. After the precipitate is removed by centrifugation , the mixture is chromatographed and the amount of theophylline calculated for the ratio between peak heights for theophylline and the internal standard[10]. GLC technique is used for determination of theophylline in plasma and saliva. The method selectively measures theophylline in the presence of its metabolites and other Xanthines. The GLC method is sufficiently sensitive to detect 1 µg theophylline/ml plasma or saliva . Analysis for theophylline in plasma and saliva samples obtained from patients showed a saliva – plasma ratio of approximately 0.5[11]. Enzymatic determination of theophylline in test samples. The methodology employs enzymes that utilize or recognize theophylline as a substrate to measure the concentration there of in sample, including body fluids. This new approach utilize enzymes as opposed to traditional methods which use antibodies for the recognition of theophylline[12]. This paper describe a square wave voltammetry (S.W.V) for trace determination of theophylline in serum. The interaction of theophylline with albumin was also considered.

EXPERIMENTAL

Apparatus

All experiments were performed using the [EG and G princeton (USA) mode (384 B)] computerized polarographic analyser equipped with (303 A) hanging mercury drop electrode and RE 0093 digital plotter. A three electrode systems were used.The working electrode was (HMDE) ; the reference electrode was Ag/AgCl,sat KCl electrode and the counter electrode was a Pt-wire electrode.pH measurement were made using pw 9421-Philips pH-meter. Temperature control was made by the use of haake NK 22 water thermostate (±0.1 Cº).

Reagents:

All the chemical used analytical reagents grade theophylline which was obtained from fluka, solutions of (1.0×10^{-4})M was prepared. All solutions were prepared with deionised distilled water . Bovin serum albumin (BSA) was obtained from merck, 1.0×10^{-5} M solution was freshly prepared. Phosphate buffer was prepared by mixing certain amounts of (0.2) M of each of K_2HPO_4 and KH_2PO_4. Kanaphylline syrup from Syria theophylline a tablet from samara.

Procedure :

The square wave voltammetry mode was used with deposition time 100 sec; condition time 10 sec ; equilibrium time 15 sec; frequency 120 Hz;scan rate 3 mv/sec. The voltammetric cell was thermostatic at 37 °C , the solution was de-aerated by passing through it a slow stream of purified nitrogen gas for 240 seconds to remove the dissolved oxygen. The square wave voltamogram was recorded on a degassed phosphate buffer solution at (pH=7.0) (5 ml).The back current was recorded, appropriate amount of theophylline stock solution were added to this solution to yield the desired concentration and the current – voltage current was recorded again. The calibration curve was then constructed . To study the binding of theophylline with albumin, the voltammetric measurement was performed on (5 ml) phosphate buffer at (pH = 7.0), containing (0.7936×10^{-5}) M of theophylline, the square wave voltamogram was recorded then successive amount of (10^{-5})M of albumin was added to the cell and the square wave voltamogram were recorded, at different temperature in the range (293-315)K° in order to calculate binding constant (k), and the thermodynamic quantities (ΔG, ΔS and ΔH).

RESULT AND DISCUSSION

Typical square wave voltamogram of (0.99×10^{-6}) M theophylline in phosphate buffer at (pH=7.0). Is shown in fig. (2).

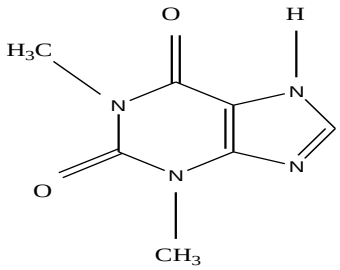

Fig (1) : Chemical structure of theophylline

M. wt = 180.2 gm/mole
m.p = (270 – 274) ˚C

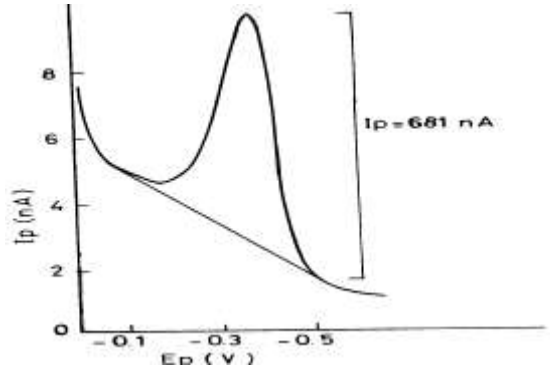

Fig (2) : Square wave voltamogram of ($0.99x10^{-6}$) M theophylline.

It can be seen from Fig 2 a well-defined s.w.v peak appeared at (-0.312V) versus (Ag/AgCl,Sat KCl) electrode was obtained.

Optimum condition :

The SWV voltammogram of ($1.996x10^{-6}$) M of theophylline was investigated in phosphate buffer (pH=7) variation all the parameter that it depend on the measurement the optimum value obtained are tabulated in Table (1).

Table (1) : Show the optimum values obtained which give either the highest peak current or the best resolution of the peak .

Condition	Value	Condition	Value
initial pot.	0.0 V	frequency	120 Hz
final pot.	- 0.6 V	scan increament	2 mV/sec
deposition time	100 second	cond. potential	1 mV
condition time	10 second	pulse height	0.06 mV
equilibrium time	10 second		

Effect of pH :

The square wave voltamogram of ($1.380x10^{-5}$) M of theophylline were investigated at different pH values (3-9) using the optimum condition in phosphate buffer show in Table(1).The peak current (Ip) and peak potential (Ep) obtained is shown in Table (2).

Table (2): Effect of pH on SWV peak and peak current of ($1.380x10^{-5}$) M theophylline.

pH	Ep (v)	Ip (nA)
3	- 0.084	342.1
4	- 0.135	208.1
5	- 0.195	387.7
6	- 0.255	342.6
7	- 0.316	487.0
8	- 0.370	355.8
9	- 0.432	103.1
	R	- 0.9997
	R^2	0.9995
	Slope	- 0.0584
	Intercept	0.0951

The peak current (Ip) is clearly dependent the pH. maximum current response were found at (pH=7.0). Which is chosen for the present study, on the other hand the peak potential (Ep) is found to be greatly dependent on pH and moves to more negative with increasing the pH values. Linear plot of Ep versus pH were obtained (fig 3). With slopes (-0.0584 VpH^{-1}). The value of correlation coeff. (R= - 0.9997) which it very near to theoretical value obtained (0.059).

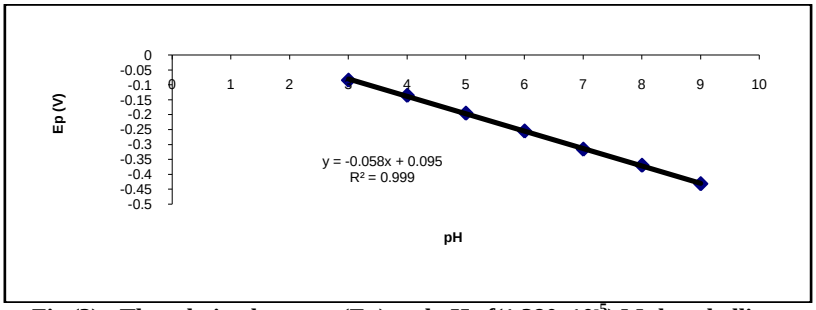

Fig (3) : The relation between (Ep) and pH of(1.380×10^{-5}) M theophylline.

Stability of theophylline in aqueous Phosphate buffer at (pH=7.0):

The square wave voltamogram of (2.343×10^{-6})M of theophylline were recorded at different time in phosphate buffer at (pH = 7). The result obtained is tabulated in Table (3). It can be seen from the Table (3) that theophylline is stable for more than (60) minute. Which is quite enough for voltammetric measurement.

Table (3): Effect of time on S.W.V. peak of (2.343×10^{-6}) M of theophylline at (pH = 7.0) in aqueous solution.

Time (min)	Ip (nA)
16	**400.3**
20	**412.0**
24	**416.0**
28	**422.0**
32	**426.0**
36	**428.0**
40	**429.0**
44	**429.0**
48	**436.0**
52	**441.0**
56	**444.0**
60	**446.0**

Analytical Consideration:

Using The optimum condition showing in Table (1), the calibration curve were constructed using a serial dilution of a standard theophylline in aqueous-phosphate buffer (pH=7.0) (5ml).Some typical result are listed in Table (4) .These solutions were prepared by adding appropriate aliquots of standard theophylline to the phosphate buffer (5 ml) at (pH=7.0).

Table (4): Effect of concentration on peak current of (7.9365×10^{-7} - 36.608×10^{-7}) M of theophylline at (pH=7.0) in aqueous solution at (Ep= - 0.312 V).

Addition × 10^{-6} M	Ip (nA)
0.793	**55.4**
0.990	**111.6**
1.185	**157.0**
1.380	**202.0**
1.574	**264.0**
1.768	**309.4**
1.960	**358.0**
2.152	**393.4**
2.343	**434.0**
2.534	**471.0**

2.912	**543.0**
3.288	**620.0**
3.660	**681.0**

From the table (4) we can see the value of (Ip) is increase with increasing concentration of theophylline. The drawing the correlation between diffusion current (Ip) with concentration we get a straight line with (R = 0.9965) as explain in fig (4) .

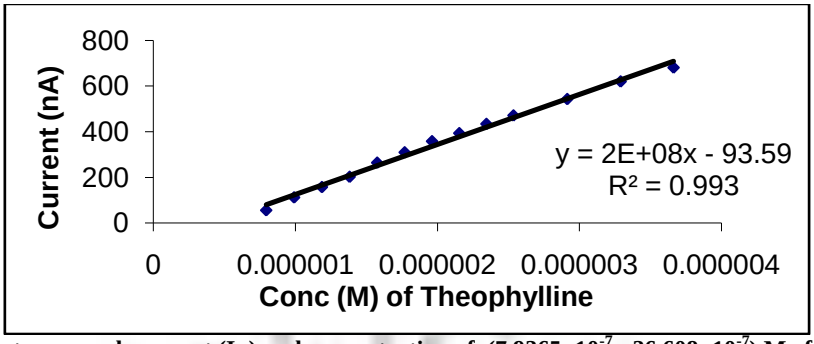

Fig (4) : the relation between peak current (Ip) and concentration of (7.9365×10^{-7} - 36.608×10^{-7}) M of theophylline at (pH = 7.0) phosphate buffer in aqueous solution

The plot peak current (Ip) of versus molar conc. Of theophylline is showing in fig.(4). Regression analysis on standard indicated a straight line. The lowest experimental detection limit was (0.793×10^{-6}).
Effect of concentration (calibration curve of theophylline) with human serum . Using The optimum condition showing in Table (1), the calibration curve were constructed using a serial dilution of a standard theophylline in human serum. Some typical results are listed in Table (5).These solutions were prepared by adding appropriate aliquots of standard theophylline to the phosphate buffer (5 ml) at (pH=7.0).

Table (5): Effect of concentration on peak current of (5.9×10^{-6} - 27.1×10^{-6}) M of theophylline at (pH=7.0) in human serum at (Ep= - 0.304 V).

Conc (M) × 10^{-6}	Ip (nA)
5.90	**170.8**
7.29	**262.8**
9.88	**384.0**
11.83	**556.0**
13.77	**664.0**
15.71	**877.0**
17.64	**984.0**
19.56	**1140.0**
21.14	**1273.0**
23.39	**1418.0**
27.18	**1620.0**

From the table (5) we can see the value of (Ip) is increase with increasing concentration of theophylline. The drawing of the correlation between diffusion current (Ip) with concentration we get a straight line with (R = 0.9972) as explain in fig (5).

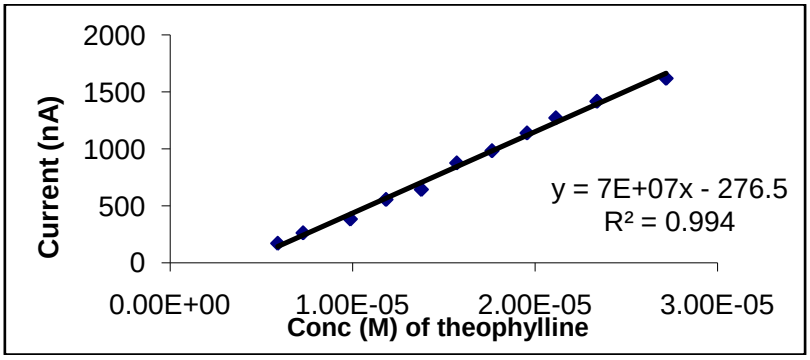

Fig (5) : the relation between peak current (Ip) and concentration of (5.9×10^{-6} -27.1×10^{-6}) M of theophylline at (pH= 7.0) phosphate buffer in human serum .

Voltammetric Behaviour of theophylline in the presence of Albumin:

The square wave voltamogram of (0.7936×10^{-5}) M theophylline was recorded in 5 ml phosphate buffer at (pH=7). then successive amount (10^{-5}) M albumin was added, the square wave voltamogram was recorded after each addition of albumin using the optimum condition in Table (6). The peak current Ip_1, were found to decrease gradually during the addition of albumin which indicate the binding of theophylline with albumin the result are shown in Table (7) at (293 K°). The plot of Ip_1/Ip_o, versus the conc. of albumin added are showing in fig (6) equation of 2nd order given a best fitting curve from which the binding constant (k) was calculated (tangent of the curve).

The effect of temperature on the binding of theophylline with albumin :

The square wave voltamogram of (0.7936×10^{-5}) M theophylline was recorded in phosphate buffer at (pH=7) at different temp. (298,303,310,315) Ip_o was optained at each temperature. The square wave voltamogram was also recorded with successive addition of albumin, the decrease of peak current Ip_1, Ip_o where followed and tabulated in Tables (7-10). For the four temperature respectively all the tables show the gradual decrease of peak current Ip with the additional albumin and a constant value of Ip/Ip_o obtained and the end of additions . From the plot of Ip_1/Ip_o, versus the conc of albumin, (k) where calculated and shown in Table (11). The vant hoff plot of log K versus 1/T gives a straight line as shown in fig (7).

Ip_o : Diffusion current of peak theophylline alone ; Ip_1: Diffusion current of peak theophylline with albumin .

Table (6) : The optimum condition of binding theophylline with albumin

Condition	Value	Condition	Value
Initial pot.	- 0.1 V	Condition time	5 Second
Final pot.	- 0.85 V	Equilibrium time	5 Sec
Deposition time	60 Second	Frequency	120 Hz

Table (7) : The peak current of (0.7936×10^{-5})M theophylline in the presence of albumin nearest ($1.976 \times10^{-5} - 21.317\times10^{-5}$)] M in phosphate buffer (pH=7.0) at 298 K°.

Conc of Albumin	Ip_1 (nA)	Ip_1/ Ip_0
0	227	**1.0000**
1.97628×10^{-5}	179	**0.7910**
3.94477×10^{-5}	174	**0.7672**
5.90551×10^{-5}	172	**0.7584**
7.85855×10^{-5}	165	**0.7275**
9.80392×10^{-5}	149	**1.6570**
11.7414×10^{-5}	150	**1.6614**
13.6719×10^{-5}	152	**0.6702**
17.5097×10^{-5}	155	**0.6834**
19.4175×10^{-5}	153	**0.6746**
21.3178×10^{-5}	154	**0.6790**

Table (8) : The peak current of (0.7936 × 10^{-5})M theophylline in the presence of albumin nearest (1.976×10^{-5} – 19.417×10^{-5}) M in phosphate buffer (pH=7.0) at 303 K°.

Conc of Albumin	Ip_1 (nA)	Ip_1/ Ip_0
0	306	1.0000
1.97628×10^{-5}	273	**0.8906**
×10^{-5}**2.9615**	253	**0.8260**
3.94477×10^{-5}	236	**0.7698**
4.92611×10^{-5}	234	**0.7627**
5.90551×10^{-5}	218	**0.7127**
7.85855×10^{-5}	**216**	**0.7052**
9.80392×10^{-5}	212	**0.6921**
11.7415×10^{-5}	212	**0.6921**
13.6719×10^{-5}	210	**0.6856**
15.5945×10^{-5}	208	**0.6791**
17.5097×10^{-5}	**214**	**0.6987**
19.4175×10^{-5}	**215**	**0.7019**

Table (9) : The peak current of (0.7936 × 10-5)M theophylline in the presence of albumin nearest (1.97×10^{-5}–17.50×10^{-5}) M in phosphate buffer (pH=7.0) at 310 K°.

Conc of Albumin	Ip_1 (nA)	Ip_1/ Ip_0
0	310	1.0000
1.97628×10^{-5}	208	**0.6718**
2.9615×10^{-5}	194	**0.6276**
3.94477×10^{-5}	186	**0.6008**
4.92611×10^{-5}	134	**0.4315**
5.90551×10^{-5}	127	**1.4102**
6.88299×10^{-5}	106	**0.3424**
7.85855×10^{-5}	95	**0.3068**
9.80392×10^{-5}	90	**0.2909**
11.7414×10^{-5}	93	**0.3004**
13.6719×10^{-5}	95	**0.3068**
15.5945×10^{-5}	**94**	**0.3036**
17.5097×10^{-5}	**95**	**0.3068**

Table (10) : The peak current of (0.7936 × 10^{-5})M theophylline in the presence of albumin nearest (0.989×10^{-5} – 11.741×0^{-5}) M in phosphate buffer (pH=7.0) at 315 K°.

Conc of Albumin	Ip_1 (nA)	Ip_1/ Ip_0
0	245	1.0000
0.98912×10^{-5}	149	**0.6061**
1.97628×10^{-5}	139	**0.5686**
2.9615×10^{-5}	130	**0.5318**
3.94477×10^{-5}	98.3	**0.4012**
4.92611×10^{-5}	96.9	**0.3955**
5.90551×10^{-5}	90	**0.3676**
6.88299×10^{-5}	66.6	**0.2718**
7.85855×10^{-5}	63	**0.2571**
8.83219×10^{-5}	51.7	**0.2110**
9.80392×10^{-5}	50	**0.2041**
11.7417×10^{-5}	**48**	**0.1959**

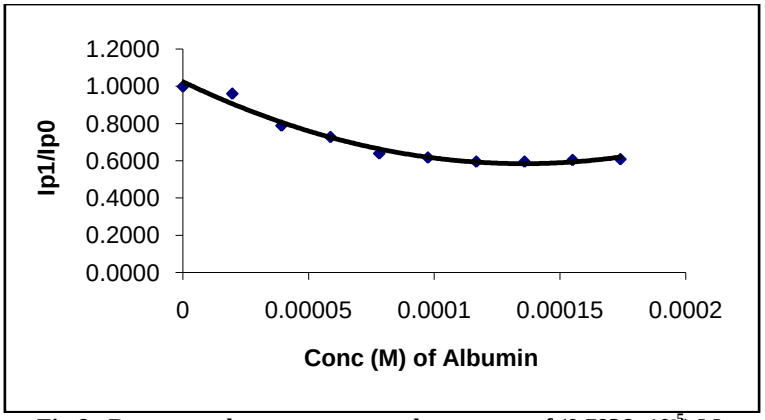

Fig 6 : Represent the square wave voltamogram of (0.7936×10^{-5}) M.

Theophylline in the presence of (10^{-5}) M albumin which shows the gradual decrease of I_p with albumin addition in phosphate buffer (pH=7). The binding constant of the interaction between theophylline and albumin at different temperature as shown in Table (12).

Table (11): The binding constant of the interaction between theophylline with albumin at different temperature .

Temp. (K) ˚	1/T	K	Log K
298	**0.00335**	**0.6674**	- 0.175613
303	**0.00330**	**0.6639**	- 0.177897
310	**0.00322**	**0.2443**	- 0.612076
315	**0.00317**	**0.1993**	- 0.700492

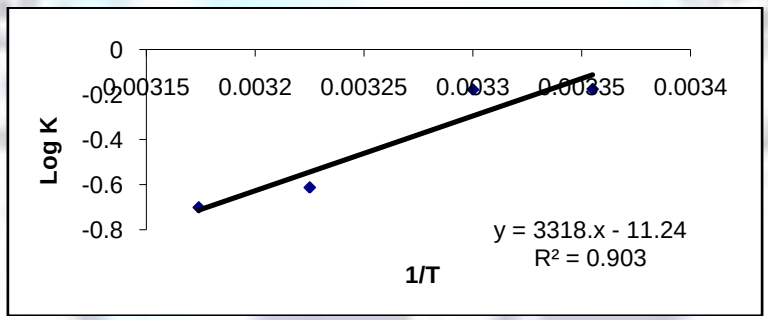

Fig 7 : The relation between Log constant binding (K) against 1/T .

From the result in the Table (11) we can calculate the thermodynamic quantities ΔG, ΔH and ΔS for interaction of theophylline with albumin which was shown in table (12), as follow .

Slope = – ΔH/2.303R

ΔH = – Slope × R × 2.303

ΔG = – 2.303 RT Log K

ΔG = ΔH – T ΔS

Table (12) : The thermodynamic quantities for interaction of theophylline with albumin.

Temp. (K) ˚	ΔH (KJ/mole)	ΔG (KJ/mole)	ΔS (KJ/mole.K)
298	**- 63.7657**	**1.0020**	**- 0.2173**
303	=	**1.0320**	**- 0.2138**
310	=	**3.6330**	**- 0.2174**
315	=	**4.2249**	**- 0.2158**

The low and positive value of ΔG indicate that the interaction of theophylline with albumin are of the type ion – ion. The negative value of ΔH indicate that the interaction process proceed exothermic process during the interaction. The negative values of ΔS indicate that the more ordered complex during the interaction of theophylline with albumin.

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