International Journal of Enhanced Research in Science Technology & Engineering, ISSN: 2319-7463
Vol. 3 Issue 4, April-2014, pp: (68-76), Impact Factor: 1.252, Available online at: www.erpublications.com

Voltammetric trace determination of betamethasone in human serum and urine-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 (betamethasone) in a direct method in the aqueous solution. The substance has revealed a clear and major two reduction peaks at potential (-1.136) volt and (-1.455) volt against the reference electrode (Ag/AgCl/SatKC). The calibration curve of the Betamethasone in the phosphate buffer (pH=7) has been studied. The relationship has been linear within the scope of concentration) for one and two peaks. Also the 05), (0.9969] molar. The correlation coefficient is (0.99$(10^{-7}\times1.99)$-$(10^{-6}\times6.01)$**[calibration curve has studied with human serum and urine. The way of standard addition is used successfully to determine the drug in serum and urine of the patients. The way has been a cheered successfully to determine the drug in tablets. Also the study of molecular binding of betamethasone with albumin and calculate the constant binding (k), through vant hoff equation to calculate thermodynamic variables. (ΔG,ΔS,ΔH), we conclude the binding of (Ion-Ion) type .**

INTRODUCTION

Betamethasone is the chemical compound with the formula $[C_{22}H_{29}FO_5]$. Betamethasone is a moderately potent glucocorticoid steroid with anti- inflammatory -org and immunosuppressive properties. Unlike other drugs with these effects, betamethasone does not cause water retention. It is applied as a topical cream, ointment foam, lotion or gel to treat itching. Betamethasone sodium phosphate is sometimes prescribed as an intramuscular injection (I.M) for itching from various elements including allergic reactions to poison ivy and similar plants [(1)]. Betamethasone dipropionate is a synthetic gluco corticoid that is used topically on the skin. The naturally-occurring gluco corticoid is cortisolor hydrocortisone which is produced by the adrenal Gland .Glucocorticoids have potent anti-inflammatory actions and also suppresser the immune response[(2)]. Betamethasone is used to treat the crusting, scaling, inflammation, and discomfort of various skin condition [(3)]. The suppression of inflammation and the immune response caused by glucocorticoid [(4)]. Excess of the drug also allows infection to occur more easily, betamethasone can use in patients with pegronies disease[(5)]. Other uses of this drug to treat a variety of skin, conditions such as eczema, dermatitis, allergies and rash can be reduced the swelling, redness that can occur in these types of conditions . This medication is a medium-strength corticosteroid [(6)]. Betamethasone solution is used for treating arthritis , asthma and skin conditions [(7)]. Several methods have been described for its determination , a sensitive and rapid extraction – spectrophoto metric method for the determination of betamethasone , based on the formation of a charge transfer complex with benzocaprol red (reagent I) and acid ethyl blue (reagent II) is described. The calibration graph of the absorbance of the chloroform and benzene extracts (10ml) at 588 and 677 nm using (reagent I) and (reagent II), respectively, is linear over the range (0-16) and (0-20) µg ml^{-1} of betamethasone with relative standard deviations (R.S.D.) of 1.6 and 1.3 % for 5 µg ml^{-1} [(8)]. A new separation method based on a reversed-phase sequential injection chromatography technique for simultaneous determination of pharmaceutic of eye drops, with detection limits from the range

(0.5–1.0 ng/ml)[(9)]. A stability–indicating high-performance liquid chromatographic method with on-line cleam-up has been developed for the analysis of betamethasone [(10)]. The electrochemical reduction of betamethasone valerate (BV) in a pharmaceutical formulation containing neomycin has been carried out in britton robenson buffer (BRB) (0.04 $moll^{-1}$) by differential – pulse polarography (DPP). BV exhibits a well- defined irreversible reduction peak at -1.03 V . The influence of pH on the reduction of BV was studied in (BRB) (pH range = 1.7-10). A method for the analysis of BV in (BRB) (0.04 $moll^{-1}$) , which allows quantification over the range $(3.9\times10^{-6} - 1.1\times10^{-4})moll^{-1}$, was proposed and successfully applied to the determination of BV in tablets with mean recovery and relative standard deviation of 100.81 % and 0.45 %, respectively [(11)]. The present work involved the use of square wave voltammetric method for trace determination of betamethasone and studying the molecular interaction of betamethasone with albumin.

EXPERIMENTAL

Apparatus:

All experiments were performed using the (EG and G princeton (USA) mode (384 B) computerized polarographic analyses 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 measurements were made using pw 9421-Philips pH-meter. Temperature control was made by the use of haake NK 22 water thermostate (±0.1 C).

Reagents:

All the chemical used analytical reagents grade betamethasone 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, 0.1 % 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.

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 10 minutes 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 betamethasone 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 betamethasone with albumin , the voltammetric measurement was performed on (5 ml) phosphate buffer at (pH = 7.0), containing (1.96×10^{-6}) M of betamethasone, the square wave voltamogram was recorded then successive amount of (10^{-6})M of albumin was added to the cell and the square wave voltamogram were recorded, at different temperature in the range (290-315)K˚ in order to calculate binding constant (k), and the thermodynamic quantities ΔG, ΔS and ΔH.

RESULT AND DISCUSSION

Typical square wave voltamogram of ($7.936\text{x}10^{-6}$) M betamethasone in phosphate buffer at (pH=7.0), as shown in fig. (2).

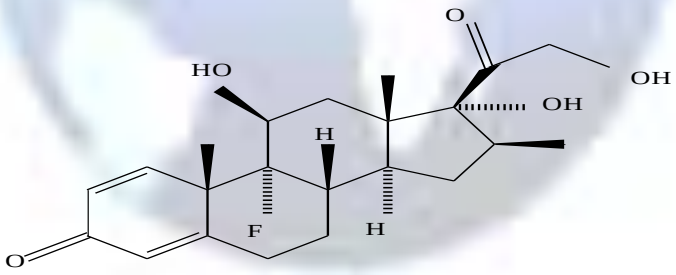

Fig (1) : Chemical structure of betamethasone
M. wt = 392.5 gm/mole
m.p = 240 ˚C

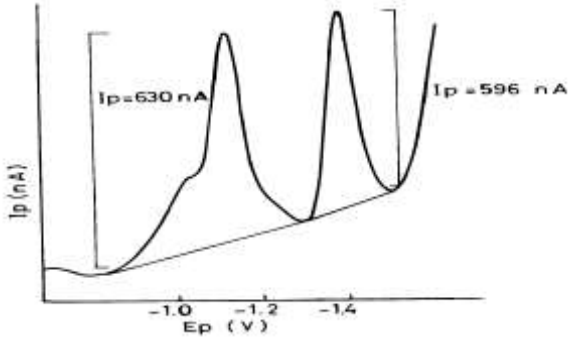

Fig (2) : Square wave voltamogram of ($7.936\text{x}10^{-6}$) M betamethasone.

It can be seen from Fig 2 a well-defined two peak appeared at (-1.136 V) and (-1.455 V) versus (Ag/AgCl,Sat KCl) electrode.

Optimum condition :

The SWV voltammogram of ($9.9x10^{-6}$) M of betamethasone was investigated in phosphate buffer (pH=7) variation all the parameter that it depend on the measure-ment 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.	- 0.7 V	frequency	120 Hz
final pot.	- 1.65 V	scan increament	3 mV/sec
deposition time	100 second	cond. potential	0.002 V
condition time	10 second	pulse height	0.04 mV
equilibrium time	15 second		

Effect of pH :

The square wave voltamogram of ($9.9x10^{-6}$) M of betamethasone were investigated at different pH values (3-10) using the optimum condition in phosphate buffer show in Table (1).The two peak current (Ip_1),(Ip_2) and peak potential (Ep_1),(Ep_2) obtained are shown in Table (2).

Table (2) : Effect of pH on ak and peak current of ($9.9x10^{-6}$) M Betamethasone

pH	Ep_1 (v)	Ip_1 (nA)	Ep_2 (v)	Ip_2 (nA)
3	- 0.908	147.9	.....	.....
4	-0.980	84.0	.....	.....
5	-1.020	444.0	- 1.408	213.0
6	-1.084	633	- 1.440	622
7	- 1.136	672	- 1.455	688
8	-1.192	504	- 1.480	522
9	- 1.256	462	- 1.516	221
10	- 1.312	442	- 1.524	63.5
	R	- 0.9992		- 0.9907
	R^2	0.9984		0.9815
	Slope	- 0.0568		- 0.0238
	Intercept	- 0.7414		- 1.292

The peak current (Ip_1) and (Ip_2) are clearly dependent the pH. maximum current response were found at (pH=7.0). On the other hand the peak potential (Ep_1) and (Ep_2) are 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 (-0.0568 VpH^{-1}) for first peak and (-0.0238 VpH^{-1}) for the second peak . The value of correlation coefficient (R_1 = 0.9992) and (R_2 = -0.9907) respectively which it very near to theoretical value obtained by (Hammett)[12].

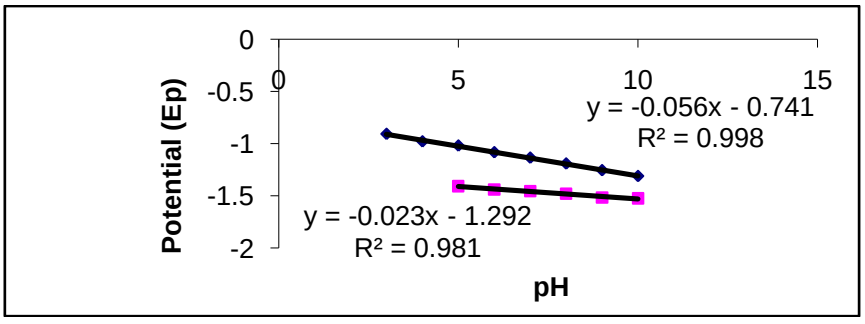

Fig (3) : The relation between (Ep_1 , Ep_2) and pH of($9.9x10^{-6}$) M Betamethasone

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

The square wave voltamogram of ($9.9x10^{-6}$)M of betamethasone were recorded at different time in phosphate buffer at (pH = 7.0). The result obtained are tabulated in table (3) . It can be seen from the Table (3) that betamethasone is stable for more than 40 minute. Which is quite enough for voltammetric measurement .

Table (3) : Effect of time on SWV peak of ($9.9x10^{-6}$) M of Betamethasone at (pH=7.0) in aqueous solution.

Time (min)	Ip_1 (nA)	Ip_2 (nA)
4	683	389.8
8	697	402.3
12	691	396.4
16	711	398.4
20	723	400.4
24	720	400.0
28	775	386.9
32	737	408.9
36	737	403.8
40	751	419.0

Analytical Consideration:

Using The optimum condition showing in (Table 1), the calibration curve were constructed using a serial dilution of a standard betamethasone 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 betamethasone to the phosphate buffer (5 ml) at (pH=7.0).

Table (4) : Effect of concentration on peak current of [(1.99×10^{-7})–(6.01×10^{-6})] M of betamethasone at (pH=7.0) in aqueous solution at (Ep_1= -1.136 V) and (Ep_2 = -1.455 V).

Conc. (M) 10^{-7}	Ip_1 (nA)	Ip_2 (nA)
1.99	293.9	...
3.98	342.8	...
7.93	501.0	156.8
11.85	609.0	349.9
15.74	697.0	510.0
19.60	825.0	650.0
23.43	949.0	770.0
27.23	989.0	831.0
31.00	1253.0	1005.0
34.74	1405.0	1080.0
38.46	1476.0	1080.0
42.14	1576.0	1190.0
45.80	1675.0	1280.0
49.42	1795.0	1376.0
53.03	1850.0	1630.0
60.15	...	1850.0

From the table (4) we can see the value of (Ip_1) and (Ip_2) are increase with increasing concentration of betamethasone .The drawing the correlation between diffusion current (Ip_1 , Ip_2) with concentration we get two straight line with (R_1=0.9969) for first peak and (R_2 = 0.9905) for second peak as explain in fig. (4).

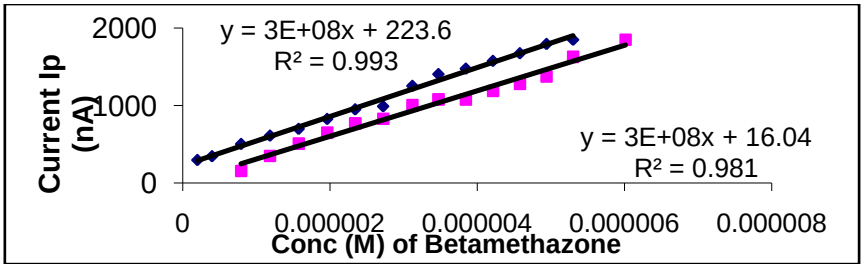

Fig (4) : the relation between two peak current (Ip_1 , Ip_2) and concentration of [(1.99×10^{-7}) – (6.01×10^{-6})] M Betamethasone at (pH = 7.0) phosphate buffer in aqueous solution

The plot two peak current (Ip_1,Ip_2) of versus molar conc. of betamethasone are showing in fig. (4). Regression analysis on standard indicated two straight line. The lowest experimental detection limit was (1.99×10^{-7}).

Effect of concentration (calibration curve of betamethasone) with human serum.

Using The optimum condition showing in Table(1), the calibration curve were constructed using a serial dilution of a standard betamethasone in human serum. Some typical result are listed in Table (5). These solutions were prepared by adding appropriate aliquots of standard betamethasone to the phosphate buffer (5 ml) at (pH=7).

Table (5) :Effect of concentration on peak current of[(1.992×10^{-6})–(19.569×10^{-6})] M of betamethasone at (pH=7.0) in human serum at (Ep1= -1.332 V) .

Conc. (M) 10^{-6}	Ip_1 (nA)
1.992	146
3.976	208
5.952	273
7.92	328
9.881	396
11.857	449
13.806	515
15.717	575
17.647	645
19.569	707
R	0.9997
R^2	0.9994
Slope	31731490.7
Intercept	80.5289

From the Table (5) we can see the value of (Ip_1) is increase with increasing concentration of betamethasone .The drawing the correlation between diffusion current (Ip_1) with concentration we get a straight line with (R_1 = 0.9997) as explain in fig (5).

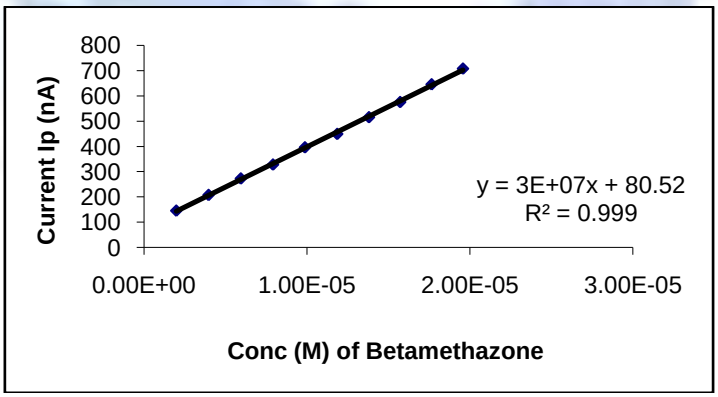

Fig (5) : the relation between peak current (Ip_1) and concentration of [(1.992×10^{-6}) – (19.569×10^{-6})] M betamethasone at (pH = 7.0) phosphate buffer in human serum .

Effect of concentration (calibration curve of betamethasone) with human urine .

Using The optimum condition showing in Table (1), the calibration curve were constructed using a serial dilution of a standard betamethasone in human urine. Some typical result are listed in Table (6) These solutions were prepared by adding appropriate aliquots of standard betamethasone to the phosphate buffer (5 ml) at (pH=7).

Table (6) :Effect of concentration on peak current of[(1.992×10^{-6})–(19.569×10^{-6})] M of betamethasone at (pH=7.0) in human urine at (Ep1= -1.162 V) and (Ep_2 = -1.450 V).

Conc. (M) 10^{-6}	Ip_1 (nA)	Ip_2 (nA)
1.992	176.5	246.4
3.976	390.5	565.0
5.952	590.0	865.0
7.920	815.0	1195.0
9.881	1014.0	1485.0
11.857	1218.0	1789.0
13.806	1443.0	2110.0
15.717	1653.0	...
17.647	1855.2	...
19.569	2056.0	...
R	0.9999	0.9999
R^2	0.9998	0.9998
Slope	1.07×10^8	1.57×10^8
Intercept	-42.1699	-62.9892

From the Table (6) we can see the value of (Ip_1) and (Ip_2) are increase with increasing concentration of betamethasone .The drawing the correlation between diffusion current (Ip_1 , Ip_2) with concentration we get two straight line with (R_1 = 0.9999) for first peak and (R_2 = 0.9999) for second peak as explain in fig (6).

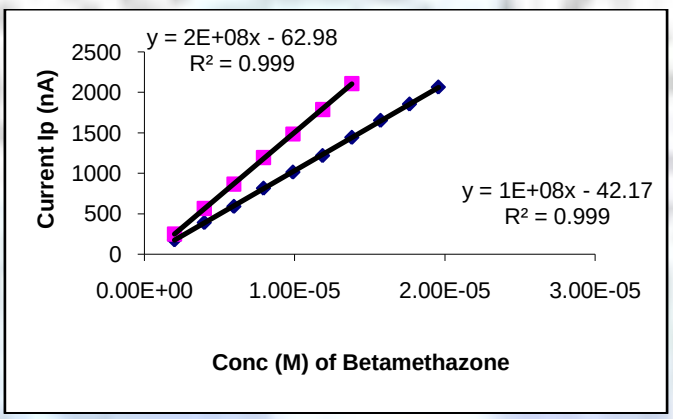

Fig (6) : the relation between two peak current (Ip_1 , Ip_2) and concentration of [(1.992×10^{-6}) – (19.569×10^{-6})] M Betamethasone at (pH = 7.0) phosphate buffer in human urine

Voltammetric Behaviour of betamethasone in the presence of Albumin :

The square wave voltamogram of (1.96×10^{-6}) M betamethasone was recorded in 5 ml phosphate buffer at (pH=7). then successive amounted (10^{-6}) M albumin was added, the square wave voltamogram was recorded after each addition of albumin using the optimum condition in Table (7). The peak current Ip_1, Ip_2 were found to decrease gradually during the addition albumin elute binding of betamethasone with albumin the result are shown in table (8) at (290 K°). The plot of Ip_1/Ip_o , Ip_3/Ip_2* versus the conc. Of albumin added are showing in fig. (7) equation of 2nd order given a best fitting curve from which the binding constant (k) was calculated.

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

The square wave voltamogram of (1.96×10^{-6}) M betamethasone was recorded in phosphate buffer at (pH=7.0) at different temp. (290,300,305,310,315) 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_3 where followed and tabulated in Table (8). respectively from the plot of Ip_1/Ip_o, Ip_3/Ip_2 versus the conc. of albumin, (k) where calculated and shown in Table (9). The vant- hoff plot of log k versus 1/T gives a straight line as shown in fig.(7). * Ip_o : Diffusion current of first peak betamethasone alone .Ip_1: Diffusion current of first peak betamethasone with albumin .Ip_2 : Diffusion current of second peak betamethasone alone . Ip_3 : Diffusion current of second peak betamethasone with albumin .

Table (7) : The optimum condition of binding betamethasone with albumin .

Conditions	Value
Deposition time	60 Second
Condition time	5 Second

Equilibrium time	5 Second
Frequency	120 Hz
Scan increment	2 mV/second
Condition potential	0.000 V
Pulse height	0.02 mV

Table (8) : The peak current of (3.846×10^{-6})M betamethasone in the presence of albumin nearest [(0.195×10^{-8}) – (2.6717×10^{-8})] M in phosphate buffer (pH=7.0) at 290 K°.

Albumin conc. × 10^{-8}	Ip_1(nA)	Ip_1/ Ip_0	Ip_3(nA)	Ip_3 / Ip_2
0.0	461	1.0000	599	1.0000
0.1956	398	0.8629	420	0.7012
0.5847	360	0.7811	390	0.6511
0.9708	340	0.7368	305	0.5092
1.5444	319	0.6926	214	0.3573
2.1112	301	0.6536	200	0.3339
2.6717	289	0.6269	190	0.3172
3.2321			195	0.3255

From the result in Table (8) , the peak current Ip_1, Ip_3 were found to decrease gradually with the present of (10^{-6}) M of albumin .

Table (9) : The peak current of (3.846×10^{-6})M betamethasone in the presence of albumin nearest [(9.794×10^{-6}) – (97.087×10^{-6})] M in phosphate buffer (pH=7.0) at 300 K°.

Albumin conc. × 10^{-6}	Ip_1(nA)	Ip_1/ Ip_0	Ip_3(nA)	Ip_3 / Ip_2
0.0	625	1.0000	873	1.0000
9.7943	548	0.8768	681	0.7801
19.5695	504	0.8064	590	0.6758
29.3255	482	0.7712	449	0.5143
39.0625	398	0.6360	214	0.2451
48.7805	395	0.6326	197	0.2257
58.4795	356	0.5688	102	0.1168
68.1597	323	0.5166	95	0.1088
77.821	300	0.4797	96	0.1100
87.4636	275	0.4402		
97.0874	247	0.3944		

From the result in Table (9) , the peak current Ip_1, Ip_3 were found to decrease gradually with the present of (10^{-6}) M of albumin .

Table (10) : The peak current of (3.846×10^{-6})M betamethasone in the presence of albumin nearest [(9.794×10^{-6}) – (97.087×10^{-6})] M in phosphate buffer (pH=7.0) at 305 K°.

Albumin conc. × 10^{-6}	Ip_1(nA)	Ip_1/ Ip_0	Ip_3(nA)	Ip_3 / Ip_2
0.0	745	1.0000	997	1.0000
9.794	619	0.8309	900	0.9027
19.569	542	0.7275	820	0.8225
29.325	491	0.6591	730	0.7322
39.062	407	0.5463	580	0.5817
48.780	366	0.4913	500	0.5015
58.479	302	0.4052	490	0.4915
68.159	260	0.3483	495	0.4965

77.821	220	0.2950	495	0.4965
87.463	185	0.2483		
97.087	165	0.2217		

From the result in Table (10), the peak current Ip_1, Ip_3 were found to decrease gradually with the present of (10^{-6}) M of albumin.

Table (11) : The peak current of (3.846×10^{-6})M betamethasone in the presence of albumin nearest [(9.794×10^{-6}) – (87.463×10^{-6})] M in phosphate buffer (pH=7.0) at 310 K°.

Albumin conc. × 10^{-6}	Ip_1(nA)	Ip_1/ Ip_0	Ip_3 (nA)	Ip_3 / Ip_2
0.0	812	1.0000	1140	1.0000
9.794	686	0.8448	789	0.6933
19.569	591	0.7278	568	0.4911
29.325	484	0.5961	290	0.2548
39.062	418	0.5148	140	0.1230
58.479	279	0.3436	126	0.1107
68.159	211	0.2595	115	0.1011
77.821	166	0.2044	116	0.1019
87.463	130	0.1606		

From the result in Table (11), the peak current Ip_1, Ip_3 were found to decrease gradually with the present of (10^{-6}) M of albumin .

Table (12) : The peak current of (3.846×10^{-6})M betamethasone in the presence of albumin nearest [(9.794×10^{-6}) – (87.463×10^{-6})] M in phosphate buffer (pH=7.0) at 315 K°.

Albumin conc. × 10^{-6}	Ip_1(nA)	Ip_1/ Ip_0	Ip_3 (nA)	Ip_3 / Ip_2
0.0	743	1.0000	1330	1.0000
9.794	612	0.8237	1220	0.9152
19.569	560	0.7537	1110	0.8252
29.3255	355	0.4773	995	0.7464
39.062	293	0.3941	850	0.6377
48.780	242	0.3260	830	0.6227
58.479	128	0.1723	820	0.6152
68.159	109	0.1464	825	0.6189
77.812	83	0.1117	820	0.6152
87.463	80.9	0.1089		

From the result in Table (12), the peak current Ip_1, Ip_3 were found to decrease gradually with the present of (10^{-6}) M of albumin.

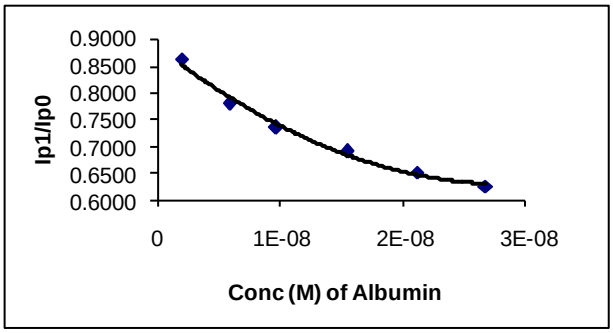

Fig 7 : Represent the square wave voltamogram of (3.846×10^{-6}) M betamethasone in the presence of (10^{-6}) M albumin which shows the gradual decrease of I_p with albumin addition in phosphate buffer (pH=7) .

The binding constant of the interaction between betamethasone and albumin at different temperature as shown in Table (11).

Table (13) : The binding constant of the interaction between betamethasone with albumin at different temperature.

Temp.(T) K°	1/T	K_1	Log K_1	K_2	Log K_2
290	0.00345	0.6341	- 0.197842	0.2980	- 0.525783
300	0.00333	0.3461	- 0.460798	0.0867	- 1.061980
305	0.00328	0.1853	- 0.732124	0.4731	- 0.325047
310	0.00323	0.0913	- 1.039529	0.0786	- 1.104577
315	0.00317	0.0929	- 1.031984	0.6103	- 0.214456

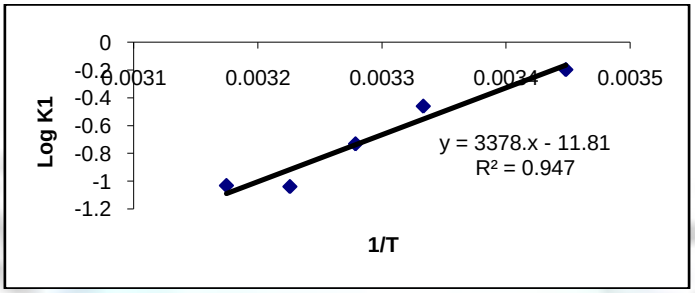

Fig 8 : The relation between Log constant binding (K) against 1/T .

From the result in the table (13) we can calculate the thermodynamic quantities ΔG , ΔH and ΔS for interaction of betamethasone with albumin which was shown in table (14) .

Slope = – ΔH/2.303R

ΔH = – Slope × R × 2.303

ΔG = – 2.303 RT Log K

ΔG = ΔH – T ΔS

Table (14) : The thyrmodynamic quantities for interaction of betamethasone with albumin .

Temp. (K) °	ΔH_1 (KJ/mole)	ΔG_1 (KJ/mole)	ΔS_1 (KJ/mole.K)
290	- 64.6830	1.0985	- 0.2268
300	=	2.6467	- 0.2244
305	=	4.2755	- 0.2260
310	=	6.1702	- 0.2285
315	=	6.2242	- 0.2251

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

References

[1]. http://en.wikipedia.org/wiki/Betamethasone (2008).
[2]. http://www.medicinenet.com/betamethasone_dipropionate/article.htm. (2008)
[3]. http://www.nlm.nih.gov/medlineplus/druginfo/medmaster/a682799.html (2008)
[4]. http://www.drugs.com/pro/betamethasone.html (2006)
[5]. Athanasios Lamprakopoulos etal ., Scandinavian journal of urology and nephrology , vol. 34 , Issue 6 , pages 355 – 360 November (2000) .
[6]. http://www.healtagrades.com/drug-ratings/drug/information/4781/betamethasone %20valerate.(2008).
[7]. http://www.goldbamboo.com/relate-t11083-tr4958.html (2008).
[8]. http://www.cat.inist.fr/?aModele=afficheN@cpsidt=2570433 (2003).
[9]. http://www.ncbi.nlm.nih.gov/pubmed/17154130 (2006).
[10]. http://www.eprints.ru.ac.za/395/.(2007).
[11]. http://www.springerlink.com/content/g8eyevrbq0twba20/.(2003).
[12]. L.P. Hammett, Physical Organic chemistry, Ms Graw-Hill, New- york , pp.184- 199 (1940).