Studies on The Molecular Interaction of Glutamine Amino Acid with Cu (π) Ions at Different pH Conditions Using S.W.V. Method

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ABSTRACT

The voltammetric behavior of pure Glutamine Amino acid was studied in a direct method in aqueous phosphate solution at (pH=7.0). Glutamine Amino acid give a well defined square wave voltammetric peak at (-0.552) volt against the reference electrode (Ag/AgCl/SatKCl) . The determined calibration curve is linear within the range of concentration $[(1.298\times10^{-6})–(8.778\times10^{-6})]$ M. with a correlation coefficient is $(R^2=0.9983)$. The molecular interaction of Glutamine Amino acid with Cu π ions has been studied, the interaction constant (K) were calculated at different temperature. Vant˙hoff equation applied to calculate the thermodynamic variables $(\Delta G,\Delta S,\Delta H)$,the result indicate that the interaction of Glutamine Amino acid with Cu (π) ions is of the type (Ion-Ion).

Key words: Glutamine, Interaction, Cu, Amino acid, pH condition.

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INTRODUCTION

Glutamine Amino Acid (GAA) is the chemical compound with the formula $C_5H_{10}N_2O_3$ as shown in (Fig.1) .Chiral recognition of d- and L- amino acids is achieved and mixtures of enantiomers quantified in the gas phase ,using the kinetics of competitive unimolecular fragmentations of trimeric Cu (π) -bond complexes. Singly charged Copper (π) -amino acid cluster ions [$Cu^π$ (A)(ref*)$_2$-H]$^+$ [A=amino acid ;ref*=chiral reference ligand ,selected from among the natural α –amino acids] undergo competitive collision induced dissociation (CID) in a quadrupole ion trap to form the dimeric complexes[$Cu^π$ (A)(ref*)-H]$^+$ and [$Cu^π$ (A)(ref*)$_2$-H]$^+$ [1].The tripeptide acts as a quadridentate ligand to give complexes with Copper and Nickel with an amino group ,two deprotonated amide groups and an imidazole pyridine nitrogen (im-N3) as donors. At 1:1 ligand –to- meal ratios the purple copper π complex [Cu LH-2]$^-$ is essentially 100 % abundant above pH (7.0) and the planar yellow [Ni LH$_2$] above pH (8.0).The displacement of the tripeptide ligand from the nickel (π)complex by L-histidine has been studied kinetically over the pH range (7-8).There is a small solvolytic reaction and reaction which is first –order in the hydrogen ion concentration [2].An initial rate approach was used to study the reaction of peroxy nitrite with human serum albumin (HSA) through stopped-flow spectrophotometry .At pH (7.4) and 37˙C,the second order rate constant for peroxy nitrite reaction with HAS was $(9.7 \pm1.1\times10^9)M^{-1}S^{-1}$.The reactivity of sulfhydryl –blocked HAS increased at acidic pH, whereas plots of the rate constant with the sulfhydryl versus pH were bell-shaped[3].A direct approach has been developed for quantitative identification of the calcium – binding amino acid gamma-carboxy glutamate in protein .

This should be advantageous for the study of numerous systems where specific roles for the binding of calcium or other divalent cations are suspected[4].A detailed study has been made of the kinetics of interaction between amino acids and esters of amino acids and O-phthaldialdehyde in the presence of mercapto ethanol. The reaction products have been characterized. A spectrophotometric method for quantitative analysis of all amino acids except proline and hydroxyl proline, has been developed. The possibility of determination of amino acid esters in mixture containing free amino acids has been demonstrated[5].Chemical kinetics ,also known as reaction kinetics, is the study of rates of chemical process. Chemical kinetics includes investigations of how different experimental conditions can influence the speed of a chemical reactions mechanism and transition state, as well as the construction of mathematical models that can

describe the characteristics of a chemical reaction[6]. Chemical reaction rates are the rates of change in concentrations or amounts of either reactants or products. Thermodynamics and kinetics are two factors that affect reaction rates. The study of energy gained or released in chemical reactions is called thermodynamics, and such energy data are called thermodynamic data. Thermodynamic data have no direct correlation with reaction rates, for which the kinetic factor is perhaps more important. Many factors influence rates of chemical reactions nature of reactants, temperature, concentration effect, heterogeneous reactions and catalysts[7].The present work involve the use off square wave voltammetric method for trace determination of Glutamine amino acid and studying the molecular interaction with Cu (п) Ions.
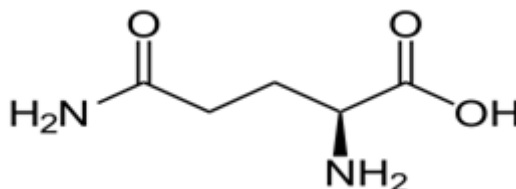

Fig 1: Chemical structure of Glutamine amino acid. M. wait = 146.15 gm/mole.

Experimental Apparatus:

All experiments were performed Using the EG and G pAR 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 thermostat $(\pm 0.1 \ ^\circ C)$.

Reagents:

All the chemical used analytical reagents grade GAA which was obtained from Fluka company, solutions of (1.0×10^{-3}) M was prepared .All Solutions were prepared with deionised distilled water . Cu (п) Ions from Cu-acetate was obtained from Merk company , 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 squre wave voltammetry mode was used with deposition time 60 sec; condition time 5 sec ; equilibrium time 5 sec; frequency 120 Hz;scan rate 2 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[8].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 GAA 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.The same procedure was also used to calculate the interaction constant (K) , using the optimum condition in (Table 1). For voltammetric measurement ,the sample cuvett contained (5ml)of phosphate buffer at(pH=7.0);final concentration(9.424×10^{-6})M of GAA. The square wave voltamogram was recorded to give the GAA peak .Then, appropriate amount of (10^{-5}) M of Cu (п) Ions was added to the cell and the square wave voltamogram were recorded at different temperature in the range (295-315) ˚K in order to calculate the thermodynamic quantities ΔG, ΔS and ΔH .

RESULTS AND DISCUSSION

Typical square wave voltamogram of (8.578×10^{-6})M GAA in phosphate buffer at (pH=7.0).is shown in (Fig.2)
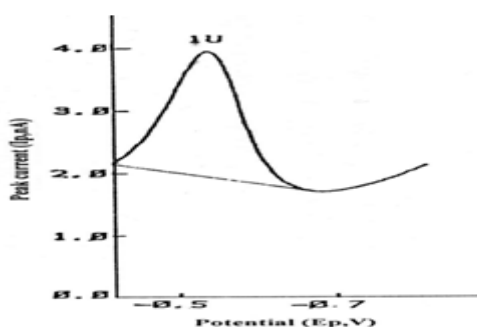

Fig 2: Square wave voltamogram of (8.578×10^{-6}) M GAA.

It can be seen from (Fig 2) a well-defined peak appeared at (-0.552 V) versus (Ag/AgCl,Sat KCl) electrode.

Optimum condition:

The SWV voltammogram of (2.96×10^{-6}) M of GAA was investigated in phosphate buffer(pH=7.0)variation all the parameter that it depend on the measurement in(Table1).

Table 1: Show the optimum values obtained which give either the highest peak current and the best resolution of the peak

Condition	Value	Condition	Value
initial pot.	- 0.1 V	frequency	120 Hz
final pot.	- 0.85 V	scan increament	2 mV/sec
deposition time	60 second	cond. potential	0.000 V
condition time	5 second	pulse height	0.025 mV
equilibrium time	5 second		

Effect of pH :

The square wave voltamogram of (3.152×10^{-6}) M of GAA were investigated at different pH values (3 - 9) using the optimum condition in phosphate buffer show in (Table1).The peak current (Ip) and peak potential (Ep) obtained are shown in (Table 2) .

Table 2: Effect of pH on S.W.V. peak and peak current of (3.152×10^{-6}) M of GAA

pH	Ep (v)	Ip (nA)
3	- 0.475	104.1
4	- 0.528	123.2
5	- 0.575	142.3
6	- 0.628	166.2
7	- 0.648	185.7
8	- 0.688	175.6
9	- 0.703	160.7

The peak current (Ip) is clearly dependent the pH. Maximum current respouse was found at (pH=7.0).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 (-0.064 V/pH) , (R^2=0.9934) which it very near to theoretical value (0.059 V/pH) [9].
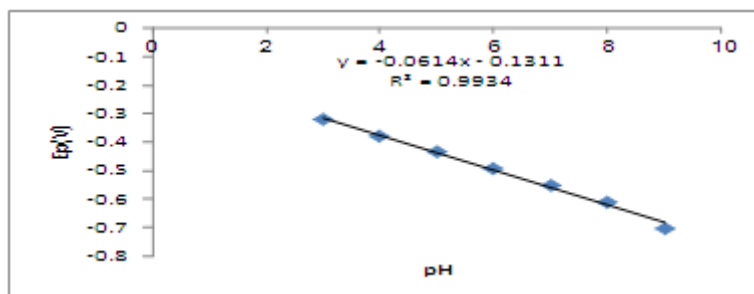

Fig 3: The relation between Ep and pH of(3.152×10^{-6}) M of Glutamine amino acid.

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

The square wave voltamogram of (3.1×10^{-6}) M of GAA were recorded at different time in phosphate buffer at (pH=7.0).The result obtained are tabulated in (Table 3).

Table 3: Effect of time on SWV peak of (3.1×10^{-6}) M of GAA at (pH=7.0) in aqueous solution.

Time (min)	Ip (nA)
5	190.2

10	193.2
15	192.4
20	193.5
25	192.5
30	193.6
35	194.2
40	193.4
45	192.6
50	193.2

It can be seen from the (Table 3) that GAA is stable for more than 50 minute.

Analytical Consideration:

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

Table 4: Effect of concentration on peak current of $[(1.298 \times 10^{-6}) – (8.778 \times 10^{-6})]$ M of GAA at (pH=7.0) in aqueous solution at Ep = - 0.552 V .

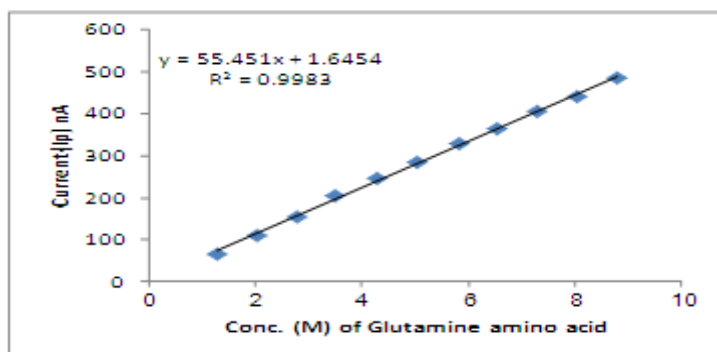| Conc. (M) 10^{-6}	Ip (nA)
1.298	65.0
2.048	110.0
2.768	155.0
3.508	205.0
4.288	245.0
5.018	285.0
5.828	330.0
6.548	365.0
7.288	405.0
8.028	440.0
8.778	485.0

Fig 4: The relation between peak current (Ip) and concentration of GAA at (pH = 7.0) phosphate buffer in aqueous solution

The plot peak current Ip versus molar concentration of GAA are showing in (Fig.4). Regression analysis on standard indicated a straight line on the conc. Range $[(1.298 \times 10^{-6}) – (8.778 \times 10^{-6})]$ M with correlation coefficient (R^2=0.9983).The lowest experimental detection limit was (1.298×10^{-6})M .

Voltammetric Behaviour of GAA in the presence of Cu п ions:

The square wave voltammogram of (9.9×10^{-6}) M of GAA in phosphate buffer at (pH = 7.0) was recorded at room temperature (295 ˚K) . Successive amount of Cu (п) ions (10^{-5}) M were then added and the square wave voltammogram were recorded after each addition . The results are shown in (Table 6). The peak current Ip of GAA were found to decrease gradually with the addition of Cu (п) ions as shown in (Fig.5). The plot Ip / Ip_0 versus concentration of Cu (п) ions added is shown in (Fig.6) (Ip_0 = The peak current of GAA only without Cu (п) ions. From which the Interaction constant (k) of GAA with Cu (п) ions was then calculated using quadratic equation where (k) obtained from the tangent.
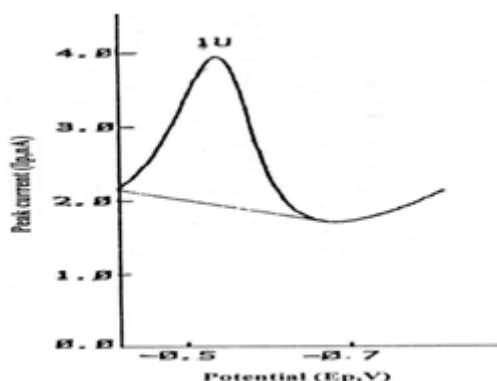
Fig 5: Square wave voltamogram of (8.578x10⁻⁶) M GAA in the presence of Cu(п) ions.
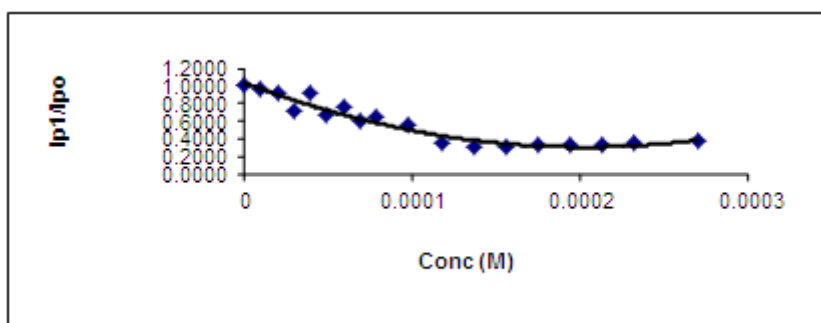

Fig 6: The plot of Ip/Ip_0 versus the concentration of Cu (п) ions added .

The effect of temperature on the interaction of Glutamine Amino Acid with Cu (п) ions :

The square wave voltammogram of (8.7×10^{-6}) M of GAA in the presence of Cu (п) ions (successive addition) were recorded at different temperature (295 – 315) °K , using the optimum condition shown in (Table 5) . The variation of peak current Ip at different temperature are shown in (Tables 6 , 7 , 8 , 9) . The interaction constant (K) for the interaction of GAA with Cu п ions were calculated and tabulated in (Table 10) . The vant hoff plot of log (K) versus 1/T gave a straight line (Fig.7) from which the thermodynamic parameters were calculated as follow ; ΔH from the slope = – Slope × R ; ΔG = – 2.303 RT Log K and from the gibes equation ΔS was calculate (ΔG = ΔH – T ΔS) . The values of tend for ΔH , ΔG and ΔS are tabulated in (Table 11) .

Table 5: The optimum condition of binding GAA with Cu (п) ions .

Conditions	Value
Deposition time	55 Second
Condition time	5 Second
Equilibrium time	5 Second
Frequency	120 Hz
Scan increment	2 mV/second
Condition potential	0.000 V
Pulse height	0.025 mV

Table 6: The peak current of (8.7×10^{-6})M GAA in the presence of different amount (10^{-5})M of Cu (п) ions in phosphate buffer (pH=7.0) at 295 °K.

Conc (M) $\times 10^{-8}$	Ip (nA)	Ip / Ip_0
0	1399.4	1.0
0.09	1395.0	0.9605
1.9	1366.2	0.9423
2.9	1087.0	0.7122
3.9	1032.5	0.9155
4.9	997.9	0.6654
5.9	1076	0.7566

6.8	847.7	0.5889
7.9	933.7	0.6445
9.8	786.5	0.5435
11.7	535.5	0.3656
13.6	483.4	0.3255
15.5	448.5	0.3223
17.5	465.8	0.3266
19.4	477.9	0.3354
21.3	520.5	0.3345
23.2	523.8	0.3496
26.9	527.8	0.3556

Table 7: The peak current of (8.7×10^{-6})M GAA in the presence of different amount (10^{-5})M of Cu (п) ions in phosphate buffer (pH=7.0) at 300 ˚K.

Conc (M)$\times 10^{-8}$	Ip (nA)	Ip/ Ip$_0$
0	1193	1.0
1.9	1098	0.195
3.9	938	0.7652
5.9	755	0.5967
7.8	464	0.3685
9.8	326.3	0.2556
11.7	321.8	0.2545
13.6	366.9	0.3005
15.5	405.3	0.3216
17.5	426.0	0.3345
19.4	456	0.3675
21.3	476	0.3767

Table 8: The peak current of (8.7×10^{-6})M GAA in the presence of different amount (10^{-5})M of Cu (п) ions in phosphate buffer (pH=7.0) at 305 ˚K.

Conc (M)$\times 10^{-8}$	Ip (nA)	Ip/ Ip$_0$
0	865.2	1.0
1.9	742.0	0.8565
3.9	466.0	0.5374
5.9	259.0	0.3245
7.8	229.5	0.2656
9.8	214.8	0.2473
11.7	208.5	0.2321
13.6	261.5	0.3032

Table 9: The peak current of (8.7×10^{-6})M GAA in the presence of different amount (10^{-5})M of Cu (п) ions in phosphate buffer (pH=7.0) at 315 ˚K.

Conc (M)$\times 10^{-8}$	Ip (nA)	Ip/ Ip$_0$
0	768.0	1.0
0.9	735.0	0.9498
1.9	445.0	0.5765
3.9	296.0	0.3778
5.9	256.0	0.3321
7.8	189.0	0.2492
9.8	167.0	0.2114
11.7	152.0	0.1998
13.6	132.0	0.1657
15.5	117.5	0.1489
19.4	97.4	0.1293

The interaction constant (K) of the interaction between GAA and Cu (п) ions in at different temperature as shown in (Table 10).

Table 10: The interaction constant (K) of the interaction between GAA with Cu (п) ions at different temperature .

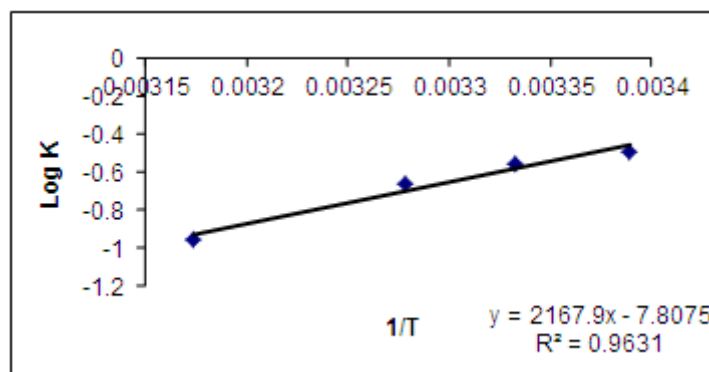| Temp. (T) K	1/T	K	Log K
295	0.003389	0.3179	- 0.497709
300	0.003333	0.2755	- 0.559878
305	0.003278	0.2199	- 0.657774
315	0.00317	0.111	- 0.954677

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

From the result in the (Table 10) we can calculate the thermodynamic quantities for interaction of GAA with Cu (п) ions which was shown in (Table 11).

Table 11: The thermodynamic quantities for interaction of GAA with Cu (п) ions.

Temp. (K) ˚	ΔH (KJ/mole)	ΔG (KJ/mole)	ΔS (KJ/mole.K)
295	- 18.02	2.8112	- 0.0706
300	=	3.2160	- 0.0707
305	=	3.8413	- 0.0716
315	=	5.7579	- 0.0779

The negative values of ΔS indicate that the complex formed after the interaction of GAA- Cu (п) ions are more ordered . The negative values ΔH indicate that the interaction process liberate heat (exothermic) . The low and positive values of ΔG indicate that the interaction are of the type ion-ion .

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