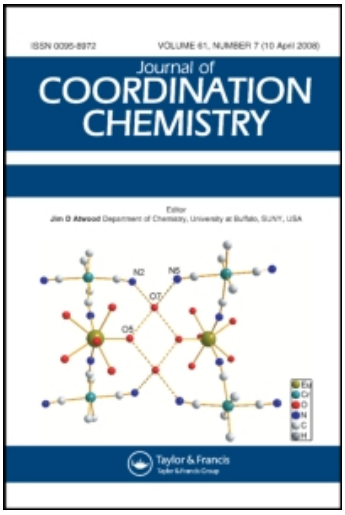

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Formation constants and coordination thermodynamics for binary complexes of Cu(II) and some α-amino acids in aqueous solution

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In this study, the formation constants of 1 : 1 binary complexes of Cu(II) with L-glutamic acid, L-aspartic acid, glycine, L-alanine, L-valine, and L-leucine and 1 : 2 binary complexes of L-glutamic acid, glycine and the protonation macro- and microconstants of all these amino acids were determined potentiometrically in aqueous solutions at 5.0, 20.0, and 35.0°C at a constant ionic strength of $I = 0.10\,\text{mol}\,\text{L}^{-1}$ ($NaClO_4$). The thermodynamic parameters ΔG_f°, ΔH_f°, and ΔS_f° were determined for the protonation of all amino acids used in this study and for the complex formation reactions of them with Cu(II). The results were analysed by means of *Principle of hard and soft* [*Lewis*] *acids and bases*. Additionally, in order to confirm the complex formation and determine the stability constants of complexes, UV-Vis spectroscopic studies were carried out. The stability constants obtained by spectrophotometrically are confirmed by those determined potentiometrically.

Keywords: Formation constants; α-Amino acids; Cu(II); Thermodynamics; Hard and soft [Lewis] acids and bases principle

1. Introduction

Amino acids have significance as "building blocks" of living systems finding applications in many fields (and in industry) including foods, animal feed supplements, and pharmaceutical production. In biological systems, clarification of various phenomena may frequently be possible only by physical and chemical properties, like protonation macro- and microconstants of the amino acids as well as their formation constants of complexes formed with metal ions [1–3]. Numerous studies have been carried out on the complexation of 3d transition metal ions with amino acids and other biologically important substances (bioligands) [4].

Determination of equilibrium constants and other thermodynamic parameters of metal complexes of bioligands would provide useful information about the metabolism

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of these substances. Most of these determinations have been carried out in aqueous media and at room temperature, especially using potentiometric pH titration method. Since the number of studies using both potentiometry and spectrophotometry to determine formation constants is very limited in the literature, we report the results of combined potentiometric and spectrophotometric measurements of Cu(II) complexes formed by L-Glutamic acid (2-amino-pentan-dioic acid; hereafter Glu), L-Aspartic acid (2-amino-butane-dioic acid; Asp), Glycine (2-amino-etanoic acid; Gly), L-Alanine (2-amino-propanoic acid; Ala), L-valine (2-amino-3-methyl-butanoic acid; Val), and L-Leucine (2-amino-4-methyl-pentanoic acid; Leu) which are amino acids (α-amino acids) of human protein. Amino acids contain two functional groups of contradictory properties within their own structures: an acidic carboxylate group and a basic amino group. They are bound to the same carbon (known as α-carbon) in α-amino acids. Under physiological conditions, amino acids undergo double ionization: deprotonation of the –COOH group and the protonation of $–NH_2$ group *via* internal proton transfer. This species is known as a "twin ion" or "zwitterions." Some amino acids carry additional functional group (R– group). It may also be a carboxylate group (acidic amino acid; e.g., Asp and Glu; in this case triple ionization occurs and at high pH range, dianionic species exists).

The α-amino acids used in this study include two kind of electron-pair donors; oxygen of carboxylate and nitrogen of amino group. They act as didentate ligands and can form *mono*, *bis* or *tris* complexes with Cu(II) like the other transition metal ions. The coordination takes place *via* α-amino-N and carboxylate-O donors and five-membered chelate rings form. These blue-colored complexes began to form between pH = 3 and 4 at which the hydrolysis of the cupric ions is largely suppressed.

In this work, the formation constants and the other thermodynamic parameters of the 1 : 1 binary complexes of Cu(II) with Glu, Asp, Gly, Ala, Val, Leu and 1 : 2 binary complexes of Cu(II) with Glu and Gly and the protonation macro- and microconstants of amino acids were calculated from potentiometric pH titration data in aqueous solutions at 5.0°C, 20.0°C, and 35.0°C (as most literature values for proton or metal–amino acid complexes are limited to room temperature) and constant ionic strengths ($I = 0.10\,\text{mol}\,\text{L}^{-1}\,NaClO_4$).

The results were analysed by means of *HSAB* (*hard and soft* [*Lewis*] *acids and bases*) *principle* [5, 6].

Formation constants were also calculated from visible absorption spectral data using Job's Method for 1 : 1 Cu(II)–amino acid complexes.

2. Experimental

2.1. *Reagents and materials*

$HClO_4$, $NaClO_4 \cdot H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, NaOH solution (titrisol), Gly, Ala, Val, Leu, and their methyl esters (Mee) and Glu, Asp were all *pro analysi* from Merck AG, FRG. All solutions were prepared with twice-distilled CO_2-free water. The metal salt solution was standardized by titration with EDTA. Aqueous stock solutions of amino acids were prepared and stored in the dark and cold room at 4°C.

2.2. *Potentiometric measurements*

The pH-potentiometric titrations were performed on an automatic titrator (Radiometer Analytical, TIM 860 Titration Manager) with a pH combination electrode (Red Rod PHC2085). The electrode was also equipped with a temperature probe. The electrode was calibrated daily with buffer solutions of Radiometer Analytical at pH = 4.00 and 7.00 at 25.0°C. The titration vessel of the titrator was a magnetically stirred, water-jacketed cell of 100 mL fitted with a special cover, through which the tip of titrator's dosing unit, the electrode and nitrogen inlet and outlet tubes could be inserted. By circulating water from a thermostat (ThermoHaake DC10) through the jacket, the temperature of the solution to be titrated was kept constant. The titrations were carried out in an inert atmosphere by bubbling purified nitrogen through the solutions before the titration and through the titration vessel during the titration.

The experimental procedure involved potentiometric titration of the following solutions with 0.100 mol L^{-1} sodium hydroxide solution: (1) 10.0 mmol L^{-1} $HClO_4$; (2) 10.0 mmol L^{-1} $HClO_4$ + 2.00 mmol L^{-1} Ligand [(a) Glu (b) Asp (c) Gly (d) Ala (e) Val (f) Leu (g) Gly Mee (h) Ala Mee (i) Val Mee and (j) Leu Mee]; (3) 10.0 mmol L^{-1} $HClO_4$ + 4.00 mmol L^{-1} Ligand [(a) Glu and (b) Gly]; (4) 10.0 mmol L^{-1} $HClO_4$ + 2.00 mmol L^{-1} Cu + 2.00 mmol L^{-1} Ligand (same as item 2 a–f); and (5) 10.0 mmol L^{-1} $HClO_4$ + 2.00 mmol L^{-1} Cu + 4.00 mmol L^{-1} Ligand (same as item 3).

These solutions were prepared according to Irving and Rossotti's method [7, 8]. The total volume of each titration solution was 50 mL. The ionic strength of the solutions was maintained at 0.10 mol L^{-1} by addition of the appropriate amount of 1.0 mol L^{-1} sodium perchlorate stock solution. Potentiometric titrations were performed as reported previously [9]. For each solution at least five replicate titrations were made.

Using potentiometric pH titration data of solutions (1) and (2), the values of average proton–ligand formation number, n_A, at various pH levels were determined by the equation given earlier [9].

Then values of the protonation constants, pK_a, were calculated by a computer (using an electronic spreadsheet software, e.g., MS Excel) from the $n_A = f(pH)$ relationship or read directly from n_A – pH curve at $n_A = 0.5$, 1.5 (and 2.5 if necessary) [9, 10].

For the calculation of the formation constant of 1 : 1 and 1 : 2 binary complexes using the titration data of solutions (1)–(5), the values of average metal–ligand formation number, n_L, at various pH values and the values of pL were calculated using the equations given elsewhere [9, 10].

For the corresponding values of n_L and pL, formation curves of the metal–ligand systems were drawn and the stability constants were read directly on curve or calculated by a computer at $n_L = 0.5$ and 1.5 [9, 10].

The ΔH_f°, ΔG_f°, and ΔS_f° values for protonation and complexation reactions were calculated from the formation constants determined at 5.0°C, 20.0°C, and 35.0°C by equations given previously [10].

2.3. *Spectrophotometric measurements*

UV-Vis spectrophotometric measurements were carried out at pH 4 and 20.0°C in a Shimadzu 1700 spectrophotometer. The concentrations of amino acid and Cu(II) stock solutions were 2.0×10^{-4} mol L^{-1}. The absorbance measurements of the solutions of

(1) Cu(II) ion alone, (2) amino acid alone, and (3) Cu(II)–amino acid complex were performed in the wavelength range of 300–900 nm. According to Job's method the stability constant of 1 : 1 binary complexes was calculated using the equation given previously [11].

3. Results and discussion

3.1. *Proton–amino acid systems*

Typical potentiometric titration curves and proton–ligand formation curves of amino acids used in this study at 20.0°C are shown in figures 1 and 2.

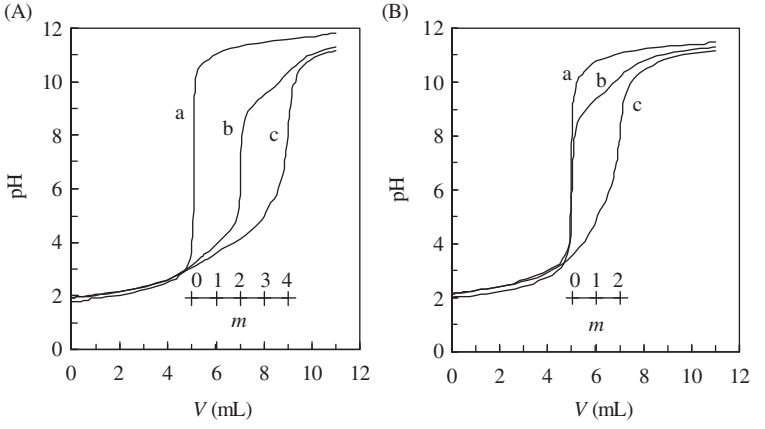


Figure 1. Potentiometric titration curves of (a) $HClO_4$, (b) $HClO_4$ + ligand (4.00 mmol L^{-1}) and (c) $HClO_4$ + ligand (4.00 mmol L^{-1}) + Cu (2.00 mmol L^{-1}) solutions. (A): for Asp (or Glu). (B): for Gly (Ala, Val, or Leu) at 20.0°C and $I = 0.10$ mol L^{-1} ($NaClO_4$) (*m*: the moles of NaOH added after neutralization of $HClO_4$ and 1 *m* corresponds 1 proton dissociated).

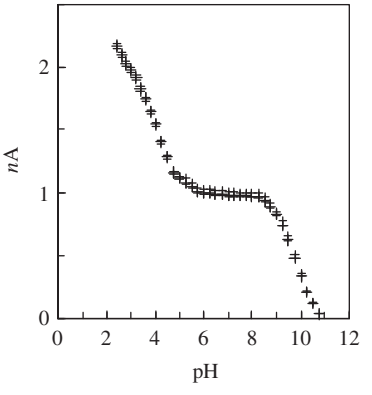
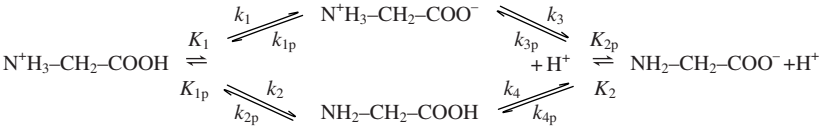


Figure 2. Proton–ligand formation curves of Glu (or Asp) at 20.0°C (including data of three replicate experiments).

The protonation constants, $pK_{\gamma\text{-COO}}$ and pK_{NH_2} for Glu, Asp, and $pK_{\alpha\text{-COO}}$ and pK_{NH_2} for Gly, Ala, Val, and Leu were determined but $pK_{\alpha\text{-COO}}$ of both Glu and Asp could not be determined with the method used in this study. The latter may be estimated approximately from the relationship of $pK_{\alpha\text{-COO}}+pK_{NH_2}=2pH$ at $n_A=1$. Calculated pK_a values are given in table 1.

Detailed equilibrium for protonation and deprotonation of neutral amino acids [19] show that each possesses two macroscopic (pK_1 and pK_2) and four microscopic acidity (protonation) constants (pk_1, pk_2, pk_3 and pk_4) [19]. This detailed equilibrium for Gly and proton system is given as follows:



(Subscript p denotes *protonation* process)

Table 1. Protonation macro- and microconstants of amino acids and amino acid esters used in this study at various temperatures and $I=0.10\,mol\,L^{-1}$ ($NaClO_4$).[a]

Ligand	T (°C)	$pK_{\alpha\text{-COO}}$	pK_{NH_2}	$pK_{\gamma\text{-COO}}$	$pK_{NH_2(E)}$	pk_4
Gly	5.0	2.32(1)	10.07(4)	–	8.14(3)	4.25(1)
	20.0	2.35(1)	9.74(1)	–	7.82(1)	4.26(1)
	(25)	2.37[b,c]	9.60[b,c]	–	7.67[b,j]	–
	35.0	2.40(2)	9.33(3)	–	7.46(3)	4.27(1)
Ala	5.0	2.34(1)	10.12(5)	–	8.18(4)	4.28(2)
	20.0	2.31(1)	9.76(2)	–	7.83(1)	4.24(1)
	(25)	2.33[b,c,d]	9.72[b,c,d,e]	–	7.75[b,j]	–
	35.0	2.28(1)	9.42(4)	–	7.51(2)	4.20(2)
Val	5.0	2.23(2)	9.99(4)	–	7.96(3)	4.26(1)
	20.0	2.27(1)	9.63(1)	–	7.63(2)	4.27(1)
	(25)	2.28[b,c]	9.54[b,c,e]	–	7.55[b,j]	–
	35.0	2.30(1)	9.29(3)	–	7.30(2)	4.29(1)
Leu	5.0	2.33(2)	10.08(5)	–	7.98(4)	4.42(3)
	20.0	2.30(1)	9.70(2)	–	7.64(3)	4.37(1)
	(25)	2.32[b,c]	9.66[b,c,e]	–	7.65[b,j]	–
	35.0	2.28(1)	9.33(4)	–	7.28(4)	4.32(2)
Glu	5.0		10.09(5)	3.99(2)		
	20.0	2.2[f]	9.65(3)	4.05(1)		
	(25)	2.16[b,g,h]	9.58[b,g,h]	4.15[b,g,h]		
	35.0		9.34(5)	4.10(2)		
Asp	5.0		10.14(5)	3.56(3)		
	20.0	2.1[f]	9.72(4)	3.62(1)		
	(25)	2.10[b,i]	9.82[b,i]	3.86[b,i]		
	35.0		9.41(5)	3.68(2)		

[a]The pK_a values are accurate to ±(0.01–0.05) pK_a units and the error limits on the last significant figures are shown in brackets ($2\alpha=0.05$).
[b]The literature values at 25°C.
[c]Ref. [12].
[d]Ref. [13].
[e]Ref. [14].
[f]These values could not be detected with the method used in this study, calculated approximately as mentioned above.
[g]Ref. [15].
[h]Ref. [16].
[i]Ref. [17].
[j]Ref. [18].

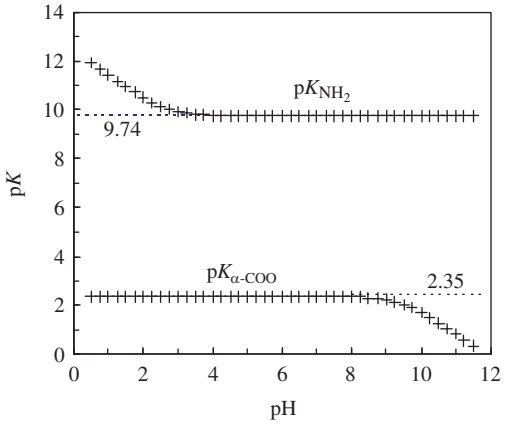


Figure 3. pK_a values of Gly *vs.* pH using exact expressions relating macro and micro acidity constants at 20.0°C.

From macroscopic acidity constants (K_1 and K_2 or K_1 only) of Gly, Ala, Val, Leu, and their methyl esters and using the well-known microscopic/macroscopic acidity constant relationships [19]

$$K_1 = k_1 + k_2 \quad \text{and} \quad 1/K_2 = 1/k_4 + 1/k_3$$

and assuming the macroscopic acidity constant of neutral α-amino acid's methyl ester ($K_{NH_2(E)}$), to be equal to microconstant k_2, the other microscopic acidity constants were calculated. For neutral α-amino acids: $k_1 \cong K_{\alpha\text{-COO}}$, $k_2 \cong K_{NH_2(E)}$ (of ester) and $k_3 \cong K_{NH_2}$ [19].

Previously suggested expressions relating macro and micro acidity constants are given as follows [19]:

$$K_1 = k_1 + k_2 + k_3k_1/[H^+] \quad \text{and} \quad 1/K_2 = 1/k_4 + 1/k_3 + [H^+]/k_1k_3 + [H^+]/k_2k_4$$

According to these exact relationships, significant deviations are observed at lower values of pH for pK_{NH_2} and at higher values of pH for $pK_{\alpha\text{-COO}}$ [19]. This is shown graphically in figure 3 for Gly.

The pK_{NH_2} values of all ligands decreases with increasing temperature (table 1). Corresponding enthalpy changes are exothermic. Enthalpic and entropic contributions to the free energy of proton–amino acid (and –ester) systems are given in table 2.

According to *HSAB* principle and its developed form (using Frontier Molecular Orbital Theory and Density Functional Theory) [5], amine group, $-NH_2$, of amino acids is a hard Lewis base. Due to a lone-pair of electrons, amino-N donor is classified as *Lobe–HOMO* (Highest Occupied Molecular Orbital) *Lewis base*. The proton is always transferred from one Lewis base to another in *HOMO–LUMO* interactions giving covalent to polar–covalent complexes [5, 6, 22]. These complexations are entirely *entropy*- or both *entropy*- and *enthalpy-stabilized* processes. Thus, the driving force is decreased in both enthalpy and entropy with major contribution from the enthalpy decrease to the free energy decrease during protonation of $-NH_2$, in accord with *HSAB* and related theories [22, 23].

The average enthalpic contribution to the free energy decrease for the protonation of $-NH_2$ of the amino acids was found as approximately 74% (84% in the case of esters), in agreement with *HSAB* principle [22].

Table 2. Thermodynamic parameters for the proton–ligand systems.[a]

								Contribution %	
		ΔH_f°(kJ mol^{-1})		ΔG_f°(kJ mol^{-1})		ΔS_f°(J K^{-1} mol^{-1})		ΔH_f°	$-T\,\Delta S_f^\circ$
Asp	p$K_{\gamma\text{-COO}}$	6.0(6)		−20		90(7)		Counteract	100
	p$K_{\alpha\text{-COO}}$		–[c]	−12			–[c]	–[c]	
	pK_{NH_2}	−41(2)	−41.2[b,d]	−55	−58[b,d]	48(4)		74	26
Glu	p$K_{\gamma\text{-COO}}$	5.5(5)	−2.4[b,e]	−23	−25.0[b,e]	96(8)	75.8[b,e]	Counteract	100
	p$K_{\alpha\text{-COO}}$		–[c]	−12			–[c]	–[c]	
	pK_{NH_2}	−42(2)	−40.1[b,e]	−54	−57.2[b,e]	43(3)	57.6[b,e]	77	23
Gly	p$K_{\alpha\text{-COO}}$	3.8(3)		−13		58(4)		Counteract	100
	pK_{NH_2}	−41(1)	−44.0[b,d]	−55		47(3)		75	25
	pk_4	1.6(1)		−24		87(6)		Counteract	100
Gly Mee	p$K_{NH_2(E)}$	−37(1)		−44		25(2)		83	17
Ala	p$K_{\alpha\text{-COO}}$	−3.0(2)	−2.9[b,f]	−13		34(2)		23	77
	pK_{NH_2}	−39(1)	−45.0[b,f]	−55		55(5)		71	29
	pk_4	−4.4(3)		−24		66(6)		18	82
Ala Mee	p$K_{NH_2(E)}$	−36(1)		−44		27(2)		82	18
Val	p$K_{\alpha\text{-COO}}$	3.3(2)		−13		55(4)		Counteract	100
	pK_{NH_2}	−39(1)	−45.3[b,f]	−54		52(4)		72	28
	pk_4	1.4(1)		−24		86(7)		Counteract	100
Val Mee	p$K_{NH_2(E)}$	−36(1)		−43		25(2)		83	17
Leu	p$K_{\alpha\text{-COO}}$	−2.9(2)		−13		34(3)		22	78
	pK_{NH_2}	−41(2)	−44.9[b,f]	−54		43(4)		77	23
	pk_4	−4.9(4)		−25		67(6)		20	80
Leu Mee	p$K_{NH_2(E)}$	−38(1)		−43		18(1)		88	12

[a]The ΔH_f° values are accurate to ±1 kJ mol^{-1} (average) and the ΔS_f° values to ± 4 J K^{-1} mol^{-1} (average) choosing $2\alpha = 0.05$. The error limits on the last significant figures are shown in parenthesis.
[b]The literature values.
[c]Could not be calculated because of insufficient data.
[d]Ref. [20].
[e]Ref. [21].
[f]Ref. [12].

The p$K_{\alpha\text{-COO}}$ and pk_4 of Gly, Val and also p$K_{\gamma\text{-COO}}$ of Glu and Asp increase with increasing temperature, while p$K_{\alpha\text{-COO}}$ and pk_4 of Ala and Leu decrease. The carboxylate oxygen is harder than –NH_2's N [22] and classified as *Lobe–HOMO Lewis base* also. The protonation of carboxylate is a hard–hard interaction (mainly electrostatic) and should be an endothermic, i.e., entirely entropy-controlled. But some of these interactions may be slightly exothermic since proton forms bonds of partial covalent nature although it is a hard acceptor [22]. Therefore, in most of the interactions between oxygen donor and proton acceptor, enthalpy change is positive and counteracts the protonation process found in this study.

Distribution diagrams for the species derived from (H–Glu) and (H–Ala) systems as a function of pH at 20.0°C are shown in figure 4. These protolytic equilibria diagrams represent zwitterionic species for all amino acids used in this study in pH region of *ca* 2.5 and 10.

3.2. *Binary Cu(II) and amino acid complex systems*

The potentiometric titration curves of Cu–Asp (or Cu–Glu), Cu–Gly (Cu–Ala, Cu–Val or Cu–Leu), Cu–Asp_2, and Cu–Gly_2 systems and formation curve of Cu–Glu_2 at 20.0°C are shown in figures 1 and 2 (a, b, and c curves). Similar titration and formation curves were obtained at the other temperatures studied.

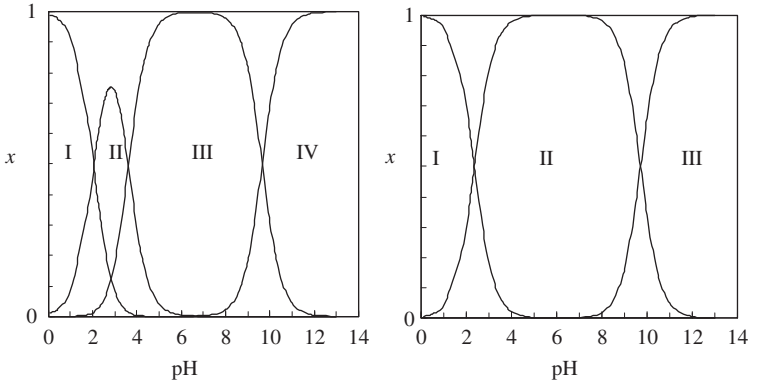


Figure 4. Diagrams of protolytic equilibria of Glu (left; I: H_3L^+, II: H_2L, III: HL^- and IV: L^{2-}) and Ala (right; I: H_2L^+, II: HL and III: L^-) as a function of pH at 20.0°C. The amount of each species present is represented by mole fraction x (similar distribution curves were obtained for the other amino acids used in this work).

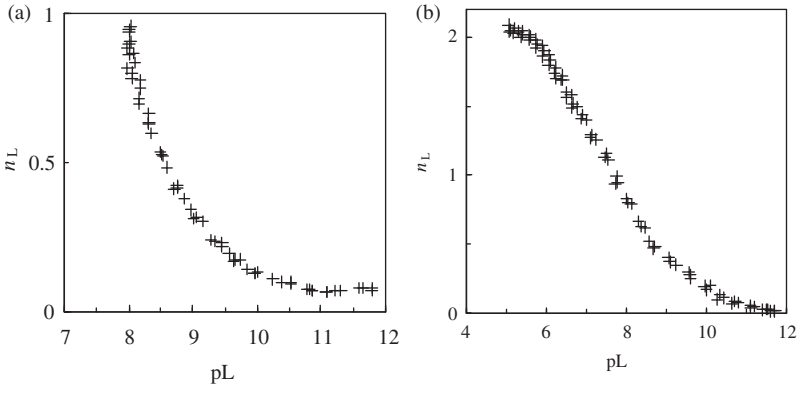


Figure 5. Complex formation curves ((a): Cu–Glu, (b): Cu–Glu_2) at 20.0°C (including data of three replicate experiments).

Complex formation curves of Cu–Glu and Cu–Glu_2 are shown in figure 5. The color of the solutions was light blue above pH 3 for 1 : 1 Cu–Glu and 1 : 1 Cu–Asp systems and above pH 3.5 for the remainder of the systems. Slight turbidity was observed above pH 6.0–6.5 for all systems.

The formation constants of Cu–Asp and Cu–Glu systems increase with increasing temperature, while stability constants of Cu–Gly, Cu–Ala, Cu–Val, or Cu–Leu systems and also Cu–Glu_2 and Cu–Gly_2 systems decrease with increasing temperature (table 3). Corresponding thermodynamic parameters for all these systems are given in table 4.

The enthalpy change of Cu–Glu or Cu–Asp complexation is positive. The driving force is entropy for this complexation and positive enthalpy changes counteract their formation.

Table 3. Formation constants of binary complexes of amino acids with Cu at $I = 0.10\,\text{mol L}^{-1}$.[a]

	$\log K^{Cu}_{Cu-L}$						$\log K^{Cu-L^c}_{Cu-L_2}$	
T (°C)	Glu	Asp	Gly	Ala	Val	Leu	Glu	Gly
5.0	8.49(7)	8.93(6)	8.56(5)	8.50(6)	8.34(6)	8.63(6)	6.95(5)	7.10(5)
20.0	8.57(5)	9.03(5)	8.34(4)	8.30(4)	8.13(3)	8.40(5)	6.75(4)	6.85(3)
(25.0)	8.32[b,d]	8.76[b,e]	8.20[b,f]	8.14[b,f]	7.98[b,f]	8.26[b,f]	6.60[b,d]	6.87[b,f]
35.0	8.66(5)	9.11(5)	8.16(5)	8.11(5)	7.94(5)	8.21(5)	6.53(5)	6.65(4)

[a]The values are accurate to average ±0.05 log K units ($2\alpha = 0.05$). The error limits on the last significant figures are shown in parenthesis.
[b]The literature values at 25°C.
[c]$\log K^{Cu-L}_{Cu-L_2}$ are 7.08, 6.80, 6.64, and 6.78 for Asp, Ala, Val, and Leu, respectively, at 20.0°C.
[d]Ref. [15].
[e]Ref. [24].
[f]Ref. [12].

Table 4. Thermodynamic parameters for Cu–amino acid systems at $I = 0.10\,\text{mol L}^{-1}$ ($NaClO_4$).[a]

					Contribution (%)	
System	ΔH_f° (kJ mol^{-1})		ΔG_f° (kJ mol^{-1})	ΔS_f° (J K^{-1} mol^{-1})	ΔH_f°	$-T\,\Delta S_f^\circ$
Cu–Glu	8.7(8)		−48	194(6)	Counteract	100
Cu–Asp	9.3(9)		−51	205(7)	Counteract	100
Cu–Gly	−21(2)		−47	87(4)	46	54
Cu–Ala	−21(2)	−22.2[b,c]	−47	88(4)	45	55
Cu–Val	−21(2)	−22.8[b,c]	−46	83(4)	47	53
Cu–Leu	−23(2)	−23.2[b,c]	−47	83(4)	48	52
(Glu–Cu)–Glu	−24(3)		−38	49(3)	62	38
(Gly–Cu)–Gly	−24(3)		−38	49(3)	63	37

[a]The ΔH_f° values are accurate to ±2 kJ mol^{-1} and the ΔS_f° values to ±4 J K^{-1} mol^{-1} ($2\alpha = 0.05$). The error limits on the last significant figures are shown in parenthesis.
[b]The literature values.
[c]Ref. [12].

The enthalpy change of Cu–Gly, Cu–Ala, Cu–Val, or Cu–Leu complexation is negative. The driving force is both entropy and enthalpy for these reactions. This interaction is a soft or borderline medium hardness like Cu(II). The contribution of enthalpy decrease to the free energy decrease is between 45% and 48% (average: 46%). The enthalpy change of Cu–Glu_2 and Cu–Gly_2 complexation is also negative with the 62% and 63% contribution to the free energy decrease.

In all these complexations, –COO^- (in case of Asp or Glu, one –COO^-) of amino acids binds to Cu^{2+} through O and amine through N (showing only bonding atoms: Cu[N, O] or Cu[N, $O]_2$ is formed). This O donor is hard and a *Lobe–HOMO Lewis base* whereas N donor is less hard than oxygen [9, 10, 22]. Acceptor Cu^{2+} is borderline between the classification of hard and soft. Cu^{2+} interacts with –COO^- and deprotonated –NH_3^+ and two coordination bonds [9, 10, 22] are formed. Cu–O may be a preferential binding in accord with "Principle of Maximum Hardness" [5, 6]. For Cu–Glu and Cu–Asp systems, entropy was the only factor which stabilizes these complexes. The hard oxygen of isolated –COOH group bound to γ-carbon plays an important role in complexation during its deprotonation (substitution between Cu^{2+} and proton).

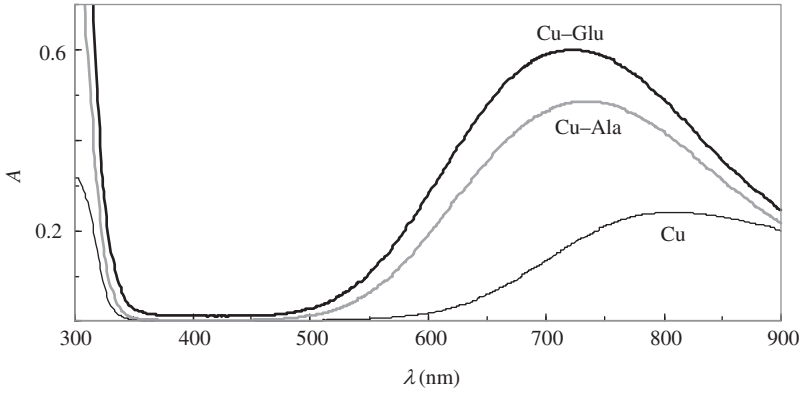


Figure 6. UV spectra of Cu, 1:1 Cu–Glu and 1:1 Cu–Ala solutions at pH 4 and 20.0°C ($c = 2.0 \times 10^{-4}$ mol L^{-1}).

On the other hand, both enthalpic and entropic stabilizations occur together for formation of Cu–Gly, Cu–Ala, Cu–Val, or Cu–Leu and also Cu–Glu_2 or Cu–Gly_2, implying that –COO^- bound to α-carbon participates more effectively in complexation, i.e., as a borderline Lewis acid, Cu(II) prefers to bind to one oxygen of –COO^- which were identical due to stabilization by resonance in acidic aqueous solution [5, 6, 10, 11, 23].

UV-Vis spectra of Cu, 1:1 Cu–Glu and 1:1 Cu–Ala solutions at pH 4 and 20.0°C (the concentrations of all species is 2.0×10^{-2} mol L^{-1}) are shown in figure 6. These spectra verify the Cu–Glu and Cu–Ala formation. From 300 to 900 nm all amino acid solutions used in this study do not absorb light. UV spectra of Cu–Asp solution at pH 4 and 20.0°C (2.0×10^{-2} mol L^{-1}) are more similar to those of Cu–Glu and Cu–Ala than those of Cu–Gly, Cu–Val, or Cu–Leu solution. All these species represent (1) blue shifting of 80–90 nm and (2) for neutral amino acids approximately 2.5-fold light absorption in the wavelength of absorbance maxima and for acidic amino acids three-fold to that of Cu(II) alone.

Applying Job's method to all 1:1 systems of this study, the formation constants of the complexes were obtained as $\log K^{Cu}_{Cu-Asp} = 9.11$, $\log K^{Cu}_{Cu-Glu} = 8.60$, $\log K^{Cu}_{Cu-Gly} = 8.46$, $\log K^{Cu}_{Cu-Ala} = 8.40$, $\log K^{Cu}_{Cu-Val} = 8.24$, and $\log K^{Cu}_{Cu-Leu} = 8.28$ at 20.0°C. A sample Job plot for 1:1 Cu–Glu complexation is shown in figure 7. These results confirm the ones determined potentiometrically [11, 12, 20, 23, 25]. Thus, the results of the potentiometric studies were also validated by Job's method. The tendency of Gly, Ala, Val, and Leu to make 1:1 complexes with Cu(II) is lower than those of Asp and Glu in aqueous solution. The complex formation tendency of Gly, Ala, Val, and Leu with Cu(II) can be supported by comparing blue-shifted values obtained by spectrophotometrically. In the presence of Cu(II), the λ_{max} nm^{-1} values of Gly, Ala, Val, and Leu obtained (720–770 nm) follow the order: Leu > Gly > Ala > Val.

4. Conclusion

Protonation macroconstants of Glu, Asp, Gly, Ala, Val, and Leu (and methyl esters of the last four) and protonation microconstants of Gly, Ala, Val, and Leu and formation constants of Cu(II) complexes of Glu, Asp, Gly, Ala, Val, and Leu were determined

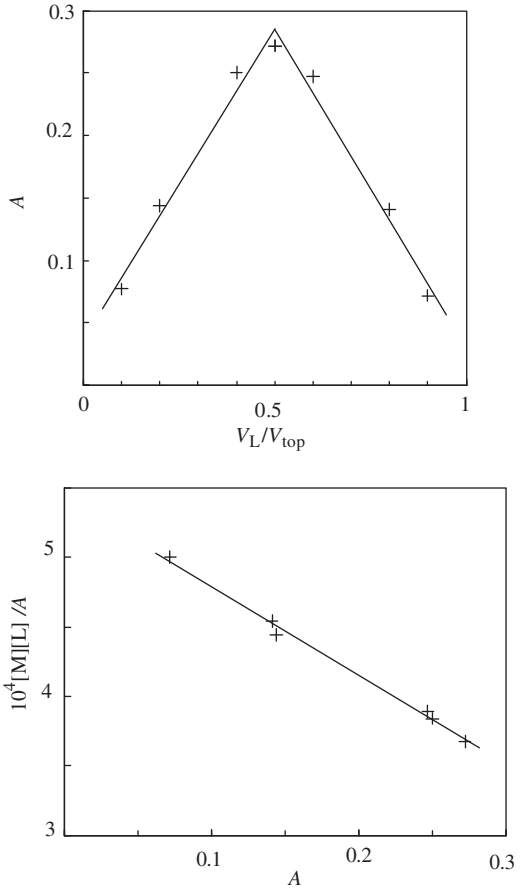


Figure 7. Job graphs for Cu–Glu system (the slope of the line yields εl and the values of εl and the intercept yields K; ε: the extinction coefficient of the complex and the l: optical path length in the cell of the spectrophotometer).

potentiometrically in aqueous solutions at 5.0°C, 20.0°C, and 35.0°C at ionic strength of $I = 0.10\,\text{mol}\,\text{L}^{-1}$ ($NaClO_4$). The pH titration technique modified by Irving and Rossotti [7] has been applied to determine these equilibrium constants. The ΔG_f°, ΔH_f°, and ΔS_f° were determined for all these complexations. The pK_{NH_2} of all ligands decreases with increasing temperature. During the protonation of $-NH_2$ the driving force is the decrease in both enthalpy (approximately 74%) and entropy contributing to the free energy decrease. The $pK_{\alpha\text{-COO}}$ and pk_4 of Gly, Val, and $pK_{\gamma\text{-COO}}$ of Glu and Asp increase and the $pK_{\alpha\text{-COO}}$, pk_4 of Ala and Leu decrease with increasing temperature. For most of these O and proton interactions $\Delta H_f^\circ > 0$ and counteracts the protonation process. These results are in agreement with *HSAB* principle [5, 6, 9–11, 22, 23].

The formation constants of Cu–Asp or Cu–Glu increase and those of Cu–Gly, Cu–Ala, Cu–Val, Cu–Leu, Cu–Glu_2, and Cu–Gly_2 decrease with increasing temperature. The driving force is both entropy and enthalpy for these complexes. Only ΔS_f° stabilizes the Cu–Glu and Cu–Asp complexes.

In all these complexes, calculated thermodynamic quantities imply that $-COO^-$ and $-NH_2$ binds to Cu^{2+}. These results are in agreement with *HSAB* also [5, 6, 9–11, 22, 23].

UV-Vis spectroscopic studies were also carried out and the formation constants obtained spectrophotometrically (using Job's method) confirmed those determined potentiometrically.

The tendency of Gly, Ala, Val, and Leu to make 1 : 1 complexes with Cu(II) is lower than those of Asp and Glu. The complex formation tendency of Gly, Ala, Val, and Leu with Cu(II) and their blue-shifts obtained spectrophotometrically gave the same order: Leu > Gly > Ala > Val.

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