Mixed-Ligand Complex Formation Equilibria of Cobalt(II), Nickel(II), and Copper(II) with *N*,*N*-Bis(2-hydroxyethyl)glycine (Bicine) and Some Amino Acids

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The formation of binary and ternary complexes of Co(II), Ni(II), and Cu(II) with bicine [*N*,*N*-bis(2-hydroxyethyl)glycine] and some selected mono- and dicarboxylic amino acids (glycine, α-alanine, leucine, valine, phenylalanine, asparagine, *β*-alanine, aspartic acid, and glutamic acid) was studied potentiometrically at (298.15 ± 0.1) K and $I = 0.1$ mol·dm^{-3} ($NaNO_3$) in aqueous solution. The acid−base properties of ligands were investigated and discussed. The formation of the 1:1 binary and 1:1:1 ternary complexes is inferred from the corresponding titration curves. The acidity constants of the ligands were determined and used for determining the stability constants of the complexes formed under the experimental conditions. The ternary complexes are formed in a stepwise mechanism. The stability constants of the binary and ternary systems were evaluated. The stability of the ternary complexes is also discussed in relation to that of the binary complexes of secondary ligands. An evaluation of the effects of ionic strength and temperature of the medium on the stability of the ternary system Cu(II) + bicine + amino acids has been studied. The thermodynamic parameters were calculated and discussed. The stability constant of the above-mentioned ternary system has been investigated in a dioxane−water solution. The complexation behavior of the ternary complexes was ascertained using differential pulse polarography (DPP) and square wave voltammetry.

Introduction

The standardization of pH and control of the acidity in the physiological region, pH 7 to 9, as found in blood and plasma, are of special importance in clinical diagnosis.

In 1966, Good et al.[1] fulfilled some partial needs by suggesting the use of zwitterionic buffers compatible with common clinical media of physiological interest. We have, therefore, investigated an ampholyte bicine [*N*,*N*-bis(2-hydroxyethyl)glycine], a derivative of the simple amino acid glycine. Bates et al.[2] have found that bicine is a useful buffer standard in the range of physiological interest.

Inorganic and organic buffers used in environmental studies involving trace-metal-ligand speciation may complex a number of trace metal. Thus, the degree to which they bind must be determined for their use in speciation studies. Ternary complexes of various metal ions with bicine have been studied.[3−7]

The present work concerns a study of the solution equilibria involved in the formation of binary and ternary metal complexes involving bicine and some selected amino acids because these systems mimic many biological reactions (enzyme−metal ion−buffer interactions).

Experimental Section

Materials and Solutions. Bicine [*N*,*N*-bis(2-hydroxyethyl)glycine] of analytical reagent grade (Sigma) was used without further purification. Chromatographically pure amino acids were analytical reagent grade, BDH products. The metal salts and dioxane were also provided by BDH as nitrates or chlorides. All solutions were prepared in deionized water. A stock solution of bicine was prepared by dissolving an accurate amount by mass in the appropriate volume of deionized water. The metal ion solutions were standardized by EDTA using suitable indicators.[8] A carbonate-free sodium hydroxide solution was prepared by dissolving the Analar pellets in deionized water, and the solution was standardized potentiometrically with potassium hydrogen phthalate (Merck AG). Nitric acid, sodium hydroxide, and sodium nitrate were from Merck p.a.

Apparatus and Procedure. Potentiometric pH titrations were performed using a model SM 702 Metrohm automatic titrator with a combined pH glass electrode equipped with a 665 dosimat and a magnetic stirrer (Switzerland). The accuracy of the instrument was (±0.001) pH unit. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities. It is to be assumed that the activity coefficient is constant, an assumption usually justified by working in a medium of a constant ionic strength (0.10 mol·dm^{-3} $NaNO_3$).[9] The electrode system was calibrated by periodic titrations of HNO_3 (or NaOH) solution (0.10 mol·dm^{-3} in $NaNO_3$) with a standard NaOH (or HNO_3) solution. Thus, all constants determined in this work are concentration constants.

The solutions were prepared (total volume 50 cm^3) and were titrated potentiometrically against standard CO_2-free NaOH (0.10 mol·dm^{-3}). Each solution was thermostated at the required temperature with an accuracy of (±0.1) K, where the solutions were left to stand for about 15 min before titration. The pH-metric titrations were carried out at the desired temperature in a purified nitrogen atmosphere. A magnetic stirrer was used during all titrations. The titration was repeated at least four times for each system.

The pH titrations were terminated when the pH readings became unstable, showing a downward drift. In all cases,

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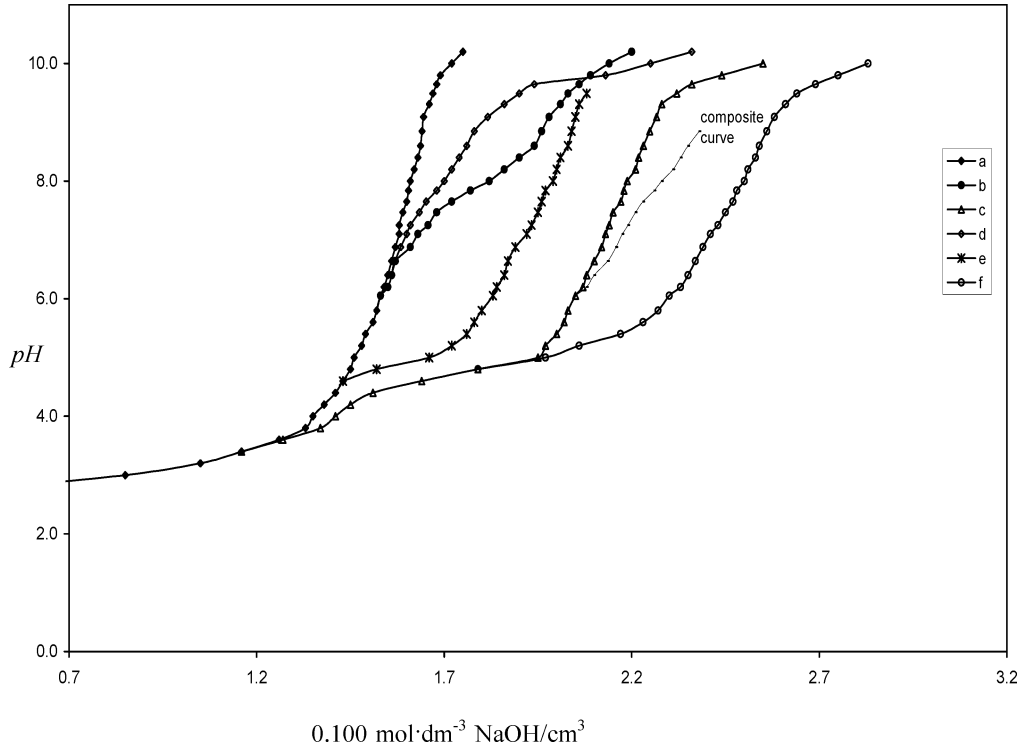

Figure 1. Potentiometric titration curves for the Ni(II) + bicine + glycine system at (298 ± 0.1) K and I = 0.1 mol·dm^{-3} $NaNO_3$. (a) 0.003 mol·dm^{-3} HNO_3 + 0.1 mol·dm^{-3} $NaNO_3$; (b) solution a + 0.001 mol·dm^{-3} bicine; (c) solution b + 0.001 mol·dm^{-3} Ni(II); (d) solution a + 0.001 mol·dm^{-3} glycine; (e) solution d + 0.001 mol·dm^{-3} Ni(II); (f) solution a + 0.001 mol·dm^{-3} bicine + 0.001 mol·dm^{-3} glycine + 0.001 mol·dm^{-3} Ni(II).

no calculations have been performed beyond the precipitation point; hence, the hydroxyl species likely to be formed after this point could not be studied. In analyzing the titration data for the determination of the proton dissociation constants of the free ligands and the stability constants of binary and ternary metal-ligand complexes in solution, Bjerrum−Calvin's pH titration technique,[10,11] as adopted by Irving and Rossotti[12,13] for binary systems and by Chidambaram and Bhattacharya[14] for ternary systems, has been used at (298.15 ± 0.1) K. Standard deviations were also evaluated for the corresponding equilibrium constants. The clinp 2.1 computer program[15,16] based on unweighted linear least-squares fits was used for all calculations.

To account for the differences in acidity, basicity, dielectric constant, and ionic activities for partially aqueous solutions relative to pure aqueous solutions, we corrected the pH values of the former solutions by making use of the procedure described by Douhéret

$$\mathrm{pH}^* = \mathrm{pH}_{(\mathrm{R})} - \delta \qquad (1)$$

where pH* represents the corrected value and $pH_{(R)}$ represents the meter readings. The value of δ for various proportions of the dioxane solvent was determined.[17,18]

Electrochemical Measurements. Square wave voltammetry and differential pulse voltammetry measurements were collected using an EG and G Princeton Applied Research potentiostat/galvanostat model 263 with a single compartment voltammetric cell equipped with a glassy carbon (GC) working electrode (area = 0.1963 cm^2) embedded in a resin, a Pt-wire counter electrode, and an Ag/AgCl reference electrode. The scan rate was 36.6 mV·s^{-1} from (+250 to −300) mV, the frequency was 20 Hz, the pulse height was 25 mV, and the scan increment was 2.0 mV.

The solutions were prepared (total volume 25 cm^3) and purged with nitrogen for 180 s. The ionic strength of the studied solutions was adjusted to that of the 0.1 mol·dm^{-3} $NaNO_3$ solution.

Results and Discussion

Representative potentiometric titration curves are shown in Figure 1 for the Ni(II) + bicine + glycine system.

The dissociation constant (pK_{a1}) of cationic bicine, H_2A^+, could not be calculated potentiometrically under the present experimental conditions because of the highly acidic nature of the associated proton. The second proton dissociation constant (pK_{a2}) of bicine, corresponding to the cationic ($\equiv N^+H$) group, was determined potentiometrically from curves a and b using the clinp 2.1 computer program. Details regarding the potentiometric method are reported in the Experimental Section. The acid−base behavior of bicine, in aqueous solution and in different solvent mixtures, has been studied by us.[19] It was found that pK_{a2} = (8.121 ± 0.004), which agrees quite well that previously reported[2,20] after allowing for changes in experimental conditions as well as the method of calculation.

The proton dissociation constants of α-amino acids studied have also been determined potentiometrically from curves a and d. The values of pK_{a2} for monocarboxylic amino acids and pK_{a2} and pK_{a3} for dicarboxylic aspartic acid and glutamic acid, respectively, although already reported in ref 21, have been redetermined at (298.15 ± 0.1) K and I = 0.10 mol·dm^{-3} $NaNO_3$ to obtain values using the same experimental procedures as used in the study of binary and ternary systems and are in agreement with data found in the literature. It is worth mentioning that the pK_{a1} values of the amino acids investigated are too low (≤2.30)[22] and exist only in strongly acidic solutions. Therefore, these values are not used in calculations because the pH-metric data are measured in the range of 2.6 ≤ pH ≤ 10.5.

An analysis of complexed ligand curves c and e, as shown in Figure 1, indicates that the addition of a metal ion to

Table 1. Stability Constants of 1:1:1 Ternary Complexes of Bicine with Amino Acids at (298.15 ± 0.1) K and I = 0.1 mol·dm^{-3} $NaNO_3$

	$\log K^{M(bicine)}_{M(bicine)(aa)}$			$\log \beta^{M}_{M(bicine)(aa)}$			$\Delta \log K$		
ligands	Co(II)	Ni(II)	Cu(II)	Co(II)	Ni(II)	Cu(II)	Co(II)	Ni(II)	Cu(II)
glycine	6.43 ± 0.02	7.61 ± 0.06	9.41 ± 0.03	12.34	14.46	17.72	1.21	1.42	1.52
α-alanine	5.60 ± 0.04	6.71 ± 0.02	9.08 ± 0.05	11.51	13.56	17.39	0.97	1.23	1.24
leucine	4.82 ± 0.06	5.75 ± 0.04	8.20 ± 0.02	10.73	12.6	16.51	0.25	0.33	0.39
valine	4.69 ± 0.04	5.66 ± 0.04	7.97 ± 0.03	10.60	12.51	16.28	0.17	0.29	0.21
phenylalanine	4.51 ± 0.06	5.47 ± 0.06	7.61 ± 0.05	10.42	12.32	15.92	0.06	0.24	0.31
asparagine	3.93 ± 0.02	5.34 ± 0.03	7.55 ± 0.02	9.84	12.19	15.86	−0.10	0.18	0.28
glutamic acid	6.75 ± 0.05	7.81 ± 0.02	9.56 ± 0.06	12.66	14.66	17.87	1.49	1.57	1.64
aspartic acid	7.13 ± 0.03	8.07 ± 0.08	9.72 ± 0.03	13.04	14.92	18.03	1.58	1.63	1.75
β-alanine	3.39 ± 0.02	4.63 ± 0.05	7.06 ± 0.06	9.30	11.48	15.37	−0.19	0.17	0.25

the free-ligand solutions shifts the buffer region of the ligand to lower pH values. This shows that the complexation reaction proceeds by releasing protons from such ligands. Generally, it is observed that the binary metal complexes of bicine and amino acids begin to form in the pH ranges of 2.8 to 4.6 and 3.0 to 5.0, respectively. The complex solutions of such binary systems do not show any precipitation due to hydrolysis up to higher pH values, where nearly complete complex formation takes place. The stability constants of 1:1 binary complexes of bicine with the metal ions selected have been determined at (298.15 ± 0.1) K and I = 0.1 mol·dm^{-3} $NaNO_3$. The values obtained are more or less in good agreement with the literature data.[4,7]

The formation of a ternary complex is ascertained by comparing the mixed-ligand titration curve with the composite curve obtained by graphical addition of the amino acids' titration data to that of the (1:1) M(II)−bicine titration curve. The mixed-ligand system was found to deviate considerably from the resultant composite curve, indicating the formation of a ternary complex (Figure 1). Therefore, it is assumed that, in the presence of both ligands, bicine interacts first with the metal ion, followed by the interaction of the amino acid; that is, the ternary complex formation could be considered in stepwise complexation equilibria (eqs 2 and 3).

$$M^{2+} + (\text{bicine})^{-} \rightleftharpoons [M(\text{bicine})]^{+} \quad (2)$$

$$[M(\text{bicine})]^{+} + (\text{aa})^{-n} \rightleftharpoons [M(\text{bicine})(\text{aa})]^{1-n} \quad (3)$$

where aa represents amino acids, and the formation constants are then given by

$$K^{M}_{M(bicine)} = \frac{[M(\text{bicine})]}{[M][\text{bicine}]} \quad (4)$$

$$K^{M(bicine)}_{M(bicine)(aa)} = \frac{[M(\text{bicine})(\text{aa})]}{[M(\text{bicine})][\text{aa}]} \quad (5)$$

The overall stability constant $\beta^{M}_{M(bicine)(aa)}$ may be represented by eq 7.

$$M + \text{bicine} + \text{aa} \rightleftharpoons M(\text{bicine})(\text{aa}) \quad (6)$$

$$\beta^{M}_{M(bicine)(aa)} = \frac{[M(\text{bicine})(\text{aa})]}{[M][\text{bicine}][\text{aa}]} = K^{M(bicine)}_{M(bicine)(aa)} K^{M}_{M(bicine)} \quad (7)$$

The mean log $K^{M}_{M(bicine)}$, log $K^{M}_{M(aa)}$, and log $K^{M(bicine)}_{M(bicine)(aa)}$ values are determined from the corresponding experimental formation curves using the average value and straight line methods. The values obtained along with the estimated error using the least-squares method are given in Table 1.

Careful consideration of all presented data reveals that the stability constants of binary and ternary metal(II) complexes with the ligands studied follow the order Co(II) < Ni(II) < Cu(II), which is in accordance with Irving−William's order.[23]

An examination of stability constant values of the same metal ion ternary complexes (Table 1) reveals the following:

(i) Glutamate complexes are less stable than aspartate complexes, which might be a result of ring size because the glutamate formed five- and seven-membered rings whereas aspartate formed five- and six-membered rings in complex formation. As the ring size increases, the stability of the complexes decreases.[24] The stabilities of the glutamate and aspartate complexes are greater than those of glycine, α-alanine, leucine, valine, phenylalanine, and asparagine as a result of large difference in their basic strengths as well as their tendency to act as tridentate ONO.

(ii) The stability of ternary complexes involving glycine is higher than that of complexes containing α-alanine. This behavior does not follow the basicities as expected, probably because the pK_{a2} values of the amino acids are so similar. It is suggested that the steric hindrance, caused by the presence of a methyl group on the carbon bearing the amino group (α-alanine), is responsible for the lower stability of its ternary complexes.

(iii) The complex stability of same metal ion ternary complexes containing leucine, valine, phenylalanine, and asparagine follows the order leucine > valine > phenylalanine > asparagine. This behavior can be explained in terms of the effective basicity of the free conjugate base of these monocarboxylic amino acids (i.e., their propensity to act as σ donors).

(iv) The stability of β-alaninate complexes is minimum owing to the fact that β-alaninate formed six-membered rings.

Let us now consider the ability of a metal(II) ion to form ternary complexes with amino acids. This tendency may be appreciated by examining the specific increments of stability that account for the formation of mixed-ligand species relative to the corresponding parent species. The calculation of these increments is possible using eq 8.[25]

$$\Delta \log K = \log K^{M(bicine)}_{M(bicine)(aa)} - \log K^{M}_{M(aa)} \quad (8)$$

In general, positive Δ log K values for the systems indicated favored the formation of the M(bicine)(aa) ternary complexes over the corresponding binary complexes. This can be ascribed to interligand interactions or some cooperation between the primary and secondary ligands such as H-bond formation.

The concentration distribution of various complex species existing in solution as a function of pH can be obtained by means of the so-called (SPECIES program).[26] The species

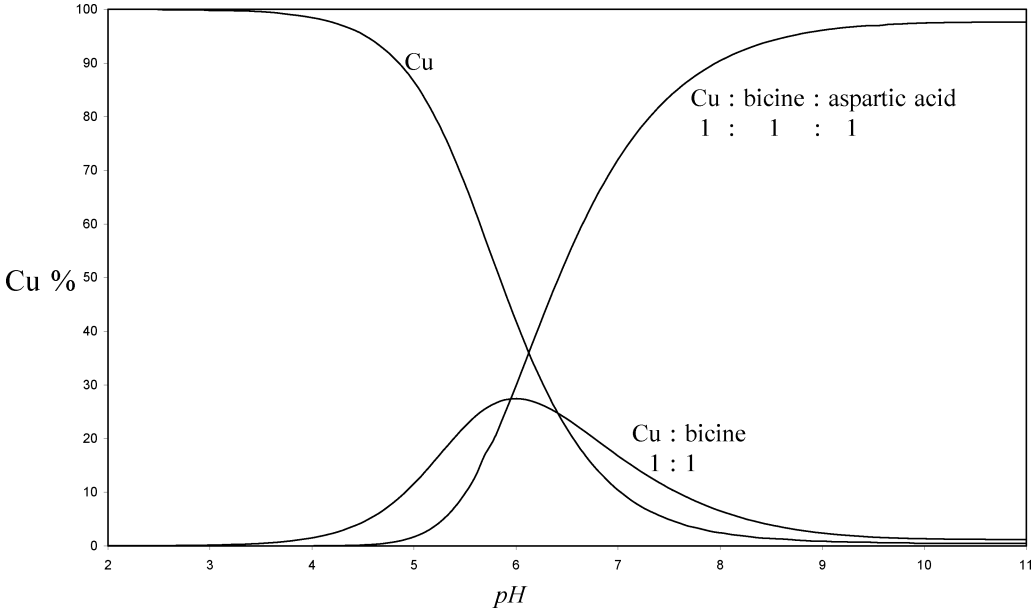

Figure 2. Concentration distribution of various species as a function of pH in the Cu(II) + bicine + aspartic acid system at (298.15 ± 0.1) K and I = 0.10 mol·dm^{-3} $NaNO_3$.

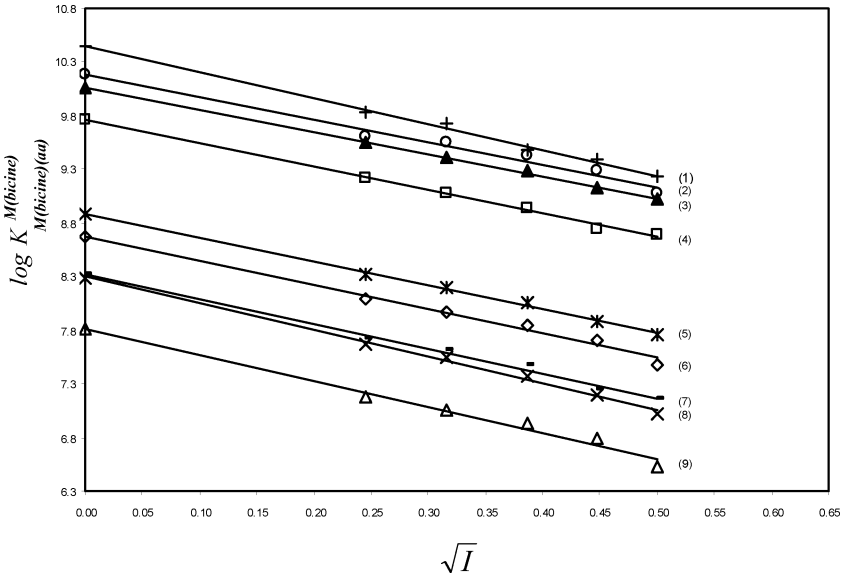

Figure 3. Plot of $K^{M(bicine)}_{M(bicine)(aa)}$ versus $\sqrt{I}$ at (298.15 ± 0.1) K. (1) aspartic acid; (2) glutamic acid; (3) glicine; (4) α-alanine; (5) leucine; (6) valine; (7) phenylalanine; (8) asparagine; (9) β-alanine.

Table 2. Stability Constants of Cu(II) + Bicine + Amino Acids at Different Ionic Strength ($NaNO_3$) and (298.15 ± 0.1) K

	I/mol·dm^{-3}					
ligands	0	0.06	0.1	0.15	0.2	0.25
glycine	10.06 ± 0.05	9.55 ± 0.03	9.41 ± 0.03	9.28 ± 0.02	9.13 ± 0.05	9.02 ± 0.03
α-alanine	9.76 ± 0.03	9.22 ± 0.05	9.08 ± 0.05	9.93 ± 0.05	8.74 ± 0.08	7.76 ± 0.06
leucine	8.88 ± 0.06	8.32 ± 0.04	8.20 ± 0.02	8.05 ± 0.02	7.88 ± 0.04	7.76 ± 0.06
valine	8.68 ± 0.02	8.09 ± 0.05	7.97 ± 0.03	7.84 ± 0.06	7.71 ± 0.08	7.48 ± 0.05
phenylalanine	8.32 ± 0.03	7.73 ± 0.02	7.61 ± 0.05	7.48 ± 0.08	7.25 ± 0.06	7.16 ± 0.04
asparagine	8.29 ± 0.08	7.67 ± 0.04	7.55 ± 0.02	7.37 ± 0.05	7.19 ± 0.05	7.02 ± 0.06
glutamic acid	10.18 ± 0.05	9.61 ± 0.05	9.56 ± 0.06	9.43 ± 0.04	9.28 ± 0.08	9.07 ± 0.08
aspartic acid	10.45 ± 0.06	9.84 ± 0.02	9.72 ± 0.03	9.49 ± 0.08	9.39 ± 0.06	9.23 ± 0.02
β-alanine	7.81 ± 0.04	7.18 ± 0.08	7.06 ± 0.06	6.93 ± 0.02	6.79 ± 0.02	6.53 ± 0.04

distribution for the Cu−bicine−aspartic acid system, taken as a representative, is given in Figure 2.

The ternary systems Cu(II) + bicine + amino acids were chosen for studying the effect of ionic strength on the stability of 1:1:1 ternary complexes. log $K^{M(bicine)}_{M(bicine)(aa)}$ values were determined at ionic strengths of 0.06, 0.10, 0.15, 0.20, and 0.25 (Table 2). Linear plots were obtained when log $K^{M(bicine)}_{M(bicine)(aa)}$ values were plotted against $\sqrt{I}$ (Figure 3) or $\sqrt{I}/(1+\sqrt{I})$ in accordance with the Debye−Hückel equation.[10]

$$\log K = \log K^{\circ} + \frac{A\Delta Z^2\sqrt{I}}{1+\sqrt{I}} + eI \qquad (9)$$

Table 3. Stability Constants of the Ternary System Cu(II) + Bicine + Amino Acids at Different Temperatures and I = 0.10 mol·dm^{-3} $NaNO_3$

	T/K			
ligands	298.15	310.15	318.15	328.15
glycine	9.41 ± 0.03	9.17 ± 0.02	9.01 ± 0.07	8.84 ± 0.08
α-alanine	9.08 ± 0.05	8.80 ± 0.03	8.68 ± 0.05	8.53 ± 0. 02
leucine	8.20 ± 0.02	7.97 ± 0.06	7.79 ± 0.05	7.63 ± 0.05
valine	7.97 ± 0.03	7.72 ± 0.05	7.53 ± 0.02	7.37 ± 0.08
phenylalanine	7.61 ± 0.05	7.40 ± 0.03	7.23 ± 0.05	7.07 ± 0.03
asparagine	7.55 ± 0.02	7.28 ± 0.05	7.08 ± 0.06	6.95 ± 0.05
glutamic acid	9.56 ± 0.06	9.31 ± 0.02	9.12 ± 0.03	9.00 ± 0.06
aspartic acid	9.72 ± 0.03	9.48 ± 0.07	9.32 ± 0.05	9.21 ± 0.08
β-alanine	7.06 ± 0.06	6.80 ± 0.05	6.64 ± 0.05	6.46 ± 0.05

Table 4. Thermodynamic Quantities of the Ternary System Cu(II) + Bicine + Amino Acids at I = 0.10 mol·dm^{-3} $NaNO_3$

ligands	$\Delta G°$/kJ·mol^{-1}	$\Delta H°$/kJ·mol^{-1}	$\Delta S°$/J·K^{-1}·mol^{-1}
glycine	53.72 ± 0.05	40.61 ± 0.11	44.28 ± 0.07
α-alanine	51.84 ± 0.03	39.12 ± 0.08	42.63 ± 0.08
leucineW	46.81 ± 0.06	37.01 ± 0.05	19.82 ± 0.10
valine	45.50 ± 0.05	33.34 ± 0.12	16.56 ± 0.06
phenylalanine	43.44 ± 0.08	28.68 ± 0.08	9.38 ± 0.12
asparagine	43.10 ± 0.03	24.05 ± 0.12	7.98 ± 0.10
glutamic acid	54.58 ± 0.02	41.17 ± 0.09	45.15 ± 0.07
aspartic acid	55.49 ± 0.06	47.35 ± 0.08	60.94 ± 0.08
β-alanine	40.30 ± 0.07	22.64 ± 0.08	7.56 ± 0.15

where $K°$ is the stability constant at infinite dilution, A is the Debye−Hückel constant, I is the ionic strength, ΔZ^2 is the difference in the sums of the squares of charges on product and reactant species, and e is an empirical parameter.

Thermodynamic equilibrium constants (at I = 0.00) were determined by extrapolation to zero ionic strength.

The thermodynamic quantities associated with the formation of 1:1:1 ternary complexes in the systems Cu(II) + bicine + amino acids were also studied at I = 0.1 mol·dm^{-3} $NaNO_3$.

The values of log $K^{M(bicine)}_{M(bicine)(aa)}$ at different temperatures (Table 3) show that the stability constants of the complexes decrease with increasing temperature. This behavior can be mainly ascribed to the thermal hydrolysis of the metal complexes.[10]

$\Delta H°$ values were calculated. $\Delta G°$ and $\Delta S°$ values were calculated from the following equations:

$$\Delta G° = -2.303TR \log K \quad (10)$$

and

$$\Delta G° = \Delta H° - T\Delta S° \quad (11)$$

It is shown from Table 4 that the strong ternary complexes are evidenced by the large magnitude of the stability constants and the exothermic nature of $\Delta H°$. The negative values of $\Delta G°$ and hence the spontaneity of ternary complexes of Cu(II) with the amino acids studied are attributed to the high, positive $\Delta S°$ term, hence the reaction is entropy favored.

It is known that solutions in biochemical microenvironments such as active sites of enzymes and side chains in proteins have dielectric constant values of 30 to 50. It was suggested that these properties approximately correspond to those (or can be simulated by those) existing in (water + dioxane).[27] Consequently, the investigation of stability constant of the ternary system Cu(II) + bicine + amino acid studied in (water + dioxane) is of biological significance.

The dielectric constant,[28] hydrogen bonding, solvent basicity, dispersion forces, and proton−solvent interaction effects are commonly recognized as influencing factors in the ionization constant of a ligand in a partial aqueous medium[29] and consequently the stability of a M−ligand complex. It was found that the log $K^{M(bicine)}_{M(bicine)(aa)}$ values increase as the amount of dioxane increases (i.e., the dielectric constant value decreases) (Table 5); therefore, the dielectric constant plays an important role in the determination of these values.

Confirmation of the ternary complexes of the type Cu(II) + bicine + glycine in solution has been carried out using differential pulse polarography (DPP) and square wave voltammetry (SWV).

A representative differential pulse polarogram for the system Cu(II) + bicine + glycine is given in Figure 4. The differential pulse polarograms of the Cu(II) solution show one cathodic peak at E_p = −68 mV. This peak may be described as a result of the reduction of Cu(II) to Cu (in a two-electron-transfer process) at the glassy carbon electrode.

The addition of primary or secondary ligands caused a slight shift of the cathodic peak to a more negative potential, indicating the formation of the binary and ternary complexes in solution.

The most interesting observation during the square wave voltammetric reduction of the Cu(II) + bicine + glycine ternary system is the discrimination of the two individual one-electron steps of Cu(II/I) and Cu(I/0) couples. Figure 5

Table 5. Stability Constants of the Ternary System Cu(II) + Bicine + Amino Acids in a Water (1) + Dioxane (2) Mixture at (298.15 ± 0.1) K and I = 0.1 mol·dm^3 $NaNO_3$

	$100w_1$			
ligands	00	10	30	50
glycine	9.41 ± 0.03	9.62 ± 0.03	9.83 ± 0.02	9.98 ± 0.03
α-alanine	9.08 ± 0.05	9.21 ± 0.05	9.51 ± 0.05	9.67 ± 0.08
leucine	8.20 ± 0.02	8.36 ± 0.04	8.68 ± 0.02	8.93 ± 0.06
valine	7.97 ± 0.03	8.17 ± 0.05	8.34 ± 0.06	8.55 ± 0.05
phenylalanine	7.61 ± 0.05	7.79 ± 0.02	7.93 ± 0.08	8.12 ± 0.04
asparagine	7.55 ± 0.02	7.69 ± 0.04	7.87 ± 0.05	8.04 ± 0.06
glutamic acid	9.56 ± 0.06	9.75 ± 0.05	9.91 ± 0.04	10.06 ± 0.08
aspartic acid	9.72 ± 0.03	9.89 ± 0.02	10.03 ± 0.08	10.22 ± 0.02
β-alanine	7.06 ± 0.06	7.37 ± 0.08	7.54 ± 0.02	7.74 ± 0.04

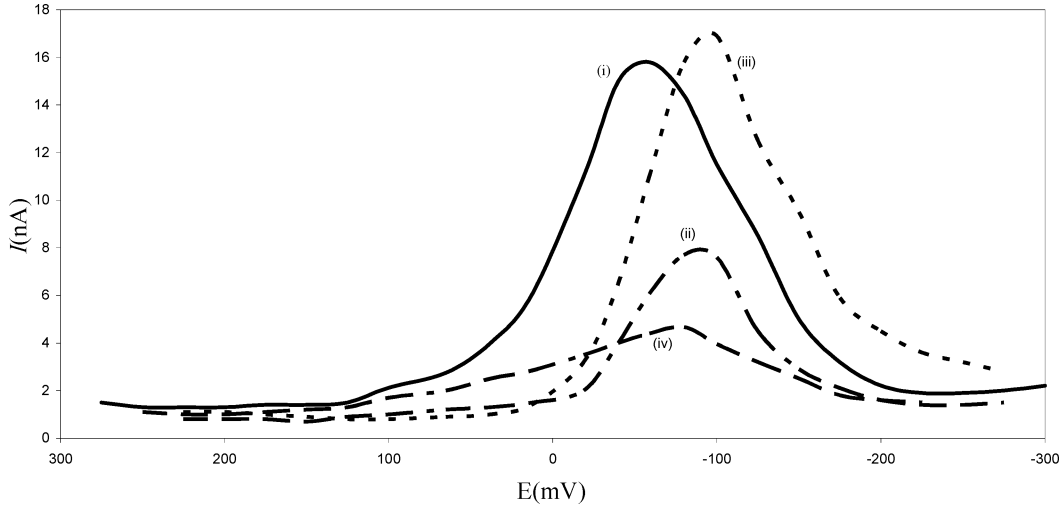

Figure 4. Differential pulse polarograms for the Cu(II) + bicine + glycine system at $I = 0.1$ mol·dm^{-3} $NaNO_3$, pH = 6.5 and (298.15 ± 0.1) K. (i) 0.001 mol·dm^{-3} Cu(II); (ii) 0.001 mol·dm^{-3} Cu(II) + 0.001 mol·dm^{-3} bicine; (iii) 0.001 mol·dm^{-3} Cu(II) + 0.001 mol·dm^{-3} glycine; (iv) 0.001 mol·dm^{-3} Cu(II) + 0.001 mol·dm^{-3} bicine + 0.001 mol·dm^{-3} glycine.

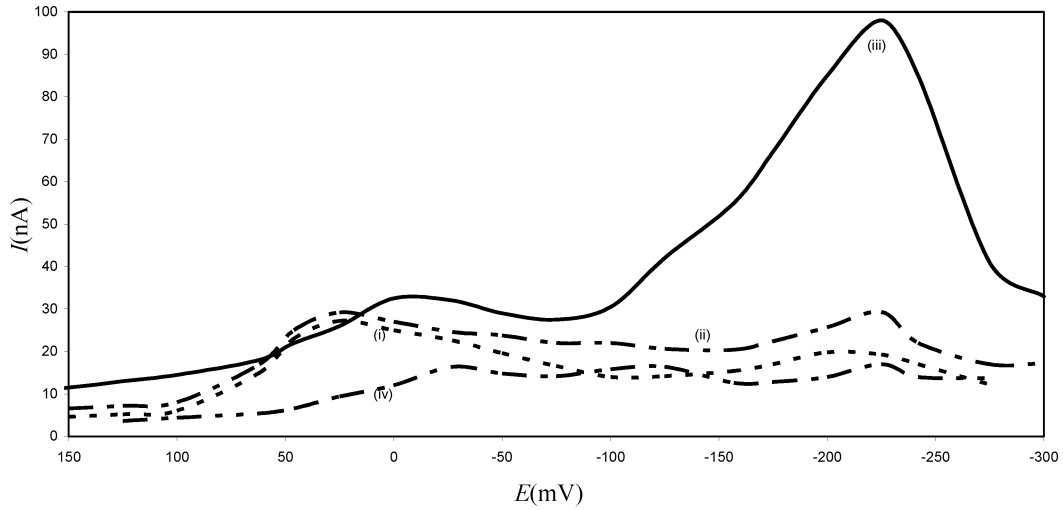

Figure 5. Square wave polarograms for the Cu(II) + bicine + glycine system at $I = 0.1$ mol·dm^{-3} $NaNO_3$, pH = 6.5 and (298.15 ± 0.1) K. (i) 0.001 mol·dm^{-3} Cu(II); (ii) 0.001 mol·dm^{-3} Cu(II) + 0.001 mol·dm^{-3} bicine; (iii) 0.001 mol·dm^{-3} Cu(II) + 0.001 mol·dm^{-3} glycine; (iv) 0.001 mol·dm^{-3} Cu(II) + 0.001 mol·dm^{-3} bicine + 0.001 mol·dm^{-3} glycine.

shows the square wave voltammogram for 1×10^{-3} mol·dm^{-3} Cu(II) in the absence and in the presence of primary and secondary ligands in the above-mentioned ternary complexes. By adding primary and secondary ligands, we observed a more electronegative peak, and the two simple Cu(II) peaks become smaller. The new peaks confirm the formation of ternary Cu(II) + bicine + glycine. An increase in the current was observed on the addition of the ligand because of the adsorption of Cu(II)−bicine complexes. Then, the current is constant when the surface coverage is reached. Therefore, the shift in peak potential may be due to the complex formation. Further addition of the secondary ligands modified the square wave voltammograms because of the formation of the ternary complexes in solution.

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