# Metal Ion−Buffer Interactions. Complex Formation of *N,N*-bis(2-Hydroxyethyl)glycine (Bicine) with Various Biologically Relevant Ligands

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Ternary 1:1:1 complexes of some metal ions Cu(II), Ni(II), and Co(II) with *N,N*-bis(2-hydroxyethyl)glycine (bicine) and some aliphatic carboxylic acids (succinic, oxalic, malic, maleic, and tartaric), aromatic carboxylic acids (5-sulfosalicylic, salicylic, and phthalic), and hydroxamic acids (benzohydroxamic acid and salicylhydroxamic acid) are described. The formation constants of the 1:1:1 ternary complexes were determined potentiometrically at (298.15 ± 0.1) K and 0.10 mol·dm$^{-3}$ ($NaNO_3$) ionic strength. The ternary complex formation was found to take place in a stepwise manner. The stability of the ternary complex is also discussed in relation to that of the binary complexes of secondary ligands. An evaluation of the effects of temperature and ionic strength of the medium on the stability of the ternary system Cu(II) + bicine + ligands has been studied. The thermodynamic parameters ($\Delta H°$, $\Delta G°$, and $\Delta S°$) were calculated and discussed. The stability constant of the above-mentioned ternary system has been investigated in a dioxane−water solution. Confirmation of the ternary complexes in solution has been carried out using differential pulse polarography, square wave voltammetry, conductometric measurements, and V-visible spectroscopic measurements.

## Introduction

In the laboratory, buffer solutions are used to calibrate pH meters and to control the pH of solutions in which pH-dependent chemical reactions take place. Similarly, life processes, for instance, those involving enzymatic reactions, can only take place in a narrow range of pH. Because some of these reactions either consume or produce hydronium ions, it is essential that the pH be strictly controlled by buffer systems. The use of buffers based on inorganic or organic salts is limited because of the interference of buffer cations and anions with the biological reaction under study. The development and introduction of the zwitterionic biological buffers by Good et al.[1] did much to change this situation. The p$K_a$ and the buffer range of the zwitterionic substances lie within the physiological limits (p$Ka$ 6.15 to 9.55). We have, therefore, investigated an ampholyte [*N,N*-bis(2-hydroxyethyl)glycine; bicine], 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, and it has also been recommended as part of buffer combinations, for example, for column isoelectric focusing. Furthermore, it has been employed in color photographic processes, in analytical methods, as a stabilizing agent, and so forth.[3]

Inorganic and organic buffer 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. For many years it has been recognized[1] that using bicine as a buffer in systems also containing metal ions requires that the interactions of bicine with these metal ions must be considered. However, the awareness that buffer substances may form not only binary but also ternary metal ion complexes is of much more recent origin.[3−7] Recently, stabilities of binary and ternary complexes involving bicine and some selected amino acids have been reported by us[8] using a potentiometric technique.

## Experimental Section

***Materials and Solutions.*** Bicine and benzohydroxamic acid were Sigma products. Salicylhydroxamic acid was purchased in pure form from Nasr Pharmaceutical Chemicals Co., Egypt. Phthalic acid, salicylic acid, 5-sulfosalicylic acid, succinic acid, oxalic acid, malic acid, maleic acid, and tartaric acid were analytical-grade (Aldrich or Merck) products. The metal salts were provided by BDH as nitrate or chlorides. Stock solutions of the metal salts were prepared in deionized water, and the metal concentration was obtained by standard analytical methods.[9] A carbonate-free sodium hydroxide (titrant, prepared in 0.1 mol·dm$^{-3}$ $NaNO_3$ solution) was standardized potentiometrically with KH phthalate (Merck AG). A nitric acid solution (≈ 0.03 mol·dm$^{-3}$ ) was prepared and used after standardization. Sodium hydroxide, nitric acid, 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. 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$^{-3}$ $NaNO_3$).[10] 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 following solutions were prepared (total volume 50 cm$^3$) and titrated potentiometrically against a standard

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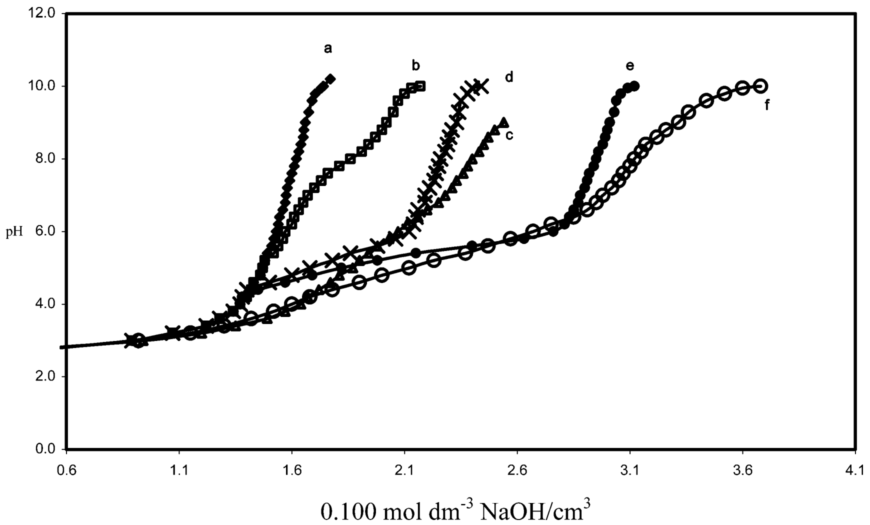


**Figure 1.** Potentiometric titration curves for the Cu(II) + bicine + succinic acid system at (298.15 ± 0.1) K and $I = 0.1$ mol·dm$^{-3}$ $NaNO_3$, 0.003 mol·dm$^{-3}$ $HNO_3$ + 0.1 mol·dm$^{-3}$ $NaNO_3$, a; solution a + 0.001 mol·dm$^{-3}$ bicine, b; solution b + 0.001 mol·dm$^{-3}$ Cu(II), c; solution a + 0.001 mol·dm$^{-3}$ succinic acid, d; solution d + 0.001 mol·dm$^{-3}$ Cu(II), e; and solution a + 0.001 mol·dm$^{-3}$ bicine + 0.001 mol·dm$^{-3}$ succinic acid + 0.001 mol·dm$^{-3}$ Cu(II), f.

$CO_2$-free NaOH (0.10 mol·dm$^{-3}$): 0.003 mol·dm$^{-3}$ $HNO_3$ + 0.1 mol·dm$^{-3}$ $NaNO_3$ (a), solution a + 0.001 mol·dm$^{-3}$ bicine (b), solution b + 0.001 mol·dm$^{-3}$ M(II) (c), solution a + 0.001 mol·dm$^{-3}$ the other ligands studied (d), solution d + 0.001 mol·dm$^{-3}$ M(II) (e), and solution a + 0.001 mol·dm$^{-3}$ bicine + 0.001 mol·dm$^{-3}$ the other ligands studied + 0.001 mol·dm$^{-3}$ M(II) (f). 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. A magnetic stirrer was used during all titrations. The pH-metric titrations were carried out at the desired temperature in a purified nitrogen atmosphere. The titration was repeated at least four times for each titration curve.

The pH titrations were terminated when the pH readings became unstable, showing a downward drift. In all cases, no calculations have been performed beyond the precipitation point; hence, the hydroxyl species likely to be formed after this point could not be studied. The initial estimates of the stability constants of the binary and ternary complexes formed in solution have been determined using the Irving and Rossotti formula.[11,12]

Initial estimates of the stability constants of the binary and ternary complexes formed in solution have been refined with the clinp 2.1 computer program.[13,14] Standard deviations were also evaluated for the corresponding equilibrium constants. The concentration distribution of various complex species existing in solution as a function of pH was obtained using the SPECIES program.[15]

The pH meter readings have been corrected in accordance with the method described by Douhéret.[16,17] Thus, if the pH meter is standardized using aqueous solution, the meter reading $pH_{(R)}$ obtained in a partially aqueous medium differs by an amount $\delta$ from the corrected reading pH* which is referred to the standard state in the partially aqueous solvent ($pH^* = pH_{(R)} - \delta$). In a typical experiment, a sample volume of 50 cm$^{-3}$ containing 0.003 mol·dm$^{-3}$ $HNO_3$ in the presence of 10, 30, and 50% (w/w) of dioxane (a), 0.003 mol·dm$^{-3}$ $HNO_3$ + 0.001 mol·dm$^{-3}$ bicine in the presence of 10, 30, and 50% (w/w) of dioxane (b), 0.003 mol·dm$^{-3}$ $HNO_3$ + 0.001 mol·dm$^{-3}$ bicine + 0.001 mol·dm$^{-3}$ M (II) in the presence of 10, 30, and 50% (w/w) of dioxane (c), 0.003 mol·dm$^{-3}$ $HNO_3$ + 0.001 mol·dm$^{-3}$ the other ligands studied in the presence of 10, 30, and 50% (w/w) of dioxane (d), 0.003 mol·dm$^{-3}$ $HNO_3$ + 0.001 mol·dm$^{-3}$ the other ligands studied + 0.001 mol·dm$^{-3}$ M(II) in the presence of 10, 30, and 50% (w/w) of dioxane (e), and 0.003 mol·dm$^{-3}$ $HNO_3$ + 0.001 mol·dm$^{-3}$ bicine + 0.001 mol·dm$^{-3}$ the other ligands studied + 0.001 mol·dm$^{-3}$ M(II) in the presence of 10, 30, and 50% (w/w) of dioxane (f) was used. The ionic strength of the studied solutions was adjusted at 0.10 mol·dm$^{-3}$ using a $NaNO_3$ solution and titrated individually against 0.10 mol·dm$^{-3}$ NaOH, prepared in the ionic medium used for the test solution.

***Electrochemical Measurements.*** Square wave voltammetry (SWV) 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.

***Conductometric Measurements.*** Conductometric titrations were followed with a SUNTEX conductivity meter SC −170.

The following mixture was titrated conductometrically against a 0.10 mol·dm$^{-3}$ NaOH solution: 0.01 mol·dm$^{-3}$ Cu(II) (10 cm$^3$) + 0.01 mol·dm$^{-3}$ salicylhydroxamic acid (10 cm$^3$) + 0.01 mol·dm$^{-3}$ bicine (10 cm$^3$).

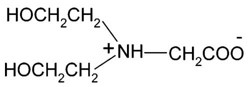
***Spectrophotometric Measurements.*** Electronic spectra were recorded using a Perkin-Elmer lambda 2 spectrophotometer.

## Results and Discussion

The typical titration curves a−f for the system Cu(II) + bicine + succinic acid are shown in Figure 1. Similar curves were observed for other systems as well. For brevity, the titration curves for other metal ion systems are not included.

***Proton Ligand Systems.*** The potentiometric titration curve of bicine (curve b, Figure 1) shows a sharp inflection

**Chart 1**



around pH 8 indicating the dissociation of only one proton (p$K_{a2}$), corresponding to the cationic ($\equiv N^+H$) group. The acid−base behavior of bicine, in aqueous solution and in different solvent mixtures, has been studied by us.[18] It was found that the p$K_{a2}$ equals (8.121 ± 0.004), and it agrees quite well with that previously reported.[1]

Bicine, being a zwitterionic amino acid, may be expected to exist in the form of its zwitterions, which may structurally be represented as follows in Chart 1.

It is possible that the hydroxyl groups are able to form a hydrogen bond with the basic nitrogen atom and, thus, assist in the dissociation of ammonium hydrogen.

The protonation constants of the aliphatic carboxylic acids, aromatic carboxylic acids, and hydroxamic acids have also been redetermined at (298.15 ± 0.1) K and $I = 0.1$ mol·dm$^{-3}$ $NaNO_3$ to obtain values using the same experimental procedures as those used in the binary and ternary system and are in agreement with data found in ref 19.

***Binary Systems.*** Potentiometric pH titrations of Cu(II), Ni(II), and Co(II) were performed at 1:1 metal/ligand molar ratios. Analysis of the complexed ligands curves c and e as shown in Figure 1 indicates that the addition of metal ion to the free ligand solutions shifts the buffer region of the ligand to lower pH values. This shows that the complex formation reaction proceeds by releasing protons from such ligands.

The stability constants of 1:1 binary complexes of the ligands studied have been determined at (298.15 ± 0.1) K and $I = 0.10$ mol·dm$^{-3}$ $NaNO_3$ (Table 1). The values obtained are more or less in good agreement with the literature data.[19]

***Ternary Systems.*** The use of bicine as a buffer substance in biochemical studies, which are normally carried out under multiligand conditions and which often also contain metal ions, shows the need to consider also the formation of mixed ligand complexes involving bicinate as a second ligand. Therefore, we studied as an example of the ternary systems with some aliphatic carboxylic acids (succinic, oxalic, malic, maleic, and tartaric), aromatic carboxylic acids (5-sulfosalicylic, salicylic, and phthalic), and hydroxamic acids (benzohydroxamic acid and salicylhydroxamic acid), because these systems mimic many biological reactions.

The formation of ternary complexes was inferred from the pH-metric curves. It was deduced that bicine acts as a primary ligand in the ternary complexes involving aliphatic carboxylic acids and hydroxamic acids, whereas it behaves as a secondary ligand in ternary systems containing aromatic carboxylic acids and the Cu + salicylhydroxamic acid + bicine system; that is, the ternary complex formation could be considered in stepwise complexation equilibria:

$$M + A \rightleftharpoons MA \tag{1}$$

$$MA + L \rightleftharpoons MAL \tag{2}$$

$$K_{MAL}^{MA} = \frac{[MAL]}{[MA][L]} \tag{3}$$

where A represents primary ligand and L represents the secondary ligand; that is, examining Figure 1, one may observe that the curves obtained for the different 1:1:1 ternary complex solutions (curve f) overlap with the titration curve of the 1:1 binary Ni(bicine)$^-$ (curve c) at low pH values and a divergence of the ternary complex titration curve from that of the binary Ni(bicine)$^-$ is observed. This shows the coordination of the succinic acid to the Ni(bicine)$^-$ binary complex in a stepwise manner as represented by the following equations:

$$Ni^{2+} + (\text{bicine})^- \rightleftharpoons [Ni(\text{bicine})]^+ \tag{4}$$

$$[Ni(\text{bicine})]^+ + (\text{succinic acid})^{-2} \rightleftharpoons [Ni(\text{bicine})(\text{succinic acid})]^- \tag{5}$$

The overall stability constant $\beta_{MAL}^{M}$ may be represented by eq 6.

$$M + A + L \rightleftharpoons MAL \tag{6}$$

$$\beta_{MAL}^{MA} = \frac{[MAL]}{[MA][L]} = K_{MAL}^{MA} K_{MA}^{M} \tag{7}$$

Stability constants in terms of divalent metal ions (Table 1) fall in the Irving−Williams order:[20] Co(II) < Ni(II) < Cu(II). The higher values of log $K$ in the case of Cu(II) chelates are due to Jahn−Teller effect.

The complex stability of the same metal ion ternary complexes containing aliphatic and aromatic acids follows the order phthalic > salicylic > 5-sulfosalicylic > succinic > malic > maleic > tartaric > oxalic acid.

The higher stability of the phthalic acid complex than that of salicylic acid may be explained as follows: because the carboxylate oxygen is not directly bound to the benzene ring, it, therefore, adjusts stereochemically more easily than the phenolate oxygen which is directly attached to the benzene nucleus. The Coulombic repulsion between the end oxygens will be more when both O,O donor atoms are phenolic oxygens than when they are carboxylic oxygens.

The relative stabilities of the ternary complexes of salicylic and 5-sulfosalicylic acid follow these relative basicities. The lower stability of the complexes involving 5-sulfosalicylic acid may be ascribed to the presence of the electron-withdrawing sulfonic group.

With respect to aliphatic acids, the order of stabilities of their mixed-ligand complexes is in accord with the basicities (p$K_{a1}$ + p$K_{a2}$) of the ligands. It is well-know that the increase in basicity of a ligand increases the stability of its metal complexes.

The observed order of stability of ternary systems with respect to the ligand hydroxamic acid is salicylhydroxamic acid > benzohydroxamic acid. The increased stability of the salicylhydroxamate ternary complexes relative to those of the other ligands may be ascribed to an additional interaction of the phenolic group with the metal ion.

The relative stability of the ternary, as compared to that of the corresponding binary, complexes can be quantitatively expressed in different ways. A review of those methods[21] has shown that, for a variety of reasons, the most suitable comparison is in the stabilities of the ternary complexes in terms of Δ log $K$ as defined by eq 8.

$$\Delta \log K = \log K_{MAL}^{MA} - \log K_{ML}^{M} \tag{8}$$

In general, positive Δ log $K$ values for the systems indicated favored formation of the M(A)(L) ternary complexes over the corresponding binary ones. This can be

**Table 1. Stability Constants of the Binary and Ternary Complexes of Bicine with the Ligand Studied at (298.15 ± 0.1) K, $I$ = 0.10 mol·dm$^{-3}$ NaNO$_3$**

| | $K^{M}_{MA}$ or $\log K^{M}_{ML}$ | | | $\log K^{MA}_{MAL}$ | | | $\log \beta^{M}_{MAL}$ | | | $\Delta \log K$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ligand | Cu(II) | Ni(II) | Co(II) | Cu(II) | Ni(II) | Co(II) | Cu(II) | Ni(II) | Co(II) | Cu(II) | Ni(II) | Co(II) |
| bicine | 8.31 ± 0.04 | 6.85 ± 0.03 | 5.91 ± 0.03 | | | | | | | | | |
| phthalic | 3.45 ± 0.06 | 3.26 ± 0.04 | 3.00 ± 0.08 | 8.88 ± 0.03 | 7.62 ± 0.06 | 6.07 ± 0.04 | 12.33 | 10.88 | 9.07 | 0.27 | 0.77 | 0.16 |
| salicylic | 10.63 ± 0.02 | 8.65 ± 0.06 | 7.22 ± 0.06 | 8.39 ± 0.04 | 7.24 ± 0.04 | 5.98 ± 0.05 | 19.02 | 15.89 | 13.20 | 0.08 | 0.39 | 0.07 |
| 5-sulfosalicylic | 9.51 ± 0.03 | 6.35 ± 0.05 | 6.16 ± 0.05 | 7.88 ± 0.06 | 7.03 ± 0.06 | 5.76 ± 0.03 | 17.39 | 13.38 | 11.92 | −0.43 | 0.18 | −0.15 |
| succinic | 3.20 ± 0.04 | 3.12 ± 0.03 | 2.96 ± 0.03 | 6.42 ± 0.04 | 5.49 ± 0.04 | 4.88 ± 0.04 | 14.73 | 12.38 | 10.79 | 3.22 | 2.37 | 1.92 |
| malic | 5.14 ± 0.06 | 4.60 ± 0.08 | 3.13 ± 0.03 | 6.11 ± 0.03 | 5.17 ± 0.06 | 4.62 ± 0.05 | 14.42 | 12.02 | 10.53 | 0.97 | 0.57 | 1.49 |
| maleic | 4.07 ± 0.05 | 3.70 ± 0.02 | 3.02 ± 0.05 | 5.95 ± 0.02 | 5.08 ± 0.05 | 4.31 ± 0.06 | 14.26 | 11.93 | 10.22 | 1.88 | 1.38 | 1.29 |
| tartaric | 5.16 ± 0.03 | 4.68 ± 0.08 | 3.17 ± 0.04 | 5.71 ± 0.06 | 4.86 ± 0.02 | 4.08 ± 0.04 | 14.02 | 11.71 | 9.99 | 0.55 | 0.18 | 0.91 |
| oxalic | 4.60 ± 0.08 | 3.46 ± 0.03 | 3.11 ± 0.03 | 5.37 ± 0.02 | 4.61 ± 0.08 | 3.87 ± 0.03 | 13.68 | 11.46 | 9.78 | 0.77 | 1.15 | 0.76 |
| salicylhydroxamic | 13.03 ± 0.04 | 6.02 ± 0.04 | 6.59 ± 0.02 | 8.60 ± 0.02 | 6.92 ± 0.05 | 6.78 ± 0.06 | 21.63 | 13.77 | 12.69 | 0.29 | 0.90 | 0.19 |
| benzohydroxamic | 7.63 ± 0.06 | 5.05 ± 0.02 | 4.75 ± 0.03 | 8.05 ± 0.03 | 6.44 ± 0.06 | 5.83 ± 0.04 | 16.36 | 13.29 | 11.74 | 0.42 | 1.39 | 1.08 |

**Table 2. Stability Constants of the Ternary System Cu(II) + Bicine + the Ligand Studied at Different Temperatures and $I$ = 0.10 mol·dm$^{-3}$ NaNO$_3$**

| | $T$/K | | | |
|---|---|---|---|---|
| ligand | 298.15 | 310.15 | 318.15 | 328.15 |
| phthalic | 8.88 ± 0.03 | 8.61 ± 0.02 | 8.29 ± 0.06 | 8.02 ± 0.03 |
| salicylic | 8.39 ± 0.04 | 8.04 ± 0.02 | 7.78 ± 0.05 | 7.47 ± 0.05 |
| 5-sulfosalicylic | 7.88 ± 0.06 | 7.54 ± 0.03 | 7.20 ± 0.03 | 6.99 ± 0.03 |
| succinic | 6.42 ± 0.04 | 6.15 ± 0.06 | 5.88 ± 0.02 | 5.64 ± 0.02 |
| malic | 6.11 ± 0.03 | 5.75 ± 0.03 | 5.54 ± 0.04 | 5.37 ± 0.05 |
| maleic | 5.95 ± 0.02 | 5.55 ± 0.05 | 5.36 ± 0.03 | 5.11 ± 0.04 |
| tartaric | 5.71 ± 0.06 | 5.34 ± 0.03 | 5.09 ± 0.06 | 4.89 ± 0.05 |
| oxalic | 5.37 ± 0.02 | 5.06 ± 0.05 | 4.76 ± 0.04 | 4.43 ± 0.06 |
| salicylhydroxamic | 8.60 ± 0.02 | 8.29 ± 0.02 | 7.98 ± 0.05 | 7.80 ± 0.03 |
| benzohydroxamic | 8.05 ± 0.03 | 7.67 ± 0.05 | 7.42 ± 0.04 | 7.13 ± 0.02 |

ascribed to interligand interactions or some cooperativity between the primary and secondary ligands such as H-bond formation.

The equilibrium concentration distribution diagrams of various complex species provide a useful picture of metal ions binding in the biological system. In all species investigated the concentration of 1:1:1 ternary complexes increases with increasing pH under our experimental conditions (2.6 < pH < 10.0), which might be a result of the ternary metal complex solutions investigated not showing precipitation even at high pH values. This reveals that the different 1:1:1 ternary metal complexes studied have no tendency to form hydroxo complex species. That makes complex formation more favorable in the physiological pH range. To indicate the main features observed in the species distribution plots in these system, the speciation diagram obtained for the Cu(II) + salicylhydroxamic acid + bicine system is shown in Figure 2.

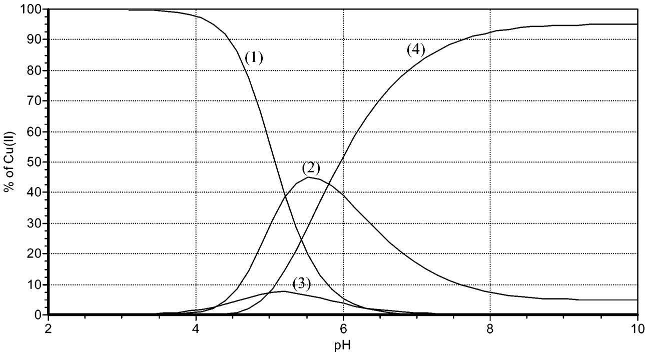


**Figure 2.** Concentration distribution of various species as a function of pH in the Cu(II) + salicylhydroxamic + bicine system at (298.15 ± 0.1) K and $I$ = 0.1 mol·dm$^{-3}$ NaNO$_3$, Cu(II), 1; Cu(salicylhydroxamic), 2; Cu(bicine), 3; and Cu(salicylhydroxamic)(bicine), 4.

**Table 3. Thermodynamic Quantities of the Ternary System Cu(II) + Bicine + the Ligand Studied at $I$ = 0.10 mol·dm$^{-3}$ NaNO$_3$**

| ligand | $-\Delta G$/kJ mol$^{-1}$ | $-\Delta H$/kJ mol$^{-1}$ | $\Delta S$/J K$^{-1}$ mol$^{-1}$ |
|---|---|---|---|
| phthalic | 50.69 ± 0.03 | 66.42 ± 0.04 | 36.08 ± 0.03 |
| salicylic | 47.90 ± 0.06 | 65.03 ± 0.02 | 32.75 ± 0.05 |
| 5-sulfosalicylic | 44.98 ± 0.03 | 59.43 ± 0.06 | 67.89 ± 0.04 |
| succinic | 36.65 ± 0.04 | 55.90 ± 0.02 | 63.81 ± 0.03 |
| malic | 34.88 ± 0.03 | 53.76 ± 0.03 | 63.26 ± 0.04 |
| maleic | 33.97 ± 0.02 | 51.39 ± 0.05 | 85.13 ± 0.06 |
| tartaric | 32.60 ± 0.05 | 50.52 ± 0.02 | 90.02 ± 0.05 |
| oxalic | 30.66 ± 0.06 | 66.42 ± 0.03 | 118.91 ± 0.03 |
| salicylhydroxamic | 49.09 ± 0.05 | 65.44 ± 0.05 | 45.15 ± 0.05 |
| benzohydroxamic | 45.96 ± 0.03 | 64.14 ± 0.02 | 63.83 ± 0.02 |

The values of $\log K^{MA}_{MAL}$, at different temperatures (Table 2), 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.[22]

The thermodynamic quantities associated with the formation of 1:1:1 ternary complexes in the systems Cu(II) + bicine + other ligands were also studied at $I = 0.1$ mol·dm$^{-3}$ NaNO$_3$.

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

$$\Delta G° = -2.303TR \log K \tag{9}$$

and

$$\Delta G° = \Delta H° - T\Delta S° \tag{10}$$

It is shown from Table 3 that the strong ternary complexes are evidenced by the large magnitude of 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 ligands studied are attributed to the high positive $\Delta S°$ term; hence, the reaction is entropy-favored.

The ternary systems Cu(II) + bicine + the other ligands were chosen for studying the effect of ionic strength on the stability of 1:1:1 ternary complexes. The $\log K^{MA}_{MAL}$ values were determined at the ionic strengths of 0.06, 0.10, 0.15, 0.20, and 0.25 mol·dm$^{-3}$ NaNO$_3$ (Table 4). Linear plots were obtained when $\log K^{MA}_{MAL}$ values were plotted against $I^{1/2}$ or $I^{1/2}/(1 + 2)^{1/2}$ in accordance with the Debye−Hückel equation.[22]

$$\log K = \log K° + \frac{A\Delta Z^2 I^{1/2}}{1 + I^{1/2}} \tag{11}$$

**Table 4. Stability Constants of Cu(II) + Bicine + the Ligands Studied at Different Ionic Strengths ($NaNO_3$) and (298.15 ± 0.1) K**

| | $I$/mol·dm$^{-3}$ | | | | | |
|---|---|---|---|---|---|---|
| ligand | 0 | 0.06 | 0.1 | 0.15 | 0.2 | 0.25 |
| phthalic | 9.72 ± 0.02 | 9.04 ± 0.03 | 8.88 ± 0.03 | 8.66 ± 0.03 | 8.50 ± 0.02 | 8.36 ± 0.03 |
| salicylic | 9.00 ± 0.03 | 8.53 ± 0.06 | 8.39 ± 0.04 | 8.27 ± 0.05 | 8.16 ± 0.03 | 8.03 ± 0.04 |
| 5-sulfosalicylic | 8.63 ± 0.04 | 8.03 ± 0.04 | 7.88 ± 0.06 | 7.74 ± 0.03 | 7.56 ± 0.03 | 7.42 ± 0.08 |
| succinic | 7.17 ± 0.03 | 6.65 ± 0.03 | 6.42 ± 0.04 | 6.26 ± 0.05 | 6.17 ± 0.04 | 6.05 ± 0.03 |
| malic | 6.88 ± 0.05 | 6.34 ± 0.02 | 6.11 ± 0.03 | 5.95 ± 0.04 | 5.86 ± 0.08 | 5.72 ± 0.05 |
| maleic | 6.68 ± 0.03 | 6.18 ± 0.02 | 5.95 ± 0.02 | 5.79 ± 0.06 | 5.70 ± 0.03 | 5.60 ± 0.03 |
| tartaric | 6.45 ± 0.03 | 5.94 ± 0.05 | 5.71 ± 0.06 | 5.55 ± 0.03 | 5.44 ± 0.05 | 5.36 ± 0.03 |
| oxalic | 6.10 ± 0.06 | 5.60 ± 0.08 | 5.37 ± 0.02 | 5.21 ± 0.03 | 5.12 ± 0.04 | 5.02 ± 0.02 |
| salicylhydroxamic | 9.32 ± 0.02 | 8.79 ± 0.04 | 8.60 ± 0.02 | 8.44 ± 0.06 | 8.36 ± 0.02 | 8.20 ± 0.02 |
| benzohydroxamic | 8.80 ± 0.03 | 8.25 ± 0.03 | 8.05 ± 0.03 | 7.92 ± 0.04 | 7.79 ± 0.08 | 7.65 ± 0.03 |

**Table 5. Stability Constants of the Ternary System Cu(II) + Bicine + the Ligands Studied in the Water (1) + Dioxane (2) Mixture at (298.15 ± 0.1) K, $I = 0.1$ mol·dm$^{-3}$ $NaNO_3$**

| | $100W_1$ | | | |
|---|---|---|---|---|
| ligand | 00 | 10 | 30 | 50 |
| phthalic | 8.88 ± 0.03 | 9.06 ± 0.02 | 9.35 ± 0.04 | 9.68 ± 0.06 |
| salicylic | 8.39 ± 0.04 | 8.55 ± 0.03 | 8.83 ± 0.02 | 9.10 ± 0.03 |
| 5-sulfosalicylic | 7.88 ± 0.06 | 8.05 ± 0.02 | 8.32 ± 0.03 | 8.59 ± 0.02 |
| succinic | 6.42 ± 0.04 | 6.57 ± 0.04 | 6.90 ± 0.03 | 7.17 ± 0.06 |
| malic | 6.11 ± 0.03 | 6.30 ± 0.06 | 6.61 ± 0.05 | 6.94 ± 0.03 |
| maleic | 5.95 ± 0.02 | 6.11 ± 0.05 | 6.42 ± 0.04 | 6.71 ± 0.08 |
| tartaric | 5.71 ± 0.06 | 5.90 ± 0.04 | 6.17 ± 0.02 | 6.44 ± 0.03 |
| oxalic | 5.37 ± 0.02 | 5.57 ± 0.06 | 5.94 ± 0.03 | 6.28 ± 0.03 |
| salicylhydroxamic | 8.60 ± 0.02 | 8.83 ± 0.08 | 9.06 ± 0.06 | 9.33 ± 0.08 |
| benzohydroxamic | 8.05 ± 0.03 | 8.22 ± 0.03 | 8.47 ± 0.02 | 8.72 ± 0.04 |

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

Thermodynamic equilibrium constants (at $I = 0.00$) were determined by extrapolation to 0 ionic strength.

Nowadays it is well-established that in proteins in the active-site cavities of enzymes the "effective" or "equivalent solution" dielectric constant is reduced compared to the presence of aliphatic and aromatic amino acid side chains at the protein−water interface. Estimates for the dielectric constants in such locations range from about 30 to 70.[23] It was suggested that these properties approximately correspond to those (or can be simulated by those) existing in the water + dioxane.[24] Consequently, the investigation of the stability constant of the ternary system Cu(II) + bicine + other ligands studied in water + dioxane is of biological significance.

The dielectric constant,[25] 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[26] and consequently the stability of a M−ligand complex. It was found that the log $K^{MA}_{MAL}$ 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) + salicylhydroxamic acid + bicine in solution has been carried out using differential pulse polarography, SWV, and conductometric measurements.

A representative differential pulse polarogram for the system Cu(II) + salicylhydroxamic acid + bicine is given in Figure 3. 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 GC electrode.

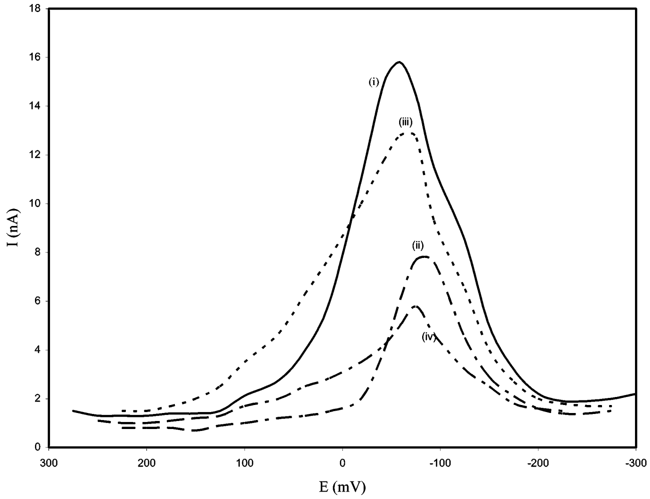


**Figure 3.** Differential pulse polarograms for the Cu(II) + salicylhydroxamic + bicine system at $I = 0.1$ mol·dm$^{-3}$ $NaNO_3$, pH = 6.5, at (298.15 ± 0.1) K, 0.001 mol·dm$^{-3}$ Cu(II), i; 0.001 mol·dm$^{-3}$ Cu(II)+ 0.001 mol·dm$^{-3}$ bicine, ii; 0.001 mol·dm$^{-3}$ Cu(II) + 0.001 mol·dm$^{-3}$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

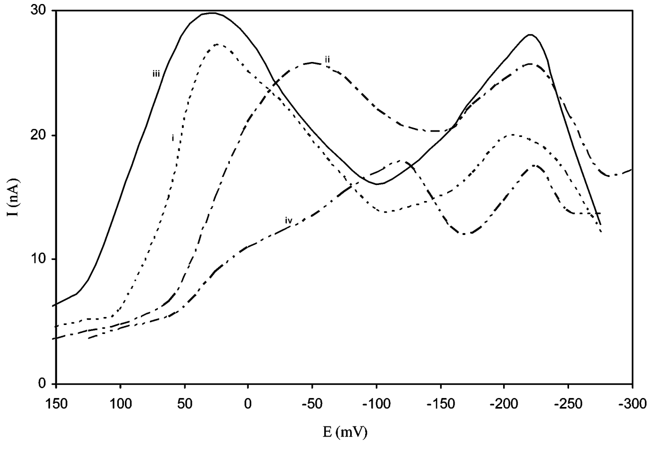


**Figure 4.** Square wave polarograms for the Cu(II) + salicylhydroxamic + bicine system at $I = 0.1$ mol·dm$^{-3}$ $NaNO_3$, pH = 6.5, at (298.15 ± 0.1) K, 0.001 mol·dm$^{-3}$ Cu(II), i; 0.001 mol·dm$^{-3}$ Cu(II) + 0.001 mol·dm$^{-3}$ bicine, ii; 0.001 mol·dm$^{-3}$ Cu(II) + 0.001 mol·dm$^{-3}$ salicylhydroxamic,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

The addition of primary or secondary ligands caused a slight shift of the cathodic peak of the binary and ternary complexes in solution.

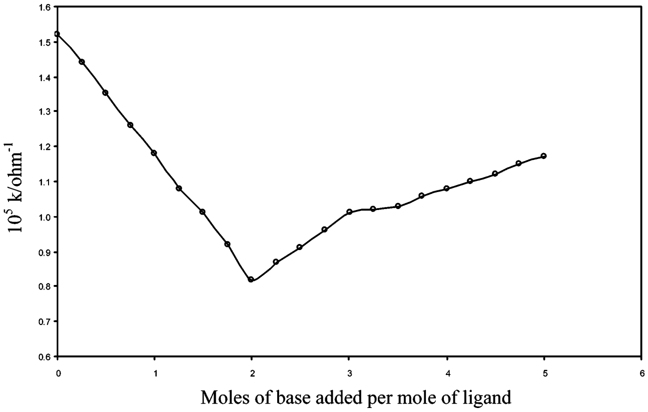


**Figure 5.** Conductometric titration of the Cu(II) + salicylhydroxamic + bicine system at $I = 0.1$ mol·dm$^{-3}$ NaNO$_3$, (298.15 ± 0.1) K, 0.01 mol·dm$^{-3}$ Cu(II) + 0.01 mol·dm$^{-3}$ salicylhydroxamic + 0.01 mol·dm$^{-3}$ bicine.

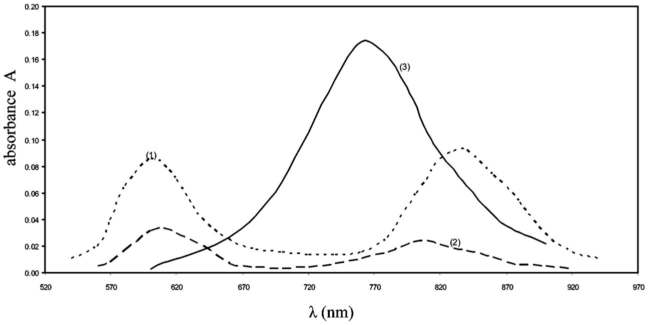


**Figure 6.** Visible absorbance spectra for the Cu(II) + bicine + succinic acid system at $I = 0.1$ mol·dm$^{-3}$ NaNO$_3$ and at (298.15 ± 0.1) K, 1 × 10$^{-3}$ mol·dm$^{-3}$ Cu(II) + 1 × 10$^{-3}$ mol·dm$^{-3}$ bicine, 1; 1 × 10$^{-3}$ mol·dm$^{-3}$ Cu(II) + 1 × 10$^{-3}$ mol·dm$^{-3}$ succinic acid, 2; and 1 × 10$^{-3}$ mol·dm$^{-3}$ Cu(II) + 1 × 10$^{-3}$ mol·dm$^{-3}$ bicine + 1 × 10$^{-3}$ mol·dm$^{-3}$ succinic acid, 3.

The most interesting observation during the square wave voltammetric reduction of the Cu(II) + salicylhydroxamic acid + bicine ternary system is the discrimination of the two individual one-electron steps of the Cu(II/I) and Cu(I/0) couples. Figure 4 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. The new peaks confirm the formation of ternary Cu(II) + salicylhydroxamic acid + bicine in solution.

The conductometric titration curve for the ternary complex of Cu(II) with salicylhydroxamic acid and bicine (Figure 5) shows an initial decrease and an inflection at $a = 2$. This probably corresponds to the neutralization of H$^+$ ions originating from the formation of the Cu(II) + salicylhydroxamic acid binary complex. In the $3 \geq a \geq 2$ range, the conductance increases slightly due to the formation of a ternary complex associated with the release of a H$^+$ ion from bicine. Beyond $a = 3$, the conductance increases appreciably due to the presence of an excess of NaOH.

Figure 6 shows the visible absorption spectra of the binary and ternary Cu(II) systems at the given pH values. The spectra of the ternary systems are quite different from those of the binary systems, emphasizing the formation of the former in solution.

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