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# Mixed Ligand Complexes Involving Ligands of Biological Importance [Cu(II)—1,10-Phenanthroline or 2,2′-Bipyridyl—α-Amino acids]

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The formation of mixed ligand complexes of Cu(II) with 1,10-phenanthroline or 2,2′-bipyridyl in presence of an α-amino acid (glycine, alanine, phenylalanine or norleucine) have been studied by the pH-metric method. The pH-titrations of the reaction mixtures in equimolar concentrations have shown the simultaneous addition of the ligands resulting 1:1:1 ternary complex formation. The equilibrium and formation constants of the resulting ternary complexes have been calculated ($30 \pm 1\,^{\circ}C$; $\mu = 0.1\,KNO_3$). The order of stability in terms of the secondary ligands has been found to be: *Phe* > *Ala* > *Gly* > *Norleu.*

*[Keywords: α-Amino acids; Copper(II) complexes; pH-Metric studies]*

*Komplexe mit gemischten Liganden von biologischer Bedeutung [Cu(II)—1,10-Phenanthrolin oder 2,2′-Bipyridyl—α-Aminosäuren]*

Die Bildung gemischter Komplexe von Cu(II) mit 1,10-Phenanthrolin oder 2,2′-Bipyridyl in Gegenwart einer α-Aminosäure (Glycin, Alanin, Phenylalanin oder Norleucin) wurde pH-metrisch untersucht. Die pH-Titration der Reaktionsmischungen in äquimolaren Konzentrationen zeigten, daß der gleichzeitige Zusatz der Liganden zu ternären 1:1:1-Komplexen führt. Die Gleichgewichts- und Bildungskonstanten der resultierenden ternären Komplexe wurden bestimmt ($30 \pm 1\,^{\circ}C$; $\mu = 0{,}1\,KNO_3$). Die Reihenfolge der Stabilität bezüglich der sekundären Liganden ist *Phe* > *Ala* > *Gly* > *Norleu.*

## Introduction

Copper(II) is supposed to play an important role in the biological systems[1,2]. Copper(II) salts are toxic to micro-organisms, especially to protozoa, fungi and algae, and also have antiseptic properties.

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Copper(II) is also known to form very stable binary as well as ternary complexes with various organic ligands[3–7]. 1,10-Phenanthroline (*Phen*) and 2,2′-bipyridyl (*Bipy*) are well known chelating agents and have been reported to be biologically active either alone or in presence of metal ion. The bacteriostatic effects of *Phen* or *Bipy* and their metallic chelates have been studied on rumen bacteria, acid fast bacteria and a number of gram positive micro-organisms (the mixed ligand complexes have been found to be more effective).

The present investigations deal with the determination of equilibrium and formation constants of the mixed ligand complexes involving Copper(II), *Phen* or *Bipy* and α-amino acids; such studies may prove to be quite useful in understanding many bio-chemical problems.

## Experimental

The stock solution of copper nitrate (AnalaR, BDH) was prepared in doubly distilled water and standardized with a standard solution of disodium salt of *EDTA* using murexide as an indicator. The solution of hydrochlorides of 1,10-phenanthroline and 2,2′-bipyridyl were prepared by direct weighing and their strengths were checked by pH-metric titrations against a standard solution of potassium hydroxide. The solutions of α-amino acids were also prepared by direct weighing in doubly distilled water.

The pH-titrations were carried out at $30 \pm 1$ °C using a constant temperature bath and a Systronics pH-meter standardized with a 0.05 *M* solution of potassium hydrogen phthalate (AnalaR, BDH).

The following pH-metric titrations were carried out:

1. 10 ml (0.025 *M*) copper(II) nitrate.
2. 10 ml (0.025 *M*) ligand.
3. 10 ml (0.025*M*) ligand in presence of 10 ml (0.025*M*) copper(II) nitrate [Cu(II):primary ligand, 1:1].
4. 10 ml (0.025 *M*) each of primary and secondary ligands in presence of 10 ml (0.025 *M*) copper(II) nitrate [Cu(II):primary ligand:secondary ligand, 1:1:1).

The ionic strength of all the solutions was kept approximately constant ($\mu = 0.1\,KNO_3$) by adding 5 ml of 1.0 *M* potassium nitrate to the each set of solutions and the total volume was always kept constant (50 ml) by adding requisite volume of doubly distilled water.

## Results and Discussion

Curve 0 (Fig. 1) represents the pH-metric titration of copper(II) nitrate with 0.1 *M* KOH. In this case the precipitation from the very beginning and a sharp inflection at $m = 1.5$ indicates the formation of precipitate containing basic salt.

Curves 1–4 (Figs. 1 and 2) represent the pH-metric titrations of *Gly*, *Ala*, *Phe*, and *Norleu* with 0.1 *M* KOH. The dissociation constants of *Phe* and *Norleu* were calculated using the method of *Chaberek* and

*Martell*[8] and those of *Gly*, *Ala*, *Phen* and *Bipy* were taken from the literature[9–11] (Table 1).

As reported earlier[12], potentiometric titration curves (Curve 5, Figs. 1 and 2) of copper nitrate with KOH in presence of an equimolar

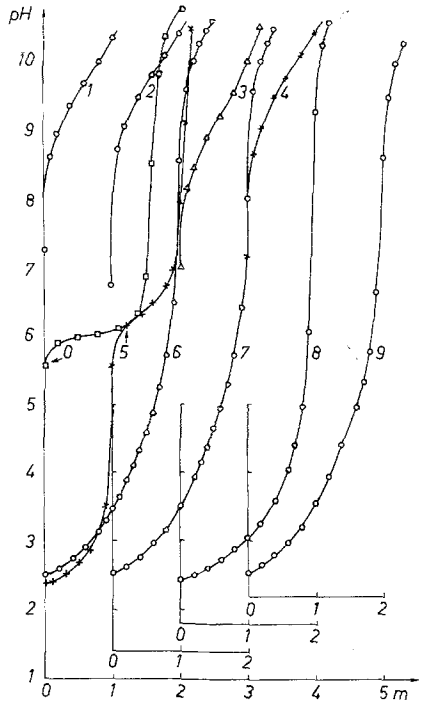


Fig. 1. Titration curves for Cu(II)—1,10-phenanthroline—α-amino acids. 0, $Cu(NO_3)_2$; 1, glycine; 2, α-alanine; 3, phenylalanine; 4, norleucine; 5, 1:1 Cu(II)-*Phen*; 6, 1:1:1 Cu(II)-*Phen*-glycine; 7, 1:1:1 Cu(II)-*Phen*-α-alanine; 8, 1:1:1 Cu(II)-*Phen*-phenylalanine; 9, 1:1:1 Cu(II)-*Phen*-norleucine; *m*, moles of base added per mole of metal ion

concentration of *Phen* or *Bipy* indicate the formation of 1:1 binary chelates and their conversion into the corresponding monohydroxo derivatives.

When the equimolar systems, Cu(II)-*Phen*-amino acid (*Gly*, *Ala*, *Phe* or *Norleu*) (Curves 6–9, Fig. 1) and Cu(II)-*Bipy*-amino acid (*Gly*, *Ala*, *Phe* or *Norleu*) (Curves 6–9, Fig. 2) are titrated, only one inflection at $m = 2$ is observed. Comparison of these curves with the corresponding composite curves [which can be drawn by adding the horizontal

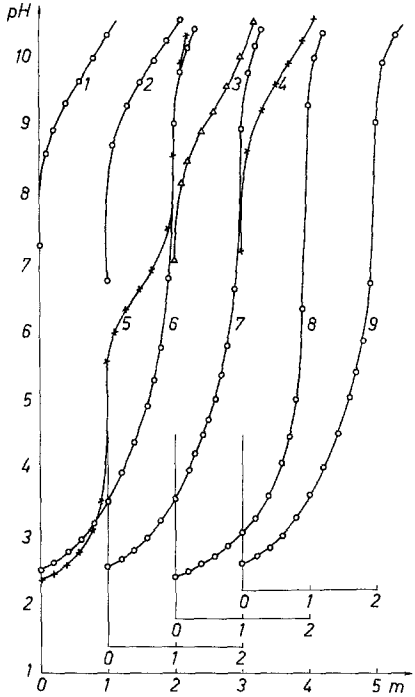


Fig. 2. Titration curves for Cu(II)—2,2′-bipyridyl—α-amino acids. 1, glycine; 2, α-alanine; 3, phenylalanine; 4, norleucine; 5, 1:1 Cu(II)-*Biby*; 6, 1:1:1 Cu(II)-*Bipy*-glycine; 7, 1:1:1 Cu(II)-*Bipy*-α-alanine; 8, 1:1:1 Cu(II)-*Bipy*-phenylalanine; 9, 1:1:1 Cu(II)-*Bipy*-norleucine; *m*, moles of base added per mole of metal ion

*Table 1*

| Ligand | Dissociation constant ($pK$) | 1:1 Chelate | Formation constant ($\log K_{MA}$) |
|---|---|---|---|
| Glycine | 9.69[9] | Cu(II)—*Phen*(1:1) | 7.40[12] |
| α-Alanine | 9.86[9] | Cu(II)—*Bipy* (1:1) | 6.33[12] |
| Phenylalanine | 9.05 ± 0.07 | | |
| Nor-leucine | 9.60 ± 0.06 | | |
| 1,10-Phenanthroline | 5.00[10] | | |
| 2,2′-Bipyridyl | 4.44[11] | | |

distance of the secondary ligand curve to the horizontal distance of Cu(II)-*Phen* or *Bipy* curve at the same pH] indicates that the formation of 1:1:1 mixed ligand complexes is the only possibility in the ternary systems studied.

Initially, in the lower buffer region all these curves run slightly above the corresponding (1:1) Cu(II)-*Phen* or *Bipy* curves and this behaviour may be attributed to the zwitter ion formation tendency of α-amino acids. Further, in the beginning, 1:1 Cu(II)-*Phen* or *Bipy* complex is formed and the lowering of the curves in the case of ternary systems starts after $m = 0.4$ and 0.8 in Cu(II)-*Phen* or *Bipy-Gly*, *Ala* or *Norleu* and Cu(II)-*Phen* or *Bipy-Phe* systems, resp. No inflection in these cases at $m = 1$ and only one inflection at $m = 2$ indicates that addition of the secondary ligand starts before the complete addition of primary ligand.

The formation of 1:1:1 mixed ligand complex in the above systems may be represented as:

$$\mathrm{Cu}A^{2+} + \mathrm{H}L \rightleftharpoons CuAL^{+} + \mathrm{H}^{+} \tag{i}$$

and the overall reaction for the formation of 1:1:1 ternary complex may than be written as:

$$\mathrm{Cu}^{2+} + \mathrm{H}A^{+} + \mathrm{H}L \rightleftharpoons \mathrm{Cu}AL^{+} + 2\,\mathrm{H}^{+} \tag{ii}$$

(where $A$ = *Phen* or *Bipy* and $\mathrm{H}L$ = α-amino acid).

If $K'$ and $K''$ are the equilibrium constants of the reactions (i) and (ii) resp. then:

$$K' = \frac{[\mathrm{Cu}AL^{+}][\mathrm{H}^{+}]}{[\mathrm{Cu}A^{2+}][\mathrm{H}L]} \tag{1}$$

and

$$K'' = \frac{[\mathrm{Cu}AL^{+}][\mathrm{H}^{+}]^2}{[\mathrm{Cu}^{2+}][\mathrm{H}A^{+}][\mathrm{H}L]} \tag{2}$$

Assuming that only mononuclear metal chelate species are formed in the system, the following equations are obtained for maintaining the material balance:

$$T_M = [Cu^{2+}] + [CuA^{2+}] + [\mathrm{Cu}AL^{+}] \tag{3}$$

$$T_{\mathrm{OH}} + [\mathrm{H}^{+}] - [\mathrm{OH}^{-}] = [\mathrm{Cu}A^{2+}] + 2[\mathrm{Cu}AL^{+}] + [A] + [L^{-}] \tag{4}$$

$$T_A = [\mathrm{H}A^{+}] + [A] + [\mathrm{Cu}A^{2+}] + [\mathrm{Cu}AL^{+}] \tag{5}$$

$$T_L = [\mathrm{H}L] + [L^{-}] + [\mathrm{Cu}AL^{+}] \tag{6}$$

where $T_M$, $T_A$ and $T_L$ represent the total concentrations of all the metal, primary ligand and secondary ligand species and $T_{\mathrm{OH}}$ is concentration of the base added to the reaction mixture during titration. In the above systems, it may be shown that:

$$a[\mathrm{Cu}^{2+}]^2 + b[\mathrm{Cu}^{2+}] - c = 0 \tag{7}$$

where

$$a = \frac{K_{MA} \cdot k}{[H^+]},\ b = X + Y,\ c = \{(2\text{-}m)T_M - [H^+] + [OH^-]\}X \cdot Y$$

$$X = \left(1 + \frac{k}{[H^+]}\right) \text{ and } Y = \left(1 + \frac{k'}{[H^+]}\right)$$

where $k$ represents the dissociation constant of *Phen* or *Bipy* and $k'$ is that for the α-amino acid; $K_{MA}$ is the formation constant of (1:1) Cu(II)-*Phen* or *Bipy* complex.

The equilibrium concentration of free metal ions present in the reaction mixture may be determined by solving equation (7). The concentrations of other species involved in the equilibrium relations can then be calculated from the above equations and also the values of equilibrium constants $K'$ and $K''$.

### *Stability of 1:1:1 Mixed Ligand Complexes*

The stability constant of a ternary complex in these systems may be defined as:

$$K_{MAL} = \frac{[CuAl^+]}{[CuA^{2+}][L^-]} \tag{8}$$

and can be determined by the expression:

$$K_{MAL} = \frac{K'}{k'} \tag{9}$$

Further, the overall stability constant of the mixed ligand complex:

$$K'_{MAL} = \frac{[CuAL^+]}{[Cu^{2+}][A^-][L^-]} \tag{10}$$

Table 2. *Equilibrium and chelate formation constants of mixed ligand complexes*

| System | $-\log K'$ | $-\log K''$ | $\log K_{MAL}$ | $\log K'_{MAL}$ |
|---|---|---|---|---|
| Cu(II)—*Phen*—*Gly* | 1.98 ± 0.14 | $\bar{1}$.58 ± 0.14 | 7.71 ± 0.14 | 15.11 ± 0.14 |
| Cu(II)—*Phen*—*Ala* | 2.03 ± 0.15 | $\bar{1}$.63 ± 0.15 | 7.83 ± 0.15 | 15.23 ± 0.15 |
| Cu(II)—*Phen*—*Phe* | 1.11 ± 0.17 | $\bar{2}$.71 ± 0.17 | 7.94 ± 0.17 | 15.34 ± 0.17 |
| Cu(II)—*Phen*—*Norleu* | 2.06 ± 0.08 | $\bar{1}$.66 ± 0.08 | 7.54 ± 0.08 | 14.94 ± 0.08 |
| Cu(II)—*Bipy*—*Gly* | 2.03 ± 0.12 | 0.14 ± 0.12 | 7.66 ± 0.12 | 13.99 ± 0.12 |
| Cu(II)—*Bipy*—*Ala* | 2.07 ± 0.08 | 0.18 ± 0.08 | 7.79 ± 0.08 | 14.12 ± 0.08 |
| Cu(II)—*Bipy*—*Phe* | 1.09 ± 0.14 | $\bar{1}$.20 ± 0.14 | 7.96 ± 0.14 | 14.29 ± 0.14 |
| Cu(II)—*Bipy*—*Norleu* | 2.11 ± 0.10 | 0.22 ± 0.10 | 7.49 ± 0.10 | 13.82 ± 0.10 |

may be calculated from the expression:

$$K'_{MAL} = \frac{K''}{kk'} \tag{11}$$

The values of the formation constants of the ternary complexes are recorded in Table 2. It can be observed that the mixed ligand complexes involving *Phen* as primary ligand are more stable than those involving *Bipy*. The order of stability of the ternary complexes in terms of secondary ligands has been found to be: *Phe* > *Ala* > *Gly* > *Norleu*.

The greater stability of the complexes containing phenyl-alanine and α-alanine in comparison to glycine may be attributed to the presence of electron repelling phenyl and methyl groups in phenyl-alanine and α-alanine. The lower stability of the complexes containing norleucine may probably be due to the presence of bulky *n*-butyl group.

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