

Metal Complexes of Some Model Peptides: Part III—Equilibrium Study of Complex Formation of Copper(II) with N-(N'-Benzenesulphonyl)-glycylanthranilic Acid

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pH-metric and spectrophotometric studies of the interaction of copper(II) with the model peptide ligand, N-(N'-benzenesulphonyl)glycylanthranilic acid (NBSGA or LH_2) and infrared spectral measurements provide evidence of formation in solution of only 1:1 Cu – NBSGA complexes, viz. $\text{Cu}(\text{LH})^+$, $\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})$, $\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})$ in the pH range 2.5–5.5 and the complex $\text{Cu}(\text{H}_{-1}\text{L})(\text{OH})^{2-}$ at $\text{pH} > 9$. Successive deprotonation constants of the ligand, LH_2 and formation constants of its Cu(II) complexes have been determined pH-metrically at 20°, 30° and 40° C in 75% (v/v) aq. ethanol medium at a constant ionic strength ($\mu = 0.2 \text{ mol dm}^{-3} \text{ NaNO}_3$). Thermodynamic parameters, viz. changes of free energy, enthalpy and entropy have been evaluated and correlated with the mode of binding of the ligand. Monodentate binding by the ligand ion, LH^- using its carboxylate oxygen atom in the complex $\text{Cu}(\text{LH})^+$ and tridentate (O, N, N) chelation by the ligand ions, $\text{H}_{-1}\text{LH}^{2-}$ and $\text{H}_{-1}\text{L}^{3-}$ using the carboxylate oxygen, deprotonated N-peptide and N-sulphonamide atoms in the complexes $\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})$, $\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})^-$ and $\text{Cu}(\text{H}_{-1}\text{L})(\text{OH})^{2-}$ have been established.

Complexes of copper(II) with polypeptides and their derivatives have been extensively studied^{1–8} to explore the role of this metal ion in enzymatic reactions. It has been concluded that a peptide binds metal ions such as Cu(II) at $\text{pH} < 4.5$ using the carbonyl oxygen atom, and at higher pH values it binds metal ions using the amide deprotonated N-peptide atom^{4–6,9}. Much work is still left to be done to evaluate the denticity of polypeptides, their modes of bonding with metal ions, effects of adjacent donor sites on the metal-peptide interactions^{10,11} and the nature of the complexes formed. The present paper describes potentiometric and spectrophotometric studies of the complex formation of Cu(II) with the newly synthesized modified peptide ligand, N-(N'-benzenesulphonyl)glycylanthranilic acid, (*o*)- $\text{HOOC}-\text{C}_6\text{H}_4-\text{NHCOCH}_2\text{NH}\text{SO}_2\text{C}_6\text{H}_5$, (hereafter referred to as NBSGA or LH_2) in 75% v/v aq. ethanol medium at 20°, 30° and 40°C maintaining a constant ionic strength, $\mu = 0.2 \text{ mol dm}^{-3} (\text{NaNO}_3)$.

Materials and Methods

The ligand NBSGA was prepared by condensing anthranilic acid (0.02 mol) with N-benzenesulphonylglycyl chloride (0.02 mol) in the presence of aq. alkali ($\text{pH} \approx 9$) following the usual procedure¹². The product was purified by recrystallisation from hot aq. ethanol and dried in air, m.p. 206°; eq. wt, 335 (potentiometric) (calc. 334) (Found: C, 53.9; H, 4.2; N, 8.4%. $\text{C}_6\text{H}_5\text{SO}_2\text{NHCH}_2\text{CONHC}_6\text{H}_4\text{COOH}$ requires C, 53.9; H, 4.2; N, 8.4%).

The copper(II) complex, $\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})$, was prepared by digesting an aq. suspension of freshly

precipitated alkali-free copper hydroxide (0.01 mol) with an ethanolic solution of NBSGA (0.01 mol) on a steam-bath maintaining ethanol content of the mixture 75% (v/v). The resulting blue solution was filtered hot and allowed to cool and evaporate at room temperature. The resulting shiny blue crystals were washed with ethanol and air-dried (Found: Cu, 15.4; N, 6.7; $\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})$ requires Cu, 15.4; N, 6.8%). The complex showed no loss of water at 100–105°C, but decomposed to a black mass at 115°C and above indicating strong binding of H_2O with Cu(II) in this complex. It exhibited the following IR spectral features: $\nu_{\text{as}}\text{COO}$ (1600), $\nu_{\text{s}}\text{COO}^-$ (1400), $\nu\text{O}=\text{CNH}$ (1630), $\nu\text{N}-\text{SO}_2$ (1330) as against νCOOH (1700), $\nu\text{O}=\text{CNH}$ (1675), $\nu\text{N}-\text{SO}_2$ (1350) of the ligand NBSGA indicating binding of Cu(II) by the carboxylate oxygen atom, amide deprotonated N-peptide atom and N-sulphonamide atom of the ligand^{13,14}. Bending mode of coordinated water molecule in the Cu(II) complex was observed at 1680 cm^{-1} .

All the other reagents were either of AR quality or properly purified and their solutions were made in doubly distilled CO_2 -free water and purified ethanol¹⁵. Carbonate-free NaOH was prepared¹⁶ from A R sample. Copper and free HNO_3 in the copper nitrate solution were estimated by ion-exchange separation coupled with combined acid-bases and complexometric (EDTA) titrations¹⁷.

pH measurements for the determination of proton-ligand and metal-ligand constants were made with a SYSTRONICS digital pH-meter, model 335 (accuracy $\pm 0.01 \text{ pH}$) using a glass electrode in conjunction with an SCE following a procedure described earlier^{10,11}.

In the present work such measurements were made at three temperatures, 20°, 30° and 40°C in 75% v/v aq. ethanol medium maintaining a constant ionic strength, $\mu = 0.2 \text{ mol dm}^{-3}$.

The concentration variables, viz, $\bar{n}_H$ (average number of protons bound to the ligand ion L^{2-}), $\bar{n}_{LH}$ (average number of ligand ion, LH^- , bound to the Cu^{2+} ion), $\bar{n}_h$ (average number of protons liberated due to the reaction of Cu^{2+} ion with the ligand LH_2) and $[LH]$, the equilibrium concentration of free ligand LH^- ion were calculated¹⁸ using the pH titration curves. Hydrogen ion concentration, $[H]$ at any stage of the titrations was found out by the usual procedure¹⁹⁻²² after incorporating corrections for mixed solvent and using the following values for pK_w of water: 14.16 (20°C), 13.80 (30°C) and 13.54 (40°C) as required.

Visible absorption spectra of a series of solutions containing Cu(II) (0.002 M) and NBSGA (0.01 M) adjusted to pH values corresponding to $\bar{n}_h$ values of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and $\bar{n}_h > 3$ at 30°C in the same solvent medium as in the case of pH-metric measurements were recorded with a Hilger UVISPEK spectrophotometer. Absorption maxima of Cu(II) shifted to lower wavelengths with rise of pH of the solutions. Metal:ligand ratios in the complexes formed in solution were determined^{23,24} spectrophotometrically where only 1:1 Cu-NBSGA complexes were detected in the entire pH range.

Results and Discussion

Deprotonation constants (K_{a2} and K_{a1}) of the ligand NBSGA (LH_2)

In the absence of Cu(II) the ligand NBSGA (LH_2) offered two buffer regions, viz. pH 2.5-6 and pH 9-12. In each of the two buffer regions one mol of base per mol of the ligand was consumed (Fig. 1). The lower pH buffer region was definitely contributed by deprotonation (Eq. 1) of the carboxylic acid group of the ligand. The higher pH buffer region was due to deprotonation (Eq. 2) of the sulphonamido group, $-SO_2NH-$, and not due to that of the peptide group, $-CONH-$, as acetylglycine, benzoylglycine etc. behave as monoprotic acids (HL) in the entire pH range²⁵⁻²⁷

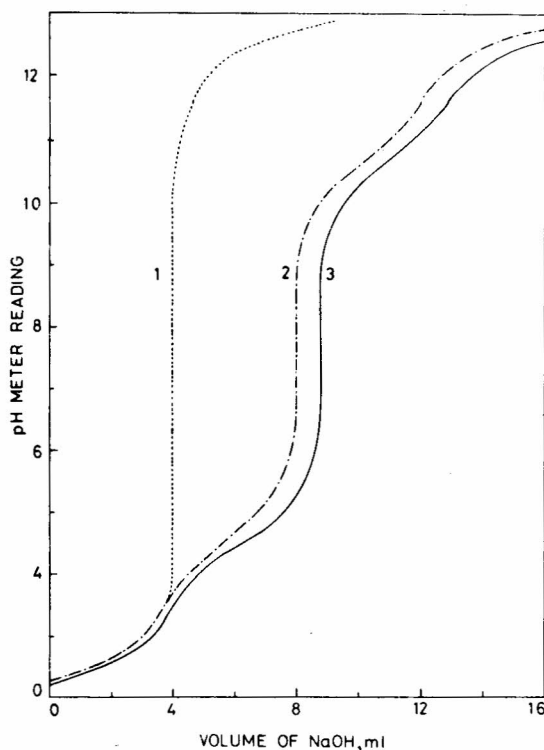
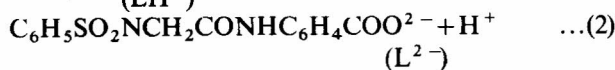
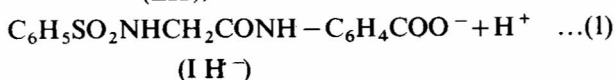
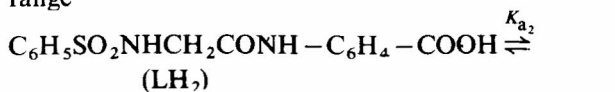


Fig. 1—pH-metric titration curves at 30°C [Curve 1: 0.01 M HNO_3 ; curve 2: 0.01 M HNO_3 + 0.01 M NBSGA (LH_2); and curve 3: 0.01 M HNO_3 + 0.01 M NBSGA (LH_2) + 0.001 M $Cu(NO_3)_2$; Titrant: 0.125 M NaOH; initial volume of each solution 50 ml; medium 75% v/v aq. ethanol; ionic strength, 0.2 mol dm^{-3} ($NaNO_3$)]

The deprotonation constants K_{a2} and K_{a1} were evaluated by computing^{18,28} the $\bar{n}_H$ and $p[H]$ ($= -\log [H^+]$) data. Refined pK_{a2} and pK_{a1} values at 20°, 30° and 40°C and the associated thermodynamic parameters, viz. ΔG , ΔH and ΔS at 30°C are given in Table I.

Formation constants of Cu(II)-NBSGA complexes

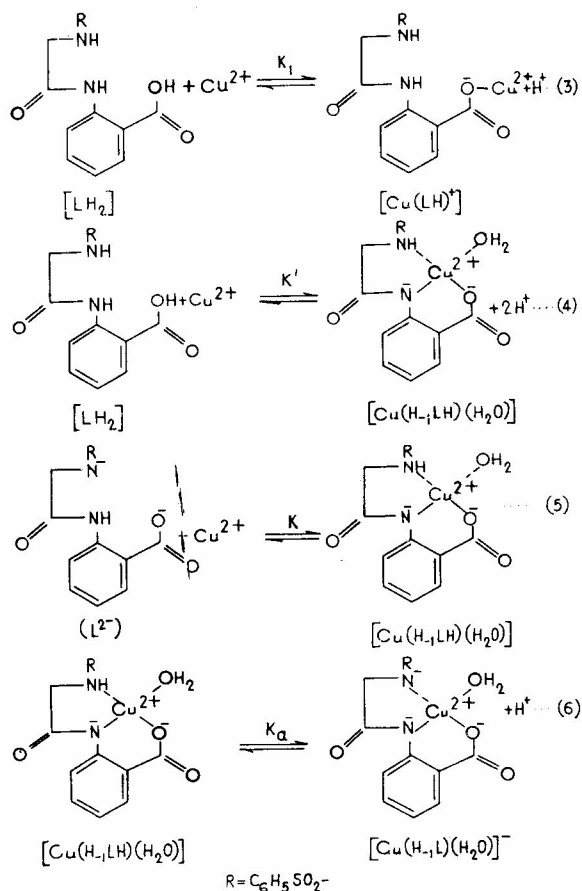
pH titration curve (curve 3, Fig. 1) of solution of NBSGA in the presence of Cu(II) showed two well-

Table I—Deprotonation Constants of Ligand NBSGA and Formation Constants of Its Complexes with Cu(II) and Thermodynamic Functions at 30°C

[Solvent: 75% (v/v) aq. ethanol; ionic strength = 0.2 mol dm^{-3}]						
Parameter	20°C	30°C	40°C	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
pK_{a2}	4.58	4.50	4.44	26	-15	33
pK_{a1}	10.08	9.94	9.78	58	-24	113
$\log K_1$	3.35	3.30	3.25	19	-8	38
pK'	5.88	5.74	5.59	33	-26	25
$\log K$	8.78	8.70	8.63	51	-13	125
pK_s	4.35	4.18	4.00	24	-30	-21
pK'_w	9.86	9.58	9.28	56	-48	25

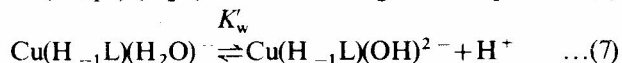
defined buffer regions, one in the pH range 2.5-5.5 and the other in the pH range 9-12. The overall reaction in the pH range 2.5-5.5 involved the release of three protons per Cu^{2+} and there was an initial step in which only one proton per Cu^{2+} was released. An additional proton per $Cu(II)$ was released in the higher pH buffer region, pH 9-12. As only 1:1 Cu -NBSGA complexes were detected by spectrophotometric measurements, liberation of three protons per Cu^{2+} definitely involved deprotonation of the $-COOH$, $-CONH-$ and SO_2NH- functions of the ligand, LH_2 , as a consequence of tridentate ($\bar{O}$, $\bar{N}$, $\bar{N}$) chelation of Cu^{2+} by the ligand thereby forming the complex $Cu(H_{-1}L)(H_2O)^-$. The initial reaction below $pH \approx 4$, where only one proton ($0 < \bar{n}_h < 1$) per Cu^{2+} was released, obviously involved deprotonation of the $-COOH$ group of the ligand as a consequence of monodentate binding of Cu^{2+} by the ligand ion LH^- with the formation of the complex $Cu(LH)^+$ ion. Such a proposition of monodentate binding in $Cu(LH)^+$ was found obvious as (i) the buffer region corresponding to the deprotonation of the $-COOH$ group of the ligand (Eq. 1) was lowered in the presence of Cu^{2+} ions (Fig. 1, curves 2 and 3); (ii) bidentate ($\bar{O}$, O) chelation by the carboxylate oxygen atom and O -peptide atom would be stereochemically unfavourable due to angle strain arising out of eight-membered ring formation; (iii) bidentate ($\bar{O}$, $\bar{N}$) chelation by the carboxylate oxygen atom and N -peptide atom could not occur as this would liberate two protons per Cu^{2+} as N -peptide atom, like the $-COOH$ function binds metal ion only on deprotonation^{4,6} and it does^{4,6,9} so at $pH > 4.5$; but here only one proton per $Cu(II)$ was released at much lower pH values 2.5-4.0; and (iv) chelation by the carboxylate oxygen atom and N -sulphonamide atom ($-SO_2NH-$), would require participation of the N -peptide atom for stereochemical reason. Considering all these factors, complex formation of $Cu(II)$ with the ligand NBSGA in the pH range 2.5-5.5 was assumed to take place according to equilibria (3) to (6) as shown in Scheme 1, in which the structures and modes of bonding of the ligand ions viz. LH^- , $H_{-1}LH^{2-}$, $H_{-1}L^{3-}$ and L^{2-} have been clearly shown.

Formation of the complex, $Cu(LH)^+$ involved release of one proton per Cu^{2+} and the absorption maxima of $Cu(II)$ in the presence of ligand at $pH \leq 4$ was fairly close to that in the absence of the ligand (Fig. 2), as the ligand field around $Cu(II)$ remained practically unchanged when one coordinated water molecule was displaced by a carboxylate oxygen atom. Blue shift of the absorption maxima of $Cu(II)$ in the presence of the ligand at pH values 4-6, when there was a sharp increase of $\bar{n}_h$ from 1 to 3 indicated an increase of the ligand field strength around $Cu(II)$ due to the formation of $Cu(O,N,N)(O)$ complexes, $Cu(H_{-1}L)$



Scheme 1

(H_2O) and $Cu(H_{-1}L)(H_2O)^-$ from $Cu(O)$ complexes $Cu(LH)^+$ or $Cu(II)$ aq. ions. At higher pH values, corresponding to $\bar{n}_h > 3$, absorption maxima of $Cu(II)$ practically did not undergo any shift from 650 nm. The higher pH (9-12) buffer region corresponding to $\bar{n}_h > 3$ was, therefore, assumed to be due to the deprotonation of a coordinated water molecule in the complex, $Cu(H_{-1}L)(H_2O)^-$ ion according to the equilibria (7)



as there would be practically no change of the ligand field on replacing one H_2O by one OH^- ion.

As the ligand ions $H_{-1}LH^{2-}$ and $H_{-1}L^{3-}$ offer tridentate chelation, the complexes $Cu(H_{-1}LH)$ and $Cu(H_{-1}L)^-$ in solution will take up and strongly bind a molecule of solvent water in the coordination plane of the other three donor sites (O , N , N) offered by the ligand ions due to the inherent tendency of $Cu(II)$ d^9 -ion to have a square planar geometry with increase of ligand field strength. Isolation and characterisation of the complex, $Cu(H_{-1}LH)(H_2O)$ and the blue shift of absorption maxima with increase of intensity with rise of pH in the region corresponding to $1 < \bar{n}_h < 3$ provided sufficient evidence of such a proposition.

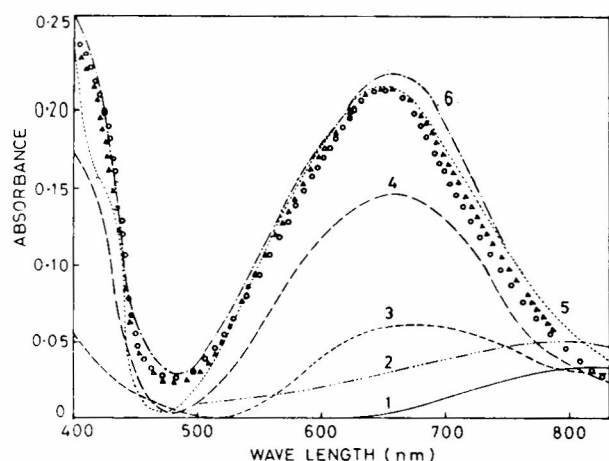


Fig. 2—Electronic spectral curves of Cu(II)-NBSGA mixtures at different pH values in 75% (v/v) aq. ethanol and ionic strength 0.2 mol dm⁻³ (NaNO₃) at 30°C [Cu²⁺] = 0.002 M, [NBSGA] = 0.01 M: pH 3.50 (1); 4.08 (2); 4.5 (3); 5.0 (4); 5.45 (5); 6.3 (6); 6.90 (Δ-Δ); 8.0 (○-○-○)

The equilibrium constants, K_1 , K' , K , K_a , K'_w were defined by

$$K_1 = [\text{Cu}(\text{LH})^+]/[\text{Cu}^{2+}][\text{LH}^-] \quad \dots(8)$$

$$K' = [\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})][\text{H}^+]^2/[\text{Cu}^{2+}][\text{LH}_2] \quad \dots(9)$$

$$K = [\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})]/[\text{Cu}^{2+}][\text{L}^{2-}] = K'/K_{a_2}K_{a_1} \quad \dots(10)$$

$$K_a = [\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})][\text{H}^+]/[\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})] \quad \dots(11)$$

$$K'_w = [\text{Cu}(\text{H}_{-1}\text{L})(\text{OH})^{2-}][\text{H}^+]/[\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})] \quad \dots(12)$$

where K_{a_2} and K_{a_1} were the stepwise deprotonation constants of the ligand LH_2 (Eqs 1 and 2). The following mass balance equations for metal ion, ligand and hydrogen ion were considered for calculation of $\bar{n}_{\text{LH}}$, $\bar{n}_{\text{h}}$ and $p[\text{LH}]$ values:

$$T_{\text{Cu}} = [\text{Cu}^{2+}] + [\text{Cu}(\text{LH})^+] + [\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})] + [\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})] + [\text{Cu}(\text{H}_{-1}\text{L})(\text{OH})^{2-}] \quad \dots(13)$$

$$T_{\text{L}} = [\text{LH}_2] + [\text{LH}^-] + [\text{L}^{2-}] + [\text{Cu}(\text{LH})^+] + [\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})] + [\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})] + [\text{Cu}(\text{H}_{-1}\text{L})(\text{OH})^{2-}] \quad \dots(14)$$

$$[\text{H}^+] = A + [\text{OH}^-] - B + [\text{LH}^-] + 2[\text{L}^{2-}] + [\text{Cu}(\text{LH})^+] + 2[\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})] + 3[\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})] + 4[\text{Cu}(\text{H}_{-1}\text{L})(\text{OH})^{2-}] \quad \dots(15)$$

where T_{Cu} , T_{L} , A and B were the total concentrations of Cu(II) ion, the ligand LH_2 , free HNO_3 and added NaOH respectively and the terms in brackets, [] were the equilibrium concentrations of the respective

species. The formation constant, K_1 , of the complex $\text{Cu}(\text{LH})^+$ was evaluated by computing the $\bar{n}_{\text{LH}}$ and $p[\text{LH}]$ data in the $p[\text{H}]$ range 2.5-4.0 corresponding to $0 < \bar{n}_{\text{h}} < 1$ assuming the concentrations of the species $[\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})]$, $[\text{Cu}(\text{H}_{-1}\text{L})(\text{H}_2\text{O})^-]$, $[\text{Cu}(\text{H}_{-1}\text{L})(\text{OH})^{2-}]$ and L^{2-} negligibly small compared to those of the other species. The constants k' and k'_a were computed from $\bar{n}_{\text{h}}$, $p\text{LH}_2$, $p[\text{H}]$ data in the pH range 3.8-5.2, corresponding to $1 < \bar{n}_{\text{h}} < 3$, assuming the concentration of free Cu^{2+} ion negligibly small in comparison to those of the other species and using the values of K_1 already determined. All the constants were subjected to least square refinements²⁸ and the refined values of $\log K_1$, pK' , pK'_a , $\log K$ and pK'_w at 20°, 30° and 40°C together with the associated thermodynamic parameters ΔG , ΔH and ΔS at 30°C are presented in Table 1.

Increase of entropy accompanying the stepwise deprotonation of the ligand suggests increase in the number of species and increase of their flexibility due to breakdown of intermolecular bonds, probably hydrogen bonds. Close agreement of the stability constant (K_1) of the complex $\text{Cu}(\text{LH})^+$ with that of Cu(II)-benzoate and Cu(II) acetate complexes²⁹⁻³⁰ under nearly identical condition justified the assumption of monodentate $-\text{COO}^- - \text{Cu}^{2+}$ binding by the ligand LH^- ion in this complex. Formation of the complex, $\text{Cu}(\text{H}_{-1}\text{LH})(\text{H}_2\text{O})$ involved tridentate ($\bar{O}$, $\bar{N}$, NH) chelation of Cu^{2+} ion by the ligand ion, $\text{H}_{-1}\text{LH}^{2-}$ and consequently ΔS was positive. Small positive value of ΔS for the equilibrium (4) was probably due to the loss of configurational entropy of the ligand LH_2 on coordination. Entropy change for the overall reaction (5) was entirely due to chelation and charge neutralization of Cu^{2+} by the ligand ion L^{2-} ; consequently ΔS was large positive as the configurational entropy change of the ligand was negligible in that case.

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