Binary and Ternary Complexes of Copper(II) involving Imidazole, Histamine, and L-Histidine as Ligands

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The complete species distribution and stability constants of the binary and ternary complexes of Cu^{II} with imidazole, histamine, and L-histidine as ligands were determined from pH titration data in aqueous perchlorate medium at I = 0.15 mol dm^{-3} ($Na[ClO_4]$) and 37 °C. The results show that imidazole, though a unidentate ligand, forms very stable binary and ternary complexes with Cu^{II}. The stability-constant data for the CuAH and CuA_2H complexes (A = histamine or histidine) suggest that their structures are similar. The ambidentate nature of the histidine ligand *i.e.* the ability to co-ordinate both in histamine-like and glycine-like modes is indicated from the results obtained.

INVESTIGATIONS on the copper(II) complexes of biologically important ligands have received intermittent attention over a number of years.[1] Of these, copper(II) complexes containing an imidazole group as a ligand are of considerable interest since this group forms part of several proteins which interact with Cu^{II}. The importance of these complexes is obvious from the π-acceptor property of the imidazole ring and the availability of its donor group at physiological pH. The stability constants of copper(II) binary complexes with imidazole, histamine, and histidine ligands have been reported by several workers.[2] However, a systematic study and computer-based analysis of the experimental data obtained under identical conditions for these binary systems has not been undertaken. Hence, in this paper, we report systematic investigations on the formation equilibria of copper(II) binary and ternary complexes with imidazole, histamine, and histidine ligands at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$).

EXPERIMENTAL

The methods of preparation and determination of $Cu[ClO_4]_2$ and of the other reagents are as described by Ramamoorthy and Santappa.[3] All the ligands used were obtained from Fluka. Doubly-distilled water was used for the preparation of all the solutions.

The pH titrations were carried out with a digital pH meter (Bhagyanagar Electronics, Hyderabad, India) with a glass and calomel electrode assembly with an accuracy of ±0.01 pH units. The pH standards taken were 4.02 for 0.05 mol dm^{-3} potassium hydrogen phthalate and 9.08 for 0.05 mol dm^{-3} borax, at 37 °C. The electrode system was calibrated by the method of Irving *et al.*[4] The titrations were performed at 37 °C in a Pyrex double-walled cylindrical glass cell (100 cm capacity) with an inlet and outlet for water circulation, the solution being stirred by a magnetic stirrer.

The formation constants of the species were determined by the titration of a 50-cm^3 solution containing $Cu[ClO_4]_2$ and the ligands at different ratios with known volumes of standard carbon dioxide-free Na[OH]. Nitrogen was used throughout the titration to maintain an oxygen-free atmosphere. The calculations have been restricted to pH <8 in both the binary and ternary systems, since the region of pH >8 is complicated due to the hydrolysis of the complex. Calculations were made with the aid of the computer program: [5] MINIQUAD-75 on an IBM 370 computer and the results obtained are recorded in Tables 1 and 2.

RESULTS AND DISCUSSION

Binary Systems of Copper(II) with Imidazole, Histamine, and L-Histidine Ligands.—Imidazole functions as a unidentate ligand with the tertiary nitrogen in the ring, generally known as imidazole nitrogen, as the binding group. Copper(II) forms four binary complexes *viz.*, CuA, CuA_2, CuA_3, and CuA_4 with imidazole (A). The stability-constant data obtained in the present study (Table 1) are in agreement with literature values.[2] Formation of CuA_3 and CuA_4 was found to be more favoured in a solution containing a higher concentration of imidazole. The distribution of various species as a function of pH for a 1 : 5 solution of Cu^{II} and imidazole is given in Figure 1.

The stability constants of CuA and CuA_2 complexes in the Cu^{II}–histamine binary system have been reported by several workers.[2] Our results from the detailed titration studies below pH 8 show that the 1 : 1 and 1 : 2 solutions of Cu^{II} and histamine contain CuAH, CuA, CuA_2H, and CuA_2 as the major species. In a 1 : 1 solution, the CuA complex predominated, accounting for *ca.* 85—95% of the total Cu^{II} in the pH range 6—8. Below pH 5, the species which contributed most was CuAH, accounting for *ca.* 25% of the total metal at pH 4.2. Formation of CuA_2H and CuA_2 was found to be more favoured in a 1 : 2 solution as shown in Figure 2.

The Cu^{II}–histidine system is of considerable interest as different geometrical arrangements are possible when the tridentate ligand co-ordinates with Cu^{II} to form 1 : 1 and 1 : 2 complexes. The stability constants of the various binary species such as CuAH, CuA, CuA_2H_2, CuA_2, $CuAH_{-1}$, CuA_2H_{-2}, and CuA_2H_{-1} have been reported by several workers.[2-12] The formation of these complexes is highly pH sensitive and below pH 8 in the present investigation, the presence of CuAH, CuA, CuA_2H_2, CuA_2H, and CuA_2 complexes was detected. In a 1 : 1 solution of Cu^{II} and histidine, the

CuAH and CuA complexes predominated, while the 1 : 2 solutions contained CuA_2H_2, CuA_2H, and CuA_2 as the major species. The contribution of various species in the Cu^{II}–histidine system at various metal-to-ligand ratios has been portrayed graphically by many workers.[6-12]

It must be pointed out that more than one structure is possible for a single species and hence the structure suggested as the predominant one is not the only one present in solution. Copper(II) forms a six-membered chelate ring in the CuA (A = histamine) complex through imidazole and primary amino-nitrogens. The higher log K value of 10.27 in the CuA (histidine) complex compared to that of 9.24 in the CuA (histamine) complex indicates that the histidine is bonding in a tridentate manner. Comparable stability-constant values of 4.22 and 4.11 for the CuAH histamine and histidine complexes respectively to that of 4.21 in the CuA imidazole complex suggest that metal–ligand binding for all these complexes is similar *i.e.*, the co-ordination of Cu^{II} with imidazole nitrogen, as in the CuA imidazole complex, is also expected for the CuAH histamine and histidine complexes. Thus, the site of protonation in the CuAH histamine complex must be the primary amino-group of the histamine ligand. Hence, in the CuAH histidine complex too, the proton would be attached to its primary amino-group, the carboxylate group remaining free.

TABLE 1

Stability constants for the parent binary complexes of imidazole, histamine, and L-histidine with Cu^{II} {37 °C, I = 0.15 mol dm^{-3} ($Na[ClO_4]$)}. The figures in parentheses are the standard deviations in the last decimal figures

(*a*) Cu^{II}–imidazole system

log β							
HA (pK_{NH^+})	CuA (log K)	CuA_2	CuA_3	CuA_4	$\log K^{CuA}_{CuA_2}$	$\log K^{CuA_2}_{CuA_3}$	$\log K^{CuA_3}_{CuA_4}$
6.95(2)	4.21(9)	7.55(14)	10.73(16)	12.91(24)	3.34	3.17	2.19

(*b*) Cu^{II}–histamine and –L-histidine systems

	log β							
	HA	H_2A	H_3A	CuAH	CuA	CuA_2H_2	CuA_2H	CuA_2
Histamine	9.39(8)	15.34(1)		13.46(4)	9.24(18)		21.82(6)	16.16(4)
L-Histidine	8.96(3)	14.96(5)	17.37(9)	14.38(4)	10.27(2)	27.41(21)	23.96(3)	18.49(4)

	log K							
	HA ($pK_{NH_3^+}$)	H_2A (pK_{NH^+})	H_3A (pK_{COOH})	pK^H_{CuAH}	$\log K^{Cu}_{CuA}$	$pK^H_{CuA_2H_2}$	$pK^H_{CuA_2H}$	$\log K^{CuA}_{CuA_2}$
Histamine	9.39	5.95		4.22	9.24		5.66	6.92
L-Histidine	8.96	6.00	2.41	4.11	10.27	3.44	5.47	8.22

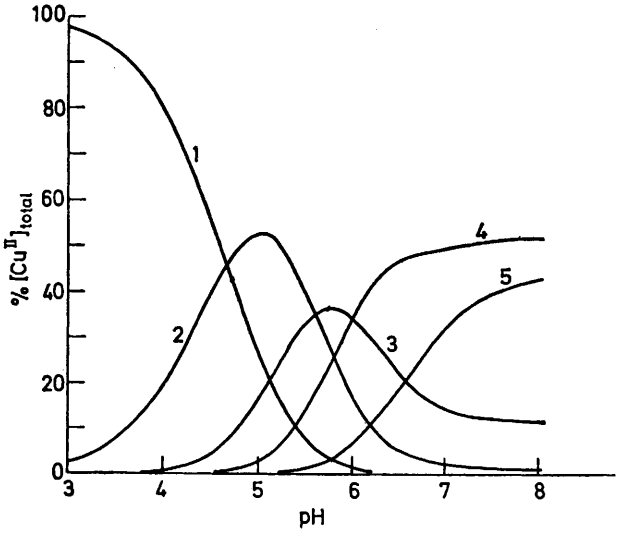

FIGURE 1 Species distribution for Cu^{II}–imidazole (A) system; [Cu] = 0.003, [A] = 0.015 mol dm^{-3}. Unbound Cu^{II} (1), CuA (2), CuA_2 (3), CuA_3 (4), and CuA_4(5)

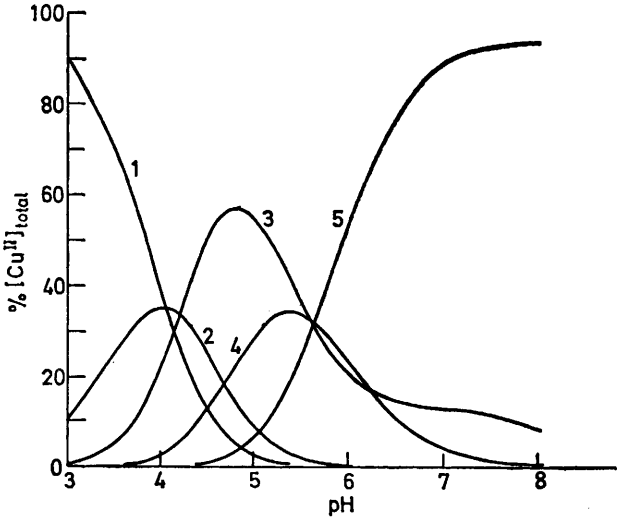

FIGURE 2 Species distribution for Cu^{II}–histamine (A) system; [Cu] = 0.003, [A] = 0.006 mol dm^{-3}. Unbound Cu^{II} (1), CuAH (2), CuA (3), CuA_2H (4), and CuA_2 (5)

This conclusion on the CuAH histidine complex is in agreement with the reports by Carlson and Brown.[10] However, other interpretations *e.g.* (*i*) a protonated primary amino-group with *N*-imidazole and *O*-carboxyl chelation [7,8] and (*ii*) a protonated imidazole group with

chelation as a substituted glycine [9] for the CuAH histidine complexes are put forward by some workers.

Thus, since the site of protonation is the primary amino-group of histamine in the CuAH histamine complex, it can be concluded that in the CuA_2H histamine complex one histamine binds through the imidazole nitrogen, with the primary amino-group being protonated, and the other histamine binds *via* imidazole and primary amino-nitrogens. The CuA_2H histidine complex may also have a similar structure, since its stability-constant value of 5.47 is nearly identical with that of 5.66 for the CuA_2H histamine complex. However, other interpretations are available [8-11] about the binding in the CuA_2H histidine complex. Of these, the co-ordination of two histidines copper(II) complex. Others [15,16] favour a histamine-like co-ordination. From the structures of the CuA_2H and CuA_2H_2 histidine complexes, it is clear that one imidazole group is involved in the CuA_2 histidine complex. Hence, the possibility of both histidines in the CuA_2 complex binding in a glycine-like mode may be considered unlikely. Considering all these factors and two further points [17,18] *viz.*, (*i*) the stability-increasing effect of the imidazole group on the formation of the ternary copper(II) complexes provided an *O*-donor ligand is present, and (*ii*) the preference for copper(II) complexes containing five-and six-membered chelate rings, it appears reasonable to suggest two equilibrium structures for the CuA_2 histidine complex where in one structure,

TABLE 2

Stability constants for the ternary systems of Cu^{II} involving imidazole, histamine, and L-histidine as ligands {37 °C, I = 0.15 mol dm^{-3} ($Na[ClO_4]$)}. The figures in parentheses are the standard deviations in the last decimal figure

(*a*) Cu^{II}–imidazole(A)–secondary ligand (B) systems

Secondary ligand	$\log \beta^{Cu}_{CuAB}$	$\log \beta^{Cu}_{CuA_2B}$	$\log K^{CuAB}_{CuA_2B}$	$\log K^{CuA}_{CuAB}$	$\log K^{CuB}_{CuAB}$	$\Delta \log K$	$\log X$	$\log \beta^{Cu}_{CuAB}$ (calc.)	$\Delta \log \beta$
Histamine	12.72(5)	16.29(4)	3.57	8.51	3.48	−0.73	1.73	12.14	0.58
L-Histidine	13.89(5)	17.51(4)	3.62	9.68	3.62	−0.59	1.74	13.32	0.57

(*b*) Cu^{II}–histamine (A)–L-histidine (B) system

$\log \beta^{Cu}_{CuABH_2}$	$\log \beta^{Cu}_{CuABH}$	$\log \beta^{Cu}_{CuAB}$	$pK^{H}_{CuABH_2}$	pK^{H}_{CuABH}	$\log K^{CuA}_{CuAB}$	$\log K^{CuB}_{CuAB}$	$\Delta \log K$	$\log X$	$\log \beta^{Cu}_{CuAB}$ (calc.)	$\Delta \log \beta$
27.88(25)	23.34(16)	17.78(11)	4.54	5.56	8.54	7.51	−1.73	0.91	17.63	0.15

with one histamine-like and the other having *N*-imidazole and *O*-carboxyl chelation, the primary amino-group being protonated, is the most popular view. The probability for a structure of this type seems lower since it involves a six-and a seven-membered ring, which is less favoured for Cu^{II} due to well known steric reasons.

Similarly, as the results in the present study suggest Cu^{II} binding to the imidazole nitrogen in the CuAH histidine complex, one may expect a structure for the $Cu(AH)_2$ histidine complex in which the two histidines bind through their imidazole nitrogens, the protons being attached to their primary amino-groups. However, Liberman and Rabin [11] proposed that in the CuA_2H_2 histidine complex two seven-membered chelate rings through the *N*-imidazole and *O*-carboxyl groups in the two histidine molecules are involved. The probability for a structure of this type, however, seems slight because copper(II) chelates with two seven-membered rings are sterically less favoured.

With regard to the solution structures of CuA_2 histamine and histidine complexes, their respective log K values of 6.92 and 8.22 clearly indicate different structures. A square-planar structure involving imidazole and primary amino-nitrogens in the two histamine ligands is expected for the CuA_2 histamine complex. The same structural characteristics have been shown by crystal-structural analysis [12] to be present in dihistaminecopper(II) perchlorate. There has been considerable confusion over the structure of the CuA_2 histidine complex. Several workers [13,14] suggest that the two histidines are bound in a glycine-like manner to form a square-planar both histidines bind histamine-like, forming two six-membered chelates (I) and in the second structure, one histidine binds histamine-like and the other histidine binds glycine-like, forming six- and five-membered chelate rings (II). By n.m.r. [19] and circular dichroism [20] studies, it was shown that the CuA_2 histidine complex with the histamine-like and glycine-like mode of binding [structure (II)] is more favoured. The higher log K value for the histidine complex compared to that for the histamine complex is then clearly due to this structural characteristic of the CuA_2 histidine complex.

Copper(II) Ternary Systems involving Imidazole, Histamine, and L-Histidine as Ligands.—Three ternary systems *viz.*, (*i*) Cu^{II}–imidazole (A)–histamine (B), (*ii*) Cu^{II}–imidazole (A)–L-histidine (B), and (*iii*) Cu^{II}–histamine (A)–L-histidine (B) are discussed in this section.

The ternary systems (*i*) and (*ii*) showed the presence of two ternary complexes (CuAB and CuA_2B) in addition to the binary complex species [CuA, CuA_2, CuA_3, CuA_4, CuBH, CuB, CuB_2H, CuB_2, and CuB_2H_2 in the histidine (B) ligand system]. The presence of CuA_2B species in both the systems indicates that Cu^{II} prefers to be four-co-ordinate. The values of log X and Δ log K (parameters generally used for indicating the stabilization of the ternary complex with respect to the binary one) and Δ log β, defined according to the expressions [21] (1)—(5), are included in Table 2. The log X values of 1.73 and 1.74 (both being higher than the statistically expected value of 0.6) [21] and Δ log β values of 0.58 and 0.57 (both being positive) for systems (*i*) and (*ii*) respectively

suggest higher stability for the ternary complex species compared to the binary ones. This may be explained on the basis of π-acceptor properties of the imidazole rings in both the primary (A) and secondary (B) ligands. However, the Δ log K values in Table 2 for these systems do

$$CuA_2 + CuB_2 \rightleftharpoons 2\ CuAB \quad (1)$$

$$\log X = 2 \log \beta_{CuAB} - (\log \beta_{CuA_2} + \log \beta_{CuB_2}) \quad (2)$$

$$CuA + CuB \rightleftharpoons CuAB + Cu \quad (3)$$

$$\Delta \log K = \log \beta_{CuAB} - (\log K_{CuA} + \log K_{CuB}) \quad (4)$$

$$\Delta \log \beta = \log \beta_{CuAB}\ (exp.) - \log \beta_{CuAB}\ (calc.) \quad (5)$$

not differ much from their statistically expected values.[21] The higher log β_{CuAB} value of 13.89 in the histidine (B) ligand system compared to that of 12.72 in the histamine (B) ligand system indicates that the histidine (B) ligand functions in a tridentate manner in the ternary species CuAB. The concentration distribution diagrams for both these ternary systems are given in Figures 3 and 4.

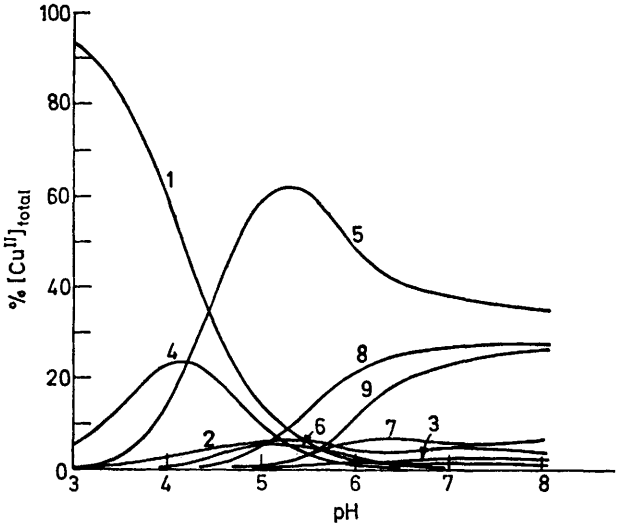

FIGURE 3 Species distribution for Cu^{II}–imidazole (A)–histamine (B) system; [Cu] = [A] = [B] = 0.003 mol dm^{-3}. Unbound Cu^{II} (1), CuA (2), CuA_2 (3), CuBH (4), CuB (5), CuB_2H (6), CuB_2 (7), CuAB (8), and CuA_2B (9). The CuA_3 and CuA_4 species are not shown due to their very low concentration

In system (*iii*), three ternary species ($CuABH_2$, CuABH, and CuAB) in addition to the binary species (CuAH, CuA, CuA_2H, CuA_2, CuBH, CuB, CuB_2H_2, CuB_2H, and CuB_2) were detected. The log X value of 0.91 (slightly higher than the statistical value of 0.6), Δ log K value of −1.73 (more negative), and Δ log β value of 0.15 (very near to zero) in Table 2 suggest that there is no marked stabilization in the copper(II) ternary complex formation with histamine and L-histidine ligands. This may be due to the mutual interactions between histamine (A) and histidine (B) ligands in the CuAB complex, since they are of similar type. However, all three ternary species *viz.*, $CuABH_2$, CuABH, and CuAB, were found in appreciable amounts (13%, 22%, and 56% of the total Cu^{II} respectively) compared to the binary species in this system (Figure 5). This suggests that ternary complex formation is favoured and hence some other factors must be involved in it. One possible explanation comes

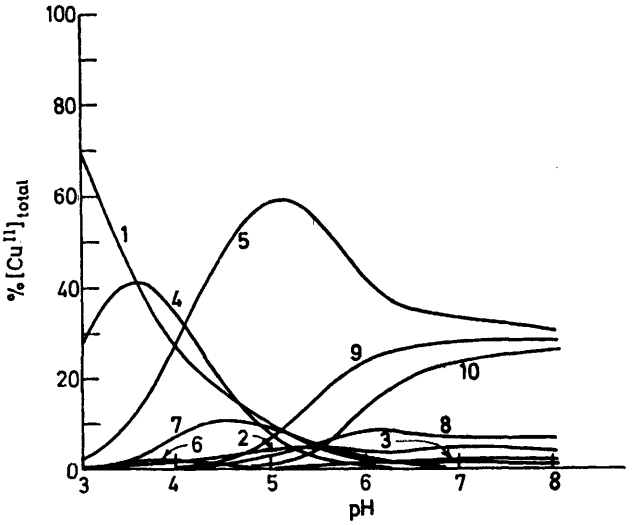

FIGURE 4 Species distribution for Cu^{II}–imidazole (A)–L-histidine (B) system; [Cu] = [A] = [B] = 0.003 mol dm^{-3}. Unbound Cu^{II} (1), CuA (2), CuA_2 (3), CuBH (4), CuB (5), CuB_2H_2 (6), CuB_2H (7), CuB_2 (8), CuAB (9), and CuA_2B (10). The species CuA_3 and CuA_4 are not shown due to their very low concentration

from considering the ambidentate nature of the histidine (B) ligand *i.e.*, two equilibrium structures may be suggested for the CuAB species in the Cu^{II}–histamine (A) –histidine (B) system, where in one structure (III),

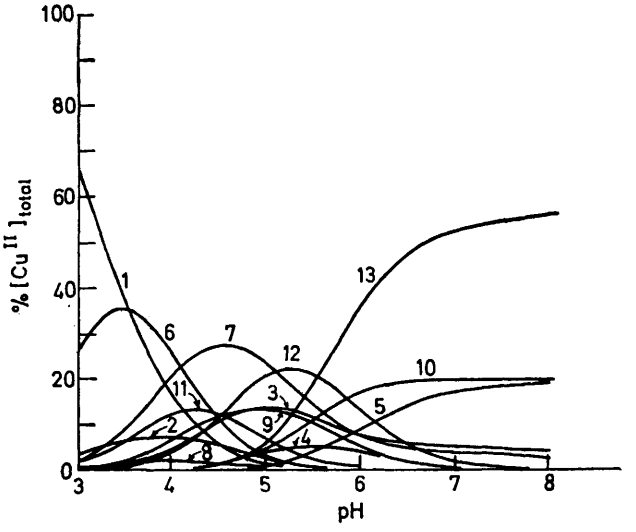

FIGURE 5 Species distribution for Cu^{II}–histamine (A)–L-histidine (B) system; [Cu] = [A] = [B] = 0.003 mol dm^{-3}. Unbound Cu^{II} (1), CuAH (2), CuA(3), CuA_2H (4), CuA_2 (5), CuBH (6), CuB (7), CuB_2H_2 (8), CuB_2H (9), CuB_2 (10), $CuABH_2$ (11), CuABH (12), and CuAB (13)

histidine (B) binds histamine-like and in the other (IV), histidine binds glycine-like, thus the CuAB species in structure (III) has two six-membered chelate rings and in structure (IV) one six- and one five-membered chelate

rings. Structure (IV) is more favoured due to (*a*) the stability-increasing effect of the imidazole group on the formation of ternary copper(II) complexes, provided an *O*-donor ligand is present, and (*b*) the preference for copper(II) ternary chelates containing six- and five-membered chelate rings.[17,18]

With regard to the protonated ternary species CuABH and $CuABH_2$, one can suggest locations for the protons by considering their pK_a values. A comparable pK^{H}_{CuABH} value of 5.56 in Table 2 to the $pK^{H}_{CuA_2H}$ values of 5.66 and 5.47 for the CuA_2H histamine and histidine complexes respectively in Table 1 suggests that the site of protonation in the CuABH complex is the primary amino-group of the histamine (A) or histidine (B) ligand, as in the CuA_2H histamine or histidine complex. However, it is not possible to predict the exact ligand being protonated, *i.e.*, whether the proton in the CuABH species is

$$CuA_2 + H^+ \xrightleftharpoons{pK^{H}_{CuA_2H} = 5.66} CuA_2H \text{ (A = histamine)} \quad (6)$$

$$CuA_2 + H^+ \xrightleftharpoons{pK^{H}_{CuA_2H} = 5.47} CuA_2H \text{ (A = histidine)} \quad (7)$$

$$CuAB + H^+ \xrightleftharpoons{pK^{H}_{CuABH} = 5.56} CuABH \text{ (A = histamine, B = histidine)} \quad (8)$$

attached to histamine (A) or to histidine (B). Naturally in the $CuABH_2$ species, one may expect the sites of protonation to be the primary amino-groups of the histamine (A) and histidine (B) ligands. The protonation reactions may be represented as equations (6)—(8).

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