Pattern of Transition-metal Chelates with Biological Activity. Complex Formation Equilibria between α-Amino-*N*-hydroxy-1*H*-imidazole-4-propanamide and Cobalt(II), Nickel(II), Copper(II), and Hydrogen Ions in Aqueous Solution

Enrico Leporati
Istituto di Chimica Generale ed Inorganica, dell'Università degli Studi di Parma, Via M. D'Azeglio 85, 43100 Parma, Italy

The complex-formation equilibria between Co^{II}, Ni^{II}, and Cu^{II} and α-amino-*N*-hydroxy-1*H*-imidazole-4-propanamide (ahip) have been investigated by potentiometric titrations in 0.5 mol dm^{-3} KCl solution at 25 °C. Their acid–base and metal–ligand reaction stoicheiometrics have been determined, and the equilibrium constants of the corresponding proton and metal ion association constants have been calculated from potentiometric data with the aid of the programs BETAREF and SUPERQUAD. The parameters relating to potentiometric calibration curves and the equilibrium chemical models are selected on the basis of a critical evaluation of the least-squares results and of a statistical analysis of the weighted residuals. Protonated and hydrolysed complexes were formed in the pH range 3.0—11.0. The following cumulative formation constants $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$ were obtained: ahip, $\log \beta_{011} = 8.942(2)$, $\log \beta_{021} = 16.009(2)$, $\log \beta_{031} = 21.400(4)$; Co^{II}–ahip, $\log \beta_{111} = 14.036(23)$, $\log \beta_{122} = 26.633(45)$, $\log \beta_{112} = 20.861(30)$, $\log \beta_{102} = 12.784(50)$; Ni^{II}–ahip, $\log \beta_{111} = 15.009(7)$, $\log \beta_{122} = 28.821(17)$, $\log \beta_{112} = 22.793(12)$, $\log \beta_{101} = 9.27(7)$, $\log \beta_{102} = 15.644(13)$, $\log \beta_{1-12} = 4.961(17)$; Cu^{II}–ahip, $\log \beta_{111} = 16.916(12)$, $\log \beta_{122} = 29.15(9)$, $\log \beta_{112} = 22.08(9)$, $\log \beta_{101} = 10.698(38)$, $\log \beta_{102} = 13.73(8)$, $\log \beta_{1-12} = 3.34(8)$. The probable structures of the chelated compounds formed in aqueous solution were deduced and their protonation and stability constants compared with those of analogous co-ordination compounds. The ligand is bound to the metal ion *via* N(3) of the imidazole ring, the α-amino nitrogen, and the hydroxyl oxygen of the deprotonated NHO^- group. Equilibrium constants for their formation and visible spectra of the Cu^{2+}–ahip system are discussed in terms of the metal chelate structures and their possible significance to biological systems.

This paper is the second devoted to describing chemical equilibria which can be used as indicators of biological activity with the eventual aim of designing metal chelates as suitable sources of various trace elements essential in animal nutrition. Hydroxamic acids are very important chelating agents in biological systems and are also potent inhibitors of urease activity.[1] From time to time hydroxamic acids have become important as therapeuticals in the treatment of hepatic coma.[2] It has been shown recently that the inhibition of Jack bean urease[3] by hydroxamic acids is reversible, and the spectral changes observed have been explained in terms of a reversible binding mode to the active-site nickel ion. It has also been suggested that aminohydroxamic acids may be particularly active because of a possible surface-active role by an unco-ordinated amino group;[4] however, in the case of Co^{II}, Ni^{II}, and Cu^{II} complexes of 2-amino-*N*-hydroxyacetamide (aha) and 2-amino-*N*-hydroxypentanamide (ahp), I have shown recently[5] that co-ordination clearly involves not only the deprotonated NHO^- group but also the α-amino group. In this paper, the only characteristic that the trace elements (Co^{2+}, Ni^{2+}, Cu^{2+}) have in common is that they are present or exert activity in plant and animal tissues in low concentrations, relative to the major mineral elements, and thus I discuss the complex chemical equilibria of metal–histidinehydroxamic acid systems as a possible source of these elements. In addition to the essential elements that are required for different life forms (*e.g.*, Co, Cu, *etc.*), nickel has recently been indicated as an essential element in animal nutrition. Its physiological role has not been delineated as yet. Nickel is now considered to be among the newer essential metals.[6,7]

Experimental

Reagents.—α-Amino-*N*-hydroxy-1*H*-imidazole-4-propanamide (ahip) was obtained from Sigma (St. Louis) and its purity checked potentiometrically. Concentrations of stock solutions of bivalent metal chlorides (AnalaR Products) were determined by inductively coupled plasma (i.c.p.) atomic emission spectrometry. The starting solutions for each potentiometric experiment were prepared by adding successively to the titration compartment a known volume of ahip solution, and an exact volume of metal chloride; then the required quantities of potassium chloride (Merck), which was used as supporting electrolyte, and a sufficient amount of doubly distilled water were added to make up the total volume V_0, which was 25.00 ± 0.015 cm^3. Doubly distilled and deionized water was used throughout, and all experiments were carried out under an atmosphere of purified nitrogen. All other chemical reagents were of the highest grade available and were used as previously described.[8–10] The ionic medium was 0.5 mol dm^{-3} KCl at the beginning of each potentiometric titration. The use of a constant medium was necessary in order to minimize variations of the activity coefficients in spite of wide changes in the concentration of the reagents.

Potentiometric Measurements.—The e.m.f. measurements were performed by using a Metrohm Titroprocessor E 636, equipped with an H 268 glass electrode (Schott-Jena glass) and a B 343 Talamid reference electrode (Schott-Jena glass). The potentiometric titrations were carried out at 25 ± 0.1 °C and $I = 0.5$ mol dm^{-3} KCl as previously reported.[9] The titration cell was maintained at 25.0 ± 0.1 °C by the circulation of thermostatted water. A slow nitrogen stream, presaturated by bubbling it through an appropriate KCl solution (0.5 mol dm^{-3}), was maintained in the titration vessel. In the e.m.f. experiments the standard electrode potential (E°), the coefficients of the correction terms for the effects of both liquid junction potential in acid (A_j) and basic (B_j) solution, the concentration of the potassium hydroxide solution (N), and K_w were determined before and after each experiment by dynamic titration [the density of the measuring points with a step of variable volume means that at each point on the titration curve, excepting the first three, the amount of added KOH (Δv) is inversely proportional to the last difference (ΔE) in the electromotive force] of a known amount (*ca.* 2.0 cm^3) of hydrochloric acid (0.2969 mol dm^{-3}) in 0.5 mol dm^{-3} KCl at 25 °C with potassium hydroxide solution, using the computer program NBAR[11,12] following the calculation procedures described previously.[8] For all the potentiometric titrations containing metal ions and/or ahip at different molar ratios, the monotonous titration technique [density of the measuring points with a step of constant volume means that at each point on the titration curve the small amount of added KOH is constant ($\Delta v = 0.05$ cm^3)] was employed. Quantities of titrant KOH were added with the use of a Metrohm Dosimat E 635 autoburet (total volume = 5.0 cm^3). E.m.f. readings and titration curves were recorded graphically by using an E 636 automatic titrator and a thermoprinter.

Spectrophotometric Measurements.—Absorption spectra in the region 440—800 nm were obtained on a Jasco Uvidec-505 spectrophotometer. Solutions containing ahip and copper ion maintained with ionic strength 0.5 mol dm^{-3} (KCl) and at 25 °C were scanned at a series of pH from 3.0 to 11.0 by using 5-mm cells.

Calculations.—Careful attention has been paid to the calculation and critical evaluation of some parameters relating to potentiometric calibration curves. In the potentiometric experiments E°, A_j, B_j, N, K_w, and the equivalence point (v_e) were determined under the same experimental conditions, according to Gran's method[11] using different calculation procedures as previously described.[8] The results obtained from two distinct sets of calibration curves are collected in Table 1. The first set (runs 1—6) refers to a solution of KOH, used as titrant, prepared several days previously, while the second set (runs 7—12) refers to a freshly prepared KOH solution. From the results obtained it is possible to verify excellent agreement between the parameters (E°, v_e, N, v_e' and v_e'') calculated for acidic solution and those calculated for alkaline solution for the second set. The statistical analysis of the results relating to the first set shows how the parameters (v_e and N, Table 1) obtained from the two regions (acid and basic) of the same titration curves are practically indistinguishable and the error in v_e or N between one titration and another (intertitration error) is smaller than that found in a single titration (intratitration error), as proof of the fact that all the points of all the titrations form a well behaved normal population. On the contrary the intertitration error in E°, A_j, B_j, and K_w is greater than the intratitration error, thus showing that unexpected factors differing from one titration to another alter the e.m.f. values and are the main source of error. In some titration curves considerable shifting in the parameters [E° (acid), E° (basic), A_j, B_j, and K_w] can be observed (see runs 1 and 4). In addition, the equivalence points v_e' and v_e'' are slightly larger than v_e (acid or basic) of the same titration curves, perhaps because of different methods of calculation applied. Thus the precision of the standard electrode potential and of the ionic product of water can be calculated only from the intertitration variability. This result is perfectly in keeping with the conclusion drawn from an analysis of the results obtained in previous papers.[5,9,10] In particular, values of K_w (Table 1) differ significantly from one experiment to another. This could be due to various factors: deterioration in the KOH solution or a non-linear function of the electrodes in the experimental range of pH (7.0—12.2), due to the appearance of the 'potassium error' (competition of K^+ with H^+) which starts mostly only above pH 11.0 and increases with the ionic strength. It is generally accepted that values of E° can change from one titration to another; however, treating the data of each experiment separately and then averaging and assessing the results is recommended because it gives a more reasonable measure of the dispersion of the errors. The stability constants (β_{pqr}), which are defined by equation (1) (charges are omitted

$$pM + qH + rL \rightleftharpoons M_pH_qL_r \qquad (1)$$
$$\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$$

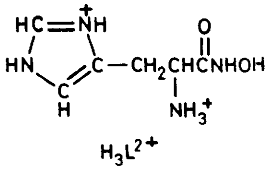
for simplicity), were refined by the method of rigorous least squares using the computer programs BETAREF[13] and SUPERQUAD;[14] p, q, and r are the number of metal(II), proton, and ligand, respectively, in the complex $M_pH_qL_r$. These programs calculate the values of the overall protonation and formation constants which minimize the sum of the squared residual between observed and calculated e.m.f. values, equation (2). The parameter Z is the total number of

$$U = \sum_{i=1}^{Z} w_i(E_i^{\text{obs.}} - E_i^{\text{calc.}})^2 \qquad (2)$$

potentiometric data and w_i is the weighting factor assigned to the ith observation [σ_E (= 0.2) is the error in the e.m.f. and σ_v (= 0.015) the error in the volume used in the refinement]. For other important features of BETAREF and SUPERQUAD the previous programs MINIQUAD[15] and MIQUV[16] and ref. 17 should be examined. All calculations were performed on the CRAY X-MP/12 computer of the Consorzio per la Gestione del Centro di Calcolo Elettronico Interuniversitario dell'Italia Nord Orientale, Casalecchio, Bologna, with financial support from the University of Parma. The compositions of the starting solutions for each potentiometric titration are quoted in Table 2.

Results and Discussion

Protonation Equilibria.—Initially the protonation constants of the ligand and the initial amounts (mmol) of reagents (T_L, T_H) were determined at the same time through the refinement of several sets of potentiometric data by SUPERQUAD. After the refinement the maximum variation in the quantities (T_L, T_H) with respect to the initial parameters was 1.12 and 1.33%, respectively. BETAREF was then used to refine the same parameters (T_L, T_H) in addition to protonation constants by introducing into the calculations the liquid-contact potentials (A_j and B_j). The maximum variation in the initial amount of the reagents (T_L, T_H) was 1.17 and 1.45%, respectively. The standard deviations of the refined quantities (Table 3) were very small and the results obtained, also in the light of the statistical analysis of the data, were in excellent agreement with the corresponding protonation constants obtained by SUPERQUAD. Since the liquid-junction potentials, relative to the cell engaged in the measurements, are fairly small and not



H_3L^{2+}

Table 1. Evaluation of E° (mV),[a] A_j (mV mol^{-1}),[a] B_j (mV mol^{-1}),[a] v_e (cm^3), N (mol dm^{-3}) (normality of KOH), and K_w (ionic product) from two sets of several potentiometric titrations of HCl (0.2969 mol dm^{-3}) with KOH at 25 °C and I = 0.5 mol dm^{-3} (KCl) both in acidic and alkaline media using the NBAR program

	E°				v_e		N				
Run	Acid	Basic	A_j	B_j	Acid	Basic	Acid	Basic	$10^{14}K_w$	v_e'[b]	v_e''[c]
1	1 170.84(8)	1 174.04(9)	−37.40(4.28)	−162.68(6.92)	2.054	2.054	0.289 11	0.289 10	2.1332	2.065	2.061
2	1 171.21(5)	1 171.68(10)	−41.45(3.12)	−54.78(9.85)	2.054	2.051	0.289 03	0.289 47	1.9631	2.067	2.062
3	1 170.95(8)	1 170.90(15)	−32.24(6.48)	−84.25(12.40)	2.056	2.050	0.288 86	0.289 62	1.9238	2.068	2.065
4	1 170.89(14)	1 174.22(15)	−46.45(7.50)	−204.19(10.91)	2.057	2.049	0.288 71	0.289 84	2.1943	2.061	2.063
5	1 169.75(13)	1 168.05(11)	−56.11(8.56)	−38.36(8.22)	2.057	2.051	0.288 66	0.289 56	1.8041	2.069	2.072
6	1 169.62(10)	1 167.99(6)	−50.20(6.21)	−34.24(4.93)	2.055	2.053	0.288 91	0.289 17	1.8090	2.065	2.066
Mean with standard deviation					2.056(1)	2.051(2)	0.288 88(18)	0.289 46(28)	1.9713(1628)	2.066(3)	2.065(4)
Variance					1.899 × 10^{-6}	3.466 × 10^{-6}	3.076 × 10^{-8}	7.876 × 10^{-8}	0.026	8.164 × 10^{-6}	1.577 × 10^{-5}
Standard error					5.626 × 10^{-4}	7.601 × 10^{-4}	7.160 × 10^{-5}	1.146 × 10^{-4}	0.066	1.166 × 10^{-3}	1.621 × 10^{-3}
7	1 159.57(9)	1 158.72(13)	−18.04(13.15)	−19.22(10.07)	1.950	1.942	0.304 59	0.305 75	1.8648	1.950	1.946
8	1 159.03(5)	1 157.47(7)	−11.47(4.72)	−11.57(6.52)	1.958	1.956	0.304 79	0.305 03	1.8140	1.960	1.959[d]
9	1 161.50(10)	1 160.23(7)	−21.15(5.77)	−27.34(5.02)	1.949	1.945	0.304 72	0.305 33	1.8346	1.949	1.949
10	1 161.33(8)	1 160.26(8)	−1.98(8.79)	−37.28(6.09)	1.949	1.945	0.304 74	0.305 29	1.8489	1.951	1.947
11	1 160.50(8)	1 159.79(7)	−5.76(6.72)	−46.34(5.75)	1.949	1.945	0.304 67	0.305 33	1.8750	1.945	1.944
12	1 160.70(7)	1 160.59(11)	3.37(4.72)	−74.40(7.52)	1.958	1.955	0.304 74	0.305 25	1.9193	1.955	1.952[d]
Mean with standard deviation					1.949(1)	1.945(1)	0.304 71(7)	0.305 33(23)	1.8594(366)	1.948(3)	1.946(3)
Variance					5.656 × 10^{-7}	1.867 × 10^{-6}	4.770 × 10^{-9}	5.483 × 10^{-8}	0.0013	7.069 × 10^{-6}	7.766 × 10^{-6}
Standard error					3.070 × 10^{-4}	5.578 × 10^{-4}	2.820 × 10^{-5}	9.559 × 10^{-5}	0.0149	1.085 × 10^{-3}	1.138 × 10^{-3}

[a] Standard deviations in E°, A_j, and B_j are given in parentheses. [b] Equivalence point calculated following the Metrohm method. [c] Equivalence point calculated following the principles of Gran by using all experimental data (also those near the equivalence point) in acidic solution. [d] Initial amount of hydrochloric acid was 2.01 cm^3, for all the others 2.0 cm^3.

Table 2. Protonation and complex-formation constant determinations. Initial concentrations (T_L, T_M, T_H/mmol) of the reagents* for the alkalimetric titrations of α-amino-*N*-hydroxy-1*H*-imidazole-4-propanamide with bivalent metal ions at 25 °C and $I = 0.5$ mol dm^{-3} (KCl)

Run	System	T_L	T_M	T_H	pH
1	H^+–ahip	0.195 16		0.671 07	2.6—11.3
2		0.204 92		0.614 77	4.4—11.1
3		0.219 56		0.658 68	3.8—11.3
4		0.185 41		0.556 22	4.3—11.0
5		0.214 68		0.644 04	4.2—11.6
6	Cu^{II}–ahip	0.197 47	0.065 88	0.591 86	3.0—11.1
7		0.222 15	0.055 49	0.665 84	3.0—10.1
8		0.197 47	0.039 51	0.591 86	3.1—11.5
9		0.172 79	0.173 10	0.517 87	2.9—7.4
10		0.207 34	0.101 83	0.621 45	2.9—10.7
11	Ni^{II}–ahip	0.197 47	0.098 50	0.591 86	3.5—10.8
12		0.197 47	0.066 15	0.591 86	3.5—10.7
13		0.197 47	0.049 25	0.591 86	3.5—11.1
14		0.172 79	0.086 00	0.517 87	3.5—11.1
15		0.222 15	0.044 10	0.665 84	3.5—11.0
16		0.207 34	0.069 09	0.621 45	3.5—10.7
17	Co^{II}–ahip	0.197 47	0.066 86	0.636 39	2.7—10.8
18		0.197 47	0.049 79	0.636 39	2.7—11.2
19		0.172 79	0.071 13	0.517 87	3.4—10.9
20		0.222 15	0.045 52	0.665 84	3.3—10.5
21		0.246 84	0.069 71	0.739 82	3.2—10.2

* T_L = mmol of ligand, T_M = mmol of metal, T_H = mmol of hydrogen ion in the titration vessel.

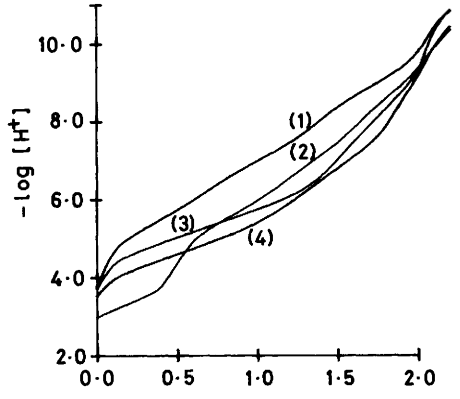


Figure 1. Titration curves of pH as a function of the volume of KOH added, calculated by the HALTAFALL program, equipped with PLOTTER Calcomp 936. ahip (1), with Cu (2), Co (3), or Ni (4). $c_L = 8.0 \times 10^{-3}$, $c_H = 2.4 \times 10^{-2}$, $c_M = 2.7 \times 10^{-3}$, and $c_{KOH} = 0.2891$ mol dm^{-3}; $V_o = 25.0$ cm^3

prominent in the calculations of the equilibria (pH interval used 2.6—11.6, see also the results relating to Ni^{II}–ahip system, Table 3), they have been neglected in other calculations.

The potentiometric titration curve of the completely protonated form of ahip (H_3L^{2+}) is shown in Figure 1. Three protons per ligand molecule dissociate between $a = 0$ and 2.0 [where a = volume (cm^3) of KOH added]. The β values for the protonation equilibria (Table 3) for L^- are not the same as those obtained by Brown and Sekhon[18a] who report values of log $\beta_{011} = 9.11$, log $\beta_{021} = 16.33$, and log $\beta_{031} = 21.73$ at an ionic strength of 0.15 mol dm^{-3} (NaCl) at 25 °C. In particular, the greatest differences, as regards the data reported in the literature,[18a] occur for log K_1^H and log K_2^H. This might be caused by the influence of the ionic strength on the protonation constants which change in a manner similar to those of acetic acid[18b] or glycine[19] and of other analogous compounds.[20–22] The compound ahip has three titratable protons. The log β_{011} or log K_1^H value corresponding to protonation of the primary amine group was estimated to be 8.942(2). On the basis of the results and by analogy with known values for analogous functional groups, log $K_2^H = 7.067(2)$ and log $K_3^H = 5.391(3)$ can be assigned to protonation of the oxygen of the NHO^- group and the nitrogen atom, N(3), of the imidazole ring, respectively. The presence of the α-amino group in aminohydroxamic acids increases remarkably the acidic character of the OH group [log K_2^H for aha,[5] ahp,[5] and ahip, and log K_3^H for α-amino-*N*-hydroxy-*p*-hydroxyphenylpropanamide (ahhpp)[23]] compared to that of acetohydroxamic acid (log $K_1^H = 9.342$[24]) in the order aha < ahp < ahip < ahhpp. This behaviour can be well explained by taking into account also the influences of the $CH_3CH_2^-$ group, the imidazole moiety, and the *p*-hydroxyphenyl group, respectively. Similarly, by comparing log K_1^H and log K_3^H for ahip with those of histidine[25] [log $K_1^H = 9.31(4)$, log $K_2^H = 6.02(2)$] it can be seen that substitution of an NHOH group for the OH group of a carboxyl rather lowers the protonation constants according to the electron-withdrawing character of the NHOH group.

Metal(II) *Complex Equilibria*.—The presence of likely metal–proton–ahip species was tested for by using the following values: $p = 1, 2, 3$; $q = 1, 2, 3, -1, -2, -3$; and $r = 1, 2$, and 3. The equilibrium models were selected in successive trials according to the best agreement between experimental and calculated potentiometric data. The values of the standard deviations (σ) of the parameters refined (log β_{pqr}), the agreement factor (σ^2), the goodness of fit statistic (χ^2), and the chemical significance of the species identified in solution were taken into account for each hypothesis. At this point all the protonation constants were kept constant and the computer programs SUPERQUAD and BETAREF (only for Ni^{II}–ahip system) were employed for a second stage of refinement in which solutions containing various metal ion–ahip ratios were processed in order to explore the binary systems. In the final section of calculations to determine the formation constants of the binary complexes, the initial amounts (mmol) of reagents (T_L, T_H) were minimized only for titration run 12 (Table 2) regarding the Ni^{II}–ahip system; the greatest variation in these quantities by comparison with the initial parameters was 0.22 and 0.62%, respectively. The calculated formation constants, log β_{pqr}, of the ligand with different metals, are given in Table 3. Sample potentiometric titration curves in the presence of Co^{2+}, Ni^{2+}, and Cu^{2+} are shown in Figure 1. The shapes of the curves clearly show different types of reactions involving the formation of simple mononuclear chelates, as well as various protonated complexes. The most common initial reaction involving divalent transition-metal ions is a two-proton displacement to form a monohydrogen complex, equation (3). Thus, the curves

$$M^{2+} + H_3L^{2+} \rightleftharpoons [MHL]^{2+} + 2H^+ \qquad (3)$$

show that all the metals investigated react with the bivalent cation (H_3L^{2+}) according to equation (3). This is generally followed by addition and subsequent dissociation reactions, in which one or two additional moles of base are required for each mole of metal chelate, equations (4) and (5).

$$[MH_2L_2]^{2+} \rightleftharpoons [ML_2] + 2H^+ \qquad (4)$$

$$[MHL_2]^+ \rightleftharpoons [ML_2] + H^+ \qquad (5)$$

In the cases of Co^{2+}, Ni^{2+}, and Cu^{2+}, the buffer regions of the above reactions do partially overlap, so that each reaction

Table 3. Cumulative and stepwise protonation complex-formation constants of α-amino-*N*-hydroxy-1*H*-imidazole-4-propanamide with bivalent metal ions at 25 °C and $I = 0.5$ mol dm^{-3} (KCl). Standard deviations (σ values) are given in parentheses

	SUPERQUAD				BETAREF	
	H^+	Co^{II}	Ni^{II}	Cu^{II}	H^+	Ni^{II}
log β_{011}	8.942(2)				8.947(3)	
log β_{021}	16.009(2)				16.017(6)	
log β_{031}	21.400(4)				21.409(9)	
log $K_2^{H\,a}$	7.067(2)^b				7.070(5)^b	
log $K_3^{H\,a}$	5.391(3)^b				5.392(8)^b	
log β_{101}			9.27(7)	10.698(38)		9.28(9)
log β_{111}		14.036(23)	15.009(7)	16.916(12)		15.009(9)
log β_{122}		26.633(45)	28.821(17)	29.15(9)		28.819(21)
log β_{112}		20.861(30)	22.793(12)	22.08(9)		22.793(14)
log β_{1-12}			4.961(17)	3.34(8)		4.962(22)
log β_{102}		12.784(50)	15.644(13)	13.73(8)		15.643(15)
Z^c	256	226	289	238	256	289
U	8.129	4.995×10^2	8.369×10^1	6.668×10^2	1.374×10^1	1.325×10^2
$\chi^{2\,d}$	7.50	5.08	12.04	6.57	12.38	15.14
$\sigma^{2\,e}$	0.18	1.50	0.55	1.69	0.24	0.69

[a] log K_n^H = log β_{0n1} − log β_{0n-1}. [b] $\sigma(\log K_n^H) = \{[\sigma^2(\log \beta_{0n1}) + \sigma^2(\log \beta_{0n-11})]/2\}^{\frac{1}{2}}$. [c] Total number of data points used in the refinement. [d] Observed χ^2; calculated value (degrees of freedom 6, confidence coefficient 0.95) should be 12.6. [e] $\sigma^2 = \sum_{n=1}^{Z} w_i(E_i^{obs.} - E_i^{calc.})^2/(Z - m)$; m is the number of parameters to be refined.

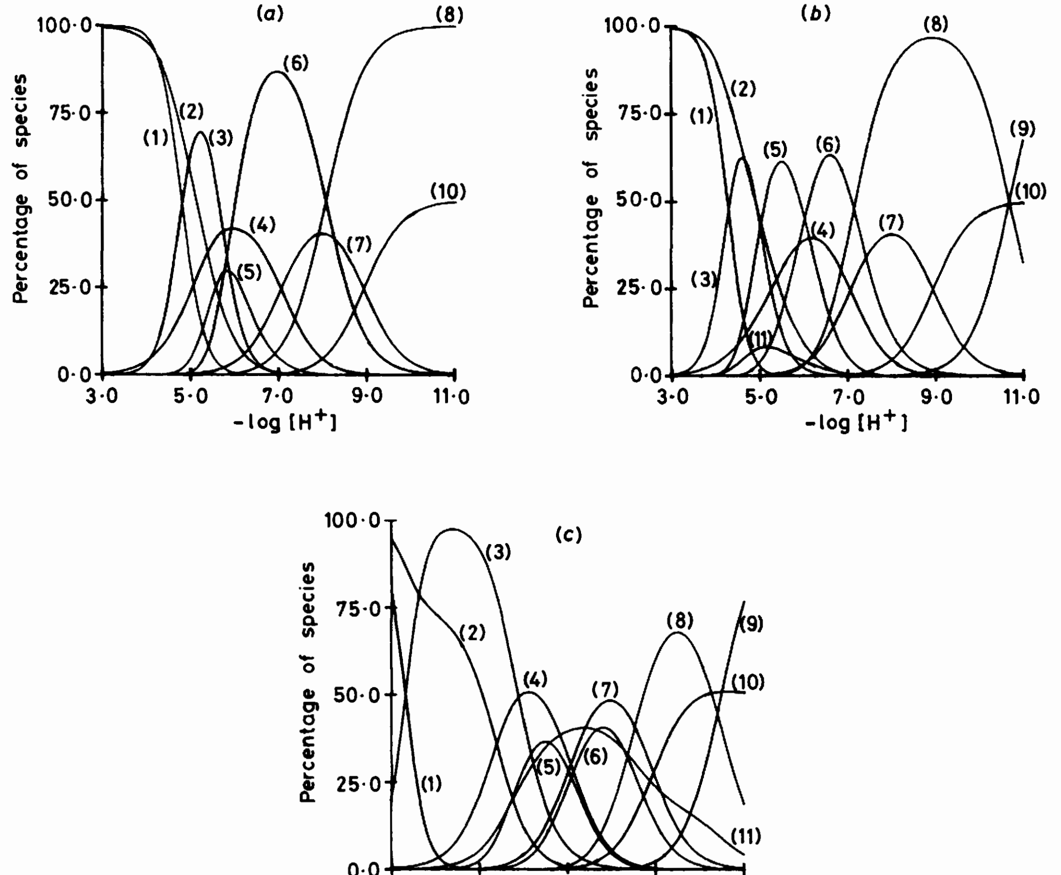
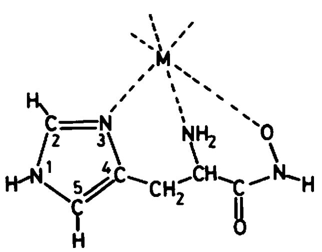


Figure 2. Typical distribution diagrams for M^{II}-ahip systems. The percentage of each species has been calculated from the data of a hypothetical solution of metal ions (0.002 mol dm^{-3}) and ahip (0.008 mol dm^{-3}) by the HALTAFALL program (N. Ingri, W. Kakalowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261), equipped with a PLOTTER Calcomp 936. The concentrations of the species not containing metal were calculated as percentages of the total ligand, those containing metal as percentages of the total metal: (*a*) Co^{2+}-ahip, (*b*) Ni^{2+}-ahip, (*c*) Cu^{2+}-ahip; (1) M^{2+}, (2) H_3L^{2+}, (3) $[MHL]^{2+}$, (4) H_2L^+, (5) $[MH_2L_2]^{2+}$, (6) $[MHL_2]^+$, (7) HL, (8) $[ML_2]$, (9) $[M(OH)L_2]^-$, (10) L^-, (11) $[ML]^+$

step is not apparent in the titration curves, and formation constants for each step may be refined together. The positions of the metal curves also indicate all the chelates to be quite stable with the exception that some complexes of Cu^{2+} have intermediate stability with respect to Co^{2+} and Ni^{2+} chelates. In the case of Cu^{2+}, the first dissociation step [equation (3)] does not overlap to give a single sloping buffer region. In the presence of metal ions, the species distribution diagram (Figure 2) shows that complexation begins at low pH values. The two-proton displacement at pH 3.0 presumably gives an $[CuHL]^{2+}$ species which reaches a maximum concentration of 97.2% total metal at pH 4.5 ($[NiHL]^{2+}$, 61.2% at pH 4.7; $[CoHL]^{2+}$, 69.6% at pH 5.2). In quite acidic solution, bonding through N(3) of the imidazole ring and the oxygen of the NHO^- group is favoured, and the α-amino nitrogen is protonated so that the complex $[MHL]^{2+}$ occurs. At higher pH the two amino groups [N(3) of imidazole ring and the α-amino group] act as donors with the oxygen of the NHO^- group, and the complexes are $[ML]^+$ and $[ML_2]$ (see below). At intermediate pH values the protonated complexes $[MH_2L_2]^{2+}$ and $[MHL_2]^+$ are also formed. At pH 9.2 the hydroxamic acid functional groups are completely ionized and the predominant species is $[CuL_2]$ which reaches a maximum concentration of 68.0% total metal at pH 9.5 ($[NiL_2]$, 96.6% at pH 9.0; $[CoL_2]$, 99% at pH 10.0). In the physiological pH range (6.7—7.3) $[CuH_2L_2]^{2+}$, $[CuHL_2]^+$, and $[CuL]^+$ account for over 92.4% total metal of the species present (61.2% for species $[NiH_2L_2]^{2+}$, $[NiHL_2]^+$, and $[NiL]^+$) and there is no evidence for any polymeric species. The complexes of Co^{2+} and Ni^{2+} (Figure 2) almost exhibit similar behaviour to those of Cu^{2+}, but sometimes have lower stabilities as expected. The stepwise stability constant of the complex $[CuL_2]$ (log K_2) is relatively small; this shows that the second molecule of ahip enters the co-ordination sphere of Cu^{II} with difficulty, probably because of the greater bulkiness of the ahip molecule, unlike the corresponding complexes of aha and ahp with copper. The fact that the $[NiL_2]$ chelate is much more stable than that of Cu^{II} is probably due to the sterically more favourable arrangement of bonds, where all six donor groups of the two ligands are involved in co-ordination of the metal ion by forming five- and six-membered chelate rings. A reasonable explanation, which indeed is consistent with the observed enhanced stability, is that the $[NiL_2]$ species is a mononuclear complex with the nickel co-ordinating to the N(3) atom of the imidazole ring and to the N atoms both of the α-amino and of the deprotonated NHO^- groups, in the same way as Ni^{II}–aha and Ni^{II}–ahp complexes. As expected, even though ahip is slightly less basic than aha or ahp it forms more stable complexes ($[ML]^+$ or $[ML_2]$), with the difference between the formation constants (log β_{101} or log β_{102}) varying in the order $Ni^{II} > Cu^{II} > Co^{II}$. The mode of bonding of the $[MHL]^{2+}$ and $[MH_2L_2]^{2+}$ species of ahip is less clear. That the metal is co-ordinated solely to one kind of site [N(3) of imidazole moiety and NHO^- group] in the complexes $[MHL]^{2+}$ and $[MH_2L_2]^{2+}$ of both the Ni^{II}–ahip and Co^{II}–ahip systems can be illustrated using a principle introduced by Sigel.[26] According to this, the difference in the logarithms of the stepwise formation constants for the mono and bis complexes formed from a bidentate ligand should have a value between 1 and 2. With K_{111} and K_{122} defined as $[MHL^{2+}]/[HL][M^{2+}]$ and $[MH_2L_2{}^{2+}]/[HL][MHL^{2+}]$, respectively, log K_{111} − log $K_{122} \approx 1.2$ for Ni^{II}–ahip and ≈ 1.4 for Co^{II}–ahip, thereby confirming a common site of co-ordination within each system. On the other hand, for Cu^{II}–ahip, log K_{111} − log K_{122} = 4.68 which differs markedly from the observed values for the Co^{2+}– and Ni^{2+}–ahip systems and probably suggests a different arrangement of ligand donor groups and co-ordinate bonds. The Irving–Williams order is followed for the complexes $[ML]^+$, $[MHL]^{2+}$, and $[MH_2L_2]^{2+}$.



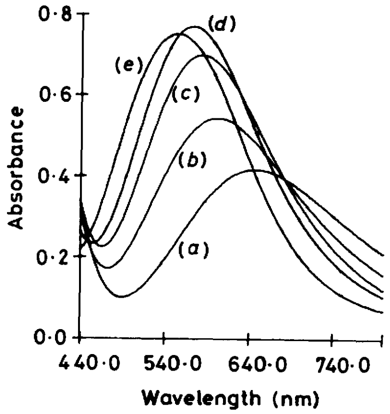


Figure 3. Plots of experimental absorbance data *versus* wavelength for some solutions of Cu^{2+}–ahip using the program VISION with the PLOTTER Calcomp 936: (*a*) pH 4.11, $[H_3L^{2+}] = 3.966 \times 10^{-2}$, $[Cu^{2+}] = 9.851 \times 10^{-3}$ mol dm^{-3}; (*b*) pH 4.37, $[H_3L^{2+}] = 4.078 \times 10^{-2}$, $[Cu^{2+}] = 1.013 \times 10^{-2}$ mol dm^{-3}; (*c*) pH 5.2, $[H_3L^{2+}] = 4.157 \times 10^{-2}$, $[Cu^{2+}] = 1.033 \times 10^{-2}$ mol dm^{-3}; (*d*) pH 7.15, $[H_3L^{2+}] = 3.894 \times 10^{-2}$, $[Cu^{2+}] = 9.673 \times 10^{-3}$ mol dm^{-3}; (*e*) pH 8.05, $[H_3L^{2+}] = 3.825 \times 10^{-2}$, $[Cu^{2+}] = 9.501 \times 10^{-3}$ mol dm^{-3}

A spectrophotometric investigation was carried out on a series of solutions containing ahip and copper(II), with concentrations and pH values selected from those used in the potentiometric titrations. Figure 3 shows typical absorption spectra obtained. With increasing pH the total colour change was from green to blue in acid solution, to purple in alkaline media, and blue-violet in the neutral region. The 4:1 ahip:Cu^{II} solution spectrum consists of one peak which shifts to higher energy upon addition of base. When the pH is increased a high combined hypsochromic–hyperchromic shift is observed from 647.0 to 572.0 nm. The increase in absorption and shift to smaller wavelengths of the unique band [647.0 for (*a*), 602.0 for (*b*), 583.0 for (*c*), 572.0 for (*d*), and 555.0 nm for (*e*)] with increasing pH indicates greater complexation, and above pH 9.0 both $\lambda_{max.}$ and $\varepsilon_{max.}$ do not change further, consistent with formation of the $[CuL_2(OH)]^-$ species which predominates in this range. Two types of absorption maximum may be distinguished. Type (1), in the range 570—700 nm, can be ascribed to *d–d* transitions of copper(II) complexes;[27–30] the maximum shifts towards shorter wavelengths with increasing pH and the variation of absorbance with pH is complicated. With type (2), the absorption maximum at *ca.* 555 nm is almost independent of pH, metal, or ligand concentration. The maximum of type (2) is probably a charge-transfer band and is evidently characteristic of *O,O* and *N,O* bonded copper(II). The present ligand (as well as amines) produces a stronger ligand field than does $[Cu(H_2O)_6]^{2+}$, which causes the absorption band to move from the far red to the middle of the red region of the spectrum. However, I suggest a d_{xy} ground state for the Cu^{II}–ahip system with square-planar geometry. Interestingly, square-planar complexes of Cu^{II} are sensitive to axial perturbation; as the distortion towards a square continues it is predicted that one band will move to higher energy.

The main conclusions to be drawn are that at low pH the copper(II) ion is co-ordinated *via* N(3) of the imidazole ring and the oxygen of the NHO^- group, probably with participation of the ketonic oxygen, whereas at higher pH binding to the α-amino group, N(3) of the imidazole moiety, and the NHO^- group is evident. As mentioned above, aminohydroxamic acids are very important chelating agents in biological systems and are also potent inhibitors of urease activity. It has also been suggested that aminohydroxamic acids may be particularly active because of a possible surface-active role by an unco-ordinated amino group in the complexes, $[MHL]^{2+}$, $[MH_2L_2]^{2+}$, and $[MHL_2]^+$ which are present at physiological pH values.

Acknowledgements

Grateful acknowledgement is made to the Ministero della Pubblica Istruzione (Rome) and National Research Council of Italy (C.N.R.) for generous grants, and to Professor A. Vacca for kindly supplying the program SUPERQUAD.

References

1 J. Hase and K. Kobashi, *J. Biochem.* (*Tokyo*), 1967, **62**, 293.
2 W. N. Fishbein, C. L. Streeter, and J. E. Daly, *J. Pharmacol. Exp. Ther.*, 1973, **186**, 173.
3 N. E. Dixon, R. L. Blakely, and B. Zerner, *Can. J. Biochem.*, 1980, **58**, 1323.
4 W. F. Anderson and M. C. Hiller, DHEW Publ. (NIH) (U.S.) 1976, NIH 76-994.
5 E. Leporati, *J. Chem. Soc., Dalton Trans.*, 1986, 2587.
6 F. W. Sunderman, jun., *Am. J. Clin. Pathol.*, 1965, **44**, 182.
7 W. E. C. Wacker and B. L. Vallee, *J. Biol. Chem.*, 1959, **234**, 3257.
8 E. Leporati, *Anal. Chim. Acta*, 1985, **170**, 287.
9 E. Leporati, *J. Chem. Soc., Dalton Trans.*, 1985, 1605.
10 E. Leporati, *J. Chem. Soc., Dalton Trans.*, 1986, 199.
11 G. Gran, *Analyst* (*London*), 1952, **77**, 661.
12 H. S. Harris and R. S. Tobias, *Inorg. Chem.*, 1969, **8**, 2259.
13 A. Sabatini and A. Vacca, personal communication.
14 P. Gans, A. Sabatini, and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195.
15 A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.
16 A. Vacca and A. Sabatini, MINIQUAD and MIQUV: 'Two Approaches for the Computation of Stability Constants from Potentiometric Data, in Computational Methods of the Determination of Stability Constants,' ed. D. J. Leggett, Plenum, New York, 1985.
17 P. Gans, A. Sabatini, and A. Vacca, *Inorg. Chim. Acta*, 1976, **18**, 237.
18 (*a*) D. A. Brown and B. S. Sekhon, *Inorg. Chim. Acta*, 1984, **91**, 103; (*b*) A. Braibanti, E. Leporati, and F. Dallavalle, *Inorg. Chim. Acta*, 1970, **4**, 529.
19 E. J. King, *J. Am. Chem. Soc.*, 1945, **67**, 2178.
20 A. Braibanti, E. Leporati, F. Dallavalle, and G. Mori, *Inorg. Chim. Acta*, 1972, **6**, 395.
21 A. Braibanti, G. Mori, F. Dallavalle, and E. Leporati, *Inorg. Chim. Acta*, 1972, **6**, 106.
22 A. Braibanti, F. Dallavalle, E. Leporati, and G. Mori, *Inorg. Chim. Acta*, 1971, **5**, 449.
23 E. Leporati, *J. Chem. Soc., Dalton Trans.*, in the press.
24 D. A. Brown, M. V. Chidambaram, J. J. Clarke, and D. M. McAleese, *Bioinorg. Chem.*, 1978, **9**, 255.
25 R. W. Hay and P. J. Morris, *J. Chem. Soc. A*, 1971, 1518.
26 H. Sigel, in 'Metal Ions in Biological Systems,' ed. H. Sigel, Dekker, New York, 1973, vol. 2, p. 65.
27 A. Gergely and T. Kiss, *Inorg. Chim. Acta*, 1976, **16**, 51.
28 W. L. Kwik, E. Purdy, and E. Stiefel, *J. Am. Chem. Soc.*, 1974, **96**, 1638.
29 A. P. B. Lever, 'Inorganic Electronic Spectroscopy,' Elsevier, New York, 1968, pp. 355—361.
30 F. A. Walker, H. Sigel, and D. B. McCormick, *Inorg. Chem.*, 1972, **11**, 2756.

Received 25*th March* 1986; *Paper* 6/595