Design of Transition Metal Chelates with Biological Activity. Potentiometric Study of Complex Formation Equilibria between 2-Amino-*N*-hydroxy-3-(*p*-hydroxyphenyl)propanamide and Nickel(II), Copper(II), and Hydrogen Ions in Aqueous Solution

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The species distribution and relevant stability of species present in aqueous solutions of nickel(II) and copper(II) with L-tyrosinehydroxamic acid [2-amino-*N*-hydroxy-3-(*p*-hydroxyphenyl)propanamide, ahhpp] were obtained by potentiometric titrations in 0.5 mol dm^{-3} KCl solution at 25 °C. The protonation constants of the ligand and the formation constants of several metal complexes have been calculated from potentiometric data with the aid of the SUPERQUAD program. The ligand forms with metals the complexes $[Cu(HL)]^+$, $[Cu(HL)_2]$, $[CuL_2]^{2-}$; and $[Ni(HL)_2]$, $[Ni(HL)L]^-$, $[NiL_2]^{2-}$, $[Ni(OH)L_2]^{3-}$, where the ligand $L^{2-} = {}^-OC_6H_4CH_2(H_2N)CHC(O)NHO^-$. The following cumulative formation constants $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$ were obtained: ahhpp, log β_{011} = 9.931(2), log β_{021} = 18.850(2), log β_{031} = 25.806(4); Cu^{II}–ahhpp, log β_{111} = 20.162(22), log β_{122} = 33.123(59), log β_{102} = 14.064(50); Ni^{II}–ahhpp, log β_{122} = 33.668(14), log β_{112} = 25.700(46), log β_{102} = 16.186(58), log β_{1-12} = 6.646(48). The H^+ of the 'protonated' complexes, which appear at intermediate pH values (5.5—9.0), is probably bound to the *p*-oxygen of the phenyl group, not involved in the chelate ring. The probable structures of the chelated compounds formed in aqueous solution are deduced by structural analysis of the solid state, and their stability constants are compared with those of analogous chelated compounds.

This paper is part of a series devoted to describing the 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. Generally hydroxamic acids are very important chelating agents in biological systems[1–3] and have become important as therapeuticals[4,5] for inhibition of urease activity. Although the mechanism of clinical action of L-tyrosinehydroxamic acid [2-amino-*N*-hydroxy-3-(*p*-hydroxyphenyl)propanamide, ahhpp] is not fully understood, there is reason to suspect that the interaction of this ligand with metal ions is significant. It has also been pointed out that aminohydroxamic acids may be particularly active in biological reactions because of a possible surface-active role by an unco-ordinated amino group;[6] however, in the case of copper and nickel complexes of ahhpp, it has been found that co-ordination clearly involves not only the deprotonated NHO^- group[7,8] (*via* the oxygen or nitrogen atom) but also the amino group. Copper is an essential trace element which plays an important role in many biological systems.[9] The major portion of copper (*ca.* 90%) in mammalian plasma is in the form of ceruloplasmin which is not exchangeable *in vivo*.[10] However, the remainder (*ca.* 10%) is bound to albumin and amino acids or aminohydroxamic acids which are in rapid equilibrium with copper in tissues.[11] In addition to the essential elements which are required for different forms of life (*e.g.* Co, Cu, Si, V, Cr, *etc.*), there are others, such as nickel, fairly recent studies[12] of which have indicated it to be an essential element in animal nutrition. A current fundamental approach to investigating the role of metal ions in biological systems involves computer simulation of the equilibria between these and low-molecular-weight ligands, including those which occur naturally and those administered as medication. The data required for such studies comprise the specific stoicheiometric identities of the metal complexes that coexist in equilibrium together with their formation constants. To continue these investigations, I now examine the interaction of Ni^{II} and Cu^{II} with ahhpp in solution. To establish an appropriate chemical model to describe aqueous solutions containing this ligand and copper(II) and nickel(II) ions potentiometry has been used as the main experimental approach. Solutions of ahhpp and copper(II) have also been examined by visible spectrophotometry.

Experimental

Materials.—L-Tyrosinehydroxamic acid (ahhpp) was obtained from Sigma (St. Louis), and its purity was checked potentiometrically. Concentrations of stock solutions of bivalent metal chlorides (AnalaR Products) were determined by inductively-coupled plasma (i.c.p.) atomic emission spectrometry. Doubly distilled and deionized water was used throughout, and all titrations were carried out under an atmosphere of purified nitrogen. All the reagent solutions for potentiometric measurements were prepared, and experimental titrations carried out following the procedures previously described.[13–15] 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 concentrations of the reagents. The base used for e.m.f. measurements was carbonate-free potassium hydroxide (0.3048 mol dm^{-3}) which was standardized according to Gran's method, using different calculation procedures as previously described.[16–18]

Potentiometric Measurements.—Potentiometric titrations were performed by using a Metrohm Titroprocessor E 636 automatic titration apparatus, equipped with a H 268 glass electrode (Schott-Jena glass) and a B 343 Talamid reference electrode (Schott-Jena glass). E.m.f. readings and titration curves were recorded on a thermo-printer using an E 636 automatic titrator. The titration cell was maintained at 25.0 ± 0.1 °C by circulation of thermostatted water and was

Table 1. Determination of protonation and complex-formation constants. Concentrations of the reagents* for the alkalimetric titrations of 2-amino-*N*-hydroxy-3-(*p*-hydroxyphenyl)propanamide (ahhpp) 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$^+$–ahhpp	0.204 84		0.614 52	3.54—11.52
2		0.230 44		0.691 34	3.44—11.46
3		0.215 08		0.645 25	3.48—11.52
4		0.220 20		0.660 61	3.45—11.79
5		0.199 72		0.599 16	3.50—11.43
6	Cu^{II}–ahhpp	0.202 74	0.095 72	0.617 95	3.15—9.61
7		0.253 93	0.040 73	0.773 98	3.20—10.24
8		0.202 74	0.040 73	0.617 95	3.26—10.32
9		0.202 74	0.061 10	0.617 95	3.19—10.24
10		0.212 88	0.030 55	0.648 85	3.29—10.26
11		0.228 08	0.031 57	0.695 19	3.27—10.41
12	Ni^{II}–ahhpp	0.202 74	0.073 51	0.617 95	3.49—10.34
13		0.202 74	0.029 40	0.617 95	3.49—9.78
14		0.202 74	0.022 05	0.617 95	3.49—10.23
15		0.192 60	0.021 32	0.587 05	3.52—10.14
16		0.212 88	0.023 52	0.648 85	3.48—10.42

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

calibrated by Gran's method using strong acid–strong base titrations in 0.5 mol dm^{-3} KCl solution. A slow nitrogen stream, presaturated by bubbling it through an appropriate KCl solution (0.5 mol dm^{-3}), was maintained in the potentiometric cell by blowing over the surface of the solution. The solution in the titration compartment was stirred by means of a mechanical stirrer. All liquid-junction potentials were checked between titrations and found to be reproducible. As the liquid-junction potentials are fairly small, relative to the measurement cell, and not significant in calculating the equilibria (pH interval used 3.0—11.5),* they have been neglected in the present calculations.

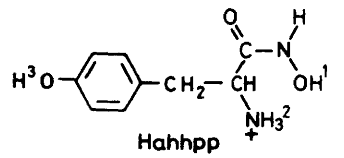
Quantities of titrant KOH were added using a Metrohm Dosimat E 635 autoburet (total volume 5.0 cm^3).

Spectrophotometric Measurements.—Absorption spectra in the region 405—800 nm were obtained on a Jasco Uvidec-505 spectrophotometer. Solutions containing ahhpp and copper ion maintained at an ionic strength of 0.5 mol dm^{-3} (KCl) and 25 °C were scanned at a series of pH values 4.80—8.5 using 10-mm cells.

Computations.—Titration data were processed initially by the NBAR program[17] in order to obtain formation curves ($\bar{n}$ against pL; $\bar{n}$ = average number of ligands bound to central metal ion, pL = $-\log$ [L^{2-}]) and subsequently by the SUPERQUAD program[19] in order to obtain best-fitting chemical models and refined formation constants (β_{pqr}) for the Ni^{II}– and Cu^{II}–ahhpp systems. By applying the formation constants to the HALTAFALL program,[20] theoretical formation functions ($\bar{n}$ *versus* pL) were regenerated so as to facilitate hypothesis testing. The formation constants of the best chemical model eventually obtained were processed by the HALTAFALL program to give profiles of the distribution of each complex species *versus* pH applicable to various concentrations of ligand and metal ion. The quantity σ^2 [equation (1)] is strongly dependent on the estimated errors in

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

the measurements of e.m.f. (E_i) and of titrant volume; Z is the total number of potentiometric data, w_i is the weighting factor assigned to the i-th observation, and ($Z - m$) is the number of degrees of freedom of the system (the number of experimental data minus the number of refined parameters). I estimate these errors to be 0.2 mV for the e.m.f. and 0.015 cm^3 for the titrant volume. All the 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 experiment are quoted in Table 1.



Scheme 1. H$_3$L$^+$; the three removable protons (1—3) are indicated

Results and Discussion

Protonation Equilibria.—A maximum number of three protons can be liberated from the ligand in the protonated form (Hahhpp = H$_3$L$^+$, Scheme 1) on titration with strong base in the pH range 3.0—11.5. All the sets of titration data indicate the presence solely of simple HL$^-$, H$_2$L, and H$_3$L$^+$ complexes. However the formation function ($\bar{n}_H$) of the ligand, which at most approaches a value of 3, suggests the existence of three protonation equilibria, the stability constants for which have been refined by the computer program SUPERQUAD.[19] At a final stage of refinement the protonation constants of the ligand and the initial amounts (mmol) of reagents (T_L, T_H) have been determined at the same time through the refinement of several sets of potentiometric data by SUPERQUAD. After the refinement the variance σ^2 as regards the initial amount of the reagents (T_L, T_H) was 6.488×10^{-7} and 8.133×10^{-5} mmol^2, respectively. The standard deviations of the refined quantities (T_L, T_H, and β_{0q1}, Table 2) 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 previously obtained by SUPERQUAD.

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* See also the results for the Ni^{II}–ahip system (ahip = α-amino-*N*-hydroxyimidazole-4-propanamide).[15]

Table 2. Cumulative and stepwise protonation complex-formation constants of 2-amino-*N*-hydroxy-3-(*p*-hydroxyphenyl)propanamide (ahhpp) with bivalent metal ions at 25 °C and $I = 0.5$ mol dm^{-3} (KCl). Standard deviations (σ values) are given in parentheses

	H^+	Ni^{II}	Cu^{II}
$\log \beta_{011}$	9.931(2)		
$\log \beta_{021}$	18.850(2)		
$\log \beta_{031}$	25.806(4)		
$\log K_2^{H\,a}$	8.919(2)^b		
$\log K_3^H$	6.956(3)		
$\log \beta_{111}$			20.162(22)
$\log \beta_{122}$		33.668(14)	33.123(59)
$\log \beta_{112}$		25.700(46)	
$\log \beta_{102}$		16.186(58)	14.064(50)
$\log \beta_{1-12}$		6.646(48)	
Z^c	276	195	237
$\chi^{2\,d}$	11.77	29.7	11.47
$\sigma^{2\,e}$	0.16	0.62	1.57
U^f	6.936	7.458×10^1	5.783×10^2

$^a \log K_n^H = \log \beta_{0n1} - \log \beta_{0n-11}$. $^b \sigma(\log K_n^H) = \{[\sigma^2(\log \beta_{0n1}) + \sigma^2(\log \beta_{0n-11})]/2\}^{\frac{1}{2}}$. ^{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.

$^e\ \sigma^2 = \sum_{i=1}^{Z} w_i(E_i^{obs.} - E_i^{calc.})^2/(Z - m)$. $^f\ U = \sum_{i=1}^{Z} w_i(E_i^{obs.} - E_i^{calc.})^2$.

The values of the cumulative and stepwise protonation constants of ahhpp at 25 °C and $I = 0.5$ mol dm^{-3} are reported in Table 2. The potentiometric titration curve of the protonated ligand H_3L^+ in the absence of metal ions has three titratable protons in the studied pH range 3.0—11.5. The assignment of the protons to the sites in the protonated forms of the ligand can be done on the basis of the results and by analogy with the known values of some aminohydroxamic acids and other analogous compounds.[1–3] The $\log \beta_{011}$ (or $\log K_1^H$) value corresponding to the protonation of the oxygen (H^3, Scheme 1) in the *para* position was estimated to be 9.931(2). By analogy with known values for similar functional groups,[14,15] the values $\log K_2^H = 8.919(2)$ and $\log K_3^H = 6.956(3)$ can be assigned to the protonation of the primary amine group (H^2, Scheme 1) and the oxygen (H^1, Scheme 1) of NHO^-, respectively.

The presence of the α-amino group in aminohydroxamic acids increases remarkably the acidic character of the OH group [$\log K_2^H = 7.484(2)$ for glycinehydroxamic acid (aha),[14] $\log K_2^H = 7.340(2)$ for DL-norvalinehydroxamic acid (ahp),[14] $\log K_2^H = 7.067(2)$ for L-histidinehydroxamic acid (ahip),[15] $\log K_3^H = 6.956(3)$ for L-tyrosinehydroxamic acid (ahhpp), and $\log K_2^H = 7.375(2)$ for DL-norleucinehydroxamic acid (ahhe)[21]]* in comparison with that of some alkylhydroxamic acids ($\log K_1^H = 9.342$ for $CH_3CONHOH$[22] and $\log K_1^H = 9.56$ for $CH_3CH_2CONHOH$[2] *etc.*). This increase of acidic character shows a trend in the order aha < ahhe < ahp < ahip < ahhpp. Similarly the substitution of a NHOH group for the carboxyl OH group in aminohydroxamic acids lowers the protonation constant of the α-amino group compared with the corresponding α-amino acids according to the electron-withdrawing character of the NHOH group [$\log K_1^H = 9.096(1)$ for glycinehydroxamic acid,[14] $\log K_1^H = 9.54(1)$ for glycine;[23] $\log K_1^H = 9.125(1)$ for DL-norvalinehydroxamic acid,[14] $\log K_1^H = 9.68(3)$ for DL-norvaline;[24] $\log K_1^H = 8.942(2)$ for L-histidinehydroxamic acid,[15] $\log K_1^H = 9.17$ for histidine;[25] $\log K_2^H = 8.919(2)$ for L-tyrosinehydroxamic acid, $\log K_2^H = 9.07(5)$ for L-tyrosine;[24] $\log K_1^H = 9.172(1)$ for DL-norleucinehydroxamic acid,[21] $\log K_1^H = 9.67(3)$ for DL-norleucine[24,26]]. A straight-line relationship of positive slope (1.59) was observed (Figure 1) by plotting the protonation constant ($\log K_2^H$) of the NHO^- group for some aminohydroxamic acids against $\log K_1^H$ (protonation constant of α-amino group).

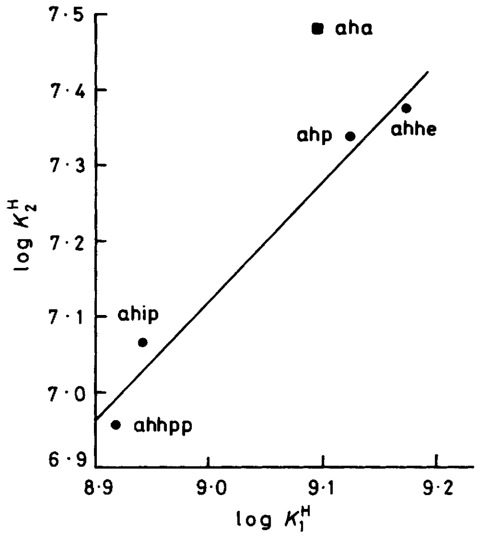
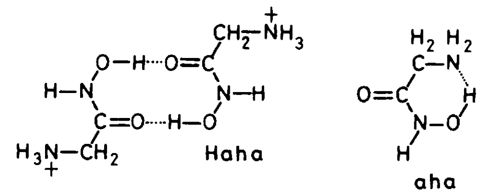


Figure 1. Relationship between $\log K_2^H$ (protonation constant of NHO^- group) and $\log K_1^H$ (protonation constant of α-amino group) in some aminohydroxamic acids. The protonation constants of aha are not included in the calculation of the straight line



Scheme 2.

An additional factor favouring, or not, protonation of functional groups ($-NH_2$, $-NHO^-$) is the presence of alkyl groups ($CH_3CH_2CH_2$- in ahp, $CH_3CH_2CH_2CH_2$- in ahhe) with inductive effect (denoted +I), or *p*-hydroxyphenyl (−I effect) and imidazole (−I effect) groups, which appear to interact with adjacent basic groups. The aromatic character of the imidazole ring on a histidine unit is evident in its resistance to different reactions. In these cases I think that the inductive effect of aromatic rings acts in opposition and prevails over the resonance effect which controls the direction of substitution in the activation of the *ortho* and *para* positions for the introduction of electrophile reagents. In the case of aha, $\log K_2^H$ (7.484) has become anomalous in the sense that the protonation constant (Figure 1) is greater than the value expected for protonation of an NHO^- group (*cf.* $\log K_2^H$ for ahp and ahhe). The abnormally low tendency for the NHOH of aha to deprotonate indicates stabilization of the species H_2L^+ (Haha) or HL (aha); this stabilization is probably due to the intramolecular or intermolecular hydrogen bonding (Scheme 2).

Metal(II) *Complex Equilibria.*—Titration data were processed initially by the NBAR program[17] to obtain formation curves ($\bar{n}$ *versus* pL). The purpose of this strategy was to facilitate the search for not only mononuclear binary complexes but also

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* Abbreviations. aha = 2-amino-*N*-hydroxyacetamide, ahp = 2-amino-*N*-hydroxypentanamide, ahhe = 2-amino-*N*-hydroxyhexanamide.

protonated and hydroxo species, particularly in the nickel(II)–ahhpp system. Subsequently all the protonation constants were kept constant and the SUPERQUAD program[19] was applied to obtain best-fitting chemical models and refined formation constants (log β_{pqr}). The presence of likely metal–proton–ahhpp species was tested for by using the following values: $p = 1$ or 2, $q = 1$ or 2, and $r = 1$—3. By applying the formation constants to the NBAR program, theoretical formation curves ($\bar{n}$ *versus* pL) were regenerated so as to facilitate hypothesis testing. The constants eventually obtained were processed by HALTAFALL[20] to give profiles of the distribution of each complex *versus* pH applicable to various concentrations of ligand and metal ion. The validity of the models is demonstrated by the excellent matching of the experimental and calculated potentiometric data. In particular, when applying SUPERQUAD to obtain consistent chemical models to explain the entire set of potentiometric data, the standard deviations (σ) of the parameters refined (log β_{pqr}), the agreement factor σ^2 (variance), the goodness of fit statistic χ^2, and the chemical meaning of the species found in solution were taken into account for each hypothesis. The refinement converged satisfactorily when the only species present were $[Cu(HL)]^+$, $[Cu(HL)_2]$, and $[CuL_2]^{2-}$ for Cu^{2+}; and $[Ni(HL)_2]$ $[Ni(HL)L]^-$, $[NiL_2]^{2-}$, and $[Ni(OH)L_2]^{3-}$ for Ni^{2+}. The most consistent set of complexes found, together with their respective formation constants, is shown in Table 2. Sample potentiometric titration curves of the protonated ligand, H_3L^+, alone or in the presence of Ni^{2+} and Cu^{2+}, are shown in Figure 2. 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 titration curves for Ni^{2+} had shapes different to those of Cu^{2+}.

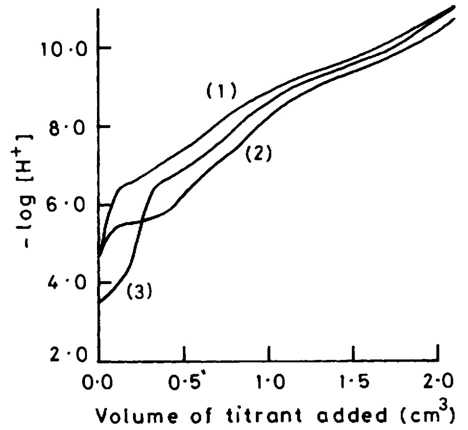

Figure 2. Titration curves of pH as a function of the volume of KOH added, calculated by the HALTAFALL program, equipped with PLOTTER Calcomp 936: ahhpp (1), with Ni (2) or Cu (3). $c_L = 8.0 \times 10^{-3}$, $c_H = 2.4 \times 10^{-2}$, $c_M = 1.3 \times 10^{-3}$, and $c_{KOH} = 0.3048$ mol dm^{-3}; $V_o = 25.0$ cm^3

The relative importance of the various species in each pH range is shown by the distribution diagrams for ahhpp with Ni^{2+} and Cu^{2+} (Figure 3). Some general observations concerning the behaviour of the various systems can be made. The representative species distribution diagrams (Figure 3) show that complexation begins at low pH values (*ca.* 3.0 for Cu^{2+}, 5.2 for Ni^{2+}) with formation of the complex $[Cu(HL)]^+$ corresponding to the displacement of two protons; the maximum fraction of the complex $[Cu(HL)]^+$ is 99.95% at pH 5.75. The initial reaction involving nickel ions is displacement of four protons from two H_3L^+ to form a bis-complex, $[Ni(HL)_2]$; the species $[Ni(HL)_2]$ reaches a maximum concentration of 97.8% total nickel at pH 6.25. At intermediate pH values, the complexes $[Cu(HL)_2]$ and $[Ni(HL)L]^-$ are also formed. $[Cu(HL)_2]$ has a maximum fraction of 73.8% at pH 8.8, while $[Ni(HL)L]^-$ reaches a peak of 73.6% at pH 8.6. The functional groups of ahhpp are completely ionized at *ca.* pH 9.5 and the predominant species complexed is $[CuL_2]^{2-}$ which reaches a maximum concentration of 98.1% at pH 10.5 ($[NiL_2]^{2-}$, 33.6% at pH 9.5). Above pH 9.0 one hydrolyzed species was detected: the maximum fraction of the complex $[Ni(OH)L_2]^{3-}$ is *ca.* 89.2% at pH 10.5. Thus, complexation can be explained by assuming that ahhpp acts as a bidentate ligand co-ordinating to metal ion (M) *via* the OH group of NHOH and the nitrogen atom of the α-amino group, in contrast to the normal co-ordination of unsubstituted monohydroxamic acids.[27] Moreover, in the various 'protonated' species of ahhpp, *e.g.* $[M(HL)_2]$ and $[M(HL)L]^-$, protonation may occur at the *p*-oxygen group of the phenyl moiety. Therefore the slightly lower value of log K_{111} for $[Cu(HL)]^+$ (log K_{111} = log β_{111} − log β_{011}) and the significantly lower value of log β_{102} for $[CuL_2]^{2-}$ correlate well with the lower basicity of the two basic groups ($-NH_2$, $-NHO^-$) compared to the Cu^{II}–aha and –ahp systems.[14]

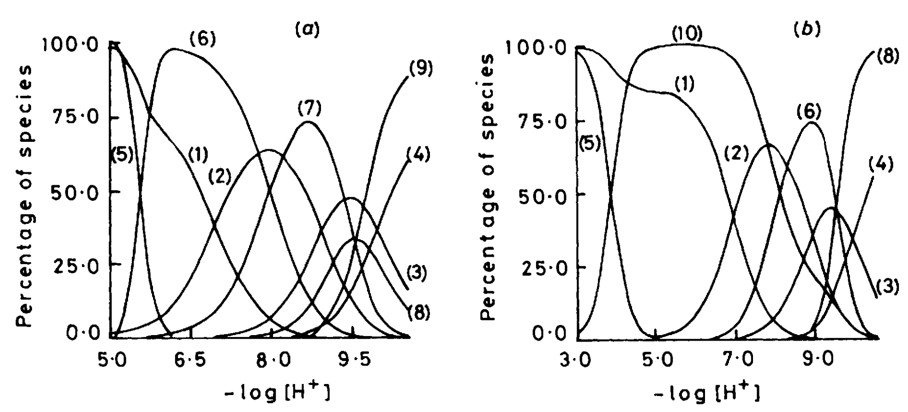

Figure 3. Typical distribution diagrams for M^{II}–ahhpp systems. The percentage of each species has been calculated from the data of a hypothetical solution of metal ions (0.0009 mol dm^{-3}) and ahhpp (0.008 mol dm^{-3}) by the HALTAFALL program, equipped with a PLOTTER Calcomp 936. The concentrations of the species not containing metal were calculated as percentages of the total ligand, those containing metal as percentages of the total metal: (*a*) Ni^{2+}–ahhpp, (*b*) Cu^{2+}–ahhpp; (1) H_3L^+, (2) H_2L, (3) HL^-, (4) L^{2-}, (5) M^{2+}, (6) $[M(HL)_2]$, (7) $[M(HL)L]^-$, (8) $[ML_2]^{2-}$, (9) $[M(OH)L_2]^{3-}$, (10) $[M(HL)]^+$

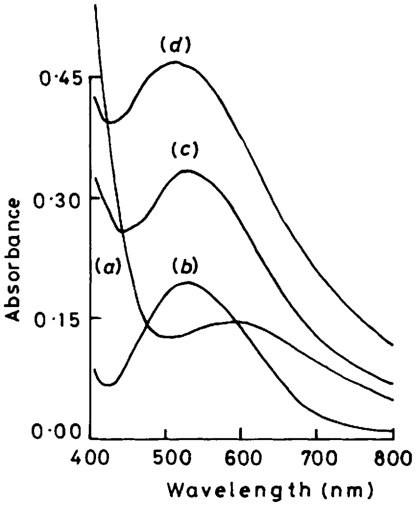

Figure 4. Plots of experimental absorbance data *versus* wavelength for some solutions of the Cu^{2+}–ahhpp system using the program VISION with the PLOTTER Calcomp 936: (*a*) pH 4.8, $c_L = 4.7 \times 10^{-2}$, $c_M = 5.8 \times 10^{-4}$ mol dm^{-3}; (*b*) pH 6.95, $c_L = 4.38 \times 10^{-2}$, $c_M = 5.48 \times 10^{-4}$ mol dm^{-3}; (*c*) pH 7.78, $c_L = 4.17 \times 10^{-2}$, $c_M = 5.2 \times 10^{-4}$ mol dm^{-3}; (*d*) pH 8.50, $c_L = 4.0 \times 10^{-2}$, $c_M = 4.94 \times 10^{-4}$ mol dm^{-3}

For the Ni^{II}–ahhpp system the general pattern of the i.r. spectra[2] and the *X*-ray crystallographic studies[8] supports preferential co-ordination *via* the nitrogen atom of the α-amino group and the nitrogen atom of the NHO^- group, as previously reported[28] for nickel complexes of glycinehydroxamic and serinehydroxamic ($HOCH_2CH(NH_2)CONHOH$) acids. Accordingly, most noticeable is an increase of the stability constant value (log β_{102}) for the $[NiL_2]^{2-}$ complex compared to the corresponding value for the $[CuL_2]^{2-}$ species.

According to a principle introduced by Sigel,[29] the difference in the logarithms of the stepwise formation constants for the 'mono- and di-protonated' complexes $\{[Cu(HL)]^+$ and $[Cu(HL)_2]\}$ formed from a bidentate ligand should have a value between 1 and 2, when the metal ion is co-ordinated solely to one kind of hydroxamic moiety (N of α-amino and NHO^- group). By using K_{111} and K_{122} defined as $[Cu(HL)^+]/[HL^-][Cu^{2+}]$ and $[Cu(HL)_2]/[HL^-][Cu(HL)^+]$, respectively, log K_{111} − log K_{122} = 7.2, which differs markedly from the observed values for other analogous systems,[15] probably suggests a different arrangement of ligand donor groups and co-ordinate bonds in the Cu^{II}–ahhpp 'protonated' forms. If the metal in $[Ni(HL)L]^-$ is tentatively assumed to be co-ordinated to the hydroxamic ends of both ligand moieties and, furthermore, if the protonation quotient $[Ni(HL)_2]/[Ni(HL)L^-][H^+]$ is assumed to approximate to log $\beta_{011} = [HL^-]/[L^{2-}][H^+]$, it follows that log β_{112} should approximate to log β_{122} − log β_{011}. In the Ni^{II}–ahhpp system, log β_{122} − log β_{011} (= 23.737) differs significantly from the observed value of log β_{112} (25.700). A reasonable explanation, which is consistent with the observed enhancement in the stability of $[Ni(HL)L]^-$, is the formation of a 'protonated' complex with the nickel co-ordinating to the hydroxamic end of a two-ligand moiety *via* the nitrogen atom of the α-amino group and the nitrogen atom of the deprotonated NHO^- group, as previously described.[14,15,28]

The spectrophotometric study was carried out on a series of solutions, containing ahhpp and copper(II), with concentrations and pH values selected from those employed in the potentiometric experiments. Some typical absorption spectra obtained for the Cu^{II}–ahhpp system at different pH values are reported in Figure 4. The only colour variation observed for this system is from green, in acid solution, to reddish purple in slightly acidic, neutral, and alkaline media. Small bathochromic and high hyperchromic shifts are observed at 512—530 nm, when the pH increases. The increase in absorption and shift to shorter wavelengths with increase in pH indicates greater complexation, until the formation of the last species complexed, $[CuL_2]^{2-}$, which predominates at basic pH. The spectra in acid and basic media show two bands: one, of low intensity at *ca.* 585 nm, and a more intense peak in the range 512—530 nm. The majority of copper(II) complexes (green) are usually tetragonally distorted with four short metal–ligand bonds in one plane (*xy*) and two longer metal–ligand bonds lying along the *z* axis above and below this plane. Such complexes give rise to one absorption band in the visible region, 625 nm (for the Cu^{II}–ahhpp system, 585 nm); molar intensity values are in the range 20—50 dm^3 mol^{-1} cm^{-1}. In the limit, which is not generally reached, the complexed species would be four-co-ordinate and square planar. The present ligand, as well as the other analogous compounds, produce a stronger ligand field than the aqua cation, $[Cu(H_2O)_6]^{2+}$, which causes the absorption band to move from the far red to the middle of the red region of the spectrum. Therefore I suggest a d_{xy} ground state for the Cu^{II}–aminohydroxamic acid system with square-planar geometry. A maximum near 585 nm is tentatively assigned to the d_{xy}–$d_{x^2-y^2}$ transition. Interestingly, square-planar complexes of Cu^{II} are sensitive to axial perturbation and as the distortion towards square planarity continues it is predicted that one band will move to higher energy.

For ligand:metal ratios up to 2:1 ($[Cu^{2+}] > 0.0038$ mol dm^{-3}) the full reaction sequence of Cu^{2+} with ahhpp could not be observed because of precipitation at *ca.* pH 3.6. Equilibria between Co^{2+} ion and ahhpp cannot be studied because at the already acid pH precipitation occurs in the titration cell.

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