Potentiometric Study on the Complex-formation Equilibria between 2-Amino-*N*-hydroxyhexanamide and α-Amino-1*H*-indole-3-*N*-hydroxypropanamide and Cobalt(II), Nickel(II), Copper(II), and Hydrogen Ions in Aqueous Solution

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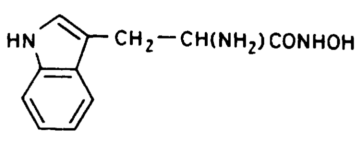
The equilibria occurring in aqueous solutions of 2-amino-*N*-hydroxyhexanamide (ahhe) and α-amino-1*H*-indole-3-*N*-hydroxypropanamide (aihp) with protons and bivalent metals have been studied by a potentiometric method. The protonation and complex-formation constants have been determined at 25 °C and I = 0.5 mol dm^{-3} KCl. The following overall formation constants $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$ were obtained: ahhe, log β_{011} = 9.172(1), log β_{021} = 16.547(2); Co$^{\text{II}}$–ahhe, log β_{101} = 6.423(25), log β_{102} = 10.964(35), log β_{1-12} = 1.77(7); Ni$^{\text{II}}$–ahhe, log β_{101} = 6.571(35), log β_{102} = 13.902(8), log β_{1-12} = 6.17(9); Cu$^{\text{II}}$–ahhe, log β_{101} = 10.29(14), log β_{102} = 19.695(26), log β_{2-12} = 20.767(18), log β_{1-12} = 9.877(43); aihp, log β_{011} = 9.088(5), log β_{021} = 16.130(7); Ni$^{\text{II}}$–aihp, log β_{102} = 14.383(19), log β_{1-12} = 5.53(7); Cu$^{\text{II}}$–aihp, log β_{102} = 20.31(6), log β_{2-12} = 21.469(38), log β_{1-12} = 10.52(7). The ligands are bound to the metals through the *N* atom of the α-amino group and the deprotonated NHO$^-$ group. Electronic spectra of the Cu$^{\text{II}}$–ahhe chelates formed have been recorded. All the above metals form mononuclear ([ML]$^+$ and/or [ML$_2$]) and hydroxo complexes with each ligand; with copper(II), polynuclear species are also present.

Following research on the protonation and complex-formation equilibria of hydroxamic acids, a study of 2-amino-*N*-hydroxyhexanamide (ahhe), $CH_3CH_2CH_2CH_2CH(NH_2)CONHOH$, and α-amino-1*H*-indole-3-*N*-hydroxypropanamide (aihp), has been undertaken. This work forms part of a comparison of stability and structure in solution of metal chelates with biological activity. The hydroxamic acids can be employed as indicators of biological activity. Although the mechanism of their chemical action is not known, there is reason to suspect that the interaction of these ligands with metal ions has a particular significance. This is based, for example, on the reports that hydroxamic acids are potent and specific inhibitors[1,2] of urease activity and have recently been employed as therapeutic substances in the treatment of hepatic coma.[3] In spite of the great interest in hydroxamic acids and metal hydroxamates and their biological function, studies on aminohydroxamic acids and their metal complexes are rather scarce.



aihp

Experimental

Reagents.—2-Amino-*N*-hydroxyhexanamide (ahhe) and α-amino-1*H*-indole-3-*N*-hydroxypropanamide (aihp) were obtained from Sigma (St. Louis) and their purity was checked by potentiometric titrations. Doubly distilled and deionized water was employed throughout, and all potentiometric titrations were carried out under an atmosphere of purified nitrogen. The 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 titration were prepared by adding successively to the titration compartment a known volume of ahhe or aihp solution, and an exact volume of metal chloride; then potassium chloride (Merck), which was employed as supporting electrolyte, and a sufficient amount of doubly distilled and deionized water were added to make up the total volume V_0, which was 25.00 ± 0.015 cm^3. All other chemicals were of the highest grade available and were used as previously described.[4–9] The ionic medium was 0.5 mol dm^{-3} KCl at the beginning of each potentiometric experiment. The use of a constant ionic strength was necessary in order to minimize variations of the activity coefficients.

Potentiometric Measurements.—Potentiometric titrations were performed by using a Metrohm Titroprocessor E 636. The electrode system consisted of a H 268 glass electrode and a B 343 reference electrode (both of Schott–Jena glass). The solution in the titration vessel was stirred by means of a mechanical stirrer and was maintained at 25 ± 0.1 °C by the circulation of thermostatted water. A stream of nitrogen, presaturated with water vapour by bubbling it through a 0.5 mol dm^{-3} KCl solution, was blown over the surface of the solution. The base used for e.m.f. measurements was carbonate-free potassium hydroxide (0.3050 mol dm^{-3}) standardized using the different procedures previously described.[10,11] In the e.m.f. titrations the concentration of the potassium hydroxide solution (N), the coefficients of the correction terms for the effects of liquid junction potential in acid (A_j) and basic (B_j) solution, the standard electrode potential (E°), and K_w were evaluated before and after each experiment by dynamic titration using different calculation procedures as previously described.[6,10,11] The collection of the experimental data (v, E) was performed by using two different methods: 'kinetics D' or 'kinetics T'. The 'kinetics' parameter determines the moment at which the value E is recorded in the computer memory (integral part of Titroprocessor E 636). In the 'kinetics D' method it is possible to choose values of drift ($\Delta E/\Delta t$) from 0.5 to 75 mV min^{-1}, subdivided in 10 steps, where ΔE changes from 0.1 to 0.5 mV and Δt from 12.8 to 0.4 s. At fixed intervals, a measurement is performed by calculating the variation of e.m.f. with reference to the last measurement and by comparing it (variation) with the

Table 1. Critical evaluation of the equivalence point (v_e/cm^3), N (mol dm^{-3} of KOH), and K_w (ionic product of water) from 19 potentiometric calibration 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

	v_e		N/mol dm^{-3}		
	Acidic	Alkaline	Acidic	Alkaline	10^{14} K_w/mol^2 dm^{-6}
Mean with standard deviation	1.948(1)	1.945(3)	0.304 87(14)	0.305 32(42)	1.811 3(55)
Variance	9.004×10^{-7}	7.769×10^{-6}	2.052×10^{-8}	1.791×10^{-7}	0.0011
Standard error	2.177×10^{-4}	6.39×10^{-4}	3.286×10^{-5}	9.708×10^{-5}	0.0039

selected limit value (ΔE). If the drift of the signal is too great, another measurement can be effected and this process is repeated until the conditions of measurement testing (drift $\leqslant$ preselected value) are performed and until it is possible to obtain a result which can be stored in the memory. The 'waiting time' owing to the drift has a lower limit of about 2 s and an upper limit of about 60 s. In the 'kinetics T' method the measured values are stored after a constant waiting time of 2 to 300 s, regulable in 10 steps. This allows, under the chosen experimental conditions, amelioration of measurement repetition in the case of a very slow response or reaction (for example titration in non-aqueous solution).

Spectrophotometric Measurements.—Absorption spectra in the region 420—700 ± 1 nm were obtained on a Jasco Uvidec-505 spectrophotometer. Samples containing ahhe and copper ion maintained at an ionic strength of 0.5 mol dm^{-3} KCl and at 25 °C were scanned at pH values from 3.91 to 11.56 using 10-mm cells.

Calculation Methods.—Great care has been paid to the calculation procedures and critical evaluation of some parameters (v_e, N, E°, A_j, and B_j) pertaining to potentiometric calibration curves, following different methods as previously described.[10,11] The statistical analysis of variance applied to a set of 19 potentiometric calibrations is reported in Table 1. The comparison of values from different experiments is a very important means of analysing the reliability. From the results obtained, excellent agreement was found between the parameters (v_e, N) calculated for acidic solution and those calculated for alkali solution. At the same time the differences in the values of these parameters were not significantly different from the mean values: even the estimated standard deviations were almost equal. The statistical analysis shows how the parameters (E°, not reported in Table 1) obtained from the two regions (acidic and alkaline) of the same calibration curves and those from one titration to another are sometimes significantly different from the mean value or from them (E° in acidic and alkaline solution), thus showing that unexpected factors differing from one titration to another can alter the e.m.f. values and are the main source of error. Moreover it is generally accepted that E° can change with the passing of time, probably due to ageing of the electrodes. The difference in the values of K_w vary significantly from one experiment to another. Thus the precision of the standard electrode potential and of the ionic product of water can be calculated only from the intertitration variability (all the points in a single titration are considered). The protonation and formation constants (β_{pqr}), which are defined by equation (1) (charges omitted for simplicity), were

$$pM + qH + rL \rightleftharpoons M_pH_qL_r \qquad (1)$$

refined by rigorous least squares using the computer program SUPERQUAD;[12] p, q, and r are the stoicheiometric coefficients of metal(II), proton, and ligand, respectively, in the complex $M_pH_qL_r$. This program calculates the values of the cumulative protonation and formation constants which minimize the sum of the squared residuals between the observed and calculated e.m.f. values [equation (2)]. The parameter w_i is a weighting

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

factor, defined by equation (3) where σ_E (= 0.2) is the error in

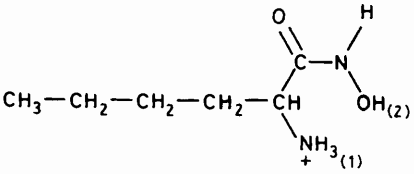
$$w_i = \sqrt{1/[\sigma_E{}^2 + (\partial E_i/\partial v_i)^2\sigma_v{}^2]} \qquad (3)$$

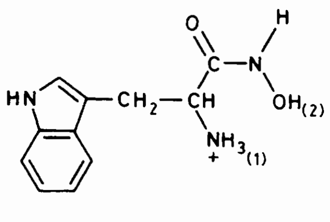
the e.m.f., σ_v (= 0.008) the error in the volume used in the refinement strategy, and Z is the total number of potentiometric data.

All calculations were carried out 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 metal-ion, hydrogen, and ligand concentrations of the starting solutions for each potentiometric experiment are listed in Table 2. Listings of experimental data and final computations from SUPERQUAD are available on request from the author.

Results and Discussion

Protonation Equilibria.—The ligands each have two titratable protons in the pH range studied (2.94—11.60) which can be attributed to hydroxide NHOH and the NH_3^+ groups. Initially





the overall protonation constants (β_{011}, β_{021}) of the two ligands and the initial amounts (mmol) of reagents (T_H, T_L) were calculated at the same time through the refinement of several sets of experimental data (v, E) by the program SUPERQUAD. At the end of this refinement the maximum variation in the quantities T_H, T_L with respect to the initial values was 0.71, 0.6%

Table 2. Determination of protonation and complex-formation constants. Initial amounts of the reagents* for the alkalimetric titrations of 2-amino-*N*-hydroxyhexanamide (ahhe) and α-amino-1*H*-indole-3-*N*-hydroxypropanamide (aihp) 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$^+$-ahhe	0.263 68		0.552 50	3.04—11.34
2		0.250 50		0.524 87	3.03—11.32
3		0.276 86		0.580 12	2.98—11.33
4		0.296 64		0.621 56	2.95—11.35
5		0.283 46		0.593 93	2.98—11.60
6	Cu^{II}-ahhe	0.264 85	0.066 19	0.555 37	2.94—11.26
7		0.198 64	0.084 52	0.416 53	3.04—10.58
8		0.264 85	0.101 83	0.555 37	3.42—10.77
9		0.231 75	0.101 83	0.485 95	2.93—10.83
10		0.231 75	0.132 37	0.485 95	2.93—11.36
11		0.165 53	0.101 83	0.347 11	3.03—10.16
12	Ni^{II}-ahhe	0.264 85	0.073 50	0.555 37	3.08— 6.14
13		0.231 75	0.124 96	0.485 95	3.61— 6.71
14		0.198 64	0.036 75	0.416 53	3.72— 7.87
15		0.231 75	0.036 75	0.485 95	3.08— 7.05
16	Co^{II}-ahhe	0.231 75	0.035 57	0.485 95	3.10—10.27
17		0.264 85	0.085 36	0.555 37	3.28— 7.74
18		0.211 88	0.055 48	0.444 30	3.11— 8.96
19		0.244 99	0.042 68	0.513 72	3.37— 8.98
20		0.278 10	0.035 57	0.583 14	2.98— 9.27
21		0.284 72	0.056 90	0.597 02	3.23— 8.92
22	H$^+$-aihp	0.173 23		0.368 82	3.05— 7.43
23		0.134 25		0.285 84	3.16— 7.74
24		0.125 59		0.267 39	3.50— 7.63
25		0.121 26		0.258 17	3.23—10.62
26		0.116 93		0.248 95	3.24—10.73
27		0.123 43		0.262 78	3.26— 7.81
28		0.129 92		0.276 61	3.18—10.99
29	Cu^{II}-aihp	0.155 59	0.030 55	0.330 95	2.97—10.16
30		0.133 36	0.020 36	0.283 67	3.08—10.61
31		0.120 03	0.010 18	0.255 30	3.16—10.41
32		0.124 47	0.013 24	0.264 76	3.21—10.67
33		0.106 69	0.022 40	0.226 93	3.19—10.19
34		0.142 25	0.014 26	0.302 58	3.14—10.64
35	Ni^{II}-aihp	0.088 91	0.007 35	0.189 11	3.36—10.13
36		0.088 91	0.009 56	0.189 11	3.37— 9.51
37		0.084 46	0.006 61	0.179 66	3.39—10.47
38		0.088 91	0.006 61	0.189 11	3.35—10.97
39		0.080 02	0.008 09	0.170 20	3.43—10.23
40		0.075 57	0.008 82	0.160 74	3.48—10.47
41		0.071 13	0.009 56	0.151 29	3.53—10.87

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

Table 3. Cumulative and stepwise protonation and complex-formation constants of 2-amino-*N*-hydroxyhexanamide (ahhe) and α-amino-1*H*-indole-3-*N*-hydroxypropanamide (aihp) with bivalent metal ions at 25 °C and $I = 0.5$ mol dm^{-3} (KCl). Standard deviations (σ values) are given in parentheses

	ahhe				aihp		
	H$^+$	Co^{2+}	Ni^{2+}	Cu^{2+}	H$^+$	Ni^{2+}	Cu^{2+}
log β_{011}	9.172(1)				9.088(5)		
log β_{021}	16.547(2)				16.130(7)		
log K_2^{H}[a]	7.375(2)[b]				7.042(6)		
log β_{101}		6.423(25)	6.571(35)	10.29(14)			
log β_{102}		10.964(35)	13.902(8)	19.695(26)		14.383(19)	20.31(6)
log β_{2-12}				20.767(18)			21.469(38)
log β_{1-12}		1.77(7)	6.17(9)	9.877(43)		5.53(7)	10.52(7)
log K_2		4.541(30)	7.331(25)	9.41(10)			
Z[c]	234	179	98	219	152	159	220
U	1.553×10	1.083×10^3	2.74×10^2	9.275×10^2	7.183	7.747×10	2.875×10^2
χ^2[d]	10.85	11.44	29.84	12.63	8.11	11.47	10.44
σ^2[e]	0.27	2.49	1.70	2.11	0.23	0.70	1.13

[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 experimental data points used in the refinement. [d] Observed value; calculated value (degrees of freedom 6, confidence coefficient 0.95) should be 12.6. [e] σ^2 is given by the expression $\sqrt{\sum_{i=1}^{Z} w_i(E_i^{obs.} - E_i^{calc.})^2/(Z - m)}$ where m is the number of parameters to be refined.

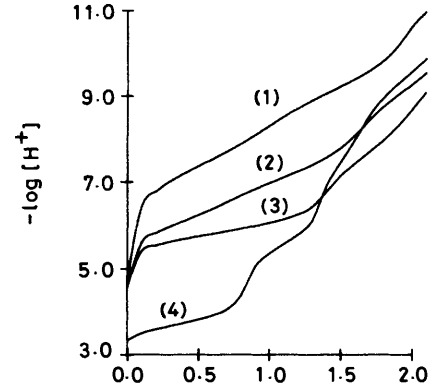


Figure 1. Titration curves of pH as function of the volume of KOH added, calculated by the HALTAFALL program (N. Ingri, W. Kakalowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261) and using a Calcomp 936 PLOTTER. Initial concentrations of solutions: $c_L = 1.2 \times 10^{-2}$, $c_M = 4.0 \times 10^{-3}$, $c_H = 2.4 \times 10^{-2}$, and $c_{KOH} = 0.3048$ mol dm^{-3}; $V_0 = 25$ cm^3. Curves: (1) ahhe, (2) Co^{2+}–ahhe, (3) Ni^{2+}–ahhe, and (4) Cu^{2+}–ahhe

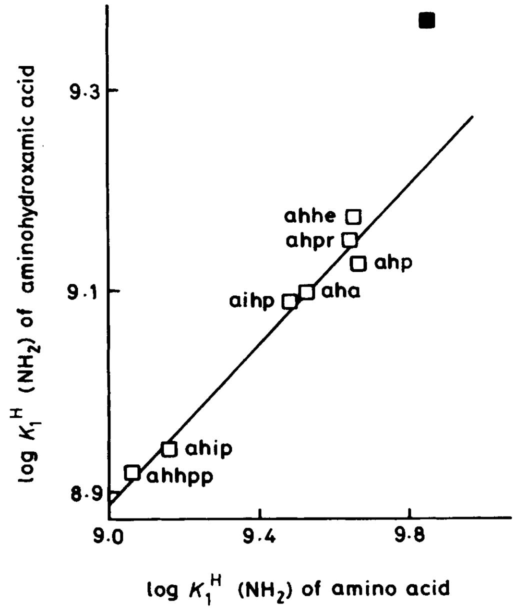


Figure 2. Relationship between log K_1^H (protonation constant of α-amino group in aminohydroxamic acids) and log K_1^H (protonation constant of α-amino group of the corresponding amino acids). The line drawn was obtained from a linear least-squares analysis of all the data ($y = 5.335 + 0.395\,x$), excluding those for DL-aspartic acid β-hydroxamate (■)[22]

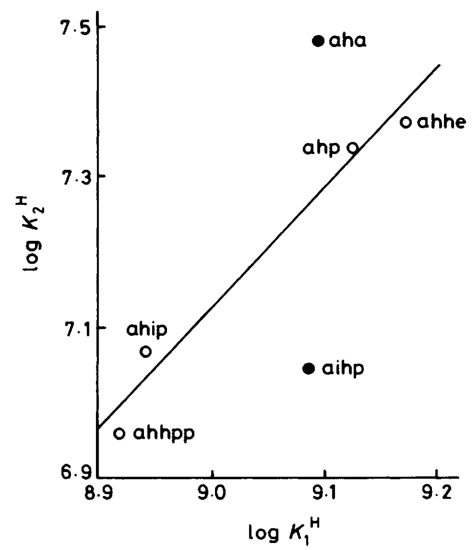


Figure 3. Relationship between log K_2^H (protonation constant of NHO^- group) and log K_1^H (protonation constant of α-amino group). The line drawn is based on the intercept (−7.389) and the slope (1.612) obtained from a linear least-squares analysis of all the data

for ahhe and 3.59, 3.47% for aihp respectively. The potentiometric titration curves for the ahhe–metal systems with strong base (KOH) are shown in Figure 1.

The refined values of the cumulative and stepwise protonation constants of the two ligands at 25 °C and $I = 0.5$ mol dm^{-3} KCl are given in Table 3. The assignment of the protons to the sites of the two ligands in the protonated form (H_2L^+) can be made on the basis of the results obtained and by analogy with the known values for some aminohydroxamic acids[10,11,13–15] and analogous compounds.[16–18] The log β_{011} or log K_1^H values corresponding to the protonation of the primary amine group (1) were estimated to be 9.172(1) for ahhe and 9.088(5) for aihp, respectively. By analogy with known values for similar functional groups,[13–15] log K_2^H [7.375(2) for ahhe, 7.042(6) for aihp] can be assigned to protonation of the oxygen atom (2) of the NHO^- group. The substitution of a NHOH group for the OH group of a carboxyl in aminohydroxamic acids lowers the protonation constant (log K_1^H) of the α-amino group as compared with log K_1^H of the corresponding α-amino acids due to the electron-withdrawing character of the NHOH group [log K_1^H = 9.096(1) for glycinehydroxamic acid (aha),[10] 9.54(1) for glycine,[19] 9.125(1) for DL-norvalinehydroxamic acid (ahp),[10] 9.68(3) for DL-norvaline,[20] 8.942(2) for L-histidinehydroxamic acid (ahip),[11] 9.17 for histidine;[21] log K_1^H = 8.919(2) for L-tyrosinehydroxamic acid (ahhpp),[13] 9.07(5) for L-tyrosine;[20] log K_1^H = 9.172(1) for ahhe, 9.67(3) for DL-norleucine,[20] 9.088(5) for aihp, 9.49 for DL-tryptophan,[19] 9.15(1) for L-α-alaninehydroxamic acid (ahpr),[15] 9.66 for L-alanine[19]].* The trend (Figure 2) is regular for all aminohydroxamic acids considered with the exception of DL-aspartic acid β-hydroxamate,[22] which deviates considerably from the straight line. This is due to the presence of one CO_2H group which significantly raises the protonation constant of the NH_2 group in the hydroxamic acid. At the same time the presence of the α-amino group in aminohydroxamic acids increases remarkably the acidic character of the OH group (log K_2^H) in the order aha [7.484(2)] < ahhe [7.375(2)] < ahp [7.340(2)] < ahip [7.067(2)] < aihp [7.042(6)] < ahhpp [6.956(3)]. By a careful study of the results obtained to date it is possible to observe the mutual influence of the basic groups (NH_2, NHO^-, Figure 3) in aminohydroxamic acids, and also of other factors (± *I* effects of groups) as previously reported.[10,11,13] Thus in Figure 3 there are two anomalous cases: the protonation constant of aha (log K_2^H = 7.484) is greater than expected for protonation of a NHO^- group, while aihp shows the opposite trend. The very high value for protonation of the NHO^- group in aha suggests that the HL molecule is associated. On the other hand, the abnormally high tendency for the NHOH of aihp to deprotonate (Figure 3) indicates destabilization of the species HL. This destabilization

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* Abbreviations: aha = 2-amino-*N*-hydroxyacetamide, ahp = 2-amino-*N*-hydroxypentanamide, ahhpp = 2-amino-*N*-hydroxy-3-(*p*-hydroxyphenyl)propanamide, ahip = α-aminoimidazole-4-*N*-hydroxypropanamide, ahpr = 2-amino-*N*-hydroxypropanamide.

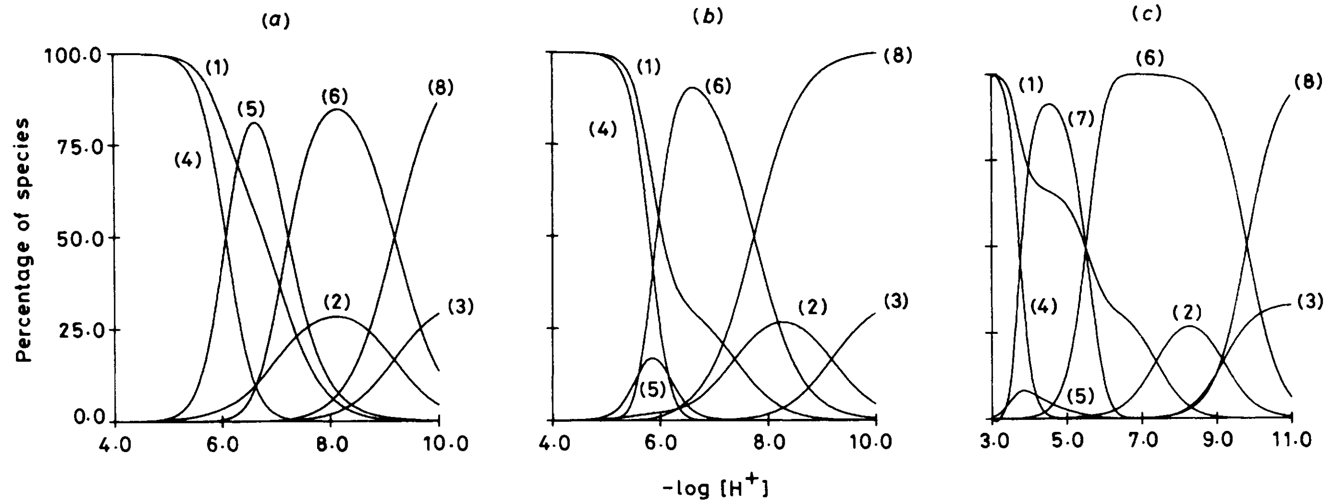

Figure 4. Species distribution diagrams for M^{2+}-ahhe systems. The percentage of each species has been calculated from the data for a hypothetical solution of metal ions (0.004 mol dm^{-3}) and ahhe (0.012 mol dm^{-3}) by the HALTAFALL program and using a Calcomp 936 PLOTTER. The concentrations of 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+}-ahhe, (*b*) Ni^{2+}-ahhe, (*c*) Cu^{2+}-ahhe; (1) H_2L^+, (2) HL, (3) L^-, (4) M^{2+}, (5) $[ML]^+$, (6) $[ML_2]$, (7) $[M_2(OH)L_2]^+$, and (8) $[M(OH)L_2]^-$

(higher acidity of the NHOH group) may be due to the greater steric effect of the indole group in aihp compared with the imidazole (ahip) or *p*-hydroxyphenyl group (ahhpp), and could result in weaker intra- or inter-molecular hydrogen bonding in the associated species.

Metal(II) *Complex Formation.*—The existence of metal–proton–aminohydroxamic acid (ahhe or aihp) complexes was examined using the values p = 1—4, q = 1, 2, 3, −1, −2, or −3, and r = 1, 2, 4, or 5. The equilibrium pattern was selected by successive attempts according to the best agreement between observed and calculated data. By means of an accurate statistical analysis the following parameters were taken into account for each hypothesis: the standard deviations (σ) of the stability constants, the agreement factor (σ^2), the goodness of fit (χ^2), and the chemical significance of the species selected. At this point all the protonation constants were kept constant and the computer program SUPERQUAD was employed for a second stage of refinement in which e.m.f. data for solutions with various metal ion–ligand ratios were processed in order to investigate the binary systems. In the final stage of refinement to calculate the formation constants of the binary complexes, the initial amounts (mmol) of metal reagent (T_M) for runs 6—11 and 17 (Table 2) were refined as special parameters. The maximum variation in (T_M) with respect to the initial values was very small.

The calculated complex formation constants, log β_{pqr}, for the two ligands with different metals are given in Table 3. Sample potentiometric titration curves of the protonated ligand (ahhe), H_2L^+, alone and in the presence of Co^{2+}, Ni^{2+}, and Cu^{2+}, are shown in Figure 1. Two protons per ligand molecule dissociate between a = 0 and 2.1 [where a = volume (cm^3) of KOH added]. The shapes of the curves clearly show that different types of reactions are occurring involving the formation of simple mononuclear chelates, $[ML]^+$ and/or $[ML_2]$, as well as of various hydrolysed species. The most common initial reaction involving bivalent transition-metal ions is a two-proton displacement to form mononuclear complexes. Thus, the curves show that all the metals investigated react with the monovalent cation (H_2L^+) according to equation (4). The positions of the curves also indicate that all the chelates in the Cu^{II}-ahhe system are more stable than those in the other metal systems.

$$M^{2+} + H_2L^+ \rightleftharpoons [ML]^+ + 2H^+ \qquad (4)$$

Starting from the stability constants given in Table 3 and the protonation constants of ahhe under the same experimental conditions, the percentage of each complex involving H^+ or OH^-, metal ion, and ligand has been calculated by using the HALTAFALL program with a Calcomp 936 PLOTTER. Typical distribution diagrams are shown in Figure 4.

The complexes of Co^{2+} and Ni^{2+} exhibit similar behaviour to those of Cu^{2+}, but the latter have higher stabilities as expected. In particular, the cumulative and stepwise formation constants for the 1:1 and 1:2 metal–aminohydroxamic acid complexes follow the Irving–Williams order. The small differences in log β_{101}, log β_{102}, and log K_2 values for ahhe and aihp compared to those for aha and ahp[10] presumably reflect the small variation in basicity of the NHO^-, NH_2 groups involved in co-ordination and the steric effects of the substituents situated in the immediate vicinity of the electron donors. This is not the case for the Ni^{2+}-ahhe system where the logarithm of the second stepwise formation constant (log K_2) is appreciably greater than that of the first, log β_{101} (see also log K_2, log β_{101} for the Ni^{2+}-ahp system[10]). This is contrary to earlier findings,[23–26] using a method of calculation based on the erroneous assumption that the formation of $[ML]^+$ is effectively complete before $[ML_2]$ begins to be formed. Other ligands have also been found to give the sequence log β_{101} < log K_2 in their metal complexes.[27,28] The explanation for this behaviour may lie in the ability of Ni^{2+} to form square-planar complexes with co-ordination of the aminohydroxamic acid *via* the N atom of the amino group and the nitrogen atom of the deprotonated NHO^- group.[29,30] It also demonstrates that individual five-membered rings in the complex $[NiL]^+$ are more stable than six-membered rings; when a second ligand is bonding facially in a bidentate manner the two five-membered rings in the complex $[NiL_2]$ are much more stable than six-membered rings formed by other metals.

Absorption spectra for the Cu^{2+}-ahhe system are shown in Figure 5 for the range pH 3.91—11.56. They consist of two bands at 490—500 and 660 nm. At low pH a broad absorption occurs near the i.r. region, its maximum shifting progressively into the visible region as the pH is increased. An addition of ligand to aqueous solutions of $[Cu(H_2O)_6]^{2+}$ leads to the

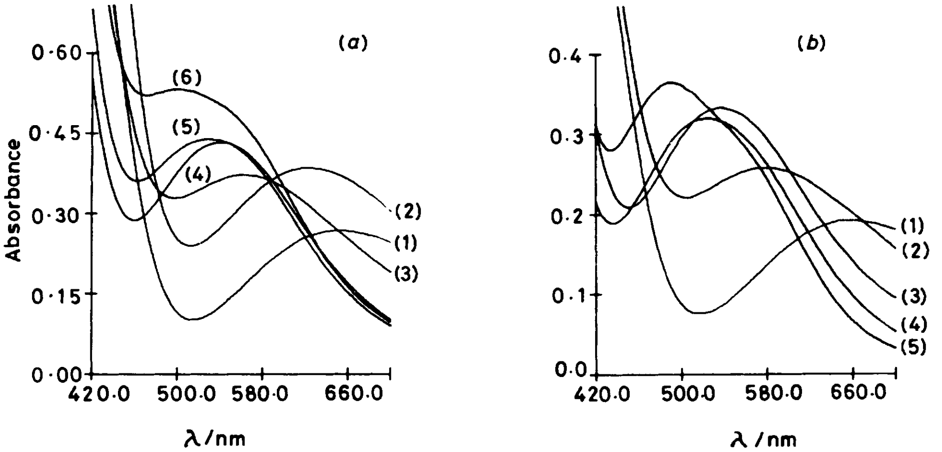

Figure 5. Plots of experimental absorbance data *versus* wavelength for some solutions of Cu^{2+}–ahhe using the program VISION and the Calcomp 936 PLOTTER. (*a*) (1) pH 3.92, $c_L = 9.270 \times 10^{-3}$, $c_M = 5.295 \times 10^{-3}$; (2) pH 5.66, $c_L = 9.088 \times 10^{-3}$, $c_M = 5.191 \times 10^{-3}$; (3) pH 6.04, $c_L = 9.017 \times 10^{-3}$, $c_M = 5.151 \times 10^{-3}$; (4) pH 8.73, $c_L = 8.948 \times 10^{-3}$, $c_M = 5.111 \times 10^{-3}$; (5) pH 10.58, $c_L = 8.879 \times 10^{-3}$, $c_M = 5.072 \times 10^{-3}$; (6) pH 11.56, $c_L = 8.712 \times 10^{-3}$, $c_M = 4.976 \times 10^{-3}$ mol dm^{-3}. (*b*) (1) pH 3.91, $c_L = 1.031 \times 10^{-2}$, $c_M = 3.962 \times 10^{-3}$; (2) pH 5.59, $c_L = 1.011 \times 10^{-2}$, $c_M = 3.886 \times 10^{-3}$; (3) pH 6.58, $c_L = 1.003 \times 10^{-2}$, $c_M = 3.857 \times 10^{-3}$; (4) pH 9.70, $c_L = 9.846 \times 10^{-3}$, $c_M = 3.785 \times 10^{-3}$; (5) pH 11.33, $c_L = 9.667 \times 10^{-3}$, $c_M = 3.716 \times 10^{-3}$ mol dm^{-3}

formation of complexes by successive displacement of water molecules, but it must be kept in mind that two of the water molecules are farther from the metal ion than the other four. A maximum at 660 nm is reached at pH 3.91, which corresponds to the maximum in the concentration of the species complexed, $[Cu_2(OH)L_2]^+$, at about this same pH [Figure 4(*c*)]. As the pH increases above 6.0 a simultaneous decrease in the absorption occurs with a maximum at 524 nm [curves (4), Figure 5] or 563 nm [curve (3), Figure 5(*a*)] and an increase in the absorption with a maximum at 490 nm. A distinctive isosbestic point appears at *ca.* 588 nm. This corresponds to an equilibrium between the species $[Cu_2(OH)L_2]^+$ and $[CuL_2]$ which are predominant from pH 4.0 to 7.0, according to Figure 4(*c*). Associated with the shift of the bands in the 490—660 nm region is a change in colour from intense green to nearly colourless in strongly acidic media, while at pH > 4.0 the colour changes from purple to reddish violet. With the pH increase, small bathochromic and high hyperchromic shifts are observed from 490 to 660 nm. For the Cu^{2+}–ahhe system, previous i.r. and magnetic results on Fe^{3+}–aha,[16] other results,[10] and also the visible spectra and the stability constants reported in the present work suggest that the co-ordination probably involves the α-amino group and the nitrogen atom of NHO^-. Equilibria between Co^{2+} and aihp cannot be studied because at acidic pH precipitation and instability in the e.m.f. measurements occur inside the titration compartment.

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