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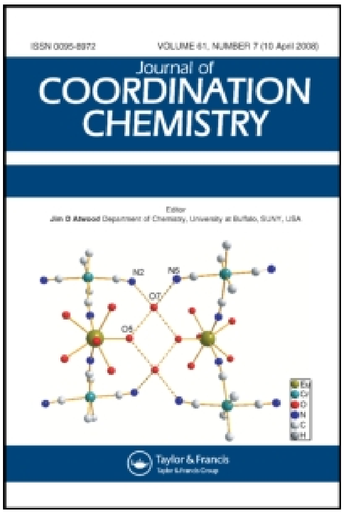

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AMINOHYDROXAMIC ACIDS AS SEQUESTERING AGENTS IN LIVING SYSTEMS. POTENTIOMETRIC DETERMINATION OF PROTONATION AND FORMATION CONSTANTS FOR 2,6-DIAMINO-N-HYDROXYHEXANAMIDE AND COPPER(II), NICKEL(II), COBALT(II), AND HYDROGEN IONS IN AQUEOUS SOLUTION

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AMINOHYDROXAMIC ACIDS AS SEQUESTERING AGENTS IN LIVING SYSTEMS. POTENTIOMETRIC DETERMINATION OF PROTONATION AND FORMATION CONSTANTS FOR 2,6-DIAMINO-*N*-HYDROXYHEXANAMIDE AND COPPER(II), NICKEL(II), COBALT(II), AND HYDROGEN IONS IN AQUEOUS SOLUTION

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The systems H^+, cobalt(II), nickel(II), and copper(II) with 2,6-diamino-*N*-hydroxyhexanamide (*L*-lysinehydroxamic acid hydrochloride = dahhe) have been investigated by potentiometric and spectrophotometric titrations at 25 ± 0.1°C and $I = 0.5\ \mathrm{mol\ dm^{-3}}$ (KCl). The protonation constants of the ligand and the overall formation constants of several metal complexes were calculated from potentiometric and spectrophotometric data with the aid of the SUPERQUAD and SQUAD programs, respectively. The following cumulative association constants (relative standard deviations are given in parentheses), $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$, were obtained: $\log\beta_{011} = 10.66$ (1), $\log\beta_{021} = 19.55$ (1), $\log\beta_{031} = 26.56$ (1); Co^{II}-dahhe, $\log\beta_{121} = 22.68$ (2), $\log\beta_{111} = 15.72$ (2), $\log\beta_{122} = 29.73$ (3), $\log\beta_{101} = 8.68$ (1); Ni^{II}-dahhe, $\log\beta_{111} = 16.93$ (8), $\log\beta_{122} = 34.87$ (1), $\log\beta_{112} = 27.70$ (4); Cu^{II}-dahhe, $\log\beta_{111} = 20.26$ (1), $\log\beta_{212} = 41.53$(2), $\log\beta_{122} = 40.56$ (3), $\log\beta_{112} = 33.33$ (10). Dahhe forms protonated complexes containing the pendant amino group in the protonated form. Electronic spectroscopy studies can be used to follow the appearance of the individual species, to estimate the coordination sphere at the metal and to observe equilibria between different complexes. Equilibrium constants for their formation and the probable structure of the chelated compounds formed in aqueous solution are discussed in terms of their possible significance to biological reactions.

Keywords: Aminohydroxamic acids, protonation, complex formation, stability constants, potentiometry, spectrophotometry

INTRODUCTION

Considerable work has been carried out during the last decade on hydroxamate chemistry. Due to their undoubted biological effects, hydroxamic acid derivatives of amino acids are of great interest. Aside from their analytical and industrial applications, they serve as metal chelators which may function as growth factors, tumor inhibitors, constituents of antibiotics, cell-division factors, antibiotic antagonists and pigments.[1,2] Some of these compounds have shown themselves to be potent inhibitors of thermolysin, elastase and aminopeptidase.[3] However, when the hydroxamic acid also contains another donor, such as an amino group, coordination could involve this group ($-NH_2$) as well as the nitrogen or oxygen of the hydroxamic function. Regarding the strong ability of this type of ligand to form chelates, the main question concerns the nature of hydroxamic binding upon coordination. Although much work has concentrated synthesis and biological and structural characterization, relatively few papers have appeared dealing with solution equilibria of proton and metal complexes of hydroxamic acids; the number of spectrophotometric studies in these systems is small.

Accordingly, the aim of the present work was to clarify equilibrium reactions involving protons, Co^{2+}, Ni^{2+}, and Cu^{2+} with dahhe, using a detailed potentiometric technique and spectrophotometric measurements.

EXPERIMENTAL

Reagents

[2,6-diamino-*N*-hydroxyhexanamide ([(dahhe,), *L*-lysinehydroxamic acid hydrochloride = H_2L^+] was obtained from Sigma (St. Louis, MO) and its purity and exact concentration of its solution checked by potentiometric titrations. All other metal (chlorides, AnalaR products) solutions were prepared and standardized using standard procedures.[4,5] Solutions were prepared by adding successively to the titration vessel a known volume of dahhe solution and an exact volume of metal chloride; then, the required quantities of potassium chloride and a sufficient amount of doubly distilled water were added to make up a total volume of 25.0 ± 0.01 cm^3. Doubly distilled and deionized water was used in all potentiometric and spectrophotometric experiments and reagent grade chemicals were used without further purification. The accurate molarity of potassium hydroxide (*ca* 0.4338 mol dm^{-3}) and hydrochloric acid (0.4326 mol dm^{-3}) stock solutions were determined by conventional potentiometric titrations according to Gran's method, with the use of different calculation procedures as previously described.[4–8]

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Potentiometric Measurements

The equilibrium studies were performed using a Metrohm E 636 Titroprocessor with a combined electrode Ross 8102SC (ORION Research). E.m.f. readings for potentiometric experiments (dynamic and static)[9] and titration curves were recorded graphically using an E 636 automatic titrator. A thermostatted stream of nitrogen, presaturated with water vapour by bubbling it through a 0.5 mol dm^{-3} KCl solution, was passed over the surface of the solution in the titration vessel. Small amounts ($\Delta v = 0.05$ or 0.025 cm^3) of titrant were added with the use of a Metrohm Dosimat E 635 autoburette (total volume = 5.0 cm^3). The system was calibrated in terms of hydrogen ion concentrations before and after a series of measurements by titrations of hydrochloric acid solution at 25.0 ± 0.1°C and $I = 0.5$ mol dm^{-3} (KCl) with a standard, carbonate-free, potassium hydroxide solution, according to Gran's method,[7] using the computer programs NBAR[8] and MAGEC[10] as previously described. The solution in the titration compartment was mixed with a mechanical stirrer. The range of concentration of ligand in the potentiometric titrations was

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0.00710–0.00841 mol dm^{-3} for the dahhe-H$^+$ system (pH range 3.50–11.79; number of titrations 4); 0.00747–0.00934 mol dm^{-3} for dahhe-Cu^{2+} system (pH range 2.87–8.28; number of titrations 6); 0.00747–0.00934 mol dm^{-3} for dahhe-Ni^{2+} system (pH range 3.35–8.62; number of titrations 6); 0.00747–0.00934 mol dm^{-3} for dahhe-Co^{2+} system (pH range 3.30–8.74; number of titrations 6). The metal concentration varied between 0.00221–0.00531 mol dm^{-3} for dahhe-Cu^{2+}, 0.00238–0.00680 mol dm^{-3} for dahhe-Ni^{2+} and 0.00237–0.00677 mol dm^{-3} for dahhe-Co^{2+} systems, and potassium chloride ($I = 0.05$ mol dm^{-3}) was used as supporting electrolyte.

Spectrophotometric Measurements

Absorption spectra in the range 400–820 (± 0.3) nm for the Cu^{2+}-dahhe system were measured with a Kontron Uvikon 860 spectrophotometer to the fourth decimal place with a step of 2 nm. Solutions containing the ligand and the cupric ion, prepared and maintained under purified nitrogen at an ionic strength of 0.5 mol dm^{-3} (KCl), were measured at pH from 3.52 to 9.49 at 25.0 ± 0.1°C using 10 mm cells.

Calculations

First, some parameters in acidic and alkaline solution (E^0, A_j, B_j, v_e, and N) were refined simultaneously by using the NBAR program, as reported in Table 1. In the present calculations, some experimental points (v, E) around the equivalence point have been neglected. Starting from the same potentiometric data, other parameters relating to calibration curves (E^0, 2.393 RT/F, K_w, and v_e) were calculated by applying the MAGEC program. Careful attention has been taken in the calculation and critical evaluation of the parameters relative to the potentiometric calibration curves, using different mathematical procedures as previously described.[4–10] In particular, by taking the results quoted in Table 1, it is possible to verify the good agreement between the parameters (v_e and N) obtained from the two computer programs (maximum variation 0.6 and 0.5%, respectively). At the same time, the differences between these parameters were not significant with respect to the mean. Additionally, accurate examination of the results in Table 1 also reveals a good agreement in the standard potential, E^0, even if it is well known that the standard potential of the glass membrane is inclined to change from day to day (owing to asymmetry effects) and that the liquid-junction potentials (A_j and B_j) do not reproduce easily, as can be seen from calculations performed with the program NBAR. A more important factor that critically affects the refinement by the program MAGEC is the value used for the dissociation constant of water, K_w, while in the calculations with the program NBAR, this parameter was kept constant ($K_w = 1.8749 \times 10^{-14}$). This parameter is very sensitive to correlations with the concentration of alkali in the burette. In those situations when K_w is uncertain, the subprogram CALIBT (MAGEC program) permits the user to systematically vary the estimate of K_w. Thus the precision of the standard electrode potential, E^0, and especially of the ionic product of water can be deduced only from the intertitration variability [all points (acid and alkaline zones) in a single titration are considered by the MAGEC program]. The statistical analysis actually shows how the parameters (E^0) 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

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TABLE 1

Evaluation of the equivalence point, v_e/cm^3, N/mol dm^{-3} (normality of KOH), K_w (ionic product of water), E^0/mV[a] (standard potential), A_j/mV[a] (junction potential in acid solution), B_j/mV[a] (junction potential in basic solution), and 2.303 RT/F (Nernstian slope)[a] from eight potentiometric titrations of HCl (0.43264 mol dm^{-3}) with KOH at 25°C and $I = 0.5$ mol dm^{-3} (KCl) using the programs NBAR and MAGEC

	MAGEC					NBAR[b]				
Run	E^0	2.303RT/F	$10^{14}K_w$	v_e	$10^{14}K_w$[c]	E^0	A_j	B_j	v_e	N
1	403.50 (8)[d]	59.20 (1)[d]	1.8450[d]	1.997[d]	1.8551	403.72 (10)[e]	−13.16 (3.93)[e]		1.997[e]	0.43326[e]
						403.86 (9)[f]		33.99 (4.12)[f]	1.996[f]	0.43345
2	403.50 (6)	59.11 (2)	1.8879	1.997	1.8937	404.26 (12)	−27.67 (4.83)		1.998	0.43312
						404.93 (7)		5.18 (3.64)	1.996	0.43359
3	404.30 (6)	59.23 (3)	1.8535	2.005	1.9123	404.24 (11)	−28.95 (15.1)		1.997	0.43319
						405.16 (8)		3.08 (5.86)	1.994	0.43392
4	403.40 (10)	59.02 (2)	1.8707	1.994	1.8746	404.48 (9)	−48.39 (8.71)		1.994	0.43389
						404.22 (8)		−14.05 (5.09)	1.993	0.43408
5	403.60 (10)	59.14 (4)	1.9409	1.994	1.9257	404.30 (8)	−38.74 (7.79)		1.995	0.43377
						404.73 (8)		−14.26 (5.88)	1.993	0.43419
6	401.30 (11)	58.99 (3)	1.9231	1.994	1.9373	402.63 (8)	−54.41 (7.49)		1.994	0.43385
						403.22 (7)		−17.27 (4.25)	1.993	0.43415
7	401.40 (13)	58.99 (4)	1.9231	1.994	1.9384	402.78 (10)	−57.64 (8.78)		1.993	0.43406
						403.38 (7)		−13.80 (3.50)	1.993	0.43416
8	400.10 (4)	59.12 (2)	1.8967	1.994	1.8871	400.65 (9)	−21.12 (3.82)		1.997	0.43325
						400.56 (6)		9.33 (7.68)	1.995	0.43370

[a] Standard deviations in E^0, 2.303 RT/F, A_j and B_j are given in parentheses.

[b] In the calculations, K_w was kept constant (1.8450×10^{-14} mol^2 dm^{-6}).

[c] Parameter calculated from the experimental data in alkaline solution following the principles of Gran by keeping E^0 as obtained in acidic zone.

[d] Using all the buffered data.

[e] Parameters calculated following the principles of Gran by using the experimental data in acidic solution.

[f] Parameters calculated following the principles of Gran by using the experimental data in alkaline solution. Initial amount of hydrochloric acid was 2.0 cm^3.

value or from each other (E^0 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 thus the main source of error.

All calculations were carried out on the CRAY Y-MP 432 and IBM 9377/90 computers of the Consorzio per la Gestione del Centro di Calcolo Elettronico Interuniversitario dell'Italia Nord Orientale, Casalecchio di Reno, Bologna, Italy, with the financial support of the University of Parma. Listings of the experimental data for the computations from SUPERQUAD,[11] NBAR, MAGEC, and SQUAD[12] programs are available as Supplementary Material and can be obtained on request from the author.

RESULTS AND DISCUSSION

Protonation Equilibria

A maximum number of three protons can be released from the ligand in the fully protonated form (Hdahhe = H_3L^{2+}) on titration with strong base in the pH range 3.50–11.79. All sets of titration data indicate the presence solely of simple HL, H_2L^+, and H_3L^{2+} complexes. Initially, the overall protonation constants (log β_{011}, log β_{021}, and log β_{031}) of the ligand and the initial amounts (mmol) of the reagents (T_H, T_L) were calculated at the same time through the refinement of several sets of potentiometric titration data, (number of experimental data points = 263, $\sigma = 0.24$, $\chi^2 = 8.3$) by SUPERQUAD without introducing the liquid-junction potentials into the calculations, as in earlier published papers.[6–9] At the end of this

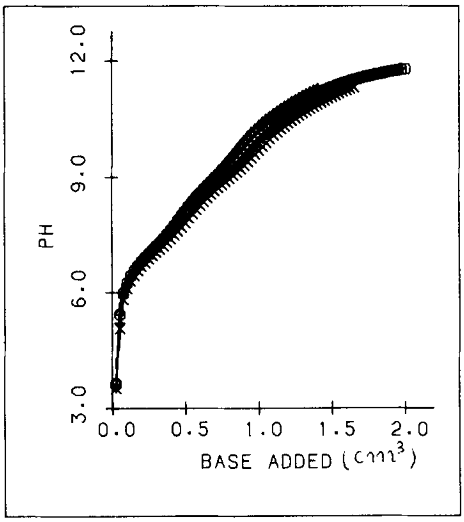

FIGURE 1 Experimental and calculated (by SUPERQUAD program) titration curves of pH as a function of volume of KOH added for the H^+-dahhe system, $V_0 = 25.0\ cm^3$, $C_{KOH} = 0.4338\ mol\ dm^{-3}$ [T_L range 0.1775–0.2103, T_H range 0.5445–0.6448]. T_L and T_H are mmol of dahhe and hydrogen ion in the titration vessel.

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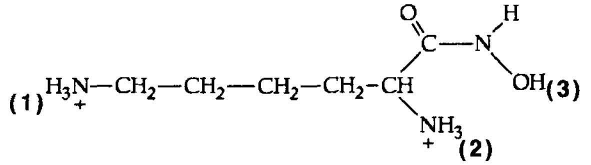
refinement the variances (σ^2) in the quantities T_H, T_L with respect to the initial values were 6.022×10^{-7} and 1.971×10^{-7}, respectively. The potentiometric titration curves [calculated (line) and observed (symbol)] of the completely protonated form of Ddahhe (H_3L^{2+}) is shown in Figure 1. The ligand Hdahhe (Scheme 1) has three macroscopic protonation centres, the corresponding equilibria involving the terminal hydroxamate group ($-NHO^{-}-$) [pH *ca* 5.20–8.00; (H^3, Scheme 1), log $K_3^H = 7.01$ (1)], the α-amino group [pH *ca* 8.10–9.70; (H^2, Scheme 1), log $K_2^H = 8.89$ (1)], and the extreme amino group [pH *ca* 9.70–11.79; (H^1, Scheme 1), log $K_1^H = 10.66$ (1)]. The ionic product of water was obtained from the MAGEC ($pK_w = 13.721$) program as previously described. All the stepwise protonation constants (log K_2^H, log K_3^H) of dahhe are a little different from the corresponding known ones of some analogous hydroxamic acids (ahhe = 2-amino-*N*-hydroxyhexanamide[13] and ahmpe = 2-amino-*N*-hydroxy-4-methylpentanamide[14]). It should be noted that the presence of the remote amino group [(1), Scheme 1] in dahhe increases the acidity of the OH (–NHOH–) and α-amino groups.



L-lysinehydroxamic acid dihydrochloride (Hdahhe), H_3L^{2+}, has three removable protons (1–3)

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Metal-complex Equilibria

Titration data obtained at different ligand-to-metal ratios were evaluated by assuming all feasible models. The equilibrium patterns were selected by successive attempts according to the best agreement between observed and calculated data and by means of an accurate statistical analysis of the agreement factor (σ^2), the goodness of fit (χ^2), the standard deviation (σ) of the formation constants, and the chemical significance of the species proposed. 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 different solutions were processed in order to investigate the ternary systems. The calculated complex formation constants, log β_{pqr}, for dahhe with different metals are given in Table 2.

Starting from the stability constants given in Table 2 and the protonation constants of dahhe 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. A typical distribution diagram is shown in Figure 2. As can be seen in Table 2, a number of protonated complexes are formed. A comparison with appropriate data for analogous aminohydroxamic acids shows that in most of the protonated complexes the remote amino group contains the dissociable proton. In the case of the Cu^{2+}-dahhe system, complexation begins at pH *ca* 3.3 with the formation of $[CuHL]^{2+}$ and the dimer $[Cu_2HL_2]^{3+}$ species, which reaches a maximum concentration of 89% at pH 4.3. Previous authors[15,16] also have concluded that the dimer is in equilibrium with the mononuclear complex $[CuHL]^{2+}$, which is present in small quantity (maximum of 4.1% at pH 3.75). The species $[CuH_2L_2]^{2+}$ reaches a maximum concentration of 93.3% total copper at pH 6.0. The Cu^{2+}-dahhe system showed two distinct buffer zones separated by a sharp equivalence point between them that would exactly

TABLE 2

Cumulative and stepwise protonation complex formation constants of 2,6-diamino-*N*-hydroxyhexanamide (dahhe) at 25°C and $I = 0.5$ mol dm^{-3} (KCl)

	SUPERQUAD			
	H^+	CO^{2+}	Ni^{2+}	Cu^{2+}
$\log \beta_{011}$	10.66 (1)			
$\log \beta_{021}$	19.55 (1)			
$\log \beta_{031}$	26.56 (1)			
$\log K_2^{Ha}$	8.89 (1)[b]			
$\log K_3^{H}$	7.01 (1)			
$\log \beta_{111}$		15.72 (2)	16.93 (8)	20.26 (1)
$\log \beta_{101}$		8.68 (1)		
$\log \beta_{122}$		29.72 (3)	34.87 (1)	40.56 (3)
$\log \beta_{112}$			27.70 (4)	33.33 (10)
$\log \beta_{212}$				41.53 (2)
$\log \beta_{121}$		22.68 (2)		
Z[c]	263	264	272	235
χ^2[d]	8.30	19.15	21.45	7.01
U	1.48×10^1	8.99×10^2	2.13×10^3	1.95×10^3
σ[e]	0.24	1.86	2.81	2.89

[a] $\log K_n = \log \beta_{0n1} - \log \beta_{0n-11}$.

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

[c] Total number of experimental data points used in the refinement.

[d] Observed χ^2; calculated value (6, 0.95) should be 12.6, where 6 is the number of degrees of freedom and 0.95 is the confidence coefficient in the χ^2 distribution.

[e] $\sigma = \sum_{i=1}^{Z} w_i(E_i^{obs} - E_i^{calc})^2/(Z - m)$, where m is the number of parameters to be refined.

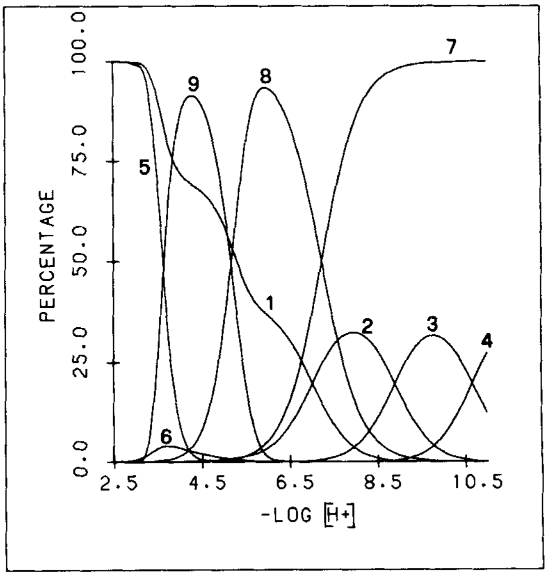

FIGURE 2 Typical distribution diagram for the Cu^{2+}-dahhe system. The percentage of each species has been calculated from the data for a hypothetical solution of copper ions (0.0033 mol dm^{-3}) and dahhe (0.011 mol dm^{-3}) by the HALTAFALL program (N. Ingri, W. Kakalowicz, L.G. Sillèn and B. Warnqvist, *Arkiv. Kemi*, **14**, 1261 (1967)). The percentages of the species not containing copper were calculated as percentages of the total ligand, those containing copper as percentages of the total metal; (1) H_3L^{2+}, (2) H_2L^+, (3) HL, (4) L^-, (5) Cu^{2+}, (6) $CuHL^{2+}$, (7) $CuHL_2^+$, (8) $CuH_2L_2^{2+}$, (9) $Cu_2HL_2^{3+}$.

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correspond to a 1.25 molar ratio (mol of base added per two mol of metal ion). The complex $[MHL]^{2+}$ reaches a maximum concentration of 13.0% total nickel at pH 5.7, 32.2% total cobalt at pH 7.1, corresponding to the displacement of two protons. The species $[MH_2L_2]^{2+}$ reaches a maximum concentration of 82.6% total nickel at pH 6.3, 12.1% total cobalt at pH 8.2. Above pH 6.2, one species was detected; the maximum fraction of the complex $[CuHL_2]^+$ is *ca* 95% at pH 8.5; $[NiHL_2]^+$ has a maximum fraction of *ca* 99.8% at pH 10.3. Typical absorption spectra for the Cu^{2+}-dahhe system are plotted in Figure 3, in the pH range 3.52–9.49. At low pH a broad absorption spectrum is present and its maximum shifts as the pH increases from 3.52 to 4.03 [maximum, 0.137 A at 728 nm (curve 1); 0.215 A at 666 nm (2); 0.318 A at 654 nm (3); 0.430 A at 648 nm (4); 0.435 A at 638 nm (5); Figure 3]. The first maximum in the range 635–650 nm occurs at pH *ca* 4.3 with a λ_{max} of 640 nm and corresponds to the maximum concentration (91.3%) of the dimeric complex [see Figure 2, curve 9]. The increase in absorption (hyperchromic effect) of the broad spectrum near the i.r. region and the shift towards shorter wavelengths (hypsochromic effect) of the same band (curves 1–5, Figure 3) with increasing pH indicates greater complexation, due to the appearance of the binuclear species, $[Cu_2HL_2]^{3+}$ (intense green colour, maximum absorption at 635–650 nm). When the pH is increased further from 5.10 to 9.49 (Figure 3) combined hyperchromic–hypochromic and large hypsochromic effects [maximum, 0.435 A at 638 nm (5); 0.359 A at 588 nm (6); 0.421 A at 542 nm (7); 0.415 A at 540 nm (8); Figure 3] are observed (change of colour from intense green to purple), while for pH > 5.2 a simultaneous increase in the absorption and a small decrease in the wavelength occurs with the formation of the complexes $[CuH_2L_2]^{2+}$ (maximum absorption at 540–545 nm) and $[CuHL_2]^+$. The colour changes from purple to reddish purple. One distinctive isosbestic point appears at 588.5 nm [Figure 3]. This corresponds to an equilibrium $[Cu_2HL_2]^{3+}/[CuH_2L_2]^{2+}$ (each species is present

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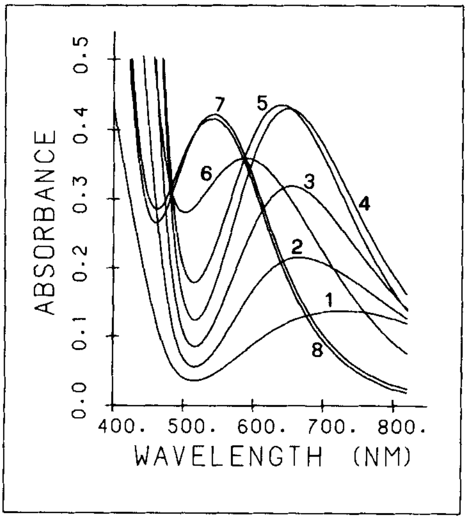

FIGURE 3 Plots of experimental absorbance data *versus* wavelength for solutions (C_L range 8.408×10^{-3}–8.862×10^{-3} mol dm^{-3}, C_M range 4.781×10^{-3}–5.039×10^{-3} mol dm^{-3}; (1) pH = 3.519, (2) pH = 3.642, (3) pH = 3.784, (4) pH = 4.033, (5) pH = 5.145, (6) pH = 5.722, (7) pH = 8.403, (8) pH = 9.495] for the Cu^{2+}-dahhe system at 25°C using the program VISION.

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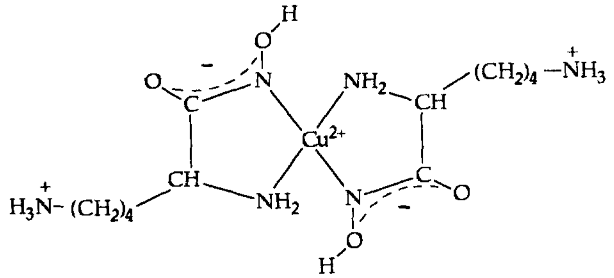
to the extent of 49.3% of the total copper at pH 5.2, [Figure 2, curves 8 and 9]. For a comparison of the spectrophotometric properties of these complexes with those formed by other analogous aminohydroxamic acids, ref. 14 should be considered. The molar absorption coefficients, ε_{pqr}, in the range 400–820 nm for the Cu^{2+}-dahhe system (all the formation constants already refined were kept constant) were determined by treatment of the specific sets of absorbance data (8 solutions, 211 wavelengths, 1688 experimental absorbance points) using the SQUAD[13] program. The standard deviation in the absorbance data of 8 solutions and 211 wavelengths was 2.797×10^{-2}, suggesting that the fit of the calculated spectra to the observed ones is very good. In general, the complexes of Co^{2+} and Ni^{2+} exhibit similar behaviour to those of Cu^{2+}, but the latter have higher stabilities, as expected. The presence of the protonated remote amino group, in addition to weakly reducing the basicity of the co-ordinated groups ($-NHOH$, $-NH_2$), contributes to a lower stability of the protonated complexes especially if compared to those of complexes obtained for analogous ligands (2-amino-*N*-hydroxyhexanamide) in which the protonated amino group is absent (see ref. 13). The small differences in stability for the various complexes of aminohydroxamic acids so far examined reflect the different structures of the ligands, in that they give rise to a significant variation of the basicity of the functional groups ($-NH_2$, $-CONHO^-$) as well as steric effects.

The bonding mode in $[MHL]^{2+}$ and $[MH_2L_2]^{2+}$, etc. complexes of the different aminohydroxamic acids is almost certainly not glycine-like, where the metal ion is bound through the carboxylate oxygen ($-CO_2^-$) or the oxygen of the hydroxamate group ($-CONHO^-$) and the α-amino group only. The much higher stability of the 1:1 and 1:2 complexes of dahhe and other analogous ligands with different metal ions compared with that of the corresponding complexes of the amino acids can be attributed to co-ordination via the nitrogen atoms of the α-amino and NHO^- groups.

In order to clarify the relationship between the functional groups of the amino acid hydroxamates and their inhibitory power, a number of derivatives conveniently modified at the hydroxamic acid function and/or at the N-terminal group were examined and the resulting effects on aminoenkephalinase activity were summarized previously.[17] The results suggest that, apart from the effect of the structure of the amino acid itself, the NH_2-terminal group must be preserved to maintain inhibitory power. Moreover, hydroxamates of amino acids and aliphatic acids are effective inhibitors of *Aeromonas proteolytica* aminopeptidase.[18] In particular, the specific inhibitory nature of *D*-amino acid hydroxamates indicates that the amino group orientation in the *D* isomers contributes to binding efficacy. The results show that, at least at physiological pH, the assumption of an uncoordinated α-amino group, which may be particularly active because of a possible surface-active role, is incorrect,[19–23] since the major species in this pH range is $[CuH_2L_2]^{2+}$, which involves co-ordination of the α-amino and hydroxamate NHO^- groups (see Scheme 2).



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