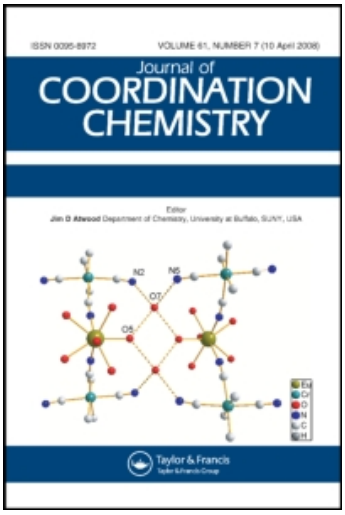

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

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To cite this Article Leporati, Enrico(1994) 'CHARACTERIZATION OF AMINO HYDROXAMIC METAL CHELATES AND THEIR ACTIVITY IN BIOLOGICAL SYSTEMS. FORMATION CONSTANTS BETWEEN 2-AMINO-N, 3-DIHYDROXYPROPANAMIDE AND 2-AMINO-N, 3-DIHYDROXYBUTANAMIDE AND IRON(III) IONS IN AQUEOUS SOLUTION', Journal of Coordination Chemistry, 33: 2, 179 — 189
To link to this Article: DOI: 10.1080/00958979408024276
URL: http://dx.doi.org/10.1080/00958979408024276

J. Coord. Chem., 1994, Vol 33, pp. 179–189
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CHARACTERIZATION OF AMINO HYDROXAMIC METAL CHELATES AND THEIR ACTIVITY IN BIOLOGICAL SYSTEMS. FORMATION CONSTANTS BETWEEN 2-AMINO-N, 3-DIHYDROXYPROPANAMIDE AND 2-AMINO-N, 3-DIHYDROXYBUTANAMIDE AND IRON(III) IONS IN AQUEOUS SOLUTION

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(Received March 21, 1994)

Complex formation equilibria and relative stability constants for species present in aqueous solutions of ion(III) with "*L*-serinchydroxamic acid" (2-amino-*N*,3-dihydroxypropanamide, adhp) and "*DL*-threoninehydroxamic acid" (2-amino-*N*,3-dihydroxybutanamide, adhb) have been investigated by potentiometric titrations at 25 ± 0.1 °C and I = 0.5 mol dm $^{-3}$ (KCl). Overall formation constants of several metal complexes were computed from potentiometric data with the hyperquad program. The following cumulative association constants (relative standard deviation values are given in parentheses) $\beta_{pqr} = [M_pH_qL_r] / [M]^p [H]^q [L]^r$ were obtained: adhp, log β_{011} = 12.72(1), log β_{021} = 21.62(1), log β_{031} = 28.38(1), log β_{122} = 32.88(6), log β_{111} = 17.07(6), log β_{112} = 28.80(6), log β_{102} = 22.15(8), log β_{123} = 41.17(7), log β_{133} = 46.26(13), log β_{113} = 34.83(7), log β_{103} = 28.06(7), log $\beta_{2\text{-}22}$ = 22.51(12); adhb, log β_{011} = 12.75(1), log β_{021} = 21.61(1), log β_{031} = 28.39(1), log β_{122} = 32.73(3), log β_{111} = 16.90(4), log β_{112} = 28.55(3), log β_{102} = 21.37(7), log β_{123} = 41.16(3), log β_{133} = 46.03(7), log β_{113} = 34.89(3), log β_{103} = 27.76(3), log $\beta_{2\text{-}22}$ = 22.34(7), log $\beta_{1\text{-}13}$ = 19.23(5). Since the two ligands show behaviour very similar to that of already investigated aminohydroxamic acids without the OH $^-$ group, it is suggested that the proton residing on the alcoholic group is not ionized when the complex formation takes place. Consequently, in the refinement process of the complex species, the H_2L^+ species with only two dissociable groups (– NHOH, – NH_3^+) has been considered, taking into account log β_{011} and log β_{021} for the two ligands, respectively. Equilibrium constants for their formation and the probable structure of the chelated compounds formed in aqueous solution are the object of discussion in terms of possible significance to biological reactions. Stability is compared with that of analogous chelated compounds.

KEYWORDS: aminohydroxamic acids, protonation, complex formation, stability constants, iron(III) complexes, potentiometry

INTRODUCTION

In recent years several studies have been carried out on hydroxamic acids and their metal complexes due to their chemical and biological importance. Hydroxamic

acid-containing compounds are ubiquitous with respect to iron-transport phenomena. Biological activities of aminohydroxamic acids can possibly be correlated with the formation of their metal complexes. Most pharmacological activity is closely related to the mechanism of iron transport in metabolism of microorganisms. Thus, hydroxamic acids can be considered as sequestering agents for a large class of metals and they have been demonstrated[1] to stabilize high oxidation state cations such as Fe^{3+}. The so-called 'siderophores' are iron chelates microbially produced; they can be divided into iron chelates based on the hydroxamic function and iron chelates based on the cathecol group, such as in *Enterobactin*. In order to mimic the properties of the best known natural siderophore, *Enterobactin*, artificial tripod-like hydroxamate binders were synthesized recently.[2] These compounds were employed to carry out *in vivo* tests as growth promoters of *Arthrobacter flavescens* which was selected as a test system since it possesses ferrichrome receptors though it does not produce ferrichrome. Thus, iron uptake can be realized by hydroxamic ligands. Adequate supply of iron is necessary for growth in all living systems and in the human body iron is an essential element in relatively large amounts (approximately 4 g in the adult), although it is very toxic if present in excess. Different ligands containing hydroxamate groups make reduction potentials for Fe(III)/Fe(II) highly negative because complexes with high oxidation states are more stabilized. However, when the hydroxamic acid contains another donor, such as an amino group, coordination could involve this group as well as the nitrogen or the oxygen of the hydroxamic function. Regarding the strong ability of this type of ligands to form chelates, a main question concerns which donors of the hydroxamic group (– CONHOH), *i.e.*, nitrogen or oxygen, are involved in coordination to iron. Aside from 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.[3,4]

Accordingly, the aim of the present work is to clarify equilibrium reactions and stabilities involving protons, Fe^{3+} and adhp or adhb, using a detailed potentiometric technique. It is clear that knowledge of stability constants and probable structures of the chelated compounds is important in determining which chelates will be useful drugs in terms of possible significance to biological reactions.

EXPERIMENTAL

Reagents

2-Amino-*N*,3-dihydroxypropanamide [(adhp), *L*-serinehydroxamic acid = H_3L^+] and 2-amino-*N*,3-dihydroxybutanamide [(adhb), *DL*-threoninehydroxamic acid = H_3L^+] were obtained from Sigma (St. Louis, MO) and their purity and the exact concentration of their solution was checked by potentiometric titrations. The iron(III) chloride (AnalaR Products) solution was prepared and standardized using standard analytical procedures.[5,6] All solutions were then set at a total volume of 25.0 cm^3 by adding successively to the titration vessel a known volume of ligand solution and an exact volume of iron(III) 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 runs and other reagent grade chemicals were used without further purification. The accurate molarities of potassium hydroxide (0.3360 mol dm^{-3}) and hydrochloric acid (0.4090 mol dm^{-3}) stock solutions were determined by conventional potentiometric titrations according to Gran's method, with the use of BEATRIX[7] and ESAB[8] programs.

Potentiometric Measurements

The data collection was performed by using the PASAT package which contains a set of programs for potentiometric automatic titrations. Potentiometric titrations were carried out with a fully automatic apparatus equipped with an ORion 720A digital voltmeter and 5 cm^3 Metrohm E 665 burette, both controlled by an Uvikon 941 PLUS equipped with a personal computer. The electrodic chain consisted of an OR 9101SC glass electrode (Orion Research) and an OR 9002 reference electrode (Orion Research). Only for the Fe^{3+}-adhb system was the reference electrode changed for an Ingold 363-S7 (type Argenthal). A thermostatted stream of nitrogen, pre-saturated with water vapour by bubbling through a 0.5 mol dm^{-3} KCl solution, was passed over the surface of the solution in the titration vessel. Small amounts (Δv = 0.05 or 0.025 cm^3) of titrant were added with a Metrohm Dosimat E 655 autoburet (total volume = 5.0 cm^3). The system was calibrated in terms of hydrogen ion concentrations before and after series of measurements by titrations of a 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,[9] by using the computer programs BEATRIX.[7] The solution in the titration compartment was agitated using a mechanical stirrer. The range of concentration of ligand in the potentiometric titrations was 0.008885 – 0.01777 mol dm^{-3} for the adhp-Fe^{3+} system (pH range 1.79–7.53; number of titrations 10); 0.008829 – 0.017069 mol dm^{-3} for the adhb-Fe^{3+} system (pH range 1.84–8.49; number of titrations 10). The metal concentration varied between 0.002166 and 0.009038 mol dm^{-3} for adhp-Fe^{3+}; 0.001716 – 0.007886 mol dm^{-3} for adhb-Fe^{3+}, and potassium chloride (I = 0.5 mol dm^{-3}) was used as supporting electrolyte.

Calculations

Careful attention has been paid to the calculation and critical evaluation of parameters ($E°$, V_e, K_w and N) relating to potentiometric calibration curves, by using different mathematical methods.[7,8] In the present calculations some experimental points (v, E) around the equivalent point have been neglected. In particular, by observing the results it is possible to verify the good agreement between the parameters (v_e and N) obtained from the two computer programs BEATRIX and ESAB (maximum variation 0.4%). At the same time differences in these parameters were non-significant in terms of mean values. The equivalence volumes, v_e, very often form a normal population, the members of which are values corresponding to individual points of any titration. Additionally, accurate examination of the results also reveals a good agreement in the standard potential, $E°$, even as 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) are not particularly constant, as can be seen from calculations performed with the

program BEATRIX. A more important factor that critically affects the refinement is the value used for the dissociation constant of water, K_w. This parameter is very sensitive to correlations with the concentration of alkali in the burette. In those situations when K_w is uncertain, the BEATRIX program permits the user to systematically vary the estimate of K_w. The error in the operational ionic product of water, $\sigma(pK_w)$ or $\sigma(E^\circ)$, may be employed to test the reliability of the electrochemical part of the system. Thus, the precision of the standard electrode potential, E°, and especially of the ionic product of water can be deduced only from inter-titration variability. All the points (acid and alkaline zones) in a single calibration were considered by using the ESAB program. This program minimizes the error squares sum in the added titrant volume, using the Gauss-Newton method,[10] and thus the parameters E°, K_w and N are refined. The system of normal equations is solved, after an appropriate scaling procedure,[11] by using the compact GAUSS method.[12] Indeed, statistical analysis shows how the parameters (E°) 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 (E° in acidic and alkaline solution), thus showing that unexpected factors differing from one titration to another can alter *e.m.f.* values and are the main source of error.

The stability constants (β_{pqr}), which are defined by equation (*1*) (charges are omitted for simplicity),

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

were refined by least-squares methods employing the HYPERQUAD computer program. This program calculates the overall formation constants which minimize the sum of squared residuals between observed and calculated e.m.f. values, equation (*2*),

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

where Z is the total number of potentiometric data and w_i is the weighting factor defined by equation (*3*),

$$w_i = 1/[\sigma^2_E + (\delta E_i/\delta V_i)^2\ \sigma^2_v] \qquad (3)$$

where σ_E(= 0.2) is the error in e.m.f. and σ_v (= 0.008) the error in the volume used in the refinement. Moreover, STBLTY[13,14] was employed to refine the formation constants (K or β). In this program a recent pH titration method (FICS) is utilized to calculate free metal ion and free ligand concentrations from experimental data without any hypothesis about the nature of species present. The fundamental relationships of the FICS method are reported in several papers.[15–17] Calculation of stability constants is then reduced to a linear least-squares problem using any of the mass balance equations. The constants obtained by the NONLINEAR LS REFINE procedure (the most commonly used refinement program) agree with the corresponding values achieved by the HYPERQUAD program. All calculations were carried out on an ActionNote 486 (Epson) computer. Listings of experimental data for the computations from HYPERQUAD, BEATRIX, and ESAB programs are available as Supplementary Material and can be obtained on request to the author.

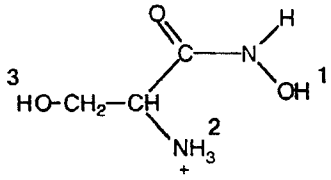
RESULTS AND DISCUSSION

Protonation Equilibria

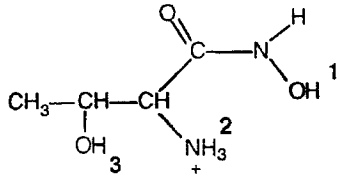
A maximum number of three protons can be released from the ligands in the fully protonated form (Hadhp or Hadhb = H_3L^+) on titration with strong base in the pH range 3.50 – 11.79. All sets of titration data indicate the sole presence of simple HL^-, H_2L, and H_3L^+ complexes. The overall protonation constants (log β_{011}, log β_{021}, and log β_{031}) of the ligands have been determined by SUPERQUAD[18] without introducing liquid-junction potentials into the calculations, as shown in earlier published papers.[19,20]

The ligands Hadhp and Hadhb (Scheme 1) have three macroscopic protonation centres, the corresponding equilibria involving the terminal hydroxamate group (– NHO^-) [pH *ca* 5.6 – 7.8; (H^3, Scheme 1), log K_3^H = 6.77(1), and log K_3^H = 6.77(1), respectively], the α-amino group [pH *ca* 7.8–10.3; (H^2, Scheme 1), log K_2^H = 8.90(1), and log K_2^H = 8.87(1)], and the remote hydroxy group [pH *ca* 12.3–13.0; (H^1, Scheme 1) log K_1^H = 12.72(1), and log K_1^H = 12.75(1)]. All stepwise protonation constants (log K_2^H, log K_3^H) of adhp and adhb are a little different from the corresponding known value for some analogous hydroxamic acids (ahhe = 2-amino-*N*-hydroxyhexanamide[21] and ahmpe = 2-amino-*N*-hydroxy-4-methylpentanamide[22]). It should be noticed that the presence of the remote hydroxy moiety [(1), Scheme 1] in adhp or adhb increases the acidity of the OH (– NHOH) and α-amino groups.

SCHEME 1 = $[H_3L]^+$ [a]



2-amino-*N*,3-dihydroxypropanamide(adhp)



2-amino-*N*,3-dihydroxybutanamide(adhb)

[a] The three mobile protons (1-3) are indicated.

Scheme 1

Iron(III) 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. Since in complex formation the adhp and adhb ligands show behaviour very similar to that of previously investigated amino hydroxamic acids[23,24] without the OH^- group, it is reasonable to suppose that the proton residing on the alcoholic group is non ionized when complex formation takes place. Consequently, in the refinement process of the species complexed, the H_2L^+ species for two ligands with only two dissociable groups (– NHOH, $-NH_3^+$) has been considered, introducing into the calculations $\log \beta_{011}$ and $\log \beta_{021}$ as constants (thus considering as free ligand the original species HL with the remote hydroxy group in the protonated form). At this point the computer program HYPERQUAD was employed to refine several sets of potentiometric titrations in which *e.m.f.* data for different solutions were processed in order to investigate the ternary systems. The calculated complex formation constants, $\log \beta_{pqr}$, for iron(III) with different ligands are given in Table 1.

Typical titration curves (observed and calculated) for Fe(III)-adhp mixtures are shown in Figures 1a and 1b. From numerous models, the ones which yielded the best fit to the titration data (see Figures 1a, and 1b) are given in Table 1. Starting from the stability constants given in Table 1 and the protonation constants of adhp or adhb under the same experimental conditions, the percentage of each complex

Table 1 Cumulative and stepwise protonation complex-formation constants of 2-amino-*N*,3-dihydroxypropanamide (adhp) and 2-amino-*N*-3-dihydroxybutanamide (adhb) with trivalent iron metal ions at 25°C and I = 0.5 mol dm^{-3} (KCl). Standard deviations are given in parentheses.

HYPERQUAD			SUPERQUAD		
	adhp	adhb	ahp[f]	ahmpe[g]	aimahp[h]
$\log \beta_{011}$	12.717(7)	12.746(10)	9.125(1)	9.152(3)	13.23(8)
$\log \beta_{021}$	21.620(8)	21.614(1)	16.465(2)	16.416(4)	22.060(6)
$\log \beta_{031}$	28.385(7)	28.387(3)			28.936(2)
$\log K_2^{H}$ [a]	8.903(8)[b]	8.868(1)	7.340(2)	7.263(4)	8.832(1)
$\log K_3^{H}$ [a]	6.765(9)[b]	6.773(2)			6.876(2)
$\log \beta_{111}$	17.07(6)	16.90(4)	16.91(2)	16.65(2)	16.057(8)
$\log \beta_{122}$	32.88(6)	32.73(3)	32.84(1)	32.60(1)	31.040(6)
$\log \beta_{112}$	28.80(6)	28.55(3)	28.50(4)	28.50(3)	26.69(2)
$\log \beta_{102}$	22.15(8)	21.37(7)	22.90(4)	22.89(9)	21.10(8)
$\log \beta_{123}$	41.17(7)	41.16(3)	41.54(7)	41.93(5)	39.72(2)
$\log \beta_{133}$	46.26(13)	46.03(7)			
$\log \beta_{113}$	34.83(7)	34.89(3)	35.03(5)	35.55(5)	33.78(1)
$\log \beta_{103}$	28.06(7)	27.76(3)	27.58(4)	28.00(9)	26.90(1)
$\log \beta_{2\text{-}22}$	22.51(12)	22.34(7)	21.87(6)	21.48(7)	20.27(2)
$\log \beta_{1\text{-}13}$		19.23(5)	18.48(9)		18.35(2)
$\log \beta_{2\text{-}13}$			35.17(12)	35.40(9)	33.34(4)
$\log \beta_{1\text{-}12}$			15.65(4)	15.98(3)	
Z[c]	561	677			
U	2.75×10^3	1.15×10^3			
χ^{2}[d]	24.37	73.48			
σ[e]	2.22	1.31			

[a] $\log K_n = \log \beta_{0n1} - \log \beta_{0n\text{-}1\,1}$. [b] $\sigma(\log K_n) = [(\sigma^2(\log \beta_{0n1}) + \sigma^2(\log \beta_{0n\text{-}1\,1}))/2]^{1/2}$. [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] $\sigma = (\Sigma_{i=1}^{Z} w_i(E_i^{calc} - E_i^{obs})^2/(Z - m))^{0.5}$ where m is the number of parameters to be refined. [f] Ref. 23; [g] Ref. 22; [h] Ref. 24.

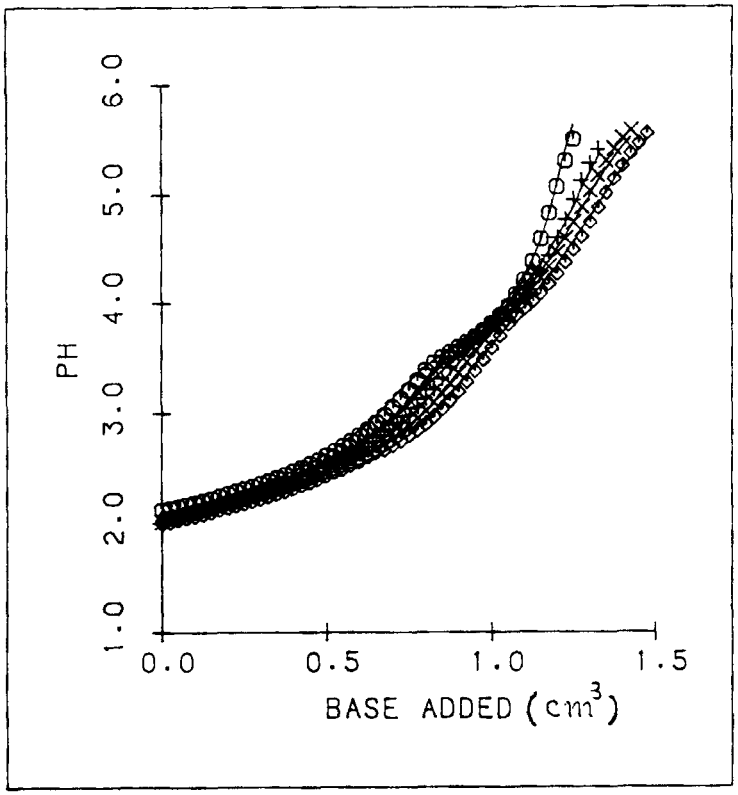


Figure 1a Experimental and calculated (by Hyperquad program) titration curves of pH as a function of volume of KOH added for the Fe^{3+}-adhp system. V_0 = 25.0 cm^3, C_{KOH} = 0.334 mol dm^{-3} [T_M range 0.05416–0.22595, T_H range of 0.68735–0.82904]. T_M and T_H are mmol of Fe^{3+} and hydrogen ion in the titration vessel. T_L = 0.33614 mmol was kept constant in all five titrations.

involving H^+ or OH^-, iron(III) and ligand has been calculated by using the HALTAFALL program with a Calcomp 936 Plotter. Typical distribution diagrams are shown in Figures 2 and 3. As can be seen in Table 1, a number of protonated complexes are formed. A comparison with appropriate data for analogous amino-hydroxamic acids shows that in most of the protonated complexes, the remote hydroxy group contains the dissociable proton. Our models are only in partial agreement with those given by Brown *et al.*[25] and El-Ezaby and Hassan[26,27] for analogous iron(III)-amino hydroxamate systems. In particular, besides other species of minor importance, the totally deprotonated species $[FeL]^{2+}$ for the two systems was not found in an acid pH range. In the presence of Fe^{3+}, the species distribution curves show that complexation begins at low pH values <1.5 for $[Fe(HL)]^{3+}$ (max. 54.5% total iron for the adhp-Fe^{3+} system and 48.0% for the adhb-Feb^{3+} system at pH 2.0, but Fe^{3+} is only present at *ca* 4% in the two systems), corresponding to the displacement of one proton. As pH increases, the data shows that complexation proceeds further and at pH 2.9 the predominant species is $[FeH_2L_2]^{3+}$ (77.4 % and

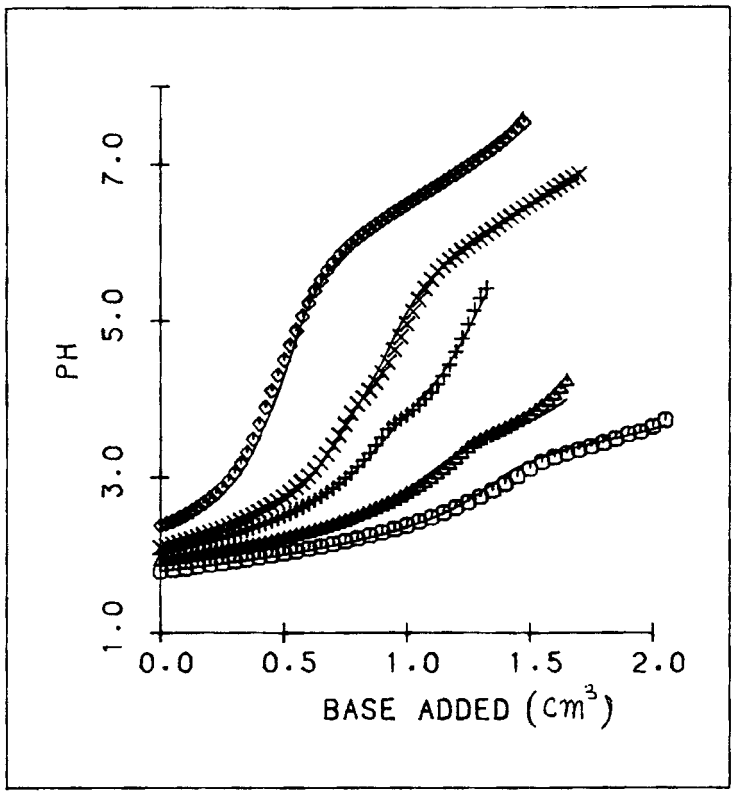


Figure 1b Experimental and calculated (by Hyperquad program) titration curves of pH as a function of volume of KOH added for the Fe^{3+}-adhp system, $V_0 = 25.0\ cm^3$, $C_{KOH} = 0.339\ mol\ dm^{-3}$ [T_L range 0.22212–0.44424, T_H range 0.49359–0.91717]. T_L and T_H are mmol of Fe^{3+} and hydrogen ion in the titration vessel. $T_M = 0.13042$ mmol was kept constant in all five titrations.

82.0 %) corresponding to the displacement of two proton. As the pH increases further, the major species present are $[FeHL_2]^{2+}$ (max. 64.5 at pH 4.9 and 56.0% at pH 4.7), $[FeH_3L_3]^{3+}$ (max. 11.8 at pH 4.9 and 14.0% at pH 4.8) and $[FeHL_3]^{+}$ (max. 33.0 at pH 6.8 and 53.0% at pH 6.9). At pH 5.9–6.0, the proton displacement value rises to about 4.0 and can be attributed to the formation of $[FeH_2L_3]^{2+}$ (30.0 % and 43.0%) when the hydroxamate group is only ionized by about 2.8 %. At pH > 7.8, three protons per mol of Fe^{3+} are liberated and the predominant species is $[FeL_3]$ which reaches a peak of 97.4 % at pH 9.0 and 72.0% at pH 7.9, respectively. Since iron(III) is a typical hard ion, we assume coordination through the O,O atoms of the hydroxamate moiety of the two ligands. Accordingly, the protonated $[FeH_3L_3]^{3+}$, $[FeHL_2]^{2+}$, $[FeH_2L_2]^{3+}$, and $[FeHL_3]^{+}$ complexes contain the amino group (besides the hydroxy group) in their protonated forms. This assumption is supported by spectrophotometric results recorded for analogous iron(III)-hydroxamate systems.[22,26,28-30] The presence of the protonated remote hydroxy group in addition to weakly basic groups (– NHOH, – CONHOH)

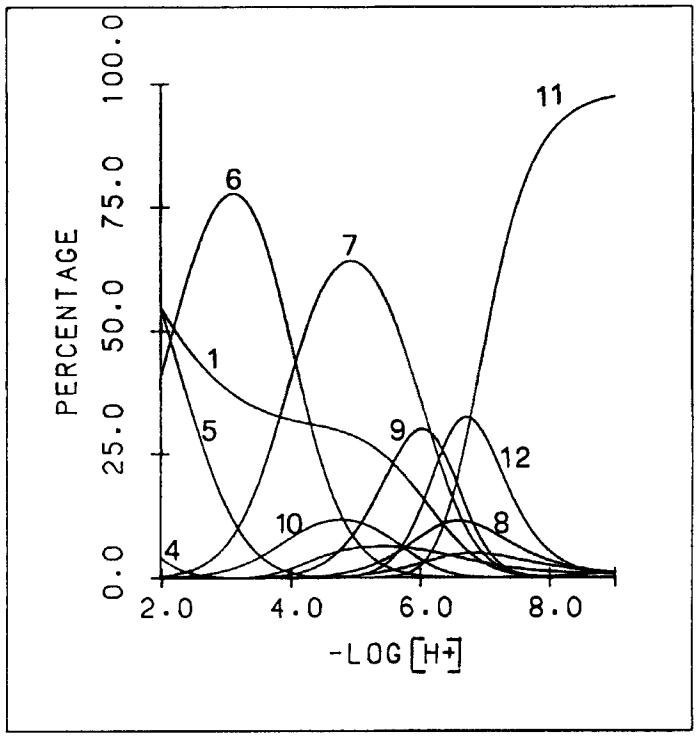


Figure 2 Typical distribution diagram for the Fe^{3+}-adhp system. The percentage of each species has been calculated from the data of a hypothetical solution of iron ions (0.0033 mol dm^{-3}) and adhp (0.010 mol dm^{-3}) by HALTAFALL program (N. Ingri, W. Kakalowicz, L.G. Sillèn and B. Warnqvist, *Talanta*, **14**, 1261 (1967). The percentages of the species not containing iron were calculated as percentages of the total ligand; those containing iron are as percentages of the total metal; (1) H_2L^+, (2) HL, (3) L^-, (4) Fe^{3+}, (5) $FeHL^{3+}$, (6) $FeH_2L_2^{3+}$, (7) $FeHL_2^{2+}$, (8) FeL_2^+, (9) $FeH_2L_3^{2+}$, (10) $FeH_3L_3^{3+}$, (11) FeL_3, (12) $FeHL_3^+$, (13) $Fe_2(OH)_2L_2^{2+}$.

contributes to a lower stability of the protonated complexes especially if compared to complexes obtained for 2-amino-*N*-hydroxyhexanamide in which the protonated hydroxy group is absent (see ref. 23). The small differences in stability for the various complexes of aminohydroxamic acids so far examined reflect their different structure, in which the introduction of various groups (hydroxy, aminoiminomethyl, etc.) into the molecule result in a significant variation of the basicity of the functional groups ($-NH_2$, $-CONHO^-$). Steric reasons are not always reflected by similar differences in stability of the coordination sphere.

In order to clarify the relationship between the functional groups of the amino acid hydroxamate 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 resulting effects on *amino-enkephalinase* activity are summarized in a previous report.[31] 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 mantain inhibitory power. Moreover, hydroxamates of amino acids and aliphatic acids are

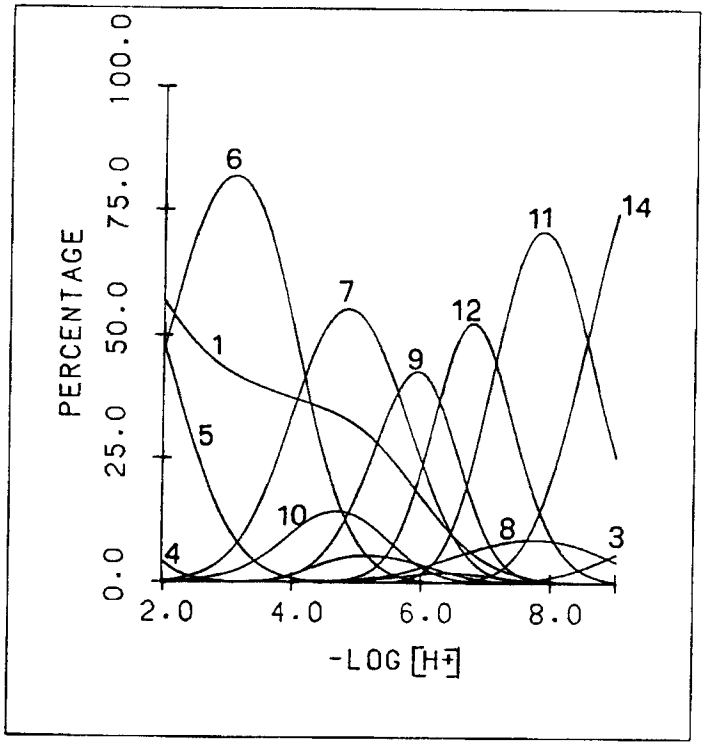


Figure 3 Typical distribution diagram for Fe^{3+}-adhb system. The percentage of each species has been calculated from the data of a hypothetical solution of iron ions (0.0033 mol dm^{-3}) and adhb (0.011 mol dm^{-3}) as with Figure 2. The percentages of the species not containing iron were calculated as percentages of the total ligand; those containing iron are as percentages of the total metal; (1) H_2L^+, (2) HL, (3) L^-, (4) Fe^{3+}, (5) $FeHL^{3+}$, (6) $FeH_2L_2^{3+}$, (7) $FeHL_2^{2+}$, (8) FeL_2^+, (9) $FeH_2L_3^{2+}$, (10) $FeH_3L_3^{3+}$, (11) FeL_3, (12) $FeHL_3^+$, (13) $Fe_2(OH)_2L_2^{2+}$, (14) $Fe(OH)L_3^-$.

effective inhibitors of *Aeromonas proteolytica* aminopeptidase.[32] 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 suface-active role, is correct.[33]

Acknowledgements

Grateful acknowledgement is made to the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T.) and National Research Council of Italy (C.N.R.) for generous grants. I am greatly indebted to Professor A. Vacca for his generous support of the program HYPERQUAD, and Professor Micheloni and Mr. Fontanelli for the software for automatic titration, PASAT.

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