Acid-Base and Complex-Formation Properties of *N*-Hydroxy-3-pyridinecarboxamide and *N*,2-Dihydroxybenzamide in Aqueous Solution

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The protonation and complexation equilibria of the iron(III) and copper(II) complexes of *N*-hydroxy-3-pyridinecarboxamide (hpca) and *N*,2-dihydroxybenzamide (dhbc) have been investigated by potentiometric and spectrophotometric techniques in 0.5 mol dm^{-3} (KCl) at 25±0.1 °C. It was shown that the properties of iron(III) and copper(II) chelates under study are similar to those of corresponding chelates of simple aromatic hydroxamic acids (without any additional donor groupings in the molecule). The hydroxamate and carbonyl oxygens may also take part in coordination in the copper(II) and iron(III) systems.

Because of their pronounced chelating properties the organic derivatives of hydroxylamine are used in analytical chemistry for a long time. Beside of oximes and *N*-alkyl or *N*-aryl derivatives of hydroxylamine there are hydroxamic acids that are known to be useful in analytical praxis.[1] The occurrence of hydroxamic acid derivatives in living organisms (where they play an important role in biochemical processes as the iron binders and transfers, growth regulators, pigments and precursors of other compounds)[2,3] and especially the discovery of antibiotic properties of aspergillic acid have initiated extensive investigation of hydroxamic acids as potential antimicrobial substances. New hydroxy- and amino-substituted benzohydroxamic acids[4] were prepared and tested along with other previously reported benzohydroxamic acids (see Table 2 of Ref. 4) for enzyme inhibition and antitumor activity. Among numerous hydroxamic acids synthesized and studied up to now, the group of hydroxamic acids derived from pyridine occupies an important place. It has been shown that various ring-substituted 4-pyridinecarbohydroxamic acids exhibit an antiviral effect.[5] Derivatives of 3-pyridinecarbohydroxamic acid (acting as the inhibitor of urease) was even patented as the domestic animal and poultry feed additive which should improve the nitrogen uptake during the meat production.[6] As a loose continuation of our previous work dealing with the properties of amino-substituted aliphatic hydroxamic acids[7—10] (which are related to 3-pyridinecarbohydroxamic acid with respect to the presence of the nitrogen atom in the vicinity of the *N*-hydroxycarbamoyl group) we decided to fill the gap in the knowledge of fundamental physicochemical properties of hpca and their iron(III) chelates by the paper presented. Moreover, we extended the study to analogous aromatic hydroxamic acid (dhbc) that has not yet been described in the literature.

Experimental

Potentiometric Measurements. Potentiometric titrations were carried out using a Metrohm E 636 Titroprocessor and a combined electrode 8102SC Ross (ORION Research). The range of concentration of ligand in the potentiometric titrations was 0.0107—0.0141 mol dm^{-3} for hpca-H^+ (pH range 2.55—11.91; number of titrations 4); 0.00673—0.01009 mol dm^{-3} for dhbc-H^+ (pH range 2.51—11.50; number of titrations 6); 0.0142—0.0283 mol dm^{-3} for hpca-Fe^{3+} (pH range 1.86—6.09; number of titrations 5); 0.0105—0.0283 mol dm^{-3} for dhbc-Cu^{2+} (pH range 2.37—6.09; number of titrations 4). The metal concentration varied between 0.002124—0.007258 mol dm^{-3} for hpca-Fe^{3+} and 0.003413—0.005784 mol dm^{-3} for dhbc-Cu^{2+} systems, and potassium chloride (I=0.5 mol dm^{-3}) was used as supporting electrolyte.

Spectrophotometric Measurements. Absorption spectra in the ranges 300—700±0.3 nm for Fe^{3+}-hpca were measured with a KONTRON UVIKON 860 spectrophotometer to the fourth decimal place with a stepping of 2 nm. Solutions containing the ligand and the iron prepared and maintained under purified nitrogen at the ionic strength 0.5 mol dm^{-3} (KCl), were measured at pH from 1.83 to 8.01 at 25.0±0.1 °C using 10 mm cells.

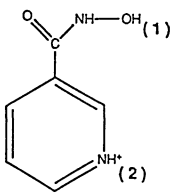
Results and Discussion

Protonation Equilibria. Starting from several sets of potentiometric data, at first the macroscopic protonation constants of the ligands (hpca and dhbc) and the initial amounts of reagents (T_L, T_H/mmol) were refined at the same time by using the SUPERQUAD[11] computer program without introducing the liquid-junction potentials (A_j and B_j) into the calculations, as shown in previous publications.[7—10] After the refinement procedure, the variances (σ^2) as regards the initial amounts of the reagents (T_L, T_H) were 4.061×10^{-7} and 5.919×10^{-7} mmol^2 for hpca and 2.753×10^{-8} and 1.074×10^{-7} mmol^2 for dhbc, respectively. The calculated cumulative and stepwise protonation and complex-formation constants (β_{pqr}) of hpca and dhbc are reported in Table 1. The potentiometric titration curves of the protonated ligand (hpca), H_2L^+ (Scheme 1), show two distinct deprotonation centers: the pyridine nitrogen (>NH$^+$) [pH 2.5—4.5; (H^2, Scheme 1), log K_2=3.189(5)], and the hydroxyl group of the -NHOH moiety [pH 7.0—9.0; (H^1, Scheme 1), log K_1=7.986(3)]; while the dhbc ligand (dhbc=H_2L,

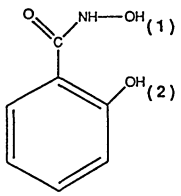
Table 1. Cumulative and Stepwise Protonation Complex Formation Constants of *N*-Hydroxy-3-pyridinecarboxamide (hpca) and *N*,2-Dihydroxybenzamide (dhbc) at 25 °C and I=0.5 mol dm^{-3} (KCl)

	SUPERQUAD			
	hpca		dhbc	
	H^+	Fe^{3+}	H^+	Cu^{2+}
$\log\beta_{011}$	7.986 (3)		9.722 (3)	
$\log\beta_{021}$	11.175 (6)		17.091 (5)	
$\log K_2^{H}$ a)	3.189 (5)[b]		7.369 (4)	
$\log\beta_{101}$				13.31 (3)
$\log\beta_{122}$		22.51 (10)		
$\log\beta_{112}$		20.03 (6)		
$\log\beta_{102}$		17.20 (9)		25.78 (9)
$\log\beta_{103}$		23.47 (8)		
Z[c]	185	267	317	245
χ^2[d]	43.02	17.85	6.66	21.18
U	4.85×10^1	1.94×10^3	7.91×10^1	5.17×10^2
σ[e]	0.53	2.72	0.51	3.07

a) $\log K_n=\log\beta_{0\ n\ 1}-\log\beta_{0\ n-1\ 1}$. b) $\sigma(\log K_n)=[(\sigma^2(\log\beta_{0\ n\ 1})+\sigma^2(\log\beta_{0\ n-1\ 1}))/2]^{1/2}$. 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) $\sigma=\sum_{i=1}^{z}w_i(E_i^{obsd}-E_i^{calcd})^2/(Z-m)$, where m is the number of parameters to be refined.



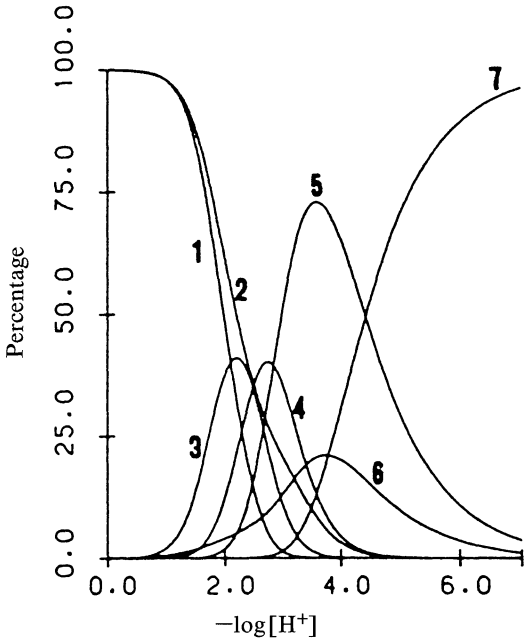
Scheme 1. (H_2L^+)=Hhpca



Scheme 2. (H_2L)=dhbc

Scheme 2) can only liberate two protons, one from the -OH group of the hydroxamic moiety [pH 6.1—8.2; (H^1, Scheme 2), $\log K_2$=7.369(4)] and one from the phenolic hydroxyl group [pH 8.4—10.6; (H^2, Scheme 2), $\log K_1$=9.722(3)]. The introduction of the CONHOH group into the pyridine ring (Scheme 1) resulted in a significant decrease of the basicity of the pyridine nitrogen from pK_a=5.3[12] to $\log K_2$=3.189(5) for hpca. This fact can be explained by a negative induction effect of the -CONHOH group on the nitrogen heteroatom. On the other hand, the substitution of an -NHOH group for the carboxyl OH^- group in the hydroxybenzohydroxamic acid examined (dhbc, Scheme 2) significantly lowers the protonation constant of the -OH group [$\log K_1$=9.722(3)] as compared to the analogous protonation constant of the corresponding -OH group in salicylic acid [$\log K_1$=13.4[13]] according to the electron-withdrawing character of the -NHOH moiety. The value for the protonation equilibrium of the hydroxamate moiety ($-NHO^-$) for the hpca is the same as obtained previously by conventional spectrophotometric method.[14] Rowland et al.[14] reported value of $\log\beta_{011}$=8.09 at I=0.1 mol dm^{-3} ($NaClO_4$) at 25 °C.

Metal-Complex Equilibria. Initial estimates of formation constants and the stoichiometries of possible complexes were obtained, in the case of the binary systems, from the features of formation curves. Following our usual approach, SUPERQUAD was employed to refine the formation constants and, on the basis of the usual numerical criteria, to select the initial sets of complexes. The final choices from the equilibrium models attempted are demonstrated by the excellent matching of the experimental and calculated potentiometric data and are reported in Table 1. The refinement included fixed contributions from the known metal hydrolysis constants of the species $[Fe(OH)]^{2+}$, $[Fe(OH)_2]^+$, $[Fe_2(OH)_2]^{4+}$, and $[Fe_3(OH)_4]^{5+}$. The relative importance of the various species in each pH range is shown by the typical distribution diagram, reported in Fig. 1. The species distribution curves show that complexation begins at low pH values: ca. 1.5 for $[FeH_2L_2]^{3+}$ (13.6%). The titration curves obtained on varying the iron and ligand concentrations were also processed by the FICS method (subprogram of STBLTY[15]) to give $(\partial C_H/\partial C_M)_{CL,pH}$ or $(\partial C_H/\partial C_L)_{CM,pH}$



Distribution diagram for system

Fig. 1. Typical distribution diagram for Fe^{3+}-hpca system. The percentage of each species has been calculated from the data of a hypothetical solution of iron ions (0.004 mol dm^{-3}) and hpca (0.012 mol dm^{-3}) by the Haltafall program (N. Ingri, W. Kakalowicz, L. G. Sillén, and B. Warnqvist, **14**, 1261 (1967)), and using a PLOTTER Calcomp 936. The percentages of the species not containing iron were calculated as percentages of the total ligand, those containing iron as percentages of the total metal. (1) Fe^{3+}, (2) H_2L^+, (3) $FeH_2L_2^{3+}$, (4) $FeHL_2^{2+}$, (5) FeL_2^+, (6) HL, (7) FeL_3.

respectively, as a function of pH. The displacement of two protons at pH 2.2 presumably gives an $[FeH_2L_2]^{3+}$ (41.0%) species and at pH 2.7 the predominant species is $[FeHL_2]^{2+}$ (40.1%) corresponding to the displacement of two protons with neutralization of one proton residing on the pyridinic nitrogen. At pH 3.6, the proton displacement value corresponding to the iron coordination rises to about 2.0 and can be attributed to the formation of the $[FeL_2]^{+}$ (72.8%) species when the hydroxamate moiety is about 79.1% ionized. On raising the pH further, $[FeL_3]$ (max. 96.35 at pH 7.0) species is formed. At pH=7.0, approximately three protons per mol of Fe^{3+} are liberated and the hydroxamic acid group is about completely ionized.

The spectrophotometric study was carried out on solutions containing hpca and iron ion, with concentrations and pH values selected from those employed in the potentiometric titrations. Some typical absorption spectra obtained for Fe^{III}–hpca system at different pH values are reported in Fig. 2. When the pH is increased from 1.830 to 5.346 (Fig. 2), small hypsochromic and high hyperchromic shifts (from 478 to 436 nm) are observed (intense reddish brown) [maximum, 0.683 A at 478 nm(1); 0.722 A at 476 nm(2); 0.803 A at 472 nm(3); 0.884 A at 464 nm(4); 0.963 A at 454 nm(5); 1.069 A at 442 nm(6); 1.141 A at 436 nm(7); Fig. 2], revealing changes previously described qualitatively by Neilands.[16] A maximum at 436 nm (curve 7, Fig. 2) is reached at pH 5.3, which corresponds to the maximum in the concentration (75.4%) of the species, $[FeL_3]$ (Fig. 1, curve 7). As the pH is raised from 1.83 to 8.01 the λ_{max} is observed to shift from 478 to 422 nm (Fig. 2). This charge-transfer band is typical of iron(III) hydroxamates but the pH dependence of the λ_{max} is not like that of the other naturally occurring siderophores as ferrichromes and ferrioxamines. A sharp color change is observed from red-brown to orange at about pH 5.5. No further changes are seen until approximately pH 8.0 when the color lightens to yellow-orange. Above pH 10.5 the iron is observed to precipitate as the hydroxide. Elaboration of the visible spectral data (300—700 nm) by a refinement procedure with the SQUAD[17] program indicates that at least four colored species are present between pH 1.83—8.01 ($[FeH_2L_2]^{3+}$, $[FeHL_2]^{2+}$, $[FeL_2]^{+}$, and $[FeL_3]$). Our best model is in partial agreement only with those given by El-Ezaby and Hassan,[18] Brown et al.,[19] and E. Farkas et al.,[20] for the iron(III)–aha (aha=2-amino-*N*-hydroxyacetamide) and iron(III)–ahpr (ahpr=2-amino-*N*-hydroxypropanamide) systems, respectively. Since iron(III) is a typical hard ion, we assume coordination through the *O,O* atoms of the hydroxamate moiety of hpca. Accordingly, the protonated $[FeH_2L_2]^{3+}$ (Scheme 3), and $[FeHL_2]^{2+}$

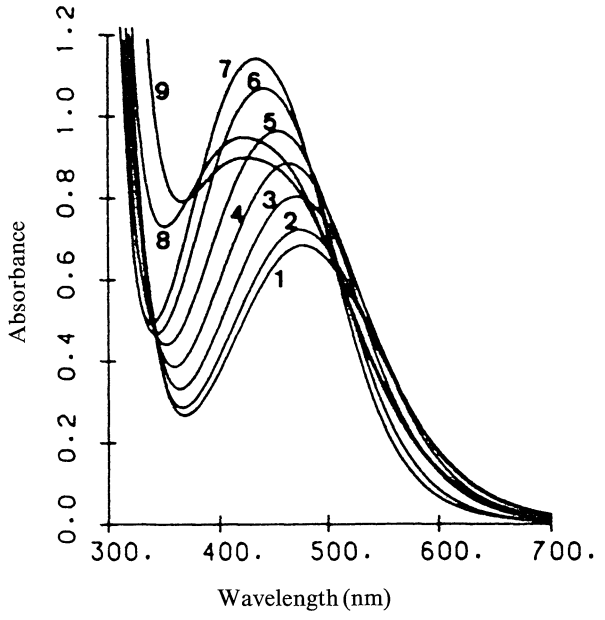
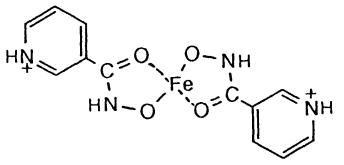
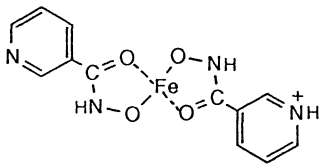


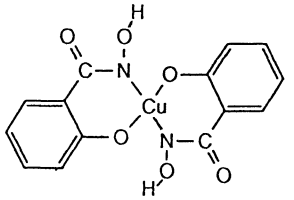
Fig. 2. Plots of experimental absorbance data versus wavelength for solutions [C_L range 2.536×10^{-2}—2.830×10^{-2} mol dm^{-3}, C_M range 3.058×10^{-3}—3.413×10^{-3} mol dm^{-3}; (1) pH=1.830, (2) pH=1.970, (3) pH=2.279, (4) pH=2.734, (5) pH=3.234, (6) pH=3.841, (7) pH=5.346, (8) pH=7.038, (9) pH=8.013] of Fe^{3+}–hpca system at 25 °C using the program VISION with the plotter Calcomp 936.



Scheme 3. $[FeH_2L_2]^{3+}$



Scheme 4. $[FeHL_2]^{2+}$



Scheme 5. $[CuL_2]^{2-}$

(Scheme 4) complexes contain the pyridinic nitrogen in its protonated form. This assumption is supported by the spectrophotometric results, and those recorded for iron(III) hydroxamate systems.[8,21–23] The pH dependence of iron(III)–hpca system differs from that of the iron(III) trihydroxamate siderophores, which have a broad visible maximum from 420 to 440 nm and do not

exhibits the marked pH-dependent spectral shifts seen for example with aerobactin.[24] Only one absorption band was observed in the pH range 1.83 to 5.35 with λ_{max} changing from 478 (pH=1.83) to 436 (pH=5.35) nm. The increase in absorption and shift to smaller wavelengths with increase in pH indicates greater complexation, and, above pH 7.3, both λ_{max} and ε_{max} remain constant, consistent with formation of the [FeL_3] species which predominates in this range. Our results concerning the main species in neutral solution of Fe^{3+}–hpca system are in good agreement with those reported earlier for iron(III) hydroxamate systems (Table 1). Nevertheless the large difference in $\log \beta_{pqr}$ over 5—6 log units reflects a very instable ligand structure of *N*-hydroxy-3-pyridinecarboxamide (hpca) probably due to the large variation in basicity of the only *N*-hydroxyamide grouping involved in coordination as simple aromatic hydroxamic acids. The Fe^{3+}–hpca system therefore probably satisfies different criteria for biological activities and analytical roles, strongly indicating Fe^{3+}–hpca complexes as suitable sources of iron as trace element essential in animal nutrition. However our results show clearly that, at least at physiological pH, the assumption of an uncoordinated α-amino group (heteroatom), which may be particularly active because of a possible surface-active role, is incorrect since the major species in this pH range is FeL_3.

In Cu^{II} complexes with dhbc it is certain that the copper hit on the ligand takes place following the *N,O* coordination (six membered chelate rings, Scheme 5). In comparison with the amino hydroxamic acids till now investigated, where the coordination to copper ion was *N,N* type, here the mononuclear complexes are much more stable ($\log K_1$ and $\log K_2$ much greater), perhaps it is due both to the kinds of atoms (*N,O* rather than *N,N*, Scheme 5) and to greater basicity of the groups involved in the coordination. The trend of potentiometric titration curves for Cu^{2+}–dhbc system has only a buffer zone (spectrophotometric titrations reveal only a intense green color), while in the systems studied till now and involving Cu^{2+} and amino hydroxamic acids were perfectly visible two buffer zones shared by inflection point, where you could observe the formation of polynuclear hydrolized species, that does not present in the Cu^{2+}–dhbc system because probably of the high stability of simple complexes.

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