Stabilities of the iron(III) chelates of 1,2-dimethyl-3-hydroxy-4-pyridinone and related ligands

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Abstract

The protonation constants and iron(III) binding constants of 1,2-dimethyl-3-hydroxy-4-pyridinone, DMHP, are reported and the effectiveness of this ligand in the complexation of iron(III) is compared with those of other iron(III) chelators at moderate and high dilution The ligand is highly effective for Fe(III) in moderately dilute solution ($\sim 10^{-3}$ M), but because of its 3 1 stoichiometry the most stable complex FeL_3 dissociates extensively in very dilute solution ($\sim 10^{-6}$ M) The relative effectiveness of DMHP for iron(III) binding is compared with those of the bidentate ligands, acethydroxamic acid and catechol, and with the multidentate ligands desferriferrioxamine, diethylenetriaminepentaacetic acid, *racemic* ethylenebis(*o*-hydroxyphenylglycine), *N*,*N*′-bis(*o*-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid, with the triscatechols MECAMS and enterobactin, as well as with the physiological iron(III) transport protein transferrin

Introduction

An important goal in coordination chemistry has been the development of effective chelating agents for iron(III) having low toxicity, for use in chelation therapy for the removal from the body of excess iron which accumulates from blood transfusions for the treatment of certain anemias such as Cooley's anemia [1] The iron chelator which has been used clinically for over ten years is desferriferrioxamine, a microbial trishydroxamic acid siderophore [2, 3] Because of its disadvantages (high cost, rapid degradation in the serum, and subcutaneous administration), considerable effort has been expended in developing synthetic substitutes for this drug The new candidates have been catecholate siderophores such as enterobactin [4, 5], and many synthetic catechol-containing ligands developed as mimics of enterobactin [6–8] Recently a number of new hydroxamate ligands has been synthesized for comparison with desferriferrioxamine (DFB) [9–11] While the latter is an open chain secondary trishydroxamate, the model compounds, which contain endocyclic hydroxamic acid functions in both macrocyclic and cryptand ligands, as well as pendent secondary hydroxamate functions joined covalently at a central point, do not seem to offer any advantage in metal affinity or selectivity over the parent siderophore This problem is not one of ligand design (i e endocyclic functional groups in macrocyclic and cryptand ligands potentially have the highest possible preorganization, providing the highest stabilities and selectivities), but is due to the synthetic difficulties of making macrocycles and cryptands with finely-adjusted cavity size Recent reports [12] of the successful clinical use of 1,2-dimethyl-3-hydroxy-4-pyridinone (DMHP, **1**) for the treatment of iron overload has focused attention on the use of this bidentate hydroxypyridinone for the treatment of β-thalassemia and related diseases It has been suggested [5] that the bidentate hydroxypyridinones produced by several microorganisms are also siderophores In addition to the above, several chelating ligands containing phenolate or oxypyridyl donor groups have also been designed and tested for iron(III) binding The effectiveness of these ligands for the treatment of iron overload in experimental animals has recently been reviewed and compared with other types of chelating ligands [13]

In spite of the interest in the hydroxypyridinones (as well as hydroxypyrones) for iron binding there have been no reports of the Fe(III) stability constants of the complexes formed, except for a brief preliminary report [14] The lack

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of dissociation of the 1:1 (bidentate) Fe(III) chelate in strongly acid solution makes direct potentiometric (p[H]) measurement of its 1:1 formation constant impossible. It has been shown by other techniques, however, that these bidentate ligands form stable tris complexes of Ga(III) and In(III) [15], and of Fe(III) [16–18]. Rough estimates of the acid dissociation constants, $\log \beta_3$ for DMHP, and octanol/water partition coefficients have appeared recently [16, 17, 19].

It is the purpose of this paper to report accurate protonation constants and Fe(III) chelate stability constants of DMHP (**1**). Comparisons will be made with other well-known iron chelators with various types of donor groups by calculation of pM values at physiological p[H] and by comparing species distribution curves for competitive two-ligand systems as a function of p[H] at high and low dilution.

Experimental

Materials

A pure crystalline sample of the ligand 1,2-dimethyl-3-hydroxy-4-pyridinone (**1**) was kindly provided by Professor Mark M. Jones of Vanderbilt University. The high purity of this material was affirmed by elemental analysis and potentiometric titration. The chemical reagents used for the preparation of standard solutions for potentiometric and spectrophotometric measurements were of the highest purity (reagent grade) available.

Potentiometric measurements

The hydrogen ion concentrations of the experimental solutions were measured with a Corning model 150 pH meter fitted with Corning glass and reference electrodes. Measurements were made at 25.00 ± 0.05 °C in a sealed water-jacketed glass vessel, under an inert atmosphere of purified argon. The electrode-pH meter system was calibrated with standard HCl and KOH to read $-\log [H^+]$ directly, designated as p[H]. Ionic strength was maintained at 0.100 M with reagent grade supporting electrolyte, and the value of K_w ($[H^+] \times [OH^-]$) was found to be $10^{-13.78}$ under the reaction conditions employed.

For the determination of protonation constants, a typical solution contained 54.09 mg (0.3888 mmol) of DMHP, 5.000 ml of 0.1029 M HCl, and 5.000 ml of 1.000 M KCl (to regulate ionic strength), and was diluted with 40.00 ml CO_2-free water. The titrant employed was 0.1027 M CO_2-free KOH solution, which was added in 0.100 ml increments to obtain over 90 equilibrium points.

The concentrations of the iron(III)-containing solutions were similar to those of the ligand alone. Potentiometric data obtained were used solely for the determination of the 2:1 and 3:1 stability constants. The 3:1 solution remained soluble as base was added through p[H] 10.8 and was observed to change from a violet color at p[H] ~ 2, to maroon at p[H] ~ 2.5, and finally to wine red after the break at p[H] ~ 5.8. In order to ensure equilibrium, the initial solution was allowed to equilibrate for more than 12 h before base was added. The titration proceeded very slowly because the initial ligand reaction was very slow. From 10 to 30 min were required per experimental point and 90 points were collected for an experimental run.

Spectrophotometric measurements

The optical absorbance spectra of the ligand and metal complex species were measured with a Perkin-Elmer model 553 fast scan spectrophotometer and matched quartz cells of light path length 1.000 ± 0.001 cm. Ionic strength was maintained at 0.100 M with reagent grade KCl. Successive spectra were obtained by the addition of standard aqueous HCl or KOH.

For spectrophotometric measurements at low p[H], solutions were prepared by ten-fold dilution of a stock solution containing 1.61×10^{-3} M Fe^{3+} and 4.92×10^{-3} M DMHP in 0.100 M KCl. Each sample was adjusted by the addition of 1.20 M HCl and 1.00 M KCl, as needed. At least several hours were allowed for equilibration at 25.0 °C before measurements were taken.

The series of curves shown in Fig. 1 were obtained on a similar solution prepared by the initial addition of 0.1027 M KOH to get to pH 9.15 then incrementally adding 1.20 M HCl while keeping the volume constant. At least 1 h was allowed for equilibration between spectra.

Calculations

Ligand protonation constants and metal chelate protonation, stability, and hydrolysis constants were calculated using the program BEST by methods described by Motekaitis and Martell [20]. Equilibrium constants and extinction coefficients were determined from the UV–Vis absorption bands with the use of BASIC program KML and FORTRAN program ABSKAS, written in this laboratory. Species distribution diagrams were calculated with the FORTRAN program SPE [20] and plotted on a Hewlett Packard Laser Jet II using program SPEPLOT [20].

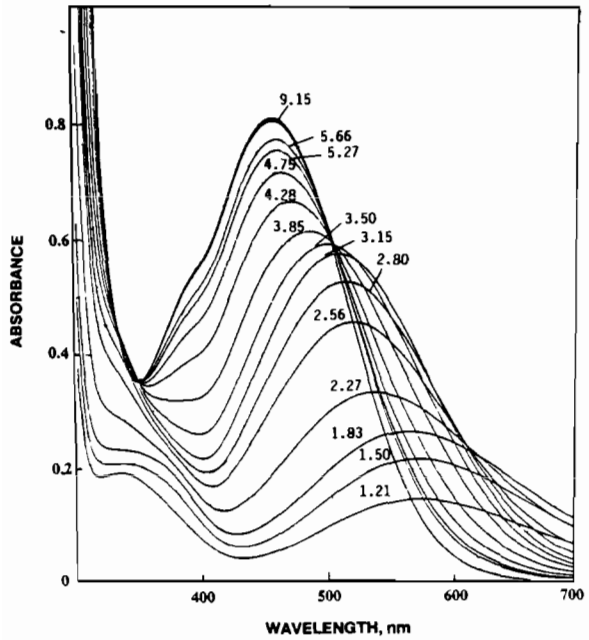


Fig. 1. Absorbance of the 1:3 Fe(III)–DMHP chelate as a function of added acid. $[Fe(III)]_0 = 1.61 \times 10^{-4}$ M; $[DMHP]_0 = 4.92 \times 10^{-4}$ M. Numbers indicate measured pH values when small increments of 1.20 M HCl were added to the 50.00 ml solution.

Results

The protonation constants of the ligand DMHP and the stability constants of the Fe(III) chelates formed are presented in Table 1, along with similar constants for other iron chelators [21]. Structural formulas of the ligands listed in Table 1 are indicated by formulas **1–9**. In order to ascertain the effectiveness of iron(III) binding resulting from the competition between hydrogen ions and Fe(III) ions at physiological p[H] the calculated pM values ($-\log[Fe^{3+}]$) are also listed, for all systems in the presence of 100% excess ligand. The additional free ligand is needed to produce a metal ion buffer solution.

It is noted that 1,2-dimethyl-3-hydroxy-4-pyridinone (formulas **1a** and **1b**) has two protonation constants, corresponding to the formation of HL and H_2L^+ (formulas **1a–1d**). The three successive β values listed correspond to the quotients $[FeL^{2+}]/[Fe^{3+}][L^-]$, $[FeL_2^+]/[Fe^{3+}][L^-]^2$, and $[FeL_3]/[Fe^{3+}][L^-]^3$. The successive log formation constants are $\log[FeL^{2+}]/[Fe^{3+}][L^-] = 15.14$; $\log[FeL_2^+]/[FeL^{2+}][L^-] = 11.54$; and $\log[FeL_3]/[FeL_2^+][L^-] = 9.24$.

Figure 1 illustrates the UV–Vis absorbance spectra obtained at the p[H] values indicated. The

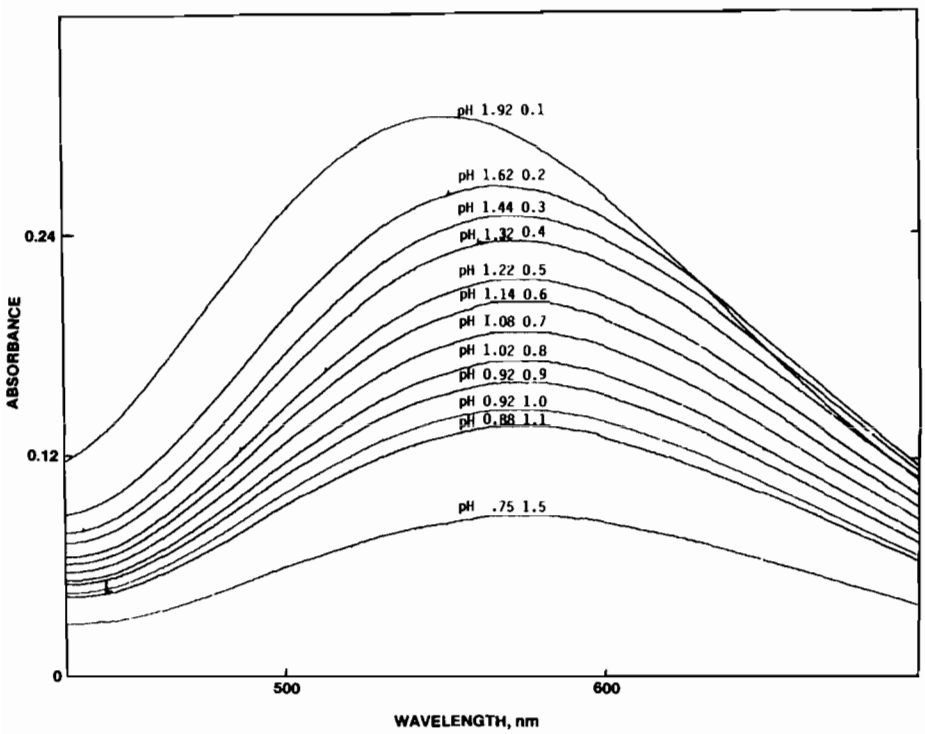


Fig. 2. Absorbance of the 1:1 Fe(III)–DMHP chelate as a function of added acid $[Fe(III)]_0 = 1.61 \times 10^{-4}$ M; $[DMHP]_0 = 4.92 \times 10^{-4}$ M. Numbers indicate ml 1.20 M HCl added to the 50.00 ml solution.

TABLE 1 Log protonation constants and Fe(III) stability constants of iron(III) chelators[a]

	Ligand, H_nK	Log $K^{H}_{H_nL}$						Log β_n			pM[b]	
		K_1	K_2	K_1	K_4	K_5	K_6	β_1	β_2	β_3	10^{-3} M	10^{-6} M
1	1,2-Dimethyl-3-hydroxy-4-pyridinone, (DMHP), HL[c]	9 76	3 62					15 14	26 68	35 92	24 3	18 3
2	Acethydroxamic acid, HL^d	9 36						11 42	21 10	28 33	17 9	12 5
3	Catechol, H_2L^d	~13 3	9 30					20 4	35 5	44 9	18 1	15 1
4	Desferrferrioxamine, (DFB), H_3L^{+d}	9 55	8 96	8 32				30 99			26 3	26 3
5	Diethylenetriaminepentaacetic acid (DTPA), H_5L^d	10 45	8 53	4 28	2 65	1 82		28 0[e]			24 6	24 6
6	*rac*-Ethylenebis(*o*-hydroxyphenyl)glycine (*rac*-EHPG), H_4L^d	12 05	10 87	8 97	6 33			35 54			25 8	25 8
7	*N,N'*-Bis(*o*-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED), H_4L^d	12 6	11 09	8 44	4 72			39 68			29 7	29 7
8	MECAMS, H_6L^{3-d}	11 5[f]	11 5[f]	11 5[f]	7 26	6 44	5 88	~41[f]			28 4	28 4
9	Enterbactin, H_6L^d	12 1[fd]	12 1[f]	9 2[f]	9 2[f]	8 4[f]	7 6[f]	~52[f]			37 6	37 6
10	Transferrin[g]							20 2	33 9[h]		20 3	20 3

[a] t = 25 0 °C, μ = 0 10 M [b]For equivalent concentration of excess free ligand [c]This work [d]Ref 21 [e]Log [ML]/[MLOH][H^+] = 9 66, ref 21 [f]Estimated [g]Conditional constants at physiological conditions [h]β_2 refers to $[M_2L]/[M]^2[L]$

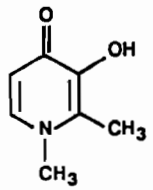
λ_{max} at 440 nm corresponds to complete formation of the 3 1 complex, FeL_3 The first isosbestic point occurs at ~500 nm and the spectrum near pH 3 4 corresponds to 50% conversion of the 3 1 complex to the 2 1 complex The second, lower isosbestic point is diffuse because more than two complex species are present (the 2 1, 1 1, and a lower concentration of 3 1) The lowest curves, at p[H] 1 50 and 1 21 correspond almost entirely to the 1 1 complex

The absorbance curves in Fig 2 illustrate formation (or dissociation) of the 1 1 Fe(III)–DMHP complex It is seen that this complex is not completely dissociated at p[H] values as low as 1 0, which is far below the limit (~2 0) at which accurate potentiometric p[H] measurements can be made Hence the 1 1 stability constant listed in Table 1 was calculated from the data in Fig 2 by the method indicated in 'Experimental'

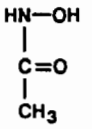
The species distributions of the complexes formed at $1\,0 \times 10^{-3}$ M Fe(III) ion and $3\,0 \times 10^{-3}$ M DMHP, and at $1\,0 \times 10^{-6}$ M Fe(III) ion and $3\,0 \times 10^{-6}$ M DMHP are presented in Figs 3(a) and (b), respectively It is seen that conversion of the 2 1 to the 3 1 complex occurs at pH 4 at 10^{-3} M, but the conversion does not occur until p[H] 7 is reached for metal complexes at $1\,0 \times 10^{-6}$ M, showing the strong effect of dilution on the formation of the 3 1 complex In fact, at 10^{-6} M, the 2 1 complex is not completely converted to the 3 1 complex, even at the highest p[H] attainable It is important to point out that the metal ion is 100% complexed at all p[H] values shown at 10^{-3} M, but that at 10^{-6} M and p[H] 2, the complexes are about 35% dissociated to the free Fe^{3+} ion and 12% dissociated to the hydroxo Fe(III) species, $Fe(OH)^{2+}$, which persist in diminishing concentrations up to pH ~4 5

Discussion

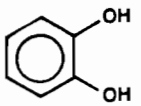
The protonation constants reported in Table 1 for DMHP ($\log K^{H_1} = 9\,76$ and $K^{H_2} = 3\,62$) differ somewhat from the values reported previously (9 7 and 3 3, respectively) [16, 17, 19] Also the iron binding constant of the 3 1 chelate reported here (Table 1, $\log \beta_3 = 35\,92$) is higher than the value previously reported ($\log \beta_3 = 34\,5$) [17] The protonation constant differences are probably due, at least in part, to differences in temperature and ionic strength, which were not uniformly specified in the previous work [16, 17, 19] for the same numerical values of the constants It is considered fortuitous that the previous Fe(III) value is as close as it is since the earlier investigations were done above pH 2 where no free dissociated Fe(III) ion is present



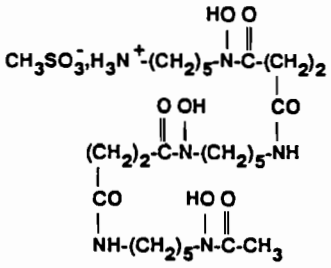
1 1,2-dimethyl-3-hydroxy-4-pyridinone, HL



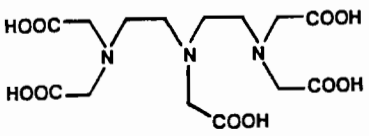
2 Acethydroxamic acid, HL



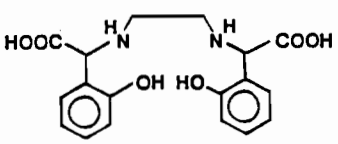
3 Catechol, H_2L



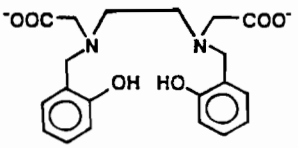
4 Desferriferrioxamine B (DFB), H_4L^+, shown as mesylate



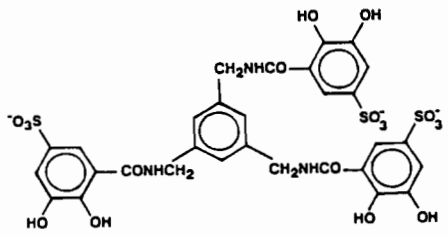
5 DTPA, H_5L



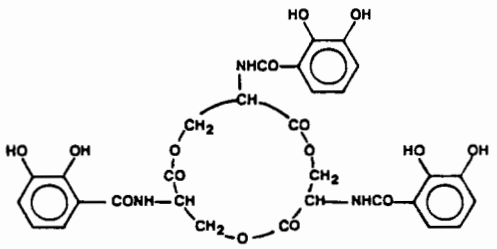
6 EHPG, H_4L



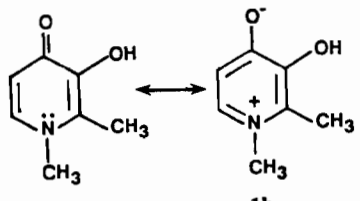
7 HBED, H_4L



8 MECAMS, H_6L^{3-}

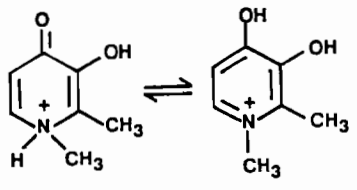


9 Enterobactin, H_6L



1a 1b

1,2-Dimethyl-3-hydroxy-4-pyridinone, HL



1c 1d

Protonated 1,2-dimethyl-3-hydroxy-4-pyridinone, H_2L^+

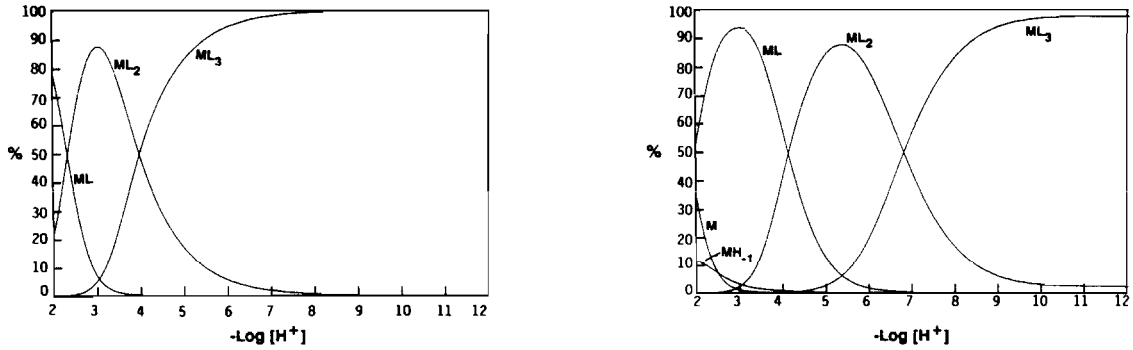
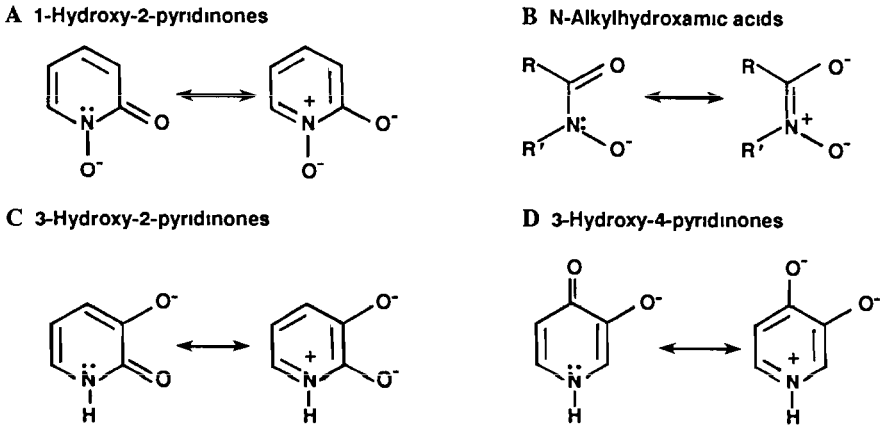


Fig 3 (a) Metal ion species distribution curves for the 3 1 DMHP–Fe(III) system as a function of pH, $t = 25\ 0$ °C, $\mu = 0\ 10$ M (KCl), T(Fe(III)) = $1\ 0 \times 10^{-3}$ M, T(DMHP) = $3\ 0 \times 10^{-3}$ M, DMHP = L % refers to total iron (b) Same as (a) except at $1\ 0 \times 10^{-6}$ and $3\ 0 \times 10^{-6}$ M

The stability constants of the DMHP–Fe(III) complexes listed in Table 1 are considerably higher than those of primary or secondary hydroxamic acids, but considerably lower than those of catechol These differences in metal ion affinity may be rationalized on the basis of the electronic structures of the ligand anions The resonance forms of the anions of the three types of hydroxypyridinones, illustrated in Scheme 1, formulas **A**, **C** and **D** shows the transfer of additional negative charge from the nitrogen lone pair to the carbonyl oxygen, so that the bidentate pair of oxygen donors carry a charge between −1 and −2 With the aid of the polarization effect of a coordinated positive metal ion, this negative charge would increase and approach −2 It is seen that **A** is a cyclic hydroxamic acid, which may be compared with an aliphatic hydroxamic acid, **B** The transfer of negative charge to the negative carbonyl oxygen of the hydroxamates **A** and **B** would be considerably greater for the aromatic hydroxamate (the 1-hydroxy-2-pyridinone) because the resonance form with two negative oxygens is further promoted by the restoration of aromatic resonance to the ring

Because of the fact that the Fe(III) binding constants of acethydroxamic acid are all considerably lower than those of DMHP, without an appreciably compensating lowering of hydrogen ion competition, there would be no point in setting up species distribution curves for a 1 1 1 system containing Fe(III) ion, DMHP and acethydroxamic acid, since the latter would not even appear on the diagram, i e there would be no competition

On the other hand, it would be of interest to compare the relative effectiveness of DFB (a trishydroxamate) and DMHP in a 1 1 3 Fe(III)–DFB–DMHP solution The species distribution curves for $1\ 0 \times 10^{-3}$ M complexes, illustrated in Fig 4, shows that at physiological p[H] DFB is an order of magnitude more effective than DMHP About pH 9, the ratio of concentrations of DMHP to DFB complexes increases somewhat as the relative hydrogen ion competition changes for the two ligands Because of the strong dilution effect on the dissociation of the 3 1



Scheme 1 Resonance forms of hydroxypridinones and hydroxamic acid anions

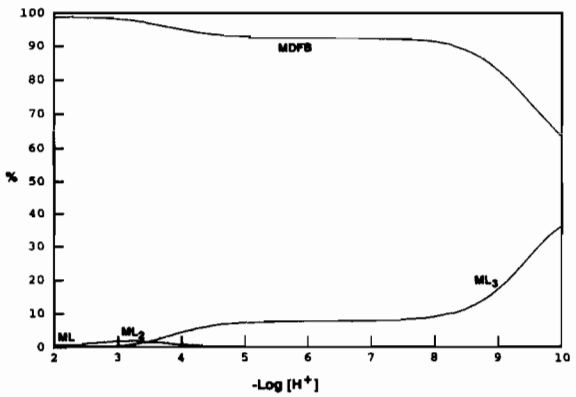


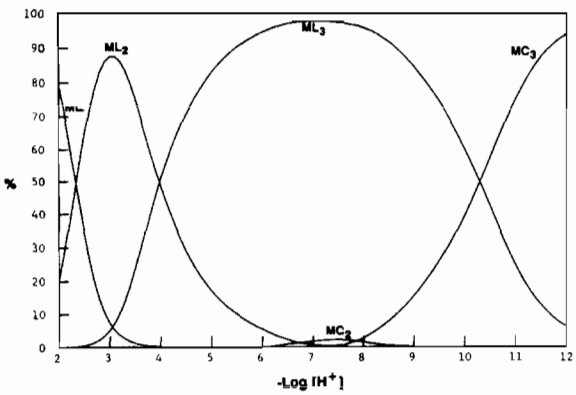
Fig 4 Iron(III) distribution curves showing competition between 1.0×10^{-3} M DFB and 3.0×10^{-3} M DMHP (HL) for 1.0×10^{-3} M Fe(III) % refers to total iron

DMHP Fe(III) complex noted above, there is no competition at all at 10^{-4} molar, and at higher dilutions In other words the DMHP complexes do not appear on the distribution diagrams at all

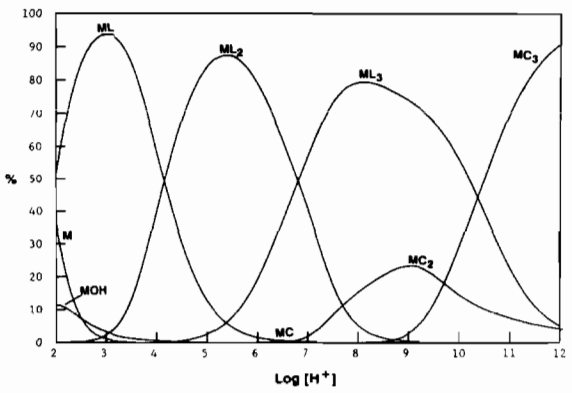
Direct comparison between DMHP and catechol is instructive These ligands form the same types of Fe(III) complexes, 1 1, 1 2 and 1 3, with the catechol constants being much higher than those of DMHP On the other hand, the DMHP protonation constants are much lower than those of catechol, giving the former ligand a considerable advantage over the latter at low pH This is clearly illustrated in Fig 5, which shows the species formed as a function of p[H] in a solution containing a 1 3 3 ratio of Fe(III) DMHP catechol Clearly, DMHP is the more effective ligand over nearly the whole p[H] range, including physiological pH At high p[H] (above p[H] 9), however, the higher stability constants favor the formation of catechol chelates, as the competition with hydrogen ions becomes less important Even in very dilute solution of the same relative stoichiometry the pH-dependence of ligand competition is preserved Figure 5(b) shows catechol dominating in the complexation of iron(III) above pH 8–9, except that in this case the complex having a 2 1 molar ratio of catechol to iron(III) gains importance because of the generally diminished concentration of the more dilution-dependent 3 1 complexes of both catechol and DMHP

The effect of dilution in reducing the stability of the 3 1 DMHP–Fe(III) complex is dramatically illustrated in the competition between DTPA and DMHP for Fe(III), at total metal species concentrations of $T_{Fe(III)}$ 10^{-3} and 10^{-6} M (Fig 6) At 10^{-3} M $T_{Fe(III)}$, the concentrations of DTPA complexes account for all of the Fe(III) in acid solution up to p[H] ~ 3 5 As the pH increases the relative amount of the 3 1 DMHP complex increases, and surpasses that of DTPA above pH 7 5, reaching a maximum at about 80% at p[H] 10 Above p[H] 10 the relative concentration of DMHP drops off as the more stable hydroxo complex of Fe(III)–DTPA becomes more important At low concentrations of iron complexes (10^{-6} M), however, the situation is quite different Under these conditions the DTPA complexes account for 100% of the Fe(III) over the entire p[H] range, except for the appearance of the 3 1 DMHP complex as a minor species (maximum concentration ~3%, or $\sim 3 \times 10^{-8}$ M) between p[H] 9 and 11

Figure 7(a) is a species distribution diagram illustrating the competition between a diphenolate hexadentate ligand, *N,N'*-ethylenebis(*o*-hydroxyphenylglycine) (EHPG) and DMHP for Fe(III) EHPG has long been used as an Fe(III)-specific chelator for iron transport in agriculture and is effective in the treatment of iron overload [13] The ratio is 3 1 1 It is seen that EHPG has a



(a)



(b)

Fig 5 (a) Iron(III) distribution curves showing competition between 3.0×10^{-3} M catechol and 3.0×10^{-3} M DMHP for 1.0×10^{-3} M Fe(III) (b) Same as (a) except at 3.0×10^{-6} M and 1.0×10^{-6} M % refers to total iron

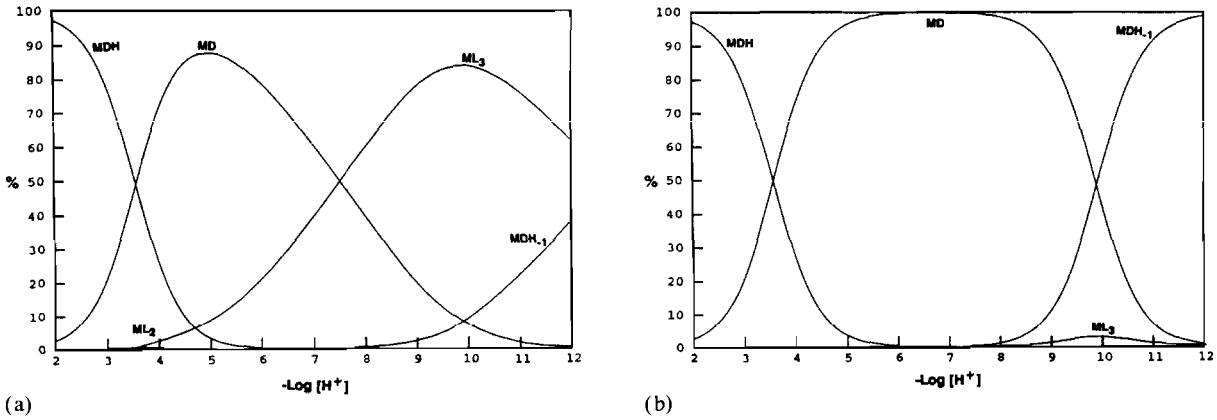


Fig. 6. (a) Iron(III) species distribution curves for the system 1,2-dimethyl-3-hydroxy-4-pyridinone:DTPA:Fe(III) 3:1:1; 1.0×10^{-3} M in total Fe(III) species. DMHP = L; D = DTPA. % refers to total iron. (b) Metal ion species distribution curves for the system 1,2-dimethyl-3-hydroxy-4-pyridinone: DTPA:Fe(III) 3:1:1, 1.0×10^{-6} M in total Fe(III) species. DMHP = L. % refers to total iron.

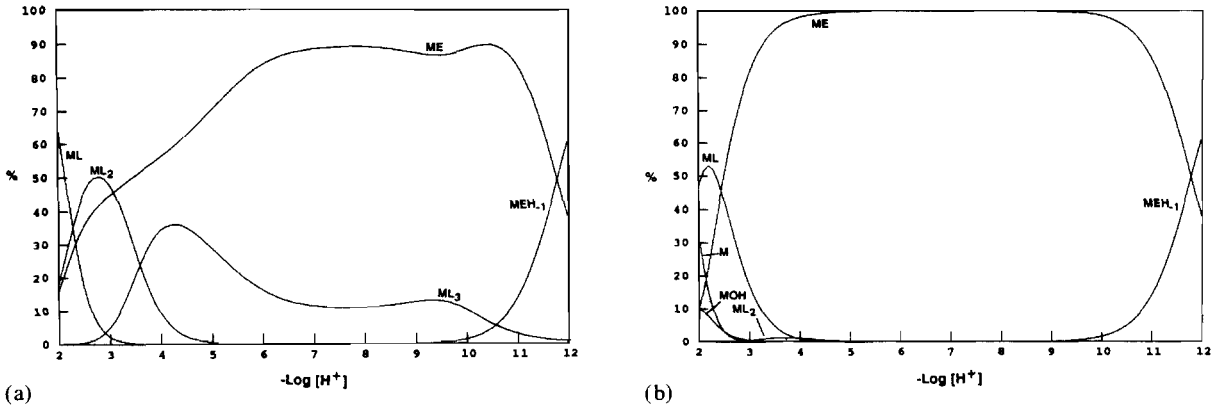
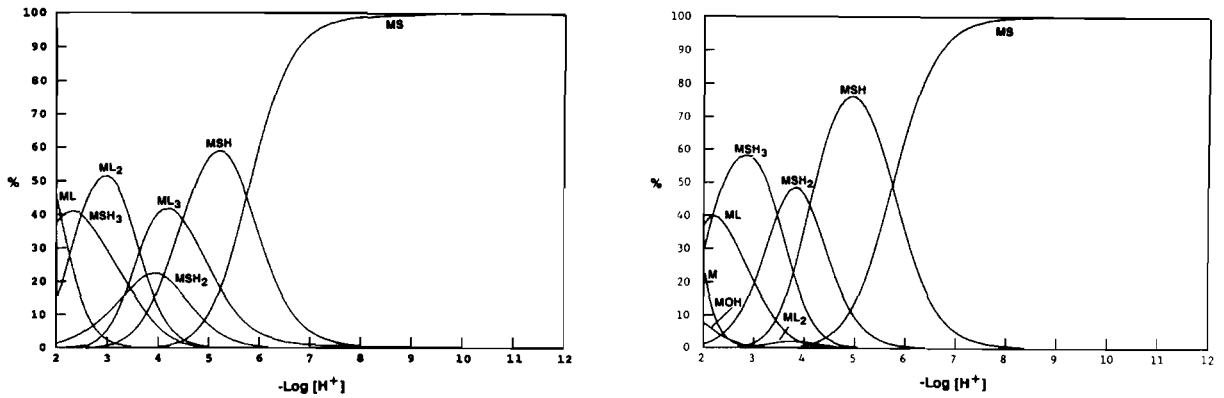


Fig. 7. (a) Metal ion species distribution curves for the system 1,2-dimethyl-3-hydroxy-4-pyridinone:*rac*EHPG:Fe(III) 3:1:1; 1.0×10^{-3} M in total Fe(III) species. DMHP = HL; EHPG = H_4E. % refers to total iron. (b) Iron(III) species distribution curves for the system 1,2-dimethyl-3-hydroxy-4-pyridinone:*rac*EHPG:Fe(III) 3:1:1; 1.0×10^{-6} M Fe(III), 3.0×10^{-6} M DMHP and 1.0×10^{-6} M EHPG. % refers to total iron.

nearly 3:1 advantage over DMHP from pH 6–10, and is even more effective at higher p[H], while below p[H] 3.5 the 2:1 and 1:1 complexes of DMHP predominate. The Fe(III) is completely complexed (complex species add up to 100%) at all p[H] values shown. When this system is diluted a thousand fold (Fig. 7(b)) we have essentially the species distribution curves characteristic of Fe(III)–EHPG alone. In other words there is no competition by DMHP at high dilution, i.e. at concentrations of Fe(III) ion below 10^{-4} M. On the other hand, if the same ratio of 3:1:1 were maintained at the 10^{-2} M level the iron(III) would be equally distributed at physiological pH between the two ligands as 1:3 and 1:1 complexes, respectively.

N,*N*′-Bis(*o* - hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED) competes even more successfully with DMHP for Fe(III) than does EHPG. Because of similar differences in hydrogen ion competition, but considerably higher Fe(III) binding by HBED, there is some Fe(III) binding by DMHP at low pH, but the shoulder indicating partial competition by DMHP between p[H] 6 and 10 is missing, with the distributions completely in favor of HBED complex species. The distribution diagram would be featureless (that of Fe(III)–HBED alone) and therefore is not shown.

Finally, competition by another ligand type, MECAMS, with three pendent catechol amide groups, is illustrated in Fig. 8. Although Fe(III) binding by the completely dissociated ligand is extremely high, as indicated in Table 1, the protonation constants are also very high, leading to



(a) (b)

Fig. 8. Species distribution curves for the system: (a) 1.0×10^{-3} M Fe(III), 1.0×10^{-3} M MECAMS, and 3.0×10^{-3} M DMHP and (b) 1.0×10^{-6} Fe(III), 1.0×10^{-6} MECAMS, and 3.0×10^{-6} M DMHP. Only Fe(III) containing species shown. % refers to total iron.

much lowered effectiveness of Fe(III) binding at low p[H]. Thus the superiority of DMHP at low pH is predictable, as well as the superiority of the catechol ligand at high p[H]. The competition by DMHP at low p[H] is wiped out at high dilution, and the distribution curves in Fig. 8(b) above p[H] 4 are identical to those obtained with MECAMS alone.

Conclusions

The stability data discussed above indicate that DMHP is a reasonably effective iron chelator at concentrations of 10^{-3} M and above. Its binding effectiveness is due mainly to the high binding constants for the 1:1 and 1:2 iron(III) to ligand complexes. In fact the 1:1 complex is so stable and involves such little hydrogen ion competition that it is still completely formed at pH values as low as 2.0. Because of the fact that three ligand anions are needed per metal ion for complete coordination to form the octahedral Fe(III) complex, there is a strong dilution effect [22], so that the ligand is much less effective at high dilution. Thus the high pM value attained at 10^{-3} M indicated in Table 1 is higher by six orders of magnitude relative to that at 10^{-6} M concentration of the 3:1 metal complex and equivalent excess ligand. Therefore one would question the effectiveness of this ligand in binding Fe(III) in biological systems at high dilution.

Comparison of the pM values maintained by DMHP at physiological pH with the value regulated by transferrin (Table 1) reinforces this estimate of poor DMHP effectiveness at high dilution. On the basis of the pM values listed, one would not expect DMHP to remove iron from transferrin at 10^{-6} M. This prediction is in agreement with the empirical findings of Kontoghiorghes [17, 23] that DMHP and other pyridinones remove iron from transferrin at high concentrations while giving up iron to transferrin at low concentrations. Clearly, this question now seems to be settled. The key to the answer lies in the concentration employed, because DMHP is more effective than transferrin (Table 1) in binding iron at 10^{-3} M. Unfortunately, in other reports on the same systems claims are based solely on the millimolar experiments without mentioning the concentration dependence [16, 19].

Perhaps another important possibility, as yet unexplored, would be the incorporation of DMHP into a suitable backbone in an endocyclic manner. The result would be a superior iron chelator with an unprecedented high effective stability constant for Fe(III). This is considered a reasonable projection simply because the ligand would have low protonation constants, suitable negative charge, and the intrinsic superiority of DMHP relative to acethydroxamic acid.

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