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Stabilities of 1,2-diethyl-3-hydroxy-4-pyridinone chelates of divalent and trivalent metal ions

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Abstract

The stability constants of the 1:1, 2:1 and 3:1 complexes of bidentate 1,2-diethyl-3-hydroxy-4-pyridinone (DEHP) with divalent and trivalent metal ions have been determined by potentiometric and spectrophotometric measurements in KCl supporting electrolyte (0.100 M) at 25.0 °C. The overall log stability constants ($\beta_{ML_3} = [ML_3]/[M^{3+}][L^-]^3$) for the Fe(III), Ga(III) and In(III) complexes of DEHP are high, 36.75, 36.05 and 32.38, respectively, while that for the relatively weak complex with Gd(III) is 19.76. The crystal structure of the 3:1 indium(III) chelate of DEHP is described. The divalent metal ions, Cu(II), Ni(II), Co(II) and Zn(II), form moderately stable 1:1, 2:1 and 3:1 complexes with DEHP. The thermodynamic stabilities of the Fe(III) chelates of DEHP are only moderately greater than those of 1,2-dimethyl-3-hydroxy-4-pyridinone (DMHP, L1). While this difference may suggest a small difference in physiological properties of the two compounds, the main difference in their effectiveness must be due to differences in their behavior *in vitro*.

Keywords: Stability constants; Metal complexes; Divalent ion complexes; Trivalent ion complexes; Bidentate chelate ligand complexes; Crystal structures; Hydroxypyridinones

1. Introduction

The development of new orally effective iron chelators for the treatment of iron overload has been an important objective for some years. Many compounds have been rejected because they lack sufficient oral activity to produce negative iron balance in animals hypertransfused with iron, or because of unacceptable toxicity. The hydroxypyridinones, a family of bidentate chelators which coordinate iron(III) with high specificity and selectivity [1–3], appear promising in this respect, with oral activity demonstrated in mice, rabbits and rats [4–8]. One of these compounds 1,2-dimethyl-3-hydroxy-4-pyridinone (DMHP), has been tested clinically and shown to be active in humans [9] but has a marked potentiating effect on the action of barbiturates in animals that may limit its potential use in humans [10]. A wide range of 3-hydroxy-4-pyridinones has been synthesized [2,4,11,12], and their physico-chemical properties, toxicity and effectiveness have been studied in detail [2,10,13,14]. Several investigators found that 1,2-diethyl-3-hydroxy-4-pyridinone (DEHP), which differs from DMHP in the replacement of methyl groups in the 1 and 2 positions by ethyl groups, has significantly less toxicity and is more effective in mobilizing iron than DMHP [10,14–16] but contradictory results have also been reported [17,18]. Two extensive studies of the oral efficacy and acute toxicity of 1,2-dialkyl-3-hydroxy-4-pyridinones indicated that these ligands are very important for treatment of iron overload and different alkyl groups binding in 1 and 2 positions of 3-hydroxy-4-pyridinone may have considerable effect on their efficacy and toxicity. An early study reported the protonation constants of DEHP (**1**) and indicated qualitatively that stable complexes are formed with iron(III) [2]. There are several reports of the stability constants of DMHP chelates, but the stability constants of DEHP with metal ions have not been studied. It is of interest to investigate the stability constants of the complexes formed by DEHP with Fe(III) as well as with other metal ions to see if there are differences in the metal ion affinities of DEHP and DMHP. It is also noted that there are no stability constant data for other alkyl derivatives of the 3-hydroxy-4-pyridinones. Hydroxy-

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pyridinones also form stable complexes with Al(III) and are of interest for the treatment of aluminium overload [19–22]. Further, Ga(III) [22, 23] and In(III) [23, 24] form hydroxypyridinone chelates which are of interest for the radioisotopic imaging of tumors, and it would therefore be useful to determine the affinities of DEHP for these metals. In this paper the stabilities of the DEHP chelates of divalent and trivalent metal ions are reported.

2. Experimental

2.1. Characterization of ligand

A sample of DEHP (**1**) was kindly supplied by Dr Hider of King's College, London. The sample was titrated and found to contain nearly one mole of HCl per mole of ligand. The NMR spectra showed that there was no organic impurity in the sample. In order to determine the formula weight, the sample was sent to Galbraith Laboratories, Knoxville, TN, for elemental analysis (found: C, 48.75; H, 7.28; N, 6.26%). On that basis the sample is considered to be $C_9H_{13}O_2N \cdot HCl \cdot H_2O$ ($FW = 221.0$). The calculated analysis is C, 48.87; H, 7.24; N, 6.23%, which is in good agreement with the titration and elemental analysis results. The hydrochloride in the sample was taken into consideration in the metal ion titrations.

2.2. Other reagents and standard solutions

Metal ion solutions were prepared at about 0.025 M from analytical grade chloride salts with distilled water and were standardized by titration with Na_2H_2EDTA (disodium salt of ethylenedinitrilotetraacetic acid) [25]. The Ga^{3+}, Fe^{3+} and Al^{3+} solutions were stored with a small excess of hydrochloric acid in order to prevent hydrolysis of the metal ions. The exact amount of excess acid was checked by titration of 1:1 ratios of these metal ions with EDTA. The amount of KOH consumed in excess of the amount needed to neutralize all of the ligand protons represents the excess of HCl.

Colorless crystals suitable for X-ray diffraction studies were obtained by slow evaporation of a 3:1 aqueous solution of DEHP and In(III) chloride at pH 10.5 and room temperature.

2.3. Potentiometric determinations

Equilibrium potentiometric determinations of the ligand protonation constants and its metal binding constants, in molar ligand to metal ratios of 1:1, 2:1 and 3:1, were carried out by the glass electrode method at 25.0 °C, 0.100 M (KCl). Stability constants were calculated with the program BEST [26]. Details of the potentiometric method have already been described [27].

The potentiometric apparatus consists of a glass jacketed titration cell, a temperature bath (Haake, 25.0 °C), glass electrodes, reference electrodes, and a 10 ml capacity Metrohm piston buret, for which the buret tip was sealed in the cap of the titration cell with a clamp and O-rings. The electrodes were calibrated in a thermostated cell with standard acid and base to read p[H] directly ($p[H] = -\log[H^+]$). The ionic strength was adjusted to 0.100 M with KCl. Atmospheric CO_2 was excluded from the titration cell with a purging stream of purified argon gas at low positive pressure. The value of $K_w = [H^+][OH^-]$ used in the computations was $10^{-13.78}$ [25]. The total number of data points for determination of protonation constants of the ligand and each metal chelate stability constant of DEHP was 210 (3 titrations) and 182 (2 titrations), respectively. The pH ranges used for the determination of protonation constants and stability constants were 2.3–11.1 and 2.0–11.4, respectively.

The metal chelates of DEHP were prepared as 3:1 complexes at 2×10^{-3} M concentration in metal ion and with three molar equivalents of ligand in 50 ml water solution. Most of the metal binding constants were calculated from direct potentiometry, but the first binding constants ($K_{ML} = [ML^{2+}]/[M^{3+}][L^-]$) of the 1:1 complexes of Fe(III) and Ga(III) chelates were determined at 10^{-4} M in separate experiments by spectroscopic measurements at low p[H] since at higher concentration ($> 10^{-3}$ M) their 1:1 complexes were formed completely at pH 2.00. Once the value of K_{ML} was determined for each trivalent metal ion complex, the values of the binding constants for ML_2 and ML_3 species were calculated by BEST [26] from direct titration of the 3:1 solutions while holding the value of K_{ML} constant. On the other hand, the 1:1 stability constants for the Al(III) and In(III) complexes were determined directly by potentiometric measurements since the complexes were not completely formed at p[H] 2.0. For the purpose of determining whether the metal ions hydrolyze during metal ion titrations, the metal ion hydrolysis constants [28] were used in the calculation. However, no hydrolysis was found to occur.

In order to provide potential eight-coordination to the Gd(III) metal ion, a solution of Gd(III)–DEHP which contained 2×10^{-3} M Gd(III) and four equivalents of ligand was investigated potentiometrically. Potentiometric titration of the divalent metal ion complexes of Cu(II), Co(II), Ni(II) and Zn(II) were carried out in 2:1 and 3:1 solutions to allow for the possible formation of ML_3 species.

2.4. Spectrophotometric evaluation of stability constants

Spectral determinations were made for the Fe(III) and Ga(III) chelates of DEHP with a Perkin-Elmer 553 fast scan UV–Vis spectrophotometer equipped with 1.000 cm matched quartz cells at 25.0 °C ($\mu = 0.100$ M (KCl)). The spectra of the 3:1 Fe(III)–DEHP system are distributed into three well defined zones corresponding to the formation of 1:1, 2:1, and 3:1 species. For Fe(III)–DEHP (1:1), log K_{ML} was determined by spectrophotometric evaluation of the dissociation of the Fe(III) complex at 568 nm ($\epsilon_{FeL} = 1660\ M^{-1}\ cm^{-1}$). Fe(III) was induced to dissociate from the Fe(III) complex by lowering the p[H] of the 1:1 iron(III) complex in a series of solutions containing 10^{-4} M Fe(III) and one equivalent of ligand with added increments of 0.128 M HCl down to p[H] 1.00. For purposes of calculation of the Fe(III) stability constant, the absorbances at 568 nm were used in the p[H] range 1.00 to 1.50, for which the ionic strength was adjusted to 0.100 M in [HCl] + [KCl]. The concentrations of the appropriate metal, ligand and complex species were calculated from mass balance equations.

Similarly, a series of solutions containing 1×10^{-4} M Ga(III)–DMHP (as a 1:1 complex) was analyzed spectrophotometrically over the p[H] range from 1.20 to 2.60 (by addition of HCl to the reaction solution), for which the most useful equilibrium points were selected between p[H] 1.20 and 1.70. Ga(III)–DEHP absorbs at 293 nm ($\epsilon_{ML} = 5300\ M^{-1}\ cm^{-1}$) while the free ligand absorbs at 275 nm in the monoprotonated form and at 244 nm as the diprotonated species. The concentrations of the appropriate metal, ligand and complex were calculated from mass balance equations as described above for the iron(III) chelate.

2.5. Crystal structure determination

A colorless crystal (0.2 mm × 0.2 mm × 0.3 mm) was mounted on a glass fiber with epoxy cement, at room temperature (formula $C_{27}H_{42}N_3O_9In$, formula weight 667.5 AMU). Preliminary examination and data collection were performed on a Rigaku AFC5R X-ray diffractometer (oriented graphite monochromator; Mo Kα $\lambda = 0.71073$ Å radiation). Cell parameters were calculated from the least-squares fitting of the setting angles for 50 reflections. Omega scans for several intense reflections indicated acceptable crystal quality.

Data were collected for $3.0 \leqslant 2\theta \leqslant 50.0°$. Scan width for the data collection was $1.51 + 0.30*\tan(\theta)°$, with a fixed scan rate of 16.00° min^{-1}. The weak reflections were rescanned (maximum of 2 rescans) and the counts for each scan were accumulated. Three control reflections, collected every 150 reflections, showed no significant trends. Background measurement by stationary crystal and stationary counter technique was made at the beginning and end of each scan of 0.50 of the total scan time.

Lorentz and polarization corrections were applied to 2028 reflections. An empirical absorption correction was applied ($T_{max} = 0.9999$, $T_{min} = 0.9867$). A total of 1917 unique reflections ($R_{int} = 0.0228$), with $|I| \leqslant 2.0\sigma I$, was used in further calculations. The structure was resolved by direct methods [29]; full-matrix least-squares anisotropic refinement on F^2 for all non-hydrogen atoms [30] yielded $[I > 2\sigma(I)]R(F) = 0.0325$, $R_w(F^2) = 0.0829$ and $S = 1.05$ at convergence (largest positive peak in the final Fourier difference map = 1.05 e$^-$ Å^{-3}; largest negative peak in the final Fourier difference map = −0.393 e$^-$ Å^{-3}). Hydrogen atoms were placed in idealized positions with isotropic thermal parameters fixed at 0.08 Å^2. Neutral atom scattering factors and anomalous scattering correction terms were taken from International Tables for X-ray Crystallography [31].

The crystal data and structure refinement are summarized in Table 1.

3. Results and discussion

3.1. Protonation constants of DEHP

The neutral ligand, HL, has two protonation constants, corresponding to the formation of HL and H_2L^+. The first proton binds to the oxygen at the 3 position (log $K_1^H = \log[HL]/[L^-][H^+] = 9.93$). A second proton (supplied by excess mineral acid) becomes bound to the pyridyl nitrogen atom (log $K_2^H = \log[H_2L^+]/[HL][H^+] = 3.81$). The equilibrium p[H] profile of the ligand is presented in Fig. 1 and the protonation constants obtained do not differ greatly from those previously reported (Table 2). Alkyl groups which are substituted on the 1 or 2 positions in 3-hydroxy-4-pyridinone do not differ very much in their influence on the protonation constants. DEHP, which differs from DMHP in the replacement of methyl groups in the 1 and 2 positions by ethyl groups, has protonation constants which are a little higher than but close to those of DMHP (Table 2).

3.2. Stability constants of complexes of trivalent metal ions

The shapes of the potentiometric p[H] curves in Fig. 1 show a break for the trivalent metal ion complexes at $m = 3$ (m = moles of base added per mole of metal ion present). The Fe(III) and Ga(III) titration curves indicate complete formation of the 1:1 metal complex over the entire p[H] range studied. Spectrophotometric evaluation of the dissociation of the 1:1 Fe(III)–DEHP complex at low p[H] (HCl added to reaction solution) gave log $K_{ML} = 15.21$ (Table 2). Fe(III)–DEHP forms

Table 1
Crystal data and structure refinement for the indium(III) complex of DEHP

Identification code	am31
Empirical formula	$C_{27}H_{42}InN_3O_9$
Formula weight	667.46
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system	rhombohedral
Space group[a]	$R3$
Unit cell dimensions	
a (Å)	15.447(3)
b (Å)	15.477(3)
c (Å)	23.026(5)
α (°)	90
β (°)	90
γ (°)	120
Volume (Å^3)	4758(2)
Z	6
Density (calc.) (Mg/m^3)	1.398
Absorption coefficient (mm^{-1})	0.797
Absorption correction, T:	
max., min.	0.9999, 0.9876
$F(000)$	2076
Crystal size (mm)	0.2×0.2×0.3
θ Range for data collection (°)	1.76–25.01
Index range	$-18 \leq h \leq 0$, $-15 \leq k \leq 15.0 \leq l \leq 27$
Reflections collected	2028
Independent reflections	1917 (R_{int} = 0.0228)
Refinement method	full-matrix least-squares on F^2
Data/restraints/parameters	1912/1/241
Goodness-of-fit on F^2	1.015
Final R indices ($I > 2\sigma(I)$)	
R^1	0.0325
R_w^2	0.0829
R indices (all data)	
R^1	0.0448
R_w^2	0.1023
Absolute structure parameter	0.05(6)
Largest differenec peak and hole (e Å^{-3})	2.051 and −0.393

[a]Both the centrosymmetric space group $R3m$ and the non-centrosymmetric s pace group $R3$, are acceptable for the observed systematic absences, however the examination of the intensity distributions indicates the choice of the non-centrosymmetric space group $R3$. The structure was solved and refined in both space groups and their final weighted residuals were compared statistically. The non-centrosymmetric space group was seen to be statistically preferred.

red complexes (Fig. 2); and FeL and FeL_2 complexes are distinctly discerned at 568 (Fig. 2) and 510 (not shown) nm, respectively. Values for the second and third stepwise binding constants, determined potentiometrically, as well as the overall binding constants (log β), are presented in Table 3 for all trivalent metal ions investigated. It can be seen that changing the alkyl groups substituted on the 1 and 2 positions of the 3-hydroxy-4-pyridinone has only a small effect on the stability constants of Fe(III) chelates (Table 3). The log K_{ML} values for the 1:1 complex of Fe(III) and DEHP is a little higher but close to that of DMHP–Fe(III) (Table 3), but the overall log stability

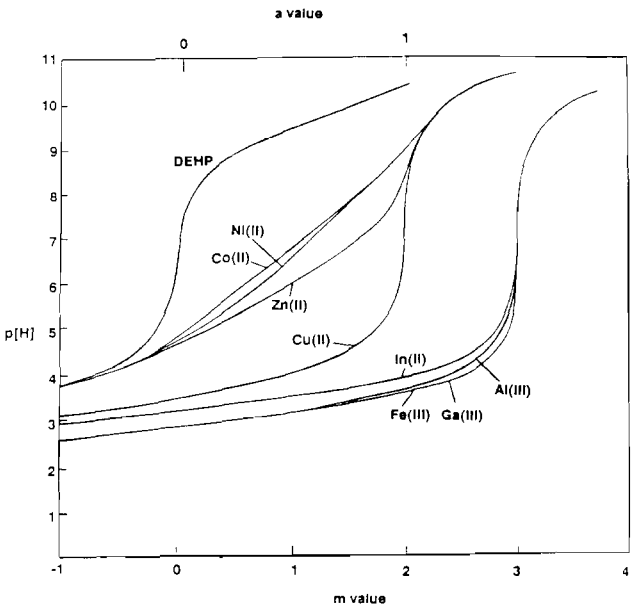


Fig. 1. p[H] profile of DEHP complexes with metal ions; m = moles of base added per mole of metal ion; a = moles of base added per mole of ligand; $T_M = 2.00 \times 10^{-3}$ M; $T_L = 4.00 \times 10^{-3}$ M (for divalent metal ions); $T_L = 6.00 \times 10^{-3}$ M (for trivalent metal ions); for ligand alone $T_L = 2.00 \times 10^{-3}$ M; 25.0 °C, $\mu = 0.100$ M (KCl).

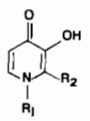
constant of DEHP–Fe(III) ($\sim 10^{37}$) is somewhat larger than that of DMHP–Fe(III) ($\sim 10^{36}$) [3]. Similar effects were observed in HBED derivatives [32]. Replacing the two methyl groups in Me_4HBED by two tert-butyl groups (t-BuHBED) causes the stability constants of Fe(III) chelates to increase about one log unit (Table 2). This result is probably due to the fact that the alkyl groups have a slightly different electron-donating ability: H < methyl < ethyl < tert-butyl. Therefore a hard acid such as Fe(III) will have a somewhat higher affinity for the anion of DEHP than for that of DMHP.

The 1:1 complex of Ga(III)–DEHP has an absorbance peak at 290 nm (Fig. 3) and the dissociation of the Ga(III) complex was followed by measuring the absorbance decrease of the complex ML with decrease in p[H] (by addition of excess HCl to the reaction solution). This spectrophotometric determination of dissociation of the 1:1 Ga(III)–DEHP complex was used to calculate the 1:1 formation constants, $\log K_{ML} = 14.58$. The first stepwise binding constants (log K_{ML}) for the Al(III) and In(III) chelates of DEHP were determined by equilibrium potentiometric titration of 1:1 solutions, and are log $K_{ML} = 13.42$ and 11.06, respectively (Table 3).

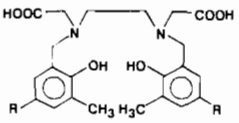
Comparison of the first stepwise binding constants of Ga(III) and In(III), shows that log K_{ML} of Ga(III)–DEHP is about 2.5 log units larger than that of In(III)–DMHP. This difference is mainly due to the larger ionic radius of In(III) and the lower 'hardness' of In(III) compared to Ga(III). The ionic radius effect on stability has been well documented [33]. The In(III)

Table 2
Log protonation constants and stability constants for trivalent metal ions ($\mu = 0.100$ M, 25.0 °C)

	K_1	K_2	K_3	K_4	K_5	β_1					Ref.
						Fe^{3+}	Ga^{3+}	In^{3+}	Al^{3+}	Gd^{3+}	
DEHP	9.93	3.81				15.21	14.58	12.04	13.42	8.09	this work
DEHP	10.1	3.70									[2]
DMHP	9.77	3.68				15.10	13.17	11.85	12.20	7.82	[3]
	9.76	3.62				15.14					[13]
	9.86	3.70					17.07	13.60			[23]
	9.86	3.70							11.91		[20]
EMHP	9.81	3.64					17.24	13.53			[23]
	9.81	3.64							11.75		[20]
	10.3	3.59									[2]
HMHP	9.932	3.59							11.51		[20]
Me_4HBED	13.12	12.99	7.94	4.09	1.4	37.41	34.19	30.72		20.27	[32]
t-BuHBED	13.21	12.95	7.87	4.39	1.7	38.52	36.30	31.26		20.38	[32]



	R_1	R_2
DEHP	C_2H_5	C_2H_5
DMHP	CH_3	CH_3
EMHP	C_2H_5	CH_3
HMHP	C_6H_{13}	CH_3



Me_4HBED R = CH_3
t-BuHBED R = C_4H_9

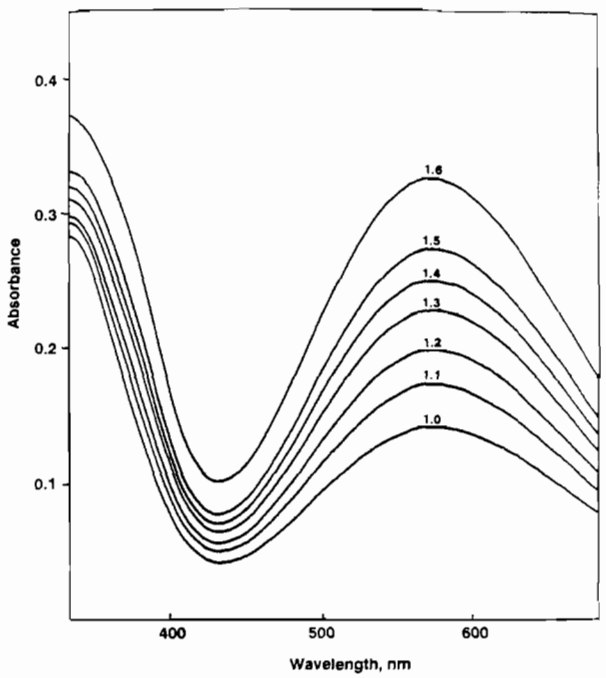


Fig. 2. Absorbance of the 1:1 Fe(III)–DEHP complex at indicated p[H] values; $T_M = 2.467 \times 10^{-4}$ M, $T_L = 2.510 \times 10^{-4}$ M; 25.0 °C, $\mu = 0.100$ M (KCl + HCl).

ion has an approximately 24% higher ionic radius than does Ga(III), which correlates with a decrease in stability constant relative to those of the corresponding Me_4HBED–Ga(III) and t-BuHBED–Ga(III) complexes of some four and five orders of magnitude, respectively (Table 2). Other examples include corresponding In(II) and Ga(III) complexes of EHPG [33], HBED [34] and HPED [35].

Table 3
Stability constants for chelates of DEHP[a] and DMHP with Fe(III), Ga(III), Al(III) and In(III); $\mu = 0.100$ M, 25.0 °C

Quotient	Log Q	
	DEHP	DMHP
[HL]/[H][L]	9.93	9.77
$[H_2L]/[HL][H]$	3.81	3.68
[FeL]/[Fe]/[L]	15.2	15.10
$[FeL_2]/[FeL][L]$	11.76	11.51
$[FeL_3]/[FeL_2][L]$	9.78	9.27
$[FeL_3]/[Fe][L]^3$	36.8	35.88
[GaL]/[Ga][L]	14.6	13.17
$[GaL_2]/[GaL][L]$	11.63	12.26
$[GaL_3]/[GaL_2][L]$	9.84	10.33
$[GaL_3]/[Ga][L]^3$	36.0	35.76
[AlL]/[Al][L]	13.42	12.20
$[AlL_2]/[AlL][L]$	11.64	11.05
$[AlL_3]/[AlL_2][L]$	8.48	9.37
$[AlL_3]/[Al][L]^3$	33.54	32.62
[InL]/[In][L]	12.0	11.85
$[InL_2]/[InL][L]$	11.0	10.63
$[InL_3]/[InL_2][L]$	9.4	9.23
$[InL_3]/[In][L]^3$	32.4	31.71
[GdL]/[Gd][L]	8.09	7.82
$[GdL_2]/[GdL][L]$	6.69	6.04
$[GdL_3]/[GdL_2][L]$	4.98	3.47
$[GdL_3]/[Gd][L]^3$	19.76	17.33

[a]Estimated errors in this research (DEHP) are one digit or less in the last decimal number shown.

As was observed for the Fe(III) chelates, alkyl groups in the 1 and 2 positions of the 3-hydroxy-4-pyridinone ring have a small but measurable effect on the stability constants of Ga(III) and In(III) chelates. The log K_{ML}

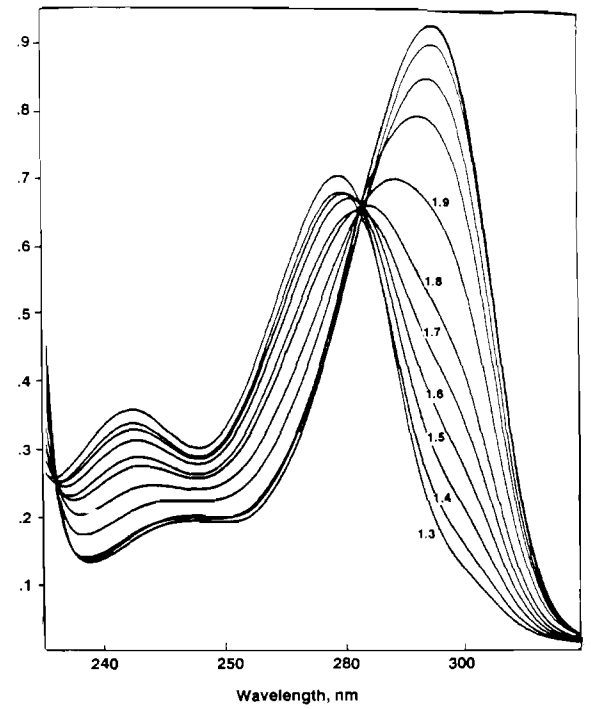


Fig. 3. Absorbance of the 1:1 Ga(III)–DEHP complex at indicated p[H] values; $T_M = 1.500 \times 10^{-4}$ M, $T_L = 1.518 \times 10^{-4}$ M; 25.0 °C, $\mu = 0.100$ M (KCl + HCl).

values for both DEHP–Ga(III) and DEHP–In(III) complexes are a little larger than those of the corresponding values of DMHP determined by Clarke and Martell [3] (Table 2), because the ethyl groups in DEHP have a slightly stronger electron-donating ability than the methyl groups in DMHP. Also it is noted that the present log K_{ML} values for both DEHP–Ga(III) and DEHP–In(III) are significantly lower than those of the corresponding values determined for DMHP chelates by Clevette *et al.* [23] (Table 2). A possible reason for this discrepancy has already been discussed [3].

The species distribution curves of the systems Fe(III)–DEHP and In(III)–DEHP are shown in Figs. 4 and 5. It can be seen that for Fe(III) crossover of the 2:1 to the 3:1 complexes occurs at p[H] 3.5, and that 2:1 complexes are completely converted to the 3:1 complexes around p[H] 7. It is important to point out that the Fe(III) ion is 100% complexed when the p[H] is equal to or greater than 2. For In(III) complexes, which have considerably lower stabilities than those of Fe(III), crossover of the 1:1 to the 2:1 complex occurs at p[H] 2.9 and crossover of the 2:1 to the 3:1 complex occurs at p[H] 4.4. The In(III) ion is only 12% complexed at p[H] 2.

The log stability constant of the 1:1 complex of DEHP and Al(III) is 13.42 (Table 3). Although aquo aluminium(III) is the 'hardest' of the trivalent ions commonly found in biological systems and the environment, and its effective radius of 0.54 Å [19] is considerably

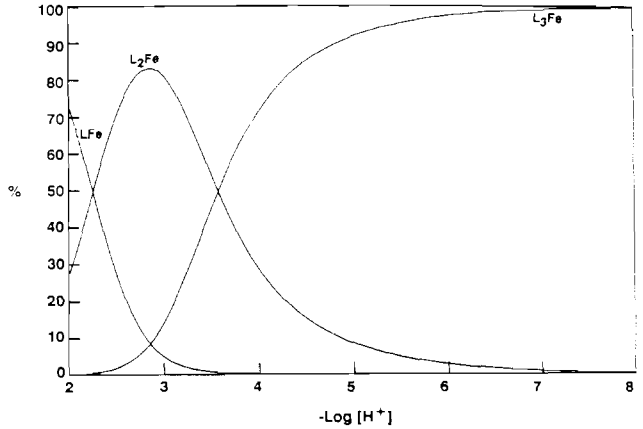


Fig. 4. Distribution of Fe(III) in 1:1, 2:1 and 3:1 complexes with DEHP.

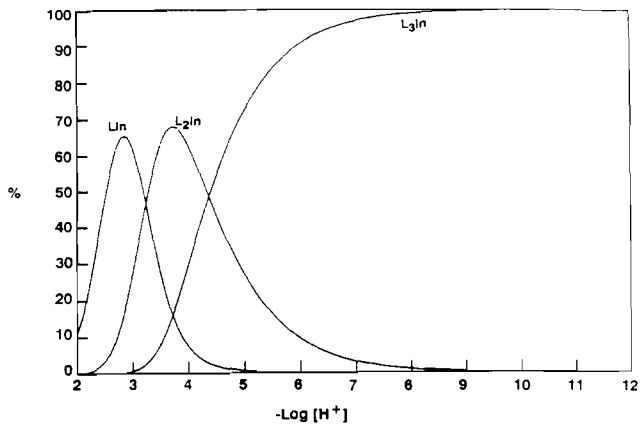


Fig. 5. Distribution of In(III) in 1:1, 2:1 and 3:1 complexes with DEHP; 25.0 °C, $\mu = 0.100$ M KCl; $t = 25.0$ °C, $\mu = 0.100$ M KCl for 2×10^{-3} M In(III).

smaller than those of other commonly encountered trivalent metal ions (such as Fe(III), 0.64 Å; Ga(III), 0.62 Å [19]), its stability constants with trivalent metal ions are usually lower than those of Fe(III) and Ga(III) [28]. Other examples of lower stabilities of Al(III) complexes include corresponding complexes of DMHP [3], EHPG [33], HBED [34] and HPED [35].

By contrast, Gd(III) forms considerably less stable 1:1, 2:1 and 3:1 complexes with DEHP (Table 3). There was no evidence for an ML_4 species of Gd(III)–DEHP even when sufficient excess ligand was provided. Log β_{ML_3} for Gd(III) = 19.76, considerably lower than those of the other trivalent metal ions investigated in this research. Excess ligand prevents the dissociation of the metal complex at alkaline p[H]. Similar results were observed for complexes of Gd(III)–DMHP [3].

3.3. Stability constants of complexes of divalent metal ions

The stability constants of the 1:1 and 2:1 complexes of DEHP with divalent metal ions were determined in

2:1 solutions by potentiometric titration. The p[H] profiles obtained for titration of 2:1 solutions of divalent metal ions with DEHP (Fig. 1) show a strong inflection at $m=2$ for Cu(II), and relatively weak inflections for Ni(II), Co(II) and Zn(II). In order to provide potential six-coordination to the divalent metal ions, 3:1 solutions of DEHP with divalent metal ions were also titrated. The values of the stepwise binding constants were computed by BEST and are presented in Table 4. It was found that Ni(II), Co(II) and Zn(II) also form 3:1 complexes, ML_3. Only 1:1 and 2:1 metal complexes with DEHP are formed with Cu(II). Thus Cu(II) probably has the preferred four-coordinate geometry in the

Table 4
Stepwise stability constants for chelates of DEHP[a] and DMHP with Cu(II), Zn(II), Ni(II) and Co(II); $\mu=0.100$ M, 25.0 °C

Quotient	Log Q	
	DEHP	DMHP
[CuL]/[Cu]/[L]	10.74	10.62
[CuL_2]/[CuL][L]	9.07	8.99
[CoL]/[Co]/[L]	6.84	6.60
[CoL_2]/[CoL][L]	4.97	5.13
[CoL_3]/[CoL_2][L]	3.96	–
[ZnL]/[Zn]/[L]	7.70	7.19
[ZnL_2]/[ZnL][L]	6.09	6.34
[ZnL_3]/[ZnL_2][L]	5.12	–
[NiL]/[Ni]/[L]	7.07	6.92
[NiL_2]/[NiL][L]	5.04	5.21
[NiL_3]/[NiL_2][L]	3.54	2.54

[a]Estimated errors in this research (DEHP) are one digit or less in the last decimal number shown.

Table 5
Atomic coordinates ($\times10^4$) and equivalent isotropic displacement parameters ($Å^2\times10^3$) for **1**

	x	y	z	U_{eq}[a]
In(1)	0	0	2929(1)	33(1)
In(2)	3333	−3333	2931(1)	35(1)
O(1)	1327(9)	390(8)	2433(4)	36(3)
O(2)	800(10)	−523(10)	3487(5)	45(3)
O(3)	2556(9)	−2820(9)	2403(4)	37(3)
O(4)	2032(9)	−3692(9)	3443(5)	44(3)
O(5)	2195(8)	−4421(9)	4529(4)	138(4)
O(6)	2510(27)	−2409(29)	1212(4)	332(15)
C(4)	2215(13)	−754(11)	3461(6)	48(5)
C(13)	1152(9)	−2583(11)	2380(6)	39(4)
C(1)	1903(13)	69(12)	2658(5)	36(4)
C(2)	2664(14)	114(15)	2378(8)	45(5)
N(1)	3243(10)	−247(14)	2684(9)	39(4)
C(3)	2999(10)	−663(11)	3190(6)	37(4)
C(5)	1616(12)	−402(11)	3233(7)	34(4)
C(6)	2975(13)	547(16)	1822(8)	65(6)
C(7)	2516(19)	−75(16)	1336(9)	100(8)
C(8)	4101(16)	−180(16)	2391(8)	63(6)
C(9)	5043(17)	766(20)	2547(10)	106(9)
C(10)	1493(11)	−3336(12)	3193(7)	35(4)
C(11)	601(12)	−3457(12)	3460(7)	35(4)
N1	98(12)	−3073(15)	3244(8)	46(4)
C(12)	367(14)	−2649(13)	2696(9)	60(6)
C(14)	1776(13)	−2882(12)	2639(7)	35(4)
C(15)	326(14)	−3979(13)	4067(6)	46(4)
C(16)	810(16)	−3192(17)	4563(6)	81(6)
C(17)	−829(14)	−3130(17)	3502(8)	57(5)
C(18)	−1763(15)	−4046(13)	3298(9)	66(5)

[a]U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table 6
Bond lengths (Å) and angles (°) for **1**

In(1)–O(1)#1	2.153(11)	In(1)–O(1)	2.153(11)
In(1)–O(1)#2	2.153(1)	In(1)–O(2)	2.198(12)
In(1)–O(2)#1	2.198(12)	In(1)–O(2)#2	2.198(12)
In(2)–O(3)	2.121(12)	In(2)–O(3)#3	2.121(12)
In(2)–O(3)#4	2.121(12)	In(2)–I(4)	2.150(11)
In(2)–O(4)#3	2.150(11)	In(2)–O(4)#4	2.150(11)
O(1)–C(1)	1.32(2)	O(2)–C(5)	1.32(2)
O(3)–C(14)	1.28(2)	O(4)–C(10)	1.34(2)
C(4)–C(3)	1.31(2)	C(4)–C(5)	1.39(2)
C(13)–C(12)	1.37(2)	C(13)–C(14)	1.40(2)
C(1)–C(2)	1.31(2)	C(1)–C(5)	1.47(2)
C(2)–C(6)	1.41(2)	C(2)–N(1)	1.45(2)
N(1)–C(3)	1.29(2)	N(1)–C(8)	1.44(2)
C(6)–C(7)	1.41(3)	C(8)–C(9)	1.50(3)
C(10)–C(14)	1.41(2)	C(10)–C(11)	1.43(2)
C(11)–N(1)	1.29(2)	C(11)–C(15)	1.56(2)
N(1)–C(12)	1.39(3)	N(1)–C(17)	1.51(2)
C(15)–C(16)	1.56(2)	C(17)–C(18)	1.50(2)
O(1)#1–In(1)–O(1)	94.4(3)	O(1)#110In(1)9O(1)#2	94.4(3)
O(1)–In(1)–O(1)#2	94.4(3)	O(1)#1–In(1)–O(2)	101.2(4)
O(1)–In(1)–O(2)	77.4(4)	O(1)#2–In(1)–O(2)	162.8(4)
O(1)#1–In(1)–O(2)#1	77.4(4)	O(1)–In(1)–O(2)#1	162.8(4)
O(1)#2–In(1)–O(2)#1	101.2(4)	O(2)–In(1)–O(2)#1	89.2(4)
O(1)#1–In(1)–O(2)#2	162.8(4)	O(1)–In(1)–O(2)#2	101.2(4)
O(1)#2–In(1)–O(2)#2	77.4(4)	O(2)–In(1)–O(2)#2	89.2(4)
O(2)#1–In(1)–O(2)#2	89.2(4)	O(3)–In(2)–O(3)#3	90.4(4)
O(3)–In(2)–O(3)#4	90.4(4)	O(3)#3–In(2)–O(3)#4	90.5(4)
O(3)–In(2)–O(4)	77.3(4)	O(3)#3–In(2)–O(4)	162.3(4)
O(3)#4–In(2)–O(4)	102.1(4)	O(3)–In(2)–O(4)#3	102.1(4)
O(3)#3–In(2)–O(4)#3	77.3(4)	O(3)#4–In(2)–O(4)#3	162.3(4)
O(4)–In(2)–O(4)#3	92.8(4)	O(3)–In(2)–O(4)#4	162.3(4)
O(3)#3–In(2)–O(4)#4	102.1(4)	O(3)#4–In(2)–O(4)#4	77.3(4)
O(4)–In(2)–O(4)#4	92.8(4)	O(4)#3–In(2)–O(4)#4	92.8(4)
C(1)–O(1)–In(1)	114.3(9)	C(5)–O(2)–In(1)	111.2(10)
C(14)–O(3)–In(2)	114.5(9)	C(10)–O(4)–In(2)	110.6(9)
C(3)–C(4)–C(5)	122.7(14)	C(12)–C(13)–C(14)	119(2)
C(2)–C(1)–O(1)	122.8(14)	C(2)–C(1)–C(5)	121(2)
O(1)–C(1)–C(5)	116.5(14)	C(1)–C(2)–C(6)	124(2)
C(1)–C(2)–N(1)	117(2)	C(6)–C(2)–N(1)	120(2)
C(3)–N(1)–C(8)	119(2)	C(3)–N(1)–C(2)	123.0(14)
C(8)–N(1)–C(2)	118(2)	N(1)–C(3)–C(4)	120.6(14)
O(2)–C(5)–C(4)	124(2)	O(2)–C(5)–C(12)	120.2(14)
C(4)–C(5)–C(1)	116.1(14)	C(7)–C(6)–C(2)	117(2)
N(1)–C(8)–C(9)	112(2)	O(4)–C(10)–C(14)	119.3(13)
O(4)–C(10)–C(11)	121(2)	C(14)–C(10)–C(11)	119.4(14)
N(1)–C(11)–C(10)	122(2)	N(1)–C(11)–C(15)	121(2)
C(10)–C(11)–C(15)	117(2)	C(11)–N(1)–C(12)	117(2)
C(11)–N(1)–C(17)	126(2)	C(12)–N(1)–C(17)	116(2)
C(13)–C(12)–N(1)	124(2)	O(3)–C(14)–C(13)	125.0(14)
O(3)–C(14)–C(10)	117.8(14)	C(13)–C(14)–C(10)	117(2)
C(11)–C(15)–C(16)	110.6(13)	C(18)–C(17)–N(1)	112(2)

Symmetry transformations used to generate equivalent atoms: #1: $-x+y$, $-x$, z; #2: $-y$, $x-y$, y; #3: $-x+y+1$, $-x$, z; #4: $-y$, $x-y-1$, z.

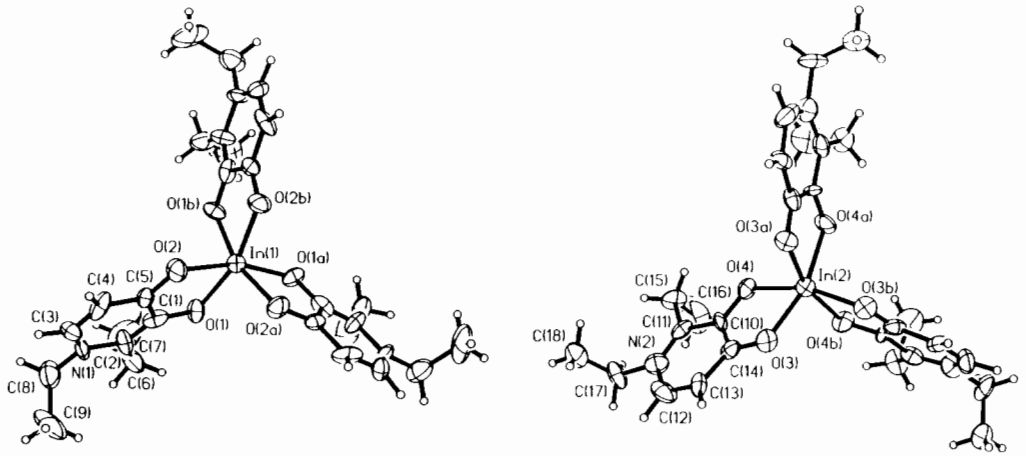


Fig. 6. ORTEP diagram of the structure of the 1:3 In(III) DEHP chelate and atom numbering scheme.

complex formed with two molar equivalents of DEHP, as has been observed for complexes with other bidentate ligands [3, 28]. Co(II), Ni(II) and Zn(II) form six-coordinate complexes with related ligands such as maltol and HMP (1-methyl-3-hydroxy-4-pyridinone) [28]. However, ML_3 species were not found for Co(II) and Zn(II) complexes with DMHP [3]. The reason for this difference between these two ligands is not obvious. The observed order of stability for the bivalent DEHP complexes, Cu(II) > Zn(II) > Ni(II) > Co(II), is unusual because most complexes show a different relative order of stability constants (Cu(II) > Ni(II) > Co(II), Zn(II)) [28]. However, the same order as the one observed with DEHP was also observed for DMHP complexes with bivalent metal ions [3].

In conclusion, it seems that DEHP is a little more basic than DMHP, and forms somewhat more stable chelates. However, this difference does not seem to be large enough to explain the difference in effectiveness of these two ligands in the treatment of iron overload. The main effect is probably due to differences in physiological properties, such as lipid solubility, membrane permeability, and bioavailability for both the ligands and their iron(III) complexes.

The crystal data and structure refinement of the 1:3 In(III) chelate of DEHP are summarized in Table 1. The atomic coordinates and equivalent isotropic displacement parameters are given in Table 5, while bond lengths and angles are listed in Table 6. Fig. 6 is an ORTEP diagram of the structure of the 3:1 1,2-diethyl-3-hydroxy-4-pyridinone complex, with the numbering scheme employed in Table 6. See also Supplementary material.

The δ and λ isomers of **1** crystallize in the space group $R\bar{3}$ with the In(III) atoms located on crystallographic threefold axes. The coordination of the In(III) atoms is best described as a distorted octahedron, with the oxygen atoms from three hydroxypyridinone groups occupying the six coordination sites. Only one of the three coordination ligands, on each isomer, is unique with the remaining ligands generated by the inherent symmetry. The ligands are specially arranged in such a fashion as to place the ethyl moieties on the same side of the complex. The bite angle (77.1(1)°) of the ligands is consistent with those reported for similar coordinated ligands [36,37]. The two isomers may be related by a pseudo inversion center. Water molecules form weak hydrogen bonds with the oxygen atoms of the ligands (O3...O6, 2.82(1); O4...O5, 2.80(1) Å), where the O5 and O6 atoms (not shown) are the oxygen atoms of the water molecules.

4. Supplementary material

The table of anisotropic displacement parameters, isotropic displacement parameters, hydrogen coordinates and the structures factor tables (11 pages) are available from the authors on request.

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