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Synthesis and characterization of metal complexes with *N,N''*-bis(2-hydroxybenzyl)-diethylenetriamine-*N,N',N''*-triacetic acid and the stabilities of its complexes with divalent and trivalent metal ions

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Abstract

The synthesis, characterization and metal ion affinities of a new multidentate ligand, *N,N''*-bis(2-hydroxybenzyl)-diethylenetriamine -*N,N',N''*-triacetic acid (HBDT) are described. Protonation constants of the ligand and stability constants of its 1:1 complexes with trivalent and divalent metal ions are determined by potentiometric, spectrophotometric methods and by potentiometric determination of ligand–ligand competition. The stabilities of complexes formed by the trivalent ions Fe^{3+}, Ga^{3+} and In^{3+} with HBDT are compared with those of structurally related ligands. While this new octadentate ligand, which contains two phenolate donor groups, forms stable complexes with trivalent metal ions its stability constants are significantly lower than those of hexadentate *N,N'*-bis(2-hydroxybenzyl)-ethylenediamine-*N,N'*-diacetic acid (HBED) which also contains two phenolate donor groups. The difference is rationalized on the basis of ligand structure and the probable arrangement of donor groups around the metal center.

Keywords: Metal ion complexes; Multidentate ligand complexes; Chelate complexes

1. Introduction

Highly stable chelates of Fe(III), Ga(III) and In(III) are of considerable interest because the complexes formed with the radioactive isotopes $^{67,68}Ga$ and ^{111}In may be employed as radiopharmaceuticals for diagnostic imaging applications [1–5], and the high spin Fe(III) complexes have possible applications as paramagnetic contrast agents for magnetic resonance imaging (MRI) [6,7]. Also, ligands that form highly stable iron complexes may be of interest as drugs for the removal of iron from the body in cases of iron overload [8]. The stabilities of trivalent metal ion complexes of the amino polycarboxylates such as ethylenediaminetetraacetic acid (EDTA, **1**) and diethylenetriaminepentaacetic acid (DTPA, **2**) are too small for the complexes to be used in vivo [9]. It was found that one way to increase the affinities of the ligands for these trivalent metal ions is to replace the carboxylate groups of EDTA with hard phenolate donors [10–12]. For example, *N,N'*-bis(2-hydroxybenzyl)-ethylenediamine-*N,N'*-diacetic acid (HBED), in which two carboxylate groups on EDTA are replaced by two phenolate groups, **3**, has stability constants for its Fe(III) and Ga(III) complexes of about 10^{39} and $10^{38.5}$ [11], which are about 14 and 13 log units higher than corresponding EDTA complexes. Other examples include EHPG [12], Me_4HBED [13] and HPED [14]. If the same strategy is employed with DTPA, with two carboxylate groups of DTPA replaced by two hard phenolate donors, one would have the ligand *N,N''*-bis(2-hydroxybenzyl)-diethylenetriamine-*N,N',N''*-triacetic acid (HBDT, **4**). This paper is a report of the synthesis of this new ligand, HBDT, and de-

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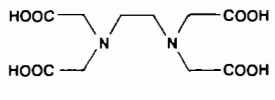
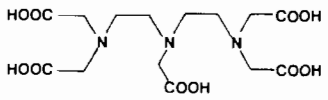
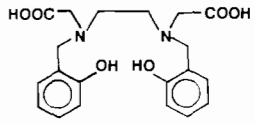
termination of the stability constants of the chelates it forms with trivalent and divalent metal ions.



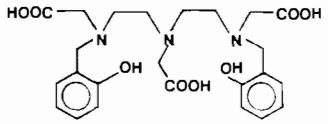
1 EDTA



2 DTPA



3 HBED



4 HBDT

2. Experimental

2.1. Synthesis and characterization of the ligand

The ligand was prepared by the procedure which is shown in Scheme 1. The starting material, *N,N''*-bis(salicyclidene)diethylenetriamine (**I**), was prepared by the method of Coleman and Taylor [15].

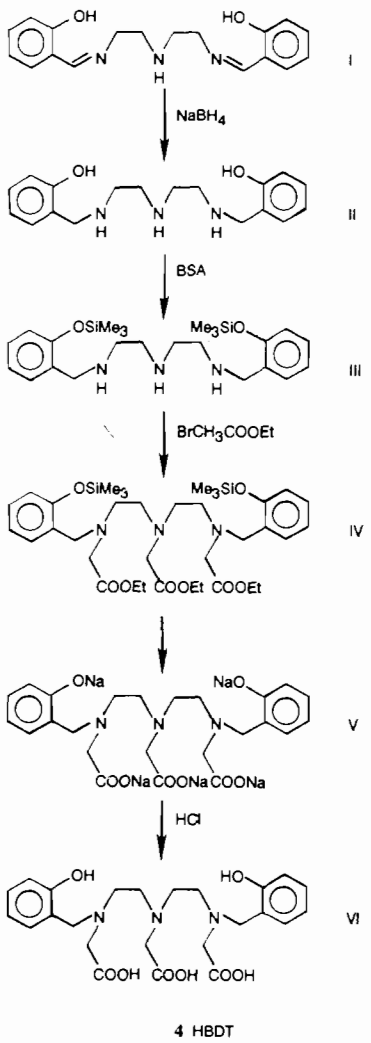
2.1.1. *N,N''-Bis(2-hydroxybenzyl)-2,5,8-triazanonane (II)*

In a 1 l flask **I** (31.1 g, 0.1 mol) was dissolved in 150 ml of ethanol and to this was added with stirring sodium borohydride (8.4 g, 0.22 mol) in batches over a period of 2 h at room temperature, and the mixture was heated on a water bath for 2 h. Ethanol was removed by evaporation and the residue was treated with water (1 l). The resulting oil was taken up in dichloromethane (200 ml), the solution was washed with water, and then dried over anhydrous magnesium sulfate. Evaporation of the solvent gave **II** as a pale yellow oil. Yield 26.8 g, 85%.

A portion of the oil was dissolved in dilute hydrochloric acid and the solution was evaporated to dryness. The trihydrochloride of **II** was obtained as a crystalline solid after the residue was treated with ethanol and it was recrystallized from dilute ethanol. *Anal.* Calc. for $C_{18}H_{25}N_3O_2 \cdot 3HCl$: C, 50.89; H, 6.66; N, 9.89. Found: C, 50.71; H, 6.70; N, 9.94%.

2.1.2. *N,N''-Bis(2-trimethylsilyloxybenzyl)-2,5,8-triazanonane-N,N',N''-triacetic acid triethylester (IV)*

The well-dried crude free base of **II** (21.4 g, 0.068 mol) was dissolved in dry benzene (330 ml) by heating on a water bath. To the turbid solution at room temperature was added *N,O*-bis(trimethylsilyl)-acetamide (BSA) (34.7 g, 0.17 mol) with stirring. The turbidity disappeared in a few minutes and the solution was heated on a water bath for 1 h. Ethyl bromoacetate (38 g, 0.22 mol) and triethylamine (25.3 g, 0.25 mol) were added to the solution at room temperature and



Scheme 1.

the mixture was heated on a water bath for 3 h with stirring. The resulting pale yellow solution containing a white precipitate was washed with water (total 1 l), and the benzene was evaporated on a rotary evaporator to give crude **IV** as a pale brown oil. Yield 32 g, 73%.

2.1.3. *N,N''-Bis(2-hydroxybenzyl)-2,5,8-triazanonane-N,N',N''-triacetic acid (VI)*

The crude **IV** (32 g, 0.049 mol) was heated with 100 ml of a solution of 10% sodium hydroxide in 50% water–methanol (vol./vol.) on a water bath for 3 h under nitrogen. From the pale brown solution which resulted, methanol was removed by evaporation and the pH of the residual solution was brought to 2 with hydrochloric acid. After the reaction mixture was allowed to stand for 12 h, the resulting pale brown oily precipitate was separated from the aqueous supernatant by decantation and the oil was washed twice with 50 ml of water. The oil was dissolved in 500 ml of hot water, treated with activated charcoal and filtered. The filtrate was concentrated to 50 ml by evaporation and

the resulting oil was separated from the supernatant solution by decantation. The oil was dried by evaporation at 60 °C, dissolved in 100 ml of ethanol and 200–300 ml of acetone was added to the solution. After the reaction mixture was allowed to stand for 12 h, the crystalline material obtained was collected by filtration, washed with acetone, and dried over phosphorous pentoxide under vacuum. Yield 12 g, 49%. The pale yellow hygroscopic crystalline powder contained acetone of crystallization. *Anal.* Calc. for $C_{24}H_{31}N_3O_8 \cdot C_3H_6O \cdot 0.3HCl$: C, 57.94; H, 6.74; N, 7.51; Cl, 2.09. Found: C, 57.77; H, 6.53; N, 7.77%.

2.2. Potentiometric measurements

Equilibrium potentiometric determinations of the ligand protonation constants and its binding constants for metal ions in 1:1 molar ligand to metal ratios were carried out at 25.0 °C, 0.100 M (KCl), and the constants were calculated from potentiometric data with the use of the program BEST. Details of the potentiometric method are described in Ref. [16].

The potentiometric apparatus consists of a glass jacketed titration cell, a constant temperature bath (Haake, 25.0 °C), glass and reference (calomel) 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 the 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.

The potentiometric determination of the ligand protonation constants was carried out in a solution with approximately 1×10^{-3} M concentration of ligand. The metal chelates of HBDT were prepared as 1:1 complexes in solutions having approximately 1×10^{-3} M concentrations of metal ion and ligand. Most of the metal binding constants were calculated from direct potentiometry, but the binding constants of the complexes formed with Fe(III) were determined at about 3×10^{-4} M by spectrophotometric titration, and with In(III) and Ga(III) ions were determined with ligand–ligand competition (1:1:1 molar ratio of Ga(III):EDTA:HBDT) potentiometric titration methods, since their complexes were formed completely over the p[H] range of accurate direct potentiometric p[H] measurements (2–12), making determination of their stabilities by direct potentiometric titration impossible. For In(III)–HBDT and Ga(III)–HBDT, the log K_{ML} values were determined by using 1:1:1 molar HBDT:Ga(III):EDTA and HBDT:In(III):EDTA systems at about 1×10^{-3} M, respectively. These mixtures were titrated potentiometrically and the p[H] profiles obtained were used for the calculation of the stability constants of the 1:1 complexes of HBDT:metal-ion, with the protonation constants of EDTA and stability constants of complexes for the metal ions with EDTA taken from the literature [17]. The protonation constants of the In(III)–HBDT and Ga(III)–HBDT chelates were determined from the p[H] profiles of the 10^{-3} M 1:1 In(III)–HBDT and Ga(III)–HBDT solutions, respectively.

2.3. Spectrophotometric measurements

Because not enough of the totally deprotonated species of HBDT was present during the potentiometric titrations over the p[H] range 2.0–12.0, UV–Vis spectrophotometric titrations at various $-\log[H^+]$ values were performed to determine the first protonation constant of HBDT. The spectrophotometric measurements were recorded with a Perkin-Elmer 553 Fast Scan UV–Vis spectrophotometer. Conditions used were approximately 1×10^{-4} M for HBDT, 25.0 °C, and cells with a 1.000 cm path length were used. The log K value for the equilibrium quotient [HL]/[H][L] was calculated from the absorbance at wavelength 295 nm and at p[H] values from 11.75 to 12.53 with the in-house FORTRAN computer program ABSPKAS. From the analytical concentration of the ligand and the log[H] values, this program determines the equilibrium constants and the extinction coefficient necessary to calculate the absorbance values that would correspond best to the observed absorbance values for a given spectrophotometric titration.

Because HBDT forms complexes with Fe(III) completely, even at p[H] as low as 2, the binding constants of the complexes with Fe(III) ions were determined by spectrophotometric titration. The complex Fe(III)–HBDT has its maximum absorbance at 522 nm ($\epsilon_{FeHL} = 1800\ M^{-1}\ cm^{-1}$). Fe(III) was dissociated from the Fe(III) complex by lowering the p[H] of a series of solutions containing about 3×10^{-4} M Fe(III) and one equivalent of ligand. Increments of 0.128 M HCl were added down to p[H] 1.00. For purposes of calculation of the Fe(III) stability constant, the absorbances at 522 nm were used in the p[H] range 1.00–1.87, for which the ionic strength was adjusted to 0.100 M in [HCl] + [KCl]. The concentrations of the metal ion, ligand and complex species were calculated from mass balance equations. The protonation equilibrium constants of the Fe(III)–HBDT complex were determined from the potentiometric p[H] profile and these values were employed for the calculation of log K_{ML}.

3. Results and discussion

3.1. Protonation constants

In the potentiometric p[H] profile for HBDT shown in Fig. 1. there are two distinct areas of interest. The

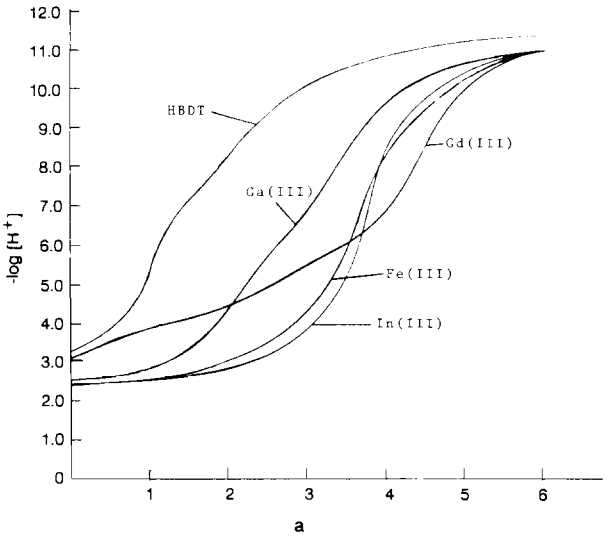


Fig. 1. p[H] profiles of HBDT and for 1:1 molar ratios of ligand to trivalent metal ion at 1.00×10^{-3} M; a = moles of base added per mole of ligand; μ = 0.100 M (KCl), t = 25.0 °C.

first, at $a<3$ (a = moles of base per mole of ligand), corresponds to protonation of the amino and carboxylate groups. The second area, at $a>3$, corresponds to protonation of the phenolate oxygens. The protonation constants are assigned on the basis of the order of basicity: phenolate > amino > carboxylate. These protonation constants (except for that of the first phenolate group) were determined by standard potentiometric titration and the results are listed in Table 1.

The protonation constant for the first phenolate oxygen was determined by spectrophotometric titration because the p[H] was found to be too high to be measured accurately by potentiometry. As shown in Fig. 2, at low p[H] values a maximum absorption at 275 nm is observed and is assigned to the protonated phenolic group. As the p[H] is increased this band shifts towards 295 nm and increases in intensity, indicating conversion of phenolic hydroxy groups into the phenolate anions. The measured absorbances and calculated p[H] values are shown in Fig. 2. This protonation constant was calculated by the computer program ABSPKAS, and the result is given in Table 1. Extinction coefficients at 295 nm ($\epsilon_L = 7890$ M^{-1} cm^{-1} and $\epsilon_{LH} = 3940$ M^{-1} cm^{-1}) were calculated from the absorbance at wavelength 295 nm (not shown) at very high p[H] so that all the phenolate groups in the ligand were deprotonated.

The first two protonation constants of HBDT, K_1^H, K_2^H, are considerably higher than those of DTPA (Table 1) because of the high basicities of phenolate oxygens in HBDT. The fact that the protonation constants are similar in magnitude to those of HBED (Table 1) reflects the fact that the phenolate oxygens of these two ligands have similar basicities and are in similar molecular environments. The phenolate protonation constants of HBDT are a little higher than those of HPED (*N,N'*-bishydroxyphenylethylenediamine-*N,N'*-diacetic acid), because of the greater electronegativity of nitrogen relative to carbon, which has been discussed previously [14]. HBDT2H (1,9-bis(2-hydroxyphenyl)-2,5,8-triazanonane), which involves the replacement of two carboxylate groups in HBDT by two hydrogens, has 11.1 and 10.4 (Table 1) as the logs of the first two protonation constants, indicating that basicities of the phenolate groups of HBDT are increased by the other functional groups present, possibly because of hydrogen bonding. A similar effect was observed for HBED relative to *N,N'*-bis(2-hydroxybenzyl)-2,5,8-triazanonane [17].

The protonation constants of HBDT, K_3^H, K_4^H, K_5^H, which are assigned to protonation of the amino groups, are significantly lower than those of DTPA (see Table 1). This is due to the fact that the protonated phenolate groups in HBDT are more electron-withdrawing than are carboxylate groups in DTPA and the protonation constants, which measure the affinities of the amino groups for hydrogen ions, are therefore lower. A similar effect was observed for EDTA and HBED [11]. The lowest measurable protonation constant of HBDT, K_6^H, which was determined by addition of excess HCl to the ligand, H_5L, is probably due to protonation of one of the carboxylate groups. A similar protonation constant was found for DTPA.

3.2. Stability constants for divalent metal ions

Direct potentiometric titration was used to determine the stability constants of the complexes formed by HBDT

Table 1
Protonation constants of HBDT[a], DTPA, HBED and EDTA (μ = 0.100 M (KCl), t = 25.0 °C)

	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6	Reference
HBDT	12.43	10.98	9.32	7.17	3.95	1.7	this work
DTPA	10.56	8.64	4.29	2.74	2.11		[9]
HBED	12.64	11.03	8.34	4.40	2.24		[11]
EDTA	10.19	6.13	2.69	2.00			[9]
HPED	12.28	10.44	6.15	3.58	1.92		[14]
HBDT2H	11.06	10.41	9.09	7.94	4.18		[18]

[a]Uncertainties in the equilibrium constants are estimated as ±0.5 of the last significant number.

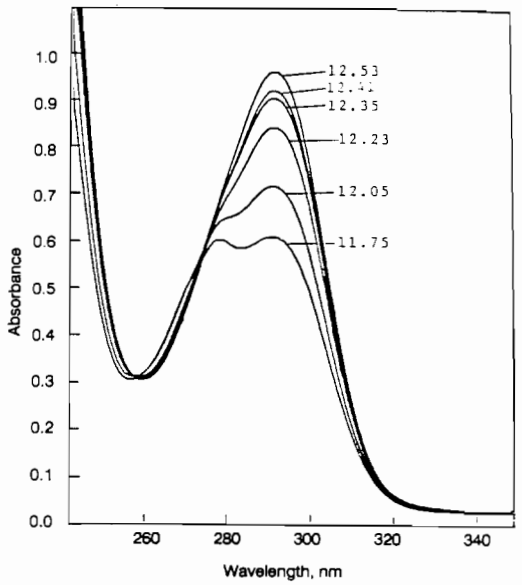


Fig. 2. Absorbance of HBDT at indicated p[H] values; $T_L = 0.844 \times 10^{-4}$ M; $\mu = 0.100$ M (KCl), $t = 25.0$ °C.

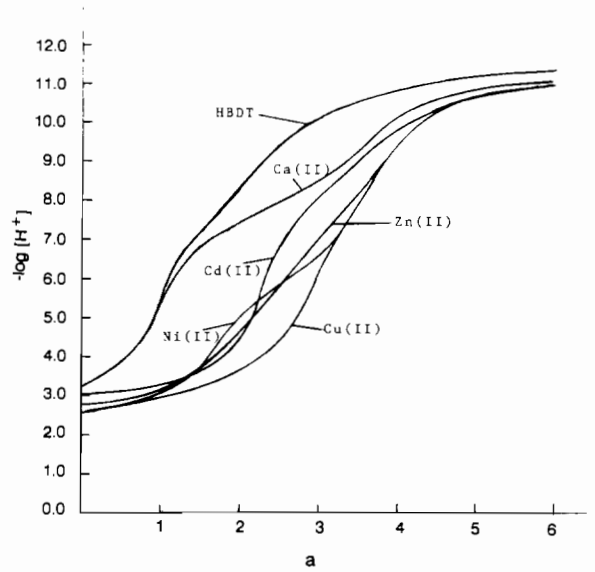


Fig. 3. p[H] profiles for 1:1 molar ratios of HBDT to divalent metal ions at 1.00×10^{-3} M; a = moles of base added per mole of ligand; $\mu = 0.100$ M (KCl), $t = 25.0$ °C.

and the divalent metal ions Zn(II), Co(II), Ni(II), Ca(II), Cu(II) and Cd(II). The experiments were run in 1:1 metal–ligand systems, and some of the p[H] profiles are shown in Fig. 3. All of the divalent metal ions but Ca^{2+} were found to combine readily with HBDT to form deprotonated (ML) and multiprotonated (MH_nL) complexes in concentrations depending on the p[H] of the solution. Calcium(II) has the lowest coordination tendency toward the ligand for the divalent metal ions studied. Its protonation constants are larger than those of the other divalent metal ions, as expected for the least stable complex. Table 2 contains the values for

Table 2
Log stability constants of divalent metal complexes of HBDT[a] ($\mu = 0.100$ M (KCl), $t = 25.0$ °C)

	Cu(II)	Co(II)	Zn(II)	Ni(II)	Cd(II)	Ca(II)
[ML]/[M][L]	20.45	18.16	17.36	19.43	16.74	8.47
[MHL]/[ML][H]	10.15	10.26	10.22	10.20	10.10	10.85
$[MH_2L]/[MHL][H]$	8.06	7.76	8.43	7.63	8.69	9.20
$[MH_3L]/[MH_2L][H]$	4.64	5.79	5.73	5.94	4.87	7.58
$[MH_4L]/[MH_3L][H]$		2.9	3.2			6.8

[a]Uncertainties in the equilibrium constants are estimated as ±0.5 of the last significant number.

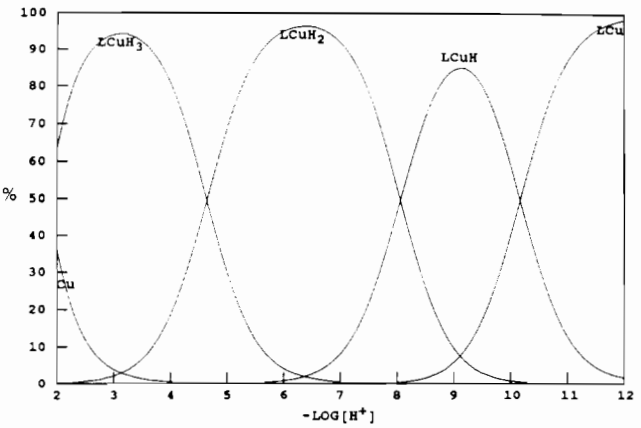


Fig. 4. Distribution curves indicating the species present as a function of p[H] in a system containing a 1:1 molar ratio of Cu(II): HBDT. $T_L = T_M = 2.00 \times 10^{-3}$ M. H_5L = HBDT, $Cu = Cu^{2+}$, % = percent of total concentration of HBDT or Cu^{2+}, set at 100%.

the log stability constants and log protonation constants of the divalent metal ion chelates.

The distribution curves of the 1:1 HBDT–Cu(II) system shown in Fig. 4 illustrate the relative importance of the protonated and deprotonated forms of the chelate. The curves indicate that the metal ion is about 65% complexed at p[H] 2. The triprotonated form, MH_3L, is converted to the diprotonated chelate at around p[H] 4.6, which in turn is converted at about p[H] 8.1 to the monoprotonated form, MHL. Above p[H] 8 the deprotonated complex ML begins to form and is the predominant species at p[H] 10.5 and above in aqueous solution.

The order of stability constants for chelates of the divalent metal ions is Cu(II) > Ni(II) > Co(II) > Zn(II). The same order of stability constants for chelates of the divalent metal ions was found for DTPA, HBED and EDTA [11,17]. Comparison of the stability constants of the divalent metal ion complexes of HBDT and DTPA shows that the HBDT ligand has a slightly higher affinity toward divalent metal ions than DTPA does. This correlates with the fact that HBDT has higher protonation constants than DTPA does [17], an indication that the ligand is more basic.

3.3. Stability constants for trivalent metal ions

In the case of Fe(III)–HBDT, which is more than 97% formed at p[H] 2, it is necessary to turn to spectrophotometry and very low p[H] to accurately determine the stability constant. Fig. 5 shows the optical absorption spectra for the L→M charge-transfer band of the Fe(III)–HBDT complex measured between p[H] 1.06 and 1.87. The absorbances at 522 nm, the calculated p[H] values, the measured extinction coefficient of 1800 M^{-1} cm^{-1}, and the value of K_{MHL} which was obtained by potentiometric titration, were employed in the calculation of K_{ML}. This was accomplished by solving the mass balance Eqs. (1)–(3)

$$A = [ML](\epsilon_{ML} + \epsilon_{MLH} K_{MLH}[H^+]) \quad (1)$$

$$T_L = [ML] + [MLH] + A_1[L] \quad (2)$$

$$T_M = [M] + [ML] + [MLH] \quad (3)$$

$$K_{ML} = [ML]/[M][L] \quad (4)$$

where A is absorbance at 522 nm, H_5L is HBDT, T_L and T_M are total HBDT and metal concentrations, respectively. A_1 is defined by Eq. (5)

$$A_1 = 1 + \beta_1[H^+] + \beta_2[H^+]^2 + \ldots + \beta_6[H^+]^6 \quad (5)$$

where β_n are overall ligand protonation constants. The absorbance maximum of Fe(III)–HBDT, 522 nm, decreases with decreasing p[H] and does not shift to higher wavelength as the p[H] is lowered. Considering the fact that Fe(III)–DTPA has no absorbance in the visible range, the absorbance (522 nm) of Fe(III)–HBDT (two carboxylate groups in DTPA were replaced by two phenolate groups) is probably due to metal–phenolate bonding. When the p[H] is lowered, the absorbance decrease corresponds to the formation of the protonated phenolate group and the loss of metal–phenolate bonding.

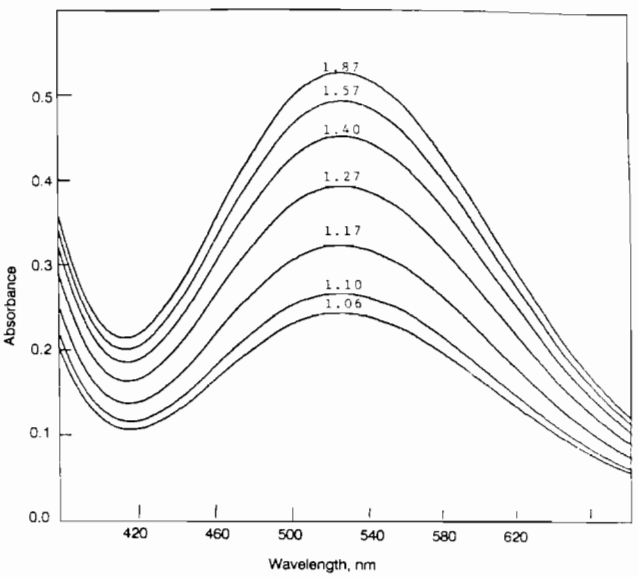


Fig. 5. Absorbance of HBDT–Fe(III) at indicated p[H] values; $T_M = 3.55 \times 10^{-4}$ M; $T_L = 3.56 \times 10^{-4}$ M; $\mu = 0.100$ M (KCl), $t = 25.0$ °C.

Table 3
Log stability constants of trivalent metal complexes of HBDT[a] ($\mu = 0.100$ M (KCl), $t = 25.0$ °C)

	Fe(III)	Ga(III)	In(III)	Gd(III)
[ML]/[M][L]	30.44	26.11	28.96	20.20
[MHL]/[ML][H]	8.81	10.41	9.63	8.37
$[MH_2L]/[MHL][H]$	4.89	7.99	4.91	5.84
$[MH_3L]/[MH_2L][H]$	1.20	5.58	1.73	4.69

[a]Uncertainties in the equilibrium constants are estimated as ±0.5 of the last significant number.

The stability constants obtained are listed in Table 3. Although the potentiometric p[H] curve in Fig. 1 does not show that the Fe(III) titration is a strong acid curve, its species distribution curves (Fig. 6) show that there is more than 97% metal complex formation with HBDT at p[H] 2, because of the formation of the multi-protonated complexes MH_3L and MH_2L. As shown in Fig. 6, MH_3L is formed at p[H] < 1 and is converted to MH_2L between p[H] 1 and 2. The diprotonated complex is converted to the monoprotonated complex which begins to predominate at p[H] 4.9, which in turn is converted to the completely deprotonated complex, but which is not the major species until the p[H] is above 8.8.

It is noteworthy that the stability constant of Fe(III)–HBDT ($10^{30.4}$) is only about 2.4 log units higher than that of DTPA ($10^{28.0}$), and that the phenolate groups do not greatly increase the affinity of the ligand for the trivalent Fe(III) ion. Replacing two carboxylate groups in EDTA by two phenolate groups (HBED) increases the stability constant of the Fe(III) chelate by about fourteen log units. Other examples include EHPG [12], HPED [14] and Me_4HED [13], all of which contain two phenolate groups, and all of which show a significant increase (8 to 13 log units) in the stability constants of the Fe(III) chelate over those of the carboxylate analogs. The hard acid, Fe^{3+}, prefers coordination with hard phenolate oxygen donors rather than with the softer carboxylate functional groups.

The phenolate Fe^{3+} complex has an intense absorption band which has a maximum at about 500 nm [11–14]. Usually the ligands which contain two phenolate groups form complexes with Fe^{3+} with an extinction coefficient around 3500 to 4100 M^{-1} cm^{-1}. However the complex of Fe(III) with HBDT has an extinction coefficient around 1800 M^{-1} cm^{-1} at low p[H]. Studies of the absorption spectra for the complexes of Fe(III) with ligands containing two phenolate groups such as EHPG and HPED showed that when the p[H] is decreased from 2.0 to 1.0 the maximum absorbance

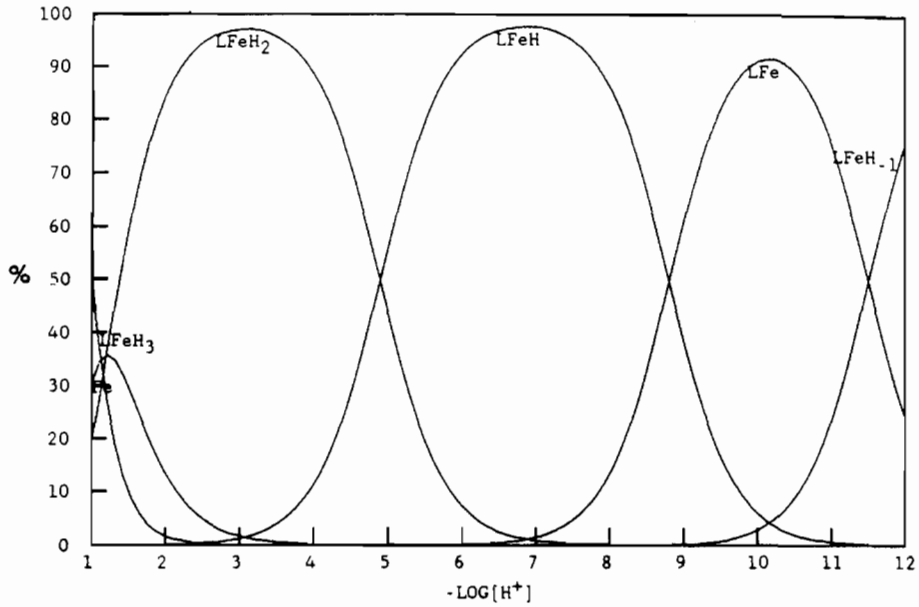


Fig. 6. Distribution curves indicating the species present as a function of p[H] in a system containing a 1:1 molar ratio of Fe(III):HBDT. $T_L = T_M = 2.00 \times 10^{-3}$ M. L = HBDT, Fe = Fe^{3+}, % = percent of total concentration of HBDT or Fe^{3+}, set at 100%.

shifts slightly to lower wavelength, corresponding to conversion of the species which has two phenolate oxygens bound to the metal ion to the protonated metal chelate species, which only has one phenolate oxygen bound to the metal ion. Also the protonated metal chelate has an extinction coefficient of about 1800 M^{-1} cm^{-1}. For the complexes of HBDT–Fe(III), when the p[H] is decreased from 1.87 to 1.06 the maximum absorbance does not shift to lower wavelength, as shown in Fig. 5. This fact, and the low extinction coefficient of about 1800 M^{-1} cm^{-1}, indicates that only one phenolate group binds to the metal ion, while the other phenolate group is protonated at low p[H] (<1.87).

Interesting questions are why the second phenolate group does not bind to the metal ion and what is the p[H] at which it begins to bind Fe(III). From the spectra of the solution at p[H] 1.9 to 11.6, it was found that the intensity of the absorbance increases with increasing p[H] and reaches a maximum at p[H] 6.9. When the p[H] is increased beyond 6.9, the intensity of absorbance decreases. Meanwhile the wavelength of the maximum absorbance also decreases when the p[H] is increased from 3.0 to 11.6. Fig. 7 shows the spectra of the 1:1 complex solution at p[H] 3.0, 6.9 and 10.2. It is suggested that the increase of the absorbance intensity at p[H] 3.0 to 6.9 is due to the second phenolate group binding to the metal ion, and that the decrease of the absorbance intensity at p[H] > 6.9 is due to the second phenolate group being displaced from coordination with the metal ion.

The coordination sites of the complexes that appear in the distribution curves (Fig. 6) may be assigned on the basis of the spectral analysis. The diprotonated

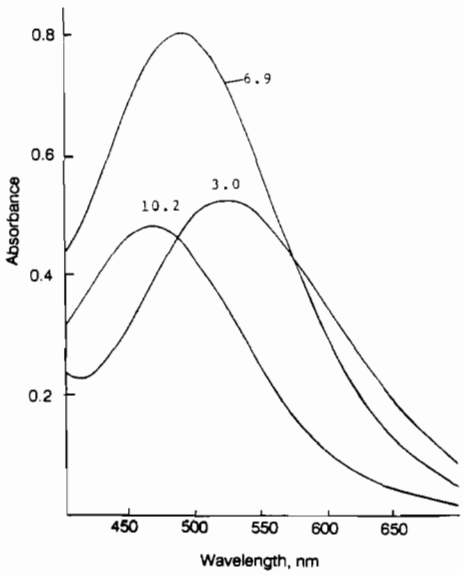
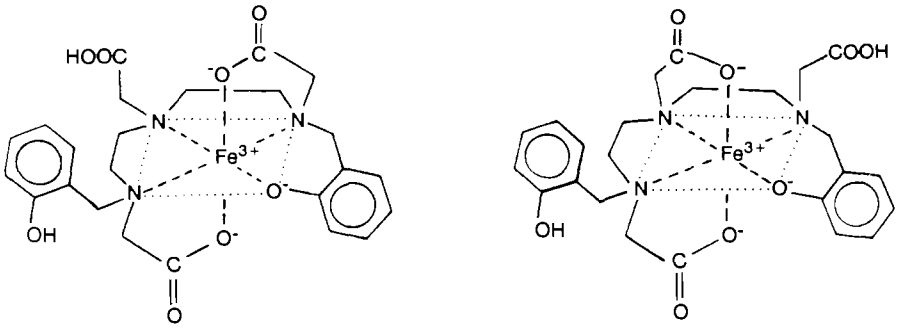
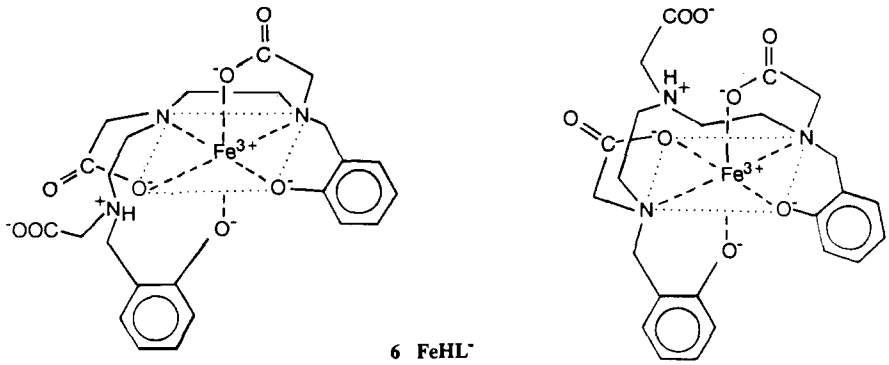


Fig. 7. Absorbance of HBDT–Fe(III) at indicated p[H] values; $T_M = 2.99 \times 10^{-4}$ M; $T_L = 2.99 \times 10^{-4}$ M; $\mu = 0.100$ M (KCl), $t = 25.0$ °C.

complex (MH_2L), which is the major species at p[H] 3.0, has one phenolate group coordinated to the Fe(III) ion while the other phenolate group is protonated, as suggested by formula **5**. There are two possible arrangements of donor groups in formula **5**, depending on which carboxylate group is considered coordinated to the metal ion. Probably both arrangements are involved. The monoprotonated complex (MHL^-), which is the major species from p[H] 5.0 to 8.5, has both phenolate groups coordinated to the metal ion. The hydrogen ion can protonate either terminal nitrogen



5 FeH_2L



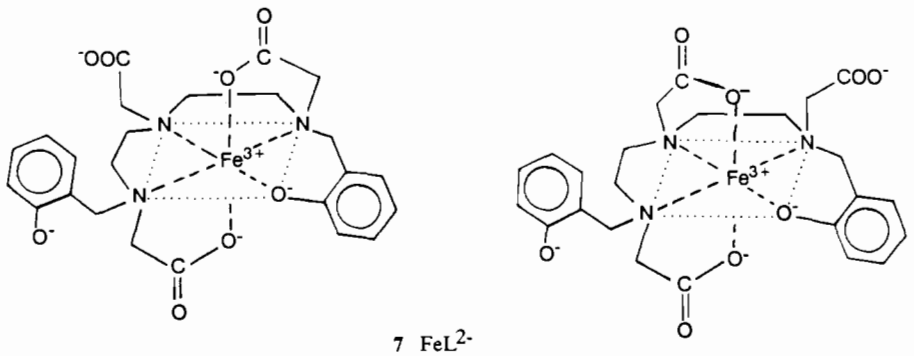
6 $FeHL^-$

or middle nitrogen as indicated by formula **6**. Because of low pK_a ($pK_a = 2$ to 3), the carboxylate groups cannot be protonated in this pH range. Further increase in p[H] results in formation of the deprotonated complex (ML^{2-}) as the major complex species present. This complex has only one phenolate group coordinated to the Fe(III) ion, as indicated by formula **7**. As with formula **5**, formula **7** has two possible arrangements of donor groups, as indicated. At high p[H], a hydroxo complex, $FeLH_{-1}^{3-}$, is seen to form. Its probable coordination sites are indicated by formula **8**. Because the absorbtivity decreases with increasing p[H] in alkaline solution and significant absorption was observed around pH 12, it is suggested that the hydroxo group replaces either the phenolate group or carboxylate group which was indicated as coordinated to the metal ion in formula **7**. At p[H] < 3.0, the triprotonated complex (MH_3L^+) is formed, probably by the binding of a hydrogen ion to a nitrogen atom, a process which would not affect the absorption spectrum.

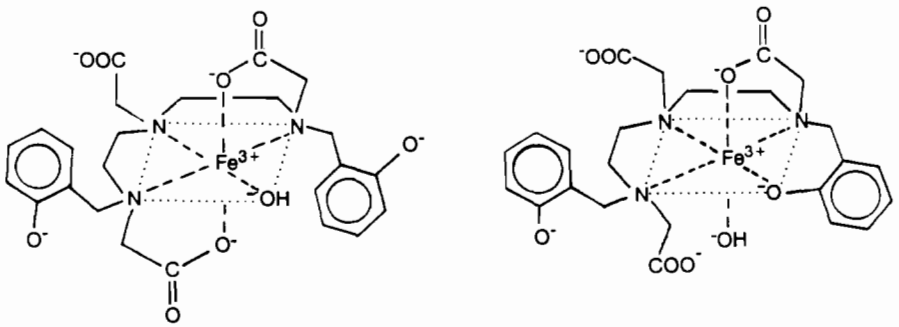
The fact that the multiprotonated complexes (MH_3L^+ and MH_2L) and the deprotonated complex (ML^{2-}) have only one phenolate group coordinated to the metal ion is due to the number and arrangement of the donor groups in the ligand and the coordination requirements of the metal ion. The ligand HBDT has eight donor groups, but Fe(III) ion can only coordinate six (Fe(III)–EHPG) [19] or seven (Fe(III)–(H_2O)EDTA) [20] of them. Therefore, there is competition among the phenolate groups, carboxylate groups, amino groups and the hydroxyl ion for the six (or seven) metal ion coordination sites. At p[H] < 3.0, the fact that one of the phenolate groups in the multiprotonated complexes is protonated may be due to the fact that its pK is much higher than that of the carboxylate group [17]. At higher p[H], the second phenolate group begins to deprotonate and coordinate to the metal ion to form the complex indicated by **6**.

The In(III) complexes of HBDT do not dissociate sufficiently at low p[H] to use potentiometric measurements to determine the stability constant of the In(III)–HBDT chelate. Potentiometric measurement of ligand–ligand competition with a 1:1:1 molar ratio of In(III):HBDT:EDTA was employed. The value of the stability constant of In(III)–EDTA in the literature [17] was used for this determination, and the results are given in Table 3.

The In(III) metal ion has a larger ionic radius and lower 'hardness' than the trivalent metal ion Fe(III) [19]. This results in a decrease in the strengths of its coordinate bonds and a corresponding decrease in the stabilities of its complexes which contain hard donor groups. Thus the stability constant for In(III)–HBED is about eleven log units lower than that of Fe(III)–HBED (Table 4). However the effect of ionic radius on the stability constant of the In(III)–HBED chelate seems to be mitigated by other factors, including the fact that only one phenolate group is coordinated to the metal ion in the species ML^{2-}, and the greater affinity of In^{3+} for the carboxylate anion, a donor group of much lower basicity. Thus the stability constant of In(III)–HBDT is only about two log units lower than



7 FeL^{2-}



8 $FeLOH^{-}$

Table 4
Comparison of log stability constants $[ML^-]/[M^{3+}][L^-]$ for trivalent metal ions ($\mu = 0.100$ M (KCl), $t = 25.0$ °C)

Metal ion	HBDT[a]	DTPA[b]	EDTA[b]	HBED[c]
Fe(III)	30.44	28.0	25.1	39.01
Ga(III)	26.11	25.5	21.0	38.51
In(III)	28.96	29.0	24.9	27.76
Gd(III)	20.20	22.5	17.3	18.89

[a]This work.
[b]Ref. [9].
[c]Ref. [11].

that of Fe(III)–HBDT and is close to that of In(III)–DTPA (Table 4).

UV spectra of the In(III) complexes show that the complexes In(III)–HBDT have an absorption peak at 275 nm when p[H] < 7.0. Increasing the p[H] shifts the peak to a higher wavelength. Fig. 8 shows the spectra of the 1:1 complex solution at p[H] 7.0, 9.2, 10.5 and 11.0. Since the protonated phenolate group has its maximum absorption at 275 nm, it is reasonable to assume that the binding of the phenolate group to the metal ion has about the same absorption area as the protonated phenolate group in the pure ligand solution. The peak at 275 nm, therefore, is assigned to the phenolate group coordinate to In(III) and the peak at 289 nm is due to the free deprotonated phenolate group which is replaced by a carboxylate group at the metal center.

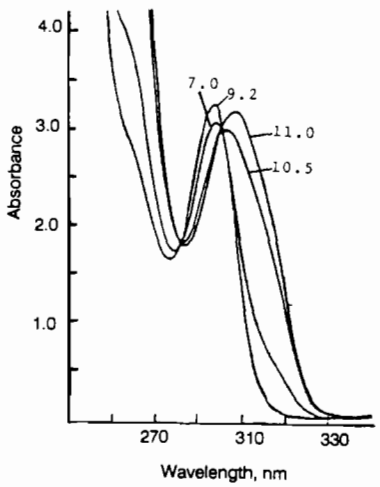


Fig. 8. Absorbance of HBDT–In(III) at indicated p[H] values; $T_M = 3.56 \times 10^{-4}$ M; $T_L = 3.66 \times 10^{-4}$ M; $\mu = 0.100$ M (KCl), $t = 25.0$ °C.

The distribution curves of In(III)–HBDT, shown in Fig. 9, indicate that more than 95% of the In(III) ion is complexed (MH_3L and MH_2L) at p[H] 2. The diprotonated complex is converted to the monoprotonated complex which predominates from p[H] 5.0 to 9.5, which in turn is converted to the completely deprotonated complex above p[H] 9.5. On the basis of the spectral analysis and result of the Fe(III)–HBDT complexes, the coordination sites of the complexes In(III)–HBDT appearing in the distribution curve may be assigned. The major species (MHL), which has an

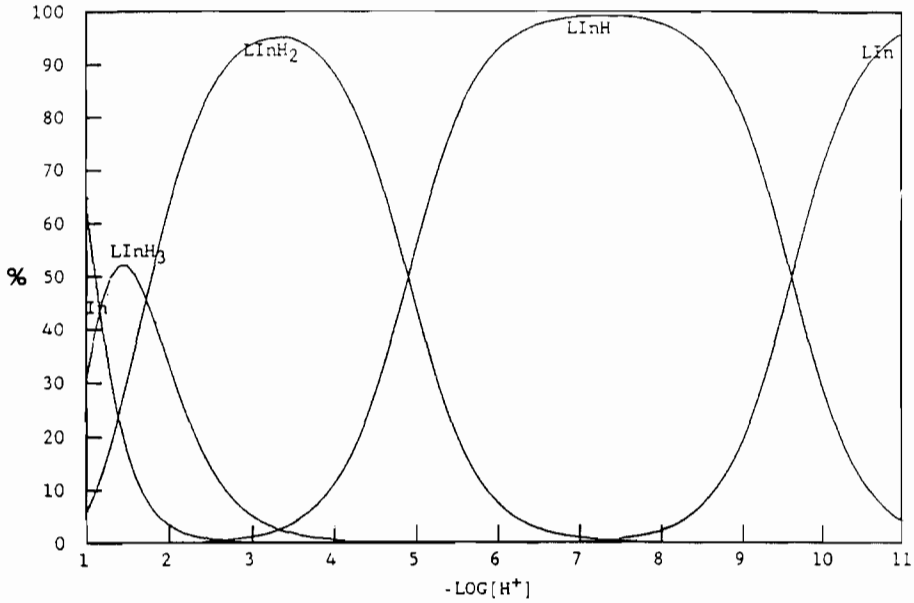


Fig. 9. Distribution curves indicating the species present as a function of p[H] in a system containing a 1:1 molar ratio of In(III):HBDT. $T_L = T_M = 2.00 \times 10^{-3}$ M. L = HBDT, In = In^{3+}, % = percent of total concentration of HBDT or In^{3+}, set at 100%.

absorption peak at 275 nm, is a complex with both phenolate oxygens coordinated to In(III). At higher p[H], complex ML^{2-} is formed, and has only one phenolate oxygen coordinated to the metal ion. The free phenolate group makes the absorption shift to longer wavelength, with a peak at 289 nm, as shown in Fig. 9.

Potentiometric measurement of ligand–ligand competition with a 1:1:1 molar ratio of Ga(III):HBDT: EDTA was employed to determinate the stability constant of Ga(III)–HBDT, and the results are given in Table 3. As with In(III), UV spectra of the solution of 1:1 molar ratio of Ga(III):HBDT indicated that a peak at 275 nm was observed at p[H] < 9.0, and with increase of the p[H] the peak shifts to 289 nm (not shown). Distribution curves of 1:1 molar ratio of Ga(III)–HBDT (not shown) indicated that the deprotonated complex, ML, begins to form above p[H] 9.0. Therefore the shift of the maximum absorption from 275 to 298 nm is assigned to the phenolate groups which are coordinated to Ga(III) in complex MHL^- begin to be displaced from the metal center and form the complex (ML^{2-}) which probably has only one phenolate group coordinated to Ga(III). The metal ion Ga^{3+} is a hard acid and has an ionic radius [21] very close to that of Fe^{3+}. It is seen that, as with Fe^{3+}, the Ga^{3+} ion has a stability constant with HBDT close to that of DTPA (Table 3) and much lower than that of HBED [11].

To summarize, the number and arrangement of donor groups in a ligand and its ability to satisfy the coordination requirements of the metal ion are important. Replacement of two acetate groups in DTPA by two phenolate donors (HBDT) at the *N* and *N″* positions does not greatly increase the stability of complexes formed with trivalent metal ions, such as Fe^{3+} and Ga^{3+}, because both phenolate groups do not coordinate the metal ion in most of the complexes formed.

The effect of the much larger ionic radius of Gd(III) is seen in the stability constants, which are about six to ten orders of magnitude lower than those of Fe(III), Ga(III) and In(III) (Table 4), indicating that hard phenolate donor groups are not suited to effective coordination of large metal ions.

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