

# Protonation constants and metal ion binding constants of *N,N'*-bis(2-hydroxyphenyl)-*N,N'*-ethylenediaminediacetic acid

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## Abstract

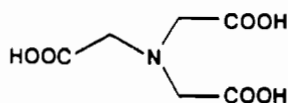
The study of a recently reported sexadentate ligand, *N,N'*-bis(2-hydroxyphenyl)-*N,N'*-ethylenediaminediacetic acid (HPED), and its hydrogen ion and metal ion affinities is described. The ligand has two phenolate donors directly attached to the aliphatic nitrogens of an *N,N'*-ethylenediaminediacetic acid backbone to impart specificity for trivalent metal ions such as those of Fe(III), Ga(III) and In(III). Potentiometric and spectrophotometric studies of the protonation constants of the ligand and its metal chelates are reported. The log stability constants for the Fe(III), Ga(III) and In(III) chelates, which are 31.81, 32.02 and 26.25, respectively, are compared with those previously reported, and with those of analogous ligands

## Introduction

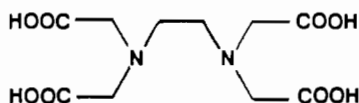
The development of synthetic high-affinity and highly selective chelating agents for Fe(III), Ga(III) and In(III) has long been an objective of coordination chemists. The stability constants for trivalent metal ion complexes of the aminopolycarboxylates such as nitrilotriacetic acid (NTA, **1**), ethylenediaminetetraacetic acid (EDTA, **2**) and diethylenetriaminepentaacetic acid (DTPA, **3**) are too small for the complexes to be used *in vivo* [1, 2]. Hard phenolate donors are employed to impart high thermodynamic stability of the metal complexes formed with hard metal ions. The sexadentate chelating agents 1,2-ethylenebis(hydroxyphenylglycine) (EHPG, **4**) and *N,N'*-bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED, **5**) have two phenolic groups replacing two of the carboxylates of EDTA and have very high affinities for Fe(III), with stability constants for *racemic*-EHPG of  $\sim 10^{35}$  and HBED of  $\sim 10^{39}$  [2–4]. These ligands were developed especially for Fe(III) sequestration and the methyl ester of HBED is now being tested for the removal of iron from test animals [5–8] and are therefore of interest for the treatment of Cooley's anemia. The corresponding gallium(III) and indium(III) chelates have received attention as radiopharmaceuticals for tumor imaging and diagnosis of biliary atresia and neonatal hepatitis [9–11]. Variations on the structure of HBED are indicated by the formulas **6** and **7**. *N,N'*-Bis(pyridoxyl)-*N,N'*-ethylenediamine-

diacetic acid (PLED, **6**) which has much less basic pseudo phenolic (hydroxypyridyl) groups derived from vitamin B<sub>6</sub>, has a lower affinity for iron, as measured by stability constants, than HBED but the lower stability is partly compensated for by the lower p*K*s of the phenolic groups [12, 13] (the decrease in p*M* is proportionally much less than the decrease in log *K*<sub>ML</sub>). *N,N'*-Bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-di-propionic acid (HBEP, **7**) which has p*K*s similar to those of HBED, has a lower stability constant of the iron(III) chelate ( $\sim 10^{31}$ ) than that of HBED [14]. This fact indicates that in HBED the carboxylate donor groups are placed in more suitable positions for coordination to the metal ion than those in HBEP, which has two beta-propionate donor groups. *N,N'*-Bis-(2-hydroxyphenyl)ethylenediamine-*N,N'*-diacetic acid (HPED, **8**) has been prepared [15]. Its amino groups are directly bound to the phenolic groups and it therefore forms complexes containing five-membered chelate rings. Its p*K*s and stability constants with Fe(III) and with divalent metal ions have been reported recently [16]. A surprising high binding constant with Fe(III) ( $\sim 10^{40}$ ) was also reported. After a detailed study of its titration curve and the p*K*s reported in the paper [16], there are reasons to doubt the accuracy of the p*K*s and thus all of the metal ion stability constants, which will be discussed below. Therefore it is necessary to reinvestigate the protonation constants of the ligand and the stability constants of the metal chelates of HPED. This paper reports the synthesis, protonation

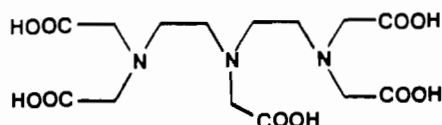
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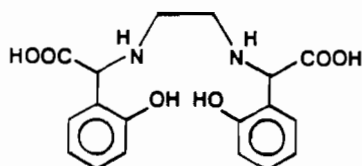
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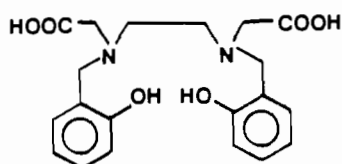
2 ethylenediaminetetraacetic acid (EDTA)



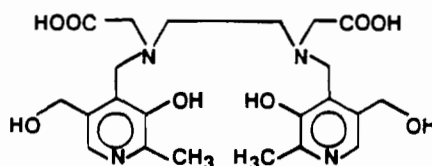
3 diethylenetriaminepentaacetic acid (DTPA)



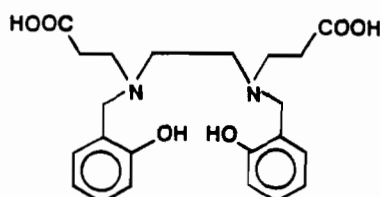
4 1,2-ethylenebis(hydroxyphenylglycine) (EHPG)



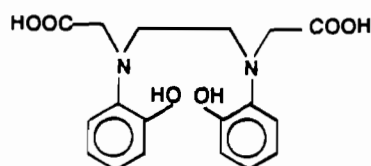
5 N,N'-(bis-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED)



6 N,N'-(bis(pyridoxyl)-N,N'-ethylenediaminediacetic acid (PLED)



7 N,N'-(bis(hydroxybenzyl)-N,N'-ethylenediaminedi-3-propionic acid (HBEP)



8 N,N'-(bis(hydroxyphenyl)-N,N'-ethylenediaminediacetic acid (HPED)

constants, the divalent and trivalent metal ion binding constants of HPED, 8.

## Experimental

### Synthesis and characterization of the ligand

The ligand *N,N'*-bis(2-hydroxyphenyl)ethylenediamine-*N,N'*-diacetic acid (HPED),  $H_4L$ , was prepared by the method previously published [15].  $^1H$  NMR (25 °C,  $D_2O$ ):  $\delta$  2.97 ( $CH_2$ , ethylene, 4H),  $\delta$  3.59 ( $CH_2$ , diacetic, 4H),  $\delta$  6–7 (phenol, 8H) and there is no organic impurity present in the sample. The compound was sent to Galbraith Laboratory for elemental analysis: Found: C, 53.79; H, 6.26; N, 6.76%. The ligand was titrated and the formula weight found is 407.0 Daltons. On that basis the sample is considered to be

$C_{18}H_{20}N_2O_6 \cdot 2.5H_2O$  (FW = 405.0). The calculated analysis would be: C, 53.33; H, 6.17; N, 6.90% which is in agreement with both elemental analysis and titration results.

### Potentiometric measurement

Equilibrium potentiometric determinations of the ligand protonation constants and its binding constants for complexes in 1:1 molar ligand to metal ratios were carried out by the glass electrode method 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 determination method are found in ref. 17.

The potentiometric apparatus consists of a glass jacketed titration cell, a 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 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.

The metal chelates of HPED were prepared as 1:1 complexes in solutions having approximately  $1 \times 10^{-3}$  M concentration in metal ion and ligand. Most of the metal binding constants were calculated from direct potentiometry, but the binding constants of the complexes with the Fe(III) and Ga(III) chelates were determined at about  $1 \times 10^{-4}$  M by spectrophotometric titration and metal-metal competition spectrophotometric titration methods since their complexes were formed completely over the range of potentiometric titration, making potentiometric determination of their stabilities impossible.

#### Spectrophotometric measurement

Because not enough of the totally deprotonated species of HPED was present during the appropriate potentiometric titrations, UV-Vis spectrophotometric titrations at various  $-\log[H^+]$  values were performed for the first protonation constant of HPED. The spectrophotometric measurements were recorded with a Perkin-Elmer 553 fast scan UV-Vis spectrophotometer. Conditions used were approximately  $1.5 \times 10^{-4}$  M for HPED in the HPED spectrophotometric titration in all instances. The spectrophotometric titrations were performed at 25 °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 pH values greater than 10.0 with the in-house FORTRAN computer program ABSPKAS. From the analytical concentration of the ligand and the  $\log[H]$  values, this program determines the equilibria and the extinction coefficients necessary to calculate the absorbance values that would correspond best to the observed absorbance values for a given spectrophotometric titration.

## Results and discussion

#### Protonation constants

The potentiometric titration curve for HPED is shown in Fig. 1. There are two distinct areas of interest. The first, at  $a < 2$  ( $a = \text{moles of base per mole of ligand}$ ), corresponds to protonation of the amino groups. The second area, at  $3 > a > 2$ , corresponds to protonation of the second phenolate oxygen. These assignments are based on the order of the basicity: phenolate >

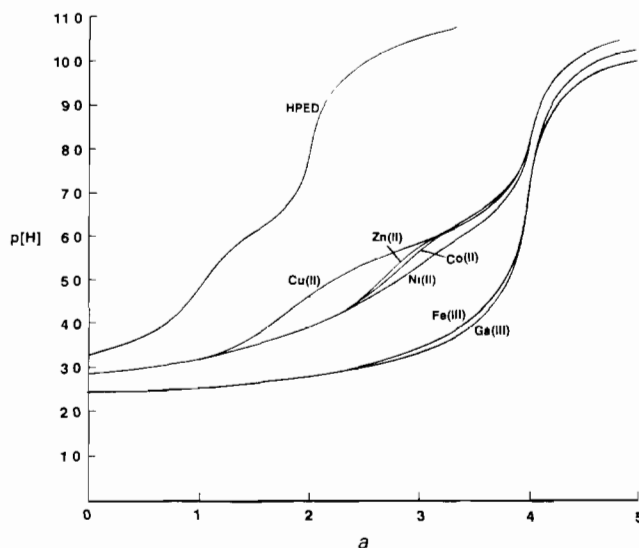


Fig. 1.  $p[H]$  profile of HPED complexes with metal ions:  $a = \text{moles of base added per mole of ligand}$ ;  $\mu = 0.1000$  M (KCl);  $t = 25.0$  °C.

amino > carboxylate groups. The protonation constants are determined by standard potentiometric titration and results are listed in Table 1. The protonation constant, at  $a > 3$ , corresponds to the protonation of the first phenolate oxygen and is determined by spectrophotometric titration because the  $p[H]$  is too high to be measured accurately in this region by potentiometry. As shown in Fig. 2, at low  $p[H]$  values only one absorption band, at 275 nm, is observed and is assigned to the protonated phenolic groups. 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 ions. 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 listed in Table 1. Extinction coefficients at 295 nm are  $\epsilon_L = 8590 \text{ cm}^{-1} \text{ M}^{-1}$  and  $\epsilon_{LH} = 4290 \text{ cm}^{-1} \text{ M}^{-1}$ .

The protonation constants of HPED,  $K_3^H$  and  $K_4^H$ , are significantly lower than those of HBED (see Table 1). This is due to the resonance between the amino groups and phenolic rings. The free pair of electrons in the amino groups which coordinate the metal ion or the proton is partly shared with the benzene ring and is thus less available. Therefore the protonation constants,  $K_3^H$  and  $K_4^H$ , involving loss of amino protons, are lower than that of HBED which has little interaction between the amino groups and the aromatic rings. The protonation constants of HPED,  $K_2^H$  and  $K_1^H$ , are also a little lower than those of HBED (Table 1). This is due to greater electronegativity of nitrogen relative to carbon. The amino groups in HPED are more electron-withdrawing and the protonation constants  $K_1^H$  and

TABLE 1. Protonation constants of HPED<sup>a</sup> and HBED;  $\mu=0.100$  M,  $t=25.0$  °C

Ligand	Log $K_1$	Log $K_2$	Log $K_3$	Log $K_4$	Log $K_5$	Reference
HPED	12.28	10.44	6.15	3.58	1.92	this work
HPED	13.5	11.5	8.38	4.47		16
HBED	12.35	11.08	8.45	4.70	2.18	4

<sup>a</sup>Uncertainties in the equilibrium constants are estimated as  $\pm 0.5$  of the last significant number.

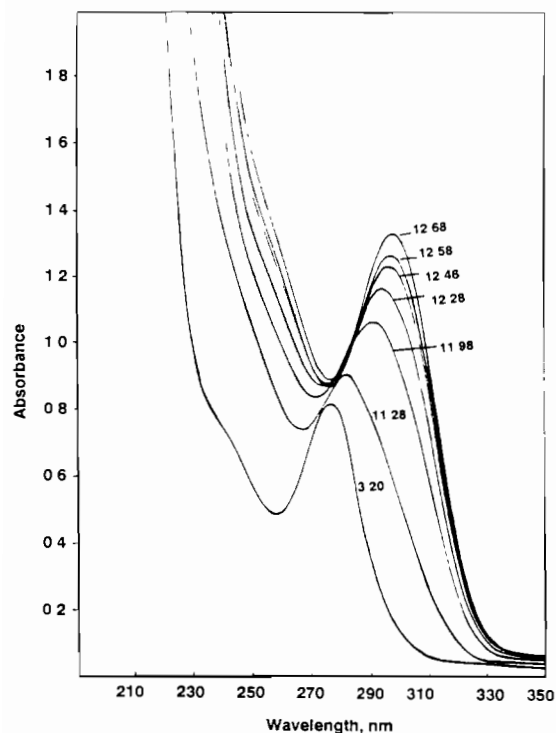


Fig. 2. Absorbance of HPED at indicated p[H] values:  $T_L = 1.975 \times 10^{-4}$  M,  $\mu = 0.1000$  M (KCl);  $t = 25.0$  °C.

$K_2^H$ , which measure the affinities of the phenolate oxygens for hydrogen ions, are therefore lower. Also, internal hydrogen bonding may be a factor. The protons of the phenolic groups of HBED are stabilized more than those of HPED. The protonation constant,  $K_5^H$ , which was determined by addition of excess HCl to the ligand (HPED,  $H_4L$ ) is probably due to protonation of one of the carboxylate groups. A similar protonation constant was found for HBED (Table 1).

The protonation constants obtained in this work are quite different from those previously reported (see Table 1). There are reasons to doubt the results in the previous paper. For example, in that paper  $K_3^H = 8.38$ , but there is a steep inflection in the potentiometric titration curves around  $pH = 8.4$ . In principle hydrogen ion dissociation cannot occur in this p[H] region. Also the value of  $K_1^H$  was recalculated with the spectral data and  $\epsilon$  values in the previous paper and  $K_1^H = 12.7$  was obtained. This

is almost one log unit lower than the value reported in the paper [16].

#### Stability constants for divalent metal ions

Potentiometric titration was used to determine the stability constants of HPED with divalent metal ions: Zn(II), Co(II), Ni(II), Ca(II), Cu(II), Cd(II) and Pb(II). The experiments were run in 1:1 metal-ligand system, and some titration curves are shown in Fig. 1. All of these metal ions were found to combine readily with HPED to form ML, MHL and  $MH_2L$  complexes in concentrations depending on the p[H] of the solution. Table 2 contains the values for the log stability constants and log protonation constants of these chelates. The distribution curves of the HPED-Ni(II) system shown in Fig. 3, indicates that the metal ion is about 12% complexed at p[H] 2. The diprotonated form,  $MH_2L$ , is converted to the monoprotinated chelate at around p[H] 4.4, which in turn is converted at about p[H] 6.2

TABLE 2. Stability constants for chelates of HPED with divalent metal ions<sup>a</sup>;  $\mu = 0.100$  M,  $t = 25.0$  °C

Metal ion	Equilibrium	Log $K$	
		HPED <sup>b</sup>	HPED <sup>c</sup>
Cu(II)	ML/M·L	23.67	23.2
	MHL/H·ML	5.81	7.6
	$MH_2L/H \cdot MHL$	5.26	5.5
Ni(II)	ML/M·L	20.10	19.4
	MHL/H·ML	6.16	9.6
	$MH_2L/H \cdot MHL$	4.28	6.7
Co(II)	ML/M·L	20.11	19.9
	MHL/H·ML	6.23	9.4
	$MH_2L/H \cdot MHL$	4.18	5.8
Zn(II)	ML/M·L	19.57	19.0
	MHL/H·ML	6.01	9.5
	$MH_2L/H \cdot MHL$	3.98	6.7
Ca(II)	ML/M·L	14.36	14.7
	MHL/H·ML	7.80	9.3
	$MH_2L/H \cdot MHL$	6.56	7.6
Pb(II)	ML/M·L	19.47	18.4
	MHL/H·ML	6.67	10.1
	$MH_2L/H \cdot MHL$	4.72	6.5
Cd(II)	ML/M·L	16.43	18.4
	MHL/H·ML	8.19	9.7
	$MH_2L/H \cdot MHL$	6.30	7.6

<sup>a</sup>Uncertainties in the equilibrium constants are estimated as  $\pm 0.5$  of the last significant number. <sup>b</sup>This work <sup>c</sup>Ref. 16.

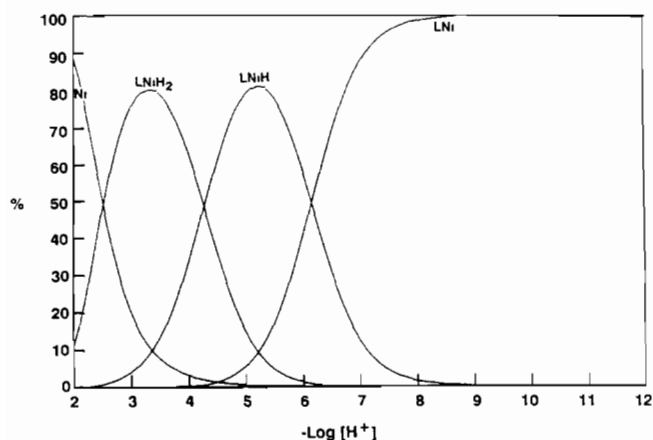


Fig. 3. Distribution curves indicating the species present as a function of  $p[H]$  in a system containing a 1:1 molar ratio of Ni(II) and HPED.  $H_4L = \text{HPED}$ ;  $N_1 = \text{Ni}^{2+}$ ; % = per cent of total concentration of HPED or Ni(II) set at 100%.

TABLE 3. Stability constants for chelate of divalent metal ions;  $\mu = 0.100 \text{ M}$ ,  $t = 25.0 \text{ }^\circ\text{C}$

Metal ion	Equilibrium	Log K		
		HPED <sup>a</sup>	HBED <sup>b</sup>	rac-EHPG <sup>c</sup>
Cu(II)	ML/M·L	23.79	23.40	25.27
Ni(II)	ML/M·L	20.10	19.99	21.33
Zn(II)	ML/M·L	19.59	19.11	18.66

<sup>a</sup>This work. <sup>b</sup>Ref. 4 <sup>c</sup>Ref. 2.

to the deprotonated form, ML. Above  $p[H]$  7 ML is the predominant form of the metal chelate in aqueous solution. It is not surprising that the results from this work are different from those previously reported [16] considering the different  $pK$ s of these two publications. Although the differences in protonation constants would account for a difference in stability constants, the present authors cannot explain why the stability constant differences, and the differences in protonation constants of the metal chelates, vary so much. The order of stability for chelates of the divalent metal ions is  $\text{Cu(II)} > \text{Ni(II)} > \text{Zn(II)}$ . The same order of stability constants for chelates of the divalent metal ions was found for HBED, EHPG (Table 3) and most metal ions [18].

#### Stability constants for trivalent metal ions

In the case of Fe(III)-HPED, which is more than 92% formed at  $p[H]$  2, it is necessary to turn to spectrophotometry and very low  $p[H]$ s to accurately determine the stability constant. Shown in Fig. 4 is the optical absorption spectra for the  $L \rightarrow M$  charge transfer band of the Fe(III)-HPED complex measured between  $p[H]$  1.05 and 2.22. From the observed absorbance

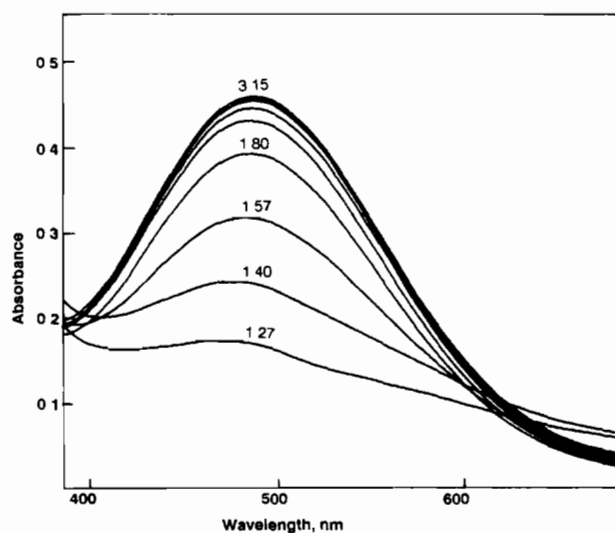


Fig. 4. Absorbance of HPED-Fe(III) complex at indicated  $p[H]$  values  $T_M = 1.794 \times 10^{-4} \text{ M}$ ;  $T_L = 1.803 \times 10^{-4} \text{ M}$ ;  $\mu = 0.1000 \text{ M}$  (KCl);  $t = 25.0 \text{ }^\circ\text{C}$ .

between 1.27 and 1.80, the calculated  $p[H]$  and measured extinction coefficient of  $3100 \text{ cm}^{-1} \text{ M}^{-1}$ , the values  $K_{ML}$  and  $K_{MLH}$  that gave the best agreement between the calculated and observed absorbances were determined. This was accomplished by solving the simultaneous mass and charge balance eqns. (1) to (4):

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

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

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

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

where  $A$  is absorbance at 485 nm,  $L$  is HPED,  $T_L$  and  $T_M$  are total HPED and metal concentration, respectively.  $A_1$  is defined by eqn. (5), and  $[MLH]$  was calculated by eqn. (6):

$$A_1 = 1 + \beta_1[H^+] + \beta_2[H^+]^2 + \dots + \beta_5[H^+]^5 \quad (5)$$

$$[MLH] = K_{MLH}[ML][H^+] \quad (6)$$

where  $\beta_n$  are overall ligand protonation constants. The absorbance maximum of the Fe-HPED, 485 nm, reveals a shift to slightly higher wavelength as the  $p[H]$  is lowered. This shift corresponds to the formation of the protonated metal chelate species MLH and its calculated extinction coefficient is  $1600 \text{ cm}^{-1} \text{ M}^{-1}$ . Considering the fact that Fe(III)-EDTA has no absorbance in the visible range, the maximum absorbance (485 nm) of Fe(III)-HPED (two carboxylate groups in EDTA were replaced by two phenolate groups) is probably due to metal-phenolate bonding. When the  $p[H]$  is lowered, the absorbance maximum decrease (Fig. 4) corresponds to the formation of protonated phenolate groups and the loss of metal-phenolate bonding.

The stability constants obtained are listed in Table 4. It is of interest to compare the high binding constants of Fe(III) for four similar phenolic ligands HPED, HBEP, *rac*-EHPG, *meso*-EHPG and HBED (Table 5). All are sexadentate and have two phenolate donors, two nitrogen donors and two carboxylate donors. The higher metal ion affinity of HBED relative to that of EHPG is due to the more favorable steric orientation of donor groups, and higher stability constants of *rac*-EHPG related to that of *meso*-EHPG is due to a geometric effect, as is discussed in detail elsewhere [3]. Increasing the chelate ring size from six members (HBED) to seven members (HBEP) causes the stability constants of Fe(III) chelates to decrease from  $\sim 10^{39}$  to  $\sim 10^{31}$  (Table 5). It is seen from these results that steric factors strongly affect stability constants. Decreasing the chelate ring size from six members (HBED) to five members (HPED) correlates with a stability constant decrease of the Fe(III) chelate from  $\sim 10^{39}$  to  $\sim 10^{32}$  (Table 5). Molecular models show that the coordination sphere is considerably more crowded and constrained in the HPED chelates than the corresponding complexes of HBED because donor groups in HPED involved in five-membered rings are less flexible than are the groups involved in six-membered chelate rings formed in HBED. The stability constants of HBED–Fe(III), therefore, are expected to be higher

TABLE 4. Stability constants for chelates of HPED with trivalent metal ions<sup>a</sup>;  $\mu = 0.100$  M,  $t = 25.0$  °C

Metal ion	Equilibrium	Log K
Fe(III)	ML/M·L	31.8
	MHL/H·ML	10
Ga(III)	ML/M·L	32.0
	MHL/H·ML	13
Al(III)	ML/M·L	25.78
	MHL/H·ML	3.73
In(III)	ML/M·L	26.25
	MHL/H·ML	3.43
Gd(III)	ML/M·L	21.18
	MHL/H·ML	6.15
	MH <sub>2</sub> L/H·MHL	3.71

<sup>a</sup>Uncertainties in the equilibrium constants are estimated as  $\pm 0.5$  of the last significant number.

TABLE 5. Stability constants for chelates of trivalent metal ions;  $\mu = 0.100$  M,  $t = 25.0$  °C

Metal ion	Equilibrium	Log K					
		HPED <sup>a</sup>	HPED <sup>b</sup>	HBED <sup>c</sup>	<i>rac</i> -EHPG <sup>d</sup>	<i>meso</i> -EHPG <sup>d</sup>	HBEP <sup>e</sup>
Fe(III)	ML/M·L	31.8	40.0	39.01	35.54	33.28	31.16
Ga(III)	ML/M·L	32.0		38.51	33.89	32.40	
In(III)	ML/M·L	26.25		27.76	26.68	25.26	

<sup>a</sup>This work. <sup>b</sup>Ref. 16. <sup>c</sup>Ref. 4. <sup>d</sup>Ref. 3. <sup>e</sup>Ref. 14.

than those of HPED. It is not surprising that  $K_{ML}$  of HPED in this work is about 8 log units lower than in the previous paper [16], considering the differences in pKs of the ligands that have been reported.

The Ga(III) complexes of HPED do not dissociate at low p[H] and it was not possible to use potentiometric measurements to determine the degree of formation of the complex of Ga(III) with HPED. However, it was possible to take advantage of strong visible absorbance bands of the ferric chelates of the ligand by employing metal–metal competition spectrophotometric titration to determine stability constants of Ga(III)–HPED. The experiments were run with a 1:1:1 molar ratio of Ga(III):Fe(III):HPED. The value of extinction coefficients of FeL and FeLH and the formation constants of  $K_{FeL}$  and  $K_{FeLH}$  determined in this work were used for this calculation. The concentration of the ferric chelate was calculated from the measured visible absorbance of the experimental solutions. With the calculated hydrogen ion concentration, and total concentration of ferric ion, gallium ion and ligand, the concentrations of the other species were determined: free Ga(III) ion, chelate of Ga(III) and free ligand. The value of  $K_{GaLH}$  was adjusted to give the best agreement between the calculated and observed absorbance, and  $\log K_{GaL}$  was determined by

$$K_{GaL} = [GaL]/[Ga][L]$$

The results are listed in Table 4. Ga(III) has a stability constant close to that of Fe(III) (Table 5). As with Fe(III), ring size also strongly affects the stability constants of Ga(III) chelates. Decreasing the chelate ring size from six members (HBED) to five members (HPED) correlates with a decrease in the stability constants of Ga(III) chelates from  $\sim 10^{39}$  to  $\sim 10^{32}$  (Table 5). The fact that Ga(III) has a stability constant close to that of Fe(III) is a reflection of the fact that the two metal ions have similar ionic radii (0.76 and 0.78 Å for Ga(III) and Fe(III), respectively) [19]. A similar correlation was observed for the corresponding EHPG chelates [3].

The stability constant of In(III)–HPED determined by normal potentiometric titration is considerably lower than those of Fe(III) and Ga(III) (see Table 4). This

result is expected from the larger ionic radius of In(III) [19] and the lower 'hardness' of In(III) compared to Ga(III) and Fe(III). The ionic radius effect on stability has been well documented [3]. The In(III) ion has an approximately 24% increase in ionic radius compared to Ga(III), which results in a decrease in stability relative to the corresponding HBED–Ga(III) complex of some eleven orders of magnitude (Table 5). The ionic radius effect on stability is also observed in HPED chelates. The stability constant of the complex In(III)–HPED is about six orders of magnitude lower than that of the corresponding Ga(III) complex. A change in chelate ring size has a much smaller effect on stability constants of In(III) chelates than on those of Ga(III) chelates because of the larger ionic radius of the In(III) ion. Decreasing the chelate ring size from six members (HBED) to five members (HPED) correlates with a decrease in the stability constants of In(III) chelates of only about a one log unit (Table 5). The distribution curve of HPED–In(III) shown in Fig. 5 indicates that the metal ion is 50% complexed at p[H] 2. The protonated form, MHL, is converted to the deprotonated form, ML, at p[H] 3.5. Above p[H] 4 ML is the predominant form of the metal chelate in aqueous solution.

In summary, it is seen that steric factors and ring size have a strong effect on the stability constants of Fe(III) and Ga(III) complexes. Because of small ionic radii, Fe(III) and Ga(III) prefer to form six-membered chelate rings. Increasing the chelate ring size from six members (HBED) to seven members (HBEP) causes the stability constants of Fe(III) chelates to decrease from  $\sim 10^{39}$  to  $10^{31}$  (Table 5). Decreasing the chelate ring size from six to five (HBED and HPED) decreases the stability constants of Fe(III) chelates from  $10^{39}$  to  $10^{32}$  (Table 5). In contrast, steric effects on the stability

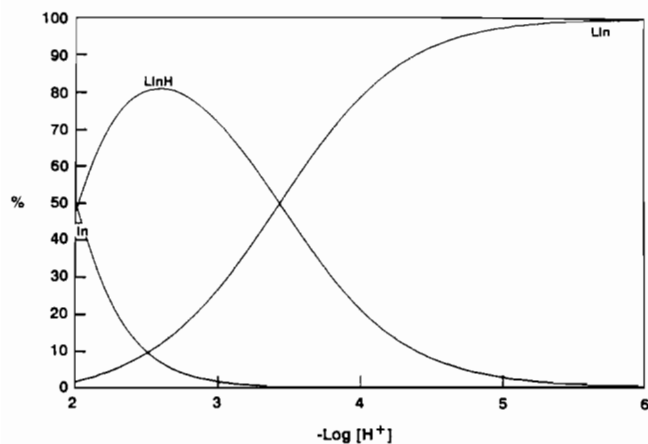


Fig. 5. Distribution curve indicating species present as a function of p[H] in a system containing a 1:1 molar ratio of In(III) and HPED. H<sub>4</sub>L=HPED; In(III)=In<sup>3+</sup>; % = per cent of total concentration of HPED or In(III) set at 100%.

constants of In(III) chelates are smaller than that of Fe(III) chelates because of the larger ionic radius of the In(III) ion. There is only about a one log unit decrease when the chelate ring size is decreased from six to five (Table 5). The effect of chelate ring size on stabilities of complexes of metal ions as a function of ionic radius was pointed out by Hancock and Martell [20].

The effect of the much larger ionic radius of Gd(III) is seen in the stability constants, which are ten and seven orders of magnitude lower than those of Fe(III) and In(III), respectively (Table 4), indicating that hard phenolate donor groups are not suited to effective coordination of large metal ions. Stability constants of Al(III)–HPED were determined by potentiometric titration, and are smaller than those of Ga(III) and Fe(III). Similar relationships are observed for other complexes of Al(III) [21–23].

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