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Note

Stability of metal ion complexes of N,N′-bis(2-hydroxybenzyl)ethylenediamine-N,N′-diacetic acid

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Abstract

The stability constants of 1:1 complexes of *N,N′*-bis(2-hydroxybenzyl)ethylenediamine-*N,N′*-diacetic acid (HBED) with trivalent and divalent metal ions have been determined by potentiometric and spectrophotometric methods in KCl supporting electrolyte (0.100 M) at 25.0 °C. The log stability constants for the Fe(III), Ga(III) and In(III) chelates are 39.01, 38.51 and 27.76, respectively, while that for the weaker complex with Gd(III) is 18.89. The results in this work are compared with those of analogous ligands.

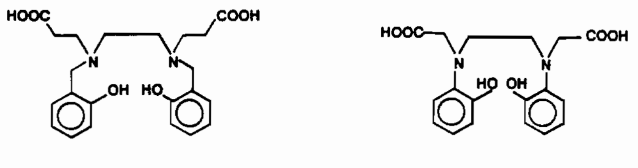
Keywords: Stability constants; Metal ion complexes; Amine complexes; Phenolic ligands

1. Introduction

N,N′-Bis(2-hydroxybenzyl)ethylenediamine-*N,N′*-diacetic acid (HBED, Fig. 1) and its esters have been found to be effective in the removal of iron from test animals [1–3] and therefore are of interest for the treatment of Cooley's anemia. The corresponding Ga(III) and In(III) chelates may also be useful as radiopharmaceuticals for tumor imaging purposes [4,5]. An early study reported the stability constants of HBED with the metal ions Co(II), Cu(II), Ni(II), Zn(II) and Fe(III) [6]. There is reason to doubt the accuracy of the reported stability constant for Fe(III) with HBED, since the early work did not take into account the protonation reactions of both the ligand and the Fe(III) chelate at low pH. Another study [7] reported the stability constants of Ga(III) with HBED, determined from a Fe(III)–Ga(III)–HBED spectrophotometric competition titration. Because the Fe(III)–HBED stability constant was used to calculate the Ga(III)–HBED stability constant in this study, any error in the Fe(III)–HBED stability constant [6] would lead to an error in the Ga(III)–HBED value. Long reported constants of Fe(III), Ga(III), Gd(III) and In(III) with HBED [8] but questions arose about his experimental methods and calculations, and his results have not been published in a refereed journal. Redetermination of the stability of HBED, therefore, is needed. This paper is a report of the reinvestigation of the stability constants of HBED with various metal ions, corrected for the inaccuracies of the previously reported stability constants of HBED with Fe(II), Ga(III), In(III) and Gd(III), and corrected for any inaccuracies in the reported stability constants of divalent metal ions.

HBED
N,N′-(bis-2-hydroxybenzyl)ethylenediamine-N,N′-diacetic acid

EHPG
1,2-ethylenebis(o-hydroxyphenylglycine)



HBEP
N,N′-bis(2-hydroxybenzyl)-N,N′-ethylenediaminedi-3-propionic acid

HPED
N,N′-bis(2-hydroxyphenyl)-N,N′-ethylenediaminediacetic acid

Fig. 1. Graphic formulas of ligands related to HBED discussed in this work.

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2. Experimental

2.1. Materials

A pure sample of HBED was kindly donated by Dr Ichiro Murase, Dojindo Laboratories, Japan.

2.2. Potentiometric measurements

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 have been described [9].

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 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.

2.3. Spectrophotometric measurements

Because not enough of the totally deprotonated species of HBED was present during the appropriate potentiometric titrations, UV–Vis spectrophotometric titrations at various $-\log[H]$ values were performed for the high p*K*s of HBED. The spectrophotometric measurements were recorded with Perkin-Elmer 553 fast scan UV–Vis spectrophotometer. Conditions used were approximately 1.0×10^{-4} M for HBED in the spectrophotometric titration in all instances. The spectrophotometric titrations were performed at 25.0 °C, and cells with a 1.000 cm path length were used. For the HBED titration, the log *K* value for the equilibrium quotient [HL]/([H][L]) was calculated from the absorbances at wavelength 292.5 nm for p[H] values greater than 10.0 with the in-house FORTRAN computer program ABSPKAS. This program determines, from the analytical concentration of the ligand and the $\log[H^+]$ values, the equilibrium constant and the extinction coefficients necessary to calculate the absorbance values that would correspond best to the observed absorbance values for a given spectrophotometric titration.

Because HBED forms complexes with Fe(III) and Ga(III) completely even at p[H] 2, the binding constants of the complexes with the Fe(III) and Ga(III) chelates were determined at about 1×10^{-4} M by ligand–ligand competition (1:1:1) molar ratio of EDTA:Fe(III):HBED) spectrophotometric titration and metal–metal competition (1:1:1 molar ratio of Ga(III):Fe(III):HBED) spectrophotometric titration methods, respectively. For the ligand–ligand spectrophotometric competition titration, in one case HBED was added to the iron(III) solution and then EDTA was added; in the other case the EDTA–Fe(III) chelate was formed before addition of HBED. The equilibrium was obtained from these two directions when thermostated experimental solutions were allowed to stand over 30 days. For the metal–metal spectrophotometric competition titration, in one case HBED was added to the iron(III) solution and then Ga(III) was added; in the other case the HBED–Ga(III) chelate was formed before addition of Fe(III). The equilibrium was obtained from these two directions when thermostated experimental solutions were allowed to stand for 2 days. The values of log K_{ML} and log K_{MLH_n} were calculated at each $-\log[H^+]$ from the absorbance at specific wavelengths, and by solving mass balance equations.

3. Results and discussion

3.1. Protonation constants

The potentiometric equilibrium curve for HBED is shown in Fig. 2. Protonation constants at $a < 2$ corresponding to the protonation of amino nitrogens were determined by standard potentiometric titration. The protonation constants at $a > 2$, corresponding to the protonation of phenolate oxygens were determined from spectrophotometric titration because the p[H} is too high to be measured accurately in this region by po-

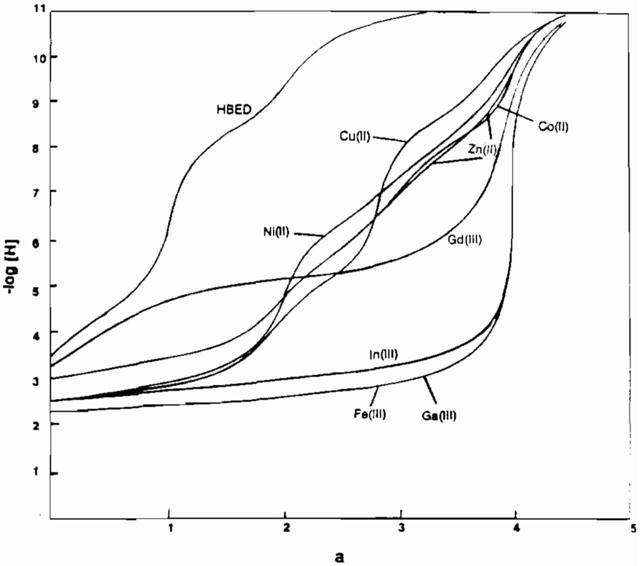

Fig. 2. p[H] profile of HBED complexes with metal ions; m = moles of base added per mole of ligand; $\mu = 0.1000$ M (KCl), $t = 25.0$ °C.

Table 1
Protonation constants of HBED, EDTA, HPED and HBEP ($\mu = 0.10$ M (KCl), $t = 25$ °C)

	log K_1	log K_2	log K_3	log K_4	log K_5	Reference
HBED	12.64	11.03	8.34	4.40	2.24	this work
HBED	12.46	11.00	8.32	4.64		[6]
HBED	12.35	11.08	8.45	4.76	2.18	[8]
EDTA	10.19	6.13	2.69	2.0		[10]
HPED	12.28	10.44	6.15	3.58	1.92	[11]
HBEP	12.66	11.16	8.65	5.73		[12]

tentiometry. These assignments are based on the order of the basicity: phenolate > amino > carboxylate groups. The protonated and deprotonated phenolates have maximum absorbance at 277 and 292 nm, respectively. The high protonation constants are determined from data at 292 nm. Results are listed in Table 1. The extinction coefficients calculated from absorbance are $\epsilon_L = 7530$ and $\epsilon_{LH} = 4310$ cm^{-1} M^{-1}. The protonation constant, $K_5{}^H$, which was determined by addition of excess HCl to the ligand (HBED, H_4L) is probably due to protonation of one of the carboxylate groups. The protonation constants of HBED are compared in Table 1 with those of analogous ligands.

3.2. *Stability constants for trivalent metal ions*

The potentiometric p[H] curve in Fig. 2 shows a break for the Fe(III) complexes at $m = 4$ (m = mole of base per mole of metal ion). The Fe(III) titration curve is a strong acid curve indicative of complete formation of the 1:1 metal complex even at p[H] 2. Ligand–ligand spectrophotometric competition, therefore, was used to determine the stability constants of HBED with Fe(III). The Fe(III)–HBED complex has its maximum absorbance at 485 nm ($\epsilon_{ML} = 4010$, $\epsilon_{MHL} = 2005$). The data at wavelength 485 nm, measured hydrogen ion concentration (p[H] = 2.3–4.1) and equilibrium constants [10] of Fe(III) with EDTA were used to calculate the equilibrium constants of Fe(III) with HBED. This was accomplished by solving the mass balance Eqs. (1)–(4).

$$A = \epsilon_{ML}[ML] + \epsilon_{MLH}[MLH] \qquad (1)$$

$$T_M = [M] + [ML] + [MLH] + [ML'] + [ML'H] \qquad (2)$$

$$T_L = [ML] + [MLH] + [L]A_1 \qquad (3)$$

$$T_{L'} = [ML'] + [ML'H] + [L']A_2 \qquad (4)$$

where A is absorbance at 485 nm, L and L′ represent HBED and EDTA, respectively. T_L, $T_{L'}$ and T_M represent total HBED, EDTA and metal concentration, respectively. A_1 and A_2 are defined in Eqs. (5) and (6).

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

$$A_2 = 1 + \beta'_1[H^+] + \beta'_2[H^+]^2 + \ldots + \beta'_4[H^+]^4 \qquad (6)$$

where β and β' are overall protonation constants of HBED and EDTA, respectively. From Eqs. (1)–(6), [M], [L] and [ML] can be calculated. K_{MLH} was adjusted to give the best agreement between the calculated and observed absorbances. The results are listed in Table 2. Distribution curves of Fe(III)–HBED–EDTA, shown in Fig. 3, indicate that about 14% of the metal forms ML (with HBED) and about 69% metal forms ML′ (with EDTA) at p[H] 2. The complex of Fe(III)–EDTA converts to Fe(III)–HBED at p[H] 3.1. Above p[H] 5 complex Fe(III)–HBED is the predominant form of the metal in aqueous solution.

The stability constant of Fe(III)–HBED (log $K_{ML} = 39.01$) obtained in this work is a little lower than

Table 2
Stability constants of metal complexes of HBED ($\mu = 0.10$ M, $t = 25$ °C)

	log K_{ML}	log K_{MLH}	log K_{MLH_2}	Reference
Cu(II)	22.95	8.78	5.12	this work
	21.38	8.63	5.18	[6]
	23.40	8.34	4.66	[8]
Co(II)	19.43	8.00	5.72	this work
	19.89	7.77	5.46	[6]
	19.18	7.93	5.97	[8]
Zn(II)	18.95	8.17	5.83	this work
	18.37	8.27	5.99	[6]
	19.11	7.91	5.79	[8]
Ni(II)	20.07	8.44	5.99	this work
	19.31	8.51	6.45	[6]
	19.99	8.54	6.65	[8]
Fe(III)	39.01	1.51		this work
	39.68			[6]
	36.74	2.16		[8]
Ga(III)	38.51	2.30		this work
	39.57			[6]
	37.73			[8]
In(III)	27.76	3.48		this work
	27.90	4.19		[8]
Gd(III)	18.89	5.68		this work
	18.78	6.06		[8]
	19.16	5.7	5.1	[13]

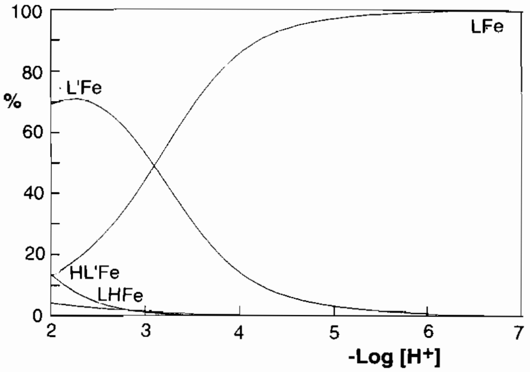

Fig. 3. Distribution curves indicating the species present as a function of p[H] in a system containing a 1:1:1 molar ratio of Fe(III):HBED:EDTA. L = HBED, L′ = EDTA, Fe = Fe^{3+}, % = percent of total concentration of HBED or Fe^{3+} set at 100%.

that of the previous report [6] (log $K_{ML}=39.68$). This difference may be due to the fact that the previous work did not take into account the fifth protonation constant of the ligand $[H_5L]/[L][H^+]^5$ and the protonation constant of the Fe(III)–HBED complex. During the experiment it was found that the equilibrium was quite slow for the system Fe(III)–HBED–EDTA. It took over 30 days to reach the equilibrium value from two directions. Two kinetic traces are illustrated in Fig. 4. The result obtained in this work is about 2.3 log units higher than Long's work [8] which was obtained by spectrophotometric titration at p[H] 1.2–1.5. There is reason to doubt the accuracy of this result because Fe(III) forms complexes with HBED completely even at p[H] 1.0. There was almost no free metal present in the solution in the experiment p[H] range 1.2–1.5. The spectrophotometric titration method, therefore, is unsatisfactory for this system.

Ligands with structures related to that of HBED have been synthesized (Fig. 1) and their stability constants with trivalent metal ions have been studied. The examples include 1,2-ethylenebis(hydroxyphenylglycine) (EHPG) [14], HBED [12] and HPED [11]. The reported stability constants of EHPG with Fe(III), Ga(III) and In(III), listed in Table 3, are lower than those of HBED. EHPG has a structure similar to that of HBED, shown in Fig. 1. However steric effects may interfere somewhat with simultaneous participation in metal ion coordination by all six donor groups in an octahedral fashion (two basic nitrogens, two carboxylate groups and two phenolate groups). HBED has much less steric strain and the stability constants of HBED with trivalent metal ions should be larger than those of EHPG, HBEP and HPED. Both have two phenolate donors, but they form seven- and five-membered chelate rings, respectively. The stabilities of their iron(III) chelates ($\sim 10^{31}$) are about 8 log units smaller than those of HBED (Table 3). These constants indicate that ring size has a strong effect on the stability constants of Fe(III) chelates. The factors involved have been discussed previously [11,15].

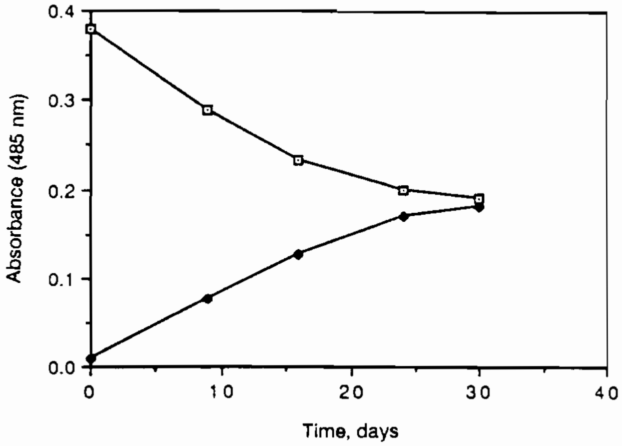

Fig. 4. Plot of kinetic traces of absorbance (485 nm) vs. time (day) in a system containing a 1:1:1 molar ratio of Fe(III):HBED:EDTA at p[H] 2.7. □: HBED was mixed with Fe(III) and then EDTA was added: ◆: EDTA was mixed with Fe(III) and then HBED was added.

Table 3
Comparison of log stability constants $[ML^-]/[M^{3+}][L^-]$ for trivalent metal ions ($\mu=0.10$ M (KCl), $t=25$ °C)

Metal ion	HBED[a]	HPED[b]	HBEP[c]	*rac*-EHPG[d]
Fe(III)	39.01	31.8	31.16	35.54
Ga(III)	38.51	32.0		33.89
In(III)	27.76	26.25		26.68

[a] This work.
[b] Ref. [11].
[c] Ref. [12].
[d] Ref. [14].

There are two possible sources of error in this work. The literature value [10] for the stability constants of the EDTA–Fe(III) system was used for the calculation of the stability constants of HBED–Fe(III), and it was assumed that the literature value of EDTA–Fe(III) is accurate. Considering the fact that the stability constant of the EDTA–Fe(III) complex has been determined by several chemists [1,16,17] and their results were in agreement, there is no reason to doubt the accuracy of this value. Another possible source of error is the formation of mixed-ligand complexes during the ligand–ligand competition titration. Based on the concept of the 'chelate effect', it can be assumed that there are no mixed-ligand complexes formed during the competition titration between these multidentate ligands. The ligand–ligand competition method has been used in many multidentate systems [18–21] and there is no report of mixed-ligand complexes formed.

Metal–metal spectrophotometric competition was used to determine the stability constant of HBED with Ga(III). The ferric chelate has strong visible absorbance while the gallium chelate has no absorbance in the visible region. The absorption data at wavelength 485 nm, measured hydrogen ion concentration, metal hydrolysis constants [10] and the equilibrium constants of Fe(III) with HBED obtained in this work, were used to calculate the equilibrium constants of Ga(III) with HBED. 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 Eq. (7).

$$K_{\mathrm{GaL}}/K_{\mathrm{FeL}}=[\mathrm{GaL}^-]/[\mathrm{FeL}^-] \qquad (7)$$

The result is listed in Table 2. The stability constant of Ga(III)–HBED in this work is somewhat different from that of the previous report (Table 2) which was also determined by metal–metal competition (Fe(III)–Ga(III)–HBED). This difference is due to the difference in equilibrium constants of Fe(III)–HBED used in the previous work. The fact that Ga(III) has

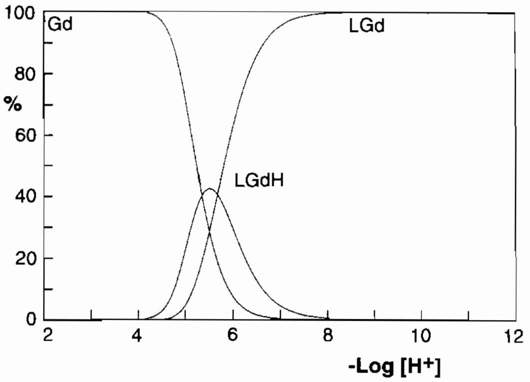

Fig. 5. Distribution curves indicating the species present as a function of p[H] in a system containing a 1:1 molar ratio of Gd(III):HBED, L = HBED, Gd = Gd^{3+}, % = percent of total concentration of HBED or Gd^{3+} set at 100%.

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) [15]. A similar correlation was observed for the corresponding EHPG [14] and HPED [11] chelates.

The stability constants for HBED with In(III) and Gd(III) obtained by potentiometric titration are listed in Table 2. The lower stability constant of the In(III)–HBED complex is expected from the larger ionic radius of In(III) and the lower 'hardness' of In(III) compared to Ga(III) and Fe(III). The ionic radius effect on stability has been described previously [11,14,18,22]. The In(III) ion has an approximately 24% increase in ionic radius compared to Ga(III), which results in a decrease in stability from the corresponding *rac*-EHPG–Ga(III) complex of some seven orders of magnitude and from the HPED–In(III) complex of some six orders of magnitude [11]. Distribution curves of Gd(III)–HBED, shown in Fig. 5, indicate that the metal ion complexes form at p[H] > 4. The monoprotonated chelate, MLH, is converted to the deprotonated chelate, ML^-, in the p[H] range 5–7. Above p[H] 7 ML^- is the predominant form of the chelate in aqueous solution. The effect of the much larger ionic radius of Gd(III) is seen in the stability constants, which are ten and nine orders of magnitude lower than those of Fe(III) and In(III), respectively (Table 2), indicating that hard phenolate donor groups are not suited to effective coordination of large metal ions.

3.3. Stability constants for divalent metal ions

The p[H] profiles for HBED complexes with Zn(II), Ni(II), Cu(II) and Co(II) are shown in Fig. 2, and the constants obtained are listed in Table 2. The stability constants for the chelates of the divalent metal ions Co(II), Zn(II) and Ni(II) are in good agreement with those of Long [8], but slightly different from those of L'Epplatenier et al. [6], which may be due to the fact that the previous work [6] did not take into account the protonation of the ligand at low p[H]. The stability constant of Cu(II)–HBED obtained in this work is a little smaller then Long's value [8] which was obtained by spectrophotometric titration at p[H] 1.4–3.6. The difference may be due to difference in ionic strength, which was adjusted by HCl substitution for KCl at p[H] < 2. The order of stability constants for the chelate of the divalent metal ions was Cu(II) > Ni(II) > Co(II) > Zn(II). The same order was found for HPED [11] and EHPG [14].

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