

N,N'-Bispyridoxylethylenediamine-*N,N'*-diacetic acid (PLED) and *N,N'*-Bis(2-hydroxy-5-sulfobenzylethylenediamine-*N,N'*-diacetic acid (SHBED)

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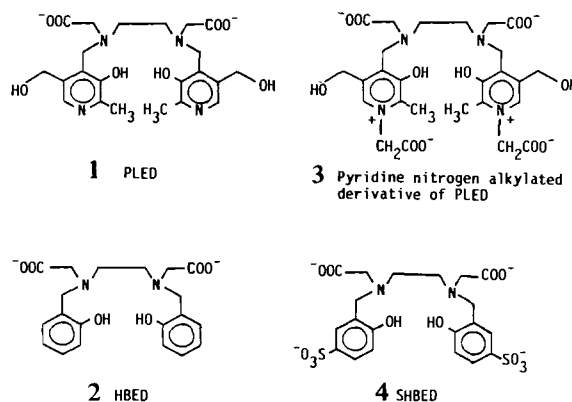
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Abstract

The sexadentate ligands, *N,N'*-bispyridoxylethylenediamine-*N,N'*-diacetic acid (PLED) and *N,N'*-bis-2-hydroxy-5-sulfobenzylethylenediamine-*N,N'*-diacetic acid (SHBED) have been synthesized for the first time in pure form, and the stability constants of the complexes formed with divalent and trivalent metal ions have been determined (Cu(II), Co(II) and Zn(II) for PLED, and Fe(III), Ga(III), In(III) and Gd(III) for PLED and SHBED). The equilibrium data in this paper are considered reliable and supercede the less accurate values previously reported in the literature.

Introduction

N,N'-Bispyridoxylethylenediamine-*N,N'*-diacetic acid (PLED) (1) is a sexadentate ligand for the coordination of divalent and trivalent metal ions. It was first conceived as an analog of HBED (*N,N'*-bis-*o*-hydroxybenzylethylenediamine-*N,N'*-diacetic acid, 2) for the removal of iron(III) from physiological systems [1]. Its main advantage would be its high solubility in water, and perhaps lower toxicity, while not sacrificing a significant degree of metal ion affinity. The original synthesis of PLED yielded a substance containing two acetate groups which at the time were considered acetates of crystallization since acetic acid was used in the isolation and final purification of the ligand. More recently, however, it has been shown in this laboratory that bromoacetic acid, a reagent used in the synthesis for alkylation of bispyridoxylethylenediamine, an intermediate, vigorously alkylates the other functional groups: the aromatic pyridine nitrogen to give quaternary pyridinium acetate groups such as 3, and more slowly, the phenolate groups of the pyridine rings to give the corresponding *O*-acetic acids. Because these covalently-bound acetates could not easily be distinguished from acetates of crystallization by elemental analysis and by NMR,



the earlier studies of PLED were probably made on one of these alkylated derivatives of PLED, or on a mixture of an alkylated derivative and PLED. In this study, an unambiguously pure sample of PLED was prepared by an improved synthetic method, and the equilibrium data now obtained are provided as replacements for all such data reported previously [1].

SHBED (*N,N'*-bis-2-hydroxy-5-sulfobenzylethylenediamine-*N,N'*-diacetic acid) (4) is the 5-sulfo derivative of the well-known ligand HBED [2, 3] which has very high affinity for trivalent metal ions such as Fe(III), but has limited water solubility. The rationale in employing the sulfonated ligand was to increase the water solubility of the parent HBED without greatly affecting affinity for the Fe(III) ion and other trivalent metal ions. Originally, SHBED was first described in a patent by Kroll [4]. However, the procedure employed yielded mainly a monosubstituted product, *i.e.* only a single phenolic group was present in the ligand. In this work, the procedure was modified so as to obtain chemically pure SHBED in reasonable yield.

Experimental

Materials

PLED. To a suspension of pyridoxal·HCl (25.0 g, 0.123 mol) in 125 ml methanol were added with

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stirring NaOH (4.88 g, 0.122 mol in 5 ml water) and ethylenediamine (3.75 g, 0.0625 mol). The yellow Schiff base precipitate was separated and washed with several 50 ml portions of MeOH and absolute ether. The Schiff base was reduced with a sodium borohydride (6.5 g) suspension in 500 ml absolute EtOH for 1 h. Suspended solid was filtered off, most of the solvent was removed, replaced by 140 ml water, and the pH was adjusted to 9.9 with HCl. The bispyridoxylethylenediamine separated as a colorless solid. It was filtered off and dried under vacuum with phosphorus pentoxide. Yield 21 g (78%).

A solution of 7.24 g (0.020 mol) of the bis(pyridoxylethylenediamine was prepared in 80 ml water, containing bromoacetic acid (5.56 g, 0.040 mol) and sodium hydroxide (1.6 g, 0.040 mol). The reaction mixture was gently warmed at 40 °C, while NaOH (16 ml, 2.5 M) was added dropwise and slowly enough to keep the pH from exceeding 11.2. The pH of the solution was raised to 11.5 with more NaOH solution and after 14 h at 25 °C it was treated with Amberlite IRC-50S (H-form), filtered, treated with Dowex 50 W-8, and filtered again, to remove the sodium salts. The solution was concentrated on a rotary evaporator at room temperature to near dryness; water and ethanol was added, producing a sticky solid precipitate, which was further purified by reprecipitation in water-methanol solution and was dried under vacuum at 50 °C for 2 h. Yield 3.3 g (35%). *Anal. Calc.* for $C_{22}H_{30}N_{55}O_8 \cdot H_2O$: C, 53.23; H, 6.45; N, 11.29. Found: C, 53.31; H, 6.21; N, 11.07%.

SHBED. To a refluxing methanol solution (60 ml) containing ethylenediaminediacetic acid (4.4 g, 25.0 mmol), sodium *p*-phenolsulfonate dihydrate (23.2 g, 100 mmol) and NaOH solution (1.8 g, 45 mmol, in 3.0 ml water), formaldehyde solution (8.2 g of 37% aq. solution, 100 mmol) was slowly added. The solution was then refluxed for 5.5 h, filtered hot, and allowed to crystallize. Yield 3.2 g (18%) as the tetrasodium salt. It may be recrystallized from water-methanol (2 ml/50 ml). *Anal. Calc.* for $C_{20}H_{20}N_2S_2O_{12}^{-4} \cdot 4Na^+ \cdot 4H_2O$: C, 33.90; H, 3.95; N, 3.95. Found: C, 34.07; H, 4.05; N, 3.80%.

Potentiometric Measurements

The details of the methods employed are described in a short monograph written by the authors [4]. The apparatus consisted of a Polysciences constant temperature bath (25.0 °C) attached to a 100 ml glass jacketed reaction vessel with an airtight cap fitted with reference and glass electrodes, appropriate gas inlet and outlets, and a sealed-in Metrohm 10.0 ml capacity piston buret tip. A Corning Model 150 pH meter was calibrated with strong acid and

strong base at 25.0 °C and $\mu = 0.100$ M (KCl) so that all pH values are directly expressed as $-\log[H^+]$, designated as p[H]. All experiments were performed under these conditions. All potentiometric data were processed on the Departmental VAX computer with the aid of Fortran program BEST [5].

Spectral determinations were made with a Perkin-Elmer Model 553 Fast-Scan spectrophotometer equipped with 1.000 ± 0.001 cm matched cells at 25.0 ± 0.1 °C. The stability constants were calculated from the data by the use of in-house programs written in Basic utilizing mass balance and known equilibrium constant constraints while minimizing the least-squares absorbance fit to the observed spectral curves. Each such determination was made on from 5 to 15 spectra.

Results and Discussion

Protonation of PLED

Because of the variety of equilibria involved and differences in behavior observed in reactions between the various metal ions investigated and PLED and SHBED, various strategies were employed, depending on the system studied. PLED protonation constants were determined by direct potentiometric p[H] measurements because all protonation reactions were observed to take place within the potentiometrically measurable p[H] range (2.0–12.0). The successive log protonation constants were found to be 10.89, 10.28, 7.20, 5.73, 3.26 and 2.31 with high relative accuracy measured by a σ_{fit} of 0.002 pH units. These protonation constants were used in the computations of the stability constants of the metal complexes of PLED. Before describing metal complex equilibria, the protonation sites of PLED deserve comment. Figure 1 shows the UV spectra of PLED measured at α values representing the maximum concentration of each protonated form. In considering the nature of the donor groups involved in successive protonation reactions, it is helpful to invoke the protonation constants of pyridoxamine (10.13, 8.01 and 3.37) [6] and pyridoxol (8.84 and 4.88) [7], analogs which are considerably less ambiguous in visualizing the nature of the functional groups involved in their protonation sequences. Thus it is known [8] that the completely deprotonated pyridoxol molecule first protonates at the pyridine nitrogen, and the resulting zwitter ion then becomes protonated on the aromatic oxygen, and that pyridoxamine protonates first at the amino group, then at the pyridine nitrogen, and finally at the aromatic oxygen. This information, taken together with the spectra shown in Fig. 1, was employed to deduce the protonation sequence illustrated in Scheme 1 which shows only the protonated forms considered to be the major species for each

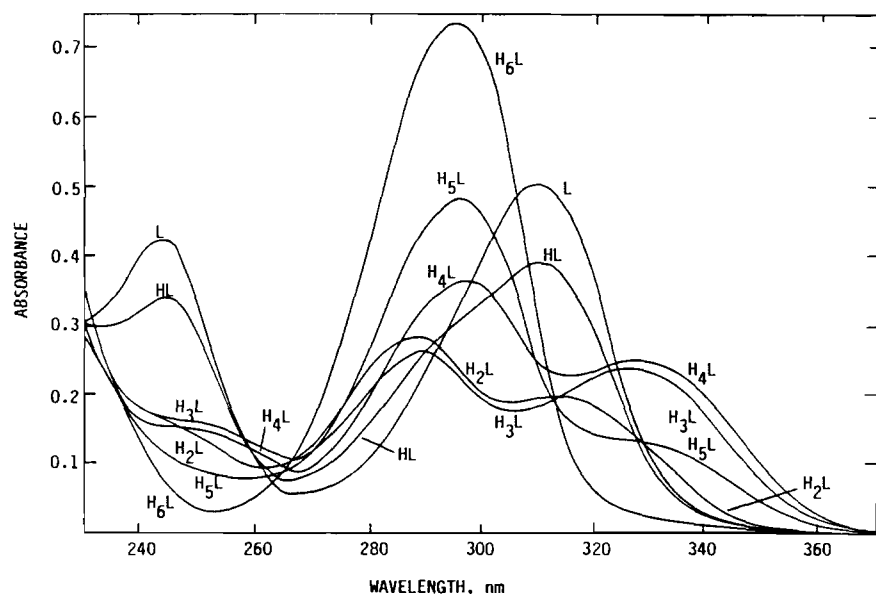


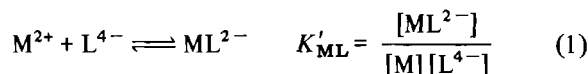
Fig. 1. Ultraviolet spectra of PLED measured at $p[H]$ values adjusted to coincide with integral a -values representing the maximum concentration of each protonated species. $T_L = 1.59 \times 10^{-4} M$, $\mu = 0.100 M$ (KCl), $t = 25.0^\circ C$.

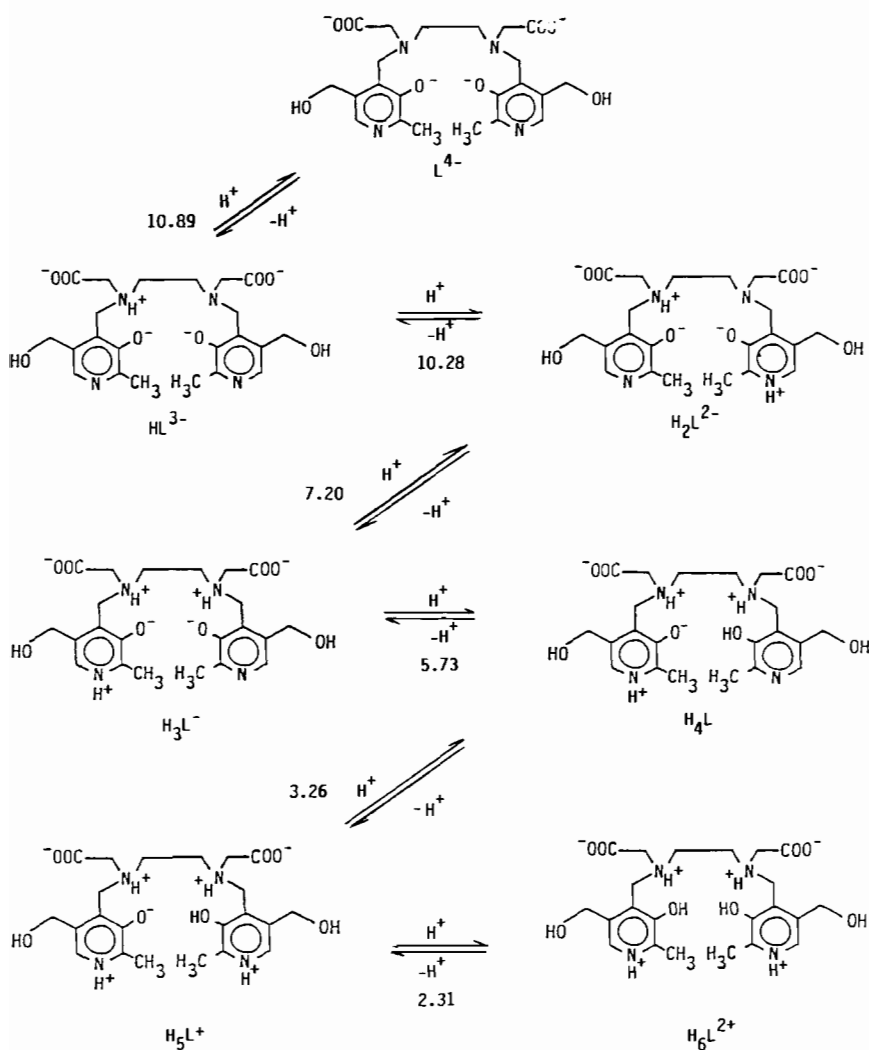
protonation step. The first protonation step occurs at one of the two aliphatic amino nitrogens, giving HL. The spectral changes are minimal in going from L^{4-} to HL^{3-} which have absorbances at ~ 250 and 310 nm, respectively. Further protonation collapses these absorbances while new bands at 290 and 310 nm appear during the formation of H_2L^{2-} . This change supports the pyridine-nitrogen site protonation, which probably occurs at the position farthest away from that of the protonated aliphatic amino nitrogen, so as to minimize coulombic repulsion between the positive charges. Because the spectrum of H_3L^- is very similar to that of H_2L^{2-} , except for a moderate shift in wavelength, it seems probable that this protonation step involves primarily the second aliphatic amino nitrogen, resulting in some increase in coulombic repulsion. Upon formation of H_4L , the band at 330 nm undergoes little change in both intensity and wavelength, while the 290 nm band grows somewhat and shifts to 295 nm, indicating the possible protonation of one of the phenolic oxygens, as indicated in Scheme 1 for H_4L . The species H_5L^+ shows a further decrease in absorbance of the 300 nm band but an increase in the 295 nm band. The ligand must at this stage become protonated at the remaining pyridine nitrogen and, with only one site left, H_6L must be the fully protonated species, with all functional groups except the carboxylate groups having undergone protonation. It is important to point out that the microspecies in the ligand protonation sequence are not limited to the major species suggested in Scheme 1, but include several other sub-

species with alternate protonation sites, and in the lower $p[H]$ range the carboxylate groups also participate. Each of the protonated forms H_nL , where $n = 1-6$, consists of a combination of subspecies, the ratios of concentrations of which are expressed by $p[H]$ -independent distribution constants. With the data currently available, it is not possible to describe the microspecies in their entirety. Also, it is important to realize that even though protonation of the pyridine nitrogens do not directly involve the coordination sphere, it has a greater effect than simply altering the overall charges of the metal complexes formed. The pyridine nitrogens are directly linked to the phenolic-type oxygens and protonation of the pyridine nitrogens greatly lowers the metal ion affinities of the phenolate oxygens.

Divalent Metal Complexes of PLED

Copper(II), cobalt(II) and zinc(II) were found to combine readily with PLED to form ML^{2-} , MHL^- , MH_2L , and MH_3L^+ , in relative amounts depending on the $p[H]$ of solution. Table 1 contains the values for the log stability constants and log protonation constants of these chelates. Without invoking additional evidence, the arrangement of the coordinating groups around the ion can be deduced from the rearrangement of the data in the Table into formation constants written in terms of specific protonated states of the ligand as in eqns. (1)–(4).

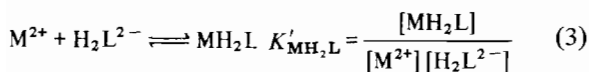
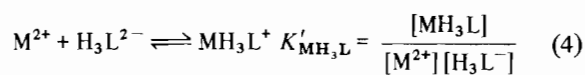




Scheme 1. Protonation of PLED.

TABLE 1. Log Stability Constants of PLED with Cu(II), Co(II) and Zn(II) at 25.0° (0.100 M KCl) Measured by Direct Potentiometry

Quotient	Symbol	Cu(II)	Co(II)	Zn(II)
$[ML]/[M][L]$	$\log K_{ML}$	21.6 ^a	17.29	16.62
$[MHL]/[ML][H]$	$\log K_{MHL}$	8.65	8.85	8.82
$[MH_2L]/[MHL][H]$	$\log K_{MH_2L}$	7.89	8.14	8.11
$[MH_3L]/[MH_2L][H]$	$\log K_{MH_3L}$	2.59	1.93	2.02
	σ_{fit}	0.004	0.003	0.003

^aAbout 95% complexed at start of titration.

The results of these calculations given in Table 2 show that as n increases from 0 to 3 in $K'(MH_nL)$,

the values of K' become lower as n increases. The reduction at $n = 3$ is five orders of magnitude compared to about two orders of magnitude for the first two steps. This is interpreted to imply that the coordinate bonding in ML^{2-} , MHL^- and MH_2L involves two carboxylates, two phenolate oxygens and two aliphatic nitrogens, with protons going to the pyridine nitrogens, while bonding in MH_3L^+ is most likely terdentate involving only one half of the PLED molecule (*i.e.* one carboxylate, one aliphatic amino group, and the phenolate oxygen, with the three protons distributed among the two pyridine nitrogens and the uncoordinated phenolate oxygen). The coordination cannot be analogous to EDDA (Table 2) because the log formation constants of EDDA are about four orders of magnitude higher than the corresponding values of $\log K'(MH_3L^+)$.

TABLE 2. Comparison of Stabilities of Protonated Complexes of PLED with those of EDDA^a

Constant	Quotient	$\log K$		
		Cu(II)	Co(II)	Zn(II)
K_{ML}	$[ML^{2-}]/[M^{2+}][L^{4-}]$	21.6	17.29	16.62
K_{MHL}^-	$[MHL^-]/[M^{2+}][HL^{3-}]$	19.4	15.25	14.55
K_{MH_2L}	$[MH_2L]/[M^{2+}][H_2L^{2-}]$	17.0	13.11	12.38
$K_{MH_3L}^+$	$[MH_3L^+]/[M^{2+}][H_3L^-]$	11.9	7.87	7.20
K_{ML}	$[ML]/[M^{2+}][L^{2-}]$	16.2 ^b	11.25 ^b	11.22 ^b

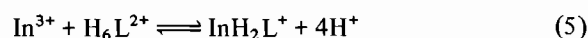
^aEDDA = ethylenediamine-*N,N'*-diacetic acid. ^bData from ref. 6.

The presence of the MH_2L type complex is important because it can be computed that at physiological pH (~ 7.4) it is the predominant species. Of some interest is the fact that this species is neutral in charge without strongly compromising thermodynamic stability of the metal complex. This suggests the possibility of improved transport of divalent metal ions across biological membranes.

Trivalent Metal Complexes of PLED

The PLED complexes of Ga(III), In(III) and Fe(III) were found to be so diverse in properties and stabilities that different experimental methods, specifically tailored to the properties of each metal complex, had to be employed in order to completely characterize the complexes and determine the essential equilibrium constants. The In(III) complex of PLED was found to form completely in the acid region below pH 2, thus excluding the possibility of direct potentiometric determination. Its stability therefore was determined by spectrophotometry as the MH_2L^+ complex was formed to different extents depending on the concentration of acid present. The conversion of MH_2L^+ to ML^- was determined by separate potentiometric $p[H]$

measurements, and $\log K_{ML}$ was then calculated by the use of eqn. (9) from the change of absorbance with $p[H]$ in the band centered at 310 nm. The actual observations were made at 315 nm ($\epsilon(MH_2L) = 4778 \text{ M}^{-1} \text{ cm}^{-1}$) where the overlap between the absorption bands of the free ligand and the metal chelate is considerably lower than at 310 nm. The values employed at 315 nm ($\epsilon(MH_2L) = 4431 \text{ M}^{-1} \text{ cm}^{-1}$ and $\epsilon(H_6L) = 961$). The equilibrium in strongly acid solution is represented by eqn. (5), with MH_2L^+ as the only indium species present. Equations (6)–(8) provide a means for evaluating the expression shown in eqn. (9); all other quantities are known or are directly measured. The log value for the displacement constant shown in eqn. (10), corresponding to eqn. (5) was found to be -0.40 , making the normal stability constant $\log K_{ML}$ for InL^- 26.54 (Table 3).



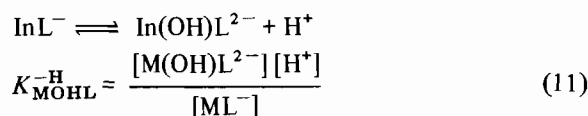
$$[MH_2L] = \frac{A - T_L \epsilon_{H_6L}}{\epsilon_{MH_2L} - \epsilon_{H_6L}} \quad (6)$$

$$[M^{3+}] = T_M - [MH_2L^+] \quad (7)$$

$$[H_6L^{2+}] = T_L - [MH_2L^+] \quad (8)$$

$$K_{ML} = \frac{[ML^-]}{[M][L]} = \frac{[MH_2L^+][H^+]^4}{[M^{3+}][H_6L^{2+}]} \times \frac{[H_6L^{2+}]}{[H^+]^6[L^{4-}]} \times \frac{[ML^-][H^+]^2}{[MH_2L^+]} \quad (9)$$

$$K' = \frac{[MH_2L^+][H^+]^4}{[M^{3+}][H_6L^{2+}]} \quad (10)$$



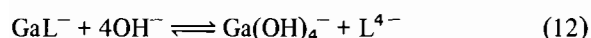
At very high pH the In(III)–PLED chelate undergoes a hydrolytic reaction, indicated by eqn. (11); the log equilibrium constant was found to be -11.21 .

The Ga(III)–PLED complex GaH_2L^+ does not dissociate even in the presence of considerable excess acid. However, the novel method developed by Motekaitis and Martell [9] involving competition by OH^- for Ga(III) to form $Ga(OH)_4^-$ resulting in dissociation of the metal chelate GaL^- , was used to determine the formation constant while monitoring the position of the equilibrium spectrophotometrically. The spectrophotometric measurements were made at 310 nm (ϵ_{ML^-}) at high pH where

TABLE 3. Log Equilibrium Constants for In(III), Ga(III) and Fe(III) Chelates of PLED (25.0 °C, $\mu = 0.100$ M (KCl))

Quotient	Constant	In(III)	Ga(III)	Iron(III)
$[\text{ML}^-]/[\text{M}^{3+}][\text{L}^{4-}]$	$\log K_{\text{ML}}$	26.54	32.31	30.78
$[\text{MHL}]/[\text{ML}^-][\text{H}^+]$	$\log K_{\text{MHL}}$	7.15	7.10	6.93
$[\text{MH}_2\text{L}^+]/[\text{MHL}][\text{H}^+]$	$\log K_{\text{MH}_2\text{L}}$	6.34	6.2	6.02
$[\text{M}(\text{OH})\text{L}^{2-}][\text{H}^+]/[\text{ML}^-]$	$\log K_{\text{MOHL}}^{-\text{H}}$	-11.21		

the only reaction that occurs is expressed by eqn. (12). Therefore in order to calculate the 'normal' stability constant, eqn. (17), eqns. (13)–(16), which are statements of the mass balance conditions, and known equilibrium constants, were employed. The molar absorbance coefficient for GaL^- was found to be $13\,300\text{ M}^{-1}\text{ cm}^{-1}$. The binding of Ga(III) by PLED is quite strong, with $\log K_{\text{ML}}$ equal to 32.31. This constant is compared with those of the other trivalent metal ions in Table 3.



$$[\text{GaL}^-] = \frac{T_{\text{L}}(\epsilon_{\text{L}} + \epsilon_{\text{HL}}K^{\text{H}}[\text{H}]) - A(1 + K^{\text{H}}[\text{H}^+])}{\epsilon_{\text{L}} + \epsilon_{\text{HL}}K^{\text{H}}[\text{H}^+] - \epsilon_{\text{ML}}(1 + K^{\text{H}}[\text{H}^+])} \quad (13)$$

$$[\text{Ga}(\text{OH})_4^-] = T_{\text{M}} - [\text{GaL}^-] \quad (14)$$

$$[\text{Ga}^{3+}] = \frac{[\text{Ga}(\text{OH})_4^-][\text{H}^+]^4}{\beta_{\text{Ga}(\text{OH})_4^-}} \quad (15)$$

$$[\text{L}^{4-}] = \frac{(T_{\text{L}} - [\text{GaL}^-])}{(1 + K^{\text{H}}[\text{H}^+])} \quad (16)$$

$$K_{\text{GaL}^-} = \frac{[\text{GaL}^-]}{[\text{Ga}^{3+}][\text{L}^{4-}]} \quad (17)$$

It is apparent that the two protonation constants of the metal chelates are associated with the stepwise protonation of the pyridine nitrogens, with no major change in the coordination sites. The phenolate oxygen donors however, coordinate the metal ions much less strongly when the pyridine nitrogens are protonated. A reformulation of the stability constants in Table 3 in terms of the types of constants in Table 2 show that the constants of the type $[\text{MH}_n\text{L}]/[\text{M}][\text{H}_n\text{L}]$, where $n = 0, 1$, and 2, decrease by four orders of magnitude for each protonation step. Unlike the divalent metal ion complexes of PLED, the trivalent metal ion chelates are not neutral in charge at physiological pH but consist of approximately 50:50 ML^- and MHL mixtures, as may be seen from the values of $\log K(\text{MH}_n\text{L})$ listed in Table 3, and the distribution curves (see Fig. 4).

Iron(III) was found to form a thermodynamically very stable chelate in both acidic and basic solutions.

Competition reactions with other ligands, and with high hydrogen ion concentrations do not result in appreciable dissociation of the complex, precluding the use of such methods for determining stability constants. Therefore, the stability constant of the iron(III)–PLED chelate was determined by the use of e.m.f. measurements to calculate the actual concentrations of the ferric ion in equilibrium with its PLED chelate in strongly acid solutions in the presence of Fe^{2+} , which does not complex the ligand under the conditions employed. Thus the determination of the reduction potential of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple in the experimental solution, together with the known value of $[\text{Fe}^{2+}]$, gave the Fe^{3+} concentration directly. Because FeH_2L^+ is the only complex present, sufficient data are available to calculate the four-proton displacement constant for the reaction $\text{Fe}^{3+} + \text{H}_6\text{L}^{2+} \rightleftharpoons \text{FeH}_2\text{L}^+ + 4\text{H}^+$, which was found to be 4.07 log units. This value is converted to a log normal stability constant of 30.78. The protonation constants of the iron(III) chelate were determined by potentiometric measurements of hydrogen ion concentrations. At physiological pH the iron(III)–PLED chelate exists only partially as the neutral chelate, MHL .

Visible spectra show that as the MH_2L^+ chelate is successively deprotonated the absorption band at 400 nm decreases in intensity while a new band at 480 nm is formed. The second absorbance, originally at 440 nm, moves 20 nm toward longer wavelengths with each deprotonation.

Protonation of SHBED

Introduction of the sulfo groups into SHBED (2) greatly increases its aqueous solubility. Although the formal charge of the deprotonated ligand is 6^- , the donor groups in the coordination sphere of a metal ion would not see the effect of the additional charge imparted by the sulfonate groups because it is counterbalanced by the electropositive character of the sulfur atoms covalently bound to the aromatic ring in 4. (For example, *p*-sulfophenol is a stronger acid than phenol.) The greater acidity of the phenolic groups (lower pKs) results in a ligand (SHBED, 4), which is more effective at lower p[H] values. This effect, however, is counterbalanced by a lower intrinsic affinity for metal ions.

The protonation constants of SHBED at 25.0 °C (0.100 M KCl) were computed by the use of two methods. The first protonation constant was determined spectrophotometrically, because it is out of range for accurate potentiometry, while the remaining constants were computed from potentiometric p[H] measurements. The logarithms of protonation constants found are 12.91, 10.42, 7.90, 4.29, 1.96 and 1.2. It is noted that the lowest value, 1.2, was determined by p[H] measurements below 2.0, which is below the cut-off at the lower end of the p[H] scale, and therefore is less accurate than the other values.

In addition to the protonation constants of SHBED listed in Table 4, extinction coefficients are presented for the various ligand species at 255 nm, which is the absorption band associated with the deprotonated phenolate group. The initial molar absorbance drops to 1/2 upon the addition of the first proton, indicating the protonation site to be a phenolate oxygen. The second protonation occurs mainly on amino nitrogen because the molar absorbance is only slightly decreased. The third protonation which forms H_3L^{3-} is associated with an extensive drop in molar absorbance indicating that it involves mainly the second phenolate group. The remaining protonations are rationalized as amino nitrogen, acetate, and a second acetate, with some mixing of the amino and acetate protonation reactions.

TABLE 4. Protonation Constants of SHBED and Change of Molar Absorbance at 255 nm with Protonation

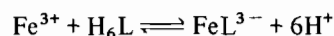
Species	Protonation constants	Molar absorbance	Comments
L^{6-}		13030	
HL^{5-}	12.91	6590	phenol protonation
H_2L^{4-}	10.42	4795	mostly nitrogen protonation
H_3L^{3-}	7.90	557	phenol protonation
H_4L^{2-}	4.29	370	nitrogen protonation
H_5L^-	1.96	227	acetate protonation
H_6L	1.2	^a	acetate protonation

^aCould not be determined.

SHBED Trivalent Metal Ion Systems

Because certain trivalent metal ions have become of considerable interest recently, it was decided to study the stabilities of the chelates of Fe(III), In(III), Ga(III), Gd(III) and Al(III) with SHBED. Iron(III) and gadolinium(III) complexes may find applications as MR imaging contrast agents, In(III) and Ga(III) are of interest as radiopharmaceuticals, and Al(III) is of interest because it has recently been implicated in brain disorders.

Iron(III) forms a deep red complex with SHBED throughout the p[H] range from 1.5–10, and takes on a yellow hue at higher p[H] values. These qualitative observations are interpreted in terms of a stable FeL^{3-} complex which forms a $FeOHL^{4-}$ species at high p[H]. This hydrolytic reaction was determined by potentiometric p[H] measurements and was found to be 50% complete at pH 10.57. Below p[H] 2 sufficient free Fe^{3+} dissociates to compute the stability constant directly. Figure 2 shows the visible spectra as a function of $-\log [H^+]$. The absorbance maximum is found to be 475 nm ($\epsilon_{ML} = 4012 \text{ M}^{-1} \text{ cm}^{-1}$). The curves indicate that the complex dissociates over a narrow pH range (half complete \sim pH 1.3–1.0) since six protons are involved in this equilibrium according to eqn. (18) (*i.e.* the ratio of concentrations of uncomplexed Fe(III) to complex FeL^{3-} varies as the sixth power of the hydrogen ion concentration). Since p[H] is as low as one can go while maintaining the formal ionic strength at 0.100, the p[H] 0.69 and 0.52 curves on the bottom of the Figure were not used in the calculation.



$$K_x = \frac{[FeL^{3-}][H^+]^6}{[Fe^{3+}][H_6L]} \quad (18)$$

The logarithm of the displacement constant shown in eqn. (18) has a value of -1.81 , which was calculated with the use of concentrations of the two most highly protonated forms of the ligand.

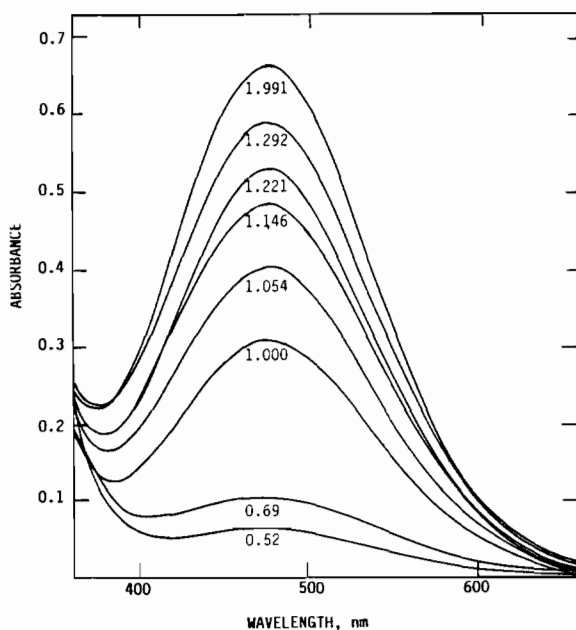


Fig. 2. Visible spectra of a series of Fe(III)-PLED solutions measured at various low $-\log [H^+]$ values. $T_{FeL} = 1.65 \times 10^{-4} \text{ M}$, $\mu = 0.100 \text{ M (KCl)}$, $t = 25.0 \text{ }^\circ\text{C}$.

The other protonated forms are out of range and they do not participate in low pH equilibria. The normal stability and hydrolysis constants obtained for the iron(III) chelates are summarized in Table 4. It was found that protonated chelates MH_nL are not present throughout the $p[H]$ range 1.0–12.0.

Because of the relatively low stabilities of the chelates formed by SHBED and gadolinium(III), all constants were obtained by potentiometric $p[H]$ measurements. However, besides the normal log stability constant of 23.36, unexpectedly four protonated chelates were found to be formed. Also unexpected is that the monoprotonated chelate MHL appears to be unstable relative to the deprotonated form (ML^{3-}) or diprotonated species, MH_2L^- .

Potentiometric $p[H]$ measurements on the In(III)–SHBED system showed the simultaneous presence of InL^{3-} , $InHL^{2-}$, as well as aquo In^{3+} ion so that the normal stability constant and the chelate protonation constant were calculated directly from the $p[H]$ data and the logarithms of the values thus determined are listed in Table 5. An analysis of the high pH data showed the presence of a hydroxo chelate. The same stability constant may be calculated from the spectrophotometric data shown in Fig. 3. In fact, a simple calculation which neglected the chelate protonation reaction gave a spectrophotometric $\log K_{InL}$ of 29.67 (0.04), which is slightly greater than the correct value in Table 5, because of this approximation. The molar absorbance at 255 nm is $20\,600\ M^{-1}\ cm^{-1}$ for InL^{3-} .

Potentiometric $p[H]$ measurements show that the protonation constant $\log K_{GaHL} = 1.18$, but

GaL^{3-} is $\sim 99\%$ formed, corresponding to a $\log K_{GaL}$ somewhat above 36. Absorption spectra show that in the pH region 1.01 to 2.14 the main reaction is expressed by eqn. (19)

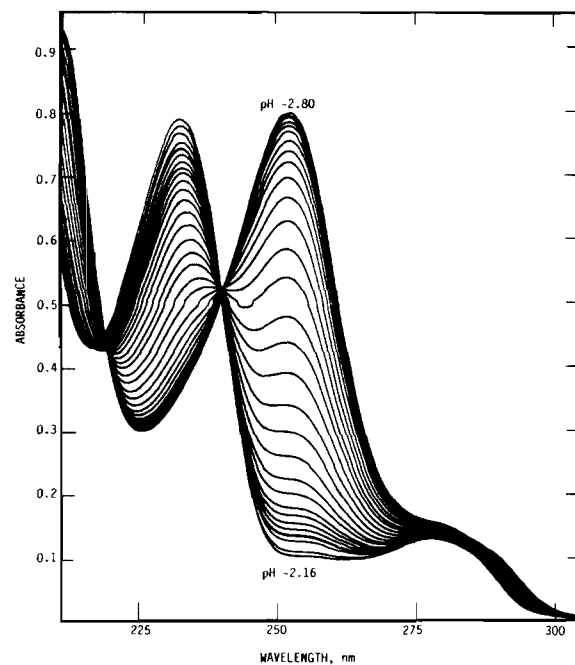


Fig. 3. Ultraviolet spectra as a function of $-\log [H^+]$ for $InSHBED^{3-}$ measured at $25.0\ ^\circ C$, $\mu = 0.100\ M$ (KCl), $T_{InL} = 1.67 \times 10^{-4}\ M$; curves measured at $0.05\ p[H]$ intervals.

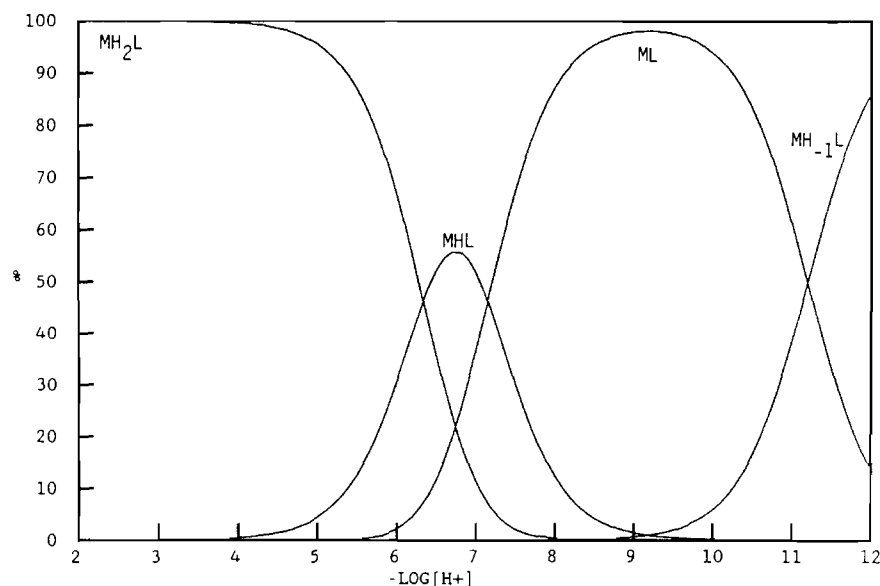


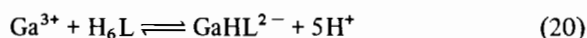
Fig. 4. Species present at various $-\log [H^+]$ values computed at $25.0\ ^\circ C$, $\mu = 0.100\ M$, for solutions containing $1.00 \times 10^{-3}\ M = [In(III)PLED]_0$, expressed as % relative to $[InPLED]$.

TABLE 5. Stability Constants of In(III), Ga(III), Fe(III) and Gd(III) Chelates of SHBED (25.0 °C and $\mu = 0.100$ M (KCl))

Symbol	In(III) ^a	Ga(III) ^b	Fe(III) ^c	Gd(III) ^d
Log K_{ML}	29.37	37.47	36.87	23.36
Log K_{MHL}	2.82	1.18		3.86
Log K_{MH_2L}				5.10
Log K_{MH_3L}				4.03
Log K_{MH_4L}				1.96
Log K_{MOHL}	-10.82		-10.56	

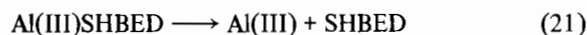
^a $\sigma_{fit} = 0.007$. ^b $\sigma_{fit} = 0.007$. ^c $\sigma_{fit} = 0.012$. ^d $\sigma_{fit} = 0.011$.

Below that p[H] range the reaction which takes place is indicated by eqn. (20).



Because of this situation log K_{GaHL} was calculated from the data measured in very acidic solutions, where the ionic strength is not completely under control, and the log stability constant was found to be 37.4 (0.3) at 25.0 °C with $\epsilon_{GaL} = 8920$ M⁻¹ cm⁻¹.

The reaction of the Al(III) ion with SHBED was found to be very slow. Solutions of metal ion and ligand set up at several initial p[H] values underwent changes in p[H] for over a week, so that it was not possible to determine the p[H] at equilibrium during this time span. However, it was possible to obtain some half lives of the dissociation of Al(III) from the preformed chelate at several p[H] values corresponding to eqn. (21).



At pH 1.30, $t_{1/2} = 43$ h whereas at pH '-0.02', the $t_{1/2} \approx 3$ h. Since these reactions slow down with increasing pH, it is apparent that a special study must be made in order to determine accurate equilibrium constants in these systems, and that the standard equilibrium techniques will simply not work.

General Comments

The differences between the stability constants of the trivalent metal ion constants in Tables 3 and 5, and those published previously [1, 10] are so large, especially in the case of In(III), that they cannot be explained on the basis of improved ligand purity alone. It is necessary to look more deeply into the source of the data and the methods of calculation [11]. The first step is to plot the degrees of formation of the complex species present as a function of p[H]. As an example, the In(III)-PLED system illustrated in Fig. 4 shows that at p[H] below 3, the complex is 100% formed, with

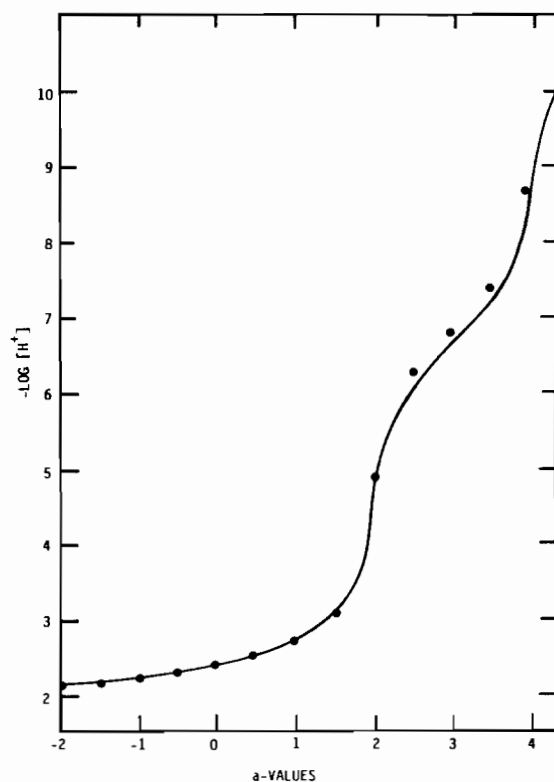


Fig. 5. p[H] profiles calculated from two sets of stability constants; the solid line was calculated for Ga(III)-PLED from the data in Table 3; solid circles were calculated from the higher stability constant (log $K_{ML} = 36.31$) in ref. 11. For both systems $T_L = T_{Ga} = 2.002 \times 10^{-3}$ M; $t = 25.0$ °C; $\mu = 0.100$ M (KCl); a = moles of standard base added per mole of ligand present.

free metal ion at a level too low to appear on the Figure. Thus a calculation carried out on the basis of the p[H] profile data in this region would be insensitive to the concentration of free metal ion, which could vary by several orders of magnitude below the level at which it would be detected in the Figure. To further illustrate the problem, the p[H] profile calculated from the data in Table 3 for Ga(III) is compared in Fig. 5 with the p[H] values calculated from the stability constants in ref. 11. The fact that the two sets of data are superimposable shows that the p[H] curves are not sensitive to the magnitude of the stability constant in the acid region, provided that they meet the requirement of complete complex formation in that region (*i.e.* for log K_{ML} values > 26.54). It is such erroneously high values which were probably employed in ref. 10 to reproduce the original titration data. In other words, accurate stability constants can be calculated only from data obtained under conditions such that each species in the equilibrium expression that applies to those conditions has an appreciable and measurable concentration.

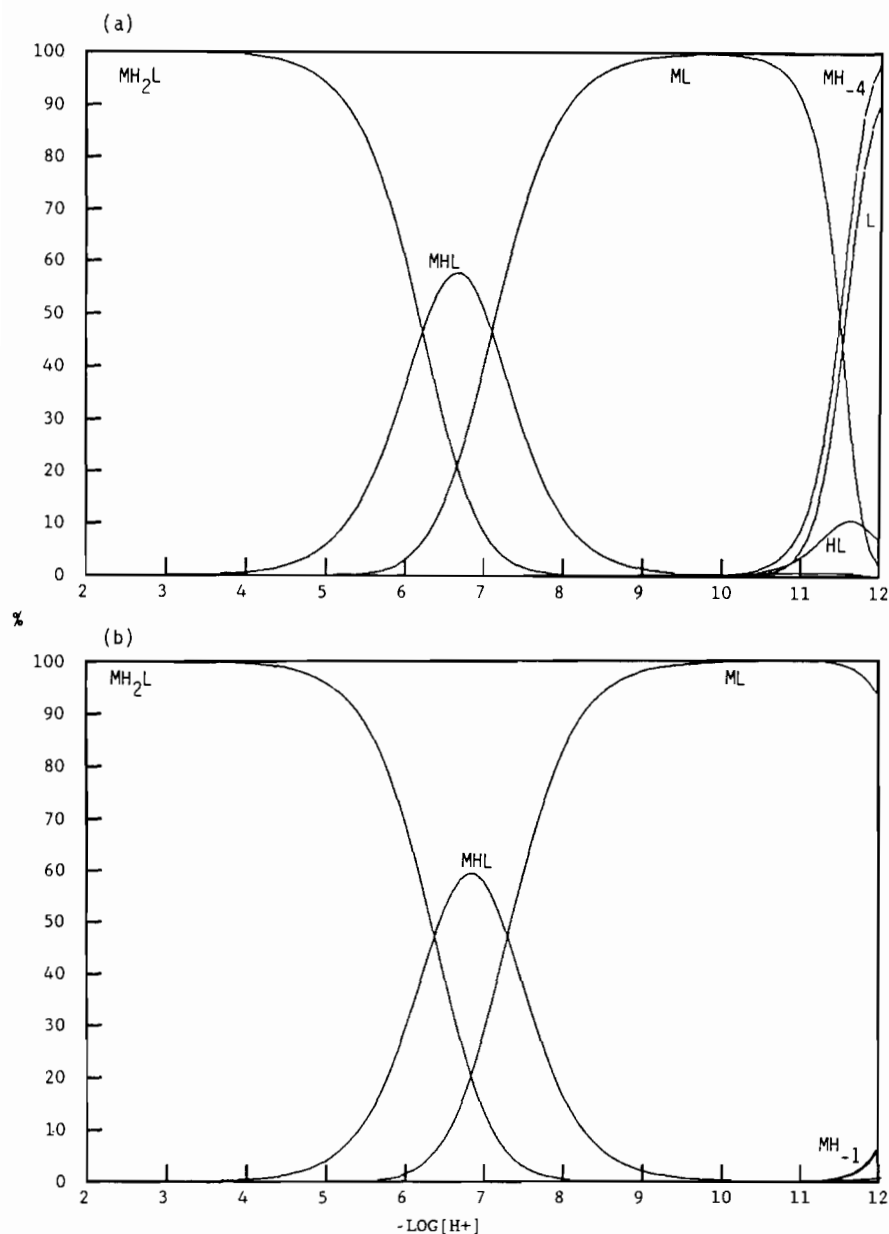


Fig. 6. Comparison of species distributions based on stability constants of Table 3 (a) with those of ref. 11; (b), illustrating the invariance of species distribution for large stability values. $\mu = 0.100 \text{ M}$ and 25.0°C in both cases; $T_{\text{ML}} = 1.00 \times 10^{-3} \text{ M}$.

Figures 6a and b illustrate the species distributions calculated from the stability constants in this paper for the Ga(III)–PLED system (6a), and from the stability constants in ref. 11. The two sets of curves seem to be identical up to $\text{p}[\text{H}]$ 10. Beyond that point, however, Fig. 6a, which is based on the corrected (lower) stability constants obtained in the present work, correctly predicts conversion of the Ga(III)–PLED complex GaL^- , to the hydroxo complex, $\text{Ga}(\text{OH})_4^-$. Also, a plot of pM versus pH based

on the corrected constants would show lower pM values than would a pM – pH plot based on the constants in ref. 11.

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References

- 1 C. H. Taliaferro and A. E. Martell, *Inorg. Chem.*, **23** (1984) 1188.
- 2 F. L'Éplattenier, I. Murase and A. E. Martell, *J. Am. Chem. Soc.*, **89** (1967) 837.
- 3 C. Pitt and A. E. Martell, *Inorganic Chemistry in Biology and Medicine*, American Chemical Society, Washington, DC, 1980, pp. 297–312.
- 4 A. E. Martell and R. J. Motekaitis, *Determination and Use of Stability Constants*, VCH Publishers, New York, 1988.
- 5 R. J. Motekaitis and A. E. Martell, *Can. J. Chem.*, **60** (1982) 2403.
- 6 R. M. Smith and A. E. Martell, *Critical Stability Constants*, Vol. 1, Plenum, New York, 1974.
- 7 R. M. Smith and A. E. Martell, *Critical Stability Constants*, Vol. 2, Plenum, New York, 1975.
- 8 B. Szpoganicz and A. E. Martell, *J. Am. Chem. Soc.*, **106** (1984) 5513.
- 9 R. J. Motekaitis and A. E. Martell, *Inorg. Chem.*, **19** (1980) 1646.
- 10 C. H. Taliaferro and A. E. Martell, *Inorg. Chim. Acta*, **85** (1984) 9.
- 11 C. H. Taliaferro, *Ph.D. Dissertation*, Texas A&M University, May, 1984.