New Multidentate Ligands.
XXIV. Disodium-N,N'-bis(2-hydroxy-5-sulfobenzyl)-ethylenediaminediacetic Acid, a New Chelating Ligand for Trivalent Metal Ions
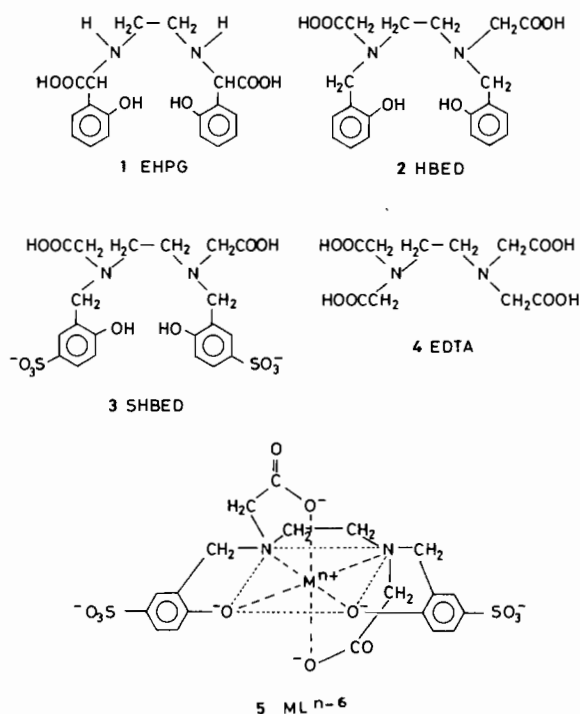
CHRISTINA H. TALIAFERRO* and ARTHUR MARTELL**

Department of Chemistry, Texas A & M University, College Station, Tex. 77843, U.S.A.

Received September 20, 1983

The study of proton and metal ion affinities is described for a new sexadentate ligand, disodium N,N'-bis(2-hydroxy-5-sulfobenzyl)-ethylenediaminediacetic acid (SHBED). The ligand has two phenolate donors 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 stability constants of its Cu(II), Ni(II), Co(II), Zn(II), Fe(III), Ga(III) and In(III) chelates are compared with those of analogous ligands. The binding of the ligand donor groups to metal ions is found to occur in several cases in a stepwise fashion, with the more basic groups requiring higher pH for activation. The order in which each phenolate group becomes coordinated to metal ions, and the structures of the intermediate complexes, are deduced by correlating the electronic absorption spectra with potentiometric data.

Introduction

Frost *et al.* [1] and Anderegg and L'Eplattenier [2] have reported the high stability of the Fe(III) chelate N,N-ethylenebis-[2-(o-hydroxyphenyl)]glycine (EHPG), *1*, to be due to the high affinity of Fe(III) for the two phenolate groups present in the ionized ligand and to the orientation of these groups so as to permit their participation in chelate ring formation. However, the structure of EHPG is such that steric hindrance may interfere somewhat with simultaneous participation in metal ion coordina-

tion by all six donor groups (two basic nitrogens, two carboxylate groups, and the two phenolate groups), with the result that the two (axial) carboxylate groups are displaced somewhat from their most favorable octahedral positions about the central metal ion. This has been clearly demonstrated in subsequent X-ray crystal structure studies [3]. For this reason, N,N'-bis(2-hydroxybenzyl)ethylenediamine -N,N'-diacetic acid (HBED) [4], *2*, was synthesized so as to present a more favorable arrangement of the same donor groups. Potentiometric studies [2] confirmed the fact that the Fe(III) chelate of HBED is extremely stable ($K_{ML} = 10^{39.68}$).

This paper describes the quantitative study of a new ligand, N,N'-bis(2-hydroxy-5-sulfobenzyl)-disodium ethylenediamine diacetic acid (SHBED), *3*, analogous to HBED, with a sulfonic acid group attached to the 5-position of each phenolic ring of HBED, in order to increase the solubility in water for its potential application as a radiopharmaceutical involving Ga(III) and In(III). The analogous ligands, HBED and EHPG and their esters have proved to be effective in test animals for the removal of iron overload [5], thus demonstrating the usefulness of aromatic hydroxy ⌐roups in the design of chelating agents having high affinity for trivalent metal ions.

Experimental

Disodium N,N'-bis(2-hydroxy-5-sulfobenzyl)ethylenediamine Diacetic Acid

This ligand was obtained commercially from Lyndal Chemical, and its synthesis has been described [6]. It was recrystallized in this laboratory, by dissolving it in a minimum amount of water, adding considerable glacial acetic acid and refrigerating the solution for 24 hours. The recrystallized product was then filtered off, washed with acetone and dried *in vacuo.* Recovery of pure product from crude material was 80%. It contained nearly two moles of acetic acid of crystallization per mole of disodium salt of the ligand. *Anal.* Calcd. for: $C_{20}H_{22}O_{12}S_2 \cdot (2Na^+) \cdot 1.9C_2H_4O_2$: C, 40.46; N, 3.96; H, 4.22. Found: C, 40.01; N, 3.92; H, 4.32.

Potentiometric Equilibrium Measurements

Potentiometric measurements of SHBED in the absence and presence of metal ions were carried out with a Corning Digital pH meter equipped with glass and calomel reference electrodes calibrated with standard aqueous HCl solution to read $-\log [H^+]$ directly. The temperature was maintained at 25.00 ± 0.05 °C, and the ionic strength was adjusted to 0.100 M by the addition of KCl. The concentrations of the experimental solutions were 2.000×10^{-3} M in metal ions and ligands. Potentiometric measurements were carried out on systems containing molar equivalents of an appropriate metal salt and the most acid form of the ligand. Additional acid may be added initially if important functional groups of the ligand are not protonated in the species employed. Small increments of standard base are then added at a concentration equivalent to the ionic strength of the solution (in this work, 0.100 M) to minimize changes in ionic strength in the course of the experiments. The reacting species were studied at concentrations low enough (2×10^{-3} M) that they did not significantly contribute to the ionic strength of the medium. For successful determination of the stability constant of the initial complex, it is essential that it be
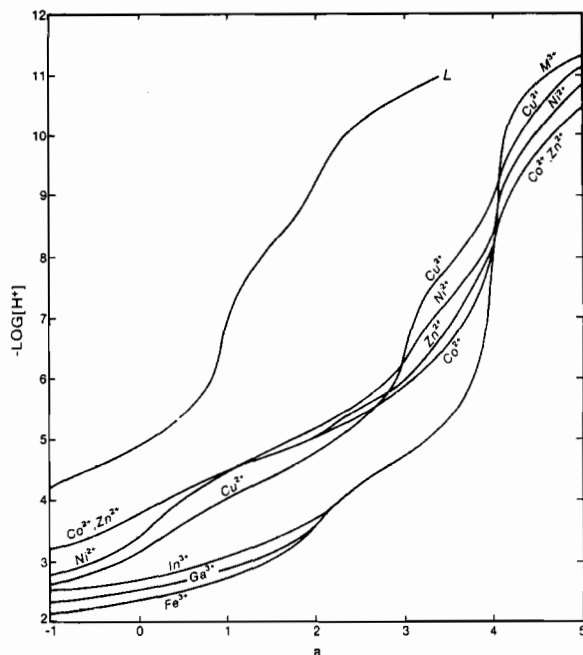

Fig. 1. Potentiometric equilibrium curves of SHBED in the absence and presence of 1:1 molar ratios of metal ion to ligand; a = mol of base added per mol of ligand present; $\mu = 0.100$ M (KCl); t = 25.00 °C; concentrations of ligand and metal ions $\cong 0.200 \times 10^{-3}$ M.

appreciably dissociated to the free metal ion (at least about 50%) at the beginning of the determination, *i.e.,* at the lowest pH measured. Further, in order to maintain the linearity of the EMF–hydrogen ion concentration calibration, it is essential that the free $[H^+]$ and $[OH^-]$ concentrations be limited to a very small fraction of that of the supporting electrolyte. This limitation restricts the $-\log [H^+]$ measurements to the range 2–12.

Since the diprotonated Cu(II) chelate is 85% formed and the Fe(III), Ga(III) and In(III) chelates are completely formed at pH 2, their formation constants could not be calculated from the normal potentiometric titration curve. Therefore, in addition to measuring the 1:1 metal–ligand system shown in Fig. 1, ligand–ligand competition studies [7] were also performed with a 1:1:1 molar ratio of the metal ion, SHBED and a reference ligand with a known metal chelate formation constant. A good reference ligand for the Cu(II), Fe(III) and In(III) systems was found to be EDTA. However, since the formation constant of the Ga–EDTA⁻ chelate is about three log units lower than the formation constants of the Fe–EDTA⁻ and In–EDTA⁻ chelates, competition by EDTA was not effective in this case, and it was necessary to use a reference ligand with a higher Ga(III) formation constant. Triethylenetetramine-

TABLE I. Protonation Constants and Metal Chelate Stability Constants of SHBED and HBED at t = 25.00 °C and μ = 0.100 M KCl.

Equilibrium Quotient	Log K		
	SHBED[b]	HBED [4]	EDTA [11]
[HL]/[L][H]	12.27	12.60[b]	10.17
[H$_2$L]/[HL][H]	10.25	11.00[c]	6.11
[H$_3$L]/[H$_2$L][H]	8.05	8.44[c]	2.68
[H$_4$L]/[H$_3$L][H]	4.50	4.72[c]	1.95
[H$_5$L]/[H$_4$L][H]	2.59	2.53[c]	1.5
[H$_6$L]/[H$_5$L][H]	1.80	1.74[c]	
[CuL]/[Cu][L]	24.44	23.69[c]	18.70
[CuHL]/[CuL][H]	7.57	8.49[c]	3.0
[CuH$_2$L]/[CuHL][H]	4.09	5.40[c]	
[NiL]/[Ni][L]	22.47	19.31	18.52
[NiHL]/[NiL][H]	7.05	8.51	3.2
[NiH$_2$L]/[NiHL][H]	5.24	6.45	
[CoL]/[Co][L]	20.66	19.89	16.26
[CoHL]/[CoL][H]	6.51	7.77	3.0
[CoH$_2$L]/[CoHL][H]	4.97	5.56	
[ZnL]/[Zn][L]	20.23	18.37	16.44
[ZnHL]/[ZnL][H]	6.90	8.27	3.0
[ZnH$_2$L]/[ZnHL][H]	5.10	5.99	
[FeL]/[Fe][L]	38.30	39.68	25.0
[FeHL]/[FeL][H]			1.3[a]
[GaL]/[Ga][L]	37.95	39.57	21.70
[GaHL]/[GaL][H]	2.62		1.80[a]
[InL]/[In][L]	37.40	39.66[c]	24.9
[InHL]/[InL][H$^+$]			1.5[a]
[InH$_2$L]/[InL][H$^+$]^2	5.31		

[a]20.00 °C.   [b]Present work.   [c]Ref. 12.

hexaacetic acid (TTHA) [8] was found to be a suitable reference ligand for the Ga(III) system.

Spectrophotometric Measurements

The visible and ultraviolet absorption spectra of the ligand and metal chelate solutions were determined with a Cary Model 14 recording spectrophotometer. The quartz absorption cells were placed in a constant temperature (25.00 ± 0.05 °C) compartment in the spectrophotometer.

Results

The results of the equilibrium determinations on SHBED are presented in Table I, along with comparable data for the analogous phenolic ligand HBED. Data for the well-known parent chelating agent, EDTA, *4*, are included for comparison.

Determination of Protonation Constants

The first protonation reaction of the ligand occurs above pH 11, where potentiometric measurements become inaccurate. Therefore, it was investigated spectrophotometrically, since it involves the protonation of a phenolic group and is therefore accompanied by an extensive change in the uv absorption spectrum. Figure 2 illustrates the measured absorbances at 255 nm, the wave-length at the maximum of the phenolate absorption band (Fig. 3), as a function of pH. The sharp increase in absorptivity between pH 9 and 12.8 may be considered a measure of the degree of dissociation of the two phenolic groups.

The ultraviolet absorption spectra of SHBED as a function of pH are shown in Fig. 3. The equilibrium involves a phenol—phenolate system, with the two species having absorption maxima at 233 and 255 nm, respectively. The measured absorbance is the sum of the products of the molar extinction coefficient
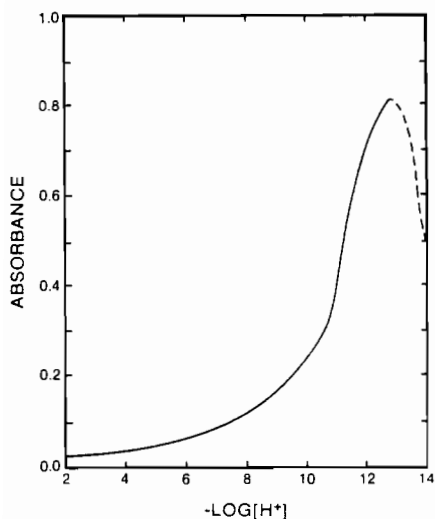
Fig. 2. The ultraviolet absorbance of SHBED as a function of pH at 255 nm [SHBED] = 1.757×10^{-4} M; μ = 0.100 M (KCl + KOH); 1.00 mm cell, t = 25.00 °C.

Fig. 3. Ultraviolet absorption spectra of SHBED as a function of pH; $-\cdot-\cdot-$, pH = 2.028; $\cdots\cdots$, pH = 4.796; $-\cdots-$, pH = 9.063; $----$, pH = 12.599; $\underline{\quad\quad}$, pH = 12.781. [SHBED]$_t$ = 1.757×10^{-4} M, μ = 0.100 M (KCl + KOH), 1.00 mm cell, t = 25.00 °C.

ϵ_{H_pL} (p = 0, 1, 2, 3, 4, 5 and 6) and the concentrations of the corresponding species, H_pL. A and ϵ_{H_pL} must correspond to the same wavelength. The total ligand concentration is also known.

$$A = \sum_{o}^{6} \epsilon_{H_pL}[H_pL] \quad\quad (1)$$

$$L_t = \sum_{o}^{6} [H_pL] = \sum_{1}^{6} K_p^{H}[H^+][H_{p-1}L] \quad (2)$$

With the aid of these relationships, the first proton association constant may be determined, as described below.

Because of inevitable experimental errors, there will always be some difference between the measured A and the sum calculated with eqn. (1). The unknown parameters are chosen so that this difference is minimized. The calculation is divided into two steps:

(a) Independent determination of K_2^{H}, K_3^{H}, K_4^{H}, K_5^{H} and K_6^{H} from the potentiometric titration curve, with the FORTRAN computer program BEST [9], which will be described briefly in the next section (Potentiometric Determination of Stability Constants). Acetic acid was titrated independently at 25.00 ± 0.05 °C and 0.100 M ionic strength and its pK_a (4.66) was calculated with the aid of the FORTRAN computer program PKAS [10]. Acetic acid, acetate and the corresponding pK_a were taken into account for the calculation of the protonation constants of SHBED.

(b) Determination of K_1^{H} using spectrophotometric measurements between pH 11.0 and pH 12.8 (at pH 12.8, decomposition of the ligand starts to occur). In this pH range,
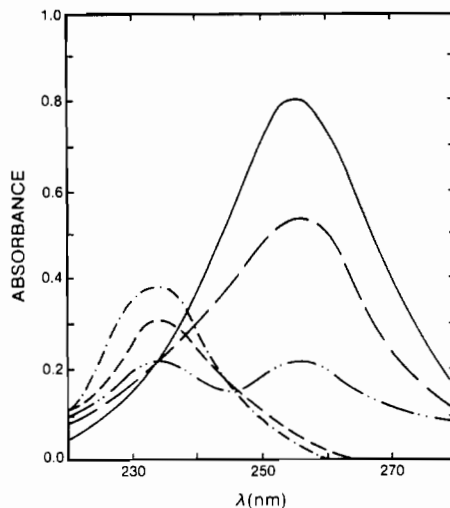
$$A = \epsilon_{HL}[HL^{3-}] + \epsilon_L[L^{4-}] \quad\quad (3)$$

$$L_t = [HL^{3-}] + [L^{4-}] = K_1^{H}[H^+][L^{4-}] + [L^{4-}] \quad (4)$$

Dividing (3) by (4) and eliminating $[L^{4-}]$, we get:

$$A = L_t \left(\frac{\epsilon_{HL}K_1^{H}[H^+] + \epsilon_L}{K_1^{H}[H^+] + 1} \right) \quad\quad (5)$$

K_1^{H} is determined so as to give constant values for ϵ_{HL} and ϵ_L for λ = 255 nm at a series of pH values.

The logarithm of the first protonation constant thus obtained for μ = 0.100 M (KCl + KOH), 25.00 ± 0.05 °C, is Log K_1^{H} = 12.27.

Potentiometric Determination of Stability Constants

The stability constants of the normal metal chelates, the protonated metal chelates and the hydrolyzed metal chelates were determined by using the FORTRAN computer program BEST. The input for BEST consists of entering the components and their concentrations, the initial estimates of the equilibrium constant for each species thought to be present in terms of these solution components, and finally the potentiometric equilibrium data determines experimentally. The program sets up simultaneous mass-balance equations for all the components present at each increment of base added and, with initial assumptions for the equilibrium constants, solves for the concentration of each species present and calculates the pH at each data point. Equilibrium constants are varied automatically in order to effect a minimization in the difference between the calculated and observed values of $-\log$ [H$^+$], thus giving ultimately a close approximation

of the original potentiometric equilibrium curve, the concentrations of the individual solution species at each data point, and the associated equilibrium constants for metal chelate formation, protonation, and deprotonation.

Discussion

Protonation Constants

A comparison of the protonation constants of SHBED and HBED shows that the first two protonation constants, corresponding to the phenolic groups and the third and fourth protonation constants, corresponding to the amino groups, are lower for SHBED. This is reasonable on the basis of the fact that the sulfonic acid group acts as an electron withdrawing group on the aromatic ring. The electron density at the phenolic oxygens is decreased somewhat by the electropositive sulfur substituent and therefore the proton affinity of SHBED is lower than that of the phenolic groups in the analogous ligand HBED.

The first protonation constant of $HBED^{4-}$ was redetermined in the present work and was found to be 0.14 log units higher than the one previously reported (12.46) [4]. A regression line was computed for the 6 protonation constants of SHBED *vs.* those of HBED. The resulting parameters ($\sigma = 0.180$, $R = 0.999$, slope = 1.06) indicate that there is a good correlation between the 6 protonation constants of SHBED and HBED, as expected from the structural similarity of these two ligands.

Stability Constants of the Metal Complexes

Correlation of the stability constants of the completely deprotonated metal chelates of HBED and SHBED yields a straight line between two clusters of points: Cu(II), Ni(II), Co(II) and Zn(II) formed one cluster while Fe(III), Ga(III) and In(III) constitute the other. The resulting parameters are: $\sigma = 0.906$, $R = 0.996$ and the slope = 1.19. These parameters indicate that there is also good correlation between the deprotonated metal chelate stability constants, as might be expected from the similarity of the structures of these two compounds, with respect to both the ligand anions and the metal complexes formed.

Another striking aspect of the stability constants of the completely deprotonated metal chelates in Table I is the fact that the formation constants of the SHBED chelates are higher than those of HBED, whereas a similar comparison of the trivalent metal ions reveal those of HBED to be higher. Considering the electron-withdrawing effect of the sulfonate groups, comparisons with metal chelates of analogous ligands such as sulfosalicylic and salicylic acid [11], and assuming that the results of the
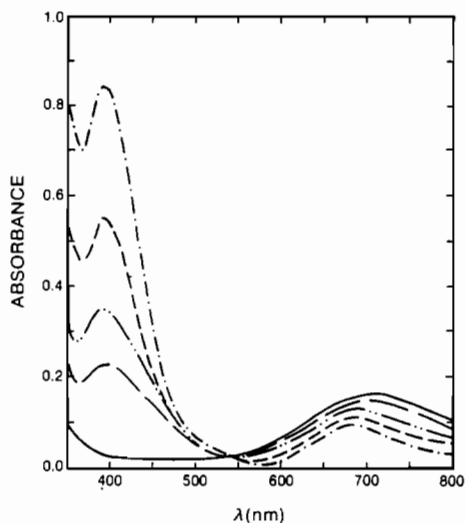

Fig. 4. Visible absorption spectra of the SHBED–Cu(II) chelate as a function of pH: ——, pH = 2.255; – – – –, pH = 4.014; –··–, pH, 5.884; ······, pH = 7.470; –·–·–, pH = 11.025. $[Cu]_t = [SHBED]_t = 1.00 \times 10^{-3}\ M$; $\mu = 0.100\ M$ KCl; 1.00 cm cell; t = 25.00 °C.

present research are correct, it seems that the HBED stability constants listed may be too low. A reinvestigation of the stability constants of HBED chelates of divalent first row transition metal ions therefore seems warranted.

The potentiometric equilibrium curves (Fig. 1) show an overlap at low pH of the buffer regions corresponding to the deprotonation of metal chelates ($MH_2L \rightleftharpoons MHL$), and to the acetic acid $\rightleftharpoons$ acetate equilibria. This makes it difficult to interpret the curves visually, but the acetic acid $\rightleftharpoons$ acetate equilibrium is easily included in the computation of the stability constants.

The probable arrangement of ligand donor groups and coordinate bonds of the completely deprotonated metal chelates of SHBED is given by 5. The initial two-proton displacement to form the diprotonated metal chelate MH_2L involves the displacement of a proton from each of the less basic tertiary amino groups. The metal ion thus is able to form a complex having three chelate rings with the tetradentate diprotonated ligand. With increasing pH, the metal ions begin to compete successfully with the protons for the phenolate groups, and appreciable amounts of complexes MHL and ML form in solution . For CuL^{2-}, there is probably strong coordination of the metal ion with the amino nitrogens and phenolate oxygens in approximately co-planar positions, and weaker coordination with the carboxylate oxygens in the fifth and sixth positions above and below the plane. The conversion $CuH_2L^{2-} \rightarrow CuL^{4-}$ is accompanied by a strong color change in the solution (Fig. 4). Below pH 3.5 the Cu(II)–SHBED

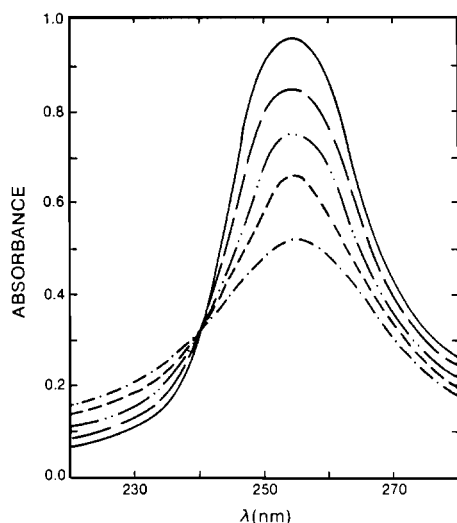Fig. 5. Ultraviolet absorption spectra of the SHBED–Ga(III) chelate as a function of pH: —·—·—, pH = 2.003; ------, pH = 2.617; —··—, pH = 3.011; — — — —, pH = 3.502; ———, pH = 11.014. $[Ga^{3+}]_t = [SHBED]_t = 1.50 \times 10^{-4}$; $\mu = 0.100$ M KCl; 1.00 mm cell; t = 25.00 °C.
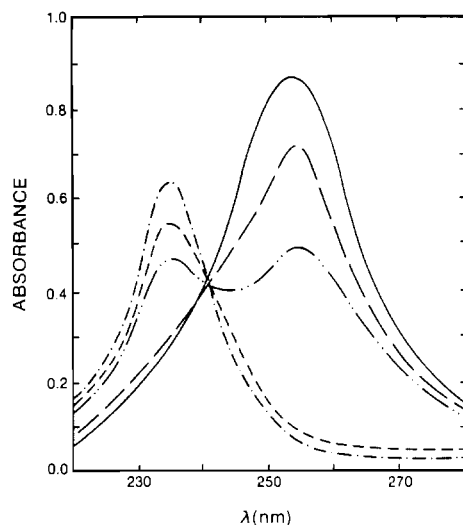

Fig. 6. Ultraviolet absorption spectra of the SHBED–In(III) chelate as a function of pH: —·—·—, pH = 2.001; -----, pH = 2.435; —··—, pH = 2.649; — — — —, pH = 3.053; ———, pH = 11.006. $[SHBED]_t = [In^{3+}]_t = 3.00 \times 10^{-4}$ M; $\mu = 0.100$ M KCl; 1.00 mm cell; t = 25.00 °C.

complex has the typical blue color normally associated with Cu(II)–amino acid chelates, characterized by a broad absorption band with a maximum at about 695 nm. The CuH_2L^{2-} complex is probably a distorted octahedron with weakly bonded water molecules in the fifth and sixth positions. As the pH is increased above 3.5 a new, much stronger band appears at 390 nm, the intensity of which increases with increasing pH and remains constant above pH 11, where the formation of CuL^{4-} is complete. This new band is certainly associated with the formation of metal–phenolate linkages and is probably caused by L → M charge transfer. Above pH 3.5, a shift in color of the complex from blue to green is observed.

The stabilities of the mono- and diprotonated chelates of SHBED were correlated with those of HBED. In each case it was found that the copper(II) complexes showed distinctive difference between the best line and the values of the protonation constants of the copper chelates of the two compounds. The correlations with copper removed are nearly perfect. The parameters for K_{MHL} are: $\sigma = 0.0192$, R = 0.999 and the slope = 1.35. For K_{MH_2L} the parameters are: $\sigma = 8.60 \times 10^{-4}$, R = 1.00 and the slope = 3.29. The exact nature of both correlations with copper removed indicates that Cu(II) behaves in a somewhat different way upon coordination, as compared to Ni(II), Co(II) and Zn(II). In the MH_2L chelate, Cu(II), Ni(II), Co(II), and Zn(II) form four strong coordinate bonds. However, the conversion $MH_2L \rightleftharpoons ML$ involves the formation of two additional strong coordinate bonds (with the carboxylate oxygens) for Ni(II), Co(II) and Zn(II), whereas Cu(II)

maintains essentially square planar geometry of its strong coordinate bonds and adds weaker coordinate bonds in the fifth and sixth octahedral positions.

If the mono- and diprotonated complexes of SHBED are compared with the analogous chelates of HBED, the protonation constants of the SHBED chelates are seen to be considerably lower. This change correlates well with the lower basicities of the phenolate oxygens compared to the values of HBED. Deprotonation of these donor groups in the course of metal ion coordination is apparently assisted by the electron-withdrawing effect of the sulfonate groups.

The potentiometric equilibrium curve for Fe(III), which is analogous to that of HBED, is unique in that four protons are displaced from the ligand in a single step, and no formation of hydrogen or hydroxo complexes is apparent from pH 2 to 11. Most of the other 1:1 Fe(III) complexes of polydentate ligands are unstable in alkaline media and form hydroxo complexes or dissociate to give ferric hydroxide. As with HBED, the high stability of the Fe(III) complex may be rationalized on the basis of the high affinity of Fe(III) for phenolate groups, and ability of all six donor groups to coordinate Fe(III) in an octahedral configuration without excessive steric strain.

As opposed to the Ga–HBED complex, the Ga-SHBED complex forms a monoprotonated chelate at low pH. At pH 2, 80% of the complex is in the monoprotonated form and 20% is in the completely deprotonated form. Figure 5 shows the ultraviolet absorption spectra of the Ga-SHBED chelate as a function of pH. The fact that the Ga(III) ion is com-

pletely complexed at pH 2 and that the absorbance at 254 nm increases with increasing pH, is enough evidence to conclude that the proton is located on one of the phenolic groups.

The behavior of the In(III)—SHBED complex is different from that of Ga(III) and Fe(III), in that it is diprotonated at low pH. At pH 2, 95% of the complex is in the diprotonated form, 5% is in the completely deprotonated form and practically no monoprotonated complex is present. This seems to indicate that the coordination of one phenolate oxygen assists the coordination of the second phenolate oxygen. Figure 6 shows the ultraviolet absorption spectra of the In(III)—SHBED chelate as a function of pH. The absorption bands at 235 nm indicate the presence of the diprotonated chelate at low pH. As the pH increases, the concentration of the diprotonated chelate decreases and the concentration of the completely deprotonated chelate increases. This is shown by the increase in absorbance at 254 nm, as the pH increases.

Acknowledgement

References

1 A. E. Frost, H. H. Freedman, S. J. Westerback and A. E. Martell, *J. Am. Chem. Soc., 80,* 530 (1958).
2 G. Anderegg and F. L'Eplattenier, *Helv. Chim. Acta, 47,* 1067 (1964).
3 N. A. Bailey, D. Cummins, E. D. McKenzie and J. M. Worthington, *Inorg. Chim. Acta, 18,* L13 (1976).
4 F. L'Eplattenier, I. Murase and A. E. Martell, *J. Am. Chem. Soc., 89,* 837 (1967).
5 A. E. Martell, in 'Development of Iron Chelators for Clinical Use', Eds. A. E. Martell, W. F. Anderson and D. G. Badman, Elsevier North Holland: New York, 1981; pp. 67–104.
6 H. Kroll, *et al., U.S. Patent* 3038793, *Chem. Ab., 57,* 16766c (1962).
7 W. R. Harris and A. E. Martell, *Inorg. Chem., 15,* 713 (1976).
8 R. J. Motekaitis and A. E. Martell, *Inorg. Chem., 19,* 1646 (1980).
9 R. J. Motekaitis and A. E. Martell, *Can. J. Chem., 60,* 2403 (1982).
10 R. J. Motekaitis and A. E. Martell, *Can. J. Chem., 60,* 168 (1982).
11 A. E. Martell and R. M. Smith, 'Critical Stability Constants', Vol. 1 & 5; Plenum: New York, (1974), (1982).
12 C. H. Taliaferro, R. J. Motekaitis and A. E. Martell, *Inorg. Chem.,* in press.