

Potentiometric and spectrophotometric determination of the stabilities of In(III), Ga(III) and Fe(III) complexes of *N,N',N''*-tris(3,5-dimethyl-2-hydroxybenzyl)-1,4,7-triazacyclononane

Eric T. Clarke and Arthur E. Martell*

Department of Chemistry, Texas A&M University, College Station, TX 77843-3255 (U.S.A.)

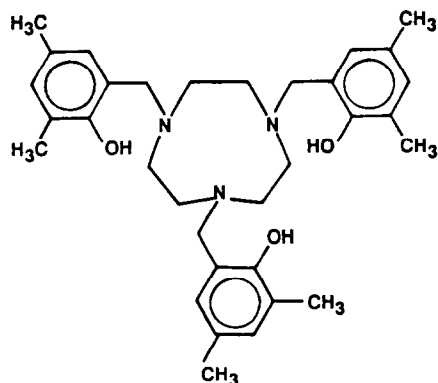
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Abstract

The protonation constants of potentially six-coordinate *N,N',N''*-tris(3,5-dimethyl-2-hydroxybenzyl)-1,4,7-triazacyclononane (TX-TACN) were determined by spectrophotometric and potentiometric methods in 75% ethanol–25% water ($\mu = 0.100$ M (KCl), $t = 25.0$ °C), and were found to be (log values) 14.2, 13.6, 12.9, 8.04 and 2.15. The stability constants of the complexes of In(III), Ga(III) and Fe(III) with TX-TACN were determined in the same medium by spectrophotometric methods, except for In(III)–TX-TACN, which was determined directly by potentiometric titration. The log stability constant for Fe(III)–TX-TACN ($K_{ML} = [ML]/[L^{3-}][M^{3+}]$), determined by competition with EDTA in 75% ethanol–25% water is 51.3. The log stability constant for Fe(III)–EDTA ($K_{ML} = [ML^-]/[L^{4-}][M^{3+}]$) was found to be 26.3 in the same medium. The stability constants for the Ga(III) and In(III) complexes with TX-TACN are $10^{44.2}$ and $10^{33.99}$, respectively. The high stabilities of the Ga(III) and Fe(III) complexes with TX-TACN are rationalized on the basis of the close fit of these hexacoordinate metal ions in the ligand cavity formed by the pseudo-octahedral arrangement of ligand donor atoms: the basic nitrogen atoms of the macrocycle and the very basic phenolate oxygen atoms.

Introduction

The sexadentate ligand *N,N',N''*-tris(3,5-dimethyl-2-hydroxybenzyl)-1,4,7-triazacyclononane (TX-TACN) (1),



1 TX-TACN

containing three tertiary amino aliphatic nitrogen donors and three phenolate oxygen donors was synthesized by Moore *et al.* [1] and investigated as a

chelating agent for trivalent radionuclides such as ^{111}In and $^{67,68}\text{Ga}$ [1]. The crystal structure reported [2] for the Ga(III) complex of TX-TACN shows it to be six-coordinate, octahedral and analogous to the six-coordinate structure of the Ga(III) complex of NOTA, 1,4,7-triazacyclononane-*N,N',N''*-triacetic acid [3]. The tendency of NOTA to form neutral six-coordinate structures [4] with metal ions such as In(III) [5, 6], Ga(III) [7] and Fe(III) [8, 9] has been of interest in view of the strong binding of these metal ions to the nitrogen and carboxylate donors of this ligand. A number of hexadentate chelating agents which contain phenolic donor groups form very stable complexes with In(II) [10] and Ga(III) [11, 12], as well as with Fe(III). The complexes of these ligands with $^{67,68}\text{Ga}$ and ^{111}In radioisotopes are of interest as imaging agents [13–15].

The objective of the current work is to determine the stabilities of complexes of trivalent metal ions in In(III), Ga(III) and Fe(III) with TX-TACN. The synthesis and crystal structures of the Cu(II) and Fe(III) chelates of tris-2-hydroxybenzyl and tris-3-tertiarybutyl-2-hydroxybenzyl derivatives of triazacyclononane, which are closely related to the complexes of TX-TACN, have been reported by Auerbach

*Author to whom correspondence should be addressed.

et al. [16], but stability constants for these related chelate compounds have not been determined.

Experimental

TX-TACN

The ligand TX-TACN used in this study was kindly donated by M. Welch and D. Moore, and was prepared by the method described previously [2]. The ligand is insoluble in water, as the result of its hydrophobic nature imparted by the six methyl substituents. In order to solubilize it sufficiently for potentiometric studies, it was found that mixed solvent of 25% H₂O and 75% (by volume) ethanol was satisfactory. The molecular weight of the TX-TACN sample employed was found to be 545 Daltons by potentiometric titration ($\mu = 0.100$ M (KCl), $t = 25.0$ °C); the theoretical weight is 537 Daltons. The elementary analyses determined by Galbraith are: Calc. for C₃₃N₃O₃H₄₅: C, 74.57; N, 7.91; H, 8.47. Found: C, 74.63; N, 7.85; H, 8.54%.

Potentiometric determinations

Potentiometric determinations of protonation constants and metal stability constants were performed in 75/25 ethanol/water in the same manner as described for aqueous media by Martell and Motekaitis [17]. The potentiometric apparatus consisted of a constant temperature bath (Haake, 25.0 °C), a Corning model 130 pH meter, and a glass-jacketed titration cell fitted with glass and calomel reference electrodes. Atmospheric CO₂ and O₂ gases were excluded from the cell by passing purified argon continuously through inlet and outlet gas-line fittings. Standard base prepared in the mixed solvent was introduced into the cell through the tip of the 10 ml capacity Metrohm piston buret. The electrodes were calibrated in a thermostated cell with standard acid and base in the mixed solvent to read p[H] directly ($p[H] = -\log[H^+]$). The ionic strength was adjusted to 0.1000 M with KCl without problems of electrolyte solubility. The pK_w of the solvent medium was found to be 14.78 ± 0.04 (on the basis of 6 determinations) by calculating the value of the pK_w necessary to bring the titration points in the alkaline range of a solution of standard acid with standard base in line with those in the acid range. Solution concentrations of ligand in the presence and absence of metal ions were in the order of 8×10^{-4} M ($\mu = 0.100$ M (KCl), $t = 25.0$ °C). Stepwise protonation constants ($K_n^H = [H_nL]/[H_{n-1}L] \cdot [H]$) within range of the potentiometric titrations (up to p[H] 11.5) were calculated by fitting the p[H] data with the help of the program BEST [18] which computes stability con-

stants by an interactive method. The stability constant (K_{ML}) of the In(III)-TX-TACN complex was determined by direct potentiometric titration.

Absorbance spectra

Spectral data were measured with 1.000 ± 0.001 cm matched quartz cells and a fast scan Perkin-Elmer 553 UV-Vis spectrophotometer thermostated at 25.0 °C. The ionic strength of the solutions in 75/25 ethanol/water was adjusted to 0.100 M with KCl. The stability constants of the TX-TACN complexes were calculated from the absorbance data under equilibrium conditions.

Electrochemical measurements

Reduction potentials of the Fe(III)/Fe(II) couple in 75% ethanol ($\mu = 0.100$ M (HCl + KCl), $t = 25.0$ °C) were measured with an uncalibrated glass-sleeved platinum electrode and a reference calomel electrode to determine the stability constant ($K_{ML} = [ML^-]/[M^{3+}] \cdot [L^{4-}]$) of the Fe(III)-EDTA complex, which is needed for determination of the stability constant of the Fe(III) complex of TX-TACN by EDTA competition.

Phenolate protonation constants

The protonation constants of TX-TACN that were above the range of accurate potentiometric p[H] measurements were determined by measurement of the phenolate absorbance at 307 nm at fixed p[H] values calculated from the amount of strong base (or acid) added to the solutions. A series of small containers with ligand at $\sim 10^{-4}$ M concentration at the specified temperature and ionic strength were adjusted with KOH (in 75/25 ethanol/water) over the p[H] range 11.5–14.0 at intervals of 0.1 p[H]. The solutions were protected against atmospheric CO₂ by adding the KOH titrant from a microburet and by rapidly sealing the containers after additions of titrant. Because equilibrium was rapid for the proton dissociation from the free ligand, the time of exposure of the solutions of atmospheric CO₂ was very brief. The absorbance data were fitted with an ABSPKAS program written in this laboratory which computes pK_a s and extinction coefficients from spectral data. The species present were L^{3-} , HL^{2-} , H_2L^- and H_3L . The maximal absorbance of L^{3-} at 307 nm gave an extinction coefficient of $13\,549\text{ M}^{-1}\text{ cm}^{-1}$ while extinction coefficients for the HL^{2-} and H_2L^- species were calculated as 9033 and $4516\text{ M}^{-1}\text{ cm}^{-1}$, respectively. The extinction of the H_3L species was set at 650 at 307 nm (its λ_{max} is 286 nm).

An additional protonation constant ($K_5^H = [H_5L^{2+}]/[H_4L^+][H]$) was determined by an analysis of the spectral data for free TX-TACN over

the pH range from 1.0 to 4.0 in increments of 0.1 p[H] ($I = 0.100$ M (KCl + HCl), 25.0 °C). Plots of absorbance at 296 nm versus p[H] gave isosbestic points at 280 and 307 nm with the $\lambda_{\max}$ of the ligand at 296 nm, which changed with p[H]. This spectral change was interpreted as a hydrogen-bonded interaction of the tertiary amino nitrogen group with the phenolic groups. A plot of this absorbance against p[H] was used to calculate K_5^H as 2.15 ± 0.03 .

Ga(III)–TX–TACN

The metal binding constant of the Ga(III)–TX–TACN complex was determined by spectrophotometric determination of the degree of dissociation of the complex below p[H] 3. Flasks containing 1.0×10^{-4} M Ga(III)–TX–TACN were adjusted to p[H] values from 1.0 to 4.0 ($\mu = 0.100$ M (KCl + HCl), $t = 25.0$ °C). Absorbances of the solutions were measured at 297 nm. The molar absorbance of Ga(III)–TX–TACN (ϵ_{ML}) and the free ligand (ϵ_L) at 297 nm are 9105 and 4746 $M^{-1} cm^{-1}$, respectively. Concentrations of M, L and ML were calculated from mass balance and absorbance equations. The existence of the free ligand in different protonated forms was taken into account by the use of the protonation constants measured separately (Table 1).

Fe(III)–TX–TACN

Direct measurement of the equilibrium between Fe(III) and TX–TACN in 75% ethanol–25% water was attempted, but the characteristic charge transfer complex formed between the protonated phenolic groups and Fe(III) at low p[H] resulted in formation of an intense blue color which masked the absorption bands of the ionized ligand (phenolate groups) and its iron(III) chelate. The formation constant of the

Fe(III)–TX–TACN complex was determined spectrophotometrically by competition with EDTA. Because the Fe(III)–EDTA complex predominates at low p[H] there is no free (solvated) Fe^{3+} , and the blue color was eliminated. In the presence of TX–TACN, the Fe(III) ion was partially displaced from the Fe(III)–EDTA complex (ML') to form the Fe(III)–TX–TACN complex (ML) and free EDTA (H_nL'). The molar absorbance of Fe(III)–TX–TACN at 495 nm in 75% ethanol–25% water ($\mu = 0.100$ M KCl, $t = 25.0$ °C) is 6375 $M^{-1} cm^{-1}$, while that of the Fe(III)–EDTA complex is about 50 $M^{-1} cm^{-1}$. Equilibrium was approached from two directions, and the p[H] and absorbance of the Fe(III)–TX–TACN complex (495 nm) were monitored over an extended period of time. Equilibrium data of the preformed Fe(III)–TX–TACN flask (3.206 p[H]; 0.213 Abs.) and the preformed Fe(III)–EDTA flask (3.254 p[H]; 0.210 Abs.) were used to determine the exchange constant ($K_{ex} = [ML][L']/[ML'][L]$) which has a log value of 25.00 . Total concentrations of metal and ligand were 1.00×10^{-4} M ($\mu = 0.100$ M KCl, $t = 25.0$ °C). The concentrations of the free ligands were calculated from the log protonation constants (Table 1) for TX–TACN, and of EDTA (Table 2) under the reaction conditions employed, which were found to be 9.37 , 6.26 , 3.46 and 3.04 , determined especially for this investigation. A fifth protonation constant, K_5^H , was estimated as 2.7 by correlation with the fifth protonation constant for EDTA in water [18] ($\log K_5^H = 1.5$).

The stability constant for the Fe(III)–EDTA complex in the solvent medium ($K_{ML} = [ML']/[M^{3+}][L'^{4-}]$) was determined as $10^{26.3}$ by measurement of its reduction potential ($\mu = 0.100$ M (KCl + HCl), $t = 25.0$ °C). The reduction potential of the Fe(III)–EDTA complex was determined in the

TABLE 1. Stability constants of the metal complexes of TX–TACN with In(III), Ga(III) and Fe(III) in 75% ethanol and 25% water ($\mu = 0.100$ M (KCl), $t = 25.0$ °C)

Quotient	Log K	Assignment
$[HL]/[L][H]$	14.2 ± 0.1^a	dimethylphenolate groups
$[H_2L]/[HL][H]$	13.6 ± 0.1^a	dimethylphenolate groups
$[H_3L]/[H_2L][H]$	12.9 ± 0.1^a	dimethylphenolate groups
$[H_4L]/[H_3L][H]$	8.04 ± 0.01^b	tertiary nitrogen atom
$[H_5L]/[H_4L][H]$	2.15 ± 0.03^a	tertiary nitrogen atom in interaction with dimethylphenolate groups
$[InL]/[In][L]$	33.99 ± 0.03^b	
$[GaL]/[Ga][L]$	44.2 ± 0.1^c	
$[FeL]/[Fe][L]$	51.3 ± 0.1^d	

^aDetermined by spectrophotometric titration. ^bDetermined by direct potentiometry. ^cSpectrophotometric determination of the dissociation of the Ga(III) complex at low p[H]. ^dSpectrophotometric determination of Fe(III) complex in competition with EDTA, and from estimate of Fe(III)–EDTA stability constant in mixed solvent medium.

TABLE 2. Protonation constants of EDTA (H_4L) and stability constants of its complexes with Fe(III) and Fe(II) in ethanol–water mixtures ($\mu=0.100$ M (KCl), $t=25.0$ °C), and reference values in 100% water

Quotient	Log K		
	100% Water	75% Ethanol 25% Water	15% Ethanol 85% Water ^a
$[HL]/[L][H]$	10.17 ^b	9.37 ± 0.03	9.77
$[H_2L]/[HL][H]$	6.13 ^b	6.26 ± 0.01	6.42
$[H_3L]/[H_2L][H]$	2.69 ^b	3.46 ± 0.03	2.92
$[H_4L]/[H_3L][H]$	2.00 ^b	3.04 ± 0.02	2.65
$[H_5L]/[H_4L][H]$	1.5 ^b	2.7 ^c	
$[ML]/[M^{2+}][L]$	14.94 ^d	15.80 ± 0.02	
$[MHL]/[M^{2+}L][H]$	2.06 ^d	3.13 ± 0.04	
$[ML]/[M^{3+}][L]$	25.0 ^e	26.3 ± 0.1^f (est.)	
$[MHL]/[M^{3+}L][H]$	1.3 ^b	1.49 ± 0.02	
$[ML(OH)]/[H][M^{3+}L]$	-7.37^b	-7.42 ± 0.04	

^aRef. 19. ^bRef. 18. ^cEstimated by regression with the 5 pK_s for EDTA in water, $\mu=0.100$ M, $t=25.0$ °C. ^dRef. 20. ^eRef. 21. ^fDetermined by reduction potential measurement in the presence of excess Fe(II) and EDTA, $\mu=0.100$ M (HCl + KCl), $t=25.0$ °C.

presence of 20% excess Fe(II) ion and 50% excess ligand. Oxidation of the $FeSO_4 \cdot 7H_2O$ employed was prevented by placing the $FeSO_4 \cdot 7H_2O$ in a plastic boat and purging the Fe(III)–EDTA solution for 3 h with argon. Then the Fe(II) salt was dissolved in the Fe(III) solution at $p[H]$ 1.5 by tipping the boat, followed by vigorous stirring of the contents of the cell. A stable potential was obtained within 4–5 h. $p[H]$ 1.5 was selected as the $p[H]$ at which free Fe(III) does not hydrolyze and free Fe(II) does not form a complex with EDTA in the solvent system employed. Proof that a Fe(II)–EDTA complex is not formed at $p[H]$ 1.5 in 75% ethanol–25% water was obtained by calculating the species distribution curves (not shown) from stability constants that were determined for Fe(II)–EDTA at higher $p[H]$ ($\mu=0.100$ M (KCl), $t=25.0$ °C). The ferrous–EDTA metal binding constant, $\log K_{ML} = [ML'^{2-}]/[M^{2+}][L'^{4-}]$, was found to have a log value of 15.80 ± 0.02 , while the log protonation constant of the Fe(II)–EDTA complex in the mixed solvent, $\log K_{MHL} = [MHL'^{-}]/[ML'^{2-}][H]$, was determined as 3.13 ± 0.04 . See Table 2 for comparison of stability constants for EDTA [18, 19] and its Fe(II) and Fe(III) complexes in mixed solvents and in water.

The stability constant (K_{ML}) of the Fe(III)–TX–TACN complex was subsequently calculated from the exchange constant (K_{ex}) and the stability constant (K_{ML}) of Fe(III)–EDTA in the solvent medium.

Results

Protonation constants of TX–TACN

TX–TACN was obtained in the form of the triprotonated neutral compound, designated as H_3L ,

with three protonated phenolic groups. Potentiometric $p[H]$ measurements are illustrated in Fig. 1. Spectrophotometric measurements of phenolic group dissociation made possible the determination of the highest protonation constants presented in Table 1. The others were determined potentiometrically. A break in the titration curve for free TX–TACN occurs at $a=0$ (a = moles of base titrated per mole of ligand),

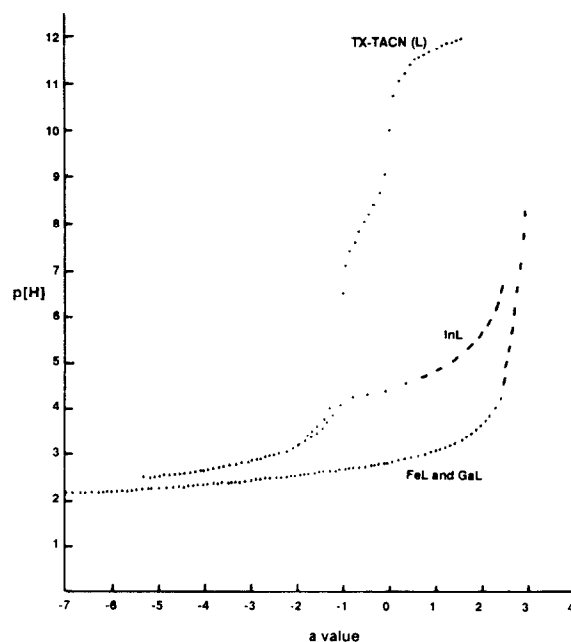


Fig. 1. Potentiometric $p[H]$ profiles of TX–TACN and its 1:1 complexes with In(III), Ga(III) and Fe(III) at 8.00×10^{-4} M ($\mu=0.100$ M (KCl), $t=25.0$ °C). Excess acid was present in each of the solutions.

corresponding to the triprotonated neutral form of the ligand. Excess mineral acid was added in order to protonate the tertiary amino nitrogens of the macrocyclic ring. The buffer region between $a=0$ and $a=-1$ involves the equilibrium for conversion of the neutral ligand (as H_3L) to its tetraprotonated form, H_4L^+ . The protonation constant for this equilibrium ($\log K_4^H = [H_4L^+]/[H_3L][H]$), has a log value of 8.04 ± 0.01 (Table 2). A second tertiary amine protonation constant ($\log K_5^H$) was calculated by spectrometric determination of the interaction of the ring nitrogen atoms with the phenolic groups, to be 2.15 ± 0.03 , as described in 'Experimental'.

Spectrophotometric analysis of the high $p[H]$ absorbance of the phenolate groups of TX-TACN (Fig. 2) with the ABSPKAS program gave three high protonation constants (i.e. $\log K_1^H = 14.2$; $\log K_2^H = 13.6$; $\log K_3^H = 12.9$, Table 1; Fig. 3) that were beyond the limit of accurate potentiometric $p[H]$ measurements. The broad isosbestic point at 288 nm indicates that the three phenolate groups may not be protonated independently of each other. The spacing of the absorbance of the phenolate groups of TX-TACN at 307 nm is slightly irregular over the alkaline $p[H]$ range from 12.6 to 13.9. Hydrogen bonding interactions between the phenolic groups and the ring nitrogens may explain the lack of uniformity in the increase in the calculated stepwise protonation constants. These are discussed in the next section.

In(III)-TX-TACN stability constant

In(III) forms a weak complex with TX-TACN with a $\log K_{ML}$ of 33.99 ± 0.03 (Table 1). The potentiometric titration curve of the In(III) complex (Fig. 1) is coincident with that of the ligand initially and diverges at $a = -2$. The In(III)-TX-TACN complex

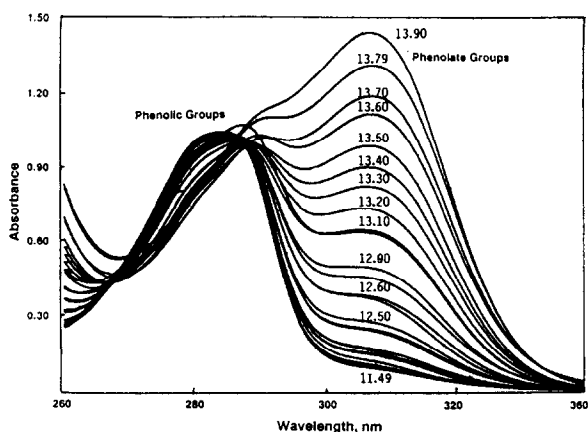


Fig. 2. UV-Vis absorbance of TX-TACN in 75% ethanol and 25% water at the $p[H]$ values shown. $[L]_T = 1.579 \times 10^{-4}$ M ($\mu = 0.100$ M (KOH + KCl), $t = 25.0$ °C).

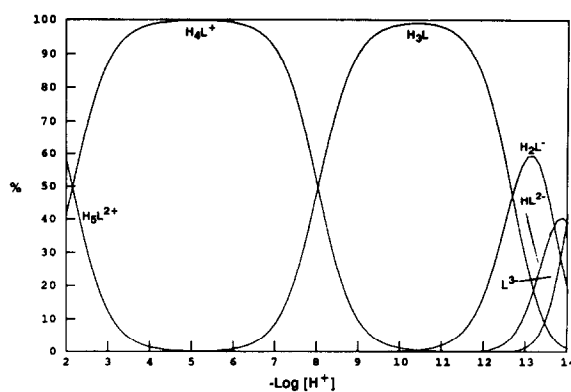


Fig. 3. Species distribution curves of TX-TACN and its protonated forms in 75% ethanol and 25% water. $[L]_T = 8.00 \times 10^{-4}$ M ($\mu = 0.100$ M (KCl), $t = 25.0$ °C). Ligand protonation constants are given in Table 1.

is formed at and beyond $a = -1$, but equilibrium is attained very slowly (up to 6–8 h per experimental point). The In(III) complex eventually dissociates to form In(OH)₃ near $p[H]$ 4.5. The species distribution curves in Fig. 4(a) assume a super-saturated solution with respect to In(OH)₃, and are representative of the results obtained in the experimental titration up to $p[H] \sim 5$, where In(OH)₃ finally precipitates. The slowness of the equilibrium may be due to incipient formation of traces of In(OH)₃. The species distribution curves (Fig. 4(b)) were calculated with the solubility product for In(OH)₃ ($\log K_{sp} \sim -37$) taken from water [22]. Note that In(OH)₃ never completely disappears once it is formed. Under experimental conditions, ML was formed between $p[H]$ 4 and 5. No MHL species was found.

Ga(III)-TX-TACN

The formation constant of the Ga(III)-TX-TACN complex was calculated from spectrophotometric data obtained at low $p[H]$ as indicated in 'Experimental'. The phenolate groups of the Ga(III) complex absorb at 298 nm (Fig. 5) with a maximal value at $p[H]$ 2.61 and higher. The absorbance of the Ga(III)-TX-TACN complex diminishes as the $p[H]$ is lowered with HCl at constant ionic strength ($\mu = 0.100$ M (KCl + HCl), $t = 25$ °C). The ligand (as $H_{(3+n)}L^{n+}$) is completely dissociated from the metal complex at $p[H]$ 1.57 and has a maximum absorption at the characteristic wavelength for phenolic groups at 286 nm. The stability constant ($\log K_{ML} = [ML]/[M^{3+}][L^{3-}]$) for Ga(III)-TX-TACN was calculated as $10.44.2$ (Table 1). Figure 6 shows the distribution of Ga(III) between the aquo ion and Ga(III)-TX-TACN (ML) below $p[H]$ 3 and complete formation of Ga-TX-TACN (GaL), which eventually precipitates, above that $p[H]$.

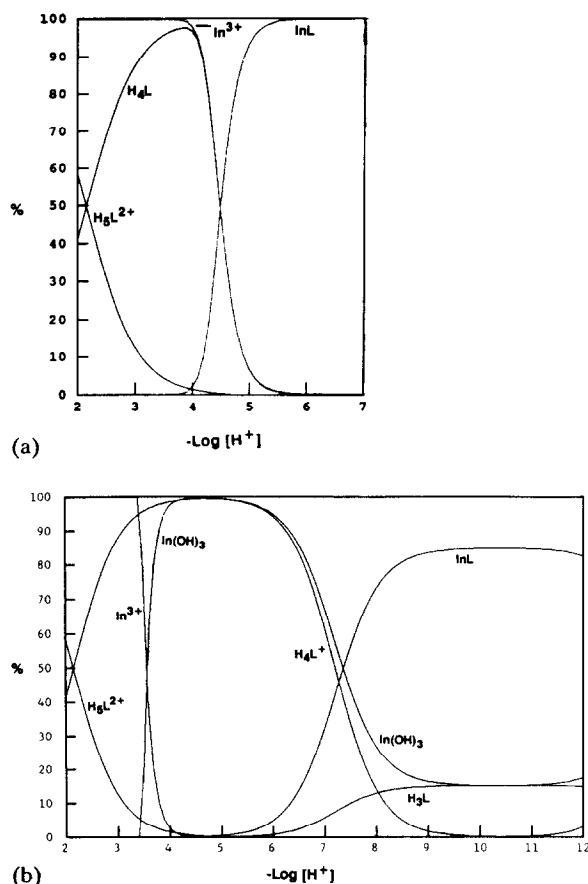


Fig. 4. (a) Species distribution curves of In(III)-TX-TACN in 75% ethanol. $[\text{L}]_{\text{T}} = [\text{M}]_{\text{T}} = 8 \times 10^{-4} \text{ M}$ ($\mu = 0.100 \text{ M}$ (KCl), $t = 25.0^\circ \text{C}$). The distribution in InL is projected above $\text{p[H]} 3.5$, as a supersaturated solution. With time, In(OH)_3 precipitates above $\text{p[H]} 4.5$. Ligand protonation constants and metal complex formation constants are given in Table 1. (b) Species distribution curves for In(III)-TX-TACN in 75% ethanol. Experimental conditions are the same as in (a). % In(OH)_3 precipitated was estimated from the solubility product for In(OH)_3 for aqueous solution [19].

The chelate (ML) came out of solution near $\text{p[H]} 5$ (Fig. 1) as a consequence of its low solubility. The ML species is sufficiently stable so that no Ga(OH)_3 or Ga(OH)_4^- hydroxo metal species were formed at significant concentrations, even at much higher p[H] . An analysis of the species present by using the stability constant (K_{ML}), the solubility constant of Ga(OH)_3 in water ($\log K_{\text{sp}} \sim -37$), and the gallate formation constant ($\log K_{\text{Ga(OH)}_4^-} = -16.81$) [22] showed that the K_{sp} of Ga(OH)_3 was never exceeded and the concentration of gallate (Ga(OH)_4^-) was never greater than 0.2% of total Ga(III) .

Fe(III)-TX-TACN stability constant

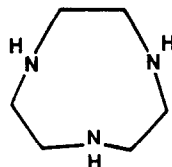
The log stability constant ($\log K_{\text{ML}}$) of Fe(III)-TX-TACN was calculated by competition with EDTA

(L') as the product of the exchange ratio ($\log K_{\text{ex}} = [\text{ML}][\text{L}']/[\text{ML}'][\text{L}] = 25.00$) and the stability constant of Fe(III)-EDTA in the solvent medium ($\log K_{\text{ML}} = 26.3$, see 'Experimental'); the log value of the stability constant for Fe(III)-TX-TACN is estimated at 51.3 ± 0.1 (Table 1). (Estimates of stability constants for Fe(III)-TX-TACN are from that of Fe(III)-EDTA which was based on the estimate of $\log K_5^{\text{H}} = 2.7$ for free EDTA in 75% ethanol-25% water.) No evidence for MHL and ML(OH) species could be found in the spectra of the Fe(III)-TX-TACN complex (not shown). The species distribution curves for the competition between TX-TACN and EDTA for Fe(III) (Fig. 7) shows that $\text{Fe(III)-TX-TACN (ML)}$ is formed to the extent of 18% of the total Fe(III) present at $\text{p[H]} 3.2$, while the competing $\text{Fe(III)-EDTA (ML')}$ accounts for the remainder (82%). The proportion of the Fe(III)-TX-TACN chelate increases rapidly as the p[H] is increased, and accounts for all of the Fe(III) at $\text{p[H]} 6$ and above.

Discussion

Influence of solvent on the protonation constants of TX-TACN

The protonation constants assigned to the tertiary amino nitrogens of TX-TACN in the 75% ethanol medium ($\log K_4^{\text{H}} = 8.04$; $\log K_5^{\text{H}} = 2.15$) are lower than those for the parent ring compound (triazacyclononane, **2**) measured in water ($\mu = 0.100 \text{ M}$ (KCl), $t = 25.0^\circ \text{C}$).



2 1,4,7-Triazacyclononane

This may be a consequence of the influence of the phenolic substituents which may have weak hydrogen-bonding effects as well as decreasing solvation by the lower dielectric solvent, which is needed to stabilize positive protonated amino groups. To obtain a better measure of this effect, the protonation constants of triazacyclononane were measured under our reaction conditions in both 75% ethanol and pure water: $\log K_1^{\text{H}}$ and $\log K_2^{\text{H}}$ have values of 9.96 ± 0.01 and 6.06 ± 0.02 , respectively, in 75% ethanol; and 10.63 ± 0.01 and 6.92 ± 0.02 , respectively, in water. Hence, the influences of both the dimethylhydroxybenzyl substituents, and the ethanolic medium seem to contribute to the lowering of proton-

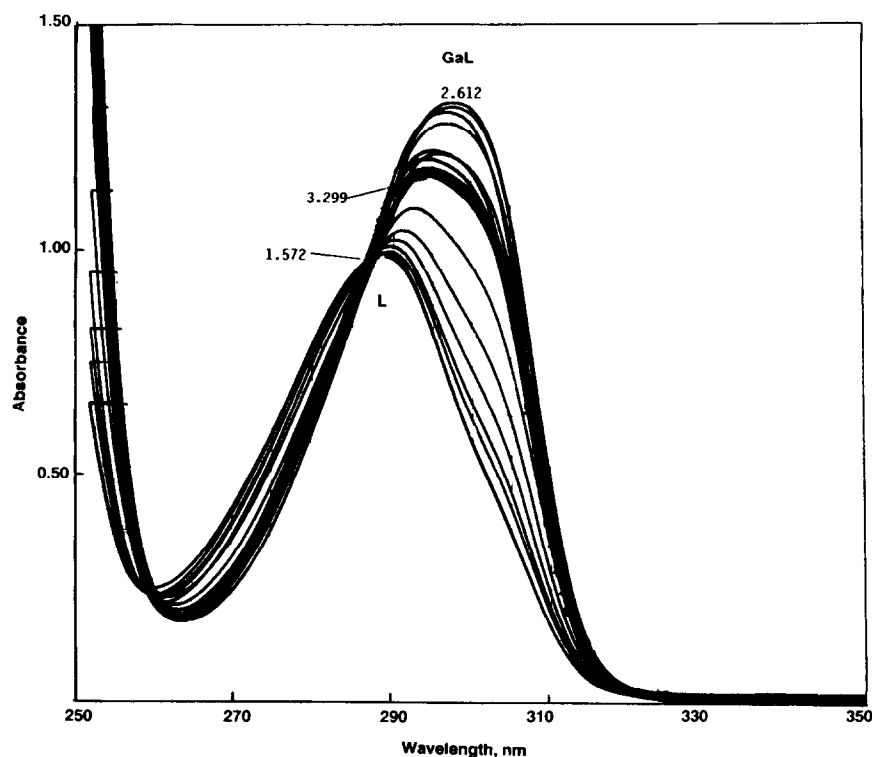


Fig. 5. Absorbance of Ga(III)-TX-TACN (297 nm) and the free ligand (288 nm) in 75% ethanol at the p[H] values shown. $[L]_T = [M]_T = 1.475 \times 10^{-4}$ ($\mu = 0.100$ M (KCl + HCl), $t = 25.0$ °C).

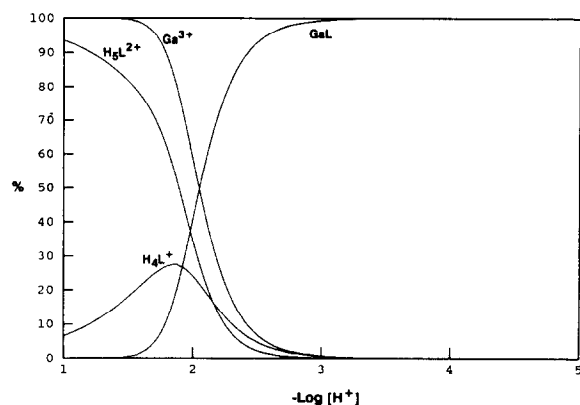


Fig. 6. Species distribution curves of Ga(III)-TX-TACN in 75% ethanol. $[L]_T = [M]_T = 8.00 \times 10^{-4}$ M ($\mu = 0.100$ M KCl, $t = 25.0$ °C). GaL is insoluble above p[H] 5. Protonation constants and metal stability constants for TX-TACN appear in Table 1.

ation constants of the tertiary amines of TX-TACN.

There are many examples in the literature on the lowering of protonation constants in low dielectric constant solvents. For example, the first protonation constant of glycylglycine is assigned to the amino group while the second is assigned to the carboxylate group. The value of K_1^H is lowered and K_2^H is

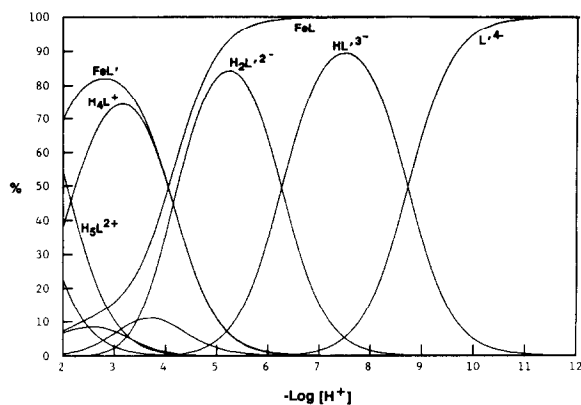


Fig. 7. Species distribution curves of equimolar Fe(III), TX-TACN (H_3L) and EDTA (H_4L') in 75% ethanol. $[L]_T = [L']_T = 8.00 \times 10^{-4}$ M ($\mu = 0.100$ M (KCl), $t = 25.0$ °C). The FeL complex is insoluble above p[H] 5. The ligand protonation constants and stability constants for TX-TACN appear in Table 1. Protonation and stability constants of EDTA are shown in Table 2.

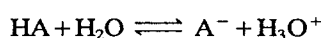
increased with increasing percentages of methanol in the solvent because the lower dielectric constant of methanol is less effective in solvating the zwitterion than is water.

More complex ligands such as EDTA (Table 2) show the influence of ethanol solvent on protonation constants less clearly. The value of K_1^H for EDTA

in 15 to 75% ethanol [19] (assigned to a tertiary amino nitrogen) are lowered with respect to that in water [18] ($\mu = 0.100$ M (KCl), $t = 25.0$ °C), but the value of K_2^H in 15% ethanol [19] and 75% ethanol (attributed to the second amino group of EDTA) are slightly higher than their counterpart in water. The observations are consistent with those of Bates [23] who noted that the protonation constants of primary and secondary amines are lowered in mixed solvent as the concentration of water is decreased:



The protonation constants for the carboxylate groups of EDTA (K_3^H and K_4^H) are higher in 75% ethanol than those measured in water because the protons are dissociated less readily in the lower dielectric constant medium:



Thus, a solvent mixture of ethanol and water solvates the conjugate base of a carboxylic acid and the conjugate acid of an amine less readily than in pure water.

Influence of solvent on metal binding constants

It is well known that the stability constants of metal ion complexes of ligands having negative donor groups increase in magnitude as the dielectric constant of the solvent is decreased. An example of this phenomenon taken from the older literature [24] is the Cu(II) complex of 8-hydroxyquinoline-5-sulfonic acid. The log binding constant ($\log \beta_2$) of Cu(II)-8-hydroxyquinoline-5-sulfonic acid is 24.1 in 20% ethanol, but is 26.1 in 60% ethanol, while it is 22.99 in water. Table 2 lists the ferrous-EDTA binding constant K_{ML} as $10^{15.80}$ in 75% ethanol while it is $10^{14.94}$ in water [23] ($\mu = 0.100$ M (KCl), $t = 25.0$ °C). Consistent with these observations, the log binding constant for Fe(III)-EDTA is 26.3 in 75% ethanol-25% water while it was reported to be lower ($\log K_{ML} = 25.0$) in pure water [21]. There are no other literature values of stability constants of EDTA complexes with Fe(II) and Fe(III) in mixed solvents.

Trends of stabilities for TX-TACN complexes of In(III), Ga(III) and Fe(III)

The stability constants for the trivalent metal complexes of TX-TACN with In(III), Ga(III) and Fe(III) in Table 1 show the usual trend where the potentially six-coordinate N_3O_3 neutral ligand forms very stable complexes with Ga(III) and Fe(III) but a less stable complex with In(III). The log stability constant ($\log K_{ML} = 51.3$) for Fe(III)-TX-TACN represents an increase in stability over that of Ga(III)-TX-TACN by 7 log units. This is a re-

markable difference, in view of the predominantly ionic nature of the coordinate bonding, and the similarity in the magnitudes of the effective ionic radii. X-ray crystal structure data for Fe(III)-TX-TACN are needed for comparison with that of the Ga(III) complex to explore the possibility that the difference may be due to differences in octahedral to triangular prismatic distortion [8], or to difference in steric effects due to the methyl substituents. The low stability of the In(III)-TX-TACN complex is rationalized by the larger size of the In(III) ion, resulting in weaker ionic bonding in the complex.

The precipitates of Ga(III)-TX-TACN and Fe(III)-TX-TACN are explained by the inherent insolubility of these metal chelates in the 75% ethanol-25% water mixture. It is expected that concentrations of 10^{-4} M in chelate would be soluble in pure ethanol as this is crucial to their possible medicinal applications.

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