Stabilities of the Fe(III), Ga(III) and In(III) chelates of *N,N′,N″*-triazacyclononanetriacetic acid

Eric T. Clarke and **Arthur E. Martell***
Department of Chemistry, Texas A&M University, College Station, TX 77843-3255 (U.S.A.)

(Received August 13, 1990; revised October 17, 1990)

Abstract

The stability constants of the complexes of Fe(III), Ga(III) and In(III) ions with the sexadentate macrocyclic ligand 1,4,7-triazacyclonane-*N,N′,N″*-triacetic acid (NOTA) have been determined in KCl supporting electrolyte (0.100 M) at 25.0 °C. The stability constants ($K_{ML}=[ML]/[L^{3-}][M^{3+}]$ of the Ga(III) and Fe(III) complexes with NOTA are high, $10^{30.98}$ and $10^{28.3}$, respectively, and indicate preferential interaction of these small metal ions with NOTA. The stability constant of the In(III)–NOTA complex is somewhat lower ($10^{26.2}$). Species distribution curves are presented to illustrate the conversion of the Ga(III) and In(III) chelates to the monohydroxo forms at p[H] 9.3 and 6.5, respectively. The Fe(III)–NOTA complex dissociates to form ferric hydroxide at and above p[H] 7.5, while the hydroxo In(III)–NOTA complex is converted to indium hydroxide at and above p[H] 7.5. The Ga(III)–NOTA system is soluble at all p[H] values, as a consequence of conversion of the hydroxo complex to the tetrahydroxo gallate ion at p[H] 10.4 and above.

Introduction

Several studies of the hexadentate ligand 1,4,7-triazacyclononane-*N,N′,N″*-triacetic acid (NOTA, **1**) and its metal chelates have been reported in recent years. Mn(II) and Gd(III) complexes of NOTA have been investigated as paramagnetic ^{1}H NMR contrast agents [1–3] for the imaging of tissues, and the In(II) and Ga(III) complexes of NOTA have been studied as radiopharmaceuticals [4]. X-ray structure determinations have shown that NOTA forms complexes with Ni(II) and Cu(II) having distorted six-coordinate geometries with compact arrangements of the N_3O_3 donors [5, 6]. X-ray crystal structures have also been reported for the NOTA complexes with Cr(III), Fe(III) [6], Ga(III) [7, 8] and In(III) [9]. Collectively, the crystal structures have demonstrated that the six-coordinate complexes formed may vary from that of a distorted octahedron for the Cr(III) and Ga(III) complexes to the pseudotrigonal-prismatic geometry of the Fe(III) complex.

Although there is considerable interest in NOTA as an effective coordinating ligand, few equilibrium studies have been reported. Although the stability constants of the alkaline earth complexes of NOTA [5, 10] have been determined, few potentiometric investigations have been carried out on the stabilities of NOTA complexes of other metal ions, perhaps partly due to the extreme slowness at which equilibrium is reached with this ligand. In addition to a spectrophotometric determination of the conditional stability constant of the Cu(II) complex [10], polarographic determinations of the stability constants for complexes of NOTA with Mn(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) have been described [11]. The objective of the present work is to determine the stability constants of the complexes of NOTA with the hexacoordinate Fe(III), Ga(III) and In(III) ions. The motivation behind this study, in addition to interest in the complexes as radiopharmaceuticals and in the ligand for removal of iron overload, is the fact that the compactness of the triazanonane ring and the steric efficiency of the pendant acetate groups [12] may lead to the formation of complexes of unusually high stability and selectivity for these trivalent metal ions.

Experimental

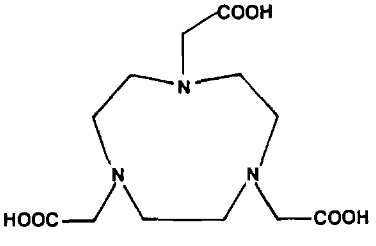
1,4,7-Triazacyclononane-N,N′,N″-triacetic acid, [9]aneN$_3$-ac$_3$ (NOTA)

The parent triazacyclononane was prepared by the method of Richman and Atkins [13] and also by Geraldes *et al.* [14]. NOTA (**1**) was synthesized by

---

*Author to whom correspondence should be addressed.

a modification of the procedure described by Desreux [15].



1 **NOTA**

In the present procedure, bromoacetic acid was slowly added to triazacyclononane in water at 0 °C, and then the temperature was raised to 60 °C while the pH was maintained near 12 for over 12 h. After the completion of the alkylation reaction, the product was purified with a strong acid Dowex-50W cation exchange resin. NOTA readily crystallized from water free of inorganic salts when concentrated in an acidified solution. Yield of NOTA in the final form was 65%; a higher yield was expected, but some loss occurred during the final recrystallization step. Proton NMR spectra were measured with a Varian XL-200 spectrometer operating at 200 MHz. The solvent was deuterium oxide and the pD was adjusted with sodium deuteroxide. Shifts are reported in ppm. Elemental analyses were performed by Galbraith Laboratories of Knoxville, Tennessee. The molecular weight of NOTA was found to be 309 Daltons by titration (303 theoretical). ^{1}H NMR: 3.060 (s, 6H, acetate protons), 2.340 (s, 12H, ring protons). *Anal.* Calc. for $C_{12}H_{21}N_3O_6$: C, 47.52; H, 6.93; N, 13.86. Found: C, 47.29; H, 7.16; H, 13.86%.

Potentiometric determinations

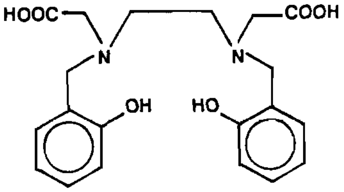
Details of the potentiometric determination employed for the measurement of protonation constants and metal complex stability constants are given in ref. 16. The potentiometric apparatus consisted of a glass jacketed titration cell connected to a constant temperature bath (Haake, 25.0 °C). The cap of the titration cell was fitted with a Schott blue stem glass electrode and a calomel reference electrode together with a pair of N_2 gas-line fittings and the tip of a 10 ml capacity Metrohm piston buret. The electrodes were calibrated in a thermostated cell with standard acid and base to read p[H] directly (p[H] = $-\log[H^+]$). The ionic strength was adjusted to 0.100 M with KCl. Atmospheric CO_2 was excluded from the titration cell by slowly passing purified N_2 gas across the top of the experimental solution in the cell. The value of $\log K_w$ was found to be -13.78.

Solution concentrations of ligand in the presence and absence of metal ion were in the order of 2×10^{-3} M (μ = 0.100 M (KCl), 25.0 °C). Protonation constants and metal ion stability constants were calculated by fitting the potentiometric p[H] data with the program BEST [17].

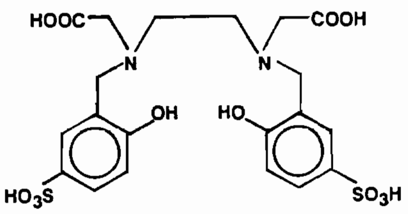
Stability constants of the hydroxo metal complexes were determined from potentiometric p[H] data measured on binary solutions containing a 1:1 molar ratio of metal ions to ligand. It was not possible to directly determine the binding constants ($K_{ML} = [ML]/[M^{3+}][L^{3-}]$) for the trivalent metal ion complexes from potentiometric p[H] data since the metal complexes formed completely at the beginning of the titration. Therefore, the metal ion affinities for NOTA were determined by competition methods. Determination of the stability constant for the Ga(III)–NOTA complex was a special case of hydroxyl anion competition to form gallate, the $Ga(OH)_4^-$ anion, at high pH [18]. The Ga(III)–NOTA complex which was formed in the experimental cell initially at low pH was titrated with standard base into the high pH region, where competition of the hydroxo gallium complex with NOTA and gallate occurs. Then the solution complex was titrated slowly in the reverse direction to allow equilibrium to be obtained at each titration point. To ensure that equilibrium had been attained, the solution complex was titrated slowly in the forward direction a second time over the same p[H] region of gallate competition (i.e., from p[H] 10.4 to 11.2). Known hydrolysis constants [18, 19] for the free Ga(III) ion ($\log K_{Ga(OH)^{2+}} = -2.97$ and $\log K_{Ga(OH)_4^-} = -16.81$) were used to compute the binding constant for the Ga(III)–NOTA complex. Competition methods that were used with reference ligands for the determination of the stability constants of the Fe(III) and In(III) complexes of NOTA are described below.

Spectrophotometric determination of metal binding constants

Spectral determinations were made for the Fe(III) complex of NOTA in the presence of *N*,*N*′-bis(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid, HBED (**2**) [20], as the competing reference ligand. The stability of the In(III) complex of NOTA was determined by spectrophotometric measurement of the displacement of the metal ion by *N*,*N*′-bis(2-hydroxy-5-sulfobenzyl)ethylenediamine-*N*,*N*′-diacetic acid, SHBED (**3**).



2 **HBED**



3 SHBED

SHBED is the 5-sulfo derivative of HBED and its complexes with In(III) have been reported by Motekaitis *et al.* [21]. The displacement of each reference ligand from its metal complex by NOTA was measured with a Perkin-Elmer model 553 fast scan UV–Vis spectrophotometer equipped with 1.000±0.001 cm matched quartz cells at 25.0 °C. The stability constants of the Fe(III) and In(III) complexes with NOTA were calculated from the changes in absorbance of the reference metal complexes (Fe(III)–HBED and In(III)–SHBED, respectively) by the use of mass balance equations and equilibrium constant expressions.

For the ternary solution that contained a 1:1:1 molar ratio of Fe(III) ion, NOTA and HBED, the equilibrium distribution of Fe(III) between NOTA (L) and HBED (L′) was measured spectrophotometrically near p[H] 2.9

$$\mathrm{FeL'} + \mathrm{H}_n\mathrm{L} \rightleftharpoons \mathrm{FeL} + \mathrm{H}_m\mathrm{L'} + (n-m)\mathrm{H}^+$$

HBED was selected as the competing ligand because it forms a singular, stable red complex with Fe(III) over a p[H] range from 2.5 to 11.0 and it is more basic than NOTA (as it has four displaceable protons). The molar absorbance of Fe(III)–HBED is 4438 M^{-1} cm^{-1} at 485 nm and is assigned entirely to the ML species of the Fe(III)–HBED complex since a protonated metal complex species was not found by direct titration [20] and spectrophotometric measurements (work not shown). In addition, hydroxo metal complexes were not present since the competition reaction was carried out at low p[H]. Hence, the species present in the Fe(III)–NOTA–HBED solution complex were ML, ML′, L and L′. An exchange ratio (K_{ex}) was computed as a ratio of the binding constants of the Fe(III)–NOTA complex and the reference Fe(III)–HBED complex

$$K_{ex} = K_{ML}/K_{ML'} = [ML][L']/[ML'][L]$$

The stability constant (K_{ML}) for the Fe(III)–NOTA complex was calculated relative to the reference stability constant ($K_{ML'}$) of the Fe(III)–HBED complex and the exchange ratio.

$$K_{ML} = K_{ML'} K_{ex}$$

A correction for the value of the Fe(III)–HBED stability constant [20] was necessary since a fifth protonation constant for HBED ($\log K_5^H = [LH_5]/[LH_4][H] = 2.35$) had been omitted in the literature determination. The corrected reference stability constant ($K_{ML'} = [ML'^-]/[M^{3+}][L'^{4-}]$) has a log value of 39.83.

Equilibrium of the ternary Fe(III)–NOTA–HBED solution was approached from both directions by adding NOTA to a preformed Fe(III)–HBED complex in one flask, and adding HBED to a preformed Fe(III)–NOTA complex in a second flask. An equivalent amount of iminodiacetic acid (IDA) was used as a catalyst. No additional acid was added to adjust the p[H] of the solutions. The total metal complex concentrations were 10^{-4} M and were measured in 0.100 M KCl. The p[H] and absorbance data were read daily for over two months until asymptotic values were found. The deep red color of the preformed Fe(III)–HBED complex eventually bleached to a faint pink color which approached that of the preformed Fe(III)–NOTA complex in the presence of HBED. However, an exact match in absorbance was not obtained because the solution developed a precipitate after 70 days. The nature of the precipitate was not determined but was probably free HBED which has been displaced from the Fe(III)–HBED complex by NOTA. The data of the preformed Fe(III)–HBED flask (0.176 abs.; 2.903 p[H]) and the preformed Fe(III)–NOTA flask (0.085 abs.; 2.931 p[H]), were used to calculate the stability constant for the Fe(III)–NOTA complex with the aid of mass balance equations.

Determination of the stability constant of the In(III)–NOTA complex by spectrometric measurement of competition between SHBED and NOTA for In(III)

SHBED was selected as the competing ligand (L′) for the determination of the stability constant of the In(III)–NOTA complex (ML) since the phenol and phenolate groups of SHBED strongly absorb in the UV region. A preliminary investigation with direct potentiometric titration of the ternary In(III)–NOTA–SHBED solution showed that In(III) was displaced from the In(III)–SHBED complex by NOTA over the range of p[H] from 6.2 to 9.0, but the rate of ligand exchange was slow. Consequently, the stock solution containing equimolar amounts of In(III) ion, NOTA and SHBED, was adjusted to p[H] 9 with standard base and then divided into eight volumetric flasks. The p[H] of each flask was adjusted to fall within the range of 6 to 9 with standard HCl and the ionic strength was maintained at 0.100 M with KCl. The species present were ML, ML(OH), ML′, L and L′.

The phenol groups of SHBED absorb at 235 nm while the ionized phenolate absorbs at 255 nm. At the p[H] relevant to the ligand exchange with NOTA (i.e., at p[H] 7.9), SHBED is present as the H_2L' and H_3L' species; both species contribute to the phenol and phenolate absorbances. On the basis of calculated species distribution curves, H_2L' and H_3L' are both formed to the extent of 50% of the total SHBED present at p[H] 7.9 (i.e., $\log K_3^H = [L'H_3]/[L'H_2][H] = 7.89$). The molar absorbances thereby represent composites of the 235 and 255 nm bands and were calculated as 17 630 and 5600 M^{-1} cm^{-1}, respectively. For reference, the molar absorbance (235 nm) of the completely protonated form of SHBED (H_4L') at p[H] less than 4.2 is 18 630 M^{-1} cm^{-1}, while that of the completely dissociated form of SHBED (L'^{4-}) at 255 nm and p[H] 12.6 is 28 710 M^{-1} cm^{-1}. The molar absorbance of the In(III)–SHBED complex is 20 310 M^{-1} cm^{-1} at 255 nm when the phenolate groups are completely dissociated to form a complex with In(III) ion over the p[H] range 3 to 9. At p[H] 10.2 and higher, the In(III)–SHBED complex forms a hydroxo metal species ML′(OH) which has a molar absorbance (255 nm) of 23 600 M^{-1} cm^{-1}. (The p[H] of the ligand exchange system with NOTA was adjusted to avoid the participation of the ML′(OH) species of In(III)–SHBED.)

For the determination of the stability constant (K_{ML}) of the In(III)–NOTA complex in the presence of competing SHBED, the concentrations of the contributing species were calculated from mass balance equations and absorbance relationships and then an exchange ratio (K_{ex}) was calculated as described previously for the Fe(III)–NOTA–HBED competition system. The reference value for the stability constant of the In(III)–SHBED complex was determined by Motekaitis *et al.* [21] ($\log K_{ML'} = 29.37$).

Results

Protonation constants of triazamacrocyclic tricarboxylate, NOTA

The protonation constants of NOTA, determined by potentiometric titration (Fig. 1) in KCl and 0.100 M tetramethylammonium chloride electrolyte are presented in Table 1. Tetramethylammonium chloride was used as the reference electrolyte for comparison to determine if the K^+ ion is weakly complexed by NOTA, since it is reasonably certain that the tetramethylammonium ion does not form a complex with NOTA. The values of the three protonation constants that were measured in KCl electrolyte (11.93 ± 0.01, 5.65 ± 0.02, 3.17 ± 0.03) and tetramethylammonium chloride (12.00 ± 0.01, 5.65 ± 0.02, 3.19 ± 0.03) are almost the same and indicate that NOTA does not form complexes with the K^+ cation (Table 1) under the conditions employed.

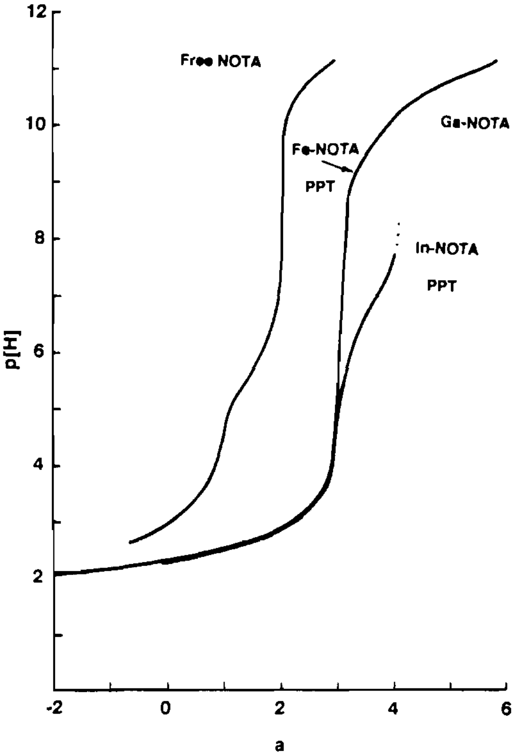


Fig. 1. Equilibrium p[H] profiles of NOTA and its 1:1 metal complexes with Fe(III), Ga(III) and In(III) at $\mu = 0.100$ M (KCl), 25.0 °C. a = moles of base added per mole of ligand present. $T_L = T_M = 2.0 \times 10^{-3}$ M. $Fe(OH)_3$ precipitate is released from the Fe(III)–NOTA complex at $a = 3$. The equilibrium curves do not begin at an integral value of $a = 0$ because a quantity of acid was added initially to each solution.

Potentiometric studies of the complexes of Fe(III), Ga(III) and In(III) with NOTA

The p[H] profiles of trivalent metal complexes of Fe(III), Ga(III) and In(III) with NOTA are shown in Fig. 1. The Fe(III)–NOTA complex formed completely at p[H] 2.1, $a = 0$ (a = moles of base added per mole of ligand present) and was titrated slowly to approach $a = 3$ (~p[H] 7.5) where the Fe(III) complex was completely converted to precipitated $Fe(OH)_3$. No MHL or ML(OH) species were found. The stability constant of the Fe(III)–NOTA complex was determined by competition with HBED, as described in ‘Experimental’. The Ga(III)–NOTA complex behaved like the Fe(III)–NOTA complex in acid solution as it is 100% formed at $a = 0$. However,

TABLE 1. Protonation constants of the triazatricarboxylate ligand (NOTA) and of its parent compound, [9]AneN$_3$ (triazacyclononane)

Equilibrium quotient	Log K			
	Present work[a]	Present work[b]	Reference 5[c]	Reference 10[c]
NOTA				
$[LH]/[L][H]$	11.96 ± 0.01	12.00 ± 0.01	11.41 ± 0.05	11.88 ± 0.02
$[LH_2]/[LH][H]$	5.65 ± 0.02	5.65 ± 0.02	5.74 ± 0.05	5.76 ± 0.01
$[LH_3]/[LH_2][H]$	3.17 ± 0.03	3.19 ± 0.03	3.16 ± 0.06	3.08 ± 0.01
Triazacyclononane				
$[LH]/[L][H]$	10.63 ± 0.01 (10.42 ± 0.02)[d]			
$[LH_2]/[LH][H]$	6.92 ± 0.02 (6.82 ± 0.02)[d]			

[a] 0.100 M KCl, 25.0 °C. [b] 0.100 M TMACl (tetramethylammonium chloride), 25.0 °C. [c] 0.100 M NaNO$_3$, 25.0 °C. [d] Ref. 22, 0.100 M KNO$_3$, 25.0 °C.

the Ga(III)–NOTA complex remained in solution between $a=3$ and $a=4$, in the p[H] range 9–10 where it formed the stable ML(OH) species ($\log K_{ML(OH)} = [ML(OH)][H]/[ML] = -9.70$). In the extreme alkaline p[H] region, the hydroxo Ga(III)–NOTA complex was converted to the gallate anion, $Ga(OH)_4^-$. The equilibrium that was established between the $ML(OH)^-$ and $M(OH)_4^-$ species of Ga(III)–NOTA resulted in a strong buffer region that lies between $a=4$ and 6, in the p[H] range from 10.4 to 11.2 or higher (Fig. 1).

The In(III)–NOTA complex formed the normal ML complex at $a=0$ and a stable ML(OH) species for which the p[H] profile of the hydroxo In(III)–NOTA complex system shows a sloped buffer region between $a=3$ and $a=4$. The equilibrium constant for formation of the ML(OH) species of In(III)–NOTA ($\log K_{ML(OH)}$) was determined by potentiometric measurements to have a log value of -6.60. The stability constant of the In(III)–NOTA complex was determined by the ligand displacement methods since the degree of complex formation was 100% at the beginning of the potentiometric determination (i.e., at $a=0$).

TABLE 2. Stability constants for Fe(III) and In(III) complexes of HBED or SHBED (25.0 °C, 0.100 M KCl)

Quotient	Log K	
	HBED	SHBED
$[LH]/[L][H]$	12.46[a]	12.27[b]
$[LH_2]/[LH][H]$	11.03	10.65
$[LH_3]/[LH_2][H]$	8.37	7.89
$[LH_4]/[LH_3][H]$	4.71	4.25
$[LH_5]/[LH_4][H]$	2.35	1.96[c]
$[LH_6]/[LH_5][H]$		1.2[c]
$[FeL]/[Fe][L]$	39.83[d]	
$[InL]/[In][L]$		29.37[c]
$[InL(OH)][H]/[ML]$		-10.82[c]

[a] Ref. 20. [b] Ref. 23. [c] Ref. 21. [d] Stability constant ($\log K_{ML} = 39.68$) from ref. 20 was corrected for the influence of a fifth protonation constant ($K_5^H = 10^{2.35}$).

TABLE 3. Stability constants for the 1:1 metal complexes of Fe(III), Ga(III) and In(III) with NOTA. Electrolyte is 0.100 M in KCl at 25.0 °C

Equilibrium quotient	Log K		
	Fe(III)	Ga(III)	In(III)
$[ML]/[M][L]$	28.3 ± 0.1[a]	30.98 ± 0.01[b]	26.2 ± 0.1[c]
$[MLOH][H]/[ML]$		-9.70 ± 0.04	-6.60 ± 0.01

[a] Determined by competition with HBED. [b] Determined by competition with gallate ion. [c] Determined by competition with SHBED.

Stability constant of the Fe(III)–NOTA complex

The stability constant ($\log K_{ML}$) for the Fe(III)–NOTA complex was calculated from the exchange constant ($\log K_{ex} = -11.5 \pm 0.1$) and the corrected reference binding constant for Fe(III)–HBED (Table 2) to have a log value of 28.3 ± 0.1 (Table 3). The species and proton formation constants that were used to calculate the concentrations of free NOTA and free HBED appear in Tables 1 and 2. The species distribution curves for the ternary Fe(III)–NOTA–HBED solution complex show that at the equilibrium p[H] of 2.9 the Fe(III)–NOTA complex accounts for 75% of the total iron(III) while the Fe(III)–HBED is 25% formed (Fig. 2).

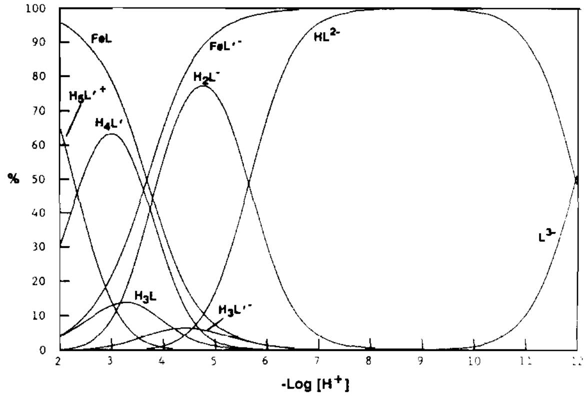


Fig. 2. Species distribution curves for the competition between NOTA (H_3L) and HBED (H_4L') for Fe(III). $[Fe]_T = [NOTA]_T = [HBED]_T = 2.0 \times 10^{-4}$ M. Stability constants for NOTA (25.0 °C, 0.100 M KCl) appear in Tables 1 and 3. Reference stability constants for HBED appear in Table 2.

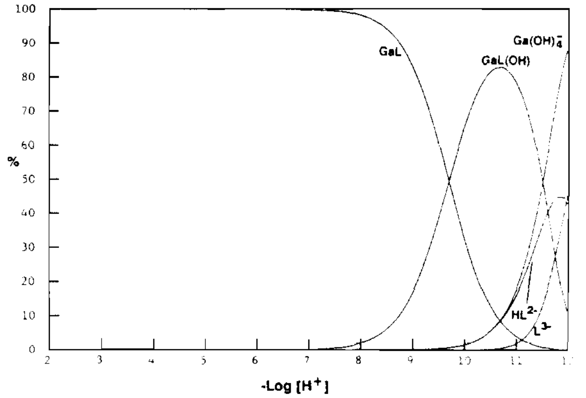


Fig. 3. Species distribution curves for the conversion of Ga(III)–NOTA complexes to the gallate ion, $M(OH)_4^-$. $[Ga]_T = [NOTA]_T = 2.0 \times 10^{-3}$ M. Stability constants (25.0 °C, 0.100 M) for NOTA appear in Table 1. Hydrolysis constants for free Ga(III) ion are $\log K_{Ga(OH)^{2+}} = -2.97$ and $\log K_{Ga(OH)_4^-} = -16.81$ [18, 19].

Ga(III)-NOTA stability constant

The equilibrium between the hydroxo Ga(III)-NOTA complex and gallate was measured by potentiometric titration between $a = 4$ and $a = 6$ (Fig. 1). The log value for the equilibrium constant for hydroxo metal complex formation ($\log K_{ML(OH)} = [ML(OH)][H]/[ML]$) had been determined independently as -9.70. Potentiometric p[H] points of the forward and reverse titrations in the gallate formation region were matched and the log stability constants for the Ga(III)-NOTA complex were computed as $\log K_{ML} = 30.97$ and 30.99 for the forward and reverse directions (see 'Experimental'), respectively, with an average of 30.98 ± 0.01 (Table 3). Species distribution curves (Fig. 3) for the gallate competition system show that gallate anion $Ga(OH)_4^-$ is formed to the extent of 30% of the total metal at p[H] 11.2, while the hydroxo Ga(III)-NOTA complex accounts for the remaining 70%. The hydrolysis constants for Ga(III) employed in the calculation, $[Ga(OH)^{2+}][H^+]/[Ga^{3+}] = -2.97$ and $[Ga(OH)_4^-][H^+]^4/[Ga^{3+}] = -16.81$ were obtained from ref. 18.

In(III)-NOTA stability constant

At equilibrium, the displacement of SHBED (H_4L') from the In(III)-SHBED complex by NOTA (H_3L) resulted in an increase in absorbance at 235 nm for the In(III)-NOTA complex, while the absorbance due to In(III)-SHBED at 255 nm was lessened in intensity (Fig. 4), in accordance with the reaction

$$InL'^- + H_nL \rightleftharpoons InL + H_mL' + (n-m)H^+$$

Ligand exchange was obtained over the equilibrium p[H] range of 6.2 to 7.7, but the displacement of free SHBED from In(III)-SHBED by NOTA resulted in overlap of the absorbances at 235 nm to a constant value near 3.0 so that only the first three data points (i.e. the absorbance that corresponded to p[H] 7.749, 7.619 and 7.301) could be used to calculate the concentrations of the metal complex

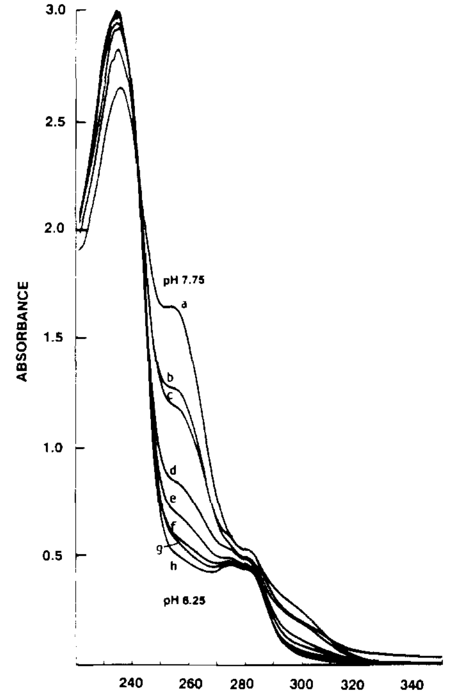


Fig. 4. Absorbance spectra of the 1:1:1 ternary In(III)-NOTA-SHBED solution. $[In]_T = [SHBED]_T = [NOTA]_T = 2.0 \times 10^{-4}$ M (25.0 °C, 0.100 M KCl). The equilibrium p[H] for curves (a)–(h) are: a, 7.75; b, 7.62; c, 7.30; d, 6.99; e, 6.88; f, 6.79; g, 6.53; and h, 6.25.

species. The exchange ratio (log $K_{ex}=[ML][L']/[ML'][L]$) was calculated as a log value of -3.2 ± 0.1, and the stability constant (log K_{ML}) calculated for the In(III)–NOTA complex is 26.2 ± 0.1 (Table 3). The protonation constants that were used to calculate the concentrations of the free NOTA and SHBED appear in Tables 1 and 2, respectively. The species distribution curves for the 1:1:1 In(III)–NOTA–SHBED system (Fig. 5) show that In(III)–NOTA was dominant as the hydroxo metal species (ML(OH)) at p[H] values higher than 7, while In(III)–SHBED is a minor species which forms to the maximum extent of 15% at p[H] 9.

Discussion

Protonation constants of NOTA

The pattern of one high protonation constant ($K_1{}^H$), one intermediate protonation constant ($K_2{}^H$) and one low protonation constant ($K_3{}^H$), is attributed to the protonation sequence of the tertiary amino nitrogens of the macrocyclic ring. The protonation constants [22] that have been reported for the parent macrocyclic compound, triazacyclononane, and those that were determined in the present work (Table 1), show only two protonation constants since the third is too low to be measured by potentiometry. Therefore, the appearance of a third protonation constant for the tricarboxylate derivative of triazacyclonone in this study and in the literature [5, 10] suggests that the third proton of NOTA may be assigned partly to one of the carboxylate groups and partly to the triaza ring in a hydrogen-bonding arrangement since the third protonation constant (log $K_3{}^H=3.17$) overlaps with the values expected for independent carboxylate groups.

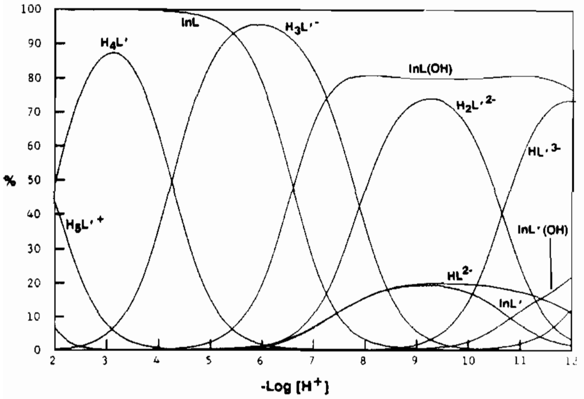


Fig. 5. Species distribution curves for the 1:1:1 In(III)–NOTA–SHBED solution. $[In]_T=[NOTA]_T=[SHBED]_T=2.0\times10^{-4}$ M. Metal complex species and hydroxo metal complex species for NOTA (H_3L) and SHBED (H_4L') are presented together with the protonated forms of the free ligands. Protonation constants and stability constants (25.0 °C, 0.100 M KCl) for NOTA appear in Tables 1 and 3, respectively. Reference stability constants for SHBED appear in Table 2.

Trends of stability constants of Fe(III), Ga(III) and In(III) complexes with NOTA

The log stability constants (log K_{ML}) of the metal complexes of NOTA with the trivalent metal ions Fe(III), Ga(III) and In(III) are 28.3, 30.98 and 26.2, respectively. Ga(III) forms a hydroxo metal complex with NOTA at high p[H], with a log equilibrium constant (log $K_{ML(OH)}$) of -9.70. In(III) forms a hydroxo metal complex with NOTA more readily (log $K_{ML(OH)}=-6.60$), which may be indicative of seven-coordinate geometry of In(III)–NOTA in solution. A seven-coordinate chloro–In(III)–NOTA complex has been described in the solid state by Craig *et al.* [9].

The stability constants of the Fe(III)- and Ga(III)–NOTA complexes are high, yet they differ by almost three orders of magnitude. While the stability constant of Fe(III)–NOTA is high, it is seen in Fig. 1 that this does not stop $Fe(OH)_3$ from precipitating at p[H] just below 9 (note that for Fe(III)–EDTA, with a stability constant of $\sim10^{25}$, $Fe(OH)_3$ precipitates at p[H] ~8.5). The conversion of Fe(III)–NOTA (ML) to solid ferric hydroxide should start to appear a little above p[H] 8 on the basis of solubility product considerations.

It is unlikely that the observed trend in complex stability constants for the trivalent metal ions can be entirely attributed to the size of the metal cation radii [24], since the ionic radii of Ga(III) and Fe(III) cations, (76 and 78.5 pm) in their complexes with NOTA are quite similar [6, 7]. On the other hand, the relatively large radius of the In(III) cation (94 pm) in the six-coordinate NOTA complex could certainly account for its much lower stability constant. A possible reason for the differences in the stability constants of the Ga(III) and Fe(III) complexes could be due to differences in the preferred orientations of the nitrogen and oxygen donor groups in the coordination spheres of these metal ions and the forced distortion of the preferred octahedral orientation toward a triangular prismatic structure [6]. There is insufficient information about such considerations to draw definite conclusions at the present time.

By way of comparison, the stability constants ($K_{ML}=[ML^-]/[M^{3+}][L^{4-}]$) of Fe(III), Ga(III) and In(III) with the tetraazamacrocyclic tetraacetates, DOTA, TRITA and TETA, show a different pattern of selectivity [25] than those of the analogous complexes with a triazamacrocyclic triacetate, NOTA.

For the series of tetraaza macrocyclic ligands, the relative order of stability of each of the trivalent metal complexes with the tetraaza macrocyclic ligands is DOTA > TRITA > TETA suggesting that the tetraaza macrocyclic rings are too large (especially the latter two) to achieve maximum stability with small trivalent metal ions. The stability constants of the Fe(III) complexes are much higher than those of In(III) and Ga(III), and the tetraaza ligands have a small preference for In(III) over Ga(III) ion. These effects are without doubt highly steric in nature. For NOTA, the stability constant of the Ga(III) complex is considerably higher than that of the Fe(III) complex, as noted above, and both are higher than the stability constant of the In(III) complex.

For the triazatriacetate ligand complexes, the apparent preferential selectivity of the Ga(III)–NOTA complex may be a consequence of the close fit of the relatively small Ga(III) cation in the pseudo octahedral cavity of the potentially six-coordinate [9]AneN$_3$-ac$_3$ or NOTA. The cavity of NOTA is defined by the facial triaza plane and an opposite facial plane consisting of three carboxylate oxygen atoms [5, 6]. It is interesting to note that the metal binding affinities that were determined for the NOTA complexes of Ga(III) and In(III) are higher than those of the tetraazatetracarboxylate ligands [25], while the stability constant of the Fe(III)–NOTA complex is comparable to that of the Fe(III)–DOTA complex. The preferred fit of trivalent metal cations to the cavity of the potentially six-coordinate triazatricarboxylate macrocyclic ligand may help to explain the high stability constants of the NOTA metal complexes and may be helpful in making structural comparisons with all other trivalent metal complexes. What is needed for a better understanding of the differences noted above are crystal structures of the DOTA, TRITA and TETA complexes of three trivalent metal ions.

Acknowledgements

E. T. Clarke expresses thanks and appreciation to the Cooley's Anemia Foundation for a Postdoctoral Fellowship. This work was also supported by the U.S. Public Health Service, National Heart, Lung and Blood Institute, Grant No. HL-42780.

References

1 S. H. Koenig, M. Spiller, R. D. Brown and G. L. Wolfe, *Nucl. Med. Biol., 15* (1988) 23.

2 C. F. G. C. Geraldes, A. D. Sherry, R. D. Brown and S. H. Koenig, *Magn. Reson. Med., 3* (1986) 242.

3 J. F. Desreux and P. P. Barthelemy, *Nucl. Med. Biol., 15* (1988) 9.

4 D. A. Moore, M. J. Welch, K. R. Wade, A. E. Martell and R. J. Motekaitis, *7th Int. Symp. Radiopharmaceutical Chemistry, Groningen, The Netherlands, July 4–8, 1988.*

5 M. J. Van der Merwe, J. C. A. Boeyens and R. D. Hancock, *Inorg. Chem., 24* (1985) 1208.

6 K. Wieghardt, U. Bosseck, P. Chaudhuri, W. Herrman, B. C. Menke and J. Weiss, *J. Inorg. Chem., 21* (1982) 4308.

7 D. A. Moore, P. E. Fanwick and M. J. Welch, *Inorg. Chem., 29* (1990) 672.

8 A. S. Craig, D. Parker, H. Adams and N. A. Bailey, *J. Chem. Soc., Chem. Commun.,* (1989) 1793.

9 A. S. Craig, I. M. Helps, D. Parker, H. Adams, N. A. Bailey, M. G. Williams, J. M. S. Smith and G. Ferguson, *Polyhedron, 8* (1989) 2481.

10 A. Bevilaqua, R. I. Gelb, W. B. Hebard and J. L. Zompa, *Inorg. Chem., 26* (1987) 2699.

11 H. Hama and S. Takamoto, *Nippon Kagaku Kaishi, 7* (1975) 1182.

12 R. D. Hancock and A. E. Martell, *Chem. Rev., 89* (1989) 1875.

13 J. E. Richman and T. J. Atkins, *J. Am. Chem. Soc., 96* (1975) 2268.

14 C. F. G. C. Geraldes, M. C. Alpoim, M. P. M. Marques, A. D. Sherry and M. Singh, *Inorg. Chem., 24* (1985) 3876.

15 J. F. Desreux, *Inorg. Chem., 19* (1980) 1319.

16 A. E. Martell and R. J. Motekaitis, *Determination and Use of Stability Constants,* VCH, New York, 1989.

17 R. J. Motekaitis and A. E. Martell, *Can. J. Chem., 60* (1982) 2403.

18 R. J. Motekaitis and A. E. Martell, *Inorg. Chem., 19* (1980) 1646.

19 C. F. Baes and R. E. Mesmer, *The Hydrolysis of Cations*, Wiley, New York, 1976.

20 F. L'Eplattenier, I. Murase and A. E. Martell, *J. Am. Chem. Soc., 89* (1967) 837.

21 R. J. Motekaitis, Y. Sun and A. E. Martell, *Inorg. Chim. Acta, 159* (1989) 29.

22 R. Yang and L. J. Zompa, *Inorg. Chem., 15* (1976) 1499.

23 C. H. Taliaferro and A. E. Martell, *Inorg. Chem., 85* (1984) 9.

24 J. E. Huheey, *Inorganic Chemistry,* Harper and Row, New York, 3rd edn., 1983, Table 3.4.

25 E. T. Clarke and A. E. Martell, to be published; E. T. Clarke, *Ph.D. Dissertation,* Texas A&M University, College Station, TX 1989.