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Gallium and Indium Imaging Agents. 2. Complexes of Sulfonated Catechoylamide Sequestering Agents[1]

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The solution equilibria for the reactions of Ga(III) and In(III) with the hexadentate ligands *N,N',N''*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-1,3,5-tris(aminomethyl)benzene (MECAMS) and *N,N',N''*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-1,5,10-triazadecane (3,4-LICAMS) and the bidentate catechol *N,N*-dimethyl-2,3-dihydroxy-5-sulfonatobenzamide (DMBS) have been determined in 0.1 M KNO_3 at 25 °C. Both Ga(III) and In(III) are coordinated by three catecholate groups at high pH and have formation constants of the order $\beta_{110} = 10^{38}$ M^{-1}. As the acidity of the medium is increased, the metal complexes of the hexadentate sequestering agents undergo protonation reactions. For the determination of the nature of the protonated metal chelates, the stretching frequency of the amide carbonyl has been monitored in D_2O by Fourier transform infrared spectroscopy (FT IR). These data support a series of two one-proton steps to form a mixed salicylate–catecholate coordination about the metal ion. In the salicylate bonding mode the metal is bound through the ortho phenolic oxygen and the amide carbonyl whereas catecholate coordination is via the adjacent phenols. In contrast, protonation of the $M^{III}(DMBS)_3$ complexes results in dissociation of a catechol moiety to form $M^{III}(DMBS)_2$. The potential use of these compounds as tumor-imaging agents in cancer diagnosis is discussed, with specific attention to the role of the gallium transferrin complex.

Introduction

The radionuclides gallium-67 and indium-111 have been used extensively as tumor-imaging reagents.[2] Injection of the citrate complexes $M^{III}(citrate)_2$ results in enhanced uptake of ^{67}Ga or ^{111}In into the faster growing tumor tissue,[3,4] which can be located by a whole body scan taken a short time after administration of the metal. There are two major drawbacks to the present radioimaging technique, however. First, with the possible exception of bleomycin derivatives,[5] no metal complexes are known to deposit the radioisotopes specifically into a cancer. As a consequence, samples of relatively large specific activity must be administered to insure that a detectable level of isotope will accumulate in the tumor. Second, ^{67}Ga not deposited in tumor tissue follows ferric ion metabolic pathways and distributes itself in the liver, spleen, and blood.[6-8] This nontumor isotope gives rise to significant background radiation, which can severely interfere with the imaging process. Development of a ligand that could effectively remove gallium or indium from the liver, spleen, and transferrin in blood, but not significantly from tumor tissue, would represent a major breakthrough in detection of soft tissue tumors.

Catechol derivatives have been shown to be excellent ligands for metals with large charge-to-radius ratios[9-16] such as Ga^{3+} or Fe^{3+}. The favorable chelate ring size, high charge, and resonance stability of the catecholate moiety contribute to the effectiveness of this ligand. We have been interested in the coordination chemistry of ferric catecholates[12-14] because of their use for treatment of transfusional-dependent iron overload associated with certain genetic blood disorders. A biomimetic approach to develop new chelating ligands has been based on the siderophore enterobactin,[17,18] which incorporates three catechoylamide moieties attached to a central ring to encapsulate ferric ion. Enterobactin has the largest formation constant (estimated to be 10^{52} M^{-1})[11] for any known ferric complex. Since Ga(III) and Fe(III) are of the same charge and of similar size,[19] it seemed likely that enterobactin would demonstrate the same strong affinity for gallium as was seen for iron. Indeed, the ^1H and ^{13}C NMR spectra of gallium enterobactin reported by Llinás et al.[20] suggest that enterobactin forms a tris(catecholate) complex with Ga(III). Enterobactin is unsuitable as a pharmaceutical reagent for a number of reasons;[11-14] however, synthetic catechoylamide sequestering agents have been designed to circumvent these disadvantages. Two synthetic ligands, MECAMS[21] and 3,4-LICAMS,[21] are shown in Figure 1. Both compounds can form six-coordinate complexes with Ga(III), are stable at physiological pH, and as a result of sulfonation of the catecholate ring, are very water soluble.

Preliminary in vivo experiments[1] suggest that similar catecholate sequestering agents lower the background level radiation in animals that have been given ^{67}Ga. We hypothesize that MECAMS and 3,4-LICAMS can effectively remove

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(21) Abbreviations used: MECAMS = *N,N',N''*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-1,3,5-tris(aminomethyl)benzene; 3,4-LICAMS = *N,N',N''*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-1,5,10-triazadecane; DMBS = *N,N*-dimethyl-2,3-dihydroxy-5-sulfonatobenzamide; EDTA = ethylenediaminetetraacetic acid; MECAM = *N,N',N''*-tris(2,3-dihydroxybenzoyl)-1,3,5-tris(aminomethyl)benzene; enterobactin = *N,N',N''*-tris(2,3-dihydroxybenzoyl)cyclotriserine ester; DMB = *N,N*-dimethyl-2,3-dihydroxybenzamide; Dip-3,4-LICAMS = *N,N',N''*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-*N,N''*-diisopropyl-1,5,10-triazadecane; TipMECAMS = *N,N',N''*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-*N,N',N''*-triisopropyl-1,3,5-tris(aminomethyl)benzene.

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Table I. Stability Constants for M^{III}DMBS Complexes (1 M Standard States)

	$\log K^{H}{}_{ML}{}^{a}$	$\log K^{2H}{}_{ML_2}{}^{a}$	$\log K^{3H}{}_{ML_3}{}^{a}$	$\log \beta_{110}{}^{b}$	$\log \beta_{120}{}^{b}$	$\log \beta_{130}{}^{b}$
Fe^{3+} [c]	7.23	1.81	−3.21	18.7 (10)	32.0 (1)	40.3 (10)
Ga^{3+}	4.0 (1)	2.0 (2)	−2.3 (2)	15.5 (10)	29 (1)	38 (1)
In^{3+}	3.5 (10)	1.4 (2)	−2.6 (2)	15 (1)	28 (1)	37 (1)

[a] $K^{n}H_{ML_n} \equiv [ML_n][H]/([M^{3+}L_{n-1}][H_nL])$. [b] $\beta_{mlh} \equiv [M_mL_lH_h]/([M^{3+}]^m[L^{3-}]^l[H^+]^h)$. [c] Stability constants from ref 13.

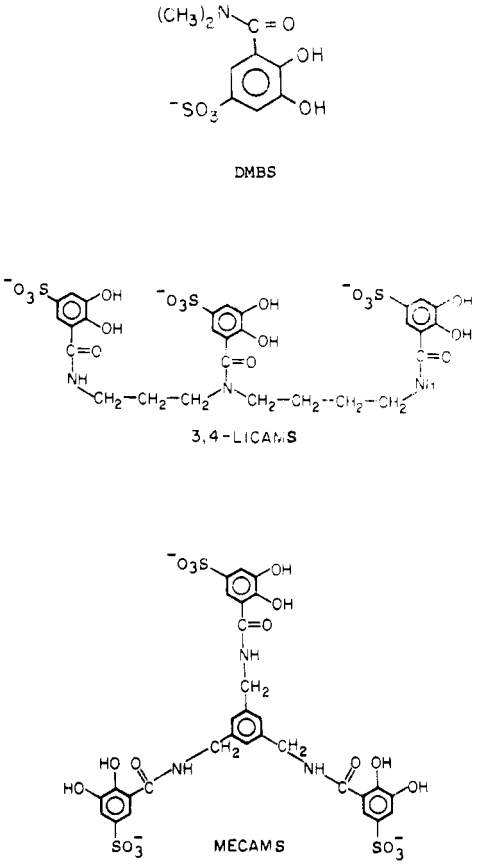

Figure 1. Sulfonated catechoylamide sequestering agents: DMBS = *N,N*-dimethyl-2,3-dihydroxy-5-sulfonatobenzamide; 3,4-LICAMS = *N,N′,N″*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-1,5,10-triazadecane; MECAMS = *N,N′,N″*-tris(2,3-dihydroxy-5-sulfonatobenzoyl)-1,3,5-tris(aminomethyl)benzene.

gallium from transferrin and other plasma proteins, which are the carriers of Ga(III) in vivo. This viewpoint is supported by data from the corresponding iron–transferrin system, from which catecholate sequestering agents are both thermodynamically[11-14] and kinetically[14,22,23] able to remove ferric ion. As the first test of our hypothesis, we report here the formation constants for the reactions of Ga(III) with MECAMS, 3,4-LICAMS, and DMBS[21] in aqueous solution at 25 °C and μ = 0.1 M. Since ^{111}In has also found use as a radioimaging isotope, solution equilibria have been investigated for In(III), which has a chemistry similar to that of Ga(III).

Materials and Methods

Ligands and Stock Solutions. All ligands were synthesized by previously published procedures of Raymond et al.[23] Stock metal ion solutions were prepared via nitric acid oxidation of Ga or In metal and maintained at pH <1.5. These solutions were standardized by following the methods of Welcher[24] using EDTA,[21] and the hydrogen ion concentration was determined by titration of the EDTA complex.

Potentiometric Measurements. We have previously given a detailed account of the apparatus used and the procedure followed for potentiometric titrations.[12] Briefly, measurements were made with a Corning 130 digital pH meter equipped with Corning glass and saturated calomel electrodes. The meter was calibrated with standard acetate and nitric acid solutions to read hydrogen ion concentration, not activity. Solutions (40 mL) were kept under inert atmosphere (argon) and were maintained at 25 ± 0.05 °C by a circulating water bath. The ionic strength was maintained at 0.1 M with KNO_3. Carbonate-free 0.0982 ± 0.0003 M KOH was prepared from Baker "Dilut-It" ampules with freshly boiled, doubly distilled H_2O. Potentiometric data were refined with use of a nonlinear least-squares analysis described previously.[12]

Spectrophotometric Measurements. Spectra were recorded on a Cary 118 spectrophotometer. Competition reactions were carried out by the following procedure for both Ga^{3+} and In^{3+}. Stock solutions, 1:1 metal to ligand ratio, of Ga(EDTA)$^-$ and Fe(catechoylamide)$^{6-}$ were mixed in varying ratios in a 10-mL volumetric flask. The pH of each competition reaction was initially adjusted to be between 6.0 and 7.0. The ionic strength of the solution was maintained at 0.1 M by addition of KNO_3. Each competition was allowed to stand for a period of 2 weeks in order to achieve thermodynamic equilibrium. No change in absorbance was observed after 10 days. The rate of the exchange reaction was monitored for one sample, and an approximate half-time of 36 h was obtained.

The Fe(catechoylamide)$^{6-}$ spectra were monitored at 487 nm. The amount of Fe bound to the catecholate ligand could be calculated from the visible spectra, on the basis of the known extinction coefficients[13] and metal protonation constants[13] of the fully formed and protonated ferric catechoylamide complexes. Spectral data were uncorrected for the absorption of M^{3+}–catechoylamide, M^{3+}–EDTA, and Fe–EDTA complexes (M = Ga, In) at 487 nm, which do not absorb significantly at this wavelength. The hydrogen ion concentration of each sample was measured with a Beckman Model 102 pH meter and Sigma combination electrode, which had been standardized at pH 7.00 and 4.01. A discussion of the method and the equations used for calculation of the formation constants given in Table I can be found in the Appendix. As a separate verification that equilibrium had been reached, the reverse reaction, between Fe(EDTA)$^-$ and Ga(catechoylamide)$^{6-}$, was taken to equilibrium. The formation constants tabulated in Table I are the average of both determinations, which agreed well.

Infrared Spectra. Spectra of Ga(MECAMS) in D_2O as a function of pD were taken on a Nicolet Fourier transform spectrometer with 0.5-mm AgCl cells. The pH meter readings in D_2O were converted to actual pH values by the method of Perrin.[25] Adjustments were made with DCl or NaOD in D_2O.

Results

The ligand protonation constants for MECAMS, 3,4-LICAMS, and DMBS have been reported previously.[13] The relevant formation constants for the Ga(III) and In(III) complexes of these ligands are described below.

Ga(III) and In(III) Complexes of DMBS. The potentiometric equilibrium curves for 1:3 solutions of Ga(III) and In(III) with DMBS are shown in Figure 2. The breaks at *a* values of 2, 4, and 6 correspond to the formation of the mono, bis and tris complexes of the ligand (*a* = moles of base added per mole of metal). Although the buffer region from *a* = 2–4 is much better resolved for In(DMBS)$_2^{3-}$ than for the Ga

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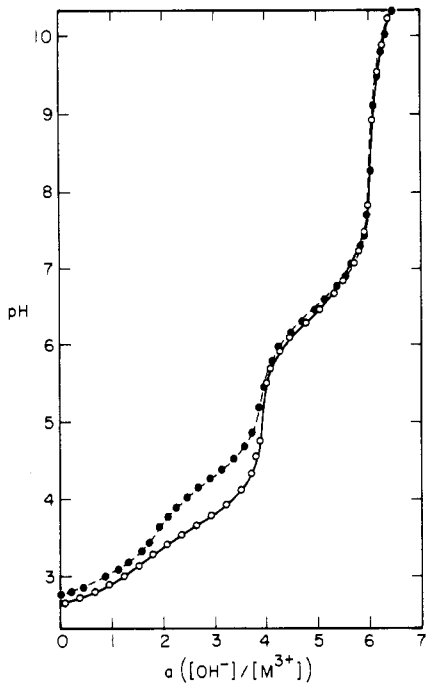

Figure 2. Potentiometric titrations of 3:1 DMBS to metal mixtures in 0.1 M KNO_3 at 25 °C. The breaks at a = 2, 4, 6 represent the formation of the mono, bis, and tris complexes of DMBS, respectively: Ga (○), In (●); $[Ga^{3+}]$ = 1.28 mM, [DMBS] = 3.88 mM, $[In^{3+}]$ = 1.62 mM, [DMBS] = 4.87 mM.

complex, the buffer region from a = 4–6 (corresponding to the formation of the tris complex) is well resolved for both metal systems.

Because it is very difficult to obtain an accurate value for the high ligand protonation constants of DMBS, we have previously chosen[13] to describe the metal complexation equilibria by a series of proton-dependent equilibrium constants expressed in terms of $H(DMBS)^{2-}$ (eq 1–3).

$$M^{3+} + H(DMBS)^{2-} \rightleftharpoons M(DMBS) + H^+$$

$$K_1 = \frac{[M(DMBS)][H^+]}{[M^{3+}][H(DMBS)^{2-}]} \quad (1)$$

$$M(DMBS) + H(DMBS)^{2-} \rightleftharpoons M(DMBS)_2^{3-} + H^+$$

$$K_2 = \frac{[M(DMBS)_2^{3-}][H^+]}{[M(DMBS)][H(DMBS)^{2-}]} \quad (2)$$

$$M(DMBS)_2^{3-} + H(DMBS)^{2-} \rightleftharpoons M(DMBS)_3^{6-} + H^+$$

$$K_3 = \frac{[M(DMBS)_3^{6-}][H^+]}{[M(DMBS)_2^{3-}][H(DMBS)^{2-}]} \quad (3)$$

The formulation, although nonstandard, avoids the introduction of error due to considerable uncertainty in determination of the first ligand protonation constant. Values of K_1, K_2, and K_3 have been calculated by nonlinear least-squares refinement of the potentiometric data in which these constants were the only parameters. These values are reported in Table I.

Ga(III) and In(III) Chelate Protonation Constants. The titration curves for the reaction of Ga(III) and In(III) with MECAMS and 3,4-LICAMS are shown in Figure 3. A metal–ligand complex is formed over the entire pH range that is accessible to potentiometric analysis. In all four cases, a well-resolved buffer region is seen between a = 4 and a = 6. Rather than representing complete dissociation of the metal–ligand complex, this buffer region represents protonation of the metal complex. There are two distinct alternatives for protonation of the metal chelate. The first, which is the protonation scheme observed for ferric catechoylamide complexes,[12-14] proceeds via two one-proton steps to form a "salicylate" complex that is described in detail below. The second protonation scheme follows a single two-proton step, which causes dissociation of a catechol moiety. A third alternative incorporates features from the first two protonation schemes; in this case, two one-proton reactions occur. However, after the first protonation (to forming a salicylate-like complex) the second equivalent of hydrogen ion displaces a catechol moiety. The different protonation schemes are illustrated in Figure 4.

Table II. Metal Chelate Protonation Constants for Catechoylamide Complexes of Ga^{3+} and In^{3+} (1 M Standard States)

	one-proton step[a]	two-proton step[b]
Ga(MECAMS)	K^{H}_{ML} = 5.7	K^{2H}_{ML} = 10.7 (1)
	K^{H}_{MHL} = 4.9 (2)	
Ga(3,4-LICAMS)	[c]	K^{2H}_{ML} = 10.2 (1)
In(MECAMS)	K^{H}_{ML} = 4.92 (3)	K^{2H}_{ML} = 9.51 (3)
	K^{H}_{MHL} = 4.70 (3)	
In(3,4-LICAMS)	K^{H}_{ML} = 5.66 (3)	K^{2H}_{ML} = 10.9 (1)
	K^{H}_{MHL} = 5.29 (3)	

[a] $K^{H}_{MH_{n-1}L} = [MH_nL]/([MH_{n-1}L][H^+])$. [b] $K^{2H}_{ML} = [MH_2L]/([ML][H^+]^2)$. [c] Potentiometric data would not refine with a model of two overlapping one-proton steps.

Table III. Carbonyl Stretching Frequency for MECAMS, Cu(MECAMS), and Ga(MECAMS)

	pM[a]	$\nu_{C=O}$
MECAMS	9	1604
	3.0	1629
Cu(MECAMS)	7.56	1602
	6.07	1628 (sh), 1600
	5.48	1622, 1600
Ga(MECAMS)	8.5	1609
	5.4	1611
	5.1	1612
	4.6	1611
	3.4	1629 (sh), 1613
	3.0	1629 (sh), 1613 (sh)
	0.8	1628

[a] The hydrogen ion concentration was calculated by the method of Perrin in ref 25.

The chelate protonation constants for the first and third models can be represented by the general equation

$$K^{H}_{MH_nL} = \frac{[MH_nL]}{[MH_{n-1}L][H^+]} \quad (4)$$

where n = 1, 2 for this system, while the constant for the two-proton step is of the form

$$K^{2H}_{ML} = \frac{[MH_2L]}{[ML][H^+]^2} \quad (5)$$

Both constants can be obtained by least-squares analysis of the potentiometric data in the same way that one would determine ligand protonation constants. In most cases good fits were found for either a one- or a two-proton model, the results of which are given in Table II.

The exact proton stoichiometry cannot be determined as was done (with use of the Schwarzenbach method[26]) for the corresponding iron complexes, because the gallium and indium compounds do not have useful vis–UV spectra. However, solution IR data suggest that in the Ga–MECAMS complex

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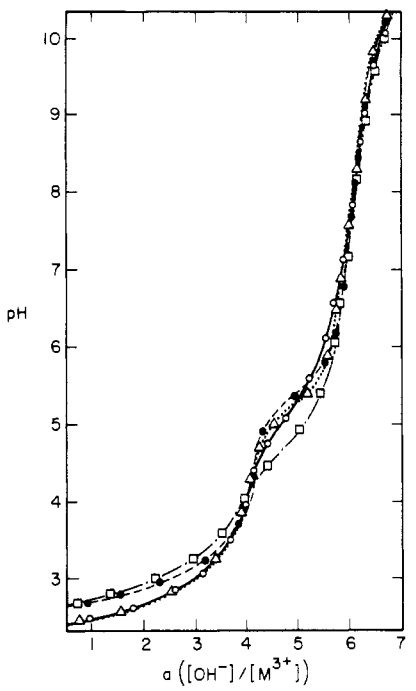

Figure 3. Potentiometric titration of 1:1 stoichiometric mixtures of In(III) and Ga(III) with MECAMS and 3,4-LICAMS in 0.1 M KNO_3 at 25 °C. The break at $a = 4$ is indicative of a metal chelate protonation reaction. All four complexes are fully formed by pH 7.5: Ga(MECAMS) (○), Ga(3,4-LICAMS) (△), In(LICAMS) (●), In(3,4-MECAMS) (□); $[Ga^{3+}] = 1.29$ mM, [MECAMS] = 1.29 mM, $[Ga^{3+}] = 1.04$ mM, [3,4-LICAMS] = 1.07 mM, $[In^{3+}] = 1.33$ mM, [MECAMS] = 1.36 mM, $[In^{3+}] = 1.65$ mM, [3,4-LICAMS] = 1.70 mM.

a catechol moiety does not dissociate, as it would by the two-proton mechanism, but rather may be shifting to a salicylate mode of bonding during protonation. These conclusions are based upon comparison with the results of a more detailed study on ferric and copper catechoylamide complexes published elsewhere.[27] The carbonyl stretching frequencies for Ga(MECAMS) are given in Table III.

Metal Ion Competition Reactions. Double-competition experiments were performed with solutions containing both gallium (or indium) *and* iron complexes of EDTA and catechoylamide:

$$FeL^{n-} + Ga(EDTA)^- \rightleftharpoons Fe(EDTA)^- + GaL^{n-} \quad (6)$$

A simple Ga(MECAMS) vs. Ga(EDTA) competition was unsuitable because, unlike the ferric catechoylamides, both the gallium and the indium complexes lack charge-transfer bands in the visible region and cannot be monitored by visible spectroscopy. In the double competitions the degree of formation of the iron catechoylamide species is determined from visible spectra. Then, using mass-balance relationship, the previously reported solution equilibria[12,18] for Fe–MECAMS, Fe–3,4-LICAMS, and (Fe^{3+}, Ga^{3+}, In^{3+})–EDTA complexes,[28] the pH, and the total iron, gallium, and ligand concentrations, one can calculate the amounts of Ga(catechoylamide), Ga(EDTA), and Fe(EDTA) present. The free ligand concentration is assumed to be negligible due to the 1:1 ratio of total metal to total ligand. After correction for protonated or hydrolyzed species, concentrations of the species in eq 6 may be used to calculate the metal catechoylamide formation constant $\beta_{110}^{M^{3+}}$ (where the subscripts represent numbers of metal, ligand, and protons, respectively), outlined by eq 7 and 8 for $[GaEDTA)]^- + [Fe(MECAMS)]^{6-}$ and described more fully in the Appendix.

$$K_{comp} = \frac{[Fe(EDTA)^-][Ga(MECAMS)^{6-}]}{[Fe(MECAMS)^{6-}][Ga(EDTA)^-]} \quad (7)$$

$$\beta_{110}^{Ga(MECAMS)} = \frac{\beta_{110}^{Fe(MECAMS)}\beta_{110}^{Ga(EDTA)}K_{comp}}{\beta_{110}^{Fe(EDTA)}} \quad (8)$$

Since the first three ligand protonations for MECAMS and 3,4-LICAMS occur at high pH, a constant that is independent of the uncertainties in the high ligand pK values can be calculated, in analogy to the case for the DMBS system. This constant is defined for $[Ga(MECAMS)^{6-}]$ as

$$K^* = \frac{[Ga(MECAMS)^{6-}][H^+]^3}{[Ga^{3+}][H_3MECAMS^{6-}]} \quad (9)$$

A comparison of K^*'s for the Ga^{3+}, In^{3+}, and Fe^{3+} complexes of MECAMS and 3,4-LICAMS is given in Table IV.

Discussion

The potentiometric data reported herein for Ga^{3+} and In^{3+} DMBS complexes are consistent with the titration curves for a 3:1 molar ratio of 3,5-disulfonatocatechol (TIRON) and Ga^{3+}, as previously reported by Martell,[29] and for DMBS with Fe^{3+}, as given by Harris et al.[13] The breaks at $a = 2$, 4, and 6 in the potentiometric titration curves, shown in Figure 2, indicate the formation of the $M^{3+}(DMBS)$, $M^{3+}(DMBS)_2^{3-}$, and $M^{3+}(DMBS)_3^{6-}$ complexes, respectively. Both the $Ga(DMBS)_3^{6-}$ and $In(DMBS)_3^{6-}$ complexes are the predominant species by pH 8.5, although formation of $Ga(DMBS)_2^{3-}$ occurs at a slightly lower pH than the corresponding $In(DMBS)_2^{3-}$. Thus, as seen from the titration curves, the relative difference in stability between $Ga(DMBS)_3^{6-}$ and $Ga(DMBS)_2^{3-}$ is greater than that observed for $In(DMBS)_3^{6-}$ and $In(DMBS)_2^{3-}$. This phenomenon may be a reflection of the larger ionic radius of In^{3+}, which could allow a larger charge separation between the three trianionic ligands.

On the basis of the pM values ($-\log [M^{3+}(H_2O)_6]$), given in Table V, one would expect $Ga(DMBS)_3^{6-}$ and, to a greater extent, $In(DMBS)_3^{6-}$ to be unstable to hydrolysis under the conditions of 10 μM total ligand and 1 μM total metal at pH 7.4. We conclude that, under our experimental conditions, the kinetics of formation of an insoluble gallium hydroxide polymer are relatively slow in the presence of DMBS, since there is no evidence from the shape or the proton stoichiometry of the titration curves indicating metal hydrolysis.

A comparison of the Ga^{3+} and In^{3+} formation constants for the tris(catechoylamide) (Table III) and DMBS complexes (Table I) shows that in sulfonated catechoylamides there is little or no chelate effect. This is in marked contrast to differences in stability of the ferric complexes of the unsulfonated hexadentate catechoylamide ligands MECAM[21] and enterobactin[21] and the monomer DMB.[21] The formation constant[9] for $Fe(DMB)_3^{3-}$ is $10^{40.2}$ M^{-3}, whereas the respective constants[11,30] for enterobactin and MECAM complexes are 10^{52} M^{-1} and $10^{45.8}$ M^{-1}. The absence of a chelate effect for the sulfonated derivatives is not unique to Ga^{3+} and In^{3+}, as similar results have been reported for the Fe^{3+} complexes.[13] We are presently determining the enthalpy and entropy of ferric ion complexation by DMBS and other sulfonated catechoylamides in order to understand better the factors that remove the chelate effect from this system.

In order to calculate the standard formation constant, β_{110}, it is necessary to have a detailed knowledge of the metal chelate

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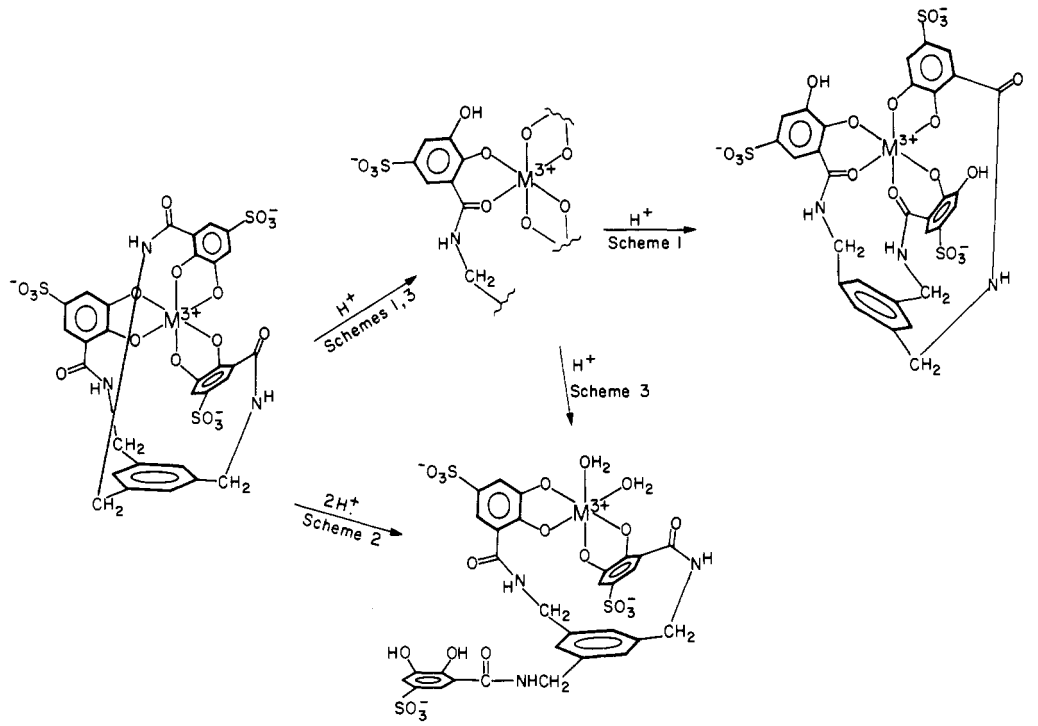


Figure 4. Alternative protonation schemes for M^{3+}(MECAMS). In scheme 1 the metal complex undergoes a series of two overlapping one-proton steps to generate a mixed salicylate–catecholate coordination about the metal ion. This differs from scheme 2, in which a single two-proton step dissociates one arm of the ligand to form a bis(catecholate) chelate. Scheme 3 incorporates features of schemes 1 and 2. In this model, the metal again undergoes a series of two overlapping one-proton reactions. However, unlike the case of scheme 1, the second proton displaces a catecholate arm, which results in a bis(catecholate) metal complex. For a detailed discussion see the text.

Table IV. K^* and β_{110} for Ga^{3+}, In^{3+}, and Fe^{3+} Complexes of MECAMS and 3,4-LICAMS (1 M Standard States)

	log K^{*a}	log β_{110} [b]
Fe(MECAMS)[c]	6.57 (1)	41 (1)
In(MECAMS)[d]	4.7 (8)	39 (1)
Ga(MECAMS)[d]	3.5 (5)	38 (1)
Fe(3,4-LICAMS)[c]	6.40 (9)	41 (1)
In(3,4-LICAMS)[d]	4.3 (7)	39 (1)
Ga(3,4-LICAMS)[d]	3.6 (6)	38.5 (10)

[a] $K^* = [ML][H]^3/([H_3L][M^{3+}])$. [b] $\beta_{110} = [ML^{6-}]/([M^{3+}][L^{9-}])$. This constant is estimated with use of an average value for the first three ligands protonations of log $\beta_{013} = 34.5$. [c] Formation constants from ref 13. [d] Constants are average values obtained from competitions in which equilibrium was approached via forward and back reactions. See text for details.

Table V. pM[a] Values for Selected Metal Ion Sequestering Agents

	Fe^{2+} [b]	Ga^{3+}	In^{3+}	ref[c]
HBED[d]	31.0	30.9		28
MECAMS	29.4	26.3	27.4	
3,4-LICAMS	28.5	26.0	26.5	
EHPG[e]	26.4	23.5		28
DTPA[f]	24.7	22.8	25.9	28
transferrin[g]	23.6			35, 36
EDTA[h]	22.2	21.6	0.7	32
TIRON[i]	19.5	19.4		29
DMBS	19.2	16.1	15.1	
hydroxide[j]	19.4[k]	17.8[k]	17.7[k]	28

[a] Conditions: $[M^{3+}]_T = 1 \times 10^{-6}$ M; [ligand] $= 1 \times 10^{-5}$ M; pH 7.4; pM $= -\log [M^{3+}(H_2O)_6]$. [b] pM values reported previously in ref 12. [c] pM values for Ga^{3+} and In^{3+} calculated from stability constants in these references. [d] *N,N'*-Bis(2-hydroxybenzyl)-ethylenediamine-*N,N'*-diacetic acid. [e] Ethylene-1,2-bis((2-hydroxyphenyl)glycine). [f] Diethylenetriaminepentaacetic acid. [g] $[HCO_3^-] = 0.024$ M. [h] Ethylenediaminetetraacetic acid. [i] 1,2-Dihydroxy-3,5-disulfonatobenzene. [j] pM values are given for $-\log [M^{3+}]_T$, representing all soluble hydrolyzed species. [k] Values of log K_{sp} used: Fe^{3+}, −38.8; Ga^{3+}, −37; In^{3+}, −36.9.

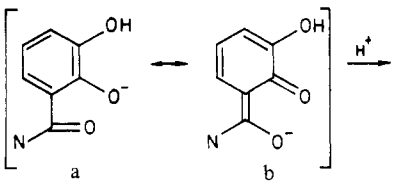
protonation equilibria. The strong complexation of Ga^{3+} and In^{3+} is indicated by the shape of the titration curves shown in Figure 3. The buffer region from $a = 4$ to $a = 6$ is attributed to the protonation of the metal chelate, rather than to weak complex formation.

There are three alternatives for the protonation of the metal chelate, as illustrated in Figure 4. From the potentiometric data alone it is difficult to determine which of these reactions is occurring. Ferric catechoylamides are believed[11,13,14,30] to follow the first protonation scheme whereby mixed "salicylate" and catecholate coordination is observed. In contrast, ferric complexes of the monomers DMB and DMBS proceed along the second pathway, in which a single two-proton step dissociates a catechol functional group. Study of the solution behavior of ferric catecholates is facilitated by intense charge-transfer bands in the 480–590 nm region. Since both Ga^{3+} and In^{3+} catechol complexes contain no usable spectral features, the Schwarzenbach analysis described previously cannot be employed.

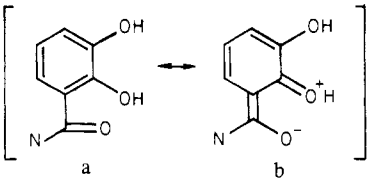
An alternative approach is to monitor the carbonyl stretching frequency of the ligand as a function of pH and accompanying changes in metal coordination chemistry. In scheme 1 (Figure 4) the carbonyl oxygen participates directly in metal ion coordination. An analysis of the C=O stretch should give an indication of the involvement of the carbonyl oxygen in metal ion coordination, as has been observed for a series of ferric complexes.[27]

For MECAMS $\nu_{C=O}$ appears at 1603 cm^{-1} at pH $\gtrsim 9$, where the ortho phenolic oxygens have been deprotonated. This band shifts to higher energy as the pH is lowered until, at pH $\lesssim 4$, where the phenols have been protonated, a peak at 1628 cm^{-1} appears. This shift is explained by the resonance structures shown for I and II. The contribution of resonance structure b is larger for I than for II. Thus, the carbon–oxygen bond has more single-bond character in the deprotonated ligand, giving rise to the lower energy stretching frequency.

Interpretation of the IR spectrum for the metal complex is not as straightforward. The peak at ~1610 cm^{-1} in Ga-



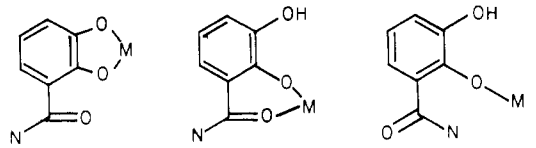
I



II

(MECAMS) at high pH is assigned to the carbonyl stretching frequency of a ligand molecule coordinated to the gallium via the phenol oxygens. As the complex is protonated, there is no shift in the carbonyl peak seen until pH 3.4, where the 1629-cm^{-1} peak of the uncoordinated catechol appears. Correlating these spectra with the titration curve in Figure 3, one sees that there is little change in the IR spectrum over the two equivalent buffer regions ascribed to metal complex protonation. Solution chemistry of the related complex Cu–MECAMS has indicated metal bonding by two catechol groups, with one catechol moiety not involved in metal coordination.[31] This free catechol arm when fully protonated can be seen by IR and gives rise to a band at nearly the same energy as that for the uncomplexed ligand.[27] Such a band was not seen for the Ga–MECAMS complex at pH values corresponding to the two-proton buffer region. This leads to the conclusion that catechol dissociation probably has not occurred, because under these pM conditions a free arm would have been fully protonated and, therefore, observable.

The IR data alone neither conclusively prove nor refute a salicylate type of coordination. For this bonding scheme to be consistent with the data, structures IIIa and IIIb *both* must



III

have carbonyl stretching frequencies between 1600 and 1615 cm^{-1}. Although this represents a relatively small difference, it appears to be true for some analogous salicylate and catecholate ferric complexes.[27] Also, we cannot rule out a structure such as IV solely on the IR and potentiometric data. However, the ready availability of a carbonyl oxygen to fill the vacant sixth coordination position, forming a stable six-member chelate ring, would suggest that IIIb is the most likely structure.

We have reported chelated protonation constants for both two one-proton and one two-proton steps, since IR spectroscopy is not sensitive to proton stoichiometries. The stability constants in Table IV have been calculated with use of the two one-proton model where possible; however, the values based upon one two-proton reaction are not significantly different in this case.

A tris(catecholate) species is believed to be present at pH 7.4 for the Ga(III) and In(III) complexes of MECAMS and 3,4-LICAMS. This formulation is supported by analogy to the ferric system and the Ga(III) and In(III) DMBS complexes and the proton stoichiometry of the potentiometric titrations.

Of the catechoylamide complexes, the ferric is the most stable. However, the Ga(III) and In(III) chelates are also very robust. For example, the Ga(MECAMS)$^{6-}$ constant is $\sim 10^{17}$ greater than that of Ga(EDTA)$^{-}$.[32] The values of β_{110} for all of the complexes are prone to some error due to the estimation of the three highest ligand protonation constants that are incorporated in β_{110}. The proton-dependent constant (eq 10) does not suffer from this and may be used at any pH below 10.

$$K^* = \frac{[ML^{6-}][H^+]^3}{[H_3L^{6-}][M^{3+}]} \qquad (10)$$

In the evaluation of the efficacy of a Ga(III) or In(III) chelating agent at physiological pH, β_{110} is not a direct measure of relative stability except under conditions when the free ligand is completely deprotonated such as in 1 M hydroxide. The effectiveness of the catechoylamide as a metal-complexing agent decreases as the pH is lowered, due to competition for the basic phenolic oxygens by protons, and follows a sixth-order hydrogen ion dependence, seen in the following equation (the principal one at physiological pH):

$$M^{3+} + H_6L^{3-} \rightarrow ML^{6-} + 6M^+$$

An alternative quantity to use for comparison is the hexaaquometal(III) concentration under standard conditions for ligands of differing acidities. These values are expressed as pM, where pM $\equiv -\log[M^{3+}(H_2O)_6]$, and are calculated for a number of sequestering agents in Table V under the conditions of 10 μM total ligand, 1 μM total metal, and pH 7.4. Generally, the larger the value of pM, the more effective the ligand is at chelating the metal ion under the prescribed conditions.

It can be seen from Table V that MECAMS and 3,4-LICAMS are among the most effective Ga(III) and In(III) chelating agents yet characterized. The importance of the high denticity of MECAMS and 3,4-LICAMS is illustrated by comparison of these hexadentate ligands with the bidentate DMBS. Although log $\beta_{130}^{Ga(DMBS)}$ is approximately equal to log β_{110} for Ga(MECAMS) or Ga(LICAMS), the pM values differ by 10 log units. This is a direct consequence of the third-order ligand concentration dependence for formation of $Ga(DMBS)_3^{6-}$, which makes DMBS less effective at concentrations less than the "standard conditions" of 1 M. This is an important consideration for a radioimaging agent, which is used in low concentrations.

Iron is transported in the plasma by the protein transferrin. When gallium citrate is injected into an animal, the gallium is also bound by this serum protein.[6,7] It would seem that mobilization of gallium from transferrin would be the most successful mechanism of metal decorporation. Gallium occupies the iron binding sites in transferrin,[33,34] and ferric ion will displace gallium from these sites.[34] Thus, the pM value for ferric transferrin must act as an upper bound for the gallium transferrin complex. A comparison of the pM values for Ga(MECAMS) and Ga(3,4-LICAMS) shows that these ligands are at least 1000 times more effective than transferrin at binding gallium. *Therefore, we can conclude that both MECAMS and 3,4-LICAMS are thermodynamically capable of removing gallium from transferrin under these conditions.*

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Derivatives of MECAMS and 3,4-LICAMS that are alkylated on the amide nitrogens have been used to investigate the clearance of gallium and indium from Sprague–Dawley rats.[1] It was shown that the metal was rapidly cleared to the kidneys but was slowly excreted into the bladder. Other unsulfonated, N-alkylated catechoylamides showed promise because of rapid liver clearance. Preliminary equilibrium data[1] were obtained from Dip-3,4-LICAMS[21] and TipMECAMS[21] which suggest these compounds are also very effective gallium and indium chelating agents. Not only must an effective metal-sequestering agent be thermodynamically able to mobilize protein-bound gallium but it must also do so on a rapid time scale. For this reason, we are presently investigating the kinetics of gallium removal from transferrin by sulfonated catechoylamides.

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Appendix

The formation constant for a gallium complex (for instance $[Ga(MECAMS)^{6-}]$) may be determined by use of the competition reaction

$$Fe(MECAMS)^{6-} + Ga(EDTA)^{-} \rightleftharpoons Fe(EDTA)^{-} + Ga(MECAMS)^{6-}$$

Experimental determination of the total concentration of $Fe(MECAMS)^{6-}$, $[Fe(MECAMS)]_T$, and knowledge of mass-balance relationships and formation constants for the three known metal–ligand combinations allow calculation of the remaining desired constant. With 1:1 stoichiometric ratios of metal to ligand, the mass-balance equations (1A)–(4A)

$$[MECAMS]_T = [Ga(MECAMS)]_A + [Fe(MECAMS)]_A \quad (1A)$$

$$[EDTA]_T = [Ga(EDTA)]_A + [Fe(EDTA)]_A \quad (2A)$$

$$[Ga]_T = [Ga(MECAMS)]_A + [Ga(EDTA)]_A \quad (3A)$$

$$[Fe]_T = [Fe(MECAMS)]_A + [Fe(EDTA)]_A \quad (4A)$$

apply. In these equations $[Ga]_T$ and $[Fe]_T$ represent the total analytical concentrations of each metal. Likewise, the total concentration of ligand is $[MECAMS]_T$ and $[EDTA]_T$. The symbolism $[Ga(MECAMS)]_A$, for example, signifies the sum of the concentrations of all Ga(MECAMS) species present in solution. Thus in the pH range 6–7

$$[Ga(MECAMS)]_A = [Ga(MECAMS)^{6-}] + [Ga(H(MECAMS))^{5-}] + [Ga(H_2MECAMS)^{4-}] \quad (5A)$$

Similar functions can be written for $[Ga(EDTA)]_A$, $[Fe(MECAMS)]_A$, and $[Fe(EDTA)]_A$. Hydrolysis can be neglected, since these ligands are known to form very strong complexes in the pH range of the competition.

The charge-transfer bands of Fe(MECAMS) in the visible region can be used to monitor the concentrations of all species, so that

$$A_{487} = \epsilon_1[Fe(MECAMS)^{6-}] + \epsilon_2[Fe(H(MECAMS))^{5-}] + \epsilon_3[Fe(H_2MECAMS)^{4-}] \quad (6A)$$

$$[Fe(MECAMS)^{3-}] = \frac{A_{487}}{\epsilon_1 + K^{H}_{ML}[H^+]\epsilon_2 + K^{H}_{ML}K^{H}_{MHL}[H^+]^2\epsilon_3} \quad (7A)$$

where K^{H}_{ML} and K^{H}_{MHL} are the stepwise complex protonation constants.

The concentrations of $[Fe(H(MECAMS))^{5-}]$ and $[Fe(H_2MECAMS)^{4-}]$ can be calculated in an analogous manner, and the sum of these three species is $[Fe(MECAMS)]_A$. Mass-balance relationships give the equations

$$[Fe(EDTA)]_A = [Fe]_T - [Fe(MECAMS)]_A \quad (8A)$$

$$[Ga(EDTA)]_A = [EDTA]_T - [Fe(EDTA)]_A \quad (9A)$$

$$[Ga(MECAMS)]_A = [Ga^{3+}] - [Ga(EDTA)]_A \quad (10A)$$

which are rearranged forms of eq 4A, 2A, and 3A, respectively.

We can next define a competition constant:

$$K_{comp} = \frac{[Ga(MECAMS)][Fe(EDTA)]}{[Fe(MECAMS)][Ga(EDTA)]} \quad (11A)$$

The quantities $[Fe(EDTA)]_A$, $[Ga(EDTA)]_A$, and $[Ga(MECAMS)]_A$ are sums of *all* the respective species present in solution, whereas K_{comp} is defined specifically in terms of $[Ga(MECAMS)^{6-}]$, $[Fe(MECAMS)^{6-}]$, $[Ga(EDTA)^{-}]$, and $[Fe(EDTA)^{-}]$. One additional calculation must be made before K_{comp} can be determined. Solving for $[Ga(MECAMS)^{6-}]$ from eq 5A gives

$$\frac{[Ga(MECAMS)]_A}{[Ga(MECAMS)]} = 1 + K^{H}_{ML}[H^+] + K^{H}_{ML}K^{H}_{MHL}[H^+]^2 \quad (12A)$$

Similar treatments can be used to generate the equalities

$$[Fe(EDTA)] = \frac{[Fe(EDTA)]_A}{1 + K^{OH}_{ML}[H^+]} \quad (13A)$$

$$[Ga(EDTA)] = \frac{Ga(EDTA)]_A}{1 + K^{OH}_{ML}[H^+]} \quad (14A)$$

$$[Ga(MECAMS)] = \frac{[Ga(MECAMS)]_A}{1 + K^{H}_{ML}[H^+] + K^{H}_{ML}K^{H}_{MHL}[H^+]^2} \quad (15A)$$

Substitution of these quantities, and $[Fe(MECAMS)^{6-}]$ from eq 7A, into eq 11 generates a value for K_{comp}. Since K_{comp} is an equilibrium constant, it can be rewritten in terms of other equilibrium constants. For this example, it is advantageous to choose

$$K_{comp} = \frac{\dfrac{[Ga(MECAMS)^{6-}]}{[Ga^{3+}][MECAMS^{9-}]}\dfrac{[Fe(EDTA)^{-}]}{[Fe^{3+}][EDTA^{4-}]}}{\dfrac{[Ga[EDTA)^{-}]}{[Ga^{3+}][EDTA^{4-}]}\dfrac{[Fe(MECAMS)^{6-}]}{[Fe^{3+}][MECAMS^{9-}]}} = \frac{\beta_{110}^{Ga(MECAMS)}\beta_{110}^{Fe(EDTA)}}{\beta_{110}^{Ga(EDTA)}\beta_{110}^{Fe(MECAMS)}} \quad (16A)$$

Rearrangement of eq 16A, using the definition

$$\beta_{110}^{ML} = \frac{[ML^{6-}]}{[M^{3+}][L^{9-}]}$$

yields the formation constant of $[Ga(MECAMS)^{6-}]$:

$$\beta_{110}^{Ga(MECAMS)} = \frac{\beta_{110}^{Ga(EDTA)}\beta_{110}^{Fe(MECAMS)}K_{comp}}{\beta_{110}^{Fe(EDTA)}}$$

Likewise, K^* may be obtained by replacing with

$$K^* = \frac{[ML^{6-}][H^+]^3}{[M^{3+}][H_3L^{6-}]}$$

Registry No. Ga, 7440-55-3; In, 7440-74-6; MECAMS, 71353-06-5; 3,4-LICAMS, 71659-79-5; DMBS, 73487-23-7; Cu, 7440-50-8.