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Carbon-13 Fourier Transform Nuclear Magnetic Resonance Study of Gallium Citrate in Aqueous Solution[1]

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Abstract: As a basis for understanding the molecular mechanism of gallium localization in tumor cells, the aqueous chemistry of gallium citrate in D_2O at 31 °C was investigated by ^{13}C FT-NMR spectroscopy (22.63 MHz). Complexes of the form Ga_n-$(citrate)_{2n}$ were detected at neutral or mildly acidic pD_c's when the molar concentration of citrate was equal to or greater than the molar concentration of added gallium. In this complex, chemical exchange of bound citrate with free citrate is slow on the ^{13}C NMR time scale. Line broadening of the citrate resonances as well as decreases in their apparent ^{13}C spin-lattice relaxation times (T_1) indicated formation of gallium citrate polymers in moderately acidic solutions at equal concentrations of gallium and citrate and in neutral solutions when the gallium/citrate concentration ratio was larger than 0.50. The "average diameter" of the polymer, estimated from the line width of the broad citrate CH_2 resonance of gallium/citrate 1 M/1 M, was 50 Å at pD_c 7.40 and 40 Å at pD_c 2.75. The properties of gallium citrate complexes have been compared with those of ferric citrate.

As part of a program to define the molecular mechanism of action of gallium tumor scanning agents,[3] this laboratory has been investigating the biological properties and aqueous chemistry of various gallium salts.[4] The citrate salt is particularly important because gallium radioisotopes are clinically administered as citrate buffered solutions, because the extent of gallium localization in certain model tumor systems depends on the citrate concentration,[4a-e] and because citrate may play a significant role in serum transport of this metal. In a recent study of gallium citrate in D_2O solution by ^1H and ^{71}Ga NMR,[4f] this laboratory demonstrated that the type of complex formed depends on both the pD_c (pH meter reading +0.40) of the solution and on the relative concentration of the metal and ligand. A 1/1 gallium citrate complex (GaCit), gallium citrate polymers, and a low molecular weight gallium citrate complex of undetermined stoichiometry were detected under various experimental conditions. The present ^{13}C FT-NMR study was undertaken to further characterize some of the gallium citrate complexes detected in the previous study.[4f] By this method it was possible to confirm the observations made in the previous study, to determine the stoichiometry of the previously uncharacterized low molecular weight complex, and to estimate the average dimensions of the gallium citrate polymers. Taken together with the previous ^1H and ^{71}Ga NMR study these investigations demonstrate some striking similarities between the aqueous chemistry of citrate complexes of gallium(III) and iron(III).

Experimental Section

Materials. $Ga(NO_3)_3 \cdot 9H_2O$ (Alfa Inorganic, Beverly, Mass.), trisodium citrate monohydrate (Fisher Scientific Co., Fair Lawn, N.J., certified reagent), citric acid monohydrate (Matheson Coleman and Bell, East Rutherford, N.J., A.C.S. reagent), and D_2O (Aldrich Chemical Co., Milwaukee, Wis.) were used as commercially supplied. In D_2O solution the pD_c was adjusted with DCl (Stohler Isotope Chemicals, Azusa, Calif.) and NaOD (Stohler Isotope Chemicals, Azusa, Calif.). Care was taken to remove paramagnetic impurities by soaking all glassware in EDTA solution before use.

NMR Spectra. Natural abundance ^{13}C FT-NMR spectra (4K transforms) were measured at 22.63 MHz with a Bruker HX-90-18 spectrometer system (single coil) equipped with a NIC-1085 data system (Nicolet Instrument Corp., Madison, Wis.). Field-frequency lock was established using the deuterium resonance of D_2O. In some experiments a Bruker B-SV2 pulse amplifier (90° pulse width 8-10 μs) was employed; other experiments were performed with an ENI 3100L broad band amplifier (Electronic Navigation Industries, Rochester, N.Y.; 90° pulse width about 30 μs). In order to ensure equilibration of spins in all FT-NMR and nuclear Overhauser effect (NOE) experiments, the recycling time between scans was at least five times the longest citrate methylene T_1. NOE's were measured by comparing peak intensities obtained with continuous ^1H decoupling and intensities obtained with ^1H decoupling gated on only during data acquisition. T_1 values were determined by means of a (180°-τ-90°-t) pulse sequence,[5] where τ is a variable delay time and t is at least four times the longest T_1 of the citrate methylene resonances. Unless otherwise specified, all spectra were measured at ambient probe temperature (31 °C) with broad band ^1H decoupling and air cooling.

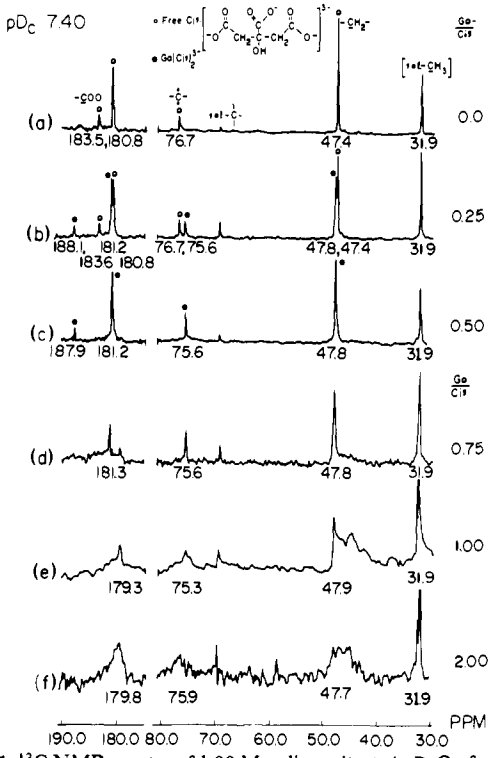

Figure 1. ^{13}C NMR spectra of 1.00 M sodium citrate in D_2O after addition of (a) 0, (b) 0.25, (c) 0.50, (d) 0.75, (e) 1.00, and (f) 2.00 equiv of gallium nitrate at neutral pD_c. (*t*-Butyl alcohol was used as a secondary standard.) (No. of scans: (a) 256, (b) 1K, (c) 1K, (d) 512, (e) 750, (f) 1K).

Table I. Methylene ^{13}C Spin-Lattice Relaxation Times (22.63 MHz) for Various Proportions of Gallium Nitrate to Trisodium Citrate (1.00 M) at pD_c 7.40 ± 0.30 at 31 °C

Ga/citrate (pD_c 7.40)	T_1, s Free $-CH_2-$	T_1, s $Ga(Cit)_2$ $-CH_2-$	T_1, s Polymer CH_2
0.00	0.409 ± 0.009	...	...
0.25	0.355 ± 0.011	0.264 ± 0.008	...
0.50	... [a]	0.186 ± 0.005	...
0.75	... [b]	0.172 ± 0.007 [b]	- - - [c]
1.00	...	0.149 ± 0.005 [b]	0.057 ± 0.010 [d]
2.00	...	0.144 ± 0.007 [b]	- - -

[a] (...) indicates absence of the corresponding CH_2 resonance. [b] CH_2 resonances of $Ga(Cit)_2$ and the polymer partially overlap; the reported T_1 is that of the sharp resonance in this complicated pattern. [c] (- - -) indicates that the CH_2 resonance was either too weak or too broad to measure. [d] See ref 7.

Samples were contained in 10 mm o.d. tubes. An external standard, *tert*-butyl alcohol (30% in D_2O), was enclosed in a 4 mm o.d. coaxial tube. Chemical shifts were corrected to internal TMS (31.9 ppm to high field of the methyl resonance of external *tert*-butyl alcohol). FT-NMR spectra and T_1 values were also measured at 67.89 MHz on a Bruker HX-270 spectrometer (Florida State University).

Results

Gallium Citrate in Neutral Solution. Carbon-13 NMR spectra of 1.00 M citrate solutions (pD_c 7.4) containing various proportions of added $Ga(NO_3)_3$ were measured (Figure 1) in order to determine the previously undetermined stoichiometry of the low molecular weight Ga citrate complex in neutral aqueous (D_2O) solution. Spectral assignments of free citrate carbons (Figure 1a) are those of Johnson and Jankowski.[6] The two equivalent methylene carbons yield the resonance at δ 47.4 ppm. The tertiary carbon resonance is at δ 76.7 ppm. Two equivalent carboxylic carbonyls are associated with the resonance at δ 180.8 ppm; the lowest field resonance is assigned to the carbonyl of the unique central carboxyl group. When 0.25 equiv of gallium are added, superposition of the spectrum of free citrate and the spectrum of the gallium complex is observed, with the citrate resonances slightly displaced from corresponding resonances of the free counterion (Figure 1b). Corresponding resonances of free and complexed citrate have equal intensity within experimental error suggesting that two citrate ligands are associated with each gallium atom. The end point of the titration occurs when 0.50 equiv of gallium have been added (Figure 1c). Only the spectrum of gallium dicitrate ($Ga(Cit)_2$) is then observed. As excess gallium is added, the sharp resonances are replaced by a set of broadened citrate resonances (Figure 1d-f).

Table I summarizes the T_1's of the citrate CH_2 at neutral pD_c for various gallium/citrate ratios.[7] The broadened citrate CH_2 peak (Ga/citrate = 1.00, 2.00) has a substantially shorter apparent T_1 (50-60 ms) than the T_1 of the corresponding resonance of either free citrate (355-409 ms) or $Ga(Cit)_2$ (186-264 ms).

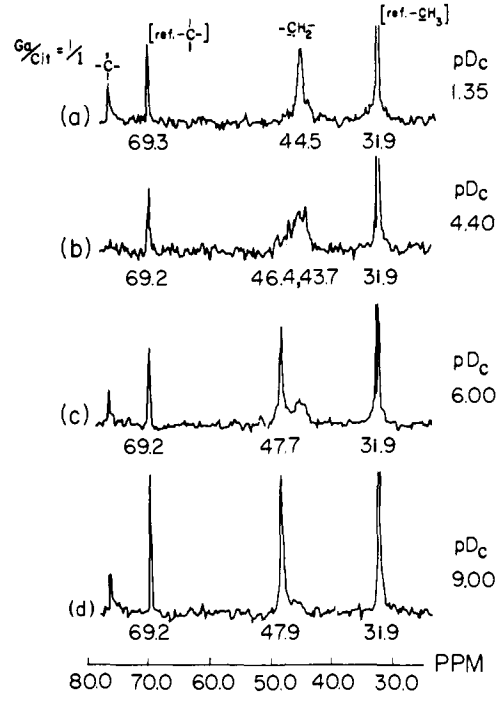

Figure 2. ^{13}C NMR spectra of 1.00 M gallium citrate at various pD_c's. (No. of scans = 512.)

Table II. Citrate Methylene ^{13}C Spin-Lattice Relaxation Times (22.63 MHz) of Ga/citrate = 1 M/1 M Solutions at 31 °C

pD_c	T_1, s		$1/\eta T_1$, $cP^{-1}\ s^{-1}$ [b]	
<0.4	0.229		1.59	
0.50	0.211		1.74	
1.10	0.091		4.40	
1.55	0.083		4.97	
2.25	0.083		5.16	
2.75	0.071		6.16	
3.10	0.093		4.76	
3.80	0.092		4.89	
4.60	0.117		3.86	
5.30	0.106		4.23	
7.20	0.149	0.059 [a]	2.80	7.06 [a]
7.70	0.157	0.054 [a]	2.59	7.53 [a]
8.60	0.217		1.77	

[a] Apparent T_1 of the citrate $-CH_2-$ resonance of the broad peak. See ref 7. [b] Viscosities were obtained from ref 4f.

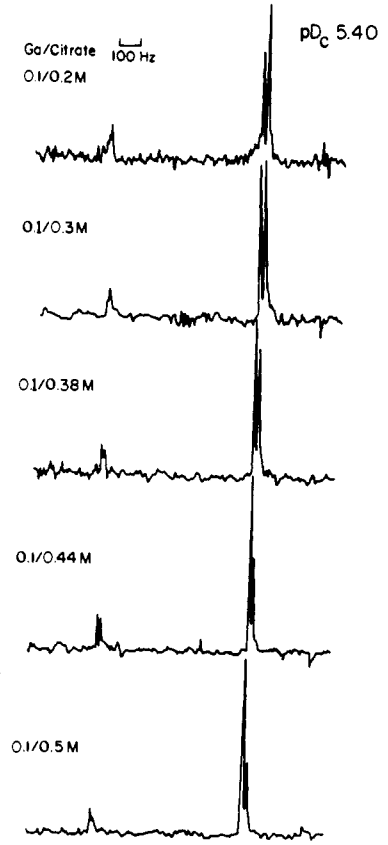

Figure 3. ^{13}C NMR spectra of 0.1 M gallium nitrate in D_2O after addition of: (a) 0.2, (b) 0.3, (c) 0.38, (d) 0.44, (e) 0.5 equiv of sodium citrate at pD_c 5.40. (No. of scans = 2K.)

Effect of pD on Gallium and Citrate Complexes. Carbon-13 NMR spectra of gallium citrate (molar ratio 1/1) at various pD_c's appear in Figure 2. At pD_c 1.35 the citrate resonances accidently coincide with those of free citrate ion. At pD_c 4.40 the citrate resonances broaden considerably, reflecting the formation of gallium citrate polymers[4f] (vide infra). At pD_c 6.00 the citrate methylene resonance consists of a broad peak roughly coincident with the broad CH_2 resonance observed at pD_c 4.40 and a sharper resonance with a chemical shift (δ 47.9 ppm) similar to the methylene peak of $Ga(Cit)_2$ in neutral solution (δ 47.9 ppm; Figure 1). At pD_c 9.00 the polymer CH_2 resonance is barely visible as a broad shoulder on the high field side of the δ 47.9 ppm peak.

The T_1's of citrate CH_2 resonances at various pD_c's at a 1/1 molar ratio of gallium to citrate are summarized in Table II. The corresponding relaxation rates corrected for viscosity changes appear in the right hand column of this table. Values obtained from the broadened resonances observed near neutral pD_c are listed separately in Table II.[7] Starting at the acidic end of this titration, the relaxation rate monotonically increases, reaches a local maximum at pD_c 2.75, and decreases monotonically toward basic solution. The broadened resonances which occur together with the sharper resonances of the $Ga(Cit)_2$ complex near neutral pD_c exhibit apparent relaxation rates somewhat greater than the relaxation rate of the broad citrate resonance at pD_c 2.75.

Effect of the Ga/Citrate Ratio on Gallium Citrate Complexes. At relatively low gallium citrate concentration ratios in moderately acidic solution (e.g., pD_c 5.40) two sharp citrate CH_2 resonances are observed (Figure 3). The relative intensities of these peaks vary with the gallium citrate concentration ratio. The lower field resonance at δ 47.0 ppm coincides with the CH_2 peak of free citrate at this pD_c, while the higher field peak originates from nonpolymeric gallium citrate complexes. A low concentration of gallium (0.1 M) was chosen in order to maximize the relative range of citrate concentrations over which the equilibrium between free citrate and nonpolymeric gallium citrate complexes could be investigated. At this gallium concentration, polymer formation, as indicated by appearance of broadened resonances, occurs at citrate concentrations below 0.2 M. At these citrate concentrations no free citrate peak is observed. Precipitation occurs at citrate concentrations approaching 0.8 M. Consequently, the stoichiometry of nonpolymeric gallium citrate complexes can be investigated over a range of citrate concentrations of about 0.2–0.8 (i.e., a fourfold variation in citrate concentration). This range is greatly diminished at higher gallium concentrations because of the relatively low solubility of the nonpolymeric gallium. Precipitation is already observed with 2.0 M citrate. At this gallium concentration and 1.0 M citrate no free citrate is detected. Thus, the useful range of citrate concentrations is no more than 1.0 to 2.0 M citrate and may be significantly less than this (i.e., at this gallium concentration the citrate concentration can be varied by less than a factor of 2). For these reasons the experiments shown in Figure 3 were performed with 0.1 M Ga. Still lower concentrations of gallium were avoided because they would have required excessive signal averaging.

NOE Measurements. The NOE's measured at 22.63 MHz are reported as $(I - I_0)/I_0$, where I and I_0 are the intensities of the citrate CH_2 resonance with and without broad band ^1H decoupling. These are: 2.0 ± 0.1 for free citrate at pD_c 2.75; 1.9 ± 0.1 for $Ga(NO_3)_3/Na_3Cit$ = 1 M/1 M in D_2O at pD_c 0.8; 2.0 ± 0.1 for $Ga(NO_3)_3/Na_3Cit$ = 0.5 M/1 M at pD_c 7.4; and 1.0 ± 0.1 for $Ga(NO_3)_3/Na_3Cit$ = 1 M/1 M at pD_c 2.75. The apparent NOE for the envelope of overlapping CH_2 resonances observed at pD_c 7.4 is 1.6 ± 0.2 ($Ga(NO_3)_3/Na_3Cit$ = 1 M/1 M). The observation of a full NOE (2.0) for the free citrate confirms the absence of any paramagnetic impurities.

Discussion

pD_c Dependence of Gallium Citrate Complexes. The present study confirms and extends the conclusions previously drawn from ^1H and ^{71}Ga NMR about the effect of pD_c and concentration on the nature of gallium citrate complexes.[4f] Titration of gallium nitrate with citrate in acidic solution (pD_c ~1) yielded a distinct end point at one equivalent of the anion as monitored by ^{71}Ga NMR, indicating formation of a 1/1 gallium/citrate complex (GaCit). The citrate ^{13}C resonances observed in Figure 2a are attributed to this species. Unfortunately, the accidental degeneracy of the chemical shifts of spectrum of free and bound citrate made it difficult to accurately determine the stoichiometry of this complex.[4f] Analysis of the ^{13}C spectrum is considerably simpler because proton decoupling eliminates the AB spin coupling pattern which obscured the ^1H NMR spectrum.

Stoichiometry of Nonpolymeric Gallium Citrate Complexes. The observation of two sets of resonances in the citrate spectrum of the nonpolymeric species at each gallium concentration (vide infra) indicates that the chemical exchange between free and bound citrate is slow on the ^{13}C NMR time scale. This slow exchange permits the measurement of the relative concentrations of both the bound and free citrate from their relative intensities in each spectrum.

(1) Mildly Acidic Solution. In mildly acidic solution the stoichiometry of nonpolymeric gallium citrate complexes is difficult to determine (see Figure 3). A Scatchard plot of these data is shown in Figure 4a. A straight line is predicted if the metal has equivalent and independent sites for the citrate.[8] Under these conditions, the value of the intercept on the abscissa is equal to the maximum number of citrate ligands on the metal. This value, 2.0, is consistent with the 1/2 gallium citrate complex being the predominant species. The slope of

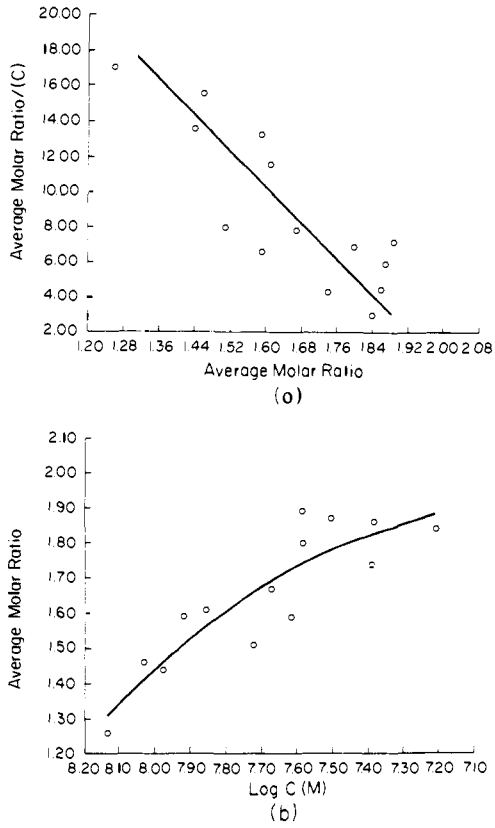

Figure 4. (a) A Scatchard plot of citrate ions binding to gallium at 31 °C and pD_c 5.40. The abscissa is the average molar ratio of bound citrate to gallium. The ordinate is the average molar ratio of bound citrate to gallium divided by the free citrate concentration. (b) A plot of the average molar ratio of bound citrate to gallium against log of the free citrate ion concentration (same data as (a)). The solid line is generated from Fletcher's nonlinear regression program.

this plot yields an apparent intrinsic stability constant of 25 ± 1 M^{-1}. This value is not a true thermodynamic stability constant because no attempt has been made to analyze the complex equilibrium involving the various states of ionization of the ligand, the metal, and the gallium citrate complexes. That the simple Scatchard model appears to fit at all may in part be a consequence of the considerable scatter of data points. Experimental error was introduced by overlap of resonances of free and bound citrate and uncertainties in the base line. Furthermore, for this simple Scatchard model to fit the data, it must be presumed that all the ionic species of citrate which bind to the metal have approximately equal intrinsic stability constants.

The data were also fit by a generalized Scatchard model developed by Fletcher et al.[9] (Figure 4b). This model requires only that the binding of ligands occur in a stepwise fashion: each step need not be independent, nor need the intrinsic stabilities of complexes formed be equal. This analysis also indicates a 1/2 Ga citrate complex with apparent stability constants $K_1 = 48 \pm 1\ M^{-1}$ and $K_2 = 13 \pm 1\ M^{-1}$. However, the results are all consistent with the formula $Ga_n(Cit)_{2n}$ (where $n = 1, 2, 3, \ldots$, etc.).

(2) Neutral Solutions. The titration shown in Figure 1 clearly indicates formation of a complex with a citrate/gallium ratio of 2.0 (i.e., $Ga_n(Cit)_{2n}$, where n is a small integer). The index n cannot be very large because rather narrow lines are observed for the complex and because the CH_2 T_1 of this complex (Table I) is comparable to, although somewhat smaller than, the T_1 of free citrate (presumable monomeric citrate) and because a maximal NOE (2.0) is observed for the complex. The preferred stoichiometry is probably 1/2 (i.e., $n = 1$). This conclusion is suggested by examination of space filling CPK models, which indicate that, whereas plausible $Ga(Cit)_2$ complexes can be constructed, higher order $Ga_n(Cit)_{2n}$ complexes appear unlikely. The decrease in the T_1 of the CH_2 peaks of free citrate and complexed citrate with increasing gallium concentration (Table I) may result from an increase in the viscosity of the solution.

Gallium Citrate Polymers. (1) Evidence for Polymer Formation. Broadening of ^{13}C NMR spectra of gallium citrate, reflecting a decrease in T_2, occurs in moderately acidic solution for 1/1 gallium citrate (Figure 2) and in neutral solution at gallium citrate ratios greater than 0.50 (Figure 1). A significant decrease in T_1 accompanies broadening of the ^{13}C resonances (Table II) and ^1H NMR spectra.[4f] These effects could, in general, result from a number of causes: (1) dipolar interactions involving long rotational correlation times, (2) intermediate rates of chemical exchange, and (3) relaxation involving scalar coupling of the magnetic dipoles of ^{13}C (or ^1H) and the quadrupolar isotopes ^{69}Ga and ^{71}Ga (whose natural abundances are 60.2 and 39.8%, respectively). Equilibrium dialysis studies of gallium citrate clearly demonstrate the formation of polymers under conditions which yield broad resonances and short T_1's.[4f] Consequently, dipolar broadening must be a significant, if not the predominant, cause of these effects. Exchange broadening appears unlikely because the spectral line widths are essentially temperature invariant. Changes in line widths at elevated temperature would have been expected if chemical exchange were the cause of broadening of the NMR spectra. The similarity of spectra obtained at 22.63 and 67.89 MHz further argues against exchange broadening.[10] A three- to fourfold increase in the apparent T_1 was observed at the higher field (pD_c 2.75), again supporting the formation of large polymers with long rotational correlation times.[11] In view of the observation that 1/1 and 1/2 gallium citrate complexes both yield the maximal NOE expected for dipolar ^1H-^{13}C interactions, a quadrupolar mechanism can be excluded for these complexes and therefore appears unlikely for gallium citrate polymers. Scalar coupling between the CH_2 group and the gallium dipole would be required for a quadrupolar relaxation mechanism. This appears unlikely, because the complexes are expected to be predominantly ionic. Further evidence against quadrupolar broadening is the observation that ^{69}Ga and ^{71}Ga NMR resonances of gallium citrate complexes are too broad to be detected. This indicates that the relaxation rate of the metal nucleus in the complex is very rapid and its magnetic dipole is unlikely to couple to the ^{13}C dipole. Consequently, dipole-dipole interactions associated with formation of large gallium citrate polymers appear to be the most likely cause of broadening of NMR resonances and shortening of their T_1's. This conclusion is consistent with the general observation that large molecules, even those containing covalently bound quadrupolar nuclei such as ^{14}N, generally relax by a predominantly dipolar mechanism[12a] unless the nucleus in question is directly bonded to nitrogen and lacks directly bonded hydrogens.[12b]

Formation of polymers when hydroxide ions are added to gallium salt solutions has been demonstrated by potentiometric titrations,[13a,14] polarography,[13b] isopiestic measurements,[13c] and light scattering.[13d,e] Polymerization of gallium perchlorate has a similar pH profile to that of gallium citrate polymerization.[4f,13e] Light-scattering studies by Craig and Tyree[13e] indicate that gallium perchlorate polymers reach a maximum weight average molecular weight of about 6670 at an OH/gallium ratio of 1.75 (pH 2.20 for 0.01 M gallium) and dissociate in more acidic or more basic solution.

Crude estimates of the average dimensions of the polymer were obtained from effective rotational correlation times (τ_{eff}) estimated from line widths of the broad CH_2 resonances of 1/1 gallium citrate ($\tau_{eff} = 3.4 \times 10^{-8}$ and 1.7×10^{-8} s at pD_c 7.40

and 2.75, respectively). For polymers with such long correlation times, negligibly small NOE's would be expected.[11] The substantial NOE (1.6 ± 0.2 and 1.0 ± 0.1 at pD_c 7.40 and 2.75, respectively) is attributed to rapid exchange between the polymer and lower molecular weight species. Assuming that the polymers are hard spheres with microviscosities equal to the bulk solution viscosity (Table II), we obtain from the Stokes-Einstein equation[15] average diameters of 50 and 40 Å at pD_c 7.40 and 2.75, respectively.

Comparison of Gallium and Ferric Citrates. Chelation by low molecular weight ligands plays a key role in the regulation and control of the metabolism of both ferric iron and gallium and in the transport of these metals across cellular membranes.[16c,4a-e] Citrate has been reported to be the principal low molecular weight serum binder of ferric iron;[17] this anion probably also plays a key role in serum transport of gallium.[4a,c,e,f] Because of the similar charge, size, and chemical properties of gallium(III) and iron(III), it is interesting to compare the structures of their citrate complexes. There is general agreement that in acidic solution the ferric *aquo ion* reacts with citrate to form a 1/1 complex.[16a,b,18] Early reports of 2/3 and 3/2 ferric citrate complexes[19] appear to be based on experimental artifacts.[18] Gallium-71 NMR experiments in this laboratory[4f] have demonstrated that gallium participates in a similar reaction, which may be summarized as

$$M^{3+} + H_4Cit \rightleftarrows MH_{4-x}Cit^{3-x} + xH^+ \qquad (1)$$

where M^{3+} is the metal aquo ion, H_4Cit is citric acid, and its four protons refer to the three carboxyl protons and the OH proton. Lanford and Quinan[18a] favor a value of $x = 2$, but this conclusion might be in error for reasons discussed by Warner and Weber.[18b] On the basis of potentiometric and spectrophotometric titration data these authors favor formation of $FeCit^-$ (i.e., $x = 4$ in eq 1). Evidence cited in support of this conclusion includes: (1) liberation of four titratable protons by formation of the ferric citrate complex,[16a,18b] and (2) the behavior of the ferric citrate complex as an anion on ion exchange resins (even at a pH of 2) and in electrophoresis experiments.[18b] Also consistent with these observations is the conclusion of Timberlake,[18c] based on iron(III)-iron(II) potentiometry, that the preferred species is $Fe_2Cit_2^{2-}$. The possibility also exists that the fourth proton liberated per citrate ligand originated from hydrolysis, i.e. from formation of species such as $Fe(OH)HCit^-$ or its dimer. Warner and Weber[18b] and Spiro,[16a] however, note that the absence of characteristic absorption bands associated with FeOH groups argues against such structures. Evidence favoring ionization of the citrate hydroxyl is, however, also suspect since interpretation of pH and UV titration data is complicated by the presence of polynuclear complexes and the $FeCit_2$ complex (vide infra).

The properties of 1/1 gallium citrate complexes closely parallel those of their ferric analogue. Thus, titration of a 1/1 mixture of $Ga(NO_3)_3$ and H_4Cit with NaOH yields an end point near neutral pH with about 4.3 titratable protons,[14] suggesting formation either of $GaCit^-$, a partially hydrolyzed complex such as $Ga(OH)HCit^-$, or oligomers of these species. Harris and Martel[14] analyzed their titration data in terms of the partially hydrolyzed species. However, interpretation of these titrations is complicated by competitive equilibria involving polymers and gallium dicitrate. Support for anionic or perhaps for neutral complexes is provided by the observation by Blanco and Perkinson[20] that gallium citrate complexes are not absorbed on Dowex-50 cation exchange resins (H^+ form) at pH values above about 3. Gallet and Paris[21] have detected the formation of various gallium citrate complexes using differential thermal analysis. However, these authors were not able to unambiguously determine the structures of these gallium citrate complexes. Thus, while the low molecular weight complexes of gallium citrate and ferric citrate appear to be very similar, definitive determination of their structure, particularly with respect to the nature of any hydroxy ligands, remains to be achieved.

Addition of base to aqueous solutions of 1/1 gallium/citrate or ferric/citrate complexes leads to formation of polymers. The remarkably stable ferric citrate polymers have been extensively characterized by numerous methods.[16a,b] This polymer has been demonstrated to be spherical with a diameter of 72 ± 9 Å and a molecular weight of 210 000 ± 10 000. The spheres contain an inner core of iron atoms linked by oxy and hydroxy bridges. The citrate counterions on the surface of the sphere serve to solubilize the spherical polymer. An essentially isomorphous structure appears likely for the gallium citrate polymer, whose dimensions appear roughly comparable to that of the iron citrate polymer if allowance is made for the contribution of low molecular weight species in estimating the average dimensions of the gallium citrate polymer. The gallium citrate polymer, however, appears to be considerably less stable, dissociating more readily in the course of equilibrium dialysis[4f] and gel filtration experiments.[22] Gallium citrate (1/1) polymers dissociate in neutral solution to form $GaCit_2$ complexes, which further dissociate in more alkaline solution to yield $Ga(OH)_4^-$.[4f] In contrast, ferric citrate (1/1) polymers are stable in basic solution until they precipitate. Spiro et al.[16a] also report formation of $Fe(Cit)_2^{5-}$ complexes in the presence of excess citrate. Similar differences with respect to stability in the presence of excess base have been noted between ferric nitrate polymers[23] and gallium perchlorate polymers[13e] and probably reflect the amphoteric nature of gallium.

It appears that just as the metabolism and transport of iron is influenced by its association into polymers and its interaction with low molecular weight chelates such as citrate, so too are the interactions of gallium with cells affected by similar molecular phenomena. However, there are important differences. Thus, there is evidence that certain ferric citrate complexes are transported across cellular membranes.[16c] Studies of gallium localization in L1210 leukemic cells suggest that the citrate salt of this metal does not penetrate membranes of these tumor cells.[4a,c,e] Further investigation of the aqueous chemistry of gallium is required to determine the molecular details of the interaction of this metal with normal and malignant cells.

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Nuclear Magnetic Resonance Studies of Metal Complexes Using Lanthanide Shift Reagents. Lanthanide-Induced Shifts in the ^1H (and ^{13}C) Spectra of Diamagnetic Metal Complexes of Quadridentate Ligands Incorporating Oxygen–Nitrogen Donor Atoms

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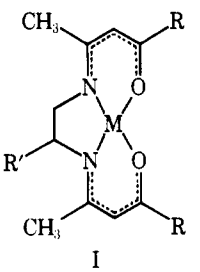
Abstract: Lanthanide shift reagents have been successfully used to induce shifts in the NMR spectra of a series of diamagnetic metal complexes of O_2N_2-donor quadridentate ligands; the ligands were prepared by condensation, in a 1:2 molar ratio, of ethylenediamine, propylenediamine, or 2,3-diaminobutane with a β-diketone. By choice of appropriate complexes it has been possible to demonstrate the influence of both steric and electronic effects on the lanthanide-induced shifts. The results indicate that 1:1 adduct formation occurs with the lanthanide ion coordinated in a bidentate fashion to the cis-oxygen donor atoms of the respective metal complexes. The technique has formed the basis for a conformational study of the nickel(II) complex of the ligand obtained by 2:1 condensation of acetylacetone and propylenediamine. The results confirm a previous observation that the backbone methyl group in this complex occupies an axial position. An NMR investigation of the interaction of an optically active shift reagent with the above complex (as well as with the rac and meso isomers of the analogue derived from 2,3-diaminobutane) indicates that the technique can be extended to the study of suitable complexes which incorporate chiral centers. Apart from their intrinsic interest, these studies also demonstrate the suitability of certain classes of inorganic coordination complexes for examination by an NMR technique which has remained primarily in the hands of organic chemists.

Lanthanide shift reagents (LSRs)[1] such as $Eu(fod)_3$ have been routinely used to simplify the NMR spectra of organic[2,3] and, to a lesser extent, organometallic[4] compounds. However, provided suitably positioned heteroatoms are present in the ligands, LSRs may also be applied to the study of coordination compounds. This has been demonstrated previously for a few isolated complexes.[5]

As part of a general investigation of the use of LSRs to study bound organic ligands in diamagnetic coordination complexes, the effects of added shift reagent on the NMR spectra of metal complexes of type I have been studied. The complexes were chosen because they are diamagnetic, soluble in $CDCl_3$, and possess two oxygen donor atoms which have lone pair electrons available for further bonding to the LSR. Further, a considerable number of different compounds of this type are readily prepared and are thus available for comparative NMR studies. We now report an investigation of the effect of LSRs on the NMR spectra of a range of such complexes.



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R	R'	Abbreviation
CH_3	H	M (Meaen)
CH_3	CH_3	M (Meapn)
C_2H_5	H	M (Etaen)
i-C_3H_7	H	M (*i*-Praen)
i-C_4H_9	H	M (*i*-Buaen)
t-C_4H_9	H	M (*t*-Buaen)
C_6H_5	H	M (Phaen)
CF_3	H	M (TFaen)