Time-Dependent Solution Speciation of the Al^{III}–Citrate System: Potentiometric and NMR Studies

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Time-dependent potentiometric and NMR spectroscopic measurements were carried out in the Al^{III}–citric acid system in an equimolar solution and at an excess of ligand in order to monitor the changes in speciation as a function of time. In fresh solutions, the mononuclear 1:1 species $[AlLH]^+$, $[AlL]$, $[AlLH_{-1}]^-$ and $[AlLH_{-2}]^{2-}$ and the 1:2 complexes $[AlL_2]^{3-}$, $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$ are formed. In agreement with earlier findings (*Inorg. Chem.* **1988**, *27*, 2565), these complexes are converted to a large extent into a thermodynamically more stable trinuclear species as the solutions age. Depending on the composition of the initially formed mononuclear species, the formation of the trinuclear species is accompanied either by the consumption or by the liberation of hydroxide ions. NMR spectroscopy was used to confirm the considerable time dependence of the species distribution. The ^1H, ^{27}Al and ^{13}C NMR spectra indicated that at equilibrium the trinuclear species $[Al_3(LH_{-1})_3(OH)]^{4-}$ predominates in the pH range 4–7. Oligomerization of the mononuclear species takes place through at least one intermediate complex. At equilibrium, the ligand-exchange reactions between the trinuclear species, the mononuclear species and free citrate are slow on the NMR time scale.

Introduction

Citrate is one of the most extensively investigated ligands for Al^{III} in aqueous solution. The reason for this is that citrate is widely found in biological systems and in natural waters. As a powerful complexing agent, citrate may solubilize the neurotoxic Al^{III}, which prefers the coordination of oxygen donor containing ligands, thus permitting the absorption of Al^{III} from the environment to the human body.[1] Since citrate occurs at about 0.1 mM in blood plasma,[2] it can also be a good low molecular mass (l.m.m.) carrier of the absorbed Al^{III} in the blood stream.

Although the Al^{III}–citrate system has previously been studied by several techniques,[3–10] there are still contradictions in the proposed equilibrium model and binding modes of the complexes. The divergence is partly due to the numerous coordination possibilities and protonation states of the donor groups (citrate contains four potential donor groups, but only three can coordinate simultaneously to the same metal ion), and partly due to the rather slow oligomerization reactions that take place in solution. An early potentiometric study by Motekaitis and Martell led to a simple model involving only the mononuclear 1:1 species $[AlLH]^+$, $[AlL]$ and $[AlLH_{-1}]^-$, both in an equimolar solution and at an excess of citrate.[6] In contrast, Gregor and Powell suggested bis(complexes) as the predominant species at a ligand excess. Besides 1:1 complexes, they proposed the formation of $[AlL_2H]^{2-}$ and $[AlL_2]^{3-}$ in slightly acidic solutions and $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$ in neutral and slightly alkaline solutions.[8] From these bis(complexes), $[AlL_2H_{-2}]^{5-}$ and $[AlL_2H_{-1}]^{4-}$ were recently isolated from slightly alkaline solutions as the first mononuclear Al^{III}–citrate complexes, and characterized by X-ray crystallography and NMR spectroscopy.[11,12] In a time-dependent potentiometric study, Öhman demonstrated that in an equimolar solution the monomeric species transform into trimeric complexes $[Al_3L_3H_{-4}]^{4-}$ and $[Al_3L_3H_{-7}]^{7-}$ in very slow processes.[10] One of these trimeric complexes was crystallized in the form of $[NH_4]_4[Al_3(LH_{-1})_3(OH)(H_2O)]$ from a neutral solution and was identified unambiguously and characterized in detail by ^1H, ^{13}C and ^{27}Al NMR spectroscopy and X-ray crystallography.[13]

Such time-dependent studies may furnish a more accurate speciation description of metal-ligand systems in biology, because thermodynamic equilibrium is never reached in biological fluids as a result of the continuous influx and efflux of the components. Thus, nonequilibrium models of metal–ligand interactions may provide more relevant information.[14]

In the present work, a time-dependent speciation study of the Al^{III}–citric acid system was made both under equimolar conditions and at an excess of citrate. The oligomerization reactions of the mononuclear species were followed by ^1H, ^{13}C and ^{27}Al NMR spectroscopy, and the formation of a trinuclear complex through intermediate species was clearly demonstrated.

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Results and Discussion

Time-Dependent Potentiometric Measurements

Potentiometric titrations indicate an overlapping stepwise dissociation of three protons from the three carboxylic groups of the ligand. The protonation constants are reported in Table 1.

Table 1. Stability constants of proton (log K)and Al^{III} complexes (log β) of citric acid at 25 °C and I = 0.2 M (KCl)

	This work	log K[a] /log β Ref.[4] [b]	Ref.[8] [c]
$K([HL]^{2-})$	5.57(2)	5.22	5.70
$K([H_2L]^{-})$	4.27(2)	4.08	4.35
$K([H_3L])$	2.87(3)	2.77	2.91
$[AlLH]^{+}$	10.18(8)	9.39	10.81
$[AlL]$	7.85(5)	7.15	8.10
$[AlLH_{-1}]^{-}$	4.27(27)	3.54	–
$[AlLH_{-2}]^{2-}$	−1.77(5)	−2.64	–
$[AlL_2H]^{2-}$	–	–	16.84
$[AlL_2]^{3-}$	12.73(31)	11.61	12.90
$[AlL_2H_{-1}]^{4-}$	7.81(19)	–	6.8
$[AlL_2H_{-2}]^{5-}$	0.4	–	−0.37
$[Al_3L_3H_{-4}]^{4-}$	16.34(8)	14.44	–
$[Al_3L_3H_{-7}]^{7-}$	–	−10.90	–
Fitting (Δ cm^3)[d]	0.0074		
No. of "batch" points[e]	78		

[a] log K values are the logarithm of the stepwise protonation constants, which are numerically equal to the negative logarithm of the acidity constants, pK_a values, of the corresponding species; ±3 s.d. values are given in parentheses. – [b] I = 0.6 M (NaCl); T = 25 °C. – [c] I = 0.1 M (KCl); T = 25 °C. – [d] Average difference between the experimental and the calculated titration curves, expressed in mL of the titrant. – [e] See Experimental Section.

In the Al^{III}–citrate system data collection runs into difficulties because of a pronounced drift in pH due to the slow complexation reactions. Öhman and Sjöberg[4] reported equilibration requiring up to 6 h in slightly acidic solutions at low Al^{III}/citrate ratios. They demonstrated that this pH drift is due to the formation of the trinuclear species $[Al_3(LH_{-1})_3(OH)]^{4-}$ during the slow oligomerization process of the mononuclear complexes.[10] Gregor and Powel minimized this drift by working at Al^{III}/citrate ratios < 1:5. The equilibrium model found at such large excesses of ligand included only the mononuclear species $[AlLH]^{+}$, $[AlL]$, $[Al(HL)L]^{2-}$, $[AlL_2]^{3-}$, $[AlL_2H_{-1}]^{4-}$ and $[Al(LH_{-1})_2]^{5-}$. In earlier work,[19] a series of titrations were carried out at Al^{III}/citric acid ratios of 1:2, 1:3, 1:4 and 1:8. Evaluation of the titration curves obtained at high ligand excesses (ratios of 1:4 and 1:8) led to a model involving only mononuclear mono- and bis(complexes). However, when the titration curves for Al^{III}/citrate ratios > 1:4 were also included in the calculation, a much better fit was obtained if, besides the mononuclear complexes, the trinuclear species $[Al_3(LH_{-1})_3(OH)]^{4-}$ was also assumed. The stability constants obtained in this way, however, cannot be regarded as fully reliable, as equilibration was not definitely attained at each titration point in the pH range 5–7. Moreover, even a slight drift in the electrode response during the very long titration process (up to 4–6 h) may cause a significant error in pH, especially near the end of the titration, when the pH increases sharply (see Figure 2).

In order to acquire an accurate speciation description of the Al^{III}–citrate system in the equilibrium state even at a small ligand excess, and to obtain qualitative information concerning the kinetic route to true thermodynamic equilibrium, the "batch method" described in the Experimental Section was used instead of the normal titration procedure. Measurements were performed at Al^{III}/citrate ratios of 1:1 and 1:3 in the pH range 2.0–8.0 (Figure 1).

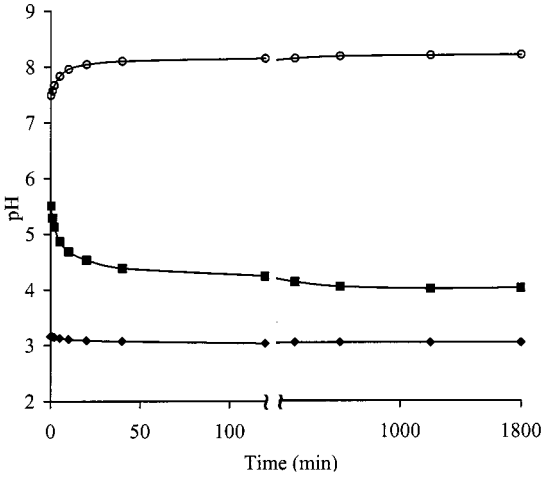


Figure 1. The change of pH as a function of time in solutions of the Al^{III}–citric acid system at a ratio 1:1, c_{Al} = 0.004 M, and at various starting pH values: (◆) 3.16, (■) 5.51, (○) 7.49

The time course of the pH changes of some selected batch solutions at different starting pH values is depicted in Figure 1. The pH changes are seen to be significant in the first 2 h; and complete equilibration can be attained in ca. 30 h. Interestingly, the direction of the pH change is dependent upon the starting pH; at pH < 6.5, the concentration of hydrogen ion increases with time, while above this pH it decreases. It has already been established[10] that this drift in pH is due to slow oligomerization reactions. Depending on the starting pH, the protonation state of the mononuclear complexes varies and their oligomerization may be accompanied by either the liberation or the consumption of protons (vide infra).

Titration curves created by using pH values relating to the same times are depicted in Figure 2. At a metal ion/ligand ratio of 1:1 the titration curves become steeper and steeper as the solution "ages". The differences between the consecutive curves are significant only in the early hours after mixing. At a metal/ligand ratio of 1:3, the differences between the titration curves are smaller than in equimolar solution, since the pH effect of the oligomerization processes is strongly buffered by the proton-dissociation reactions of the free citric acid. The 30-h titration curve represents the equilibrium state. On the basis of earlier literature reports,[4,8,10,15] the equilibrium titration data were evaluated by a speciation model including mononuclear mono- and bis(complexes) in different protonation states, i.e. $[AlLH]^{+}$, $[AlL]$, $[AlLH_{-1}]^{-}$, $[AlLH_{-2}]^{2-}$, $[AlL_2]^{3-}$,

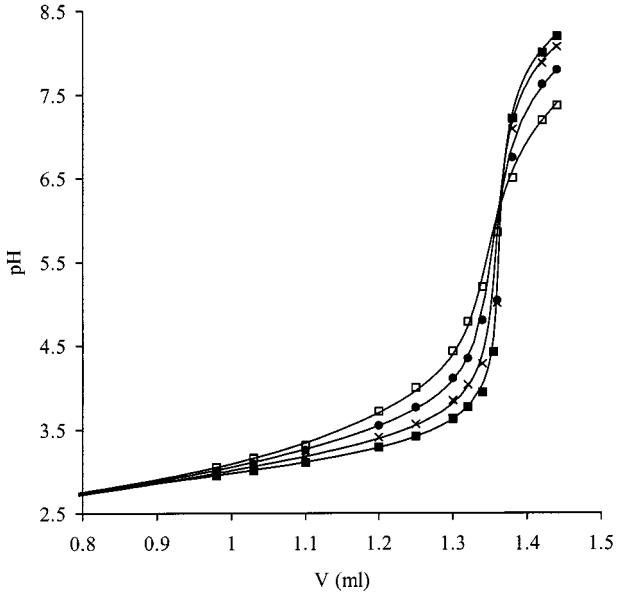


Figure 2. Titration curves (plotted as mL titrant KOH versus pH) of the Al^{III}−citric acid system at a ratio 1:1, $c_{Al} = 0.004$ M and at different times after mixing the reactants: (□) 1 min, (●) 10 min, (×) 40 min (■) at equilibrium (after 30 h)

$[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$, and the trinuclear complex $[Al_3L_3H_{-4}]^{4-}$, or more precisely $[Al_3(LH_{-1})_3(OH)]^{4-}$. The stability constants of the complexes, together with some literature data, are listed in Table 1. It can be seen from Table 1 that there is a better agreement in the stability constants of the mono(complexes) (especially the stepwise deprotonation data agree well), while there is a much higher uncertainty in the stability of bis(complexes). Our model is in reasonable good agreement with that proposed by Öhman,[4,10] except that he did not consider the species $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$, and we did not find another trinuclear complex $[Al_3L_3H_{-7}]^{7-}$ or more precisely $[Al_3(LH_{-1})_3(OH)_4]^{7-}$. This latter species, however, is not formed in a significant quantity below pH ≈ 8, the upper limit of our studies. The differences in the stability constants can be explained by the rather large differences in the experimental conditions ($I = 0.6$ M, NaCl and 0.2 M, KCl). The agreement with the stability data of the mononuclear mono- and bis(complexes) reported by Gregor and Powell[8] is much better, as they used much closer experimental conditions ($I = 0.1$ M, KCl).

In order to obtain information about the complexation route of citric acid with Al^{III}, we evaluated the titration curves relating to the same times by using the same speciation model (vide supra). Species distribution curves at different times after the mixing of the components are depicted in Figure 3 and Figure 4. We are aware that the stability data calculated from the non-equilibrium titration points are not real thermodynamic constants (for this reason we do not report their values), but they can provide a roughly qualitative picture regarding the time course of the complex formation processes. Figure 3 clearly indicates that, shortly after mixing, mononuclear complexes predominate in the system, which then transform slowly to the trinuclear complex, $[Al_3(LH_{-1})_3(OH)]^{4-}$, probably through intermediate species. The concentration of the trinuclear species increases with time at the expense of the mononuclear mono(complexes) $[AlLH_{-1}]^{-}$ and $[AlLH_{-2}]^{2-}$, and also of the bis(complexes) $[AlL_2]^{3-}$, $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$ in the case of an excess of ligand.

At pH ≈ 5, $[AlLH_{-1}]^{-}$, the predominating mononuclear complex, is formed in a fairly fast reaction and is in equilibrium with the trinuclear species. The pH of the solution decreases with time (see Figure 1), due to the slow equilibrium of Equation (1).

$$3\,[AlLH_{-1}]^{-} + H_2O \rightleftarrows [Al_3(LH_{-1})_3(OH)]^{4-} + H^{+} \quad (1)$$

At a ligand excess, bis(complexes) like $[AlL_2]^{3-}$ and $[AlL_2H_{-1}]^{4-}$ are also present and are also involved in the oligomerization processes. Several of the possible reactions are given in Equation (2), Equation (3) and Equation (4).

$$[AlL_2]^{3-} + 2\,[AlLH_{-1}]^{-} + H_2O \rightleftarrows [Al_3(LH_{-1})_3(OH)]^{4-} + HL^{2-} + H^{+} \quad (2)$$

$$[AlL(LH_{-1})]^{4-} + 2\,[AlLH_{-1}]^{-} + H_2O \rightleftarrows [Al_3(LH_{-1})_3(OH)]^{4-} + HL^{2-} \quad (3)$$

$$[AlL_2]^{3-} + [AlL(LH_{-1})]^{4-} + [AlLH_{-1}]^{-} + H_2O \rightleftarrows [Al_3(LH_{-1})_3(OH)]^{4-} + 2\,HL^{2-} \quad (4)$$

When the starting pH is ca. 7, the predominant mononuclear species is $[AlLH_{-2}]^{2-}$, and the pH increases with time according to the equilibrium of Equation (5).

$$3\,[Al(LH_{-1})(OH)]^{2-} \rightleftarrows [Al_3(LH_{-1})_3(OH)]^{4-} + 2\,OH^{-} \quad (5)$$

At a ligand excess the transformations of the species $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$ should also be considered. As an illustration some of the possible oligomerization reactions are given in Equation (6) and Equation (7).

$$[AlL(LH_{-1})]^{4-} + 2\,[Al(LH_{-1})(OH)]^{2-} \rightleftarrows [Al_3(LH_{-1})_3(OH)]^{4-} + L^{3-} + OH^{-} \quad (6)$$

$$[Al(LH_{-1})_2]^{5-} + 2\,[Al(LH_{-1})(OH)]^{2-} + H_2O \rightleftarrows [Al_3(LH_{-1})_3(OH)]^{4-} + L^{3-} + 2\,OH^{-} \quad (7)$$

It must be mentioned here that these equilibria are written in the simplest way. During the oligomerization of the mononuclear complexes, one or more intermediate species are formed, as can be demonstrated by NMR spectroscopy (vide infra).

At pH ≈ 3, there is only a slight pH decrease with time. At this pH, the species [AlL] predominates in the fresh solution and only a small amount is transformed to the trinuclear complex at equilibrium. Thus, the changes in pH are not significant (see Figure 1).

NMR Spectroscopic Measurements in Equimolar Al^{III}−Citrate Solutions

^{1}H, ^{13}C and ^{27}Al NMR spectroscopic measurements were carried out to support the potentiometric results. The spectrum recorded at pH ≈ 2 in a 0.04 M solution (Figure 5a) reveals the AB quadruplet of the free citrate (×) at δ = 3.06, 3.03, 2.89 and 2.86 ($J_{AB} = 15.7$ Hz), two broad signals at δ = 2.95 and 2.92 and two sharper signals at δ = 2.83

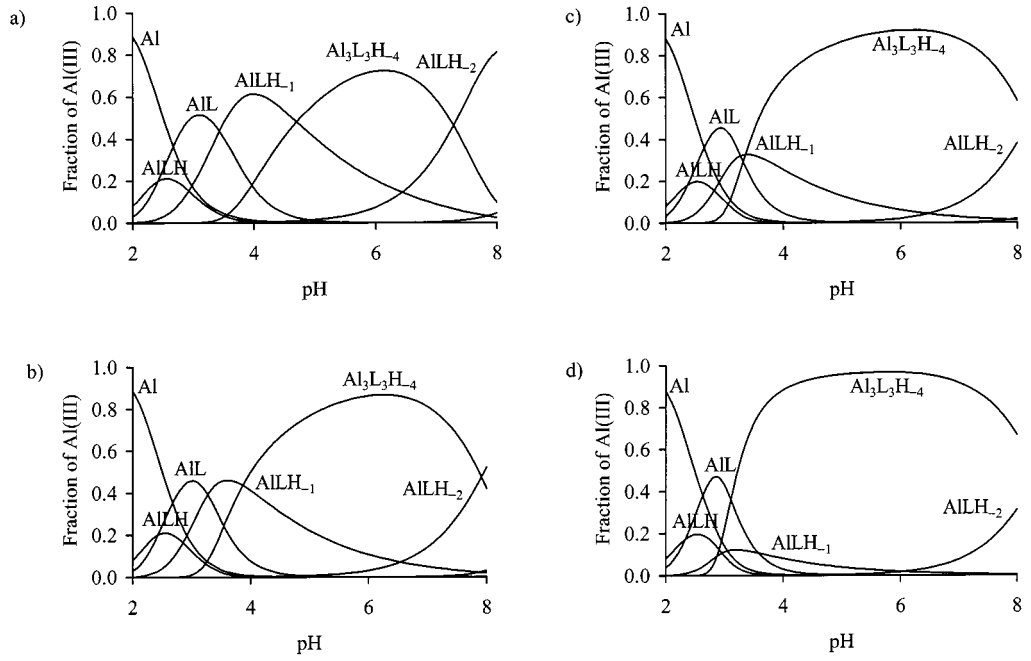


Figure 3. Species distribution curves as a function of pH in the Al^{III}–citric acid system at a ratio 1:1, c_{Al} = 0.004 M and at different times after sample preparation: (a) 1 min, (b) 10 min, (c) 40 min (d) at equilibrium (after 30 h)

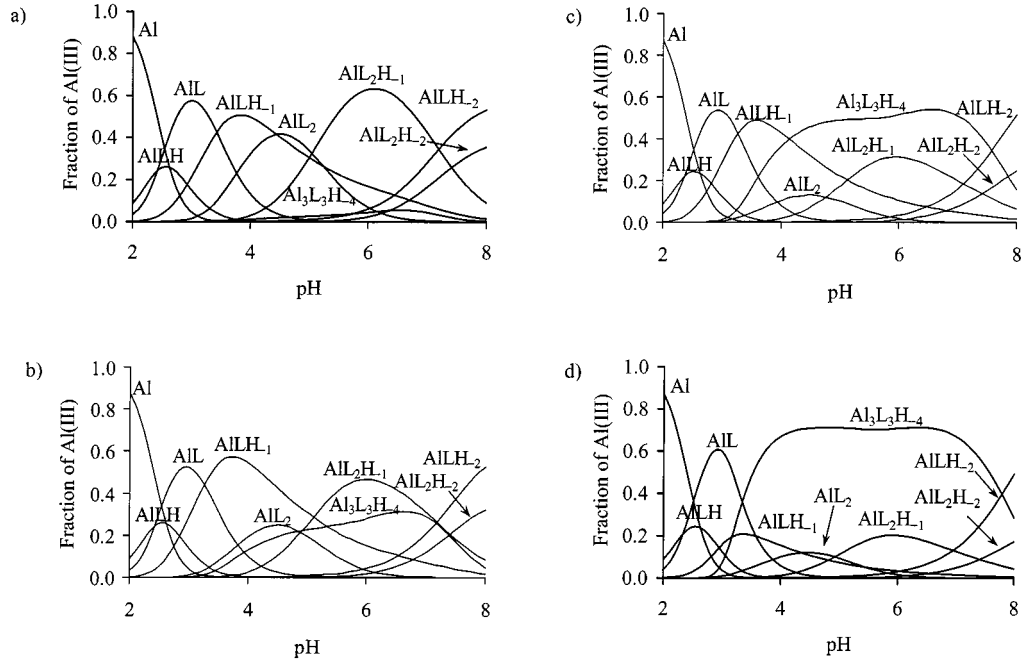


Figure 4. Species distribution curves as a function of pH in the Al^{III}–citric acid system at a ratio 1:3, c_{Al} = 0.004 M and at different times after sample preparation: (a) 1 min, (b) 10 min, (c) 40 min (d) at equilibrium (after 30 h)

and 2.80 which seem to be part of another AB quadruplet. Proton-proton correlation measurements confirm that these four signals form an AB quadruplet (•) with a coupling constant of 17.7 Hz. Potentiometry suggests that these signals belong to the mononuclear species $[AlLH]^+$.

On increasing of the pH to ca. 2.5, the ^1H NMR spectrum (Figure 5b) seems to be more complicated than expected from the speciation curves (Figure 3). From the signals appearing in the spectrum, the AB quadruplet of free citrate can unambiguously be assigned to δ = 3.03, 3.00, 2.87 and 2.84 (×), which is shifted upfield due to the deprotonation of one of the carboxyl groups. The AB quadruplet observed at pH ≈ 2 (•) is also shifted upfield. This indicates the equilibrium of two species, resulting in an average signal due to the fast proton exchange reactions between them. These two mononuclear species are probably $[AlLH]^+$ and [AlL]. The appearance of some low-intensity signals in the spectrum indicates that at 0.04 M, the oligomerization processes have already started at pH ≈ 2.5, while in more dilute solutions (e.g. at 0.004 M, under the conditions of our po-

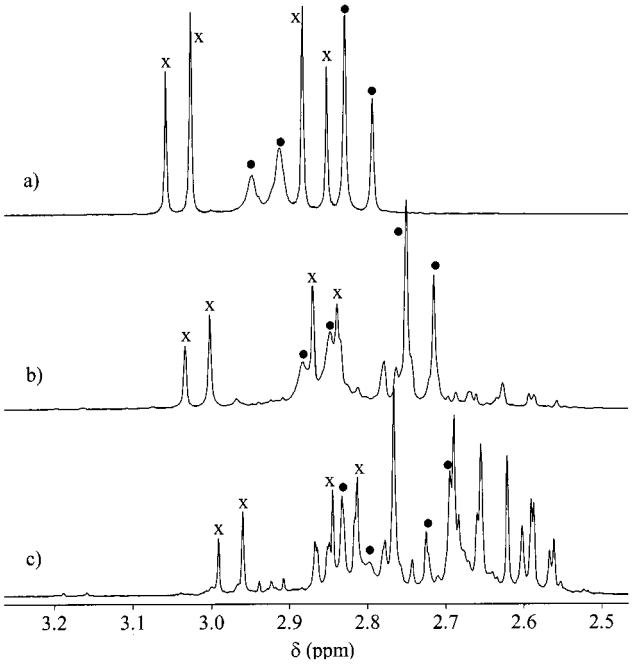
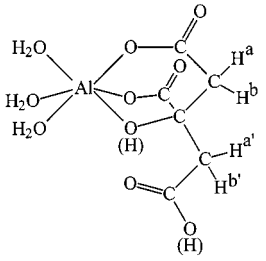


Figure 5. ^1H NMR spectra obtained in the Al^{III}−citric acid system at a ratio 1:1, c_{Al}=0.04 M and at different pH values: (a) pH ≈ 2, (b) pH ≈ 2.5, (c) pH ≈ 3; all spectra were recorded after the thermodynamic equilibrium was reached

tentiometric studies) oligomerization starts only above pH ≈ 3 (Figure 3d). The ^1H COSY NMR spectrum obtained for the same solution (see Supporting Information) indicates that these minor signals form a series of AB quadruplets, suggesting a very asymmetric structure of the oligomeric complex formed at this pH. From these AB quadruplets, three can be clearly identified at δ = 2.86, 2.83, 2.66 and 2.63 (J_{AB} = 16.6 Hz); 2.88, 2.84, 2.63 and 2.59 (J_{AB} = 20.2 Hz) and 2.70, 2.67, 2.59 and 2.56 (J_{AB} = 14.6 Hz). The signal at δ = 2.76 does not couple with any other signal.

At pH ≈ 3, the oligomerization processes are more pronounced. The characteristic AB quadruplet of the mononuclear species that appears at lower concentrations is shifted to δ = 2.83, 2.80, 2.72 and 2.69 (•), and can be assigned to the species [AlL] and $[AlLH_{-1}]^-$ (Figure 5c).

A tridentate chelation of citric acid was assumed, involving the coordination of one of the deprotonated terminal carboxylates, the deprotonated central carboxylate group and the protonated alcoholic OH group in the case of the species $[AlLH]^+$; this latter group is deprotonated in the complex [AlL]. The other terminal carboxylic group is not bound, and protonated in the species $[AlLH]^+$ and [AlL], and deprotonated in the complex $[AlLH_{-1}]^-$. The fact that there is an AB quadruplet in the ^1H NMR spectrum can be explained by a relatively fast intramolecular exchange between the bound and unbound carboxylate moieties, i.e. the citrate molecule behaves fluxionally. A more likely explanation is that one of the methylene proton pairs (e.g. a and a′, see Scheme 1) probably has a spatial arrangement such that its chemical environment changes more than in the case of the protons b and b′ during fluxional motions. For this reason, the signals given by the protons a and a′ are broader than the signals for the protons b and b′. If the rate of fluxionality were lower, separate signals could be observed for the protons a and a′. In our opinion, only this proposed binding mode of the citrate molecule with a 5+6 joined (COO^-, OH, COO^-) chelate system (see Scheme 1) and their fluxional motion can explain the asymmetric pattern of the AB quadruplet assigned to the mononuclear species. Furthermore, participation of the protonated alcoholic OH group in the coordination in the species $[AlAH]^+$ is supported by the observed increase in the stability of this complex as compared with the corresponding complexes of malonic acid (H_2L) or succinic acid (H_2L).[16,17] The basicity adjusted stability constant [log K^* = log $K([AlLH]^+)$ − log $\beta(H_3L)$] characteristic for the equilibrium $Al^{3+} + H_3L \rightleftarrows [AlLH]^+ + 2\ H^+$ is log K^* = −2.53 for Al^{III}−citrate, while it is −5.57 for the corresponding 1:1 complex $[AlL]^+$ formed with succinic acid, which involves a seven-membered (COO^-, COO^-) chelate. The corresponding proton displacement constant for malonic acid, which forms a six-membered (COO^-, COO^-) chelate, is log K^* = −1.51.



$[AlLH]^+$—[AlL]—$[AlLH_{-1}]^-$

Scheme 1

Similar fluxional behavior was found in the case of the species $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$ in aqueous solution. The solid-state structures of the isolated complexes $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$, determined by X-ray crystallography, indicates asymmetric arrangements of the citrate molecules. Both citric acids bind to Al^{III} through one of the two terminal COO^- groups, the central COO^- group and the deprotonated alcoholate function, while the other terminal carboxylic functions are unbound (one of them is protonated in the species $[AlL_2H_{-1}]^{4-}$, while both are deprotonated in $[AlL_2H_{-2}]^{5-}$).[11,12]

The time-dependent changes in the species distribution in the pH range 3−8, i.e. the slow formation of the trinuclear species $[Al_3(LH_{-1})_3(OH)]^{4-}$ (Figure 3), is clearly demonstrated by NMR spectroscopy too. The required amounts of the stock solutions of Al^{III}, citrate and base were mixed at selected pH values (pH ≈ 5.0 and 7.0) and a series of spectra was taken at predetermined times until pH equilibrium was attained. Equilibrium was considered to be reached when two consecutive spectra coincided.

Figure 6 shows the time-dependence of the ^1H NMR spectra recorded at pH ≈ 5 (similar spectra obtained at pH ≈ 7 are included in the Supporting Information). The signals are initially poorly resolved, broad and merged, indicating dynamic changes in the system. The complexation

processes and/or ligand-exchange reactions between the initially formed mononuclear complexes and intermediate species seem to be fairly fast.

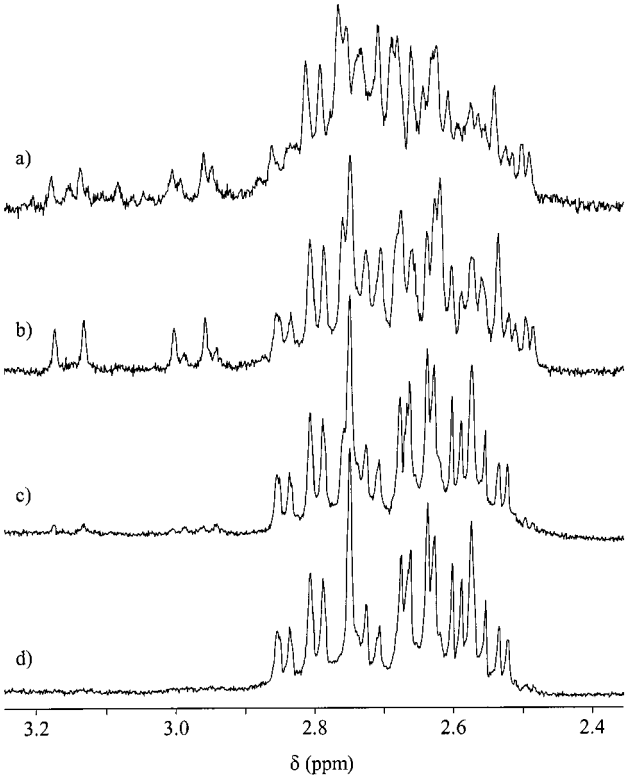


Figure 6. Time dependence of the ^{1}H NMR spectra of the Al^{III}–citric acid system at a ratio 1:1, c_{Al} = 0.04 M at starting pH ≈ 5; (a) 15 min, (b) 40 min, (c) 10 h, (d) 30 h after mixing of the components

At pH ≈ 5 in the equilibrium state, the characteristic but rather complicated spectrum of the trinuclear complex $[Al_3(LH_{-1})_3(OH)]^{4-}$ is obtained (see Figure 6d), as is expected from the potentiometric results. The speciation curves indicate that at this pH the trinuclear complex is the only species present (see Figure 3). The X-ray structure of the isolated trinuclear complex $[Al_3(LH_{-1})_3(OH)]^{4-}$ reveals an asymmetric arrangement of the carboxylate-bridged citrate molecules between the Al^{III} centers.[13] Accordingly, in the ^{13}C NMR spectrum 18 resonances appear: each carbon atom gives a separate signal. Similarly, the ^{1}H NMR spectrum is also complicated as all the methylene protons are chemically and magnetically unequivalent.[13,18] The characteristic ^{1}H resonances of the trinuclear complex are as follows: δ = 2.87, 2.85, 2.82, 2.80, 2.77, 2.74, 2.72, 2.69, 2.68, 2.65, 2.64, 2.62, 2.60, 2.58, 2.57, 2.55 and 2.54. A complete assignment of the signals was achieved by means of 2D carbon–proton correlation measurements and is under publication.[19] The appearance of the weak resonances between δ = 3.0 and 3.2 leads to the conclusion that the trinuclear complex is probably formed through at least one intermediate species. These "extra" resonances may belong to this/these intermediate species, since they reach a maximum intensity in time and are later almost completely absent from the spectrum obtained at equilibrium. It was demonstrated by ^{1}H COSY NMR measurements that they are half of the AB quadruplets, the other half lying under the signals of the trinuclear species.[12]

When the starting pH is ca. 7 (see Supporting Information), the pattern of the spectrum obtained in the equilibrium state clearly indicates the presence of the trinuclear species (the characteristic resonances are listed above), the free citrate (δ = 2.68, 2.63, 2.55 and 2.50; J_{AB} = 15.5 Hz) and a complex which gives broad resonances at δ = 2.49 and 2.45. These latter signals may be half of an AB quadruplet, the other part overlapping with signals of the trinuclear complex. According to potentiometry, this other complex is $[AlLH_{-2}]^{2-}$, which is a mixed hydroxo species with the same citrate binding mode as in the complex $[AlLH_{-1}]^-$. The broadening of the signals is probably due to the presence of the OH^- group in the coordination sphere of the Al^{III}. The appearance of free citrate can be explained by the change in the pH of the samples with time. As discussed above, at this pH the oligomerization is accompanied by a considerable release of OH^-. Thus, the pH at equilibrium is much higher than the starting value, reaching pH > 8.5. At this pH, OH^- starts to displace citric acid from the coordination sphere of Al^{III}, resulting in the parallel formation of $[Al(OH)_4]^-$.

The formation of intermediate complexes during oligomerization is clearly demonstrated by ^{13}C NMR spectroscopy too. To obtain a ^{13}C NMR spectrum of better quality, the concentration of citric acid was increased to 0.2 M and the Al^{III}/citric acid ratio was kept at 1:1. Figure 7 shows the methylene range of the spectra recorded at different times at pH ≈ 5. Spectrum (b) was obtained at equilibrium and the resonances of the six methylene carbon atoms of the asymmetric trinuclear complex are exhibited.[13] Due to the asymmetric structure and kinetically inert nature of the complex (vide extra), each methylene group yields separate signals. The upper spectrum (a) corresponds to a non-equilibrium state. Besides the signals of the trinuclear species, four new resonances can be recognized, at δ = 41.7, 43.6, 43.9 and 47.2. These signals are completely absent from the spectrum at equilibrium. The potentiometric results show that at pH ≈ 5 the complex $[AlLH_{-1}]^-$ oligomerizes to give $[Al_3(LH_{-1})_3(OH)]^{4-}$. This mononuclear complex may give at least 2 signals in the methylene range of the ^{13}C spectrum. Consequently, we cannot assign the "extra" signals in the above discussed spectrum to $[AlLH_{-1}]^-$. They are probably due to some intermediate species which forms during the oligomerization of the mononuclear complex to the final trinuclear species. These intermediate species are presumably oligonuclear complexes which contain at least two molecules of citric acid in an asymmetric arrangement. At pH ≈ 7, the same behavior is observed.

The changes in the Al^{III}–citrate system were also followed by ^{27}Al NMR spectroscopy (see Figure 8). The spectra recorded in a fresh equimolar Al^{III}–citrate solution at pH ≈ 5 and 7 consist of two components; a large peak at δ = 11.5 and a shoulder on the upfield side at about δ = 0. The intensity of the main signal increases with time, while that of the shoulder at δ = 0 initially increases and then undergoes a considerable decrease until equilibrium is re-

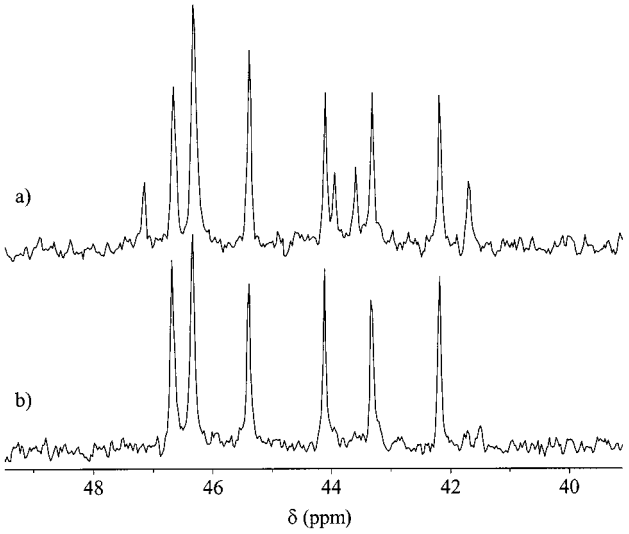


Figure 7. The methylene range of the time-dependent ^{13}C NMR spectra in the Al^{III}−citric acid system at a 1:1 ratio, c_{Al} = 0.2 M, at pH ≈ 5; (a) 12 h after mixing of the components, (b) at equilibrium

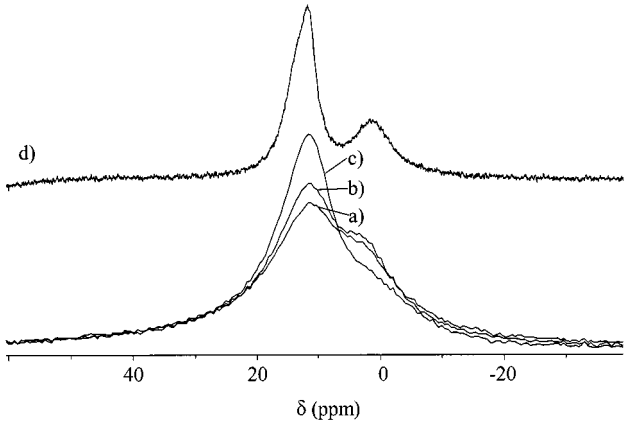


Figure 8. ^{27}Al NMR spectra (measured with a 200 MHz instrument) of the Al^{III}−citric acid system at a 1:1 ratio, c_{Al} = 0.1 M, at pH ≈ 7 and at different times after preparation of the sample; (a) 10 min, (b) 1 h, (c) 30 h, (d) the same spectra as (c) but measured with a 500 MHz instrument

ached. This behavior of the shoulder is probably related to the formation and decomposition of intermediate complexes. The fact that practically the same spectra were recorded at pH ≈ 5 and 7 suggests that a single species, formed fairly slowly, predominates in this pH range. Karlik et al.[5] assigned this spectrum to a mononuclear complex, probably $[AlLH_{-1}]^-$. It is now clear that this spectrum is that of the trinuclear species.

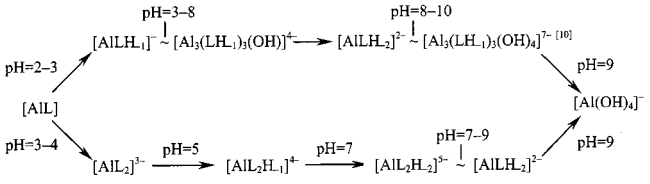
The ^{27}Al NMR spectrum of a sample recorded when the crystalline trinuclear complex was dissolved in water consisted of three signals: at δ = 0.2, at 10.7 and as a downfield shoulder on the latter, at 12.6.[13] In our case, as a result of the lower resolution of the instrument, the signals at δ = 10.7 and 12.6 are merged completely, giving a single broad peak with maximum at δ = 11.5 and the signal at δ = 0.2 becomes a shoulder. The spectral pattern described in the literature[13] was obtained when the same sample, as shown in Figure 8c, was measured with a 500 MHz instrument (see Figure 8d).

NMR Spectroscopic Measurements in the Al^{III}-Citrate System with Ligand Excess

The potentiometric results show that at an excess of ligand, besides the mononuclear 1:1 species, 1:2 complexes are also formed in freshly prepared solutions. These 1:2 and 1:1 complexes undergo slow oligomerization processes which, similarly as in equimolar solutions, result in the predominant formation of the trinuclear species $[Al_3(LH_{-1})_3(OH)]^{4-}$. The solid-state characterization and solution behavior of these 1:2 complexes have already been determined using ^1H and ^{13}C NMR spectroscopy.[11,12] The ^{13}C NMR measurements carried out by Gregor and Powell[8] in a Al^{III}−citrate solution at a 0.25 M Al^{III} and a metal ion/ligand ratio of 1:4, revealed the presence of the 1:2 species and free citrate only. The authors did not mention whether these spectra were taken in fresh solution or in the equilibrium state. In the ^1H NMR spectra measured by us at the same concentration at pH ≈ 5 and 7, but at an Al^{III}/citrate ratio of 1:3 (the ratio used in our potentiometric study), the signals of the trinuclear species could also be unambiguously recognized in the equilibrium state. It was further found that in concentrated solutions the oligomerization processes are slower than at 0.04 M or 0.004 M; equilibrium can be reached in about a week. These results lead to the conclusion that in ref.[8] the spectra were obtained using fresh solutions, and in the equilibrium state the trinuclear complex should also have formed, although in this case (because of the higher ligand excess) in a somewhat lower amount than at a ratio of 1:3. In less concentrated solutions and at higher metal/ligand ratios, the oligomerization processes may truly be negligible.

Conclusions

The time-dependent speciation study of the Al^{III}−citrate system indicates that in fresh solutions mononuclear mono- and bis(complexes) are the predominant species, although the trinuclear complex $[Al_3(LH_{-1})_3(OH)]^{4-}$ is also present, especially under equimolar conditions. However, the concentration of this complex is strongly reduced in the event of a ligand excess. The trinuclear species is formed in slow processes through the oligomerization of mononuclear complexes[10] (see the upper row of Scheme 2). An excess of



The species $[Al_3(LH_{-1})_3(OH)_4]^{7-}$ was proposed by Öhman at pH > 8[10]

Scheme 2

ligand favors the formation of the bis(complexes) (the reaction steps are indicated in the lower row of Scheme 2); under such conditions these are strongly competitive with the oligomerization processes indicated in the upper row.

These facts may be of importance when speciation model calculations on Al^{III} are performed for biological fluids, e.g. for blood serum, where citric acid may be present in a 100-fold excess as compared with Al^{III}. For the average concentrations of citrate and Al^{III} in serum, Harris[2] estimated 0.1 mM and 3 μM, respectively. However, ca. 80% of the total Al^{III} is bound to transferrin and only 20% is available for the l.m.m. binders.[2,14] As may be seen in Figure 9, under serum conditions the formation of the trinuclear species (even if the complex is very stable) is completely suppressed. In the event of an overload of Al^{III} in the organism, the metal concentration in the serum may increase to 10−100-fold, and this would increase the extent of the formation of the oligomeric species at equilibrium quite considerably. However, its formation is definitely a slow process and our calculations demonstrate that its concentration is still negligible 30 min after absorption.

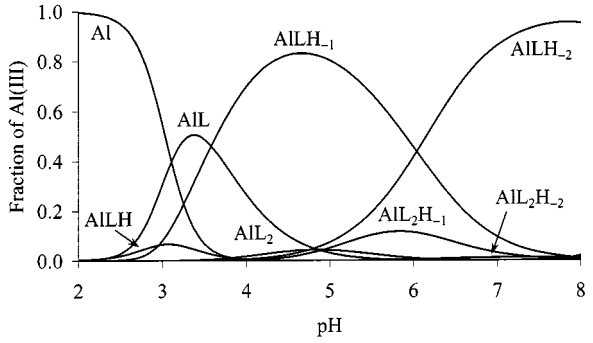


Figure 9. Speciation curves of the Al^{III}−citric acid system, at blood serum conditions, $c_{Al}{}^{III}$ = 0.6 μM, $c_{citrate}$ = 99 μM

As biological fluids are open systems, and by virtue of the continuous metabolic processes, Al^{III} may not spend enough time in the biological fluid for the equilibrium state to be reached. Accordingly, its speciation as calculated with the thermodynamic stability constants may not be totally relevant to the biological conditions. In such cases, only a complete equilibrium and kinetic description of the formation of all complexes can provide the exact biospeciation information. In such a complicated system, however, this can be achieved only in a very long and tedious way. A reasonable estimation of the speciation may be made by using stability constants calculated from measurements under non-equilibrium conditions.

Experimental Section

Reagents: Citric acid, of highest analytical purity (Sigma product), was used without further purification. The exact concentration of the ligand solution was determined by potentiometric titration using the Gran method.[20] The Al^{III} stock solution was prepared from recrystallized $AlCl_3{\cdot}6H_2O$, and its metal concentration was determined gravimetrically through its oxinate. The stock solution contained 0.1 M HCl to prevent hydrolysis of aluminium. The ionic strength of all solutions was adjusted to 0.2 M KCl and the temperature was 25.0±0.1 °C.

Potentiometric Measurements: The stability constants of the proton complexes of the ligands were determined by pH-potentiometric titrations of 10.0 mL samples. The ligand concentration was 0.004 M. The titrations were performed with a 0.2 M carbonate-free KOH solution of known concentration under purified argon. − The time-dependent speciation measurements were performed with the "batch" technique. After the appropriate amounts of stock solutions of Al^{III} were mixed with the ligand and the base, the pH of the solutions was recorded at given times until pH equilibrium was attained in the system (ca. 30 h). The procedure was repeated at different starting pH values, whereby titration curves relating to different times were obtained. The measurements were performed at metal/ligand ratios of 1:1 and 1:3, under intense stirring under argon. The pH range studied was 2−8. The time-dependent changes were monitored in this way for 39 different samples. However, in order to increase the reliability of the speciation description of the system in thermodynamic equilibrium twice as many samples were prepared, but half of them, i.e. every second was measured only 30 h after mixing, when pH equilibrium was surely attained. The stability constants listed in Table 1 were calculated from the total 78 "batch" points. The pH was measured with a Radiometer PHM 84 instrument with a CMAWL Russel combined glass electrode, which was calibrated for hydrogen ion concentration according to Irving et al.[21] − The concentration stability constants $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$ were calculated with the aid of the PSEQUAD computer program.[22] The stability constants used for the hydroxo species of Al^{III} were taken from ref.[23] and corrected to I = 0.2 using the Davies Equation: −5.49 for $[AlH_{-1}]^{2+}$, −13.54 for $[Al_3H_{-4}]^{5+}$, −97.2 for $[Al_{13}H_{-32}]^{7+}$ and −23.40 for $[AlH_{-4}]^{-}$.

NMR Spectroscopy: ^1H and ^{13}C NMR spectra were recorded at 25 °C with a Bruker AM360 spectrometer. In the ^1H and ^{13}C NMR spectra, chemical shifts were referenced to the signal of TMS as an external standard. The samples were prepared in D_2O; the pH was adjusted with concentrated NaOD and DCl, using the relation pH = pD + 0.4. The concentrations of the solutions were 0.04 M or 0.1 M for Al^{III}, and a metal ion/ligand ratio of 1:1 was usually applied. ^{27}Al NMR spectra were recorded with a Bruker AM200 instrument at 25 °C. Chemical shifts were recorded with respect to $[Al(H_2O)_6]^{3+}$. Solutions of 0.1 M Al^{III} and a metal ion/ligand ratio of 1:1 were prepared in water containing 10% of D_2O to provide an NMR lock signal.

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