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A Novel Dinuclear Species in the Aqueous Distribution of Aluminum in the Presence of Citrate

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The chemistry of aluminum was explored in the presence of the physiological ligand citric acid and in low-pH aqueous media. As a result, the first dinuclear aluminum–citrate complex $(NH_4)_4[Al_2(C_6H_4O_7)(C_6H_5O_7)_2]\cdot 4H_2O$ was isolated at low pH (∼3.5), and was characterized by FT-IR spectroscopy and X-ray crystallography. The structural analysis reveals the presence of a dinuclear assembly of two aluminum ions octahedrally coordinated to three citrate ligands of differing protonation state. The NMR solution behavior of this complex emphasizes its time-dependent transformation into a number of variable nature species, ultimately leading to the thermodynamically stable trinuclear species. It also establishes the participation of the dinuclear complex as a viable component of the aqueous Al(III)–citrate speciation. The chemical and structural features of this novel low molecular mass species provide considerable insight into citrate's ability, as a natural ligand, to influence the chemistry of aluminum in a pH-dependent fashion, and potentially affect aluminum's (bio)distribution, absorption, accumulation, and biotoxicity at sensitive biological sites.

Aluminum biotoxicity has been the subject of considerable research in recent years due to the involvement of that element in a number of health-related physiological aberrations.[1] Specifically, several diseases have been linked to the toxic influence of aluminum, including numerous dementias, neurodegenerative conditions, like Alzheimer's disease, encephalopathies, microcytic anemia, and others.[2] The association of aluminum with the aforementioned clinical conditions has raised questions concerning the processes by which biochemical pathways are influenced by that metal ion. Key to understanding the role of aluminum in such events is the aqueous speciation of that element and the requisite chemistry in biological fluids. Interactions, however, of aluminum at the biological level can occur with both high and low molecular mass biomolecules present in those fluids. Without disregard for the essential contribution of large molecular mass molecules, like transferrin, to the interactive chemistry with aluminum, the corresponding chemistry with low molecular mass molecules is equally important and significant. Prevalent among such molecules is citric acid, which is abundantly present in human plasma (0.1 mM).[3] It is capable of promoting metal-binding chemistry, solubilizing metal ions, like aluminum, and consequently raising their bioavailability and ultimate absorption by various tissues. Therefore, Al(III) speciation in the presence of the low molecular mass ligand citrate emerges as an important feature of that metal ion's involvement in interactions with biological loci. Unraveling the nature and properties of the various Al(III) species participating in such pH and concentration dependent distributions may aid in further determining Al(III) bioavailability and its link to disruption of biochemical processes and/or toxicity. Being aware of these properties, we have investigated the aqueous chemistry of citrate with Al(III) at low pH. Herein, we report on the synthesis and

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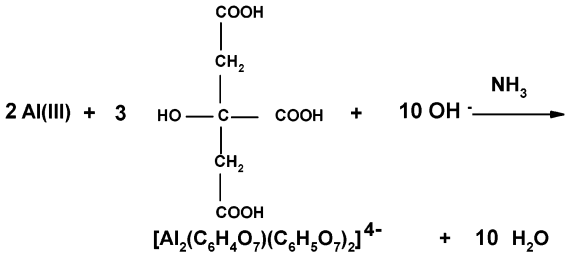
Scheme 1. Stoichiometric Reaction for the Synthesis of $(NH_4)_4[Al_2(C_6H_4O_7)(C_6H_5O_7)_2]\cdot 4H_2O$ (**1**)



characterization of the first dinuclear Al(III)−citrate complex with potential implications in the biodistribution of that metal ion in aqueous media.

Reaction of Al(III) with citric acid in water at pH ∼3.5 led to the isolation of a colorless crystalline material, the analytical data on which were consistent with the molecular formulation $(NH_4)_4[Al_2(C_6H_4O_7)(C_6H_5O_7)_2]\cdot 4H_2O$ (**1**) (Scheme 1).[4]

X-ray crystallography confirmed the presence of the first dinuclear Al(III)−citrate complex.[5] It is composed of two octahedral Al(III) centers, the coordination requirements of which are satisfied by three citrates. Two of the citrates are triply deprotonated, whereas the third one is fully deprotonated. As such, the citrates bind Al(III) through their central carboxylate and alkoxide groups as well as one of the terminal carboxylates. The remaining terminal carboxylate does not bind Al(III) and moves away from it in the protonated or deprotonated form (Figure 1).

The binding mode and structural attributes of the citrate ligands are similar to those seen in other mononuclear, $(NH_4)_5[Al(C_6H_4O_7)_2]\cdot 2H_2O$ (**2**),[6] $(NH_4)_4[Al(C_6H_4O_7)(C_6H_5O_7)]\cdot 3H_2O$ (**3**),[7] and trinuclear $(NH_4)_5[Al_3(C_6H_4O_7)_3(OH)(H_2O)][NO_3]\cdot 6H_2O$ (**4**) complexes.[8]

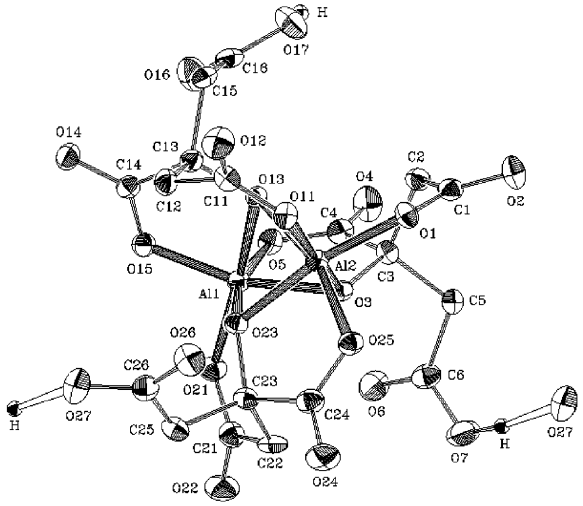


Figure 1. ORTEP structure of the anion in **1** along with the atom-labeling scheme. Thermal ellipsoids are drawn by ORTEP and represent 50% probability surfaces. Al−O bond range: 1.835(3)−1.932(3) Å. O−Al−O angle range: 77.8(1)−106.7(1)°.

A strong hydrogen-bonding interaction between one of the terminal protonated carboxylate groups and the terminal deprotonated carboxylate of an adjacent molecule in the lattice is responsible for the formation of chains of dimers directed along the *b*-axis [HO7···O27′ (x, $-1+y$, z) = 1.654 Å, O7···O27′ = 2.544 Å, O7−H···O27′ = 170.5°]. There are no direct hydrogen bonds between the assembled chains, which are linked together through an extensive network of hydrogen-bonding interactions involving the carboxylate oxygens, the water molecules of crystallization, and the ammonium counterions. This extensive hydrogen-bonding network may be responsible for the stability of the derived lattice in **1**.

FT-IR spectroscopy pointed out the presence of vibrationally active groups, contributing to the identification of complex **1**. Specifically the antisymmetric vibrations for the citrate carbonyls were observed in the range 1700−1600 cm^{-1}, with the symmetric vibrations being observed in the region 1500−1400 cm^{-1}. The difference $\Delta(\nu_{as}(COO^-)-\nu_s(COO^-))$ was greater than 200 cm^{-1}, indicating the presence of carboxylate groups free or bound to Al(III) centers in a monodentate fashion.[9] Confirmation of the above contention was afforded by the X-ray structural analysis on **1**.

Preliminary ^1H NMR spectra of **1** in aqueous solutions at the autogenous pH showed complicated patterns of signals, which could be classified in different groups. Due to the complexity of the signals, an initial assignment was attempted by following the time-dependent changes occurring in the spectra of **1** in water. Three types of groups of signals emerged in the spectra of **1**. One type of group of signals, appearing immediately upon dissolution of **1**, decreased monotonically with time. Of this type, one subgroup of signals appears in the region 2.2−2.3 ppm, and another one appears in the region 2.8−3.0 ppm, with the third subgroup being present between 2.4 and 2.5 ppm. These groups of signals were tentatively assigned to the dinuclear complex upon dissolution in water. Narrow signals of another type at

(4) $Al(NO_3)_3$ and citric acid were placed in aqueous solution with a molar ratio of 1:2. The resulting reaction mixture was stirred overnight with mild heating. On the following day, the reaction mixture was taken to dryness by means of a rotary evaporator. The residue was redissolved in the minimum amount of water, and the pH of the resulting solution was adjusted to ∼3.5 with a solution of aqueous ammonia (1:1). Stirring continued at room temperature for an additional 2 h. Subsequently, ethanol was added and the reaction flask was placed in the refrigerator. Two weeks later, a colorless crystalline material was deposited at the bottom of the flask. The crystals were isolated by filtration and dried in vacuo (yield ∼50%). Satisfactory elemental analysis was obtained for **1** (See Supporting Information).

(5) (a) Crystal data: space group, $P2_1/c$, a = 18.955(3) Å, b = 9.470(2) Å, c = 18.353(3) Å, β = 110.316(4)°, V = 3089.6(9) Å^3, ρ_{calcd} = 1.644 Mg/m^3, Z = 4, T = 298 K, $2\theta_{max}$= 115°, Cu Kα radiation (λ = 1.5418 Å), reflections collected/unique/used = 4386/4238 (R_{int} = 0.0611)/4238, parameters refined 568, $(\Delta/\sigma)_{max}$ = 0.017, $(\Delta\rho)_{max}/(\Delta\rho)_{min}$ = 0.546/−0.309 e/Å^3, final R (2976 reflections $I > 2\sigma(I)$) indices were R = 0.0551, R_w = 0.1238. Diffraction measurements were taken on a Nicolet $P2_1$ four-circle diffractometer, upgraded by Crystal Logic. Unit cell dimensions for **1** were determined and refined by using the angular settings of 25 automatically centered reflections in the range 22° < 2θ < 54°. Intensity data were measured by using $\theta-2\theta$ scans. Detailed crystallographic information has been deposited as Supporting Information. (b) Sheldrick, G. M. *SHELXS-86: Structure Solving Program*; University of Göttingen: Göttingen, Germany, 1986. (c) Sheldrick, G. M. *SHELXL-93: Structure Refinement Program*; University of Göttingen: Göttingen, Germany, 1993.

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2.82, 2.76, 2.70, and 2.64 ppm (360 MHz), the intensity of which increased monotonically with time, were clearly assigned to free citrate. Finally, a third type of group of signals, appearing at 3.1 ppm and around 2.91 ppm (this one less clearly defined due to overlapping with other peaks), exhibited a different behavior from the previous signals. Their intensity initially increased, then gradually decreased and ultimately disappeared.

HH-COSY NMR suggested that the signals of the first type belong to **1** upon dissolution in water. Signals of the third type were tentatively attributed to the presence of dinuclear Al:Cit species of 2:2 stoichiometry with different protonation states. These species may reflect intermediates of the oligomerization of AlCit toward the most stable complex **4**.[7] In AlCit, formed in this slightly acidic pH range, Cit contains a deprotonated alcoholate function and coordinates in a tridentate fashion (COO^-, O^-, COO^-), while one of the terminal carboxylates is in the protonated form.[7] The presence of AlCit species may also be inferred by some of the signals in the region 2.4−2.8 ppm. The above assignments were supported by 2D-COSY and 2D-HSQC NMR experiments.

Collectively, the NMR data propose the existence of the dinuclear complex **1** upon dissolution, and its concomitant time-dependent transformation into an Al(III) dimer (previously suggested by us)[7] and a mononuclear Al(III) species. In a complex set of equilibria, similar to those presumably occurring upon dissociation of **2** and **3**, the above basic components constitute the scaffold onto which the thermodynamically stable trinuclear species arises at the equilibrium state.[8] Thus, complex **1**, as a potential participant in the aqueous speciation of the Al(III)−citrate system, is the sole representative of complexes isolated and structurally characterized in the low-pH regime of the entire pH spectrum. Other complexes in the middle (4−5.5) and high pH (7−8) regime have been synthesized, and structurally characterized in the past.[6,7] The aforementioned complexes have been shown to interconvert in pH-dependent transformation reactions, indicating their linkage as participants in the aqueous speciation of the Al(III)−citrate system.[7]

A plethora of speciation studies were reported in the past on aqueous Al(III) systems, including the aluminum−citrate system.[10] These studies had proposed a number of Al(III)−citrate complexes as speciation components. Among such complexes were mononuclear species of varying protonation states and trinuclear species, like **4**, with the latter having been shown to be the thermodynamically stable species. On the basis of this information, **1** appears to possess solid state (octahedral Al(III), oxygen terminal ligands, etc.) and solution (time-dependent behavior consistent with that of mononuclear complexes reported before) properties, which conform to its ultimate transformation to **4**, also observed on a number of other Al(III)−citrate complexes.[7] This association of **1** with other species in solution, either proposed or observed in the past, provides clues for **1** being a viable component in the aqueous distribution of Al(III) in the presence of citrate. To further prove this point, pH-dependent interconversions between complex **1** and other well-known Al(III)−citrate complexes, such as **2** and **3**, previously established as participants in the aqueous distributions of Al(III) in the presence of citrate are currently being investigated.

Finally, the novel dinuclear complex **1** reflects the chemistry unfolding between Al(III) and the physiological ligand citrate at low pH. This chemistry reveals the potential binding properties of citrate at low pH and is exemplified through the binding modes and variable protonation state of that ligand within **1**. In this context, it sheds light into the nature of species that might exist under similar reaction conditions and possibly exert influence on biological targets. Albeit removed from the physiological conditions, **1** along with mononuclear species, like AlCit, purported to exist at low-pH values, may be good precursors from which other pH-dependent components in the aqueous Al(III)−citrate distribution(s) may be assembled at low or higher pH values. This mechanistic view of potential reaction schemes and emerging Al(III) species appears to be the basis of comprehending how this toxic metal ion reacts its way into the physiological milieu, ultimately presenting itself as a bioavailable entity to metal ion carriers like transferrin,[11] and/or eliciting interactions at the cellular level. The fact that (a) citrate has been known to increase the uptake of key metal ions like Al(III) and Fe(III), ultimately carried to biological sites by transferrin,[12] and (b) the aqueous coordination chemistry of Fe(III) has been shown to exhibit similarities to that of Al(III), may indicate a similar uptake and transfer pathway for solubilized and bioavailable Al(III). With this view in mind and taking into consideration the possibility of participation of low molecular mass Al(III)−ligand species in non-transferrin-dependent pathways, leading to the transfer and distribution of Al(III) in biological sites, it appears that the work presented on complex **1** assumes great significance. Experiments targeting the aforementioned issues as well as synthetic methodologies perusing the chemistry of Al(III) species, with potential implications in that metal ion's toxicity, are currently under development in our labs.

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Supporting Information Available: Tables of X-ray crystal structure refinement data and positional and thermal parameters for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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