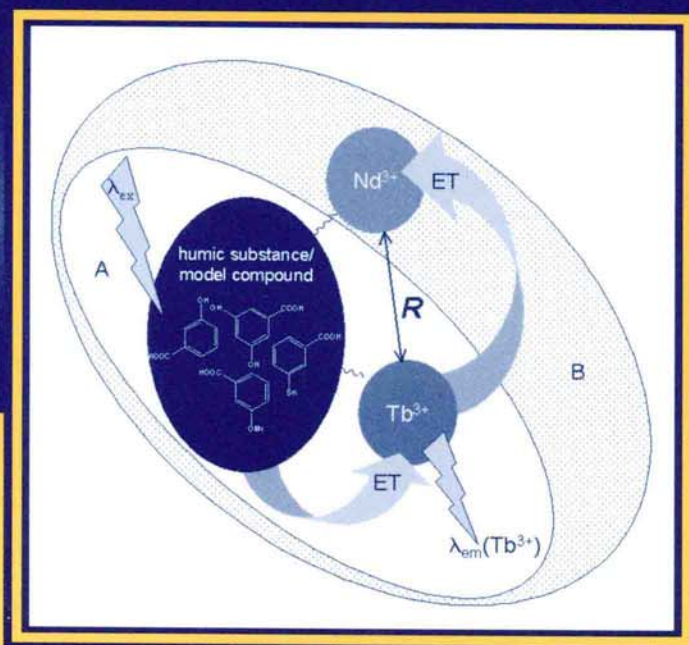# Humic Substances

## Molecular Details and Applications in Land and Water Conservation



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# QUANTITATIVE ANALYSIS OF ALUMINUM AND SOIL FULVIC ACID COMPLEXES BY SOLUTION STATE ALUMINUM-27 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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## 13.1. INTRODUCTION

The study of humic material has been ongoing for decades, yet scientists around the world still do not understand it fully [1]. It can be found in both terrestrial and aquatic environments. Humic material is an important class of molecules because it is a major reservoir of carbon in the global carbon cycle, can influence bioavailability of metals, controls solubility of both organic and inorganic compounds, binds metals and organics, and plays a major role in coagulation and aggregation in aquatic systems [2]. Therefore, having a good understanding of humic material and its properties is important in many environmental applications.

Aluminum is one of the most abundant metallic elements in the Earth's crust. It is also believed to be a leading cause of Alzheimer's Disease [3-8]. Under acidic conditions, aluminum takes its most mobile and toxic form, hexaquoaluminum(III), $Al(H_2O)_6^{3+}$, and can be transported easily in the environment. There is a growing concern with more frequent episodes of acid rain around the world and acidification of lakes, especially in countries where less attention has been paid to environmental issues. Aluminum undergoes strong hydrolysis when the pH approaches 5. Previous study [9] has shown that below pH 5, aluminum exists primarily as labile $Al(H_2O)_6^{3+}$. Between pH 5 and 7, aluminum hydrolysis products become more abundant, namely $Al(OH)_{aq}^{2+}$ and $Al(OH)_{2aq}^{+}$. The white precipitate aluminum hydroxide, $Al(OH)_3$, starts to form as pH is increased above 7. Beyond pH 8, aluminate anions $Al(OH)_4^{-}$ dominate, and aluminum hydroxide is re-dissolved and becomes soluble in basic media. Hence, our study investigates aluminum speciation at pH ≤ 5 before aluminum hydrolysis products dominate the system.

Nuclear magnetic resonance spectroscopy (NMR) has emerged as a powerful analytical technique in humic material research. Traditionally, most humic NMR studies have been concerned with $^1$H and $^{13}$C, which reveal extensive functionality on humic molecules. With new NMR instrument development and more sophisticated pulse programs, multinuclear NMR techniques are becoming popular [10-14]. In this study, $^{27}$Al NMR is applied to investigate binding between aluminum ions and soil fulvic acid in the pH range 3 to 5 and stability constants are obtained using both experimental NMR results and graphical Scatchard and Langmuir regressions. A model compound, oxalic acid, was also analyzed by the same NMR technique to evaluate the ability of this new method to analyze a known system.

## 13.2. MATERIALS AND METHODS

Humic material used in this study is a soil fulvic acid (SFA) from the B2 horizon of an organic-rich Podzol from Conway, New Hampshire, USA, which was isolated and generously given to us by Dr. James Weber. The elemental composition of this SFA is 53.1 % carbon, 3.24 % hydrogen, 0.90 % nitrogen and 0.8 % ash [15].

Stock solutions of aluminum (270 mgL$^{-1}$) and SFA (10,000 mgL$^{-1}$) were prepared by dissolving AlCl$_3\cdot$6H$_2$O and SFA in de-ionized water. Complexes of aluminum and SFA were prepared by adding 0.100 mL of aluminum stock solution and 1.00 mL of SFA stock solution in a 10.0 mL volumetric flask. Exactly 0.200 mL of 5.0 M NaNO$_3$ (Fisher) was also added to ensure an ionic strength equal to 0.1 M and 0.01 M NaOH (VWR) was added drop-wise to adjust the pH to 3.0, 4.0 or 5.0 before diluting to the mark with de-ionized water. The mixture was kept stirring for 1-12 h before NMR analysis.

In this study, oxalic acid (Fisher) was used as a model compound. The oxalic acid (Ox) solution was prepared by dissolving oxalic acid in de-ionized water to make a 100 mM stock solution. Aluminum oxalate solutions (molar ratio 1 to 1) were prepared at the 2 mM concentration level by adding 1.00 mL of each stock solution into a 50 mL volumetric flask. Also, NaNO$_3$ was added to adjust the ionic strength to 0.1 M and NaOH was added to adjust the pH to 3.0, 4.0 or 5.0. Similarly, aluminum oxalate solutions with a molar ratio of 1 to 2 were prepared.

$^{27}$Al NMR measurements were carried out on a Bruker DRX 500 MHz instrument operated at 130 MHz and equipped with a 5 mm broadband probe. All $^{27}$Al NMR spectra were referenced to a 1 M AlCl$_3$ solution at pH 2 in 20 % D$_2$O. To 0.500 mL of sample solution, 3 drops of D$_2$O were added to provide a signal lock and pH was again measured by an NMR pH electrode purchased from Cole-Parmer. Due to the broad background interference signal from metal alloy in the construction materials of the NMR probe, all spectra show a broad peak centered at 85 ppm with a width of ~100 ppm, which interferes with peaks from 40 to 135 ppm. The peaks of interests for aluminum-soil fulvic acid generally appear between 0 ppm and 30 ppm [16,17]. Therefore, a quantitative reference must appear outside the 0-135 ppm range to be useful.

In this study, a quantitative reference was achieved by using AlI$_3$ in acetonitrile. As reported previously [18], AlI$_3$ in acetonitrile forms a set of distinct peaks in the negative or upfield region on a $^{27}$Al spectrum, depending on the degree of saturation, and they do not interfere with peaks appearing in the positive or downfield region. AlI$_3$ is highly reactive with H$_2$O and O$_2$. Therefore, the AlI$_3$ solution was prepared in a glove bag (NPS) under argon for each experimental day.

Before reaching the glove bag, argon is passed through drierite ($CaSO_4$) to remove moisture and the glove bag is flushed with argon three times. After the $AlI_3$ solution had been stirred for 20 min, 0.200 mL of $AlI_3$ solution was transferred into an NMR insert (Wilmad). This insert was used as the quantitative reference between sample solutions. All manipulations were carried out in the atmospheric glove bag under argon. A sample of 1.0 mM aluminum solution was prepared from the stock solution and this solution was used to determine the concentration of $AlI_3$ complexes in the NMR insert. All subsequently generated NMR peak integrals were referenced to the same NMR insert containing the $AlI_3$ complexes. This was done every time before Al-SFA and Al-Ox solutions were analyzed. A solution of $Al(H_2O)_6^{3+}$ was also run with the insert as the first and the last experiment of each day to ensure the $AlI_3$ complex integrals remained constant. A typical spectrum for the $AlI_3$ reference is shown in Figure 13.1.
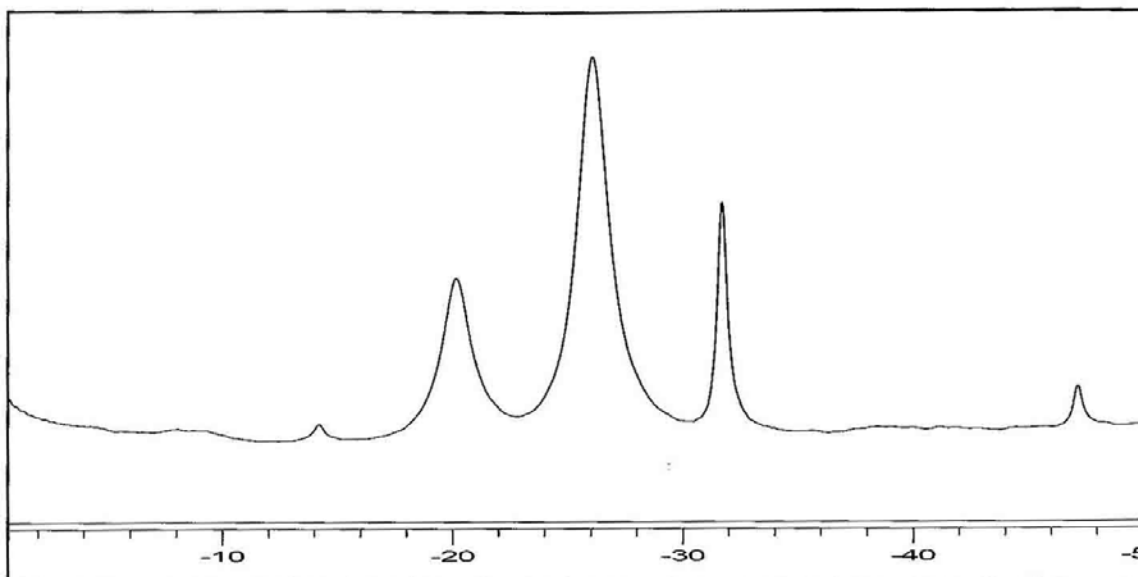


**Figure 13.1** $^{27}$Al NMR of anhydrous $AlI_3$ (0.010 mM) in dry acetonitrile

All NMR spectra were manipulated with MestRe-C v3.0 software. A polynomial function was applied to correct the baseline. $AlI_3$ reference peaks in the negative region were integrated as one single integral and calibrated to be 1.000. The remainder of the peaks in the positive region were also integrated together. Since these peaks severely overlapped each other, they were subjected to deconvolution and the intensity of each peak was then determined.

## 13.3. RESULTS AND DISCUSSION

### 13.3.1. Aluminum-Soil Fulvic Acid NMR

As we have reported previously [19], aluminum-soil fulvic acid (Al-SFA) spectra give rise to four distinct peaks that are observable between pH 3 and 5 and can be seen in Figure 13.2. The peak at approximately 0 ppm is free $Al(H_2O)_6^{3+}$ ion. The peaks at 7, 12 and 17 ppm are believed to be aluminum ions bound with one oxalic acid type site on SFA (Al-SFAox), aluminum ions bound with two oxalic acid type sites on SFA (Al-SFAox$_2$), and aluminum ions bound with three oxalic acid type sites on SFA (Al-SFAox$_3$), respectively. In Figure 13.2A at pH 3, peaks for $Al^{3+}$ (0

ppm) and Al-SFAox (7 ppm) dominate the system. As pH is increased to 4 and 5, peaks for Al-SFAox (7 ppm) and Al-SFAox$_2$ (12 ppm) become more prominent. The same behavior is observed in Figure 13.2B. Since there is more SFA in the system, peaks for Al-SFAox (7 ppm) and Al-SFAox$_2$ (12 ppm) dominate at pH 3. When pH is increased to 4 and 5, most of the peak intensity is shifted to peaks for Al-SFAox$_2$ (12 ppm) and Al-SFAox$_3$ (17 ppm).
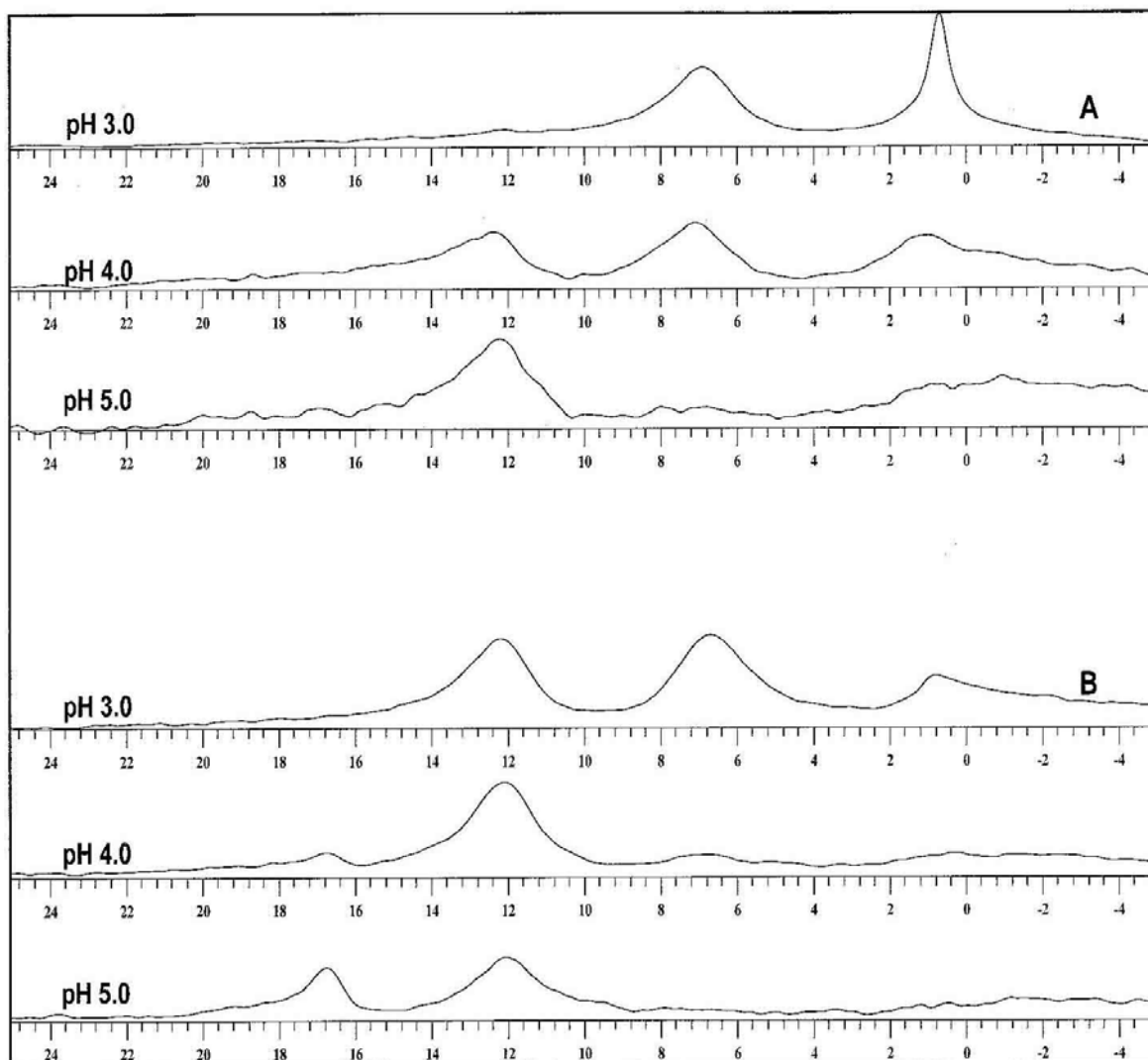


**Figure 13.2** ^{27}Al NMR spectra of (A) Al$^{3+}$(135 mg/L)-SFA(5000 mg/L) and (B) Al$^{3+}$(135 mg/L)-SFA(12500 mg/L) at pH 3, 4 and 5

Peak assignments were confirmed by studying oxalic acid as a model compound, where similar chemical shifts are observed [20]. NMR measurements were carried out on the model compound oxalic acid with aluminum. The results are shown in Figures 13.3 and 13.4 and the data are summarized in Table 13.1. Similar to the Al-SFA system, four distinct peaks are observed, namely peaks at 0, 7, 12 and 17 ppm, and they are assigned to Al$^{3+}$, Al-Ox, Al-Ox$_2$ and Al-Ox$_3$, respectively. In Figure 13.3 at pH 3, peaks for Al$^{3+}$ (0 ppm) and Al-Ox (7 ppm) dominate and as pH goes to 4 and 5, peaks for Al-Ox (7 ppm) and Al-Ox$_2$ (12 ppm) become more intense. In Figure 13.4, since more oxalic acid is in the system, peaks for Al-Ox$_2$ (12 ppm) and Al-Ox$_3$ (17 ppm) dominate. Aluminum oxalate speciation was modeled by MINTEQ [21] with conditional stability constants reported by Sillen and Martell [22]. MINTEQ was able to predict similar aluminum speciation within approximately 10 %, as shown in Table 13.1.
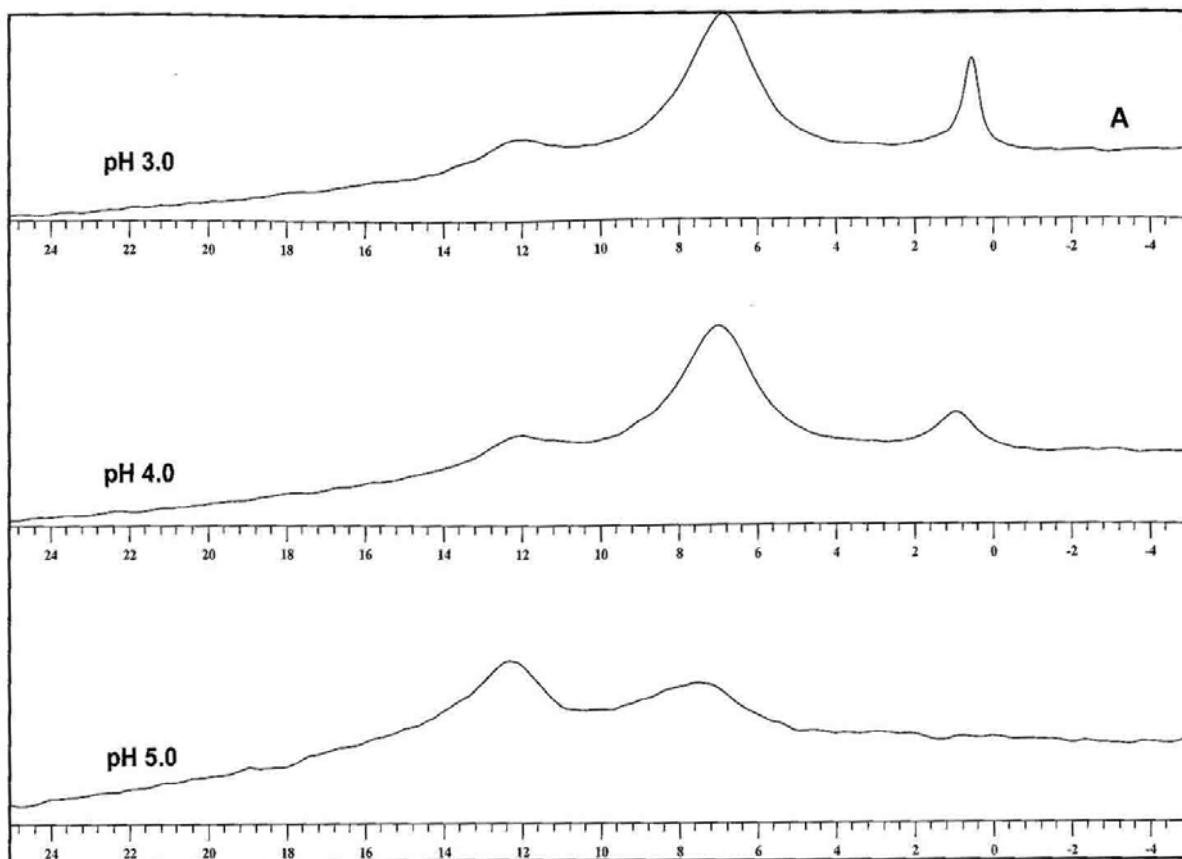
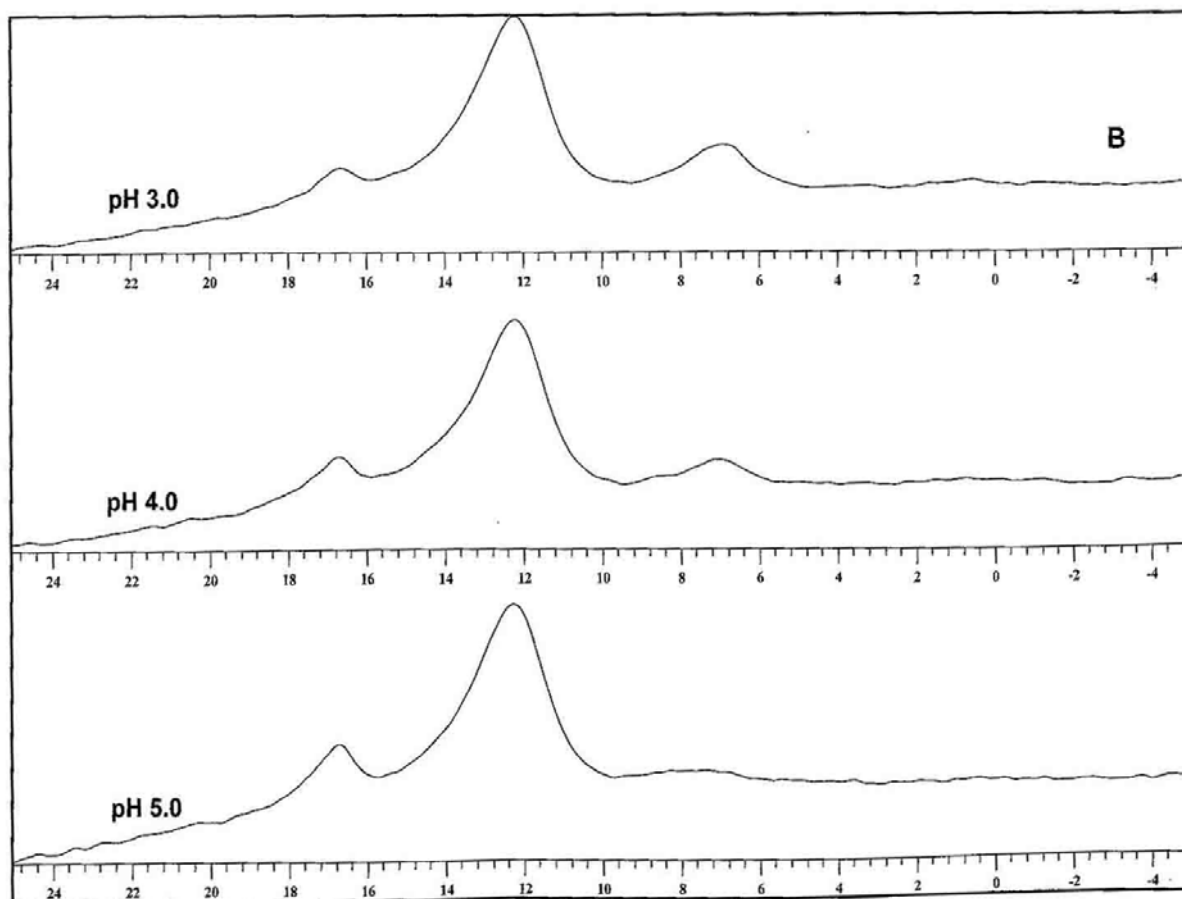**Figure 13.3** $^{27}$Al NMR spectra of Al$^{3+}$(2 mM)-Ox(2 mM) complexes



**Figure 13.4** $^{27}$Al NMR spectra of Al$^{3+}$(2 mM)-Ox(4 mM) complexes

## 13.3.2. Stability Constants

In order to determine stability constants for each of the Al-SFA binding sites, namely Al-SFAox (7 ppm), Al-SFAox$_2$ (12 ppm) and Al-SFAox$_3$ (17 ppm), quantitation of these peaks is required. Equation 13.1 allows calculation of the concentration of aluminum for peaks at 7, 12 and 17 ppm from the reference AlI$_3$ solution of known concentration in the NMR insert.

$$\frac{[Al_{sample}] \cdot V_{sample}}{[Al_{reference}] \cdot V_{reference}} = \frac{I_{sample}}{I_{reference}} \tag{13.1}$$

Here, $V_{sample}$ and $V_{reference}$ are the volume in the NMR tube and NMR insert, respectively. $I_{sample}$ and $I_{reference}$ are the integrated peak areas for the peaks of interest and AlI$_3$ reference peaks in a spectrum. [Al$_{reference}$] is the concentration of AlI$_3$, which is known. Therefore, the concentration corresponding to each peak [Al sample] can be subsequently determined. After each Al-SFA complex concentration is known, a stability constant, K, for the Al-SFA complexes can be calculated [23].

**Table 13.1** Percentage of each aluminum species in equilibrium with the oxalate ligand as measured experimentally by NMR and calculated with MINTEQ

| Al:Ox = 1:1 at 2 mM Al and 0.1 M Ionic Strength | | $Al^{3+}$ | $Al-Ox^+$ | $Al-Ox_2^-$ | $Al-Ox_3^{-3}$ |
|---|---|---|---|---|---|
| pH 3.0 | NMR | 14.2 | 71.4 | 14.3 | 0.0 |
|  | MINTEQ | 13.6 | 74.0 | 12.4 | 0.0 |
| pH 4.0 | NMR | 6.8 | 69.4 | 23.9 | 0.0 |
|  | MINTEQ | 13.0 | 73.8 | 12.9 | 0.0 |
| pH 5.0 | NMR | 0.0 | 75.3 | 24.7 | 0.0 |
|  | MINTEQ | 10.0 | 70.8 | 14.4 | 0.0 |
| Al:Ox = 1:2 at 2 mM Al and 0.1 M Ionic Strength | | $Al^{3+}$ | $Al-Ox^+$ | $Al-Ox_2^-$ | $Al-Ox_3^{-3}$ |
| pH 3.0 | NMR | 3.9 | 13.6 | 76.4 | 6.2 |
|  | MINTEQ | 0.0 | 24.2 | 75.0 | 0.0 |
| pH 4.0 | NMR | 0.0 | 11.4 | 73.8 | 14.8 |
|  | MINTEQ | 0.0 | 15.1 | 83.8 | 1.1 |
| pH 5.0 | NMR | 0.0 | 8.0 | 80.2 | 11.8 |
|  | MINTEQ | 0.0 | 13.7 | 85.0 | 1.2 |

Two selected data sets are shown for illustration. Table 13.2 shows the experimental NMR results in molar concentration for the speciation of Al$^{3+}$(1 mM) with SFA(1000 mg L$^{-1}$). At pH 3 and 4, more than 92% of the aluminum species are accounted for in the system observed by NMR. However, at pH 5, only 57 % is observed, meaning that the remaining 43 % has been removed from the system, probably by precipitates forming at pH 5. Looking at the individual peaks in Figure

13.2, as pH is increased, peak intensity shifts from $Al^{3+}$ (0 ppm) and Al-SFAox (7 ppm) to Al-SFAox$_2$ (12 ppm) and Al-SFAox$_3$ (17 ppm). Table 13.3 shows the results in molar concentration of the speciation of $Al^{3+}$(5 mM) with SFA(7500 mg $L^{-1}$). At pH 3, 4.49 out of 5 mM or about 90 % of the aluminum species are observed. However, at pH 4 and 5, some aluminum species are not observed due to precipitates forming in solution. As for individual peak intensity, since more SFA is present in the solution, peaks for Al-SFAox (7 ppm) and Al-SFAox$_2$ (12 ppm) are dominant (Figure 13.2). Also, increasing the pH from 3 to 5 causes peak intensities to shift because increasing pH promotes aluminum binding with SFA to form complexes. At higher concentrations and pH, precipitates create a problematic situation. At this time, investigation is still ongoing to determine methods to account for loss of aluminum and SFA due to precipitates in solution. A stability constant K can be defined as in Eq. 13.2.

$$K = \frac{[ML]}{[M_f][L_f]} \tag{13.2}$$

Here, [ML] is the molar concentration of a metal-ligand complex, [$M_f$] is the molar concentration of free metal and [$L_f$] is the free ligand molar concentration in the equilibrium system. $^{27}Al$ NMR allows a direct measure of [ML] and [$M_f$]. However, some calculations are necessary to obtain [$L_f$].

**Table 13.2** Speciation (in mM) of $Al^{3+}$(1 mM)-SFA(1000 mg/L)

|          | $Al^{3+}$ (0 ppm) | Al-SFAox (7 ppm) | Al-SFAox$_2$ (12 ppm) | Al-SFAox$_3$ (17 ppm) | Total Observed |
|----------|---------|---------|---------|---------|---------|
| pH 3.0 | 0.67 | 0.31 | 0.00 | 0.00 | 0.98 |
| pH 4.0 | 0.43 | 0.39 | 0.10 | 0.00 | 0.92 |
| pH 5.0 | 0.17 | 0.22 | 0.18 | 0.00 | 0.57 |

**Table 13.3** Speciation (in mM) of $Al^{3+}$(5 mM)-SFA(7500 mg/L)

|          | $Al^{3+}$ (0 ppm) | Al-SFAox (7 ppm) | Al-SFAox$_2$ (12 ppm) | Al-SFAox$_3$ (17 ppm) | Total Observed |
|----------|---------|---------|---------|---------|---------|
| pH 3.0 | 2.01 | 1.80 | 0.63 | 0.00 | 4.49 |
| pH 4.0 | 1.30 | 1.30 | 0.87 | 0.00 | 3.42 |
| pH 5.0 | 0.23 | 0.52 | 0.83 | 0.41 | 2.00 |

Based on our observations from the NMR data presented above and previously [19], Al-SFA binding can be described by oxalic acid type binding sites. We define L as a bidentate oxalic acid binding site on the soil fulvic acid. For our sample of soil fulvic acid, it has been reported that there are 8.2 carboxyl groups (meq)/soil fulvic acid (g) [15]. Since one oxalic binding site requires two carboxyl groups (bidentate), we can then calculate the total potential oxalic binding sites [$L_T$] on the fulvic acid from the mass in grams of fulvic acid in our solutions. To obtain free ligand concentration [$L_f$], ligands already complexed with metals are subtracted from the total oxalic binding site concentration as shown in Eq. 13.3.

$$[L_f] = [L_T] - [ML] - [ML_2] - [ML_3] \tag{13.3}$$

Also, because fulvic acid is a weak acid, the degree of acid dissociation $\alpha$ is important. Our model is a bidentate oxalate ligand. Hence, acid dissociation can be described by a pH-dependent $\alpha_2$, Eq. 13.4 [24].

$$\alpha_2 = \frac{K_{a1}K_{a2}}{[H^+]^2 + [H^+]K_{a1} + K_{a1}K_{a2}} \tag{13.4}$$

The acid dissociation constants assuming a diprotic SFA, $K_{a1}$ ($4 \times 10^{-3}$) and $K_{a2}$ ($2 \times 10^{-5}$) have been reported previously [15] and can be used to calculate acid dissociation for pH 3, 4 and 5. In addition, since $\alpha_2$ [L$_f$] represents the diprotic dissociated portion of the total free ligand concentration, the pH-dependent diprotic stability constant $K_1$ can be calculated from Eq. 13.5.

$$K_1 = \frac{[ML]}{[M_f]\alpha_2[L_f]} \tag{13.5}$$

Similarly, the step-wise second stability constant $K_2$ can be calculated as Eq. 13.6. The stability constants obtained are summarized in Table 13.4.

$$K_2 = \frac{[ML_2]}{[ML]\alpha_2[L_f]} \tag{13.6}$$

**Table 13.4** Comparison of stability constants calculated from experimental $^{27}$Al NMR and graphically approximated from Scatchard plots and Langmuir isotherms

|  | pH 3.0 | | pH 4.0 | | pH 5.0 | |
| --- | --- | --- | --- | --- | --- | --- |
|  | Log $K_1$ | Log$K_2$ | Log $K_1$ | Log $K_2$ | Log $K_1$ | Log $K_2$ |
| NMR | 3.6 | 2.8 | 2.8 | 2.4 | 2.5 | 2.4 |
| Scatchard | 2.4 | n/a | 3.0 | 2.7 | 3.5 | 3.6 |
| Langmuir | 2.8 | n/a | 2.6 | 3.5 | 3.7 | 4.0 |

$K_1$ is larger than $K_2$ since it is easier for aluminum to be bound with one SFAox than with two SFAox$_2$ as the ligands may hinder each other. Both $K_1$ and $K_2$ are lower than expected, which may occur because of the removal of aluminum-SFAox complexes (ML, ML$_2$ and ML$_3$) by precipitates in solution and as pH approaches 5, forming hydrolysis products that can affect the binding affinity of aluminum, which in turn causes the denominator in the stability equation to be higher and K to be smaller. Also, the acid dissociation constants ($K_{a1}$ and $K_{a2}$) used in this calculation were published [15] almost thirty years ago, so the accuracy of these numbers may be questionable. Nevertheless, preferential binding with aliphatic sites on SFA by aluminum is demonstrated.

Scatchard plot and Langmuir regression analyses were also applied to graphically deduce conditional stability constants for comparison. They are also summarized in Table 13.4. The results are in general slightly larger than the theoretical calculations and agree with each other within a reasonable discrepancy. The small difference may result from the preliminary and limited nature of the data sets, the fact that precipitates are forming at pH 5 and the relatively small concentration range used. Both Scatchard and Langmuir regressions have been traditionally applied to determine the total bound versus free or unbound metal to organic species [25,26]. Here, since we have a measure of each bound species, we can plot each bound species versus free metal to determine a conditional stability constant for that species. Having done this, the slope of the Scatchard plot is actually positive instead of negative. This suggests that in this concentration range the cooperative nature of the binding between sites is positive. In other words, the affinity of one site is affected by the affinity of other sites, which is expected since the aliphatic oxalic type of site on SFA needs to be spatially close enough to bind with aluminum. Interestingly, the slope of the Langmuir isotherm is also opposite to traditional regressions, showing a negative slope. The details of these observations are still under investigation.

## 13.4. CONCLUSIONS

Solution state $^{27}Al$ nuclear magnetic resonance spectroscopy is demonstrated to be a powerful tool in investigating the binding site environment of aluminum ions with soil fulvic acid. Four distinct peaks were observed in an Al-SFA system, namely free $Al^{3+}$ (0 ppm), aluminum ion bound with one oxalic acid type binding site Al-SFAox (7 ppm), aluminum ion bound with two oxalic acid sites Al-SFAox$_2$ (12 ppm) and aluminum ion bound with three oxalic acid sites Al-SFAox$_3$ (17 ppm). Stability constants were calculated from the experimental results and were compared with results obtained by Scatchard and Langmuir regressions. They agreed with each other reasonably well despite the limited number of data points. Currently, we are conducting experiments to minimize effects from precipitates formed in solution. In the future, we plan to investigate other fulvic acids using $^{27}Al$ NMR to better understand the binding behavior of aluminum in the natural environment.

## ACKNOWLEDGEMENTS

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