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Chapter 9

Fluorescence Spectroscopic Studies of Al–Fulvic Acid Complexation in Acidic Solutions

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The complexation interaction between a soil fulvic acid (SFA) and aluminum (III) ion has been studied using several different types of fluorescence measurements. Excitation and emission scans have been employed along with total luminescence, fluorescence lifetime and fixed wavelength experiments. Titrations of SFA with Al^{3+} at fixed pH values of 4.00 and 5.00 as well as pH titrations were used to examine the unique fluorescence behavior of humic material complexes of aluminum. SFA was found to exhibit both fluorescence quenching and enhancement effects upon complexation of Al^{3+} depending on the pH and wavelengths employed. A pronounced shift in the fluorescence spectral maximum to longer excitation wavelengths and shorter emission wavelengths was observed for the Al-SFA complex. Striking similarities between the fluorescence behavior of the model compound salicylic acid and SFA in the presence of Al^{3+} were clearly evident.

Molecular fluorescence has been shown to be a valuable tool for studying the binding of certain metal ions to both isolated humic materials (1-4) and natural organic matter present in samples collected from lakes, rivers and other bodies of water (5,6). Paramagnetic metal ions tend to reduce or quench fluorescence which has been demonstrated for Cu^{2+} (1-5), Co^{2+} (4,6), Mn^{2+} (4), Ni^{2+} (1), Fe^{3+} and Fe^{2+} (7) with various samples of humic materials. Diamagnetic metal ions, on the other hand, may quench, show no effect or even enhance humic material fluorescence depending on the metal, the source of the humic material and other experimental factors (1,8-13)

A review of the published literature for Al^{3+}-humic interactions reflects variations in fluorescence behavior (8-12,14,15). Sposito and co-workers (11,12) concluded that there was a quenching effect for aluminum ion complexed with a chestnut leaf litter extract (LLE). Philpot et al. (14) also reported fluorescence quenching of humic acid (Aldrich) upon complexation with aluminum. However, unlike these studies, Plankey and Patterson (8-10) found that the fluorescence intensity

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of fulvic acid was increased by the addition of aluminum ions. According to recent work by Luster et al. (15), natural organic matter (LLE) complexed with Al^{3+} can either decrease or increase the relative fluorescence intensity depending on the wavelength at which the measurements are made.

Humic materials have various binding sites with different complexation properties. The actual complexation interaction between Al^{3+} and humic material depends on the structural and conformational chemistry of the individual molecule, and the arrangement of functional groups for each site. Complexation with a metal ion changes the electronic polarization of both the metal ion and the binding site. This change could result in a fluorescence intensity increase or decrease at a specific emission wavelength, which is dependant upon the fluorescence properties of that particular binding site. The fluorescence spectrum is an overall result of the combination of intensities of all fluorophores versus the wavelength. Fluorophores associated with binding sites will also be affected by the presence of a metal ion, the properties of that metal ion, pH and other factors.

Therefore, it is possible to observe the fluorescence intensity increase at one emission wavelength and decrease at another wavelength when the humic material is bound to Al^{3+} or possibly other metal ions. Both kinds of fluorescence effects may be useful in calculating conditional stability constants (K) and the concentration of aluminum binding sites (C_L) on the humic material (11). The rigidity of the molecular structure may increase due to a small, highly charged cation like Al^{3+} binding with humic material. This may possibly increase the fluorescence quantum yield by reducing other possible nonradiative transitions (16).

According to the initial results reported here, a fluorescence enhancement effect for humic material binding with aluminum can be observed, while under different conditions a quenching of fluorescence predominated. Another very interesting fluorescence phenomenon described here is that after complexation with Al^{3+}, the maximum intensity of the humic material fluorescence shifts to longer excitation wavelengths and to shorter emission wavelengths in an excitation emission matrix (EEM).

This work is a preliminary investigation into the factors that influence changes in the fluorescence of a particular soil-derived fulvic acid (SFA) upon complexation with Al^{3+}. Conditions that produce quenching, enhancement and shifting of the fluorescence are examined. Fluorescence lifetime measurements of SFA and Al-SFA were also conducted and compared to previously reported results. In addition, Al^{3+} complexation with the model compound salicylic acid (SA) is examined. Significant evidence indicates that SA type sites are important for metal complexation in humic materials (17). Interestingly, SA has similar fluorescence properties to humic materials and undergoes fluorescence enhancement and a wavelength shift upon complexation with Al^{3+}.

Experimental

Apparatus. A Mark I Spectrofluorometer (Farrand Optical Co., Inc.) was utilized to collect fluorescence data for the experiments involving fluorescence titrations of SA

or SFA and EEMs of SFA. Data was collected and processed with Lab Calc and Grams 386 software packages (Galactic Industries Corp., Salem, NH) running on IBM compatible personal computers. All fluorescence measurements were performed in standard 10 mm quartz cells at room temperature except for the titrations of salicylic acid and soil fulvic acid which were done at 25 °C with a constant temperature water bath (model 1150, VWR Scientific, Boston, MA) and a jacketed titration cell (EG&G Princeton Applied Research, Princeton, NJ). An Orion 960 Autochemistry System (Orion Research, Boston, MA) coupled with an Orion 91-02 glass combination pH electrode was used to measure the pH of all solutions.

The instrument used for both SFA pH titrations and SA EEM experiments was a Perkin-Elmer model MPF-44B spectrofluorometer (Perkin-Elmer Corp., Norwalk, CT). The excitation and emission spectra were obtained by using an SLM-Aminco 48000 phase shift spectrofluorometer (Milton Roy Co., Urbana, IL).

Fluorescence lifetimes were measured by time-correlated single photon counting using a mode-locked, synchronously pumped, cavity-dumped pyridine I dye laser (343 nm) or Rhodamine 6G dye laser (290 nm). Emissive photons were collected at 90° with respect to the excitation beam and passed through a monochromator to a Hamamatsu Model R2809U microchannel plate. Data analysis was made after deconvolution (18) of the instrument response function (FWHM ~ 80 ps).

Reagents. Solutions of salicylic acid (Fisher Scientific Co., Pittsburgh, PA) were prepared by dissolving the powdered reagent in dilute NaOH followed by neutralization of the excess base with $HClO_4$. Salicylic acid stock solutions were 1 mM and freshly made for each series of experiments. The soil fulvic acid used in these studies was obtained from Dr. James H. Weber, Department of Chemistry, University of New Hampshire. The isolation and characterization of SFA (19-21) and its metal ion binding and fluorescence properties (1-4) have been reported previously. Aluminum solutions were prepared from two sources; dissolving Al metal wire (J.T. Baker Co., Phillipsburgh, NJ) in $HClO_4$ or dissolving aluminum perchlorate (Alfa/Johnson Mathey, Ward Hill, MA) in deionized water. Sodium hydroxide and perchloric acid were purchased from Fisher Scientific Company. The ionic strength of the solutions in both EEM and fluorescence titration experiments for SA and SFA was made up with 0.1 M $NaClO_4$ (Aldrich Chemical Co., Milwaukee, WI). Both SFA and SA solutions were filtered through 0.4 μm pore size filters after adjustment to the pH of the experiment.

Results and Discussion

Monitoring the fluorescence of a 15 mg/L soil fulvic acid solution while adding aliquots of an Al^{3+} solution at pH 4.00 gives rise to the data shown in Figure 1. A distinct enhancement of fluorescence occurs from the initial value, set at 100%, to a maximum of more than 180% (Figure 1). Using non linear regression to fit a one site binding model to the data as discussed in a previous chapter (22) provides a conditional stability constant (K) of 6.2×10^5 (log K=5.79) and a concentration of

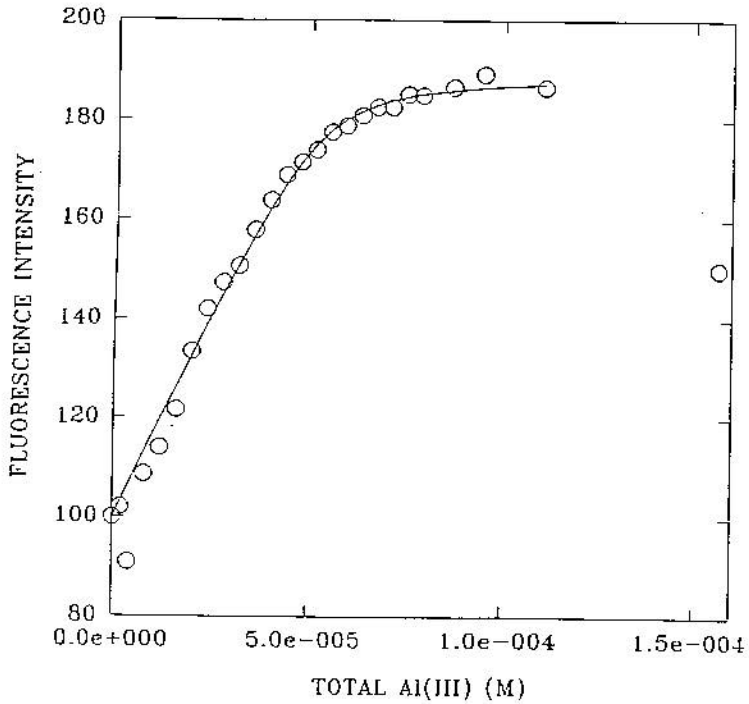

Figure 1. Fluorescence enhancement titration curve for 15.0 mg/L soil fulvic acid in 0.1 M $NaClO_4$ at pH 4.00 and 25 °C; (◯) measured fluorescence. Line is SigmaPlot fitted curve excluding last titration point. The fluorescence is monitored at an excitation wavelength of 360 nm and an emission wavelength of 420 nm.

SFA ligands sites of 50.6 μM. The solid line in Figure 1 shows the best fit of the model to the data.

SFA and Al-SFA pH Titrations. Changing the pH of the SFA solution to 5.00 and the excitation wavelength to 335 nm causes fluorescence quenching to occur when Al^{3+} is added as illustrated in Figure 2. The fluorescence emission spectrum (Figure 2) exhibits reduced fluorescence at nearly all wavelengths when Al^{3+} is present. Similar results are observed at higher pH as well. These results indicate that both pH and fluorescence wavelengths may be important in determining whether fluorescence enhancement or quenching are observed.

In order to study the effect of pH on the fluorescence of SFA and Al-SFA complexes, pH titrations were performed. Figure 3 shows the fluorescence intensity of two solutions containing of 15 mg/L SFA or 15 mg/L SFA with 200 μM Al^{3+} as the pH is varied from 2 to 10. The solution of SFA alone shows a modest increase in fluorescence from pH 2 to a maximum around pH 5 and then a decrease as pH is increased further (1). The solution containing Al^{3+} and SFA exhibits a slight increase from pH 2 to 3 and then a dramatic decrease in fluorescence (i.e., quenching) as pH is increased from 3 to 5.

This behavior is very likely caused by increased complexation of Al^{3+} as pH goes up. The Al-SFA curve in Figure 3 levels off between pH 5 and 7 where strong complexation of Al^{3+} by SFA produces a maximum fluorescence quenching or minimum fluorescence emission at these wavelengths.

As the pH is further increased above pH 7, fluorescence increases and approaches nearly the same intensity value observed at low pH (no Al^{3+} binding) which is the same intensity in the absence of Al^{3+} (Figure 3). The reason for this increase in fluorescence at high pH is probably the hydrolysis of Al^{3+} to various aluminum hydroxides. As the Al hydroxides form, Al-SFA complexes are broken up and little or no free Al^{3+} is available at pH 10 to bind with SFA and cause fluorescence quenching.

Fluorescence Peak Maxima. Figure 4 shows a total luminescence spectrum or excitation emission matrix (EEM) for 15 mg/L SFA in the form of a contour plot. Contour lines give the fluorescence intensity at essentially all values of excitation wavelength from 290 nm to 390 nm and all values of emission wavelength from 391 nm to 491 nm. The peak maximum is observed at an excitation wavelength of 335 nm and an emission wavelength of 446 nm. When 2×10^{-4} M Al^{3+} is added at pH 4 the intensity increases and the peak maximum moves to an excitation wavelength of 350 nm and an emission wavelength of 436 nm as shown in Figure 5.

The observed changes in the SFA peak maximum of 15 nm for excitation wavelength and 10 nm in emission wavelength are clearly significant. Although the cause of this change is not understood at this time, it's existence may have an effect on whether quenching or enhancement is observed. If fluorescence measurements are made at wavelengths near the 350 nm excitation and 436 nm emission maximum observed in the presence of Al^{3+}, then enhancement should be observed. Measurements made at other wavelengths may show lesser enhancement or even quenching.

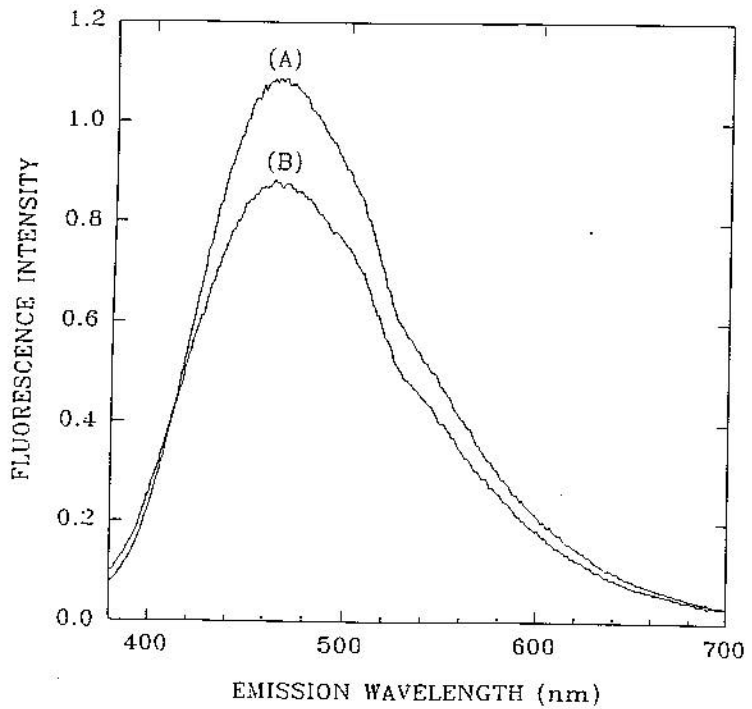

Figure 2. Fluorescence emission spectra of 100 mg/L soil fulvic acid at pH 5.00 with (A) no aluminum and (B) 100 μM Al^{3+}. The excitation wavelength is 335 nm.

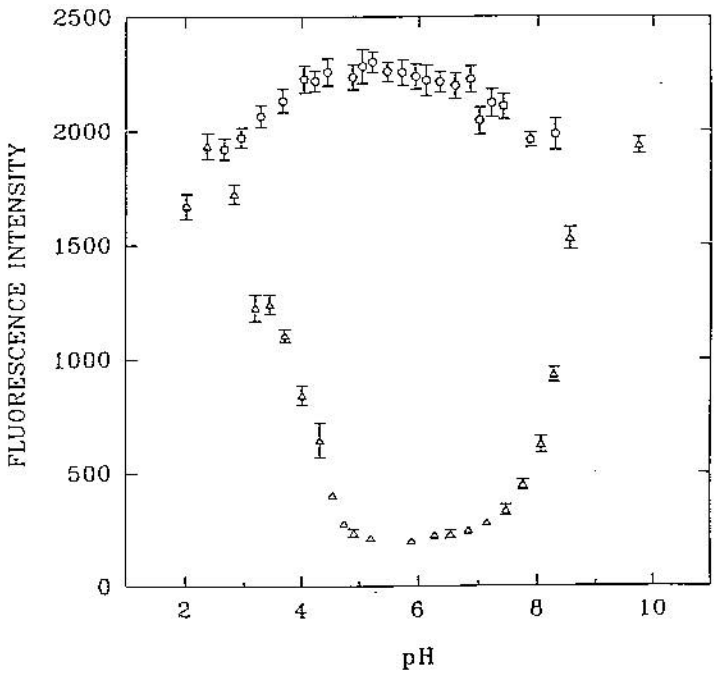

Figure 3. pH titrations of soil fulvic acid solutions with fluorescence monitored at an excitation wavelength of 340 nm and an emission wavelength of 455 nm. The samples are: (◯) 15 mg/L soil fulvic acid; (△) 15 mg/L soil fulvic acid with 200 μM Al(III) with error bars.

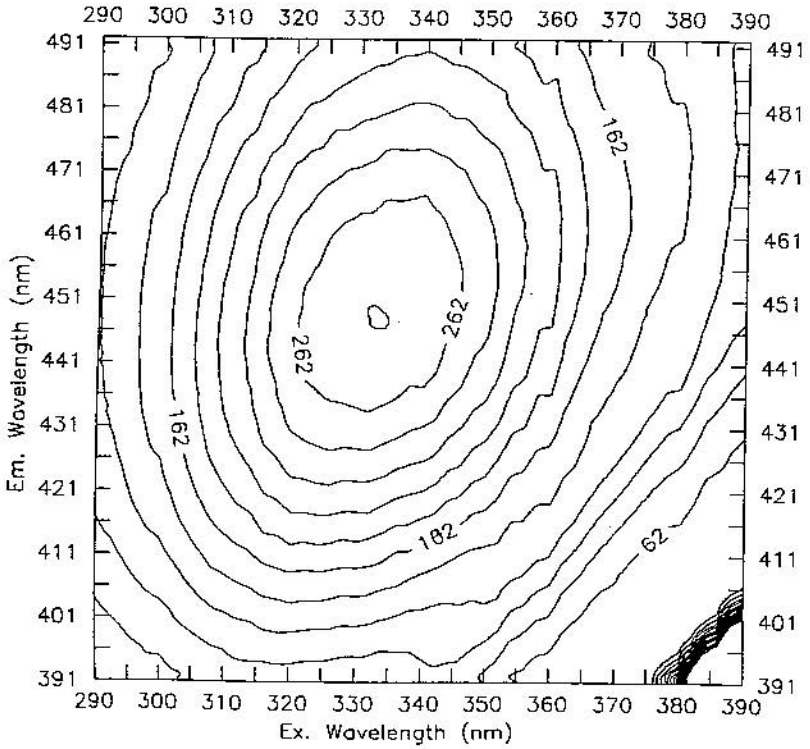

Figure 4. Excitation emission matrix contour plot of 15.0 mg/L soil fulvic acid in 0.1 M $NaClO_4$ at pH 4.00. The contour lines give fluorescence intensity in arbitrary units.

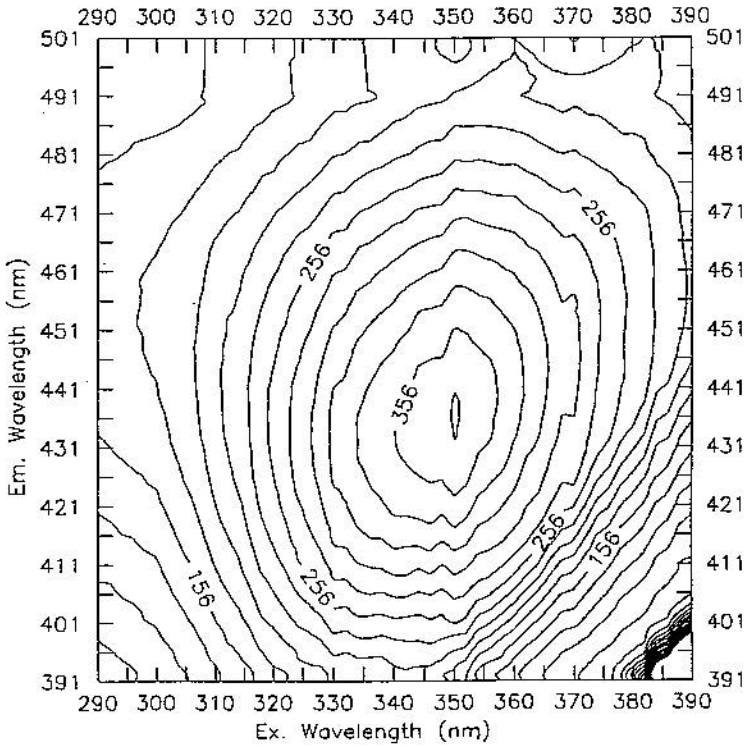

Figure 5. Excitation emission matrix contour plot of 15.0 mg/L soil fulvic acid with 2.00 x 10^{-4} M Al(III) in 0.1 M $NaClO_4$ at pH 4.00.

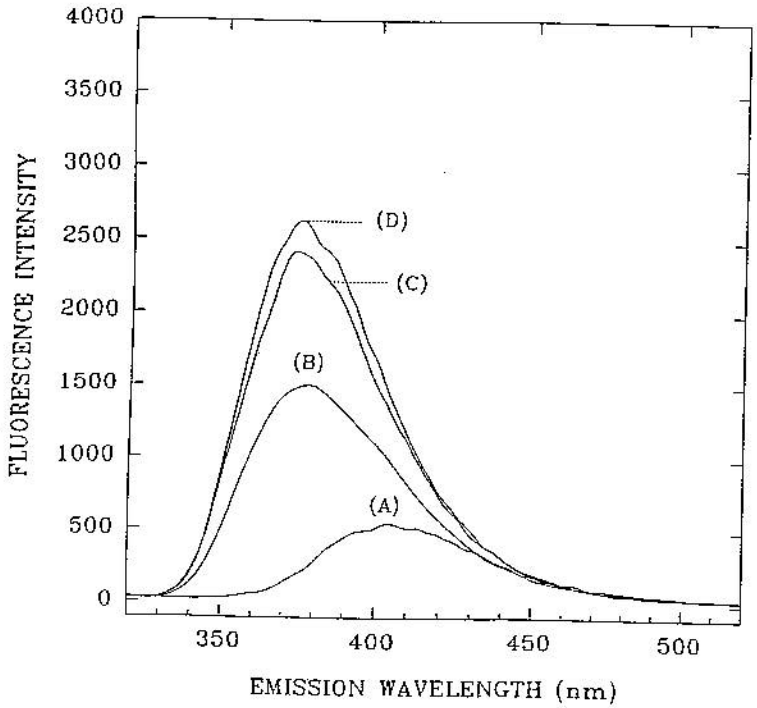

Figure 6. Emission spectra for (A) 10 μM salicylic acid (SA), (B) 10 μM SA and 20 μM Al(III), (C) 10 μM and 60 μM Al(III), and (D) 10 μM SA and 100 μM Al(III) at pH 4.00. The excitation wavelength is 317 nm.

Extrapolation of these results to other humic materials should be done with caution as they may or may not exhibit analogous behavior. However, experiments with the model compound salicylic acid under similar conditions showed nearly identical results. Enhancement of fluorescence and peak shifting are both exhibited in the salicylic acid spectrum show in Figure 6. At a fixed excitation wavelength of 317 nm, curve A (Figure 6) gives the emission spectrum of SA alone prior to any wavelength shift. The wavelength maximum for curve A is very close to 405 nm. When Al^{3+} is added at a concentration of 20 μM and the Al-SA complex forms, the peak shown in curve B of Figure 6 is not only increased approximately threefold, but its maximum also moves to less than 380 nm. Curve B also exhibits a very small shoulder near 400 nm resulting from the uncomplexed SA still remaining in solution. Further additions of Al^{3+} giving concentrations of 60 μM (curve C) and 100 μM (curve D) cause additional enhancement and clearly establish the wavelength maximum at 375 nm (Figure 6).

The overall shift in the fluorescence maximum of SA upon complexation of Al^{3+} is best seen by comparing the EEMs in figures 7 and 8. Figure 7 shows a SA fluorescence peak maximum at pH 4.00 of 301 nm for the excitation wavelength and 408 nm for the emission wavelength. When 60 μM Al^{3+} is added at pH 4.00, the resulting Al-SFA complexes give rise to a dramatically shifted peak maximum at 312 nm excitation and 381 nm emission wavelengths (Figure 8). This wavelength change is more than 10 nm in excitation wavelength and in excess of 25 nm in emission wavelength. Any quantitative work relying on fluorescence measurements of SA in the presence of Al^{3+} must carefully take into account this change in fluorescence wavelengths upon complexation. Researchers designing fluorescence experiments to measure the complexation of Al^{3+} by SA or SFA must also be cognizant of this phenomenon and make careful selection of monitoring wavelengths in order to measure the desired effect.

Fluorescence Lifetime Measurements of SFA. The time dependent decay, S(t), of SFA fluorescence can be described by three exponential terms, and the following equation fit to the data

$$S(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + A_3 e^{-t/\tau_3}$$

where τ_1, τ_2 and τ_3 are the fluorescence lifetimes and A_1, A_2 and A_3 are the relative preexponential terms for the three components. The results obtained in this study are compare to the work of others in Table I. As stated by Cook and Langford (23), direct comparison of these three lifetimes should be done cautiously since they are a minimum set of parameters to fit the decay curves within experimental error. The lifetimes of SFA may be better described as a distribution of lifetimes.

All three component lifetimes became longer after SFA complexed Al^{3+}, but the first component (shortest lifetime) almost tripled in length due to the binding. The first component also had a smaller preexponential term, whereas the exponential terms of the other two components became larger for the Al-SFA complex. This indicates that the aluminum binding is similar for those fluorophores represented by compo-

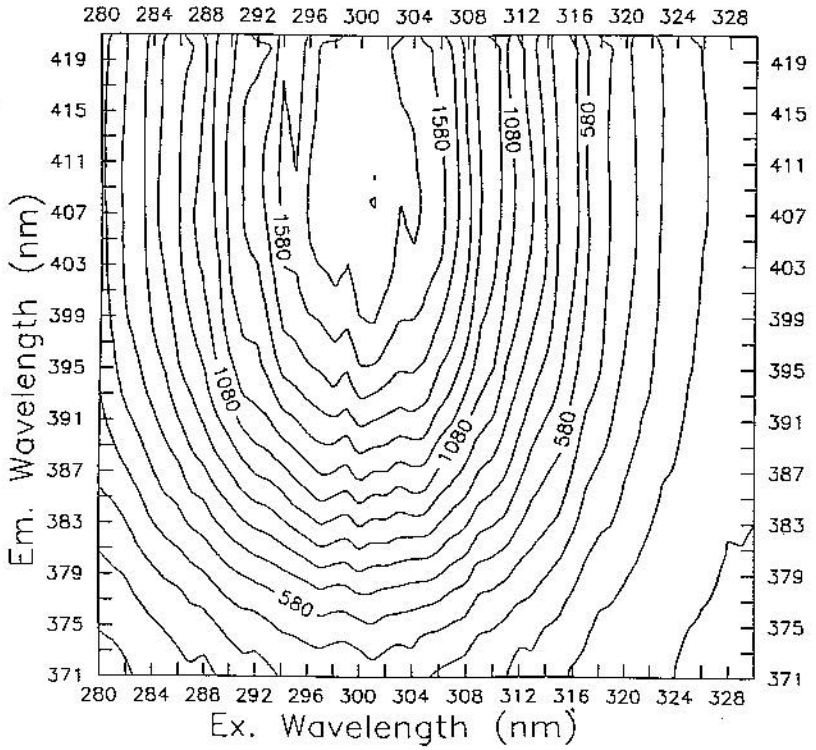

Figure 7. Excitation emission matrix contour plot of 10.0 μM salicylic acid in 0.1 M $NaClO_4$ at pH 4.00.

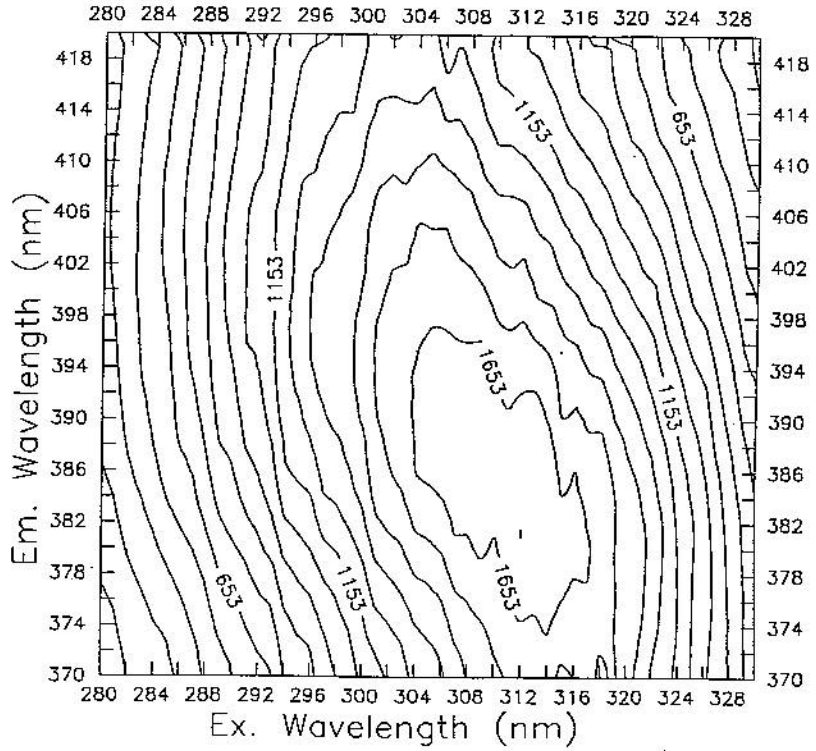

Figure 8. Excitation emission matrix contour plot of 10.0 μM salicylic acid with 60 μM Al(III) in 0.1 M $NaClO_4$ at pH 4.00.

nents 2 and 3, and the fluorophores of component 1 have distinguishably different binding properties.

Fulvic acids from different sources show a similar trend of having three components present in the fluorescence decay curves (Table I). The SFA used for this study and the Armdale FA have similar lifetime distributions. Laurentian FA, on the other hand, shows rather unique lifetime properties.

Table I. Fluorescence lifetime measurements of three fulvic acids (FA) and Al-soil fulvic acid (SFA).

	Lifetimes (ns) and Preexponential Terms						
	First Component		Second Component		Third Component		
Sample	Life-time	Pre-exponent	Life-time	Pre-exponent	Life-time	Pre-exponent	Reference
SFA	.205	.59	1.63	.30	8.17	.11	this study
Al-SFA	.593	.49	2.32	.37	8.49	.14	this study
Armdale FA	.2	.86	2.00	.11	7.00	.03	24
Laurentian FA	.05	.66	.430	.20	4.20	.14	23

Lifetime measurements of SA and the Al-SA complex gave essentially the same values of 5.4 and 5.3 ns, respectively, with an excitation wavelength of 290 nm and an emission wavelength of 400 nm. The errors on the fluorescence lifetime values given in Table I are ± 0.1 ns for the first and shortest lifetime, ± 0.2 ns for the second and ± 0.5 ns for the third.

Conclusion

The fluorescence intensity of SFA in the presence of Al^{3+} shows both quenching and enhancement behavior depending primarily on the wavelength used and to some extent the pH. The model compound salicylic acid exhibits very similar behavior showing wavelength shifts of more than 10 nm for excitation and 25 nm for emission upon complexation of Al^{3+}. Fluorescence lifetime measurements also show changes between complexed and uncomplexed SFA with the most significant change observed for the shortest lived fluorescence component.

One possible explanation of the data presented here is that fluorescence normally observed in the absence of aluminum is quenched as Al^{3+} complexes are formed. Simultaneously a new fluorescence peak for the complex may appear at slightly shifted wavelengths. The complex may have a higher quantum efficiency and therefore show greatly enhanced fluorescence. This hypothesis may be useful in explaining the fluorescence behavior of both SFA and SA in the experiments described here.

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