

# Effect of Experimental Parameters on the ESI FT-ICR Mass Spectrum of Fulvic Acid

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**Fulvic acid (FA) is a heterogeneous mixture of organic macromolecules found in the waters, soils, and sediments of the earth's surface. The ability of electrospray ionization (ESI) to effectively transfer large ions from the solution phase to the gas phase and the coupling of ESI to the high-mass-resolution capabilities of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) provide a potential method for the mass spectrometric analysis of FA. Positive- and negative-ion ESI FT-ICR MS analyses of four reference International Humic Substances Society FAs were performed. The spray solution composition was found to have a dramatic effect on the ion distributions, with high-mass aggregates ( $m/z \sim 2000$ – $4000$ ) being formed in less polar spray solutions. Positive-ion spectra for each FA obtained under optimum conditions resulted in number-average molecular weights ranging from 1700 to 1900. The mass spectra were extremely complex, with ion distributions on the order of  $m/z \sim 500$ – $3000$ . The presence of more than one ion at each nominal mass was routinely observed. Negative-ion ESI analysis of the FA samples resulted in the observation of multiply charged ions whose distributions could be affected by the acidification of the spray solution. Solution parameters which have been reported to affect molecular weight distributions of FA such as pH, ionic strength, and concentration of multivalent cations were found to have little or no effect on the observed  $m/z$  distributions.**

Humic substances are polyelectrolytes produced by the biotic and abiotic alteration of plant and animal tissue during the early diagenesis of organic matter found in the soils, sediments, and waters of the earth's surface. They are divided into three operationally defined fractions: humin, which is insoluble in an aqueous solution at all pH values; humic acid, which is soluble in alkaline aqueous solutions; and fulvic acid, which is soluble in an aqueous solution at any pH value.

Fulvic acid is generally believed to exhibit the greatest degree of aromatic character, the highest acidic functional group content (e.g., carboxylic acid functional groups), and the lowest average molecular weight (typically 800–1200) and is the most polar of the three humic fractions.<sup>1–5</sup> Because fulvic acid is soluble in water

and has the lowest average molecular weight, and ostensibly is the simplest, on a molecular level, of the three humic fractions, its molecular properties were chosen for intensive study and characterization.<sup>6</sup> For these reasons, fulvic acid was also chosen for characterization in the study described here.

The characterization of humic materials is fraught with analytical and philosophical difficulties. Elemental analysis,<sup>7</sup> <sup>13</sup>C NMR spectroscopy,<sup>8</sup> and infrared<sup>9</sup> spectroscopy, among other methods, have been used to evaluate the nature of humic materials. These methods, however, do not give information about specific components but instead describe an "average" characteristic of the bulk material. It is becoming widely accepted that these substances cannot be represented by a discrete chemical structure but are, in fact, best viewed as complex mixtures.<sup>9,10</sup> And while this view can be used to explain many of the functions of humic materials in a natural environment, and even some of their properties,<sup>10</sup> it poses significant problems when the objective of an investigation is to obtain information about the components of a humic mixture.<sup>9</sup> For example, the interpretation of data obtained from most methods of chemical characterization is based on the assumption that the material under study is a *pure substance*. The apparent fact that humic materials are, by their very nature, complex mixtures makes it exceedingly difficult to obtain information about the individual components of these mixtures from chemical characterizations. Even <sup>13</sup>C NMR spectroscopy, so powerfully applied in other areas of chemical investigation, has been relatively limited in the scope of its application to the study

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of humic materials.<sup>11</sup> It has been primarily employed to determine the relative percentages of aliphatic, aromatic, carbohydrate-type, or carboxyl carbons present in a particular sample.<sup>12</sup> An obvious way to obtain information about the components of these mixtures would be to subject a particular humic fraction to some separation method prior to or during its characterization.

As alternatives to the chromatographic separation of the components of a mixture, separations based on the mass or energy differences of molecules could be used to isolate individual components. The separation of charged molecules, or molecular fragments, based on mass or energy differences is the basis of mass spectrometry. However, the high molecular weight and polarity of fulvic acid make it difficult to transfer the sample into the vapor phase using conventional sample introduction techniques (for example, a direct-insertion probe or gas chromatography). Consequently, almost all mass spectral studies of unaltered humic or fulvic acids (that is, underivatized humic substances or humic materials which have not been subjected to chemical degradation procedures) have been performed by pyrolysis mass spectrometry.<sup>13–16</sup> Using pyrolysis mass spectra and multivariate statistical analysis, MacCarthy et al.<sup>13</sup> have shown that fulvic acid and humic acid could be distinguished from each other. It should be pointed out, however, that such distinctions can usually be made from elemental analysis, infrared spectroscopy, or <sup>13</sup>C NMR spectroscopy alone.

The development of desorption ionization techniques (e.g., fast atom bombardment, laser desorption, and matrix-assisted laser desorption) and electrospray ionization (ESI) has extended the applicability of mass spectrometry to the study of high-mass materials.<sup>17–20</sup> Previously, we reported a significant extension of the mass ranges observed for fulvic acid by mass spectrometry with laser desorption ionization.<sup>21,22</sup> In fact, the mass ranges observed in this study were higher than any recorded in the mass spectrometric analysis of humic acid, which is believed to be a higher molecular weight material than FA.<sup>1–5</sup> However, laser desorption analyses were highly dependent upon the laser wavelength and power density employed, resulting in poor reproducibility in the measurements. The extent of FA fragmentation during the desorption–ionization process could not be determined.

Like laser desorption, electrospray ionization is a “soft” ionization technique, in the sense that it results in little, if any, fragmentation of the analyte molecules during desorption and

ionization. Electrospray ionization has been found to transfer almost any molecule carrying a net charge in solution to the gas phase.<sup>23</sup> The ESI process involves the formation of small, highly charged droplets that undergo solvent evaporation with subsequent ion transfer to the mass spectrometer. The phenomenon occurs with high ionization efficiency.<sup>23</sup> These characteristics, and the ability to be coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), which has the capability for ultrahigh-mass resolution, suggest that this technique may be a powerful analytical tool in the characterization of complex mixtures such as fulvic acid. The purpose of this paper is to examine the effect of ESI spray solution conditions and spray solution composition on the mass spectrum of fulvic acid obtained by ESI FT-ICR MS.

## EXPERIMENTAL SECTION

**Instrumentation.** Electrospray ionization FT-ICRMS experiments were performed at the National High Magnetic Field Laboratory (Florida State University) using a home-built Chait-style source<sup>24</sup> interfaced to a 9.4 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR) equipped with an open-ended Penning trap.<sup>25</sup> A complete description of the instrument is given elsewhere.<sup>26</sup> Ions were externally accumulated in a 60 cm rf-only octapole ion guide for 10 s before transfer to the ion cell. The ions were transferred via a second, 2 m long, octapole to the ion source. Spectra were collected with varying transfer times to evaluate the time-of-flight effect imposed by the octapole path length.

**Effect of Transfer Time.** Optimization of ion transfer time to minimize the time-of-flight effect imposed by transferring ions through the second octapole to the ion cell can be achieved either through the time allowed for transfer or through the voltage applied to accelerate the ions through the octapole.<sup>27</sup> In preliminary positive-ion experiments, assessment of the spectra led to the adoption of a transfer time of 2.0 ms in all subsequent experiments because this value appeared to maximize ion intensity over the entire ion mass distribution. In the negative-ion mode, the time-of-flight effect was more effectively optimized through transfer voltage than through transfer time. The optimal transfer voltage was determined to be –5 V and was used in all subsequent experiments.

**Samples.** The International Humic Substances Society (IHSS) reference fulvic acids were utilized in this study. The source material preparation<sup>28</sup> and fulvic acid extraction procedure<sup>29</sup> are described elsewhere. Chemical characteristics of peat (PFA), soil (SFA), Suwanee River (SuFA), and Nordic aquatic FA (NaFA)

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samples are given by Novotny.<sup>30</sup> While data are usually presented only for the PFA sample, discussions regarding the general appearance of spectra and the effect of solution variables on the spectra are applicable to all spectra except as noted.

**Positive-Ion ESI. (a) Sample Preparation and Introduction.** Stock sample solutions were prepared for analysis as 1% 5 mg FA per milliliter/2% acetic acid/30% H<sub>2</sub>O/67% methanol (MeOH) (v/v) solutions. The H<sub>2</sub>O and MeOH used in the sample preparations were of Aldrich Optima grade and were used without further purification. Acetic acid was doubly distilled. The ion spray was produced from a 0.6  $\mu$ L/min flow from a 0.25 mm i.d. capillary. Ion current for the FA samples ranged from 700 to 850 pA under these spray conditions. When necessary, flow rate was adjusted to increase or decrease ion current. The following source voltages were used: needle, 3000 V; capillary, 72 V; tube lens, 400 V; skimmer, 20 V. The capillary current used for resistive heating was 2.3 A. (At the time these experiments were performed, it was not possible to monitor capillary temperature and current was used instead. In preliminary experiments, we found that this parameter had a small effect on the ESI process.) Chirp excitation and ion detection were performed from  $m/z$  80 to  $m/z$  5000. Instrument performance was evaluated prior to FA sample analysis by acquiring the spectrum of bovine serum ubiquitin.<sup>31</sup> Spectra were processed and are presented using a Hanning apodization.<sup>32</sup> Number-average molecular weight ( $M_n$ ) and weight-average molecular weight ( $M_w$ ) values were calculated from spectra as described by Cooper.<sup>33</sup>

**(b) Spray Solution Composition.** Solution composition and pH have been found to affect the apparent molecular weight distribution of fulvic acid obtained using other characterization techniques.<sup>34</sup> Spray solutions of varying concentrations of MeOH and H<sub>2</sub>O were prepared to assess whether the solution composition, in particular its polarity, had any effect on the mass distribution of the observed FA ions. The solutions studied included 30:70, 50:50, and 70:30 volume percent ratios of H<sub>2</sub>O:MeOH. To evaluate the effect of ionic strength, sample solutions of FA were prepared for ESI as described above with NaCl added to give ionic strengths of 0.0001, 0.001, 0.01, and 0.1 M without altering the FA concentration. Sodium hydroxide was added to produce solutions of pH 2, 4, 6, and 8, which were used to examine the effect of pH on the observed ion distributions. The effect of FA concentration on the  $m/z$  charge distribution was examined using solution FA concentrations of 0.3, 1.0, 1.7, and 2.3 mg/mL. The effect of the concentration of a divalent cation was assessed by the addition of calcium chloride to stock FA sample solutions. Calcium ion concentrations of 60 and 120 mg/L were evaluated.

**Negative-Ion ESI. (a) Sample Preparation and Introduction.** The source voltages employed in negative-ion ESI analyses were equal in magnitude to those employed in the positive-ion

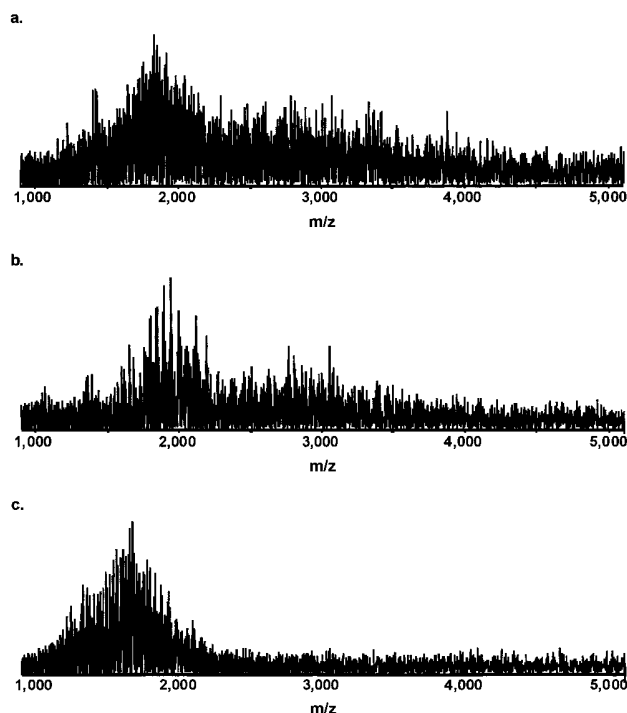


Figure 1. Effect of spray solution composition on the positive-ion spectra of PFA. Compositions are volume percents: (a) 30% H<sub>2</sub>O/70% MeOH; (b) 50% H<sub>2</sub>O/50% MeOH; (c) 70% H<sub>2</sub>O/30% MeOH. All spectra represent 64K data points.

analyses but were of opposite polarities. Oxygen was used as a scavenger gas to prevent corona discharge in the ion spray.<sup>35</sup> A flow rate of 0.8  $\mu$ L/min was employed. Samples were prepared for negative-ion analysis by mixing 32  $\mu$ L of a 5 mg/mL FA sample with 1 mL of a 50:50 isopropyl alcohol (IPA):H<sub>2</sub>O solution (v:v). The IPA was of Aldrich Optima grade and was used as received. The resulting solutions had pH values that ranged from 4.5 to 5.0.

**(b) Spray Solution Composition.** To observe the effect of pH on the negative-ion distribution, the PFA sample solution was acidified by addition of concentrated HCl to give a sample pH of 2. The sample was analyzed at a reduced flow rate of 0.6  $\mu$ L/min to reduce spray "sputtering" due the high electrolyte concentration.<sup>36</sup>

To determine the charge state in the negative-ion analyses, a region of the negative-ion distribution of PFA was selected by SWIFT excitation/ejection.<sup>37</sup> The  $m/z$  580–605 region was chosen for study because it was the lowest  $m/z$  region that had significant ion signal after the SWIFT ejection of unwanted ions (resolution decreases with increasing  $m/z$ ).

## RESULTS

**Positive-Ion ESI.** Figure 1 displays the effect of varying amounts of methanol in the spray solution on the PFA spectrum.

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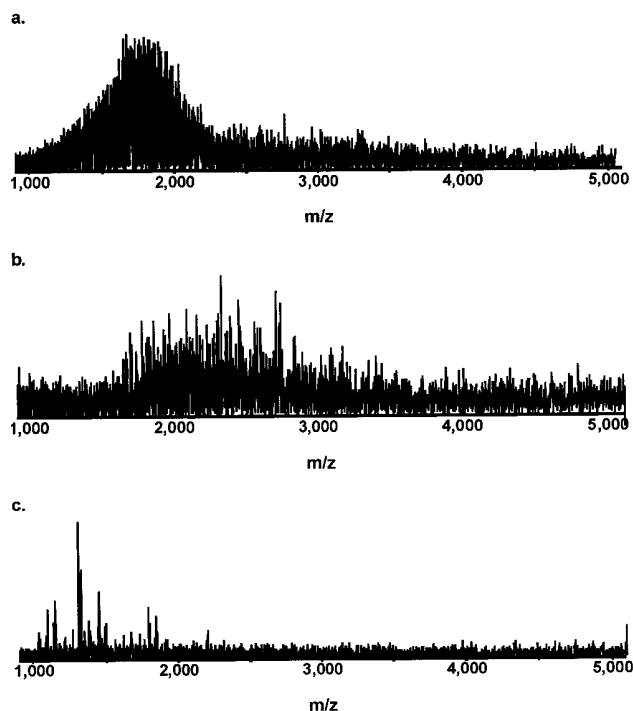


Figure 2. Effect of ionic strength (i.e., NaCl concentration) on the  $m/z$  distribution in positive-ion spectra of PFA. Ionic strengths: (a) 0.0001 M; (b) 0.001 M; (c) 0.01 M.

It is evident that varying the solution composition alters the ion distribution with an increasing percentage of water decreasing the presence of high-mass ions ( $m/z > 2500$ ). The 70:30 spray solution resulted in the most stable ion signal and as a result was utilized in all subsequent positive-ion analyses.

Figure 2 displays the effect of increasing ionic strength on the  $m/z$  distribution of PFA. With increasing ionic strength, there is a decrease in overall ion intensity. The disappearance of the distribution centered at  $m/z \sim 1800$  in 0.01 M NaCl is accompanied by the appearance of a low-intensity, slightly higher mass distribution which could be consistent with FA aggregation resulting from charge screening in high ionic strength solutions.<sup>38</sup> An alternative explanation could be cationization of FA components at high ionic strengths. However, blank ESI spray solutions with ionic strengths corresponding to 0.01 M NaCl displayed ion distributions that were essentially identical to those of the corresponding PFA-containing solutions (e.g., Figure 2c). Closer inspection of the sample and blank spectra suggests that the ions measured under high ionic strength conditions are primarily those of solvent-salt adducts. Thus, ESI sample introduction at higher ionic strengths is not possible because of aggregation of the FA which occurs under these conditions and/or the effect of high salt concentrations on the spray process.

Figure 3 shows the effect of pH on the  $m/z$  distribution of PFA. The spectra of the pH 2 and 4 spray solutions have very different ion distributions. Evaluation of a blank spray solution at pH 4 (Figure 3c), prepared in an identical manner but without the addition of PFA, shows that the ion distributions at pH 4 are due to salt-solvent adduct ions, as was observed in the ionic strength studies at higher NaCl concentrations in the spray

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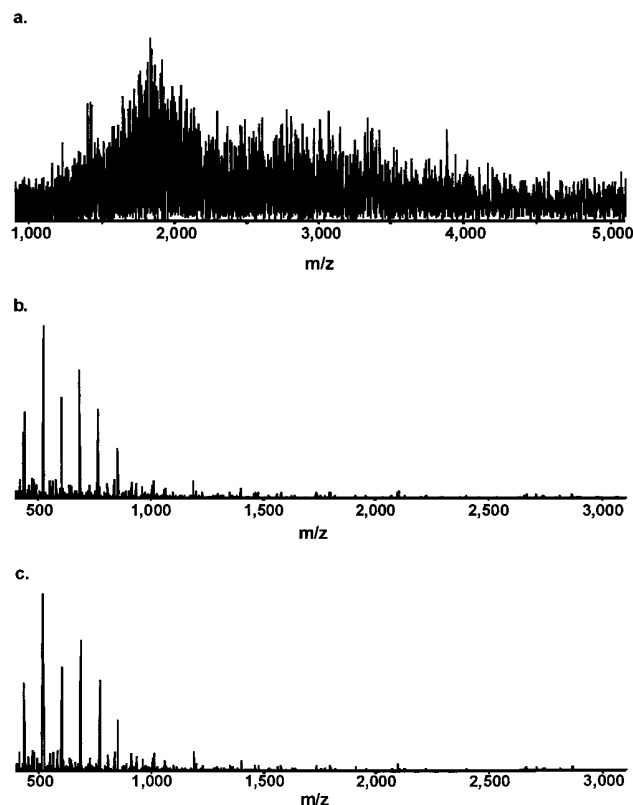


Figure 3. Effect of pH on the  $m/z$  distribution in positive-ion spectra of PFA. Spectra are presented for (a) pH 2, (b) pH 4, and (c) the blank spray solution at pH 4.

solutions. We conclude that the spectrum acquired at pH 2 is representative of PFA under these conditions. Spectra acquired at pH 6 and 8 (not shown) are essentially identical to the spectrum obtained at pH 4 and the spectrum of the pH 4 blank solution.

A 7-fold increase in the sample concentration appeared to have little effect on the  $m/z$  distribution of the ions observed. Despite solution FA concentrations that are high enough to induce aggregation,<sup>39,40</sup> the presence of high-mass ions was not apparent in the spectra. It appears that the forces responsible for the association of these ions are not strong enough to survive the ESI process.

The ion  $m/z$  distribution of PFA in the presence of  $\text{Ca}^{2+}$  did not appear to shift upon the addition of 60 mg/L  $\text{Ca}^{2+}$ . The  $M_n$  and  $M_w$  values calculated from these spectra confirm that there is no measurable change in ion distribution upon the addition of  $\text{Ca}^{2+}$ . When the  $\text{Ca}^{2+}$  concentration was increased to 120 mg/L, the ion signal was erratic due to spray sputtering resulting from the high salt concentration in the solution. Thus it is difficult to draw any conclusions about the effect of the  $\text{Ca}^{2+}$  on the  $m/z$  distribution, even though it appears that the ion mass distribution may shift to higher  $m/z$  values with 120 mg/L added  $\text{Ca}^{2+}$ .

**Negative-Ion Spectra.** Isolation of ions in the  $m/z$  580–605 region for PFA was accomplished through SWIFT ejection (Figure 4a). Figure 4b shows that there are several ions present at every

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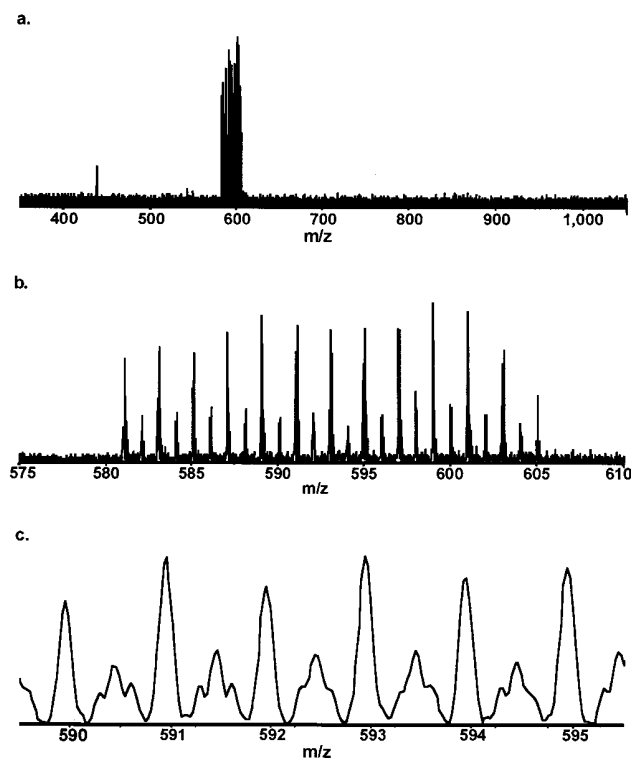


Figure 4. SWIFT-selected region in the negative-ion mass spectrum of PFA: (a) entire observed mass range; (b) selection of ions within an  $m/z$  575–610 window; (c) selection of ions within an  $m/z$  590–595 window.

nominal mass in this region. It also appears that there is a distribution of more intense ions overlapping a distribution of less intense ions. This every-other peak pattern is unusual and may be due to the presence of chloride adducts or a series of molecules that differ by their degree of saturation. And if FA, and humic materials in general, are as heterogeneous as has been proposed,<sup>9–10,41–43</sup> this pattern may simply reflect the molecular diversity in this conceptualization of these substances. Expanding this mass range even further (Figure 4c), it is evident from the broad distributions of ion peaks found between the nominal mass units that multiply charged species are present.

Figure 5 shows the effect of pH on the distribution of negative ions in the PFA spectrum. The addition of HCl to the sample solution results in a decrease in ion signal as well as an apparent shift toward higher  $m/z$  values in the ion distribution. The reduction in signal is most likely due to both the addition of the electrolyte, which results in a poor ion spray, and the neutralization of some of the acidic groups present in the FA sample, which results in some of the ions being neutralized.

**ESI FT-ICR Mass Spectra of Fulvic Acids.** The positive-ion and negative-ion spectra acquired using what we have concluded are optimum conditions for the characterization of each IHSS reference fulvic acid are given in Figures 6 and 7, respectively.

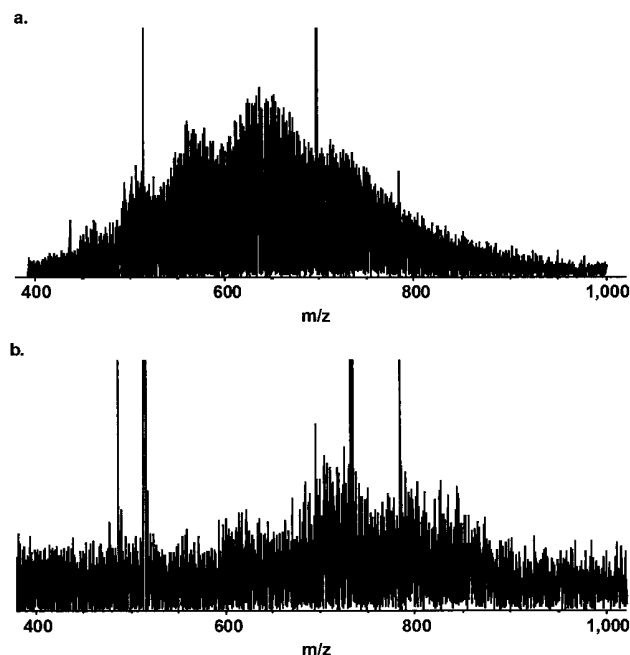


Figure 5. Effect of pH on the  $m/z$  distribution in negative-ion spectra of PFA. Spectra are presented for (a) pH 4.5 and (b) pH 2. The intense mass peak at  $m/z$  ~700 is due to an impurity in the spray solution.

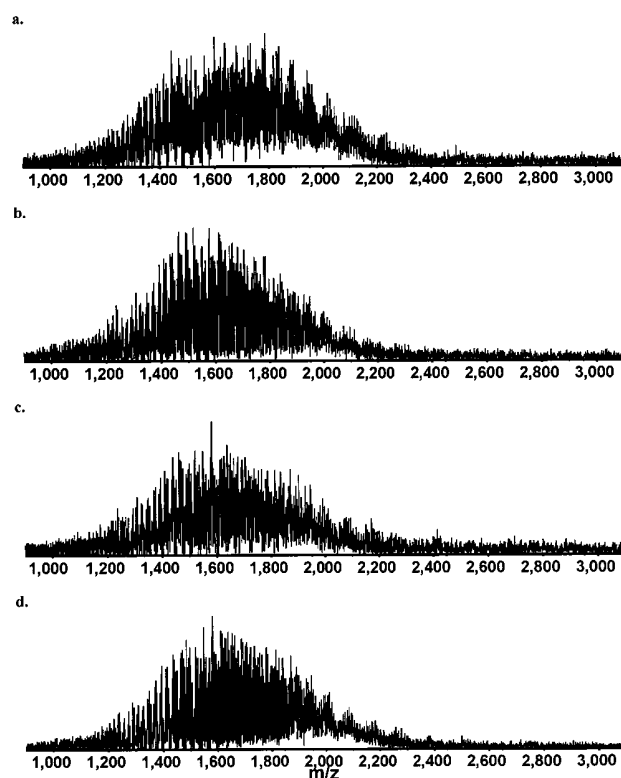


Figure 6. Positive-ion ESI FT-ICR mass spectra of IHSS reference fulvic acids acquired under optimized conditions: (a) NaFA; (b) PFA; (c) SFA; (d) SuFA. Spectra are produced from 15 summed acquisitions.

The apparent number-average molecular weight values calculated from these spectra are given in Table 1. The calculated number-average molecular weights were found to be reproducible within 5% of the average value calculated from replicate analyses.

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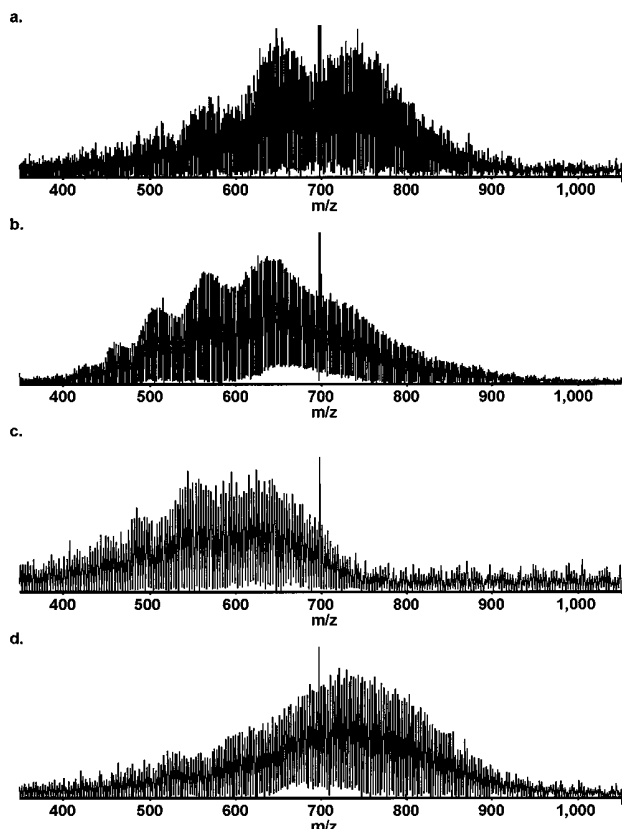


Figure 7. Negative-ion ESI FT-ICR mass spectra of IHSS reference fulvic acids acquired under optimized conditions: (a) NaFA; (b) PFA; (c) SFA; (d) SuFA. Spectra are produced from 15 summed acquisitions. The intense mass peak at  $m/z \sim 700$  is due to an impurity in the spray solution.

Table 1. Apparent Number-Average ( $M_n$ ) and Weight-Average ( $M_w$ ) Molecular Weights Calculated for the FA Samples from Replicate and Summed (15 Scans) Positive-Ion and Negative-Ion Spectra<sup>a</sup>

sample	$M_n$	$M_w$	sample	$M_n$	$M_w$
NaFA			SFA		
(+) ion	1917	1921	(+) ion	1843	1860
(-) ion	658	669	(-) ion	574	581
VPO	1042		VPO	839	
PFA			SuFA		
(+) ion	1875	1901	(+) ion	1908	1924
(-) ion	625	633	(-) ion	699	714
VPO	2377		VPO	768	

<sup>a</sup> The spray solution is 70% H<sub>2</sub>O/30% MeOH (v/v). Values are reproducible to  $\pm 5\%$ . Sample vapor pressure osmometry (VPO) number-average molecular weights reported by Novotny<sup>30</sup> are given for comparison purposes

## DISCUSSION

**Positive-Ion Spectra.** Application of ESI to the characterization of fulvic acid has produced higher mass distributions than ever before obtained by mass spectrometry for these materials or, for that matter, for any humic material. In analyzing the electrospray ionization conditions for FA in the positive-ion detection mode, we have noted the following. First, the most stable sample ions were produced under the simplest spray conditions, where FA was in a predominantly aqueous solution. Second, the

addition of electrolytes to the spray solution resulted in a sporadic spray. The masses observed under these conditions were typically those of cationized species found in the solvents used to prepare the spray solutions. The ability of these cationized background ions to "swamp" the sample ions is indicative of the low ion intensities observed for the components of FA. Given the heterogeneous nature of FA, low ion intensities for FA ions could be expected as a result of the low abundance of any given species in the mixture of components which comprise FA. The presence of salt in the spray solution will suppress analyte ion intensity because the electrospray current is carried by the salt. This leads to a further diminution in FA ion intensity. Fundamental studies of the electrospray process have investigated these phenomena extensively.<sup>36</sup>

Due to the cationization of solvent/background species during experimental studies that required the addition of electrolyte, the effect of ionic strength and pH on the ion distribution of FA could not be unambiguously determined. Sporadic spray resulting from high salt concentrations made it difficult to assess the effect of the presence of a divalent cation on the FA mass at concentrations approaching those found in a natural environmental system. Smith et al.<sup>36</sup> have reviewed this phenomenon.

The presence of high-mass ions was observed in less polar spray solutions probably because these solutions were less effective in ion solvation and shielding. As a result, oppositely charged ionic sites on various FA molecules may have interacted to stabilize the ions in the solution. The interactions produced by stabilization would then have to be strong enough to survive the electrospray ion formation process. Many noncovalent species have been successfully transferred from solution to the gas phase by ESI.<sup>19–23</sup>

Under spray conditions which resulted in more stable ions, the  $m/z$  distributions were highly reproducible from scan to scan for the various samples analyzed. As a result, enhancement of the signal-to-noise ratio resulting from scan summing allowed for the measurement of low-abundance ions with higher  $m/z$  ratios. The number-average molecular weight values determined for the FA samples with 15 accumulated scans in the 70:30 spray solution are similar to the values determined for the samples by other commonly employed techniques such as vapor pressure osmometry (VPO). From Table 1 it is evident that the values determined by ESI are more similar from sample to sample than those determined by VPO. It is difficult to assess at this time whether this is an artifact of the ESI spray process (favoring the formation of certain types of ions) or is truly indicative of the samples' molecular makeup.

In general, fulvic acid's molecular weight is thought to increase in the following order as a function of the environment in which it was formed: aquatic < soil < peat < lignite. This is a reflection of the increased contributions of lignin and cellulose to FA in soils, the reduced microbial metabolism of lignin and cellulose in the acidic and anoxic environment present in a peat bog, and the increased condensation that occurs during the diagenesis of peat to produce lignite. The positive-ion  $M_n$  values in Table 1 do not reflect these trends. We believe that this may be explained, at least in part, by the inherent difficulties in the VPO characterization of FA samples.<sup>44–46</sup> Molecular weight determination by VPO is affected by the extent of dissociation of the analyte molecules

because the dissociated proton is counted as a particle, decreasing  $M_n$ . This is not an issue in ESI. Additionally, the application of VPO is limited by the low solubility of FA in the aprotic solvents (DMF, DMSO, etc.) that are typically used in VPO analyses.

The ESI results presented here indicate that we have at least achieved stable sizes even if we cannot report that the values in Table 1 are "the"  $M_n$  values of these fulvic acids. We believe that because ESI is unaffected by the solution variables that plague VPO, the positive-ion ESI  $M_n$  values in Table 1 are better representations of number-average molecular weights for FA.

**Negative-Ion Spectra.** The negative-ion analyses reported here resulted in molecular weight averages from 570 to 700 for the FA samples (Table 1). It should be noted that the potential presence of multiply charged ions identified in Figure 4 limits the use of these molecular weight averages as such. With this caveat in mind, use of the average molecular weight values in Table 1 becomes a convenient method to characterize the distributions presented in Figures 6 and 7 in order to describe trends between the positive-ion and negative-ion modes and between samples.

The formation of multiply charged negative ions in the ESI experiment is consistent with the polycarboxylic acid nature that is usually attributed to FA.<sup>4-6,34</sup> Perdue<sup>46</sup> has shown that essentially all carboxylic acids will dissociate at pH 8, resulting in the presence of carboxylate anions in the sample solutions. Thus, multiply charged anionic species will exist in solution regardless of the pH at which the analysis is conducted.

Many models<sup>1,4-6,47-51</sup> typically conclude that there are multiple carboxylic acid groups per FA "molecule". Because the positive-ion and negative-ion results differ by a factor of approximately 3,

it is tempting to speculate that these results indicate that each fulvic acid "molecule" has, on average, three ionizable functional groups. At least one study has concluded something similar on the basis of a titrimetric analysis.<sup>50</sup> It should be pointed that the spectra alone do not provide proof of this postulate, despite the observation that the formation of multiply charged negative ions in the ESI experiment would be predicted on the basis of the polycarboxylic acid nature of FA. A plausible alternative explanation for the differences between the positive- and negative-ion experiments is that the spectra in Figures 6 and 7 are displaying the signature of entirely different components of FA.

## SUMMARY

Electrospray spray-solution composition and mass spectrometric conditions are presented for the characterization of the IHSS reference fulvic acids by ESI FT-ICR MS. Low ionic strength aqueous spray solutions were found to be preferable. Positive-ion spectra give  $M_n$  values similar to those reported by VPO. Number-average molecular weights calculated from the negative-ion spectra are significantly lower than those obtained from the corresponding positive-ion spectra. The molecular weight distributions reported in this study should be viewed as a general indication of the mass distributions of the components of fulvic acid since the presence of multiply charged ions cannot be ruled out with the data presented here.

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