The application of electrospray ionization mass spectrometry (ESI MS) to the structural characterization of natural organic matter

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Abstract

This report describes the application of electrospray ionization (ESI) mass spectrometry to the structural characterization of soil organic material, a critical component of environmental processes and the global carbon cycle. Quadrupole time-of-flight (QqTOF) mass spectrometry provided a routine screening of aqueous ions in humic and fulvic acid mixtures and MS/MS capabilities for selected ions. Fourier transform ion cyclotron resonance (FT–ICR) mass spectrometry required longer analysis time but achieved resolving powers $> 80,000$ and mass accuracies of < 1 ppm, which allowed exact molecular formula determination for selected peaks. This technique represents a significant advance in the identification of compounds within humic substances. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Soil is a complex biogeochemical system that is a component of numerous global processes (e.g. carbon and nitrogen cycling, biological productivity, and erosion). The chemistry of organic matter in this system is dependent on the interactions of biological material with mineral substrates and refractory soil organic matter (SOM) known as humic material. The inherent difficulties in defining the structure of individual components of humic material derive from the fact that they are macromolecular (>500 Daltons), polar in nature, and not easily examined by instrumentation designed to provide detailed chemical structures. Significant progress has been made recently in the structural determination of large biomolecules (e.g. proteins) using "soft" ionization

techniques such as electrospray ionization (ESI). In ESI, polar hydrophilic macromolecules are de-solvated and charged prior to acceleration into mass spectrometers. This approach appears promising for structural characterization of humic substances. Recent reports have shown that humic substances can be ionized readily, but the mass spectrometric data are very complex with multiple peaks at every m/z (Novotny et al., 1995; Fievre et al., 1997; McIntyre et al., 1997; Solouki et al., 1999; Brown and Rice, 2000; Klaus et al., 2000; Persson et al., 2000). Furthermore, insufficient resolution and mass accuracy has limited the determination of exact molecular formulas.

McIntyre et al. (1997) first demonstrated the application of ESI MS to the analysis of organic materials found in drinking water. Fievre et al. (1997) used ESI combined with an ultrahigh resolution Fourier transform ion cyclotron resonance (FT–ICR) mass spectrometer to evaluate the molecular weight distribution of humic and fulvic acids isolated from the Suwannee River, GA. However, the sheer complexity of the mixture prevented Fievre et al. (1997) from obtaining ultrahigh

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resolution mass spectra. Nonetheless, they were able to enhance the resolution by using high performance liquid chromatography (HPLC) to separate fractions of the humic and fulvic acids prior to analysis. In a more recent study, Brown and Rice (2000) selectively isolated narrow mass ranges of electrospray-generated positive ions of humic acids with tandem FT–ICR MS to obtain high resolving power. However, the reported resolution was still insufficient to assign accurate molecular weights and subsequently molecular formulas for the observed ions. To date, there have been no studies of the structural characteristics of humic acids using a quadrupole time-of-flight mass spectrometer.

Two mass analyzers are the focus of this manuscript, a quadrupole time-of-flight mass spectrometer (QqTOF MS) and the Fourier transform ion cyclotron resonance mass spectrometer. Each mass analyzer was coupled to an electrospray ionization source. The QqTOF MS consists of two quadrupoles coupled to a time-of-flight analyzer. For rapid screening analyses, the quadrupoles are operated in RF mode, allowing all ions to pass into the time-of-flight MS for analysis. The initial screening analysis requires little time (15 min or less) and the mass resolving power of $\approx 10,000$ is sufficient to determine the general mass distribution of compounds within each sample. The resolution achieved is greater than that of conventional triple quadrupole mass spectrometers. The FT–ICR MS consists of an external ESI source coupled to an ICR cell residing within a high magnetic field. Ions are accumulated in an external hexapole prior to acceleration into the ICR cell. Analysis time is often a function of sample complexity, with hours required for high resolution spectra for very complex mixtures. In general, though, FT–ICR mass spectrometry has the ability to achieve mass resolving powers $> 100,000$ routinely. MS/MS can be performed with both techniques on selected ions to elucidate exact molecular structure and formulas of particular compounds.

The increased sensitivity and resolution of FT–ICR MS are a result of the difference in detection methods between the ICR cell and the time-of-flight analyzer. In the time-of-flight analyzer, ions are directly detected through their collision with the detector plate at the end of the flight tube. In the ICR MS technique, ions are indirectly measured by the detection of cyclotron frequencies of the ions within the cell. The cyclotron frequency of each ion is a function of both the m/z of the ion and the magnetic field. Once a cyclotron frequency spectrum is attained from the Fourier transform of the time domain transient, it can be converted to a mass spectrum via a simple algebraic equation.

The inherent differences in the ion physics between the QqTOF analyzer and the ICR cell lead to different limitations in the mass spectra. The flight tube in the QqTOF has a wider mass range and constant mass resolving power across a wide m/z range. However,

minor components of complex mixtures with low absolute concentrations (e.g. high molecular weight material within humic or fulvic acids) are difficult to observe due to insufficient resolution. Averaging a number of QqTOF scans increases the signal-to-noise ratio of minor components but not significantly.

Within the ICR cell, ions are confined inside a three-dimensional ion trap in the ICR cell at very low pressure ($< 10^{-9}$ Torr) and at high magnetic field (> 3 T). The ion trap has a fixed volume and therefore has limited capacity for charged species. If the number of ions inside the cell increases beyond a certain limit, the performance of the instrument deteriorates (often called "space-charge" effects) (Marshall et al., 1998). To obtain an ultrahigh resolution mass spectrum, the ion number must remain low. During the analysis of complex mixtures, however, ion number increases rapidly as the complexity of the sample increases. For humic acid mixtures, the effect of "space charge" interactions becomes significant due to the high ion number necessary to observe a signal and as a result, only low-resolution spectra have been obtained (Marshall et al., 1998). In earlier reports, researchers attempted to reduce "space charge" effects by (1) the isolation of a selected range of ions prior to detection (Brown and Rice, 2000) and/or (2) chromatographic separation of the components prior to ionization and trapping (Fievre et al., 1997; Marshall et al., 1998). However, both procedures insufficiently reduced the ion number to the degree necessary to obtain ultra-high resolution and separate the isobaric ions present in the mass spectrum.

In this report, we have used ESI coupled with QqTOF MS and high resolution FT–ICR MS to screen a series of humic and fulvic acid samples. Several well-studied humic substances were examined by electrospray ionization coupled to the QqTOF mass spectrometer to demonstrate the applicability of the approach and to show that significant changes in structural characteristics are reflected in the "low-resolution" MS data obtained. The FT–ICR MS achieved higher resolution and was used to determine exact molecular formulas for mass analyzed peaks for two select samples: a humic acid extract of degraded wood (Hatcher, 1987) and dissolved organic matter (DOM) from Suwannee River, GA (Serkiz and Perdue, 1990).

2. Methods

2.1. Sample preparation

Five samples were examined in this study: humic acids from a degraded wood sample from Mt. Rainier, WA (Hatcher, 1987); DOM (primarily fulvic acids) from Suwannee River, GA (Serkiz and Perdue, 1990); humic and fulvic acid fractions from Armadale soil (Ogner and

Schnitzer, 1971; Matsuda and Schnitzer, 1972); and humic acids from a diluvial soil from Iwata, Japan (Matsuda and Schnitzer, 1972; Hatcher et al., 1989). Samples were provided by E.M. Purdue (Georgia Institute of Technology, Atlanta, GA—DOM) and M. Schnitzer (Agriculture Canada, Ottawa, Ontario–Armadale and diluvial soils). Each sample was obtained as dried, ash-free humic or fulvic acids. Stock solutions were made by dissolving the dried sample in either double-distilled water (Suwannee River DOM) or pH 8 NH_4OH to make solutions of 5 mg ml^{-1} (Suwannee River and Mt. Rainier) or 1 mg ml^{-1} (Armadale and diluvial). Stock solutions were diluted with methanol or isopropyl alcohol prior to mass analysis.

2.2. Instrument parameters: Qq-TOF MS

All experiments were performed on a Micromass Q-Tof™ II (Micromass, Wythenshawe, UK) mass spectrometer equipped with an orthogonal electrospray source (Z-spray) operated in positive ion mode. Polyalanine and alanine were used for mass calibration for the 100–2000 m/z range. Humic and fulvic acids were prepared in a 50:50 water: alcohol (methanol or isopropyl alcohol) solution and infused into the electrospray source at a rate of 5–10 μl min^{-1}. Optimal ESI conditions were: capillary voltage = 3 kV, source temperature = 110 °C and cone voltage = 60 V. The ESI gas was nitrogen. The first quadrupole, Q1, was set to pass ions from m/z 100–2000 and all ions transmitted into the pusher region of the TOF analyzer were scanned over m/z 100–3000 with a 1 s integration time. Data were acquired in continuum mode until acceptable averaged data were obtained (10–15 min).

2.3. Instrument parameters: FT–ICR MS

Two samples were chosen for further analysis by FT–ICR mass spectrometry. Analyses were performed on both a commercially available 7 T ESI FT–ICR mass spectrometer model Apex II 7e (Bruker, Billerica, MA) and a previously described 9.4 T ESI FT–ICR mass spectrometer (Marshall and Guan, 1996). Both were configured for external ion accumulation in the positive ion mode. For the 7 T ESI FT–ICR mass spectrometer, humic and fulvic acid samples were prepared in 25:75 water: methanol (1.25 mg ml^{-1}) and infused into a tapered 50 μm i.d. fused silica micro-ESI needle at a rate of 250 nl min^{-1}. Typical ESI conditions were: needle voltage = 2.4 kV and heated capillary temperature = 80 °C. Ions were accumulated in a linear hexapole ion trap for 1.1 s and then transferred to a 3″-Penning trap (1.5 V trapping voltage) by electrostatic ion transfer optics. Typical initial base pressure for the instrument was 9×10^{-10} Torr. A MIDAS data station controlled all experiments (Senko et al., 1996). Numerous scans (18,000–19,000) were accumulated to reduce signal-to-noise ratios

and increase resolution. The time-domain ICR signal was subjected to a baseline correction, Hanning apodization, and one zero fill before Fourier transform and magnitude calculation.

For the 9.4 T FT–ICR mass spectrometer, humic acid samples prepared in 50:50 water: methanol were infused into a tapered 50 μm i.d. fused silica micro-ESI needle at a rate of 300 nl min^{-1} and a concentration of 0.5 mg ml^{-1} for Mt. Rainier humic acid and 2.5 mg ml^{-1} for Suwannee River DOM. Typical ESI conditions were: needle voltage = 2.5 kV and heated capillary current = 2.5 A. Ions were accumulated in a linear octapole ion trap (operated at 1.8 MHz) for 10–30 s and then transferred to a 4″-Penning trap (2 V trapping voltage) through a second octapole ion guide (operated at 1.5 MHz). Typical initial base pressure for the instrument was 7×10^{-10} Torr. A MIDAS data station controlled all experiments (Senko et al., 1996). The time-domain ICR signal (average of 650 scans) was subjected to baseline correction followed by Hanning apodization and one zero-fill before Fourier transformation and magnitude calculation.

3. Results

All five samples were analyzed via ESI QqTOF MS and two (Mt. Rainier humic acid and Suwannee River DOM) were further analyzed using ESI FT–ICR MS. We will focus first on the samples analyzed with both mass analyzers to highlight the similarities and differences in the mass spectra generated by each technique. The Mt. Rainier humic acid spectra from both ESI mass analyzers are encouragingly similar (QqTOF MS in Fig. 1 and FT–ICR MS in Figs. 2 and 3). Both spectra are complex with peaks at almost every m/z. In the ESI QqTOF spectrum (Fig. 1), peaks associated with low molecular weight compounds dominate the vertical scale due to high resolution and narrow peak width. Peaks at higher molecular weight (> 600 m/z) are broader and most likely represent more than one compound. While the intense and visible signals at low m/z could be due to contaminants or specific molecules of exact composition present in significant but low amounts, the low-level signals at every nominal mass sum together to account for the majority of peak intensity throughout the mass range.

The general distribution of compounds in the 7 T FT–ICR mass spectrum (Fig. 2) is nearly identical to the ESI Qq-TOF spectrum with the exception of the 100–200 m/z range. The absence of peaks in this range in the FT–ICR spectrum has been observed previously in our lab and may be due to inefficient ion transfer and trapping of low m/z species in the FT–ICR experiment. The mass resolving power for low molecular weight compounds was 80,000 (at 321 m/z) for the 7 T instrument after 18,000 scans. Higher resolution was achieved with the
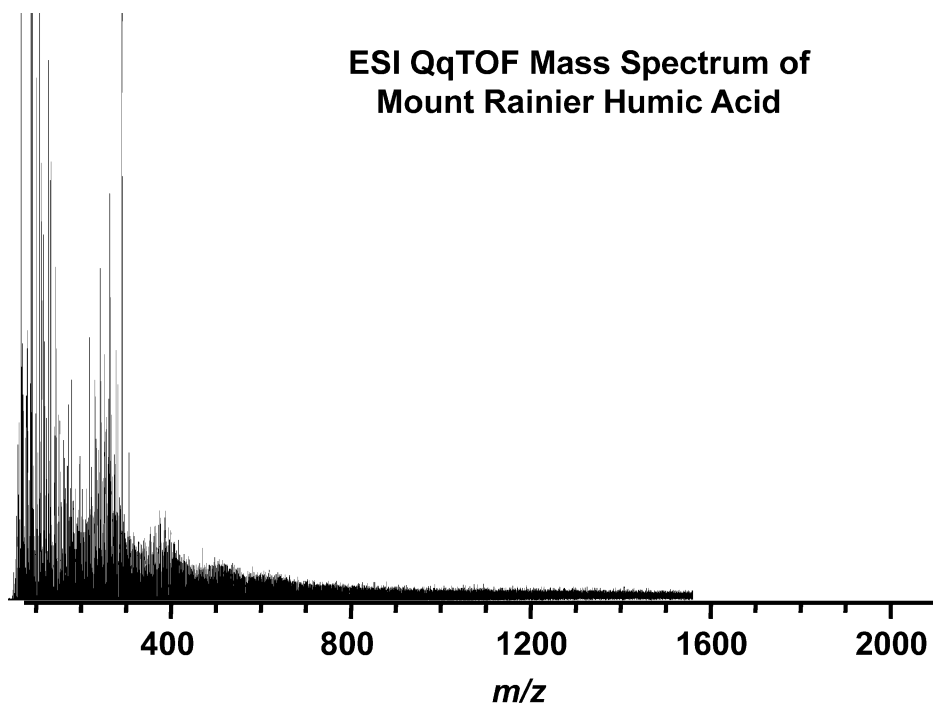
Fig. 1. ESI QqTOF positive ion mass spectrum of the humic acid extract of a degraded wood sample from Mt. Rainier, WA. The sample was prepared in 50:50 water: methanol at a concentration of 2.5 mg ml^{-1}. The spectrum represents the average of 300 scans.
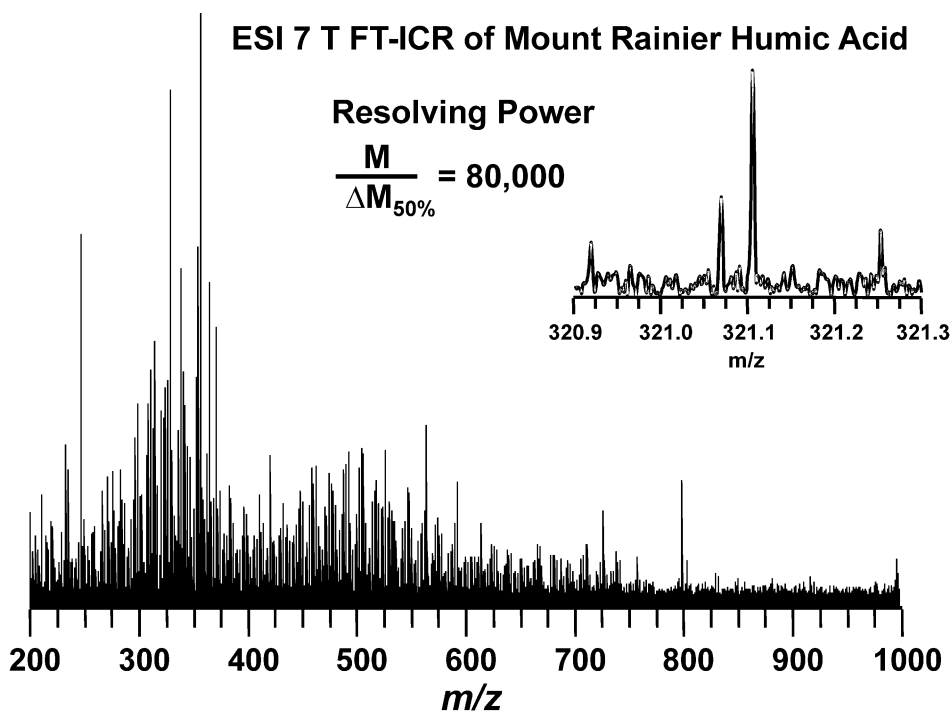

Fig. 2. ESI FT–ICR positive ion mass spectrum of the humic acid extract of a degraded wood sample from Mt. Rainier, WA, acquired on a 7 T FT–ICR MS. The sample was prepared in 25:75 water: methanol at a concentration of 1.25 mg ml^{-1}. The spectrum represents the average of 18,000 scans. The inset is an expansion of the region around 321 m/z where the mass resolving power was approximately 80,000.
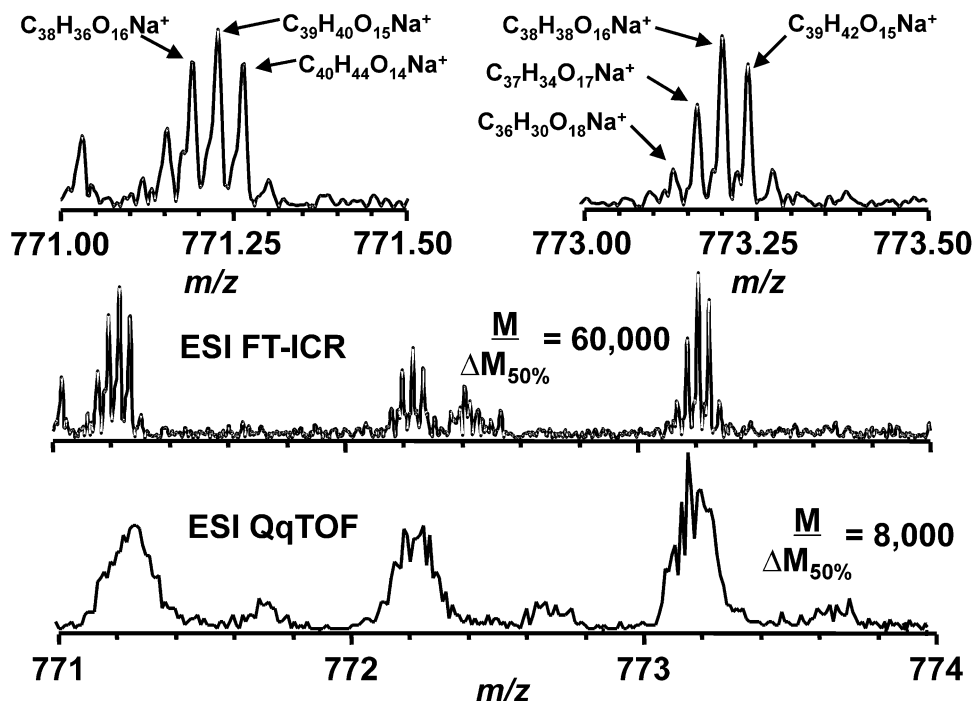
Fig. 3. Comparison of expanded spectra of Mt. Rainier humic acid. The ESI Qq-TOF spectrum (bottom) is an expansion of the 771–774 m/z range from Fig. 1. The ESI FT-ICR spectrum (middle) was acquired on a 9.4 T FT–ICR MS and the mass resolving power in this region was approximately 60,000. The top two mass spectra are further expansions of the FT–ICR spectrum. The resolution of peaks in these regions was sufficient to allow the assignment of unique molecular formulas derived from proposed structures (values and errors in Table 1).

9.4 T instrument after 650 signal transients (mass resolving power in excess of 100,000 at 500 m/z). An important characteristic of the spectra presented in Figs. 2 and 3 is that they were obtained *without* ion isolation or prior fractionation. Instead, we were able to achieve high resolution by reducing the number of ions trapped in the FT–ICR cell and performing extensive signal averaging.

The enhanced resolution of the FT–ICR MS over the QqTOF MS is evident when comparing small regions of each spectrum (e.g. 771–774 m/z in Fig. 3). Broad peaks present in the QqTOF mass spectrum are further resolved into clusters of discrete compounds by the 9.4 T FT–ICR MS. With mass resolving power of 60,000 in this region, all the species present in this mixture appear to be fully resolved. Using polyethylene glycol with an average molecular weight of 600 as an internal mass calibrant, accurate molecular weights for all the species observed in the mass spectrum were assigned with an average mass accuracy better than 1 ppm. Exact molecular masses were calculated for a series of hypothetical lignin oxidation products (specifically, oxidation of pendant alcohols at the α and γ carbons of lignin tetramer side chains) and compared to the experimentally observed molecular weight for selected components in the mixture (Table 1). Differences of approximately 1 ppm were considered to be positive identifications for

these selected compounds. Nitrogen was not included in molecular formula determinations because the sample was derived from nitrogen-free lignin.

The dissolved organic matter (DOM) sample from Suwannee River, GA was also analyzed using the 7 T FT–ICR MS (Fig. 4). This sample is comprised primarily of fulvic acids (Serkiz and Perdue, 1990). The mass spectrum is significantly more complex than that obtained for the humic acid sample from Mt. Rainier and there are multiple peaks at every nominal mass. Some of the peaks match those in the Mt. Rainier sample but a large fraction occur at higher mass defect, suggesting a source of aliphatic compounds that is not present in the Mt. Rainier humic acid.

Three other humic acid samples were examined with the ESI QqTOF MS; the diluvial humic acid sample from Iwata, Japan (Fig. 5) and the humic and fulvic acid fractions from Armadale soil (Fig. 6). In all three cases, the insets show solid-state ^{13}C nuclear magnetic resonance (NMR) data obtained by a ramp-CPMAS method. The Armadale fulvic and humic acid mass spectra are nearly identical with clusters of peaks in the 80–200, 300–400, and 550–800 m/z range. Sharp peaks stand out prominently above a background of peaks at virtually every nominal mass extending from the low mass end of the scale to 3000 m/z. As observed in both

Table 1
Accurate and measured *m/z* values for compounds proposed in Fig. 3. Accurate mass values were calculated from elemental formulas of each compound. The error on the measured *m/z* is expressed in terms of ppm

Proposed compound	Accurate mass	Measured *m/z*	Error (ppm)
$C_{38}H_{36}O_{16}Na^+$	771.189260	771.190589	+ 1.7
$C_{39}H_{40}O_{15}Na^+$	771.226848	771.227443	+ 0.8
$C_{40}H_{44}O_{14}Na^+$	771.26302	771.2638	+ 1.0
$C_{36}H_{30}O_{18}Na^+$	773.13213	773.134421	+ 3.0
$C_{37}H_{34}O_{17}Na^+$	773.16948	773.169177	-0.4
$C_{38}H_{38}O_{16}Na^+$	773.205530	773.206002	+ 0.6
$C_{39}H_{42}O_{15}Na^+$	773.24216	773.242749	+ 0.8

the Mt. Rainier and Suwannee River samples, the majority of peak intensity is contained within the broad clusters at every nominal mass. To illustrate this, Fig. 5 shows an expanded region of the spectrum for the diluvial humic acid but identical characteristics were observed in both Armadale spectra.

4. Discussion

4.1. General characteristics of ESI MS

Before discussing specific aspects of each mass spectrum, there are two general characteristics of ESI mass spectra that merit reflection. The first important question is whether the mass spectra accurately reflect the composition of the sample. Electrospray ionization ionizes only polar compounds and the purely aliphatic fraction within humic and fulvic acids will not be detected. However, this fraction is a very minor component of most humic acids (Anderson et al., 1989). Within the polar fraction, combinations of particular functional groups such as carboxylic acids, alcohols, and especially amines, result in a wide range of ionization efficiencies. The differences in ionization efficiencies will affect the relative abundances of compounds within the spectrum and limit the ability of the technique to obtain quantitative information. Until relative ionization efficiencies are determined for humic-like compounds, ESI is best suited for qualitative analyses.
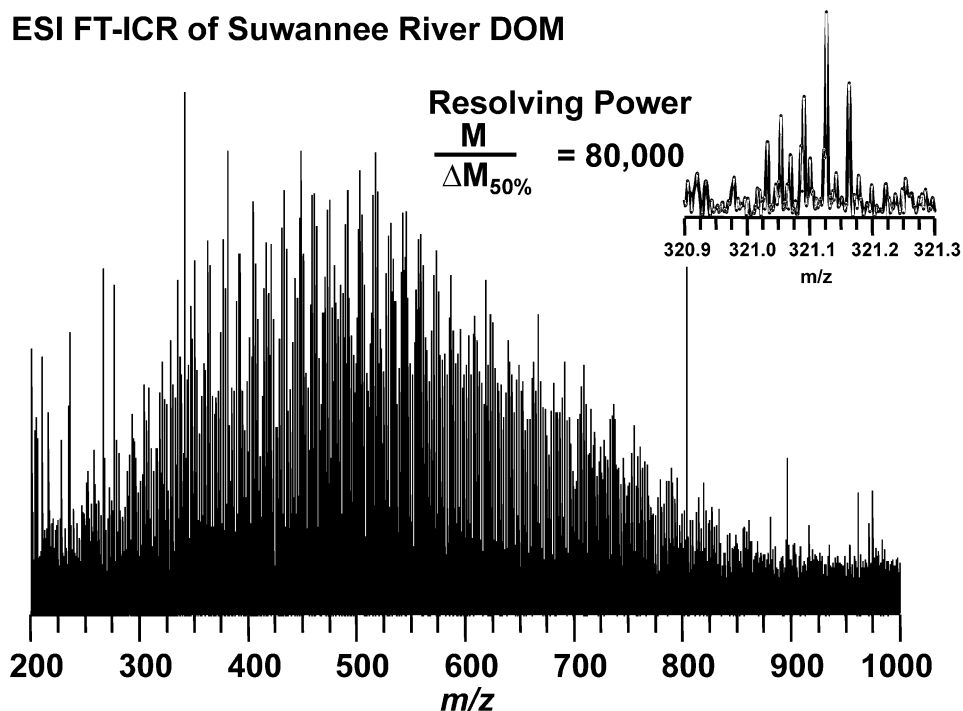

Fig. 4. ESI FT–ICR positive ion mass spectrum of dissolved organic matter (DOM) from Suwannee River. The spectrum was acquired in the same manner as that in Fig. 2.
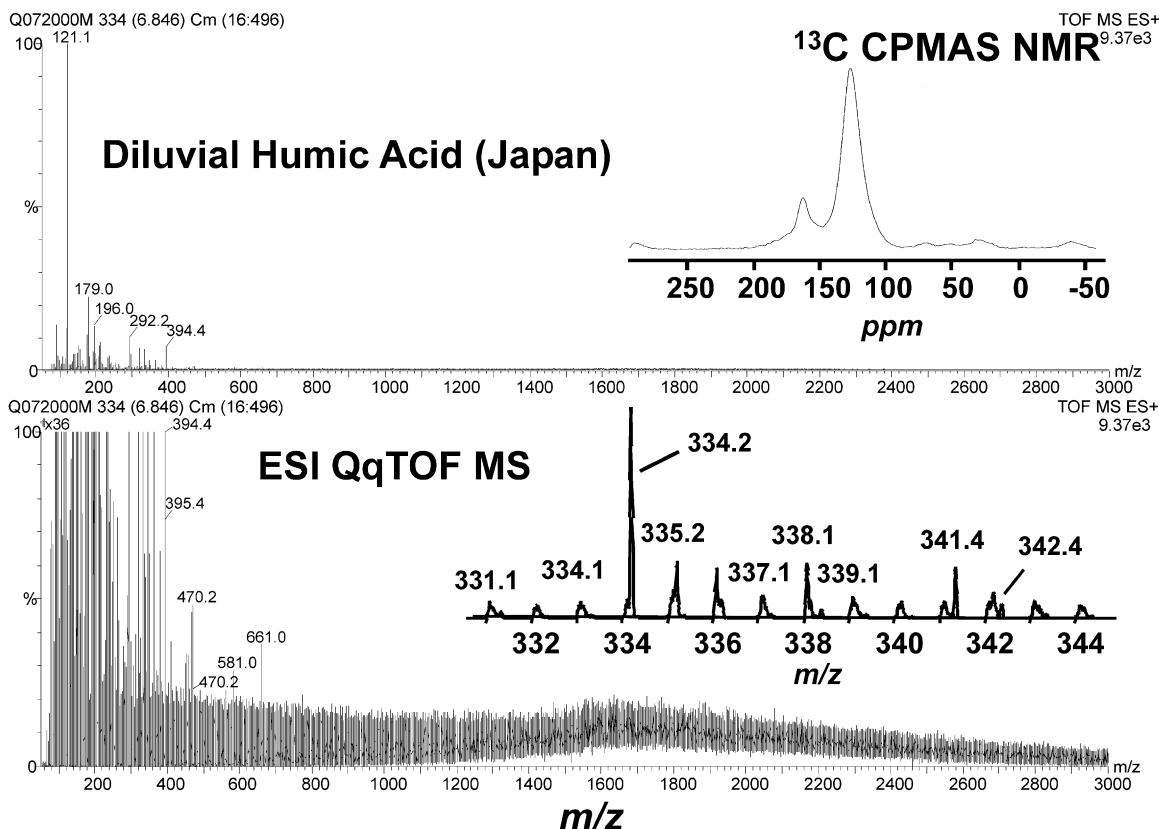
Fig. 5. ESI Qq-TOF positive ion mass spectrum of diluvial humic acids from Iwata, Japan. The full spectrum is shown in the top panel. This sample was prepared in 50:50 water: methanol solution at a concentration of 1 mg ml^{-1}. The inset on the top panel is solid-state ^{13}C NMR data using ramp-CPMAS. The bottom panel represents a 16× vertical magnification of the full spectrum. An expansion of the 332–344 m/z range is also presented.

The second question for general ESI MS is whether the observed molecular weight range and distribution are accurate. Numerous values for the molecular weight range of humic substances have been determined using size exclusion chromatography (SEC) (e.g. 1500–5000 amu (Chin et al., 1997); 800–18,000 amu (Muller et al., 2000)). It has been noted, though, that molecular weight determinations using SEC are difficult to interpret given the range of values observed (de Nobili et al., 1989; Swift, 1989). In addition, recent reports have suggested that humic and fulvic acids may be aggregates of lower molecular weight material instead of covalently-linked macromolecules (MacCarthy and Rice, 1985; Piccolo and Conte, 1999), consistent with the molecular weight ranges observed in our mass spectra obtained from two very different mass analyzers. Fragmentation of high molecular weight material is one possible explanation put forth for the relatively low molecular weight distribution observed in these mass spectra. Leenheer et al. (2001) have suggested that compounds with high carboxylic acid content may be susceptible to fragmentation during electrospray ionization. However, because

ESI is regarded as a "soft" ionization technique, more work with standards and fractionated samples is needed to ascertain the extent to which this effect is affecting the molecular weight distribution of the humic and fulvic acids in natural samples.

4.2. Comparison of spectra for Mt. Rainier humic acid

The humic acid extract of a degraded wood sample was chosen for detailed work because it is composed of partially-humified lignin (de Montigny et al., 1993) and is representative of degraded wood in forests of the Northwest Pacific region. The ESI QqTOF MS enabled us to determine quickly the general characteristics of the compounds within the Mt. Rainier degraded wood humic acids, such as molecular weight range and distribution, which were then confirmed with ultra-high resolution ESI FT–ICR MS. The high-resolution mass spectra in Figs. 2 and 3 provide sufficient resolving power and mass accuracy to determine a unique formula weight and, in some cases, a unique structure. If the mass region between 771 and 774 m/z is expanded to
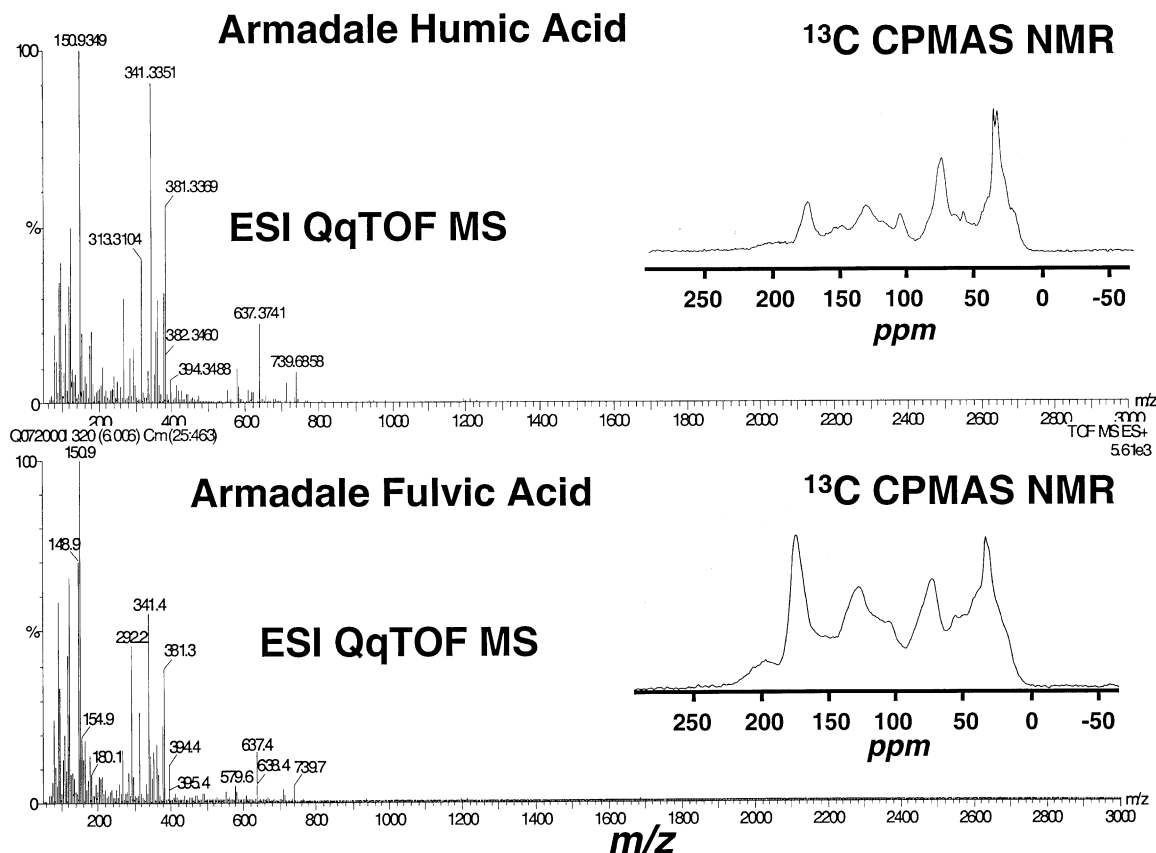
Fig. 6. ESI Qq-TOF positive ion mass spectra of Armadale humic acids (top) and fulvic acids (bottom). The spectra were acquired in the same manner as in Fig. 5. The insets for both spectra are the respective solid-state ^{13}C NMR data using ramp-CPMAS.

examine the individual peaks (Fig. 3), we observe clusters of peaks at each nominal mass unit, as well as at half mass units representing potentially doubly charged species. These characteristics are observed throughout the mass spectrum. By analyzing the clusters of ions at each nominal mass and measuring their exact mass to a precision of 1 ppm, we can calculate a molecular formula for each of the peaks (Table 1). The molecular formulas shown in Fig. 3 were derived from formulas for structures expected from degraded lignin; specifically, oxidation of pendant alcohols at the α and γ carbons of lignin tetramer side chains. It is important to note that the molecular formula is a sodium adduct, a well known artifact of positive-ion electrospray ionization methods, especially when sodium hydroxide is used during sample preparation.

We then compared the humic acids from Mt. Rainier to DOM from Suwannee River. Lignin oxidation products would presumably exist in both samples since lignin should be a common source for both types of organic matter. Thus, common peaks in both samples could be presumed to derive from similar sources, e.g. humified lignin. In addition, DOM should have a variety of non-lignin sources which should be reflected in peaks that appear only in the Suwannee River sample. Using the high resolution achieved with the FT–ICR MS, we compared selected m/z regions to determine the extent of compound similarity in the two samples. For example, one can compare the insets of Figs. 2 and 4 which show the expanded region around 321 m/z. Mt. Rainier humic acid has two peaks in this region whereas the Suwannee River DOM has at least five peaks and at least three of them could be due to non-lignin sources. Using FT–ICR MS, we can now compare humic or fulvic acid samples on a molecular level and correlate these differences to changes in space and time. Combining this work with nuclear magnetic resonance and wet chemical degradation studies will allow us to propose molecular formulas that are consistent with all three data sets and approach a true picture of the structure of complex humic material.

We have not been able to determine the cause for the discrepancy between the two spectra in the low (100–200) m/z range. Although this region is susceptible to background contamination on both instruments, proper control experiments have not been performed to ascertain

the degree to which background peaks appear in this region. In addition, QqTOF spectra for analyses of the Armadale and diluvial samples did not contain these peaks (Figs. 5 and 6), suggesting that low molecular weight peaks in the Mt. Rainier spectrum reflect actual compounds within the sample. While it is possible that the FT–ICR MS is unable to detect these low molecular weight compounds due to mass discrimination, standard peaks have been observed in this *m/z* range. In addition, we have not yet optimized the ICR cell for detection of low molecular weight compounds.

4.3. Comparison of QqTOF mass spectra of different humic and fulvic acids

The QqTOF mass spectra obtained for the diluvial humic acid (Fig. 5) and the Armadale humic and fulvic acids (Fig. 6) were compared with one another as well as with the solid-state ^{13}C NMR data for each sample. The NMR data indicate that the Armadale humic substances are rich in aliphatic structures with a low abundance of aromatic compounds. The fulvic acid is more aromatic than the humic acid and also contains increased abundance of carboxyl and carbonyl carbons. In contrast, the diluvial humic acids are predominantly aromatic carboxylic acid-like structures; specifically, carbon-linked benzene-polycarboxylic acids (Hatcher et al., 1989).

The ESI QqTOF mass spectra reflect the differences and similarities in the two sample sets predicted by the NMR spectra. In both Armadale spectra (Fig. 5), sharp peaks dominate a background of broad peaks at virtually every nominal mass throughout the range analyzed (80–3000 *m/z*). These sharp peaks are most likely due to discrete molecules such as fatty acids (consistent with data in Schnitzer and Nayroud, 1975). Fatty acids are recognized easily due to their high mass discrimination that separates them from the clustered masses. The other peaks at each nominal mass are multiplets due to a mixture of various structures with the same nominal but slightly different exact masses. The clusters occur at every nominal mass with little or no signals at fractional masses, indicating that the ions are singly charged. At high masses, additional clusters grow in at fractional masses, indicating the presence of doubly charged ions, whose probability increases with increasing mass. It is noteworthy that the intense signals for the two Armadale samples are virtually identical, as anticipated for samples from the same soil. This indicates similarity in the molecular constituents attributed to the intense peaks. It is possible that some of these peaks are derived from contaminants in the tubing lines to the electrospray unit. However, these peaks are not observed in the humic acids from the diluvial soil, except for those at 292 and 394 *m/z*.

An observation worthy of mention is the exact mass for each cluster, especially in the case of the diluvial humic acids. The clustered masses do not have a significant mass discrimination, usually centered at about 0.1 to 0.2 amu above the nominal mass. This indicates that structures assigned to these ions are generally hydrogen poor compared with structures containing long-alkyl substituents. Oxygen atoms with a negative mass discrimination and the paucity of hydrogens associated with condensed aromatic rings could explain the low mass discriminations observed for the clusters.

While a great deal more information can be gleaned from the QqTOF data set, more experiments are necessary to identify and eliminate possible contamination. In addition, instrument parameters require further optimization to provide a more quantitative representation of the various structures present in humic substances. The data can only be regarded as qualitative at the moment, due largely to the fact that little is known of the ionization efficiencies and relative detection for various structures. At the very least, the QqTOF MS technique provides us with a rapid means of examining qualitative differences among various humic substances prior to detailed studies by FT–ICR MS where exact formula weights can be discerned.

5. Conclusions

The combined techniques of ESI QqTOF MS and ESI FT–ICR MS represent an advance in the study of the structural characterization of natural organic matter. While the samples employed in our study were composed primarily of humic and fulvic acids, other organic matter samples can be studied using these techniques by simply altering instrument conditions. The simultaneous use of ESI MS with other structural characterization methods such as high-resolution multidimensional NMR will provide the basis for constructing possible structures whose exact formula weights can be calculated and compared to observed peaks in high-resolution mass spectra. Furthermore, the characterization of relatively simple humic and fulvic acid fractions may allow us to rapidly identify components in more complex samples based solely on species mass. It is our firm belief that the unique advantages offered by ESI QqTOF MS and ESI FT–ICR MS open the door to detailed molecular characterizations of natural organic matter that have eluded many previous studies.

Acknowledgements

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