# Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI FT-ICR MS): Characterization of Complex Environmental Mixtures

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**Abstract**

Structural and chemical characterization of compounds within complex environmental mixtures provides unique insights into the roles of these compounds in different environmental processes. Here, an emerging technique in environmental chemistry, electrospray ionization Fourier transform ion cyclotron mass spectrometry (ESI FT-ICR MS), is described and its applications to the molecular characterization of humic acids and petroleum products are reviewed and examined. Electrospray ionization is a low-fragmentation ionization technique that preferentially ionizes polar functional groups prior to mass spectrometric analysis. This technique allows the characterization of intact polar macromolecules that are inaccessible to standard chromatographic techniques. Ion cyclotron resonance mass spectrometry is an ultrahigh resolution and mass accuracy mass spectrometry technique based on the detection of ion cyclotron motion within a magnetic field. The combination of ESI FT-ICR MS with other structural techniques such as NMR allows the unprecedented identification and characterization of polar macromolecules in environmental mixtures and will find numerous applications within environmental chemistry and forensics.

Key words: ESI FT-ICR MS; petroleum; humic acids; natural organic matter

# 1. Introduction

## *1.1. Complex environmental mixtures*

Environmental mixtures are comprised of compounds with a variety of chemical and physical properties. Some examples of environmental mixtures include the solvent (or base) extracts of different solid materials (e.g., soil or aquatic sediments), the solvent extracts of petroleum materials (e.g, asphaltenes), and products of industrial processes (e.g, explosives). Each of these mixtures contains compounds with polar functional groups (such as carboxylic acids or heteroatoms) as well as nonpolar functional groups (such as alkyl chains). The molecular composition and structure of compounds within each mixture can be used to assign source functions such as specific environments or particular manufacturers. In addition, the structures of both the contaminant mixture components and the natural organic matter with which they interact determine the bioavailability and the efficacy of detoxification mechanisms.

Structural characterization of most environmental samples is impossible due to their heterogeneity and inaccessibility to standard analytical techniques. Molecules with hydrophilic functional groups have been under-characterized because chromatography-based techniques (such as gas chromatography / mass spectrometry) depend on compound volatility. However, naturally-occurring macromolecules and anthropogenic contaminants often contain both hydrophilic and hydrophobic functional groups within the same molecule or at least in the same mixture. Due to the variety of compounds within many environmental organic mixtures, no one technique will ever be able to fully characterize all the molecules therein. However, the small (<1000 Da) hydrophobic

molecules have been characterized preferentially to date and it is imperative to begin the characterization of other components of environmental mixtures.

This paper focuses on the role of one particular analytical technique in the structural characterization of polar or slightly polar constituents of environmental mixtures. This technique combines electrospray ionization (ESI), a low-fragmentation ("soft") ionization technique for polar compounds, with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), a mass spectrometric technique with ultra-high resolution and mass accuracy capabilities. I will focus on the application of this technique to two questions – the characterization of humic and fulvic acids and the characterization of heteroatomic petroleum components. Applications to other environmental questions will be mentioned and future directions for this technique will be proposed.

### *1.2. Standard characterization techniques*

The analytical techniques that have been used most frequently to examine compounds in organic matter and petroleum samples include solid- and liquid-state nuclear magnetic resonance spectroscopy (NMR) (for example, MacCarthy and Rice, 1985, deMontigny et al., 1993, Simpson et al., 2001, Dria et al., 2002) and gas chromatography / mass spectrometry (GC/MS) (for example, Zhukov and Vereshchagin, 1981, Krahn et al., 1993, Whelan et al., 1993, Rowland et al., 2001). NMR provides the relative quantities of different functional groups within the bulk sample, thus assessing the general chemical nature of the material. GC separates compounds based on volatility and interactions with the stationary phase of a capillary column. However, few of the

compounds within natural organic matter (NOM) such as carbohydrates or fatty acids can be volatilized and analyzed without prior derivatization of polar functional groups or fragmentation (e.g., Clifford et al., 1995, delRio et al., 1998). In addition, semi-polar compounds with similar chemical characteristics are not fully resolved by a one-dimensional GC column and appear within a hump in the middle of the spectrum, generally referred to as the "unresolved complex mixture" or UCM (Rowland et al., 2001). Other analytical techniques including high-pressure liquid chromatography (HPLC) / size exclusion chromatography (SEC) (e.g., Muller et al., 2000), and direct temperature mass spectrometry (Minor et al., 1999), have also been used to characterize different components of naturally-occurring organic matter. As with GC/MS and NMR, neither of these techniques has yielded molecular-level composition for intact macromolecules within natural organic matter.

## 2. Electrospray ionization (ESI)

### *2.1. Basic principles*

#### *2.1.1. Spray formation*

Electrospray ionization (ESI) ionizes polar, hydrophilic compounds from a variety of polar solvents by producing negatively or positively charged ions from polar molecules with acidic and basic functional groups (such as carboxylic acids and amines). Singly or multiply-charged ions are then accelerated into a mass spectrometer for analysis. The ability to multiply-charge ions reduces the *m/z* ratio so that the ion can be analyzed within the appropriate *m/z* range. Electrospray ionization begins with the application of a high voltage difference (1-4 kV) between the sample needle and the capillary at the front of

the instrument (Figure 1). The voltage difference causes the solvent at the end of the needle to explode in a mist of small charged solvent droplets. As the solvent in each droplet evaporates, the charged species on the surface are brought closer together until charge-charge (Coulombic) interactions force the droplet to explode into many smaller droplets (the Rayleigh limit – where the magnitude of the Coulombic repulsions overcome the solvent surface tension). Eventually, charge is transferred to polar functional groups within the droplets such that each ion exists within a minimum of solvent and is attracted into the capillary of the instrument (an excellent review is presented in Gaskell, 1997). The remaining solvent is removed by heat or by $N_2$ gas (or both) in the capillary entrance. The transfer of charge within the solvent droplets occurs according to the relative basicity or acidity of the functional groups present. For example, negative ions are produced more easily from carboxylic acids than from alcohol groups. Further, the sign of the ions produced is opposite to the sign of the voltage difference applied to the capillary.

### *2.1.2. Flow rates*

Solvent flow rates fall into two categories. Microelectrospray utilizes flow rates in the range of $\mu L\ min^{-1}$. Nanoelectrospray utilizes flow rates in the range of $nL\ min^{-1}$ (Emmett et al., 1998) and consumes significantly lower amounts of sample per analysis. High resolution instruments are generally interfaced with nanoelectrospray sources to achieve the highest data volume per sample. However, there has been a drive to interface electrospray ionization with HPLC columns. Flow rates for HPLC are generally too fast

(mL $min^{-1}$) for general electrospray instrumentation. Thus the HPLC effluent is often split prior to ESI (e.g., Li et al., 1999, Speir et al., 2000).

### *2.1.3. Fragmentation*

ESI is a "soft" ionization technique with low incidence of fragmentation, thus increasing the possibility that analytes remain intact. Fragmentation of parent ions can be controlled by the initial potential. Low potentials have a lower incidence of fragmentation due to lowered energy in ion formation. Since electrospray formation also depends on the magnitude of the potential difference, the potential magnitude must be optimized for each sample type such that fragmentation is minimized and a stable spray is maintained (Gaskell, 1997). Electrospray ionization has been used successfully to examine biological samples such as proteins and drug metabolites and is one of the central analytical techniques for the emerging field of proteomics (see citations contained within Loo, 1997, Marshall et al., 1998a, Marshall et al., 1998b).

## ***2.2. Analytes***

### *2.2.1. Molecule types and ionization efficiencies*

Electrospray ionization preferentially ionizes those molecules that can carry a positive or negative charge as a result of protonation, de-protonation, or complexation with metal ions. Thus purely aliphatic compounds such as hydrocarbons are not ionized by ESI. In general, the relative abilities of different functional groups to stabilize a positive or negative charge will determine a molecule's relative ionization efficiency. In the positive ion mode, basic functional groups are preferentially ionized. For example, compounds

with lone-pair nitrogen groups such as pyridines are ionized much more efficiently (approximately 10X more efficiently) than oxygenated functional groups such as alcohols or carboxylic acids (Hughey et al., 2001b). In the negative ion mode, acidic functional groups such as carboxylic acids are easily de-protonated and therefore are preferentially ionized relative to nitrogen-containing compounds. In many natural organic mixtures where N concentrations are negligible, metal complexes such as sodium adducts are observed. These adducts presumably occur at the site of an ester or another electron-donating functional group such as an alkyl amine or an alcohol. The presence of metal adducts (such as $Na^+$) complicates the mass spectrum and can hinder accurate interpretation.

The interplay of different functional groups in complex macromolecules is difficult to predict and so the relative ionization efficiencies of components of environmental mixtures are not well understood. For example, the difference in relative ionization efficiencies can generate biased data for the molecular composition of a particular sample. Some investigators have approached this issue by combining data from both positive and negative ion modes. This is somewhat difficult due to the uncertainties in relative ionization efficiencies but as long as the data is used qualitatively the method is a reasonable compromise.

### *2.2.2. Solvent systems*

A number of solvent systems can be used for electrospray ionization. The most important feature of the matrix is a low surface tension. Stable spray formation is difficult in pure water and other high surface tension solvents such as dimethyl sulfoxide

(DMSO). For aqueous samples, many investigators lower the surface tension by diluting the sample with alcohol. Methanol and isopropyl alcohol are common dilutants in any ratio (e.g. ratios from 90:10 water:methanol to 10:90 water:methanol can be used). Strong acid or base is often added to increase detection of ions and preferentially form one type of ion. Electrospray ionization can be performed also in polar organic solvents such as acetonitrile and methylene chloride, although acid or base is commonly added to enhance ion production during electrospray. A common solvent system for petroleum analyses is an acidified methylene chloride-methanol mix (ratios vary - e.g., Hughey et al., 2001b, Qian et al., 2001).

### *2.2.3. Salt effects*

Electrospray ionization is particularly sensitive to the water content and the salt concentration of the solvent system. Non-volatile salts such as NaCl adversely affect ESI by interfering with spray formation (Gaskell, 1997, Brown and Rice, 2000, Kujawinski et al., 2002b). At high salt concentrations, corona discharge can occur in the gap between the needle and the capillary entrance. In addition, salt can precipitate in the sample needle and block sample delivery altogether. Solvent extracts usually have low salt concentrations because of low salt solubility in extraction solvents such as methylene chloride. However, natural organic mixtures often have high salt concentrations either due to high background salt (as in marine samples) or high salt concentrations in extraction solvents (as in humic acids extracted with 0.1 M NaOH).

*2.2.4. Sample preparation*

Critical parameters for sample preparation are the solvent system and the salt concentration. Samples in organic solvents should be acidified to increase ion production. Aqueous samples should be diluted with an alcohol such as methanol or isopropyl alcohol to lower droplet surface tension. Those samples that require a buffered solution should be made in a volatile buffer if possible. Ammonium ($NH_4^+$) can volatilize as $NH_3$ (g) during ESI. Likewise, carbonate ($CO_3^{-2}$) can be removed as $CO_2$ (g). In both cases, extensive de-salting procedures may not be necessary unless the ionic strength is very high (mM).

Non-volatile salt concentrations can be reduced by dialysis or cation exchange (metal ion is removed and replaced with a proton). Dialysis membranes are available with various molecular-weight cutoffs and should be chosen carefully. Membranes with 1000 Da molecular weight cutoff are commonly used for humic acid preparation (e.g., Salloum et al., 2001). However, material in the <1000 Da size fraction is lost and thus the molecular weight distribution can be biased. Cation-exchange resins do not produce size-fractionation biases but may introduce polar contaminants from the resin beads (Kujawinski, unpublished results). Extensive washing of the resin is required prior to sample exchange and analysis. Salt removal is not trivial for samples derived from high salt environments (e.g., marine DOC). The specific protocols for desalting such samples should be chosen carefully to minimize sample loss and / or alteration.

## 3. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)

### *3.1. Basic principles*

#### *3.1.1. Introduction*

The critical second half to ESI MS analysis is the nature of the mass spectrometer. A number of mass spectrometers have been used in the structural characterization of NOM including ion-traps (Leenheer et al., 2001), quadrupole time-of-flight analyzers (Moulin et al., 2001, Kujawinski et al., 2002a) and Fourier transform ion cyclotron resonance (Fievre et al., 1997, Brown and Rice, 2000, Kujawinski et al., 2002a). Mass resolving power (defined as $m/\Delta m_{50\%}$ where $\Delta m_{50\%}$ is the width at half-height of peak $m$) is a critical parameter in assessing the relative resolving capability of a mass spectrometer. Resolving powers of approximately 10,000-15,000 translate into a mass accuracy of about 10 ppm. This value is fairly common for quadrupole time-of-flight instruments. Depending on the magnetic field strength, FT-ICR mass spectrometers are capable of ultrahigh mass resolving power (>100,000) and mass accuracy (<1 ppm) (Marshall and Guan, 1996, Marshall et al., 1998a).

#### *3.1.2. Ion detection*

Ion cyclotron resonance (ICR) mass spectrometry measures the $m/z$ value of ions based on their motion within an ICR cell that resides within the horizontal bore of a magnet (excellent explanations are presented in Marshall et al., 1998a, Marshall et al., 1998b). Within a spatially uniform magnetic field, an ion's velocity perpendicular to the field lines is bent into a circular orbit by a Lorentz Force (Figure 2). This motion is also called cyclotron motion. The ions are confined in the field by trapping electrodes that

confine the ions' motions along magnetic field lines. The frequency of each ion's cyclotron motion is given by:

$$f_c = \frac{zB_0}{m}$$

where $f_c$ is the cyclotron frequency, $z$ is the charge of the ion, $B_0$ is the magnetic field strength and $m$ is the mass of the ion. Thus the cyclotron frequency is inversely related to the $m/z$ ratio and directly proportional to the magnetic field strength.

Detection of the cyclotron frequencies (and thus the $m/z$ ratio in a static magnetic field) is accomplished by inducing coherent motion of the ions followed by detection of their induced image current on two detection electrodes. Since the initial radii of the ions' orbit are too small to be detected by these electrodes, they are expanded by the application of a radio frequency excitation through two excitation electrodes. The excited ions then induce an image current that is amplified and recorded. The resulting time-domain signal is Fourier-transformed to produce a frequency spectrum. The frequency spectrum can be easily converted to a mass spectrum using the relationship between frequency and $m/z$ shown in Figure 3, where A and B are constants.

### *3.2. Ion density effects*

At high ion concentration, ions with similar frequencies can interact with one another, resulting in phase-locking. Each ion is no longer resolved and instead, an intermediate frequency is observed. The deleterious ion-ion interactions within the cell are more generally referred to as "space charge effects" (e.g., Wang and Marshall, 1986). These effects can be minimized with higher magnetic field strength or by lowering ion density. Resolution increases linearly with magnetic field strength due to decreased ion-

ion interactions. This results in the ability to distinguish ever-increasing numbers of compounds at each nominal mass with increasing magnetic field strength. Ion density can also be lowered by increasing the physical volume of the cell within the magnet bore.

### *3.3. Sample considerations*

Kujawinski *et al.* (2002a, 2002b) were first to show that improved mass resolution could be obtained with an ICR MS when numerous scans of low ion concentration were accumulated prior to Fourier transformation. In this method, nanoelectrospray (flow rates = $\mu L\ hr^{-1}$) was used to introduce a minimum amount of material into the ICR cell to achieve reasonable compound separation per scan. Numerous scans [approx. 20,000 on a 7 T ICR (24 h) and 200 on a 9.4 T ICR (30 min)] were the co-added to improve the signal-to-noise ratio and overall resolution. With this resolving power, over 1400 peaks were resolved between 200 and 1000 *m/z* in a lignin-rich humic acid extracted from a piece of degraded wood (Figure 4) and over 9000 peaks were resolved within a riverine dissolved organic matter sample (Figure 5). The lack of peaks at fractional *m/z* indicates that the species within these spectra are singly-charged, consistent with the observations of previous studies (Fievre et al., 1997, McIntyre et al., 1997, Brown and Rice, 2000). The mass resolving power of compounds in the spectra improve as a function of the magnetic field strength (Figure 6 – 36,000 at 700 m/z from the 7 T ICR and 200,000 at 700 m/z from the 9.4 T ICR). This mass resolving power is high enough to enable the identification of elemental formulas for low m/z compounds.

### *3.4. Data processing*

#### *3.4.1. Molecular formulas*

Mass spectrometric data yields the *m/z* value for particular components of a mixture. These *m/z* values can be converted to molecular mass as long as the charge (*z*) is known. The charge of a compound can be ascertained by examining its isotopic distribution. The first isotope peak (e.g., one $^{13}C$ instead of all $^{12}C$) always occurs 1/*z* amu units after the original compound. Thus if an isotope peak is observed at ½ amu later, the compound is doubly charged. Once the charge is known, the molecular mass is known. A set of elemental compositions consistent with the molecular mass can be generated for each molecular mass. The number and choice of elements are often constrained by the analyst's knowledge of the sample. For example, common elements include C, N, O, S, and H. Salt cations are often used if non-volatile salts could not be removed (most common salt cation is $Na^+$). The number of possible elemental combinations depends on the error associated with the mass spectrometric measurement and increases with increasing *m/z*. For example, with ultrahigh resolution and mass accuracy at subppm levels, elemental compositions for masses <400 Da can be assigned on the basis of mass alone (Hughey et al., 2001a).

#### *3.4.2. Data visualization*

Analyses of complex environmental mixtures by ESI FT-ICR MS often yield spectra with thousands of resolved peaks and thus require sophisticated data reduction and visualization protocols. One of these protocols, Kendrick mass analysis, was developed within the petroleum community to identify compounds related by addition or subtraction

of $–CH_2$ groups (Kendrick, 1963). In this protocol, the masses of all peaks are normalized to the mass of a $–CH_2$ group, defined as 14.000 Da, as shown in the equation:

Kendrick mass = IUPAC mass X (14/14.01565)

Kendrick mass defect = nominal Kendrick mass – exact Kendrick mass

The mass defect of the Kendrick mass (KMD) will be equivalent for all compounds with the same chemical backbone but differing numbers of $–CH_2$ groups. This technique was used successfully by Hughey et al. (2001a) to identify series of heteroatomic compounds within the positive ion mode spectrum of diesel fuel. The identity of compounds >400 Da can be assigned if the compounds are part of a $–CH_2$ series that begins below 400 Da. Thus, the use of Kendrick mass analysis can extend the numbers of compounds identified on the basis of mass. Kendrick mass analysis is not as useful in non-petroleum based mixtures. For example, this technique was used to examine peaks with a humic acid and a sample of riverine dissolved organic matter (Kujawinski et al., 2002a). The series of $–CH_2$ compounds were very short (2-4 components) and thus no significant increase in identification capability was observed. Nonetheless, the use of Kendrick mass analysis can be generalized to examine series of compounds based on any functional group.

Molecular-level changes as a function of particular experimental manipulations can be assessed in ESI FT-ICR mass spectra by comparing the spectra on a peak-by-peak basis. In essence, this technique subtracts one spectrum from the others to identify those compounds that have either disappeared or appeared as a function of the experimental parameter. Rodgers et al. (1999, 2000b) used this method to assess the effects of weathering on jet fuel and other transportation fuels. One can envision using this technique to monitor a number of different experimental parameters, such as photo-

oxidation of aquatic organic matter (Kujawinski et al., 2002c) and biological remediation of polar contaminants.

### *3.4.3. Quantification of peaks*

The data generated by ESI FT-ICR MS cannot be used quantitatively at this time. The differences in relative ionization efficiencies mean that relative intensities cannot be used as proxies for relative concentrations. Changes in peak intensities between different samples cannot be interpreted as strictly quantitative at this time either. Miyabayashi *et al.* (2000) showed that space charge effects within the ICR cell and the external accumulation hexapole adversely affect the ability of FT-ICR MS to be quantitative for both a crude oil and a model standard, polyethylene glycol (MW = 600). They suggested that design modifications were necessary to circumvent the space charge effects.

Miyabayashi *et al.* (2000) also suggested that space charge effects limit the linear response of the instrument. Thus linear calibration curves may not be appropriate. Furthermore, it is not clear that charge competition (and thus compound ionization) within a water droplet is well constrained or understood. More importantly, a standard curve (with presumably a few selected components) may not adequately mimic this effect (an idea also voiced by Gaskell, 1997). An alternative possibility is the use of a standard addition curve for selected components. In contrast to the external standard method, the sample matrix would be the same for both the samples and the standards. However, with thousands of peaks to choose from, selection of appropriate standards may be difficult.

## 4. Current applications

The application of ESI FT-ICR MS to environmental mixtures is a recent development in environmental analytical chemistry. Environmental samples with polar components (i.e., that are appropriate for study by ESI MS) are usually extremely complex and difficult to examine. Thus, only those instruments with high resolution can be used effectively to characterize these samples. The work to date has focused on two types of samples in particular – heteroatomic (N, O, or S) petroleum constituents (Hughey et al., 2001a, Hughey et al., 2001b, Qian et al., 2001, Rodgers et al., 2001b) and humic and fulvic acids (Fievre et al., 1997, Solouki et al., 1999, Alomary et al., 2000, Brown and Rice, 2000, Kujawinski et al., 2002a, Kujawinski et al., 2002b).

### *4.1. Natural Organic Matter – Humic and fulvic acids*

Humic and fulvic acids are complex mixtures of refractory, polar soil organic matter. They play a key role in various soil processes, including redox chemistry and contaminant transport. The structure of humic and fulvic acids determines the strength of interactions with anthropogenic contaminants and therefore has a large impact on contaminant bioavailability and transport. Humic and fulvic acids are extracted from soil using 0.1 M NaOH (in water) and the "humic acid" fraction is precipitated at low pH. The low pH supernatant is defined as fulvic acids. Attempts to characterize the structure of this material have included techniques such as elemental analysis, NMR (Hatcher et al., 1980, Wershaw, 1985, Simpson et al., 2001, Dria et al., 2002, Simpson et al., 2002), tetramethylammoniumhydroxide (TMAH) coupled with GC/MS (Clifford et al., 1995) and pyrolysis/ GC/MS (e.g., Gillam and Wilson, 1985, Guthrie et al., 1999). Each of

these techniques has yielded interesting insights into the structure of humic and fulvic acids. Biomolecules such as carbohydrates, proteins, and lipids have all been observed in humic and fulvic acids (Anderson et al., 1989) in addition to lignin, cellulose and other plant materials.

The first attempts to examine aqueous organic matter using ESI MS were published by McIntyre *et al.* (triple quadrupole MS - 1997) and Fievre *et al.* (FT-ICR MS - 1997). Both data sets contained peaks at every nominal mass with a large mass distribution between 100-1200 m/z (McIntyre et al., 1997) and between 500-2000 m/z (Fievre et al., 1997). The ICR data of Fievre *et al.* (1997) suffers from poor mass resolution due to few scans and low humic acid concentration (approximately 0.1 mg $mL^{-1}$). These authors were able to improve the resolution of their data by fractionating their sample with HPLC prior to FT-ICR MS analysis.

ESI FT-ICR MS analyses of humic and fulvic acids have confirmed initial hypotheses of the complexity and heterogeneity of this material. Using ESI FT-ICR MS, an unprecedented amount of information can be gained about the molecular-level chemical composition of humic and fulvic acids. We can examine the elemental compositions of different components of these mixtures and identify chemical relatives (using Kendrick mass functional group analysis) (Kujawinski et al., 2002a). With high resolution mass spectra, we can use the original samples as a basis for experimental manipulations and monitor molecular-level changes as a function of specific geochemical processes.

The molecular weight distribution of humic and fulvic acids determined by FT-ICR MS data (100-2000 Da) stands in contrast to that measured by other techniques: 3.9

kDa for humic acids using flow field-flow fractionation-inductively coupled plasma mass spectrometry (Amarasiriwardena et al., 2001); 1-10 kDa for fulvic acids (Chin et al., 1997) and 3-200 kDa for humic acids using SEC (deNobili et al., 1989, Swift, 1989). The cause of this discrepancy is unknown. The theoretical upper mass limit for a 7 T FT-ICR MS is about 5.8 megaDa (Marshall et al., 1998b). Practically, large proteins in the size range of 100s of kDa have been observed (e.g., chondroitinase enzymes - Kelleher et al., 1997). Therefore large macromolecules such as those proposed for humic acids can be detected using FT-ICR MS. However, if these molecules cannot accept multiple charges, they will not be observed with the common mass window of 100-2000 *m/z*. Investigators have extended their analyses to larger mass windows but have not observed the proposed macromolecules.

There are two possible interpretations: (1) there is a small fraction of material within the larger humic / fulvic acid framework that is ionized preferentially by electrospray ionization and (2) humic and fulvic acids are actually aggregates of smaller material and simply act as macromolecules under the strong ionic strength conditions of size exclusion chromatography. While Leenheer *et al.* (2001) have suggested that the ionization process fragments humic macromolecular material, other evidence has shown that size exclusion chromatography is susceptible to extreme experimental variability and can give very different results under slightly different conditions (Piccolo and Conte, 1999). In addition, NMR studies have suggested that humic materials may be mixtures of smaller molecules that have different aggregating tendencies at different ionic strengths (Simpson et al., 2001, Simpson et al., 2002). Kendrick mass analysis has shown that the structural characteristics of the material detected in ESI FT-ICR MS is consistent with

those indicated by solid-state NMR studies. Thus, it seems that the same type of material is being analyzed in both techniques. Unfortunately, it is impossible to perfom mass balance experiments with electrospray ionization and thus these hypotheses cannot be tested and/or disproven with current ESI-based methods.

### *4.2. Petroleum products*

Another major application of ESI FT-ICR MS has been the characterization of heterocyclic compounds in petroleum and related products (Rodgers et al., 1999, Rodgers et al., 2000b, Hughey et al., 2001a, Hughey et al., 2001b, Qian et al., 2001, Rodgers et al., 2001b). The spectra are the first data with high enough resolution to identify heterocyclic components of petroleum and related products. Rodgers *et al.* (2001b) used this technique to examine a subset of crude oil, the petroporphyrins, after solvent extraction and chromatographic separation. Qian *et al.* (2001) showed the technique could be used to examine whole crude oil and identified approximately 3000 nitrogen-containing aromatic compounds, out of approximately 5000 total compounds resolved between 250 and 1250 Da. They observed an average of 10-50 peaks per nominal mass. Most of these compounds are singly-charged and so these peaks represent 10-50 different compounds with the same *m/z* value. The ability to resolve so many peaks per nominal mass is unprecedented and represents a leap forward in the structural characterization of the polar fraction of petroleum and related products.

The mass resolution achieved by FT-ICR MS can be used to monitor molecular-level changes during different experimental manipulations. For example, Hughey *et al.* (2001b) NS-, NO-, $N_2O$- and $O_2$-containing compounds were preferentially removed by

hydrotreating. With the ultrahigh resolution and mass accuracy available with this technique, these changes were assessed without prior chromatographic separation. For the petroleum work of Hughey *et al.* (2001b), the difference in relative ionization efficiencies among different components served to reduce the complexity of the spectrum. Since that study was particularly interested in which nitrogen-containing compounds were recalcitrant after hydrotreatment, the difference in relative ionization efficiencies was used to focus on the compounds of interest.

### *4.3. Other current applications*

Other emerging areas of study include forensic analyses and compositional analyses of food products and natural organic matter. In forensics, FT-ICR MS has been used to identify arson accelerants (Rodgers et al., 2001a). In addition, both explosives and their residue have been analyzed to develop a method for explosives fingerprinting. (Wu et al., 2002). Environmental contaminants such as 3,3'-dichlorobenzidine and their degradation products have been monitored by Nyman et al. (1999). Cooper and Marshall (2001) examined elemental composition differences in different types of wine and tentatively identified a number of components. Components of biological mixtures (e.g., lipids - Rodgers et al., 2000a) and of natural organic matter such as phosphorus-containing compounds (Llewellyn et al., 2002) have been studied in detail after isolation from more complex mixtures.

## 5. Future applications

### *5.1. Biomarker identification and fingerprinting of sources*

Qualitative analyses of different samples are well within the capabilities of this instrument. If the hypotheses being tested are carefully posed, this technique has enormous potential to characterize a suite of environmental samples from a variety of sources. One can envision a process of "fingerprinting" different samples to explore the environmental processes affecting different compound types. For example, terrestrial and marine organic matter has been shown to be very different both chemically and structurally (e.g., Hedges and Oades, 1997). The molecular differences between these two pools have not been examined due to the lack of appropriate analytical tools (a good review of organic matter characterization is contained in Hedges et al., 2000). With ESI FT-ICR MS, fractions of this material can be examined and compound groups specific to each pool can be identified (i.e., a suite of biomarkers). The concept of biomarkers has been used extensively in biogeochemical studies and has helped apportion source function in different environments. Since ESI FT-ICR MS data cannot be used quantitatively at this time, only the presence or absence of a particular compound (or suite of compounds) can be used to identify sources. However, using the ultrahigh resolution and mass accuracy, detection of these indicative compounds may be trivial. Interesting biomarkers would include geochemically relevant enzymes and compounds specific to particular microbial species.

### *5.2. Detection and analysis of complexes*

Another future application that is particularly appealing is the identification of complexes within natural organic matter such as aggregates and/or micelles and complexes with metals or anthropogenic organic contaminants. The nature of these interactions is not well understood in aquatic environments and it would be instructive to isolate a specific complex to study the underlying chemistry of the interaction. However, a number of methodological concerns need to be addressed. For example, the basis of the compound-compound interactions determines the extent to which the complex will survive the electrospray ionization process. Complexes based on electrostatic interactions survive the ESI process since the driving force is interactions between the components of the complex (Loo, 1997). In contrast, complexes based on hydrophobic interactions are solvent-dependent and may not be stable in the gas phase after ESI. Thus, as the relative contribution of hydrophobic interactions increases, the complex is less stable during ESI and is not always detected (an excellent review of this topic in Loo, 1997).

Characterization of humic acid / metal complexes has been attempted by Solouki *et al.* (1999) and Alomary *et al.* (2000). Using ESI FT-ICR MS, they first examined the hydrogen /deuterium exchange properties of fulvic acids. The study was then extended to explore the ability of fulvic acids to associate with Al by equilibrating a fulvic acid sample with Al and noting any mass shifts in the broadband (large mass range) ESI FT-ICR mass spectrum. Specific complexes could not be identified within the large number of peaks in both spectra (before and after Al addition). In addition, the compounds within the original spectrum could not be resolved adequately to observe the appropriate

mass shift after addition of Al. The investigators were able to show, however, that an overall mass shift consistent with the complexation of Al (+26.98 Da) had occurred. Complexation of fulvic acids with iodine was also examined by Moulin et al. (2001) using a quadrupole time-of-flight spectrometer. These studies are a good first step in the use of ESI FT-ICR MS for the characterization and identification of complexes with organic matter. However, further work is needed to establish the types of complexes that will survive ESI before reliable data on hydrophobic complexes are obtained with this technique.

### *5.3. Other potential applications*

One can imagine a host of other possible applications of ESI FT-ICR MS to environmental questions. For example, MS techniques with a laser desorption interface have been used to identify whole microbes (e.g., Fenselau and Demirev, 2002, Yao et al., 2002). Rapid identification of biological warfare organisms such as the smallpox virus or Anthrax spores is within the capabilities of a technique with ultrahigh resolution and mass accuracy. In another example, identification of contaminants and their metabolites within a complex biological extract can be difficult due to the inability to extract polar metabolites from aqueous solutions (Nyman et al., 1999). The ultrahigh resolution of ESI FT-ICR MS enables the detection of specific compounds such as polar metabolites, even if they exist in small amounts.

## 6. Conclusions

The emergence of ESI FT-ICR MS as an analytical tool in environmental chemistry has revolutionized our ability to determine the elemental composition of components of

complex environmental mixtures. The ability to examine polar compounds with ultrahigh resolution and mass accuracy has led to knowledge of the composition of polar constituents of natural organic matter and petroleum. In the case of petroleum, this knowledge will lead to improved petroleum processing. Although spectra are still qualitative, well-designed studies will be able to use this technique to answer questions of chemical composition that were inaccessible previously.

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**8. Citations**


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## 9. Figure Captions

**Figure 1.** A simplified schematic of electrospray ionization. A voltage difference is applied across the gap between the sample needle and capillary of the mass spectrometer used. This voltage difference attracts appropriately charged ions into the capillary. Within the capillary, ions are further de-solvated by either heat or $N_2$ gas (or both). After the capillary, the ions are focused by a series of ion optics and accelerated into a mass spectrometer.

**Figure 2.** Ions travel into the bore of the magnet along the z axis. The application of a static magnetic field bends the ion motion into a circular orbit within the x-y plane. The frequency of this orbit, $f_c$, depends on the magnetic field strength, $B_0$, the charge of the ion, $z$, and the mass of the ion, $m$.

**Figure 3.** The transformation of ion cyclotron motion into a mass spectrum. The radius of the ion motion is increased by the application of an rf frequency on the two excitation electrodes. As the ions pass by one of two detection plates (shown above and below the ion radius), they induce a current differential between the two plates which is then amplified and plotted versus time. The time transient is Fourier-transformed and the resultant frequency spectrum is converted to a mass spectrum using the relationship between ion frequency and ion m/z. Note A and B are constants.

**Figure 4.** A representative spectrum of a humic acid sample. This positive ion spectrum was collected with a sample of Mt. Rainier humic acid (1 mg $mL^{-1}$) in 75:25 MeOH:

water. Approximately 18,300 scans were co-added prior to Fourier transformation. Reprinted from Kujawinski *et al.* (2002b). **Need permission!**

**Figure 5.** A representative spectrum of a riverine dissolved organic matter spectrum. This positive ion spectrum was collected with a sample of Suwannee River dissolved organic matter (1 mg $mL^{-1}$) in 75:25 MeOH: water. The dissolved organic matter was collected using ultrafiltration through a reverse osmosis membrane (courtesy of E. M. Purdue, Georgia Institute of Technology). Approximately 17,000 scans were co-added prior to Fourier transformation. Reprinted from Kujawinski et al. (2002b). **Need permission!!**

**Figure 6.** The effect of magnetic field on Mt. Rainier humic acid (MRHA) MS spectra. The top panel shows the MRHA spectrum acquired with a 9.4 T magnet (150 scans); the bottom panel shows the MRHA spectrum acquired with a 7 T magnet (22,700 scans). The insets of each of the panels highlight the difference in compound resolution between the two magnets. The shift in *m/z* values of the peaks arises from the decrease in space charge effects in the higher magnetic field strength instrument. This translates to a better resolution of the compounds present and so a more accurate assessment of their molecular weight. The elemental compositions of these compounds are not known at this time. Reprinted from Kujawinski et al. (2002b). **Need permission!!**