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Organic Geochemistry 34 (2003) 1325–1335
Organic Geochemistry
www.elsevier.com/locate/orggeochem

High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C_{18} solid phase disk

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Received 16 September 2002; accepted 10 April 2003
(returned to author for revision 20 January 2003)

Abstract

We propose and demonstrate an approach involving use of C_{18} solid phase disk extraction coupled with high resolution mass spectrometry for obtaining non-invasive molecular level information on dissolved organic matter (DOM) from river water. With this approach, DOM extraction from acidified natural water can be achieved rapidly with a simple filtration setup at a remote field site. From total organic carbon and UV-Vis absorbance measurements, we show that a large portion (over 60%) of the original DOM in water is recovered without the interference of salts. NMR analysis indicates that the C_{18}-isolated material has a similar distribution of functional groups as the original DOM but 2-D NMR details are greatly enhanced. Electrospray ionization mass spectrometry and high resolution mass spectrometry were employed to study DOM at molecular level. Highly resolved mass spectra of DOM (resolving power ($\delta m/m_{50\%}$) > 80,000 at m/z <600) reveal that there are many pairs of peaks that differ by the exact masses of $-H_2$, –O, or $-CH_2$ indicative of possible homologous series of structures.

1. Introduction

Dissolved organic matter (DOM) in rivers and oceans is comprised of a complex mixture of compounds including degradation products from plants and animals. Understanding the chemistry and origin of DOM, specifically riverine DOM, is important because it is a major source of carbon flow to the oceans and plays an important role in the global cycle of carbon. However, there is currently little molecular level information available for this material (Hedges et al., 2000). This is attributed mainly to analytical difficulties arising from DOM's complexity, high polarity, low concentration in natural water (ppm or ppb level) and lack of well-suited non-invasive analytical methods. ^{13}C NMR (Wilson, 1987; van Heemst et al., 2000) and gas-chromatography/mass spectrometry (GC/MS) (Bruchet et al., 1990; Saiz-Jimenez, 1994; Schulten, 1999) connected to pyrolysis, CuO (Ertel et al., 1984; Hautala et al., 1998; Opsahl and Benner, 1995) degradation or tetramethylammonium hydroxide (TMAH) (del Rio et al., 1998; Saiz-Jimenez et al., 1993) chemolysis have been used extensively to characterize riverine DOM chemically. ^{13}C 1D NMR is very effective in obtaining an average functional group distribution of the mixture but provides little information about the individual constituents. Pyrolysis and chemolytic approaches (CuO

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doi:10.1016/S0146-6380(03)00101-3

and TMAH) are invasive techniques that are also selective in that only volatile products can be observed (Saiz-Jimenez, 1994). Considering the inherent polar nature of DOM molecules (a characteristic that strongly contributes to its solubility in water) and the fact that thermolytic or chemolytic methods can be destructive for most of the DOM, it is possible that only a small portion of the DOM may be analyzed by GC-based methods. Therefore, it is very important to develop a procedure that enables us directly to characterize the individual component molecules of DOM in a relatively non-invasive manner.

Electrospray ionization (ESI) is a soft ionization technique that has been recently used to analyze trace amounts of biomolecules (Mann et al., 2001). ESI-MS is becoming an important technique for identification and characterization of natural organic mixtures such as humic substances (Brown and Rice, 2000; Fievre et al., 1997; Hatcher et al., 2001; Kujawinski et al., 2002a,b; Leenheer et al., 2001; Plancque et al., 2001; Solouki et al., 1999; Stenson et al., 2002). Since riverine DOM is composed of polar natural organic mixtures including humic substances (Thurman, 1985), it is reasonable to assume that ESI-MS can be an important analytical method for obtaining molecular level information on DOM. McIntyre et al. (1997) studied organic material from ground water and showed that ESI-MS could be used to study the material.

Because relatively small amounts of organic material exist in natural water, sample pre-treatment is necessary to concentrate the organic material, before ESI-MS analysis can be performed. Traditionally, XAD resin extraction (Leenheer, 1981; Thurman and Malcolm, 1981), freeze-drying and ultrafiltration (Burba et al., 1995) have been used to isolate and concentrate DOM from water. XAD extraction requires an extensive and time-consuming procedure to clean the resin before adsorption. Freeze-drying is another excellent method to collect the non-volatile portion of DOM. However, it often requires a significant amount of time and the dried solids contain significant quantities of nonvolatile salts. Since salts are known to interfere in the electrospray ionization process (King et al., 2000), extensive desalting may be necessary prior to ESI-MS analysis.

Ultrafiltration is also widely used to fractionate DOM according to nominal molecular weight. A variety of membranes are used for this purpose, the lowest molecular weight cutoff membrane being usually 1000 Daltons (Da). Since a significant portion of DOM may be composed of small molecules (less than 1000 Da), it is very likely that the ultrafiltration retentate may not represent the true molecular weight distribution or composition of DOM. The low recovery of total organic matter in most studies employing ultrafiltration may be attributed to this loss of low molecular weight material. In seawater, for example, only a maximum of 35% of the total DOM is recovered (Aluwihare et al., 1997; Benner et al., 1992). The whole process of isolation and concentration requires a substantial amount of time, as does XAD resin extraction.

C_{18} solid phase extraction (SPE) has been used to concentrate and desalt trace organic molecules including herbicides (Ferrer et al., 1999; Viana et al., 1996), metal–organic complexes (Mills et al., 1982), biodegradation products (Bielicka and Voelkel, 2001), dissolved lignin (Louchouarn et al., 2000) and DOM (Amador et al., 1990; Roubeuf et al., 2000) from water. For DOM, C_{18} solid phase extraction is reported to be more efficient than XAD-2 extraction by 24–84% (Amador et al., 1990). C_{18} SPE and mass spectrometry has been used to study carboxylic acids (Frauendorf and Herzschuh, 1998), biological samples (Malmstrom et al., 2002; Yang et al., 2002) and effect of UV radiation on treated water (Magnuson et al., 2002). C_{18} SPE is available as a disk or a cartridge. A sample can be isolated and concentrated in a considerably shorter amount of time with a disk because higher flow rates can be used (Liska, 2000). Since extraction rate is relatively independent of flow rate (Liska, 2000), the experimental setup for disk SPE is more flexible than for cartridge SPE. For example, a simple filtration setup with an aspirator as a vacuum source can be used to extract a sample. Because of this flexibility and simple setup, disk SPE can be easily adapted to field studies.

We propose that the C_{18} disk SPE may be a very efficient extraction method for DOM, producing samples that are well suited for direct molecular level high resolution ESI-MS analysis of DOM. In this study, we report the demonstration of this combined approach for rapid processing by SPE disk extraction and molecular level characterization of DOM from natural water samples using high resolution ESI-MS, primarily to demonstrate that this approach can be used as an effective characterization protocol. In addition, we show that the isolated salt-free DOM provides superior data from 2-D NMR spectroscopy.

2. Experimental section

2.1. Chemicals and materials

Nano-pure water was prepared from a water purification unit (UHQ, ELGA, Lowell, MA). HPLC grade methanol was obtained from Fisher Scientific (Itasca, IL). The standard 47 mm filter apparatus consisted of a stainless steel mesh support on a Teflon® base. Solid phase C_{18} extraction disks (47 mm) were purchased from 3M Empore (St. Paul, MN). Before extraction, disks were conditioned according to the manufacturer's

recommendations that involved soaking with 10 ml of MeOH. An aspirator or vacuum pump (Laboport, Trenton, NJ) was used to draw the water samples through the extraction disk.

2.2. Sample preparation

All the water samples were filtered through 0.3 μm nominal pore size glass filters (Whatman, Clifton, NJ) prior to extraction. The pH values of the samples were adjusted to between 2 and 2.5 with tetrafluoroacetic acid (Applied Bio-Systems, Foster City, CA) or hydrochloric acid (ACS grade, Fisher Scientific) prior to extraction. DOM was extracted from water samples collected from two streams. One stream is a black-water stream located in the Pinelands of New Jersey (McDonalds Branch, DOC content averages 15–18 mg/l) and the other is a rainfall-fed mountain stream located in the Guanacaste Conservation Area in Costa Rica (Rio Tempisquito, DOC content averages 0.5–1 mg/l) (Newbold et al., 1995). The samples from New Jersey were frozen at the Stroud Water Research Center (Avondale, PA), and transported to The Ohio State University (Columbus, OH) and then thawed before DOM isolation. Aliquots of 300–500 ml of the water were sequentially loaded onto the SPE disk to prevent breakthrough due to the relatively high concentration of dissolved organic carbon. In the case of samples from Costa Rica, up to 7 l of stream water was passed through the SPE disk on site because of the relatively low concentration of DOC. The retentate was eluted twice with 10 ml of 90:10 MeOH:H_2O solution and stored in the refrigerator to reduce evaporation of solvent.

2.3. Instrumentation

To evaluate extraction efficiencies of the C_{18} disk, absorbance spectra were obtained using a total organic carbon analyzer (TC 5000, Shimadzu, Columbia, MD) and spectrophotometer (UV-2501 PC, Shimadzu, Columbia, MD). The raw water sample from McDonalds Branch, eluent (the water sample passed through the C_{18} disk) and retentate were compared. The retentate was eluted and diluted with nano pure water before spectrophotmetric analysis.

Solution-phase NMR spectra of McDonalds Branch samples were acquired using a Bruker Avance 400 MHz NMR fitted with a QNP ^{1}H, ^{13}C, ^{15}N, ^{31}P probe. To obtain enough (~30 mg) DOM for NMR analysis, extraction of DOM was repeated at least five times. The extracted samples were dissolved in D_2O (0.75 ml)/NaOD (10 μl). 1-D Proton NMR spectra (32 scans) were obtained with 2 s recycle delay and processed with 1 Hz line broadening. Total Correlation Spectroscopy (TOCSY) spectra (128 scans, 512 time domain data points in F1, 1024 time domain data points in F2) were acquired using 80 ms mixing time, with Time-Proportional Phase Incrementation (TPPI). Processing was carried out using a sine-squared function with phase shift of 90° in both dimensions.

ESI quadrupole time of flight MS (Q-TOF, Micromass) was used to analyze the C_{18}-isolated DOM. In a positive ionization mode, experimental parameters were optimized with a capillary voltage of 3 kV, source temperature of 90 °C and cone voltage of 50 V. In a negative mode, the capillary voltage was set to −3.2 kV with same source temperature. Each sample was injected by syringe pump with a flow rate of 10–20 μl/min. Data were collected for about 5 min per sample.

All ESI FT-ICR MS experiments were conducted on a Apex II 7e (Bruker, Billerica, MA) 7 T system. Sample was injected continuously with a flow rate of 11 μl/h. The time domain signal was averaged at least for 5 h (1500 scans) to obtain high resolution MS spectra. Detailed information about the instrument and optimized conditions for FT-ICR MS experiments have been reported previously (Kujawinski et al., 2002a,b). The instrument was externally calibrated with polyethylene glycol solution of 600 Da average molecular weight purchased from Sigma. Mass accuracies within 20 ppm were achieved by external calibration.

Elemental analysis on solid samples was performed by a NA 1500 C/N analyzer (Carlo-Erba, Millano, Italy). C_{18} isolated DOM samples were subjected to freeze-drying before elemental analysis.

3. Results and discussion

3.1. Extraction of DOM by C_{18} SPE disk on site

Transportation of water samples from a field site to the laboratory can be problematic. Besides the difficulty in transporting large amounts of water to the laboratory for analysis, sample treatment such as freezing before shipment can precipitate some material that can alter the composition of DOM. Therefore it is beneficial if samples can be extracted at the field site. Most procedures currently used to isolate DOM either require long extraction times or an instrument that is not easily transported (Leenheer, 1981; Thurman and Malcolm, 1981; Burba et al., 1995). Due to its simple setup, the SPE disk and filtration hardware were easily transported to a remote field station, in this case to Costa Rica. The final preserved samples (20 ml in MeOH:H_2O for each extraction) were easily transported to the laboratory. The sample from McDonald's Branch was not extracted in the field due to the fact that such an approach was not implemented at the time of collection.

3.2. Characterization of C_{18} SPE disk

To evaluate the C_{18} SPE disk as a tool for extraction of DOM from acidified natural water samples, total organic carbon (TOC) concentrations, UV-Visible absorbance measurements and ^{1}H-NMR were employed. Extraction efficiency for different techniques is usually measured by comparing total organic carbon (TOC) concentrations of DOM samples (Mills and Quinn, 1981; Roubeuf et al., 2000) before and after extraction. However TOC analysis was difficult in this study due to MeOH remaining within the C_{18} disk after activation. From a blank experiment, TOC contributed by MeOH (background TOC value) was 21 mg/l for the first rinse using 500 ml of the nano-pure water. Since the background TOC value is higher than the TOC value of the original water sample (15 mg/l), TOC measurements could not be conducted with any degree of accuracy, especially if we employed the extraction procedure that was recommended by the manufacturer (soaking the C_{18} disk in methanol right before extraction). Thus, for TOC measurements, we chose to flush as much MeOH as possible from the disk and at least 6 l of nano-pure water was used for this purpose prior to extraction. After this, 500 ml of acidified McDonalds Branch water was loaded onto the disk. The eluents were collected and TOC was measured (6 mg/l). The values were compared with the TOC values of the raw water to obtain the extraction efficiency of 60%. It should be noted that 60% might not fully represent the extraction efficiency of the C_{18} disk. This is because MeOH, the activating agent, was washed out before the extraction and measurement of TOC. Therefore 60% should be considered a lower end of the extraction efficiency.

Because of this limitation, absorbance measurements are probably a better approach to evaluate extraction efficiency. Absorbance spectra from McDonalds Branch raw water and eluent were compared to evaluate the extraction efficiency of chromophoric substances (Fig. 1a). To calculate extraction efficiency of the disk, the absorbance values for each trace were integrated from 250 to 400 nm and the values were compared. Overall, 70% of chromophoric material was retained on the disk after sample acidification. During sample loading, the retained organic material changed the color of the disk from white to brown. The color of the disk reverted to white after elution with 90:10 MeOH:H_2O. The recovery of the extracted material into the eluent solvent (90:10 MeOH:H_2O) was calculated from absorbance spectra (Fig. 1b). To calculate the recovery efficiency, the integrated absorbance values from 250 to 400 nm of the diluted and volume reconstituted retentate were compared to the integrated absorbance differences of the same range from Fig. 1a. The absorbance difference in Fig. 1a represents the amount of chromophoric molecules extracted by the C_{18} disk SPE. About

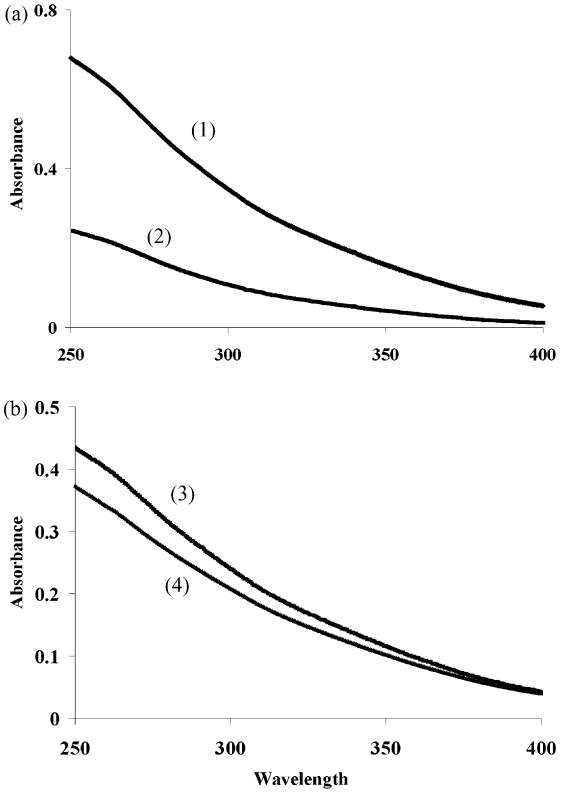

Fig. 1. Extraction and recovery of C_{18} SPE disk by absorbance experiments; each line represents absorbance values (1) from raw water, (2) from eluent (raw water after extraction), (3) of the difference between (1) and (2), and (4) from the diluted C_{18} SPE disk extracted sample reconstituted to an original volume.

90% of the colored material was recovered from the disk into eluent solvent.

Changes in functional group distribution after C_{18} extraction were investigated by ^{1}H NMR analysis. For the McDonalds Branch sample, both raw water and C_{18} extracts were subjected to freeze-drying. Recovered materials were redissolved into D_2O for NMR analysis. In general, the spectra display similar characteristics with the exception of a broad lump centered around 2 ppm which is more prominent in the untreated sample (Fig. 2). This band is likely to result from either short chain acids such acetic acid or inorganic hydroxide species. Both are known to be abundant in natural waters, will be retained during freeze-drying, and neither will be retained strongly by the C_{18} disk. The spectra show broad profiles with contributions from aromatic/amide, oxygen containing functionalities (such as sugars, methoxy, aliphatic lignin linkages, peptides, esters and ethers), and aliphatic units (Simpson, 2001; Simpson et al., 2001a). From this, it appears that the C_{18} SPE disk extracted samples retain large portion of the functional group distribution of organic material in raw water.

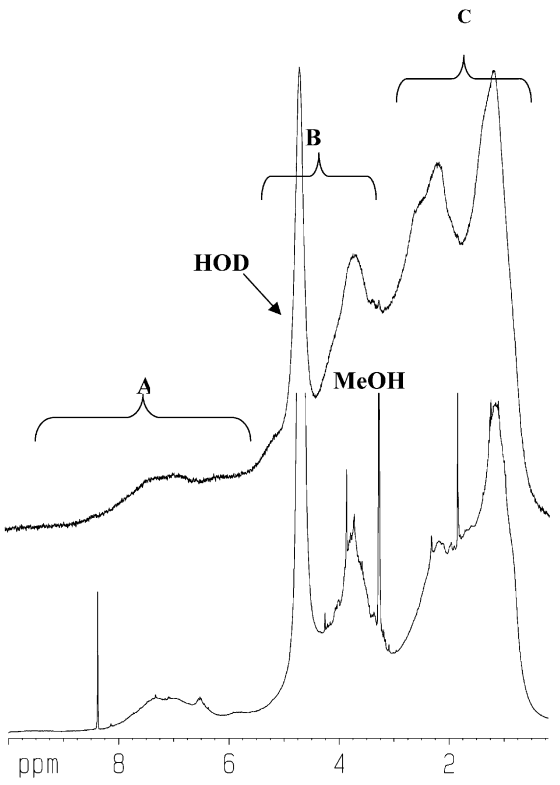

Fig. 2. Proton spectra of the DOM isolated by evaporation (top) and after isolation with C_{18} SPE disk (bottom). The major structural categories are labeled (top) as protons in aromatic and amides (A), sugars, amino acids, methoxy, aliphatic hydroxy (B) and aliphatic, amine protons (C).

Several sharp peaks are observed in the spectrum of the sample prepared by C_{18} disk in Fig. 2. Those peaks are not from C_{18} material. Methylene protons in long chains, as is present in the C_{18} resin, will resonate as a fine and sharp resonance at 1.29 ppm. Even a small quantity of C_{18} would be sufficient to completely swamp the extremely weak signals from the DOM. Thus, the NMR data indicate that there is no detectable contamination of the DOM by the C_{18} material. The appearance of sharp peaks in the treated DOM is likely attributable to the removal of metals from the DOM by the SPE. In the original DOM, metal species that are commonly associated with the DOM can induce rapid relaxation and are thought to lead to aggregation of DOM (Simpson et al., 2001a). Simple molecules such as methanol, formic acid, acetic acid and sugar species will be less likely to be bound or associated with DOM through metal bridging. Even small quantities of these small molecules free in solution will produce sharp signals that will be superimposed on the broader background characteristic of the DOM. Furthermore, before C_{18} isolation, the high concentrations of paramagnetic metal species can interfere with spectrometer shimming (homogenization of the magnetic field, carried out during experiment set up) and this tends to broaden the lines. Sharp signals will only appear in a highly homogeneous field which cannot be achieved in freeze-dried DOM due to the paramagnetic metal contents. Thus, appearance of sharp signals from small molecules within the DOM mixture is expected after a clean up procedure.

3.3. 2-D NMR analysis of DOM

Both the raw water and the C_{18} extract were further analyzed by 2-D NMR spectroscopy. Fig. 3 compares the TOCSY spectra of the materials. The TOCSY spectrum of the material obtained by freeze-drying of raw water contains few if any cross peaks and provides little structural information (Fig. 3a). The lack of cross peaks results from an abundance of paramagnetic species that cause the organic protons to relax during the mixing time of the 2-D experiment. However, the freeze-dried sample of the C_{18} isolated DOM exhibits longer relaxation times and thus results in higher signal-to-noise ratios and improved spectral definition. The improved 2-D NMR spectrum is presumably due to the considerably reduced salt content in the C_{18} isolated material. Numerous cross peaks can be identified and general assignments are given in Fig. 3b. The detailed interpretation of the components present is beyond the scope of this paper, and has been already discussed for similar samples (Simpson et al., 2001a,b).

3.4. High resolution mass spectrometry of DOM

High ionic strengths can adversely affect the electrospray ionization process (King et al., 2000) and thus decrease sensitivity and resolution of MS analysis. In a previous study (Brown and Rice, 2000), it was observed that the peaks from fulvic acid were reduced and disappeared in high ionic strength solutions (> 0.001 M). Therefore it is desirable to remove excess salt from the sample prior to ESI-MS analysis. The retentate from C_{18} SPE yielded high resolution mass spectra without further purification. Spectra of McDonalds DOM samples extracted by SPE and analyzed by Q-TOF MS are presented in Fig. 4. Both negative and positive modes of ESI-MS were attempted for McDonalds Branch DOM sample and almost identical spectra were obtained (Fig. 4a, b). Since improved ionization current was obtained in the positive mode without adding additional acid or base in our study, positive ion mode was used in this study. The sample from Costa Rica was also analyzed by Q-TOF MS and the spectrum is displayed in Fig. 4c. The two spectra (Fig. 4a, c) show different distributions of mass to charge ratio; nonetheless they clearly demonstrate that the extraction can be successfully performed at a remote field site as well as in the laboratory. In the spectra, most peaks are located below

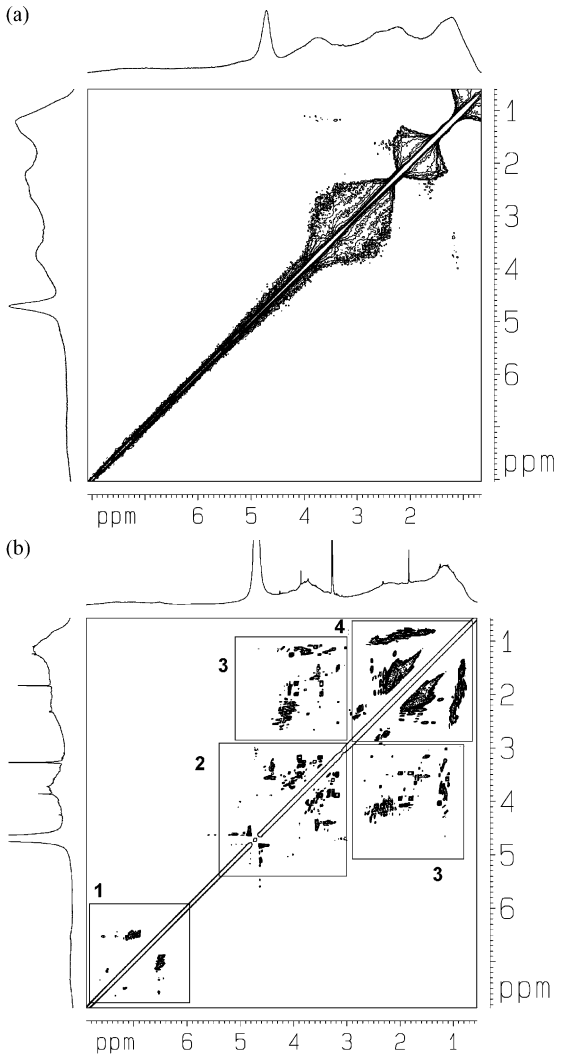

Fig. 3. TOCSY spectrum of the DOC isolated by evaporation (top-a) and after isolation with C_{18} SPE disk (bottom-b). General assignments included within text boxes are as follows (1) aromatics; (2) sugars; aliphatic units bridging lignin aromatics; amino acids α–β couplings); (3) methylene units adjacent to ethers, esters, and hydroxyls in aliphatic chains; amino acids (α–β–γ couplings); (4) methylene in aliphatic chains; and methyl units in amino acids and aliphatic chains.

1000 mass to charge ratio. This distribution of peaks is consistent with previously published spectra of fulvic acid from natural water (Plancque et al., 2001; Stenson et al., 2002); however, it is lower than what it could be expected from the previously reported average molecular weight of DOM (Beckett et al., 1987; Chin et al., 1994; Wagoner et al., 1997). The reason for the lower than 1000 m/z distribution could be rising from selective ionization and/or fragmentation in the ionization process (Leenheer et al., 2001; Stenson et al., 2002). More research is being conducted to address this issue.

The McDonalds Branch DOM sample was further analyzed by positive mode ESI Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) for enhanced resolution and sensitivity. A high resolution positive ion spectrum (mass resolving power of $m/\Delta m_{50\%} > 80{,}000$ at $m/z < 600$) was obtained (Fig. 5). This spectrum shows the molecular complexity of DOM. Not only are there clusters of peaks at every nominal mass unit up to 1000 m/z but each cluster is further resolved into several peaks. Similar results were observed in ESI FT-ICR mass spectra of other humic substances (Kujawinski et al., 2002a,b; Stenson et al., 2002). Nested within the spectrum are series of intense peaks at odd mass to charge ratio (m/z) and weak peaks at even numbered m/z. This pattern was previously reported (Brown and Rice, 2000) from negative ion spectra of fulvic acid and interpreted as either chloride adducts or a homologous series of molecules. If chloride adducts were responsible for this pattern, hydrochloric acid used to acidify the natural water samples would be the likely source. To test this possibility, two DOM samples, one acidified by trifluoroacetic acid and the other by hydrochloric acid, were respectively extracted and compared (data not shown). Identical spectra are obtained with and without added chloride ions indicating that the pattern is not necessarily due to chloride adduct formation from the HCl.

Kendrick mass defect (KMD) analysis (Kendrick, 1963) can be used to identify patterns of elemental composition within high resolution mass spectra of complex mixtures (Hughey et al., 2001; Stenson et al., 2002). The concept of Kendrick mass is to change the mass scale into a CH_2 mass-normalized scale [Eq. (1)]. Kendrick mass defect is then calculated as the difference between the normalized Kendrick mass and the nominal observed mass [Eq. (2)]. The values for Kendrick mass defect are a reflection of the deviation of an exact mass from that of homologous structures varying only by CH_2 groups. In other words, the exact mass of molecules varying by the same functional group (CH_2) would be different by multiples of the exact mass of CH_2, and, as a result, they will have the same Kendrick mass defect value. Thus, two fatty acids with elemental compositions of $C_{20}H_{40}O_2$ and $C_{21}H_{42}O_2$ will have the same Kendrick mass defect. Kendrick mass defect can be used to identify patterns of masses having the same compositional differences (Stenson et al., 2002).

$$\text{Kendrick mass} = \text{observed } m/z \times (14/14.01565) \quad (1)$$

$$\text{Kendrick mass defect} = (\text{nominal observed mass} - \text{Kendrick mass}) \times 1000 \quad (2)$$

Kendrick mass defects for the many peaks in the DOM sample were calculated from the FT ICR MS

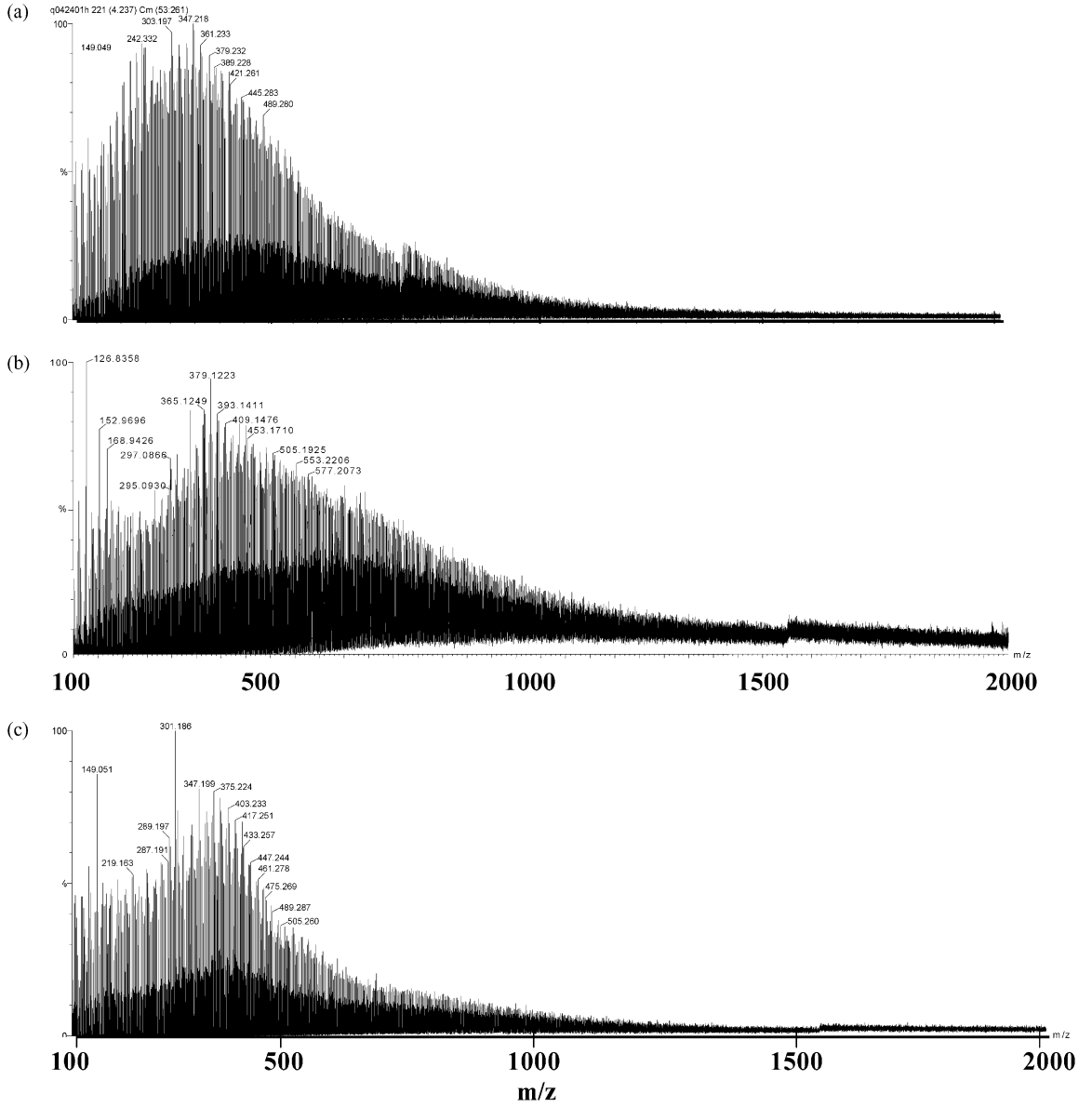

Fig. 4. Positive (a) and negative (b) ion mode ESI Q-TOF mass spectra of DOM isolated from McDonald's Branch and (c) positive ion mode spectra of DOM from Rio Tempisquito, Costa Rica.

data and plotted (Fig. 6a). Four significant figures after the decimal point were used to calculate the mass defect. In a plot of Kendrick mass defect versus nominal observed mass, molecules differing by a specific elemental composition (due to exact mass of the contributing atoms) are connected by lines. The slope of the lines can be determined by the following equation:

$$\text{Slope} = \left(\Delta \text{ Kendrick mass defect} / \Delta \text{ nominal observed mass}\right) \times 1000 \quad (3)$$

where Δ is used to represent a difference.

Molecules separated by CH_2 units will have same Kendrick mass defect [the numerator in Eq. (3) will be zero in these cases] and be connected by horizontal lines with a slope of 0. The lower expanded mass range of the spectrum of DOM is shown in Fig. 6b and numerous series of molecules differing by CH_2 can be identified. Other series of molecules differing by H_2 and O were also identified (Fig. 6c,d). In these plots, the peaks differing by the corresponding masses of H_2 or O can be identified by parallel lines, each with a specific slope (a slope of -6.7 and 1.4, respectively). The series are further verified by calculating and comparing the exact

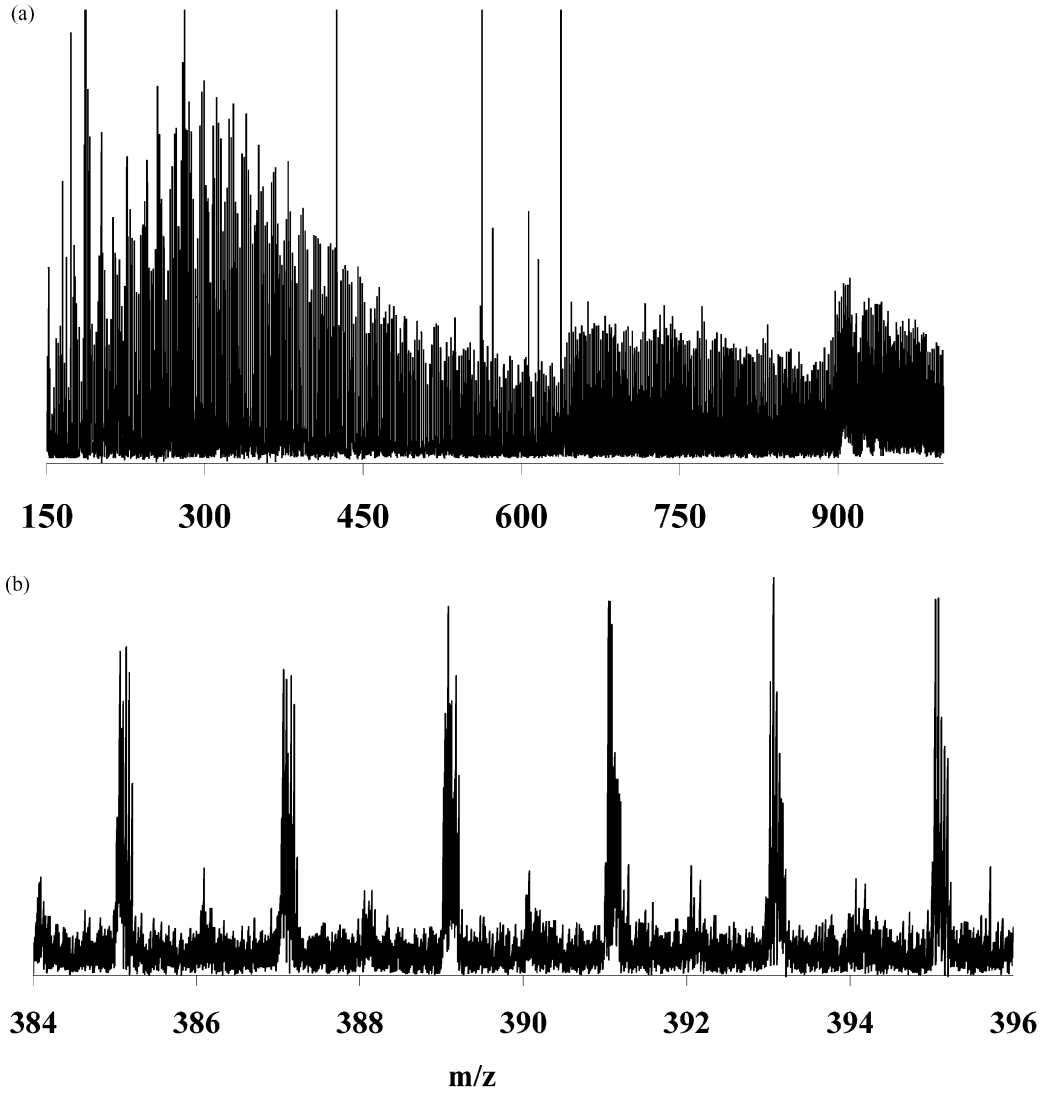

Fig. 5. Positive ion mode ESI 7 T FT-ICR mass spectrum on McDonald's Branch DOM (a) and expanded view of selected region (b).

mass difference between the peaks to the theoretical mass of H_2 and O. The series representing CH_2, H_2 and O result in even number differences between peaks and this, along with the added H^+ from ionization, is what primarily contributes to the pattern of predominantly odd mass peaks observed for DOM.

The predominant peaks in the spectrum occur at odd m/z throughout most of the mass range. In ESI MS spectra, even numbered peaks can arise from even electron ions that contain an odd integer number of nitrogen atoms. While the weak even m/z series in these spectra are likely due to the ^{13}C isotope peaks from the odd m/z series, they may also be due to ions that contain an odd number of nitrogen atoms. In order to determine the nitrogen contribution, the elemental composition of the freeze-dried McDonald's Branch DOM sample was obtained by combustion analysis (Table 1). The molar ratio between carbon, hydrogen and nitrogen is 49:59:1. While we expect that nitrogen containing ions will have very high ionization efficiencies compared to other heteroatoms, it is reasonable to expect that with low elemental nitrogen content the possibility of having compounds with an odd number of nitrogen atoms will be low. Therefore we conclude that the peaks observed at even m/z are most likely due to isotopic contribution from the more intense odd m/z series of ions. This was verified in many instances by observing that the odd m/z predominant peaks showed corresponding isotope peaks at exactly

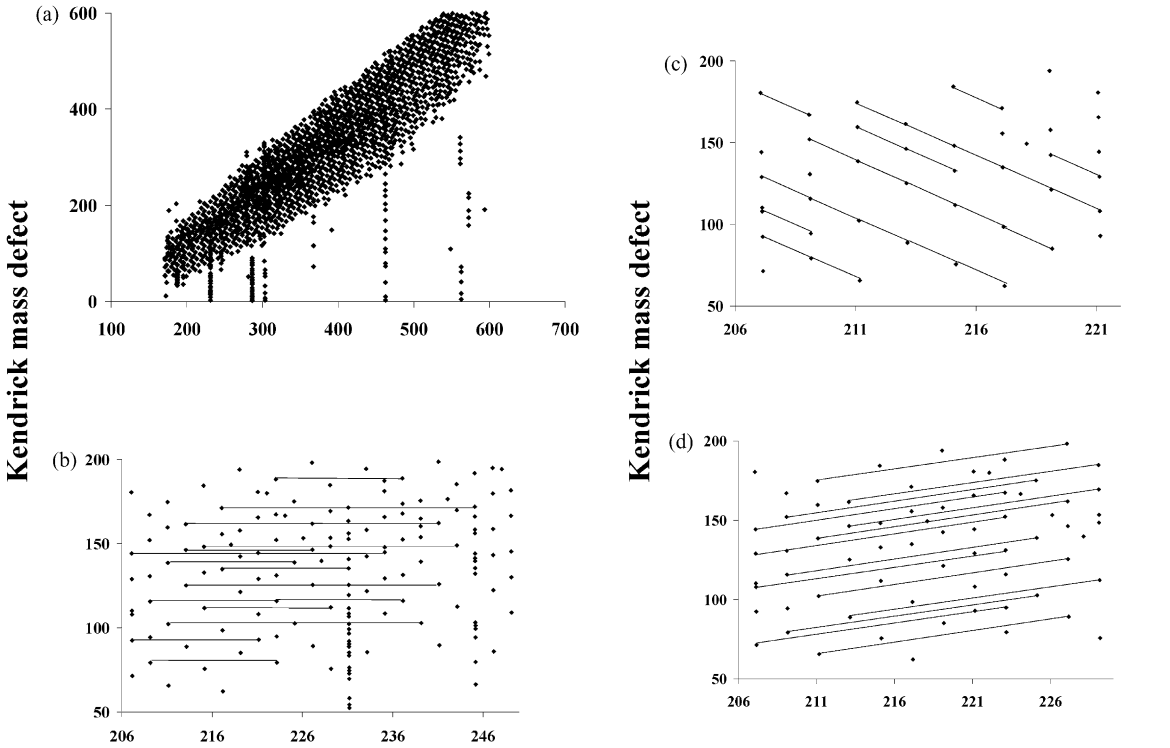

Fig. 6. Kendrick mass defect plot for the entire mass region ($170 < m/z < 600$) (a) and expanded plots with lines denoting the series of peaks separated by CH_2 (b), H_2 (c) and O (d).

Table 1
Elemental analysis of freeze-dried, extracted DOM from McDonald's Branch

Element	Weight percent (%)	Molar ratio
C	46.4	49.2
H	4.6	58.5
N	1.1	1

1.00335—the mass difference between an atom of ^{12}C and ^{13}C as has been observed by Stenson et al. (2002).

4. Conclusions

By employing a C_{18} disk SPE, DOM in acidified natural water can be isolated and desalted with a simple filtration setup either in a laboratory or at a field site. This protocol also efficiently removes inorganic materials that may be problematic for analysis by NMR or ESI-MS. The material obtained from C_{18} disk retained the majority (over 60%) of DOM and reflected the original functional groups distribution. From the high resolution mass spectrum and elemental analysis of DOM, it was found that series of molecules with a mass difference equivalent to $-CH_2$, $-H_2$ and –O and a low content of nitrogen contribute to the observed odd mass dominant peak pattern. In conclusion, the combination of C_{18} SPE and high resolution ESI MS and 2-D NMR spectroscopy is shown to be a very powerful approach to obtain molecular-level information on DOM. More studies are being conducted to obtain additional molecular level information from the high resolution spectra.

Acknowledgements

The authors thank Dr. Louis A. Kaplan for providing samples and Dr. Yu-Ping Chin and Sara Thomas for their assistance in TOC measurement. This work was supported by NSF grants (DEB-9904047, CHE-0089147, and CHE-0089172).

Associate Editor—**J. Rice**

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