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Research Article

Characterizing the properties of dissolved organic matter isolated by XAD and C-18 solid phase extraction and ultrafiltration

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Abstract. The properties of aquatic dissolved organic matter (DOM) isolated by solid phase extraction (SPE) C-18 cartridges, ultrafiltration, and XAD chromatography are compared. Samples taken from the Suwannee River, Georgia, USA and McDonalds Branch in the Pine Barrens, New Jersey, USA were chosen to represent waters where DOM originates from predominantly terrestrially-derived (allochthonous) precursors. Pony Lake, Antarctica represented an exclusively algal/microbially-derived (autochthonous) DOM. Fluorescence, UV absorption, ^{13}C NMR spectroscopy, and high-pressure size exclusion chromatography (HPSEC) were employed to discern differences and similarities between the DOM isolated by these three methods. Only subtle differences between isolation methods were observed for the terrestrially derived DOM samples when assayed by light and fluorescence spectroscopy. Conversely, the Pony Lake DOM isolates exhibit greater variability when analyzed by these methods. ^{13}C-NMR analyses showed structural differences between the methods for all samples. HPSEC analysis also revealed differences with the C-18 isolates exhibiting the highest molecular weights. Thus, it appears that each method isolates sufficiently different fractions of DOM that can only be delineated when a consortium of analytical methods are used to assay the samples. Nonetheless real differences between autochthonous and allochthonous derived DOM were observed with the algal-derived samples exhibiting high fluorescence ratios and lower aromaticity relative to the terrestrially derived materials. These results demonstrate that caution must be exercised when interpreting DOM reactivity data that rely upon the use of specific fractions.

Key words. DOM characterization; DOM isolates; DOM properties; ^{13}C NMR; HPSEC; EEM.

Introduction

Dissolved organic matter (DOM) is present in all natural waters and can participate in important biogeochemical and environmental reactions (Meier et al., 2004; Uhle et al., 1999; Breault et al., 1996; Rozan et al., 1999; Chin et al., 1997; Backhus and Gschwend, 1990; Voelker et al., 1997; Kaiser and Sulzberger, 2004). Many bioavailability and reactivity studies use either unaltered DOM or a previously isolated fraction e. g., fulvic acid. Little structural information, aside from simple light absorption or fluorescence, can be attained from unaltered DOM due to possible matrix effects (quenching by cations, light absorption of inorganic species) and low concentrations

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present in natural waters (typically < 2 mM carbon). The use of pre-isolated DOM relies heavily upon commercial sources, e.g., Aldrich or Fluka humic acids or materials provided to the scientific community from the International Humic Substances Society (IHSS). Most available DOM from the IHSS is comprised of both terrestrially and aquatic derived fulvic and humic acids. A small number of researchers isolate DOM for their own use by ultrafiltration (UF) or solid phase extraction (SPE) using a nonpolar C-18 stationary phase (Coble et al., 1990; Benner and Opsahl, 2001; McCarthy et al., 1996; Benner et al., 1997; Burdige and Gardner, 1998; Hedges et al., 2000; Louchouarn et al., 2000; Kaiser et al., 2003; Kaiser et al., 2004; Kaiser and Sulzberger, 2004). Thus, results from these DOM bioavailability and/or reactivity studies are only applicable to the fraction of DOM isolated based upon the method used and the environment from which the sample was attained.

Much of the aquatic DOM available to researchers from the IHSS has been isolated from the Suwannee River using either XAD chromatography or reverse-osmosis (Sun et al., 1995). SPE and UF constitute the other common isolation methods, but these fractions are typically not available to the research community at large. Previous research has examined the various attributes of the different types of isolation media e.g., C-8 versus C-18, C-18 versus XAD methods, or have conducted rudimentary comparison studies between XAD and UF (Peuravuori and Pihlaja, 1997; Aiken et al., 1992; Roubeuf et al., 2000). Kaiser and coworkers (2003) have used multidimensional solution-state NMR to elucidate the differences in the chemical composition of SPE and UF riverine DOM. To date we are unaware of any study that has examined in detail the structural components of DOM isolated by all three methods. Each technique fractionates the DOM matrix by a different process and presumably a component unique with respect to its reactivity, structure, and bioavailability. UF is a physical separation process that segregates based upon size even though matrix effects, e.g., ionic strength and pH influences the efficiency of the method. Molecular weight cutoff ranges for ultrafiltration membranes used to isolate DOM are typically 1 to 3 KDa, and efficiencies range from 20 to 99% of the total DOM in a whole water sample (McCarthy et al., 1996; Benner et al., 1997; Burdige and Gardner, 1998; Everett et al., 1999; Hedges et al., 2000; Kaiser et al., 2003; Kaiser et al., 2004; Kaiser and Sulzberger, 2004).

XAD and SPE are chromatographic methods that separate DOM into polar and nonpolar fractions. The Amberlite XAD-8 stationary phase is comprised of a polystyrene resin, while SPE materials are alkyl chains (C-8 to C-18) covalently bonded to a silica substrate. Fractionation of DOM by XAD-8 results in the familiar fulvic acids that many researchers use, but can be cumbersome due to the size of the columns and the care needed to prepare the resin. Conversely, SPE methods employ small columns or filtration disks, which are field portable and require minimum prep time. The major disadvantage of SPE is that the amount of material isolated is significantly less than either UF or XAD.

In this study we compared the structural characteristics of DOM isolated by UF, SPE, and XAD chromatography. We hypothesize that each method isolates a unique component of the DOM pool, resulting in materials that differ in structural characteristics and reactivity. Three sites, the Suwannee River, GA, USA, Pony Lake, Antarctica, and McDonalds Branch, Pine Barrens NJ, USA were selected for the inter-comparison between isolation methods and fluorescence instruments. DOM from these sites has been extensively studied by us (Chin et al., 1994, 1997; Meier et al., 1999, 2004; Uhle et al., 1999; Kim et al., 2003), and the Suwannee River is the source of the popular IHSS fulvic and humic acid used by many researchers. In addition, a less complete data set from two other sites (Hammonassett River, CT, USA, and Lake Fryxell, Antarctica) was also included. The two Antarctic waters represent an algal-microbial derived DOM endmember, while the McDonalds Branch and Suwannee River are acidic (pH 3–5) "blackwater" environments where the DOM is derived predominantly from terrestrial material. The Hammonassett River has significantly less DOM than the two "blackwater" sites, but is also influenced by inputs of terrestrially derived materials (Mash, 2001).

Methods

The Suwannee River was sampled in January 1997 at Fargo GA, while water samples from McDonald's Branch (site, S2) were collected within Lebanon State Forest, NJ in May 1997. All waters were sampled near the surface and transported back to Ohio State University within 36 hours. We collected water samples from Pony Lake, Antarctica in December 1997 from the surface. Ultrafiltration and XAD DOM isolation for this sample was conducted at the Crary Lab in McMurdo Station, while C-18 analysis was conducted back in Columbus, OH, on a subset of the sample.

All samples were filtered through 0.45 μm membrane filters and stored in the dark at 4 °C until analyzed. Samples for SPE C-18 were processed in the following manner: Varian 10 g Mega BondElute C-18 cartridges were cleaned with 500 mL of methanol (Optima) and then rinsed with approximately 20 L of Milli-Q water adjusted to pH 3 with HCl (Fisher, trace metal grade) (Louchouarn et al., 2000; Kaiser et al., 2003). Dissolved organic carbon (DOC) in all the samples (raw water reconstituted solutions, and blanks) was measured using a Shimadzu 5000 TOC Analyzer. Cartridges were used when the DOC

of our blanks was identical to our Milli-Q source water. Because of the highly variable initial DOC concentrations (> 3 mM to < 0.2 mM carbon) of our natural waters, sample volumes were adjusted (from 450 mL to 20,000 mL) to circumvent column overloading. Before elution, the SPE cartridge was rinsed with Milli-Q water adjusted to pH 3 to remove any residual salts remaining from the sample. Samples were then eluted using 100 mL of methanol (Optima) and were concentrated under vacuum at 30 °C. Finally, the samples were frozen and then lyophilized.

Splits of the water samples were isolated by tangential flow ultrafiltration (TFUF) using the methods described in Everett et al. (1999). Briefly, filtered and acidified water samples were passed through a tangential-flow UF system (Amicon CH2M) equipped with a 1000 Dalton membrane. The retentate was treated with a strong cation exchanger (Biorad GX-50) to remove paramagnetic metal ions, and freeze-dried. XAD isolation of the samples was conducted in Dr. George Aiken's lab at the USGS in Boulder, CO using the methods of Aiken et al. (1992) or at the Crary Lab in McMurdo Station.

Single fluorescence emission scans, synchronous scans, and Excitation Emission Matrices (EEMs) from 40 mg /L (as total mass DOM not DOC) solutions (pH 3) were obtained using a Cary Eclipse fluorescence spectrophotometer (Varian Instruments), with the following instrument parameters: bandpass of 5 nm, scan rate of 120 nm/min, and PMT voltage of 600 volts. Single emission scans were collected for DOM samples adjusted to pH 3 at an excitation wavelength of 370 nm. Both a Cary Eclipse and Hitachi F-2000 fluorescence spectrometers were utilized to calculate DOM fluorescence ratios at emission wavelengths of 450 and 500 nm to determine any possible variations between instruments (McKnight et al., 2001). Instrument parameters for the Hitachi F-2000 fluorescence spectrophotometer were as follows: bandpass of 10 nm, scan rate of 240 nm/min, and PMT voltage of 700 volts. A Milli-Q blank was subtracted from each DOM isolate scan, and each scan was corrected for inner-filter effects. Synchronous scans were acquired at pH 3, 6, and 8 with an excitation scan range of 280 nm to 530 nm and a wavelength offset ($\Delta\lambda$) of 20 nm (Cabaniss, 1992). EEMs of isolated DOM samples adjusted to pH 3, 6, and 8 were created by scanning the emission wavelength from 250 nm to 600 nm at excitation wavelengths in 10 nm increments beginning at 220 nm and ending at 600 nm. Milli-Q blanks were subtracted from each DOM EEM scan, which were subsequently normalized to carbon. All pH adjustments were made using HCl or NaOH.

Solid state ^{13}C-NMR spectra were obtained using the ramp cross polarization magic angle spinning (CPMAS) pulse program and two pulse modulated decoupling on a Bruker DSX 300 NMR spectrometer, operating at a frequency of 75.48 MHz for ^{13}C (Dria et al., 2002). Chemical shift ranges for DOM structures range between 0–60 for alkyl groups, 60–110 for O-alkyls, and 110–160 ppm for aromatic groups (Knicker et al., 1996; Hatcher and Wilson, 1991; Nelson et al., 1993). The various structural contributions were calculated as a percent total of the integrated area.

High pressure size exclusion chromatography (HPSEC) was used to determine weight averaged (M_w) molecular weights of isolates, filtrates, and whole water samples and calculated using Equation 1,

$$M_w = \sum_{i=1}^{N} h_i (M_i) / \sum_{i=1}^{N} h_i \quad (1)$$

where h_i and M_i are the respective peak height and molecular weight at retention time "i". HPSEC was performed using a Waters 510 solvent pump, a Waters 486 variable wavelength detector, and a Waters Protein-Pak 125 modified silica column. Experimental details for this method are described in Chin et al. (1994). Polystyrene sulfonate (Polysciences) standards with molecular weights ranging from 18,000 to 1,800 Daltons and acetone were used for calibration purposes. The mobile phase was composed of 0.1 M NaCl, 0.002 M K_2HPO_4, and 0.002 M KH_2PO_4 and degassed before use.

Results and discussion

Fluorescence spectroscopy

The fluorescence ratios (FR) calculated from our instruments varied from the values reported in McKnight and co-investigators (2001) and Fulton et al. (2004). These differences can be attributed to the unique optical design and light source of each instrument (McKnight, personal communication). Indeed, comparisons between our two instruments revealed values that are consistently lower for the Cary Eclipse. Nonetheless our results corroborated the trends reported by McKnight and co-workers (2001) whereby DOM from the allochthonous samples yielded the lowest ratio regardless of how they were isolated, while the two Antarctic autochthonous samples had the highest ratios. Our fluorescence ratios also correlate well with molar absorptivities (ε) at 280 nm where DOM of terrestrial origin is consistently higher than materials derived from algal and microbial precursors (Table 1). The Pony Lake UF sample retained a large amount of salt (fluorescence ratios are in parentheses in Table 1), which may affect the FR calculations through quenching effects by metals (Miano et al., 1988). Most of our isolated DOM samples are roughly 30 to 50% carbon, and have DOC concentrations that average 20 mg-C/L. The Pony Lake UF sample, however, had a DOC concentration of about a 1.0 mg-C/L, indicating extremely low organic carbon levels relative to the total mass of the dried material (40 mg).

Table 1. Molar Absorptivity, FR, MW, TOC, and ^{13}C NMR data for Suwannee River, Pony Lake, Pine Barrens S2, Hammonassett River, and Lake Fryxell raw waters and samples isolated by C-18, XAD, and ultrafiltration. Chemical shift ranges for DOM structures range between 0–60 for alkyl groups, 60–110 for O-alkyls (e.g., carbohydrates), and 110–160 ppm for aromatic groups. NA = not analyzed.

Sample Name	ε (L/mol-C cm)	FR Cary	FR Hitachi	Wt. Avg MW (Daltons)	TOC (mg-C/L)	% Aliphatic	% Aromatic	% Carbo-hydrate	Al/Ar Ratio
SR C-18	372	1.20	1.37	2029	35.19	37.9	19.5	15.8	1.94
SR XAD	401	1.15	1.33	1927	24.79	36.5	20.3	13.9	1.79
SR UF	479	1.14	1.27	2363	20.54	28.9	21.9	18.5	1.32
PL C-18	123	1.49	1.75	960	16.35	45	8.69	25.6	5.17
PL XAD	101	1.57	1.81	995	58.90	50.1	12.6	13.9	3.97
PL UF	141	(1.78)	(1.87)	NA	1.057	32.8	13.4	28.0	2.44
PB S2 C-18	432	1.13	1.26	2337	20.16	33.2	25.0	13.4	1.32
PB S2 XAD	455	1.02	1.16	2423	18.37	29.4	25.2	13.7	1.16
PB S2 UF	526	1.08	1.24	2470	19.61	28.6	23.9	17.4	1.19
HR UF	438	1.25	1.43	2350	12.32	31	25.9	14.4	1.19
LF XAD	166	1.67	1.88	922	21.47	NA	NA	NA	NA
SR raw water	NA	1.21	1.36	2284	NA	NA	NA	NA	NA
PL raw water	NA	1.70	1.91	838	NA	NA	NA	NA	NA
PB S2 raw water	NA	1.22	1.41	2200	NA	NA	NA	NA	NA

Fluorescence ratios revealed subtle variability between the C-18, XAD, and ultrafiltration isolates. Differences in the fluorescence ratio between the three isolation methods for the terrestrially derived Suwannee River and Pine Barrens samples on the Cary Eclipse were small (0.06 and 0.11), but exceeded the range of standard deviations compiled for a number of selected samples (0.01 to 0.03). The variation in Pony Lake (algal) samples isolated by each technique, however, is even larger than the observed variation in the terrestrial samples (Table 1). The ultrafiltered Pony Lake isolate yields an anomalously high ratio (parenthesis in Table 1) that is partly attributable to both the high salt content and low DOC levels present, as stated earlier. Moreover, a large disparity exists between SPE and XAD Pony Lake isolates and its raw water, indicating that some fluorophores in the raw water that emit at shorter wavelengths were not isolated by either XAD or SPE.

A comparison of EEMs (Fig. 1 and Table 2) from the three Suwannee River isolates show little variation in the location of peak intensity and overall peak shapes between the three isolation methods. For example EEM spectra of the Suwannee River isolates revealed an average excitation/emission (Ex/Em) maximum at 250/445nm (peak A) and 335/460 nm (peak B). Similarly Peaks A (235/445 nm) and B (330/455 nm) in the Hammonassett River samples closely resembles the Suwannee River EEM spectra.

In the Antarctic lake samples (Pony Lake and Lake Fryxell) emission peak maxima occur at lower wavelengths, i.e., 235/420 nm for peak A and 325/415 nm for peak B (Table 2). These EEM spectra resemble those reported for marine-derived DOM (Coble, 1996; Kowalczuk et al., 2003), but lack the tyrosine and trytophan fluorescence peaks reported by Coble and co-workers (1990). EEMs of XAD-isolated DOM from Lake Fryxell are similar to XAD-isolated Pony Lake DOM (with peak A at 235/425 nm and peak B at 315/417 nm) even though much of the DOM from the former lake is derived from both eukaryotic and prokaryotic organisms, while DOM in Pony Lake is derived from predominantly eukaryotic photo-autotrophs and mixotrophs (McKnight et al., 2000).

Unlike the other DOM samples, large differences in EEM spectra were observed between the Pony Lake XAD and C-18 samples. The Pony Lake sample isolated with C-18 does not have a well-developed peak B compared to the sample isolated by XAD. Finally, a sample of Pony Lake UF DOM was analyzed, but was not reported because the low DOC levels coupled with the high salt concentrations resulted in EEMs that can not be properly interpreted.

In all samples with the exception of one (see below), the location of peak maxima slightly shifts to longer emission wavelengths as pH increases from 3 to 8. At this point the significance of this observation is difficult to interpret. The Pony Lake C-18 isolate, however, behaved differently from the other samples (Fig. 2). As pH increases in this sample, a peak develops near 280/420 nm at pH 6, which possibly resembles the marine-humic peak discussed by Coble (1996). Given that Coble (1996) conducted her studies at a higher pH and used C-18 to isolate her seawater samples, our results may reveal the existence of a similar DOM component in Pony Lake. Finally, Pony Lake is a highly saline (~16 ppt) eutrophic coastal pond, located immediately adjacent to McMurdo Sound, and thus would presumably have some DOM components that are similar to the marine environment.

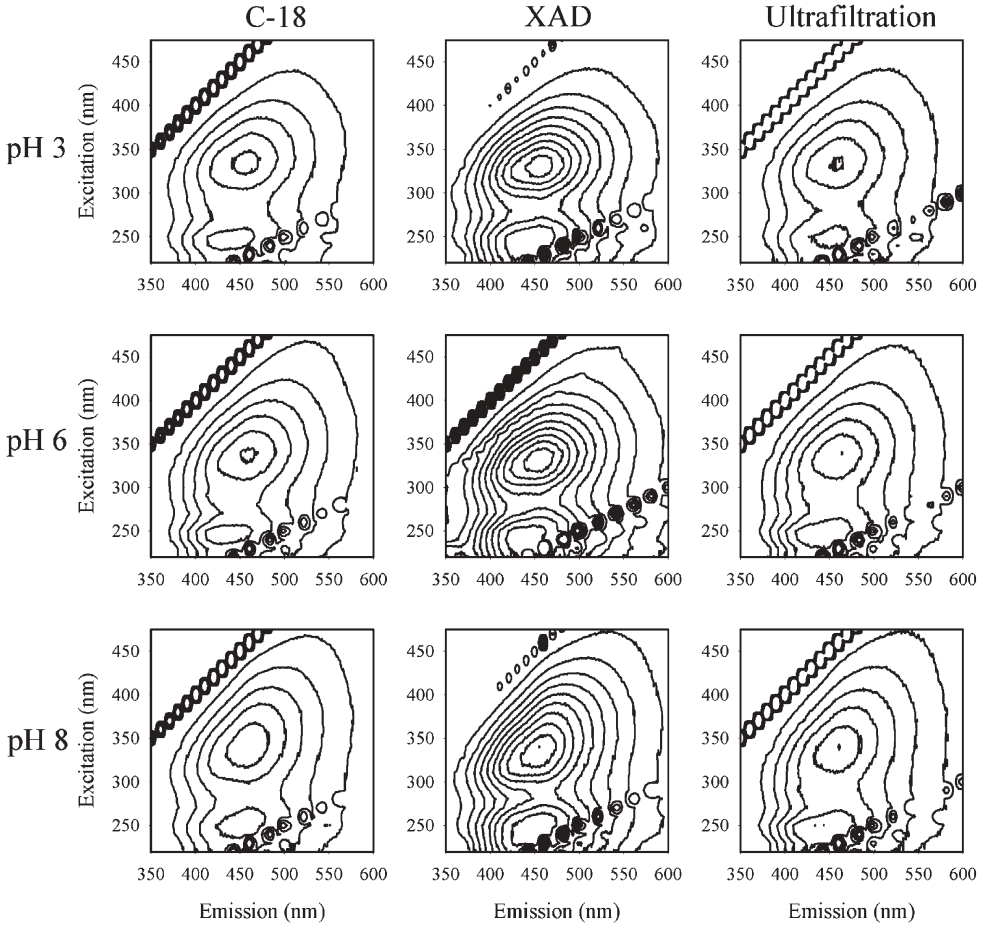

Figure 1. EEMs of isolated DOM from the Suwannee River normalized to DOC concentrations.

Synchronous scans were used to further delineate difference between the various DOM isolates. This type of fluorescence spectroscopy represents a cross-section of an EEM spectrum in two dimensions. As a result, subtleties and better-resolved peaks are revealed that are not present in an emission scan conducted at a fixed excitation wavelength. For this paper we limit our observations to strictly qualitative interpretations of the data. We observed a large pH dependent peak at 430–440 nm for our two terrestrially derived samples (Fig. 3), which is quenched with decreasing pH. This phenomenon was also observed by others (Senesi, 1990; Cabaniss, 1992; Pullin and Cabaniss, 1995) for terrestrially derived DOM samples and humic materials extracted from soils. We suspect that this shift in the emission spectra may be caused by a combination of protonation/deprotonation of active fluorophores, solvent effects, or metal interactions. In the Pony Lake samples there is a large peak at approximately 350 nm as well as two minor peaks at 380 and 400 nm that are scattering artifacts, which we verified by reconstructing the synchronous scan using a slice from its equivalent EEM. Surprisingly, we observed little to no variation in fluorescence intensity with pH for this sample in contrast to the terrestrially derived DOMs. Thus, if protonation/deprotonation reactions are accountable for much of the changes in intensity observed for the other samples, the Pony Lake sample suggests that most of the active fluorophores do not participate in these reactions, or possess pKa's that are significantly less than 3. Effects of pH on the fluorescent properties of our two terrestrial samples were not as evident in their EEM spectra, but were clearly observed by synchronous fluorescence spectroscopy. Conversely, for the Pony Lake samples, no pH effect was observed by us from the synchronous fluorescence spectroscopy scans, but our EEM spectra revealed the existence of a new peak at pH 6. Thus, these results highlight the importance of conducting different types of fluorescence spectroscopy to construct a complete picture of the DOM's fluorescence properties.

NMR spectroscopy

Quantitative solid-state ^{13}C-NMR spectra from DOM samples provided us with the most detailed structural in-

Table 2. Peak intensities from EEM scans at pH 3, 6, and 8 of samples from each site.

Sample Name	Peak A Ex/Em (nm)	Peak B Ex/Em (nm)
Suwannee River C-18 pH 3	250/430	330/457
Suwannee River C-18 pH 6	250/429	340/459
Suwannee River C-18 pH 8	260/461	340/467
Suwannee River FA XAD pH 3	240/427	330/456
Suwannee River FA XAD pH 6	250/427	330/455
Suwannee River FA XAD pH 8	250/461	340/451
Suwannee River UF pH 3	250/457	330/454
Suwannee River UF pH 6	250/429	330/460
Suwannee River UF pH 8	250/456	340/461
ranges	**240–260/427–461**	**330–340/451–467**
Pony Lake '97 C-18 pH 3	230/410	320/408
Pony Lake '97 C-18 pH 6	240/416	330/427
Pony Lake '97 C-18 pH 8	240/420	320/419
Pony Lake '97 XAD pH 3	240/428	330/419
Pony Lake '97 XAD pH 6	240/431	330/425
Pony Lake '97 XAD pH 8	250/433	330/425
Pony Lake '94 XAD pH 3	230/423	330/420
Pony Lake '94 XAD pH 6	230/430	330/421
Pony Lake '94 XAD pH 8	240/427	330/420
ranges	**230–240/410–433**	**320–330/408–427**
Pine Barrens S2 C18 pH 3	250/464	340/459
Pine Barrens S2 C18 pH 6	260/464	340/463
Pine Barrens S2 C18 pH 8	250/460	340/464
Pine Barrens S2 XAD pH 3	260/460	330/451
Pine Barrens S2 XAD pH 6	250/463	330/457
Pine Barrens S2 XAD pH 8	250/466	340/460
Pine Barrens S2 UF pH 3	250/453	340/462
Pine Barrens S2 UF pH 6	250/428	340/469
Pine Barrens S2 UF pH 8	250/468	340/464
ranges	**250–260/428–468**	**330–340/451–469**
Hammonassett River UF pH 3	240/419	330/450
Hammonassett River UF pH 6	240/423	330/462
Hammonassett River UF pH 8	230/436	330/453
Lake Fryxell FA XAD pH 3	230/417	320/415
Lake Fryxell FA XAD pH 6	230/423	310/416
Lake Fryxell FA XAD pH 8	240/430	310/419

formation of any analytical technique used in this study. The differences between the various structural components as well as the aliphatic/aromatic ratio (Al/Ar) for the three isolation methods were considerably smaller in the terrestrially derived samples (Suwannee River and the Pine Barrens) as compared to the Pony Lake isolates (Table 1). Nonetheless, the structural make-up of the Pony Lake DOM is not unique to Antarctica. Recent work by Kaiser and co-workers (2003) reported aliphatic/aromatic ratios in SPE and UF isolates from an oligotrophic near-natural river (the Tagliamento River in Italy) that were similar to our equivalent Pony Lake samples. In the Pony Lake SPE isolates, the aliphatic/aromatic ratios were also a factor of approximately 2 higher than in the UF isolates. Furthermore, we observed a large difference in the carbohydrate content between Pony Lake samples, where the XAD isolate yielded lower amounts of carbohydrates. These observations can be explained by the separation mechanisms employed by each technique. Ultrafiltration separates organic matter mechanically on the basis of size as opposed to polarity. Because carbohydrates can be considerably more polar than other DOM moieties they would less likely be retained by either the XAD or C-18 stationary phases. Conversely, no such discrimination occurs for UF where the carbohydrates are segregated only by size. Thus, both chromatographic methods are biased toward isolating the more nonpolar components. Finally, it is conceivable that the SPE C-18 method may preferentially isolate hydrophobic aliphatic groups from the Pony Lake DOM matrix, which would explain its very high Al/Ar ratio.

The ^{13}C NMR spectrum of the Suwannee River SPE isolate reveals a large peak in the aliphatic chemical shift region at 32.9 ppm (Fig. 4), which is not present in the

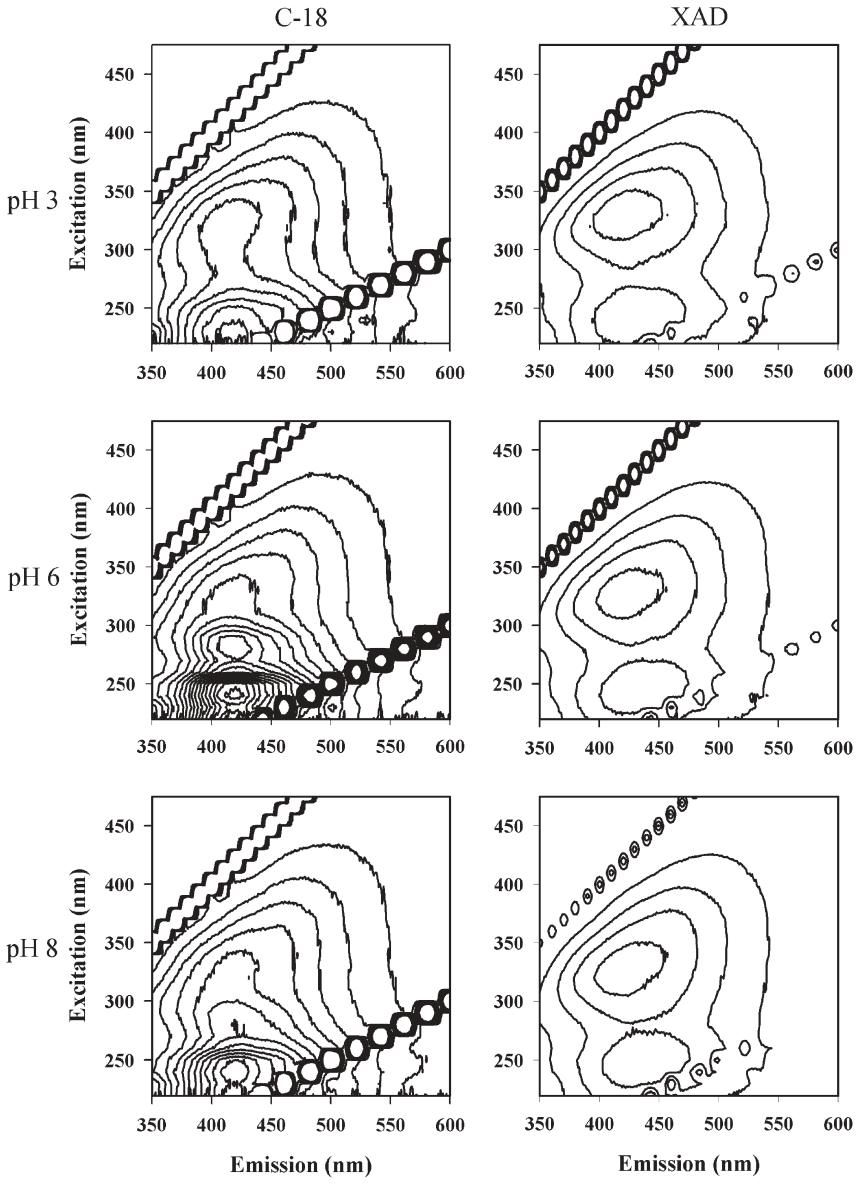

Figure 2. EEMs of isolated DOM from Pony Lake normalized to DOC concentrations.

same samples isolated by either XAD or ultrafiltration. Kaiser and co-workers (2003) observed a similar sharp peak in the C-18 isolated Tagliamento River DOM samples and they hypothesized that this peak maybe comprised of methylene groups from the C-18 stationary phase. Based upon recent work by Kim and co-workers (2003), however, high-resolution 2-D NMR spectra of DOM isolated by C-18 filter discs reveal no such contamination from the stationary phase. Moreover, Hu et al. (2000) reported similar peaks in soil humic substances (isolated by XAD), and attributed them to naturally occurring methylene moieties. With respect to the findings of Kim and co-workers (2003), the C-18 columns used here possess significantly more surface area than the filter discs and the potential for methylene cleavage from the silica support is greater for our stationary phase. Regardless of the origin of this peak it encompasses approximately 1% of the total area counts rendering it a very minor component of the DOM matrix.

Size exclusion chromatography

Weight average molecular weight (MW) (Table 1) was measured using HPSEC. All the measured molecular weights are in good agreement with values reported by others (where available) for DOM isolates from these sites (Chin et al., 1994; Uhle et al., 1999; Meier et al., 2004). Moreover, the trends are consistent with results

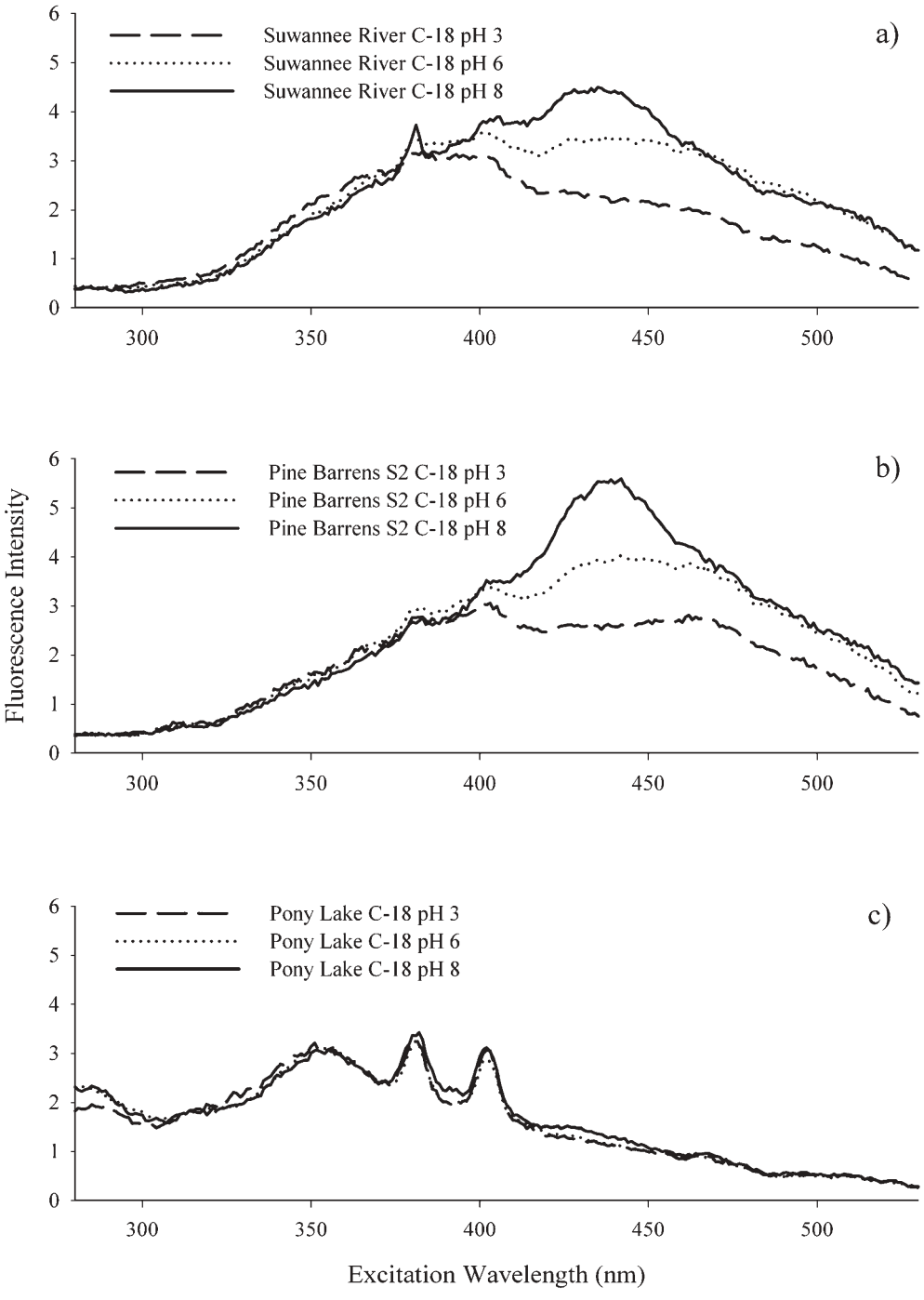

Figure 3. Synchronous scans of DOM from a) Suwannee River, b) Pine Barrens, and c) Pony Lake isolated by C-18.

observed by others where terrestrially derived DOM possessed higher molecular weights than their Antarctic counterparts (Aiken et al., 1992; Chin et al., 1994). One exception to our observations is the Suwannee River XAD fulvic acid MW, which is slightly smaller than reported values in Chin et al. (1994). This result is not surprising given that the earlier study used a Suwannee River fulvic acid that was isolated from an earlier sampling event. When comparing MW from each isolation technique, the highest MW was measured for the TFUF isolate for all samples used in the study. Unlike the XAD and C-18 chromatography, TFUF isolates DOM by physically separating molecules less than the membrane pore size. Thus, this method tends to bias DOM isolated in this manner toward higher weight average MW as manifested by our HPSEC results.

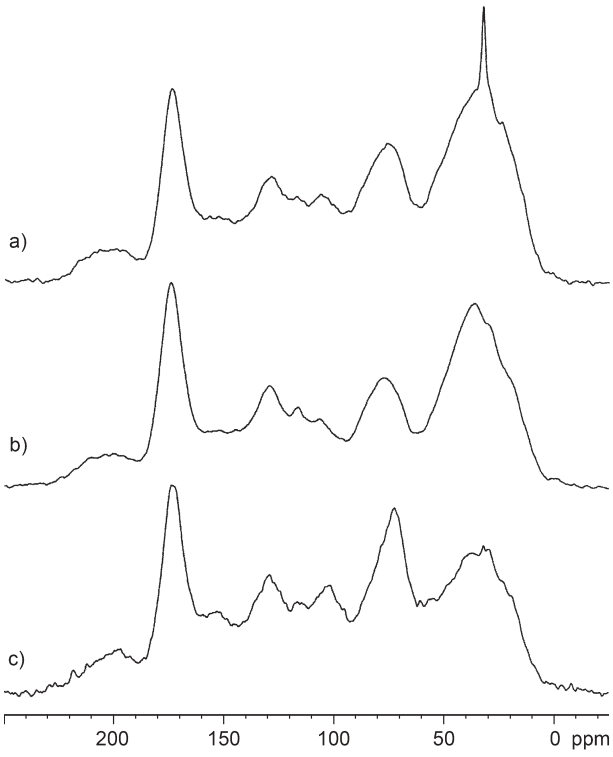

Figure 4. ^{13}C NMR spectra from Suwannee River DOM isolated by a) C-18, b) XAD, and c) ultrafiltration.

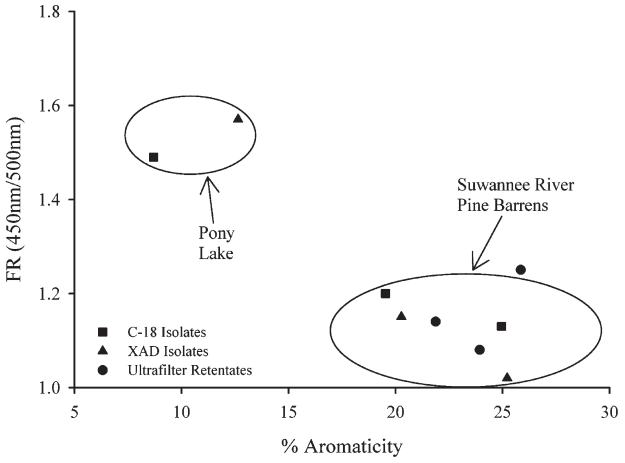

Figure 5. The relationship between the FR and aromatic content of samples from the Suwannee River, Pine Barrens, and Pony Lake.

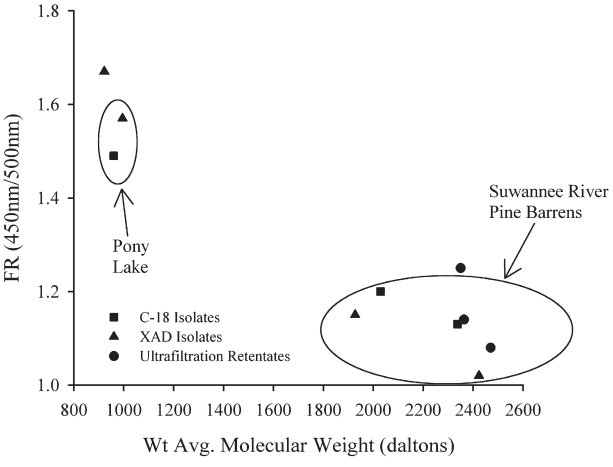

Figure 6. Molecular weight vs. FR for C-18 isolates, XAD isolates, and ultrafiltration retentates.

We observed an inverse relationship between the fluorescence ratio (Fig. 5) and aromaticity similar to those reported by McKnight and co-workers (2001). A similar relationship between the fluorescence ratio and the DOM's molar extinction coefficient (ε) can also be gleaned from the data in Table 1, where the terrestrially derived samples possess high ε and low FR values. Moreover, an inverse relationship between the fluorescence ratios of our samples and their molecular weights also exists (Fig. 6). This phenomenon was anticipated because algal derived DOM has lower weight average MW than comparable materials from terrestrial sources (Chin et al., 1994). We believe that the presence of lignin derivatives in the terrestrial DOM isolates contributes greatly to their increase in molecular weights, molar extinction coefficients, and aromaticity, while the lack of these components in the algal-derived samples results in smaller molecules that lack significant aromatic structures. These analyses provide further evidence of the usefulness of the FR index for assessing the source material of DOM in surface waters.

Conclusions

This study has shown that similarities and differences occur in DOM properties isolated by XAD and C-18 chromatography, and ultrafiltration. Many of the differences between the various isolates were observed in the autochthonous-derived samples, e.g., Pony Lake where the C-18 may be preferentially isolating the hydrophobic aliphatic components as manifested in its high Al/Ar ratio. Conversely only small differences in the molecular properties between the predominantly allochthonous DOM isolates were observed. Nonetheless, differences in the DOM isolated by these three methods, while significant for the Pony Lake sample were small in comparison to differences between the molecular properties of the different types of DOM used in this study.

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