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A comparison of surface water natural organic matter in raw filtered water samples, XAD, and reverse osmosis isolates

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Abstract

This research compared raw filtered waters (RFWs), XAD resin isolates (XAD-8 and XAD-4), and reverse osmosis (RO) isolates of several surface water samples from McDonalds Branch, a small freshwater fen in the New Jersey Pine Barrens (USA). RO and XAD-8 are two of the most common techniques used to isolate natural organic matter (NOM) for studies of composition and reactivity; therefore, it is important to understand how the isolates differ from bulk (unisolated) samples and from one another. Although, any comparison between the isolation methods needs to consider that XAD-8 is specifically designed to isolate the humic fraction, whereas RO concentrates a broad range of organic matter and is not specific to humics. The comparison included for all samples: weight average molecular weight (M_w), number average molecular weight (M_n), polydispersity (ρ), absorbance at 280 nm normalized to moles C (ε_{280}) (RFW and isolates); and for isolates only: elemental analysis, % carbon distribution by ^{13}C NMR, and aqueous FTIR spectra. As expected, RO isolation gave higher yield of NOM than XAD-8, but also higher ash content, especially Si and S. M_w decreased in the order: RO > XAD-8 > RFW > XAD-4. The M_w differences of isolates compared with RFW may be due to selective isolation (fractionation), or possibly in the case of RO to condensation or coagulation during isolation. ^{13}C NMR results were roughly similar for the two methods, but the XAD-8 isolate was slightly higher in 'aromatic' C and the RO isolate was slightly higher in heteroaliphatic and carbonyl C. Infrared spectra indicated a higher carboxyl content for the XAD-8 isolates and a higher ester:carboxyl ratio for the RO isolates. The spectroscopic data thus are consistent with selective isolation of more hydrophobic compounds by XAD-8, and also with potential ester hydrolysis during that process, although further study is needed to determine whether ester hydrolysis does indeed occur. Researchers choosing between XAD and RO isolation methods for NOM need to consider first the purpose of the isolation; i.e., whether humic fractionation is desirable. Beyond that, they should consider the C yield and ash content, as well as the potential for alteration of NOM by ester hydrolysis (XAD) or condensation/coagulation (RO). Furthermore, the RO and XAD methods produce different fractions or isolates so that researchers should be careful when comparing the compositions and reactivities of NOM samples isolated by these two different techniques. © 2002 Published by Elsevier Science Ltd.

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1. Introduction

Natural organic matter (NOM) is an important component of surface waters, soil pore waters, and

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shallow ground waters, both in terms of concentration and reactivity. NOM may control the mobilities of trace metals [1] and hydrophobic organic compounds [2–5] as well as the aggregation kinetics of colloidal particles [6]. Moreover, NOM plays an important role in a wide range of photochemical reactions [7], and serves as a source of organic carbon to microorganisms [8,9]. The reactivity of NOM is closely tied to its physicochemical properties such as molecular weight, aromaticity, elemental composition, and functional group content [10]. For example, several researchers have suggested that higher molecular weight, more aromatic components adsorb preferentially to mineral surfaces [11–16]. Other research has suggested that adsorption is dominated by an intermediate molecular weight fraction [17,18]. The bioavailability of NOM is generally believed to decrease with increasing molecular weight [19], although recent research has shown that some high molecular weight compounds (>1000 Da) also may be utilized by bacteria [20]. Clearly, characterization of NOM is fundamental to hydro-bio-geochemistry.

Although some characterization methods for dissolved NOM can be applied to raw filtered samples, isolation and concentration are required for many analytical procedures. Ultraviolet and visible absorbance and fluorescence, liquid chromatography and organic acidity can be studied in solutions of ~ 10 mg C L^{-1} [21], but an ~ 100 mg sample is typically needed for elemental composition. Furthermore, while Chin and colleagues [15,22] have applied High-pressure size exclusion chromatography (HPSEC) to raw filtered surface water (RFW) samples, the method does not always produce reliable results at NOM concentrations $\leqslant 3$–5 mg C L^{-1} [23]. This means that it cannot be applied to waters with low dissolved organic carbon (DOC) concentrations without pre-concentration. ^{13}C NMR, a fundamental technique for characterizing C distribution, requires NOM concentrations on the order of $2–5 \times 10^4$ mg C L^{-1} (or a solid sample), and aqueous FTIR typically requires $\sim 10^4$ mg C L^{-1}. Moreover, many experimental applications such as determination of adsorption isotherms are facilitated by the use of concentrated NOM isolates. For some research purposes, a specific fraction such as fulvic acid is desirable; for example, to compare the composition, metal binding, or light attenuation properties of fulvic acids from different sites. In such cases, it is necessary not only to isolate and concentrate the NOM, but to fractionate it as well.

Effort has focused on developing NOM isolation methods that simultaneously concentrate the organic matter; methods include resin adsorption (typically XAD, e.g. [24,52], ultrafiltration [25,26], reverse osmosis (RO) [21,27,28], low temperature evaporation [29] and others. Some investigators have combined methods [30–32]. The various isolation/concentration methods are only useful if they do not cause unintended fractionation or unknown alteration of the NOM samples.

The most commonly used isolation method for surface water NOM is adsorption to non-ionic macroporous resins [24], particularly the acrylic-ester XAD-8 resin recommended by Aiken [33]. This method is intended not only to isolate the NOM, but to fractionate out the humic substances, as well. XAD-8 is an excellent sorbent for humic substances at low pH, resulting in the isolation of a low-ash product [24,33]. XAD adsorption was used to produce the International Humic Substances Society (IHSS) standard and reference surface-water fulvic acid samples from the Suwannee River (SRFA). IHSS SRFA has been characterized by a wide variety of methods [34] and hence provides a standard for comparison of XAD isolates. XAD resin adsorption may be used to isolate humic substances from large quantities (10–100 s of L) of water [35], even in the field. The XAD-8 isolation method requires samples to be acidified to pH 2 with concentrated HCl and then eluted from the column with 0.1 M NaOH. These acid/base extremes could potentially alter the NOM structure [36]. The XAD adsorption procedure typically results in apparent yields of about 40–60%, consistent with fractionation of NOM to isolate humic substances [37].

Use of a portable RO system generally allows for rapid on-site isolation and concentration of many samples. Unlike the XAD sorption method(s), RO is not designed specifically to isolate humic substances. Several groups [21,28,29,38] report yields around 80–90% when applied to a variety of surface and ground waters. Crum et al. [30] reported $>90\%$ recovery of DOC from a synthetic groundwater. Serkiz and Perdue [28] found that Suwannee River RO isolates had higher O + S:C ratios than the IHSS standard fulvic and humic acids collected at the same site (although years apart), suggesting that the RO isolates had a more polar character. These authors further noted that additional cleaning procedures such as use of cation exchange were sometimes needed to decrease the ash contents of RO samples. Dissolved silica and SO_4^{2-} often were concentrated along with NOM, and it was difficult to remove them from the RO isolate without causing unwanted NOM fractionation [38]. It is not known whether concentration of inorganic components and accompanying ionic strength changes over the course of the RO procedure alter the characteristics of the NOM.

In this manuscript, we compare RFW samples with XAD-8 and RO isolates from McDonalds Branch, a small freshwater fen in the New Jersey Pine Barrens (USA). All of the samples were collected simultaneously, so that direct comparison could be made. The comparison includes the following physicochemical properties for all samples: weight average molecular weight (M_w),

number average molecular weight (M_n), polydispersity (ρ), absorption at 280 nm normalized to moles carbon (ε_{280}). For isolates, % carbon type analysis by ^{13}C NMR, functional group analysis by Fourier-transform infrared (FTIR) spectroscopy and elemental analysis were performed. We also include results of XAD-4 isolation [39] for one sample. As described below, this combined analytical approach provides new insight into the effects of RO and XAD isolation procedures on the physicochemical properties of surface water NOM. In the comparisons below, it is important to remember that the RO and XAD methods have fundamentally different intents; the RO method is designed to isolate a larger percent of the NOM whereas the XAD methods are designed to isolate different NOM fractions.

2. Methods

2.1. Site description

NOM-rich surface water was collected at McDonalds Branch, a small freshwater fen located approximately 30 km from the Atlantic coastline, in the New Jersey Pine Barrens, USA. For a detailed site description, see Refs. [15,40]. A first-order stream, McDonalds Branch has been an US Geological Survey (USGS) hydrologic bench-mark station since 1953, and was the subject of extensive hydrologic and geochemical investigations [41,40]. The surficial geology in the basin is dominated by the Miocene Age Cohansey formation, which consists primarily of pure quartz sand with localized clay lenses (fine-grained quartz, kaolinite, and illite) and Fe(III) (hydr)oxide cemented units [41,42]. The lack of highly weatherable, divalent cation-releasing minerals, coupled with the wetland environment, results in low-ionic-strength, acidic, organic-rich surface and shallow ground waters [40].

We chose to collect water from an upstream site (S2) and a downstream site (S10), because these two sites are hydrologically quite distinct and typically contain significantly different DOC concentrations. The sites are described in detail by Lord et al. [41] and Johnsson and Barringer [40]. Site S2 is located in a hardwood swamp, which is a potential ground-water recharge area, although recharge is often impeded by clay lenses underlying the streambed. Site S10 is located in an Atlantic White Cedar swamp, which is generally a ground-water discharge area. Occasionally, during periods of high flow, the water table in this area may become depressed so that recharge occurs. Site S2 receives significant drainage, primarily as interflow, from surrounding pine uplands which contain organic-rich spodosols. Hence, this site generally contains DOC in the range of 7–35 mg $C\,L^{-1}$, pH from 3.5–4.2, and relatively high Al and Fe concentrations, 0.4–2 and 0.3–8 mg L^{-1}, respectively. Owing primarily to dilution by ground-water discharge [43], the DOC decreases downstream. Site S10 generally contains DOC in the range of 1–7 mg $C\,L^{-1}$, pH 3.8–4.5, Al 0.05–0.4 mg L^{-1}, Fe 0.05–0.3 mg L^{-1} [40,41] (Maurice et al., unpublished data).

Site S2 was sampled in Spring 1997. Although we intended to sample both sites S2 and S10 in Fall 1997, site S2 was dry because of drought conditions so that we could only sample site S10. Both S2 and S10 were sampled in Spring 1998. Selected chemical characteristics of raw filtered water samples S2 Spring 1997 and S10 Fall 1997 are provided in Table 1. Metals and anions were determined by ICP-AE (optical ICP) at the Wooster water quality lab, Ohio State University. The results are consistent with the ranges described above. Unfortunately, sample bottles for inorganic analysis from the Spring 1998 were lost in transit. Samples of RFW (filtered as described below) were collected in 1 L baked glass bottles, and returned to the laboratory in ice-filled coolers.

Table 1
Selected chemical characteristics of raw filtered surface water samples from McDonalds Branch basin

Site	Temp.(°C)	Field values[a]		Lab values		Ca	Mg	Na	K	Fe[b]	Al	SO_4	Cl	DOC ($mgCL^{-1}$)
		pH	Cond. (μScm^{-1})	pH	Cond. (μScm^{-1})	($mgCL^{-1}$)								
Spring 1997														
S2	14.5	4.0	65	4.3	48	0.755	0.224	1.917	0.148	0.951	0.831	7.72	3.99	31.6
Fall 1997														
S10	10.3	4.5	41	4.5	23	0.634	0.398	2.112	0.313	0.179	0.184	8.67	4.03	3.4
Spring 1998														
S2	11.5	3.8	91	3.8	84	nm	nm	nm	nm	0.515	nm	nm	nm	24.2
S10	12.8	4.2	69	4.2	66	nm	nm	nm	nm	0.188	nm	nm	nm	9.9

[a] Field vaues were on unfiltered samples; lab values were on filtered samples.
[b] Fe concentration for Spring 1998 samples were measured by FIA analysis.

2.2. Reverse osmosis isolation

Osmosis is the phenomenon whereby water flows through a semi-permeable membrane which blocks the transport of solutes such as salts. Transport of water across the membrane is controlled by differences in composition, primarily ionic strength, between the solutions on either side of the membrane. In RO, the natural flow of water is reversed through the application of pressure. By selecting RO membranes of the right pore size and composition, NOM can be concentrated from water through an RO system, although other components such as SO_4^{2-} and silica often are concentrated along with the NOM. Small ionic salts, including the smaller and more hydrophilic NOM components, are likely to be lost (i.e., not isolated) in the RO process. The RO concentration process we used is shown schematically in Fig. 1.

We used a portable RealSoft PROS/IS RO system built by E.M. Perdue to isolate NOM on-site. This system is described in detail by Serkiz and Perdue [28] and Sun et al. [38]. Raw surface water was pumped out of the McDonalds Branch stream, in-line filtered using a series of three high-volume filter cartridges of decreasing pore size: 20 μm, Omnifilter; 1 μm, Parker Filtration; 0.4 μm, Nuclepore, and then measured using a flow meter before entering the sample reservoir. Operation of the RO system was similar to the procedure described by Serkiz and Perdue [28], and included in-line treatment with Dowex-50 cation exchange resin (Na form) to remove polyvalent cations prior to passing through the RO membrane. Following each RO processing of sample, the permeate was discarded, and the retentate solution was returned to the sample reservoir and combined with additional filtered raw water for processing. At ~200 L intervals, an ~1 L aliquot of the filtered water was collected and combined with previously collected aliquots. We collected several composite samples and thus were unable to calculate % yield precisely. If precise calculation of % yield is required for an application, then composite samples can be combined; we chose not to do so because we were using the samples for other experimental work in several different laboratories. Occasional Si fouling of the RO membranes necessitated NaOH flushing.

Concentrates were collected in 1 L baked glass bottles and returned to the lab in coolers, covered with ice. They were refrigerated in the dark for several days after which they were lyophilized for storage. At least one 1 L bottle of each sampled isolate was H^+ saturated for use in the various characterizations described herein. The liquid RO concentrate was shipped on ice to the Boulder (CO)

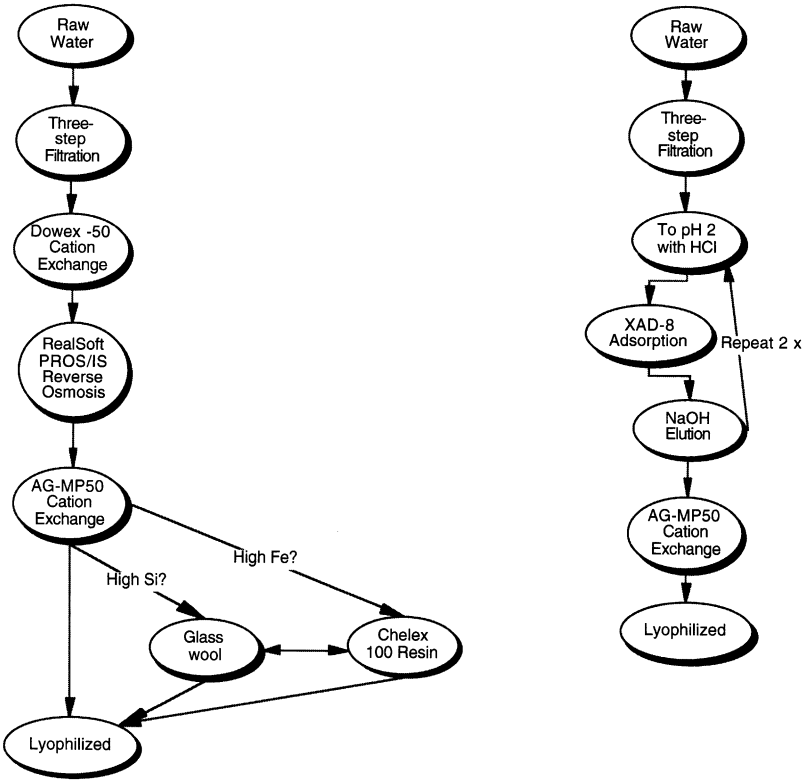

Fig. 1. Schematic illustration of the RO isolation method (left) and the XAD-8 resin fractionation technique (right) used herein.

laboratory of G. Aiken. Upon receipt, samples were refrigerated. 5–6 L of the concentrate were H^+ saturated by passing through a 300 mL column of H^+ saturated AG-MP 50 cation exchange resin (Biorad) at 20 mL min^{-1}. The cation exchange resin was cleaned by repeated base and acid rinses, followed by distilled water. Each sample of concentrate was passed though the column a second time, and then lyophilized for storage and subsequent analysis.

Later analysis indicated that the AG-MP-50 cation exchange resin did not significantly decrease the concentration of Fe in the RO isolates. For this reason, Chelex-100, a di- and tri-valent specific cation exchanger, was used to decrease the Fe concentration of the isolates in other experiments.

A portion of each H^+-saturated, lyophilized isolate was sent to Huffman Laboratories (Golden, CO) for elemental analysis. As described below, we detected high Si in some RO isolates; indeed, Si often was observable to the naked eye as a grayish-white powder. For comparison purposes, we filtered these high-Si samples through glass wool at low pH to remove Si.

2.3. XAD isolation

The XAD-8 isolation procedure is shown schematically in Fig. 1. Water samples were filtered on-site using a series of three high-volume filter cartridges of decreasing pore size, as described in the RO procedure, above. The filtered water was stored and shipped in 20 L Nalgene HDPE (high density polyethylene) carboys enclosed in coolers and covered in ice. The samples were extracted within ~1 week after collection and filtration.

The humic materials were extracted using Amberlite XAD resins, closely following the methodology of Aiken et al. [39]. For most of the samples, only the so-called 'hydrophobic acid' fraction (fulvic plus humic acid) was extracted, using XAD-8. For only the S2 Spring 1998 sample, both the 'hydrophobic' and 'hydrophilic' acids were extracted using XAD-8 and XAD-4 in series.

The XAD-8 and XAD-4 resins (20–50 mesh) were obtained from Rohm and Hass and cleaned according to Aiken et al. [39]. Glass low-pressure chromatography columns were filled with resin and cleaned further using three successive 0.1 N NaOH–0.1 N HCl rinses just prior to the extraction. 2 L of resin were used for every 70 L of water sample. This ratio of resin-volume-to-sample-volume is higher than previously used [24,39]. This change was made to prevent loss of sample through saturation of the resin by the high levels of organic material present in the Pine Barrens water (up to 30 mg C L^{-1}).

Samples were acidified to pH 2.0 using HCl and pumped through the XAD-8 column followed by the XAD-4 column (when used). The adsorbed organic matter was back-eluted from each column using 0.1 N NaOH and immediately reacidified to pH 2.0 using HCl. The eluates were then reconcentrated on the appropriate resin, rinsed with distilled water to desalt, and back eluted using 0.1 N NaOH. The eluate was immediately passed through H^+-saturated AG-MP 50 cation exchange resin (Biorad) to H^+ saturate the samples, remove sodium, and further decrease the concentrations of other metals. Finally, the cleaned, concentrated, and fractionated material was lyophilized for storage. A portion of each lyophilized isolate was sent to Huffman Laboratories, Golden, CO for elemental analysis.

Using the DOC concentration of the original sample, the sample volume, the mass of isolated material and its C content, the percent of organic carbon in the original sample removed by the resin (termed here "extraction efficiency") was calculated.

2.4. DOC, UV/Visible spectrometry, and Fe analyses

DOC was analyzed in triplicate on a Shimadzu TOC-5000 analyzer (Shimadzu Co., MD). The relative standard deviation (RSD) was $<2\%$ for DOC >2 mg L^{-1}, and $<5\%$ for DOC <2 mg L^{-1}. UV/Visible absorbance spectra of NOM were collected on a Hitachi U2000 spectrophotometer using a double beam and 1 cm quartz cells with Milliq UV water as the reference, scanned from 600 to 200 nm at 100 nm min^{-1}. The absorbance at 280 nm was used to determine the absorptivity normalized to moles C (ε_{280}, L mol^{-1} C cm^{-1}) of organic matter in solution. Dissolved Fe was measured with a graphite furnace atomic absorption spectrophotometer (GFAAS) (Perkin Elmer 5100PC), with a detection limit of 1 μg L^{-1}. Some filterable colloidal Fe also might have been included in this analysis.

2.5. HPSEC determination of molecular weight

The HPSEC was used to determine M_w, M_n, and ρ of raw filtered water and isolates. A detailed description of the HPSEC method is provided elsewhere [22] as modified by [23]. The HPSEC unit (Department of Chemistry, Kent State University) is described by Zhou et al. [18,23]. Sodium polystyrene sulfonates (PSS) 18, 8, 5.4, 4.6 K Da (PSS standards purchased from Polysciences, Inc., PA), salicylic acid (138 Da, 99.999% purity, Aldrich), and acetone (58 Da, HPLC grade, Aldrich) were used as standards to calculate the M_n and M_w of the NOM samples.

2.6. ^{13}C NMR

To prepare samples for analysis by ^{13}C NMR, 200 mg of lyophilized material were dissolved in approximately 100 mL of deionized water. The pH of the resulting solution was adjusted to 6.0 by dropwise addition of a

dilute aqueous solution of NaOH (99.99%, Aldrich Chemical Company). The solution was lyophilized again and then dissolved in approximately 2 mL of 10% deuterium oxide (Aldrich). The liquid was filtered though glass wool into a 10 mm NMR tube. The filtration step was especially necessary when preparing the RO isolates to remove colloidal silica particles that would not dissolve. While this filtration step may have removed some of the carbon from the samples, this was considered preferable to exposing the sample to the high pH values ($\sim$11) needed to dissolve the silica. The solution was stored in dark refrigeration $<$1 week until analysis.

Quantitative liquid state ^{13}C NMR spectra were collected at 75.4 MHz using a General Electric GN-300 FT-NMR with a 10 mm probe. To remove differential NOE (nuclear Overhauser enhancement) effects, the spectra were collected using inverse-gated decoupling (decoupling on during the acquisition but off during the pulse delay). A 90° pulse width with a 10 s pulse delay was used. This pulse delay is at least 3–5 times typical humic material T1 values, 0.2–2.4 s [44], which will remove differential saturation effects. The spectra were collected using a spectral width of $\pm$25,000 Hz and an acquisition time of 41 ms using the deuterium resonance for the lock signal. After collecting at least 10,000 transients, the FID was baseline corrected and subjected to exponential multiplication using a line broadening of 50 Hz. The phase shifted Fourier transform was referenced by setting the carboxyl peak maximum to a chemical shift of 175 ppm, and then integrated using the GN-series software.

The spectra were collected using 4, 8, and 32 K block sizes. Early experiments indicated that larger block sizes did not improve results, so that the 4 K block size was employed in later experiments to increase the overall rate of data acquisition. The lack of resolution enhancement with the larger block size is certainly due to the many overlapping resonances from the heterogeneous mixture of organic compounds present in the NOM.

In order to determine the effect of Fe on the NMR signal, a spectrum was collected for a sub-sample that was treated with Chelex resin and compared to the spectrum of the same sample without treatment. We chose to use a sample of S2 Spring 1997 because this NOM sample was from the water with the highest Fe concentration (Table 1) and because we had an abundance of this sample. Two 200 mg amounts of the site S2 Spring 1997 RO isolate were each dissolved in 100 mL of deionized water. 10 g analytical grade Chelex 100 (Bio-Rad, Na^+ form) were added to one solution and both solutions were adjusted to pH 6.0 as described above. The solutions were both stirred for 24 h in the dark at room temperature. Both samples were filtered through Gelman Type A/E glass fiber filters. 2 mL of each of the resulting solutions were removed for Fe analysis. The solutions were lyophilized and then analyzed by NMR as described above. No difference between the two spectra was found (within the error of the method), either in the visual comparison or in the electronic integration results. The 2 mL samples were diluted tenfold with high purity pH 2.0 HNO_3 and analyzed for Fe using a total Fe colorimetric flow-injection analysis technique described elsewhere [45,46]. The chelex treatment decreased the Fe content from 0.208% to 0.083% (by weight). This sizable decrease of Fe with no change in the NMR spectrum was taken to indicate that the Fe present in the Pine Barrens samples did not affect the quantitative analysis of the distribution of their carbon types in this study. No further attempt was made to remove Fe from the remaining humic substance samples.

An estimate of the precision of the % carbon type analysis was obtained by measuring three separate NMR spectra from the same humic substance sample (S2 RO isolate, spring 1997). The % C in each category was found to have a RSD (1F) from 1.3% to 3.7% (see Table 2). An exception is the ketone/quinone region (195–220 ppm) which had an RSD of 15%. This high relative error value, mostly the result of the low abundance of this C type ($<$10%), makes the compar-

Table 2
Quantitative solution state ^{13}C NMR data for the Spring 1997 S2 RO isolate

NMR spectrum region (ppm)	Percent carbon					
	Spectrum 1	Spectrum 2	Spectrum 3	Average	1σ	RSD (%)
0–60	21.6	20.8	20.0	20.8	0.8	3.7
60–90	12.7	12.0	12.8	12.5	0.4	3.3
90–110	8.4	8.2	8.3	8.3	0.1	1.3
90–165	34.1	32.8	34.1	33.7	0.7	2.2
165–195	24.2	24.8	23.1	24.0	0.8	3.4
195–220	7.4	9.5	9.9	9.0	1.4	15.0
110–165	25.6	24.6	25.8	25.4	0.7	2.6

ison of the ketone/quinone content of the samples difficult.

2.7. Aqueous FTIR

Solutions were prepared for aqueous FTIR spectroscopy by dissolving isolated NOM in 1–2 mL of deionized water, with a target concentration of approximately 20 mg NOM per mL. Total volume was decreased from 2 mL for isolates with limited availability. These solutions were allowed to equilibrate overnight to ensure full hydration, then brought to room temperature for analysis.

Spectra were acquired using a BioRad Excalibur FTIR spectrometer equipped with a Pike Technologies horizontal attenuated total reflectance (ATR) cell and operated by Merlin software. The horizontal ATR cell used a 45° angle ZnSe trough plate with ~10 reflections, resulting in effective pathlengths of ~4–20 μm over the range of frequencies studied (800–4000 cm^{-1}). The spectra were displayed as ATR absorbance (also referred to as pATR)–the negative log of the ratio of sample to reference intensities, uncorrected for frequency dependent pathlength. The Excalibur system contains a ceramic IR source, a KBr beamsplitter, and Peltier-cooled DTGS source for operation in the mid-IR. Each spectrum represents the average of 128 interferograms collected at 4 cm^{-1} resolution. A reference spectrum (empty ATR trough) was collected at the beginning of each session, and one deionized water blank was collected for every four sample spectra. NOM spectra were calculated by subtracting the water absorbance spectrum from the NOM solution absorbance spectrum. In a few cases, a separate water vapor spectrum was obtained and subtracted to minimize sharp water vapor peaks near 1600–1650 cm^{-1}.

Aqueous IR spectra were collected at both acidic and basic pH in order to obtain peaks for both COOH and COO^- groups, and because the alcohol and ester bands are convoluted with the COOH peaks at acidic pH but not at basic pH. The lower pH (1.6–2.0) was the initial pH of the NOM solution. The higher pH (8.4–11.0) was obtained by adding a small amount of concentrated (50%) NaOH to the low pH solution. The equilibration time at high pH was typically <2 min to minimize base-catalyzed hydrolysis.

The water-subtracted NOM spectra were exported to GRAMS 32 software for integration and peak-fitting. Integration used a zero-absorbance baseline. Two mixed Gaussian/Lorentzian peaks were fitted to the 1670–1850 cm^{-1} region of the low pH spectra, and were able to account for $>99\%$ of the variability. The peak with a maximum near 1720 cm^{-1} was assigned to carboxylic acid groups (−COOH), whereas a smaller peak with a maximum near 1770 cm^{-1} was assigned to ester groups (−COOR).

3. Results and discussion

3.1. Yields and elemental analysis

The RO process is known to be a high-yield isolation method for most surface waters [21,28,38], and this appears to be true for McDonalds Branch waters as well. Approximate yields for the four samples considered here were in the range of 80–100% of the DOC; as discussed above, we did not calculate more precise yields because the different sub-samples were not combined as some were needed for other experiments.

The % recovery as C for XAD-8 isolates ranges from 51% to 77% (Table 3). Thus, for most samples, recovery was somewhat greater than the range of 40–60% previously reported by [53]. The high recovery by XAD-8 is typical of Pine Barrens waters, which tend to be humic-rich. The S2 XAD-4 Spring 1998 isolate represented 12% of the total DOC.

Elemental analyses of the XAD-8 isolates were fairly typical, but the RO had elevated ash content (Table 4). High ash has been previously reported in RO isolation of some NOM samples [38]. Because of this, we did not send Spring 1998 RO samples to Huffman Laboratories for elemental analysis. Subsequently, we found that most (80–92%) of the Si could be removed by filtration of redissolved isolate through glass wool at pH 6. For Spring 1998 samples, we therefore compared molecular weight, absorbance, and ^{13}C NMR analyses on RO samples with and without filtration to remove Si (see Table 6, below).

McDonalds Branch XAD-8 isolates had consistently higher O:C (atomic ratios) than IHSS SRFA, indicating greater polarity. Note that the McDonalds Branch isolate would contain both fulvic and humic acids because the two were not separated following the isolation procedure. However, humic acids typically have lower O:C than fulvic acids [28]. The Spring and Fall 1997 RO isolates contained considerably more S than the corresponding XAD-8 isolates.

Serkiz and Perdue [28] observed that Suwannee River RO isolates contained relatively less C but more H, N,

Table 3
Recovery data for XAD isolates (XAD-8 unless otherwise designated)

Sample	% C[a]	% recovery as C
S2 Spring 97	48.0	51
S10 Fall 97	49.0	65
S2 Spring 98	46.4	77
S2 Spring 98 XAD-4	50.3	12
S10 Spring 98	46.4	68

[a]S2 XAD-4 % C mesaured by DOC; others by elemental analysis; % C for all are not on an ash-free basis.

Table 4
Elemental analysis of 1997 NOM isolates from McDonalds branch basin. Analyses performed by Huffman Labs, Golden, CO

Sample	Type	Weight % on ash-free basis					wt %	ppm			Atomic ratios			
		C	H	O	N	S	Ash	Al	Fe	Si	O:C	H:C	S:C	N:C
SRFA	XAD8	53.49	4.29	41.02	0.70	0.56	0.85				0.58	0.94	0.004	0.010
S2 S97	XAD8	49.17	4.05	44.45	0.62	0.71	3.04	393	1580	614	0.68	1.00	0.005	0.011
	RO	43.69	4.13	48.99	0.69	2.51	3.85	2190	2080	15900	0.84	1.13	0.021	0.013
S10 F97	XAD8	50.20	4.40	43.80	0.75	0.85	0.65	NM	NM	NM	0.66	1.66	0.006	0.012
	RO[a]	23.26	3.86	55.99	0.49	16.39	50.06	557	487	119200	—[a]	—[a]	—[a]	—[a]
S2 S98	XAD8	47.71	4.40	46.94	0.45	0.50	3.15	NM	NM	NM	0.73	1.11	0.004	0.008
S10 S98	XAD8	48.32	4.54	46.13	0.47	0.54	4.32	NM	NM	NM	0.79	1.24	0.005	0.009

[a] This sample has high ash content which may greatly affect % and ratio values. IHSS reference SRFA data from Ref. [54].

and O than IHSS standard SRFA collected using the XAD approach, although the samples were collected years apart. Similarly, we found that S2 Spring 1997 RO isolate contained relatively less C, more O and N (slightly) than S2 Spring 1997 XAD-8 isolate. However, H contents were comparable. The RO isolate contained considerably more S than the XAD-8 isolate. The differences in O, N, and S values between the two types of isolates could be due, at least in part, to the presence of a non-humic high molecular weight fraction and a hydrophilic acid fraction (see discussion below), one or both of which is enriched in O, N, and S relative to the humic fraction. For the Fall 1997 samples, it is likely that the much higher S value for the RO isolate is due to sulfate that was concentrated along with the organic matter.

3.2. Molecular weight of isolates and RFWs

Molecular weight data for RFWs, XAD-8, XAD-4, and RO isolates are presented in Table 5. An example comparing HPSEC chromatograms for RFW and isolates (S2 Spring 1998) is shown in Fig. 2a and b. M_w and M_n decreased in the order: RO > XAD-8 > RFW > XAD-4, with no consistent trend in polydispersity, ρ. The M_w, M_n trend occurred whether or not the RO samples were filtered to remove Si and/or chelexed to remove Fe. The one XAD-4 sample, which we collected from S2 Spring 1998, had M_w, M_n, and ρ all considerably less than the RFW. The high MW material is removed by the XAD-8 column before the remaining organic matter is passed through the XAD-4 column. Additionally, the low M_w values for XAD-4 isolates may be related to the small average pore diameter for the XAD-4 resin. The average pore diameter of the XAD-4 resin is 50 Å whereas the average pore diameter of the XAD-8 resin is 250 Å ([48] as referenced in [33]). It is also probable that 'hydrophilic' acids retained on the XAD-4 resin are characteristically smaller than 'hydrophobic' acids retained on the XAD-8 resin, as lower molecular weight molecules tend to be hydrophilic [39]. The M_w values for McDonalds Branch XAD-8 isolates were all within $\pm 10\%$ of the M_w of IHSS reference SRFA, as measured by our group.

The plot of HPSEC UV response versus log MW (Fig. 2a) shows that the XAD-8 isolate tracked the RFW quite well in the high MW range (above log MW ~ 3.3), but not in the intermediate to low molecular weight ranges. This makes sense from the standpoint that the XAD-8 resin retains more hydrophobic material, and hydrophobicity increases with increasing MW. On the other hand, the XAD-4 isolate tracked the RFW best in the intermediate molecular weight range, poorly in the high molecular weight range (above log MW ~ 3.3), and reasonably well but with slightly lower response in the low MW range (below log MW ~ 3.1). Since the XAD-8 resin previously removed the high MW fraction, we did not expect a correlation for XAD-4 with RFW in the HMW range. In the low MW range, the NOM material likely becomes too hydrophilic to be retained by the XAD-4 resin and passes through the column as ultrahydrophilic acids or hydrophilic neutrals. The RO peak was shifted to higher log MW, relative to RFW, throughout, indicating either the addition of high MW material during RO processing, or the condensation and/or aggregation of NOM. This was the case even for samples chelexed to remove Fe and filtered to remove Si. The shift to higher MW for XAD-8 isolate, the even more pronounced shift to higher MW for RO isolate, and the shift to lower or more intermediate MW for XAD-4 isolate can be seen in Fig. 2b which compares data normalized to maximum UV response.

3.3. ε_{280} of isolates and RFWs

Although isolates typically had ε_{280} different from that of the parent RFW (Table 5) no clear trends in direction of change or correlation with isolation method were noted. For S2 Spring 1997, both RO and XAD-8 isolates had lower ε_{280} than the RFW, while for S10 Fall 1997 the isolates' ε_{280} were higher than the RFWs ε_{280}.

Table 5
Comparison of MW and ε_{280} values for surface water bulk filtered samples and isolates[a]

Site	Sample type	DOC (mg/L)	M_w	M_n	ρ	ε_{280} (L mol C^{-1} cm^{-1})	Fe (mg L^{-1})
IHSS SRFA	XAD-8		2170	1260	1.72	415	
S2 S 97	Raw filtered	31.64	1954	1102	1.77	539	0.96
	XAD-8		2383	1387	1.72	442	
	RO		2618	1432	1.83	373	
S10 F 97	Raw filtered	2.37	BD	BD	BD	319	0.06
	XAD-8		1963	1066	1.84	347	
	RO		2358	1109	2.13	367	
	RO (chelexed)		2239	1396	1.60	298	
S2 S 98	Raw filtered	23.8	2056	1184	1.74	410	0.52
	XAD-8		2217	1193	1.86	454	
	RO		2743	1455	1.89	355	
	RO (Si filtered)		2528	1404	1.80	349	
	XAD-4		1693	1221	1.39	344	
S10 S 98	Raw filtered	9.61	1837	1048	1.75	383	0.19
	XAD-8		2028	1150	1.76	356	
	RO		2196	1263	1.74	396	
	RO (Si filtered)		2142	1179	1.82	359	

[a] *Note*: M_w = weight average molecular weight, M_n = number average molecular weight, ρ = polydispersity, ε_{280} = absorptivity at 280 nm normalized to moles C, BD = Below detection limit; the HPSEC did not provide accurate MW data for samples with DOC <3 mg C L^{-1}.

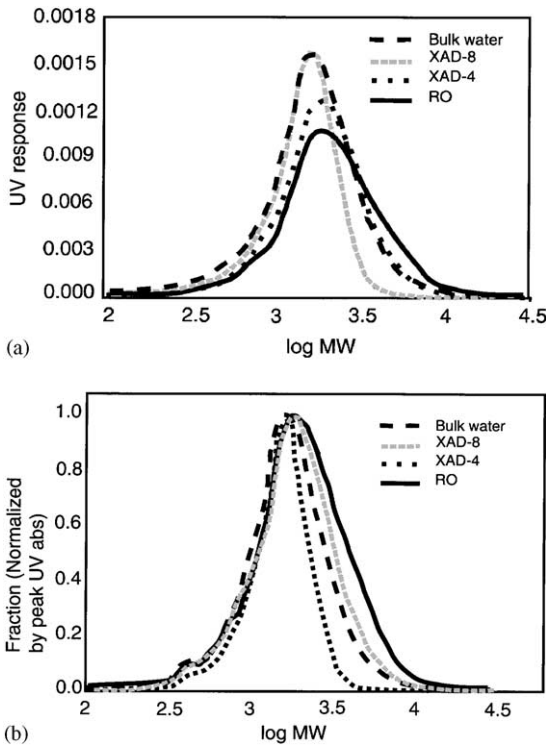

Fig. 2. (a) Comparison of HPSEC chromatograms from S2 Spring 1998. (b) HPSEC chromatograms normalized to peak UV absorbance.

For Spring 1998, one isolate ε_{280} was higher and one lower than the RFW for each sampling station, but the isolation process producing the higher ε_{280} was different at the two sites. The absence of consistent differences may be due to a number of factors in addition to the inherent chromophore content. Fe concentration (especially Fe(III) associated with NOM) and self-association have both been shown to affect NOM absorbance in the UV.

Chin et al. [22] showed that aquatic fulvic acids tended to fall along a trend line when M_w was plotted against ε_{280}. In our work, one RFW sample plotted well below the trend line observed by Chin et al. [22] this sample had high [Fe], almost 1 mg L^{-1} (Fig. 3). The other RFW samples, the XAD-8 isolates, and the XAD-4 isolates all plotted along Chin et al. [22] trend line. However, many of the RO isolates plotted above the trend line. Chin et al. [22] trend considered only XAD-8 fractions; the percent of XAD-8 extractable NOM in the RFW and RO samples may vary, as may the behavior of the other fractions with regard to UV absorbance.

3.4. ^{13}C NMR of isolates

As shown in Table 6, all of the XAD-8 and RO isolates from McDonalds Branch had at least slightly higher % aromaticities than the IHSS SRFA. The Spring 1998 S2 XAD-4 isolate had lesser % aromaticity, greater heteroaliphatic (60–90), greater ketone/quinone

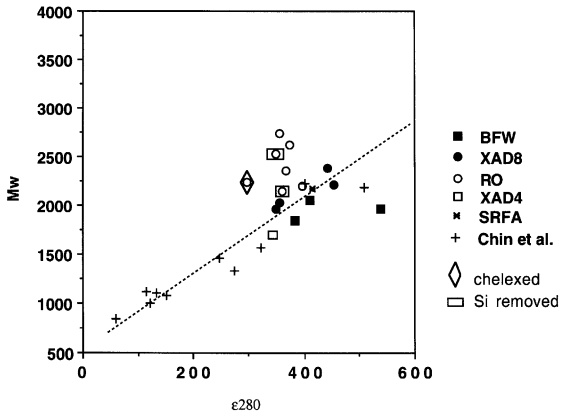

Fig. 3. Comparison of ε_{280} and M_w for all samples (RFW, RO, XAD-8, XAD-4 isolates). The Chin et al. [22] data also included for comparison. Regression line from Ref. [22] is included as a dashed line. The overall McDonalds Branch data set does not show any correlation between ε_{280} and M_w ($R^2 = 0.0108$). However, the XAD-8 data do show a linear trend ($R^2 = 0.804$) closely approximating the Chin et al. [22] trend. RO isolates plot consistently above the Chin et al. [22] trend and most RO data points are above the McDonalds Branch XAD-8 and RFW trends.

(195–220), and considerably greater carboxyl (165–195) content than the corresponding XAD-8 isolate. The increased ketone/quinone and carboxyl contents are consistent with more hydrophilic properties of the XAD-4 isolate and with previous results by Aiken et al. [39]. An example comparing ^{13}C NMR spectra for isolates is provided in Fig. 4.

The McDonalds Branch XAD-8 and RO isolates have qualitatively similar C distributions, with small but consistent differences indicating greater polarity of the RO isolates. Three of the XAD-8 isolates had greater % aromaticity, and lesser carboxyl (165–195), ketone/quinone (195–220), and heteroaliphatic (60–90) C percentages than the corresponding RO isolates. In contrast, the S10 Fall 1997 RO isolate had higher % aromaticity than the corresponding XAD-8 isolate, along with lesser carboxyl and ketone/quinone. Since this sample is extremely high in Si (~50% ash content by weight) and required additional treatment to remove silicate prior to NMR analysis, the results for this sample are considered less reliable. For the Spring 1998 S2 sample, the RO isolate is intermediate in values between the XAD-8 and XAD-4 fractions, which suggests that the RO isolate contains both of these fractions.

The conventional labels for regions of the ^{13}C NMR spectrum are only approximate. It will be useful to recall that the heteroaliphatic region includes the non-carbonyl ester carbons, and that the carboxyl region includes the ester carbonyl carbons. The slightly higher carboxyl and heteroaliphatic intensities for the RO isolates are similar in magnitude, and could potentially represent higher ester content, rather than higher acid and alcohol contents.

Generally, increased H:C is thought to reflect more aliphatic C, but we did not observe any correlation between H:C and % aromaticity for the XAD-8 isolates. We also did not observe any clear correlation between O:C and % aromatic.

4. Aqueous FTIR

Previous research by members of our group provides background for interpreting FTIR spectra of the NOM

Table 6
Solution state ^{13}C NMR data for surface water NOM isolates

% Carbon Site/sample	Aliphatic	Hetero-aliphatic	Acetal/aromatic	Aromatic	Carboxyl	Ketone/quinone	% Aromatic
Chemical shift range (ppm)	0–60	60–90	90–110	90–165	165–195	195–220	110–165
SRFA XAD-8	33	11	5	28	20	7	24
S2 XAD-8	18.4	11.0	9.0	39.9	22.8	7.9	30.9
RO	20.0	12.8	8.3	34.1	23.1	9.9	25.8
S10 XAD-8	22.3	9.9	7.9	35.2	21.8	10.8	27.3
RO	22.1	14.7	11.1	42.6	16.1	4.0	31.5
S2 XAD-8	25.5	11.6	8.9	37.6	20.6	4.8	28.7
RO	24.5	13.0	8.4	33.9	22.4	6.2	25.6
XAD-4	23.7	15.3	8.3	28.9	26.1	6.0	20.6
S2 XAD-8	24.4	10.8	8.1	36.8	22.6	5.4	28.7
RO	24.0	12.5	8.7	33.0	23.5	6.9	24.4

SRFA from Ref. [47].

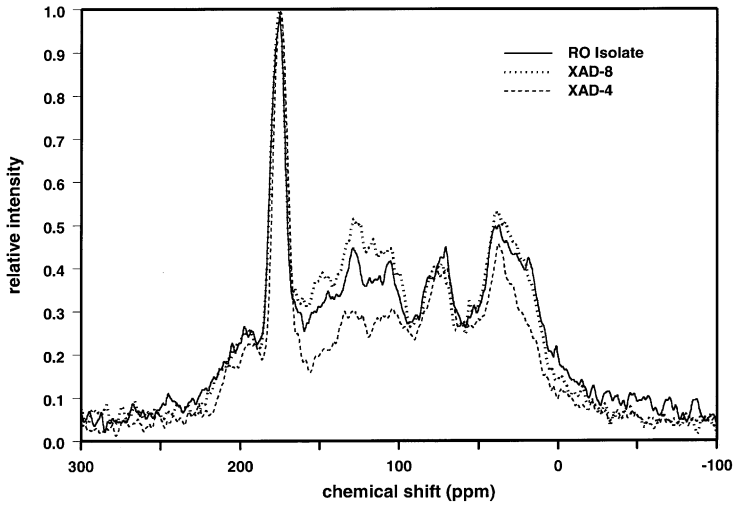

Fig. 4. The quantitative liquid state ^{13}C NMR spectra of three isolates extracted from the same Pine Barrens water sample (Site S2, Spring 1998). The spectra were referenced and normalized using the carboxyl peak (175 ppm). See text for a complete description of the NMR methodology.

Table 7
Aqueous FTIR data for surface water samples

Sample	Isolate	v C=0 COOH (cm^{-1})	v_{as} COO^- low pH (cm^{-1})	v_{as} COO^- high pH (cm^{-1})	v_{as} COH (cm^{-1})	Peak area C=O[a]	Peak area COO^-[b]	% COOR in total C=O[c]
S2 S97	XAD-8	1717	1610	1571	1095	0.125	0.255	12.5
	RO	1719	1614	1572	1094	0.125	0.240	19.3
S10 F97	XAD-8	1714	1606	1566	NA	0.122	0.218	11.2
	RO	1720	1610	1565	NA	0.133	0.182	31.2
S2 S98	XAD-8	1715	1607	1569	NA	0.148	0.261	10.1
	XAD-4	1719	1606	1567	1031	0.122	0.244	9.6
	RO	1720	1613	1580	1101	0.132	0.204	24.8
S10 S98	XAD-8	1715	1607	1566	1021	0.132	0.240	13.5
	RO	1720	1607	1565	1098	0.137	0.155	8.4

[a] Proportional to total C=O, COOH + ester + ketone, at low pH.
[b] Carboxylate only, at high pH.
[c] Based on low pH data.

samples [49] Cabaniss and McVey [50,51,55]. Aqueous infrared spectra of XAD-8 and RO isolates are also qualitatively similar with regard to the carboxyl peaks, but the RO spectra show enhanced ester bands and significant silicate interferences (Table 7). The most prominent features at acid pH (for example, Fig. 5a) are

(a) the C=O stretching peak near 1710–1720 cm^{-1}, which is largely due to carboxylic acid groups, but which includes a shoulder at higher frequency corresponding to the ester carbonyl;
(b) the asymmetric carboxylate stretch, v_{as}, near 1600–1610 cm^{-1}, which indicates that an appreciable fraction of the carboxyls are deprotonated even at this low pH;
(c) peaks or shoulders near 1200 cm^{-1}, arising from the carboxyl group C–O stretch and C–O–H scissoring modes;
(d) a peak near 1100 cm^{-1} attributed to some combination of alcohol C–O and silicate vibrations.

The maximum frequency of the C=O and v_{as} peaks varies rather little with extraction method, although the RO isolates typically have slightly higher frequencies (Table 7). The C=O peak areas (normalized to weight of isolate) are very similar.

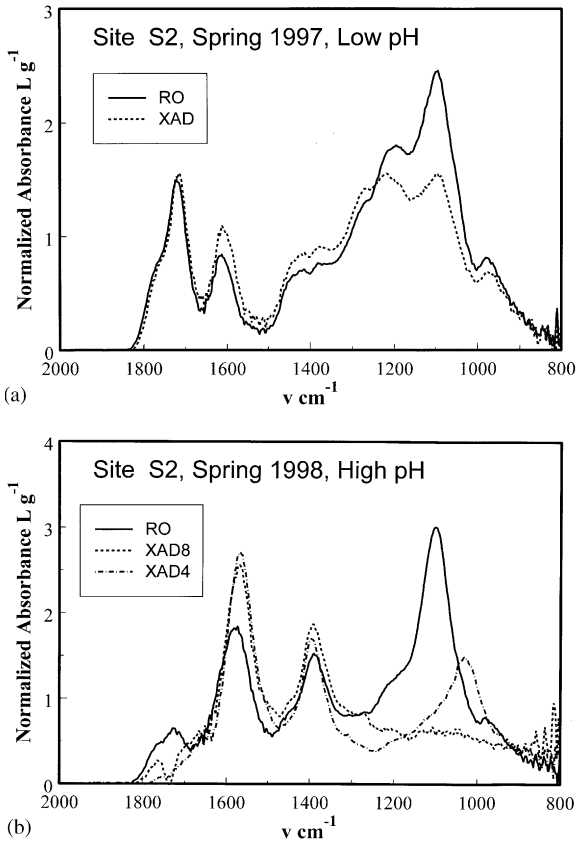

Fig. 5. (a) Aqueous FTIR spectra of XAD-8 and RO isolates at low pH. (b) Aqueous FTIR spectra of XAD-8 and RO isolates at high pH.

An interesting difference between the isolates appears in the shoulder of the C=O peak. In all the acidic spectra, the overall peak ($\sim$1650–1850 cm^{-1}) can be fitted with two mixed Gaussian/Lorentzian peaks. Both the ratio of the peak areas (last column of Table 7) and visual inspection of the peaks indicate that esters compose a greater fraction of the overall peak from the RO isolates than the corresponding peak from the XAD-8 isolates. The only RO isolate (S10S98) which fails to follow this trend was inadvertently exposed to very high (>12) pH before spectral acquisition, and this may have hydrolyzed many of its ester groups.

The most prominent features at basic pH (for example, Fig. 5b) are

(a) the v_{as} stretch, shifted to a lower frequency in keeping with the relationship between intrinsic pK_a and v_{as};
(b) the combined alcohol and silicate peak, no longer including COOH vibrations; and
(c) the symmetric carboxylate stretch, v_s, now visible as a separate distinct peak near 1390–1400 cm^{-1}.

The carboxylate stretches are generally similar in frequency, although the normalized area is larger for the XAD-8 isolates (Table 7). This difference in area, together with the presence of a small peak in the ester region, confirms that while the total carbonyl content of the isolates is very similar, the RO isolate contains a smaller proportion of acid groups and a larger proportion of esters (and possibly ketones). The presence of a large peak near 1100 cm^{-1} in several of the spectra indicates a significant silicate contamination; indeed, some of the RO isolate spectra are impossible to interpret (from an NOM standpoint) in this area because the alcohol-related vibrations are so much less intense than the silicate.

4.1. Differences between XAD-8 and RO isolates

The XAD-8 and RO methods considered here both isolate and concentrate most of the DOM from the stream water, producing broadly similar products. The RO generally has higher recovery of total C than the XAD-8. This is consistent with the fact that RO is intended to isolate a broad range of DOM whereas XAD-8 is specifically intended to isolate the (generally dominant) humic fraction. Nonetheless, significant differences do occur between the isolates, consistent not only with the fact that the RO is a broad isolation method whereas XAD-8 is an isolation and fractionation method but also with potential hydrolysis and/or condensation of the DOM during the isolation. The RO isolate has higher M_w and higher ester content than the XAD-8 isolate, as indicated by HPSEC and FTIR peak analysis, respectively. The ^{13}C NMR data indicate a greater percentage of heteroaliphatic C (which includes ester C) in the RO isolate, although the combined carboxyl and ester carbonyl percentages are similar for the two isolates.

These results suggest the possibility that:

(a) the high pH XAD-8 extraction method hydrolyzed a significant quantity of ester groups existing in the original NOM,
(b) the high DOC concentration and acidic pH of the RO isolation promoted condensation of carboxyl groups with alcohols to form esters, or
(c) both mechanisms operated.

The potential for hydrolysis is supported by previous work on ester hydrolysis at elevated pH (but not as high as the XAD-8 extraction) and by our observations that long exposure of RO isolates to high pH diminishes the ester peak. Previous work [39] suggested that the extreme pH's of the XAD-8 method could hydrolyze ester linkages. While our results are consistent with ester hydrolysis, such hydrolysis is not confirmed by our results because differences in ester content could reflect

the presence of higher ester content material isolated by the RO but excluded from the XAD-8 resin. The XAD-8 resin specifically isolates only the humic fraction.

The potential for condensation is suggested by our observation that M_w is higher for both RO and XAD-8 isolates than for the RFW. However, for the XAD-8 isolates, the higher M_w is likely due to the fact that lower MW material is not retained by the column, so that average MW is shifted to a higher value. Thus, the higher M_w of the XAD-8 isolates is probably not a result of condensation but rather of fractionation. As can be seen in Fig. 2a (right side of peaks), the RO isolate contains not just a greater fraction of higher MW material, but also a greater total amount of higher MW material than the raw filtered (bulk) water and the XAD-8 isolate. This suggests potential condensation for RO samples, but further study is needed to determine whether condensation does indeed occur.

5. Conclusions

RO and XAD are extensively used to isolate DOM from natural water samples. On the one hand, RO isolates a broad range of DOM components; on the other hand, XAD-8 specifically isolates the humic fraction (and XAD-4, the 'hydrophilic' fraction). Thus, RO may offer an advantage for researchers looking to determine the composition and reactivity of the broad DOM pool whereas XAD-8 may be most beneficial to researchers specifically interested in the humic fraction behavior as a major and often dominant component of the DOM pool. Within this context, it is important to compare the similarities and differences between the isolates in order to interpret the implications of experimental results based on the two techniques.

1. Direct comparison of surface water samples shows that RO isolation gives both a higher yield of organic C and a higher ash content than the humic-fractionating XAD-8 method, in agreement with previous work in which the methods were used on similar samples.
2. The XAD-8 yield of organic C was higher than average literature values, consistent with previous work showing McDonalds Branch to be humic-rich.
3. The M_w (and to a lesser extent, the M_n) of the XAD-8 and RO isolates are significantly higher than the RFW, and the XAD-4 isolate considerably lower, decreasing in the order RO > XAD-8 > RFW > XAD-4. On the one hand, the XAD-8 M_w is likely increased due to fractionation of the NOM favoring high MW components and excluding low MW components; on the other hand, comparison of HPSEC chromatograms indicates that the RO isolate likely underwent some condensation reaction(s) to increase MW during the isolation process.
4. ^{13}C NMR spectra show the RO isolate to be higher (15–50%) in heteroaliphatic C and ketone/quinone C than the XAD-8 isolate. The XAD-8 isolate is higher in aromatic C, consistent with the idea that it selectively isolates more hydrophobic molecules.
5. The aqueous FTIR spectra show that the RO isolate is higher in esters than the XAD-8 isolate, but comparable in total carbonyl groups. This suggests that ester hydrolysis may occur during the XAD-8 procedure. However, it is also possible that the RO procedure isolates more ester-rich organic matter.
6. Comparison of the chemical properties of RO, XAD-8, and XAD-4 isolates suggests that the RO samples contain a combination of XAD-8 and XAD-4 materials. The RO samples also may contain more hydrophobic neutrals and/or hydrophilics.
7. Selection of an appropriate isolation process for a given study should include consideration of the desirability of fractionation, and the need to compare results with previous work, the anion content of the waters, and problems due to high ash content in the RO isolates. The RO and XAD-8 methods were designed for different purposes—isolation of humics versus concentration of a broad spectrum of organic matter.
8. While elemental analysis, ^{13}C NMR, and ε_{280} data provided valuable data, the HPSEC measurements of molecular weight and the FTIR data proved to be crucial for distinguishing the properties of the various samples. We therefore recommend that these techniques be included whenever possible as part of a suite of complimentary methods used to characterize NOM samples.

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