

High-pressure size exclusion chromatography analysis of dissolved organic matter isolated by tangential-flow ultrafiltration

Christopher R. Everett and Yu-Ping Chin¹

Department of Geological Sciences, Ohio State University, Columbus, Ohio 43210

George R. Aiken

U.S. Geological Survey, 3215 Marine Street, Boulder, Colorado 80303

Abstract

A 1,000-Dalton tangential-flow ultrafiltration (TFUF) membrane was used to isolate dissolved organic matter (DOM) from several freshwater environments. The TFUF unit used in this study was able to completely retain a polystyrene sulfonate 1,800-Dalton standard. Unaltered and TFUF-fractionated DOM molecular weights were assayed by high-pressure size exclusion chromatography (HPSEC). The weight-averaged molecular weights of the retentates were larger than those of the raw water samples, whereas the filtrates were all significantly smaller and approximately the same size or smaller than the manufacturer-specified pore size of the membrane. Moreover, at 280 nm the molar absorptivity of the DOM retained by the ultrafilter is significantly larger than the material in the filtrate. This observation suggests that most of the chromophoric components are associated with the higher molecular weight fraction of the DOM pool. Multivalent metals in the aqueous matrix also affected the molecular weights of the DOM molecules. Typically, proton-exchanged DOM retentates were smaller than untreated samples. This TFUF system appears to be an effective means of isolating aquatic DOM by size, but the ultimate size of the retentates may be affected by the presence of metals and by configurational properties unique to the DOM phase.

The isolation and characterization of aquatic dissolved organic matter (DOM) in both limnological and oceanic environments has been the focus of a number of studies (Aiken et al. 1985, 1992; Orem et al. 1986; Orem and Hatcher 1987; Chen et al. 1993; Amon and Benner 1994, 1996; Chin et al. 1994, 1998; plus many others). The most common methods are comprised of either chromatographic fractionation using a hydrophobic resin (i.e., XAD 8 and 4) (Aiken et al. 1992) or physical separation by some form of ultrafiltration (Thurman et al. 1982; Aiken 1984). In particular tangential-flow ultrafiltration (TFUF, also known as cross-flow ultrafiltration) has been used extensively to isolate marine DOM from both coastal and open oceanic sites (Whitehouse et al. 1990; Benner et al. 1992; Buessler et al. 1996; Mopper et al. 1996; Guo and Santchi 1996; plus many others) and DOM from groundwater (Backhus and Gschwend 1990). The TFUF method is a physical separation process by which solvent and solute molecules smaller than the membrane pores pass through when a hydrostatic pressure is created. Molecules larger than the membrane pores are retained and concentrated. It is typically assumed that most of the retained DOM molecules have molecular weights greater than the manufacturer-specified membrane cutoff size. These isolates subsequently are used to characterize the various properties of DOM or are utilized in a wide range of geochemical and

environmental reactivity studies (Backhus and Gschwend 1990; Dunnivant et al. 1992; Amon and Benner 1994, 1996; Danielsen et al. 1995; Guo et al. 1995, 1996). It has always been assumed that the isolates are in some manner representative of the properties and reactivity of part or all of the unfractionated DOM pool. Unfortunately, TFUF methods are subject to a number of analytical problems, including loss of DOM to the membranes and breakthrough of DOM molecules larger than the specified pore sizes (Aiken 1984; Kilduff and Weber 1992; Buessler et al. 1996). Moreover, DOM may exist in different configurations, and the actual fractionation process may be ambiguous if only the manufacturer-specified operational cutoffs are used. Thus, pH and ionic strength effects may play a role in determining the actual separation that occurs in such systems.

To date, there has been no comprehensive examination of the actual molecular weights of DOM isolated by TFUF. High-pressure size exclusion chromatography (HPSEC) has been used to provide reasonable estimates of aquatic humic substance molecular weights when compared with values determined by vapor pressure osmometry (VPO), field flow fractionation (FFF), and ultracentrifugation (Chin and Gschwend 1991; Chin et al. 1994). Thus, HPSEC may be an independent means of determining the size of organic materials in TFUF retentates and filtrates for a specific water sample and could be used to ascertain the actual efficiency of the ultrafilter membrane for isolating aquatic DOM as opposed to relying upon the cutoff specifications provided by the manufacturer or by studies that utilize molecular weight standards (e.g., dextrans, polystyrene sulfonates) (Kilduff and Weber 1992; Gustaffson et al. 1996).

In this study, we examined the use of a TFUF device for isolating DOM and the use of HPSEC for quantifying the molecular weights of the retentate and filtrate materials. Wa-

¹ To whom correspondence should be addressed.

Acknowledgments

We thank Ed O'Loughlin for helping us collect the samples and Lori Ellinger for assisting us in the lab. Comments by Ron Benner and an anonymous reviewer greatly improved the manuscript. David Klarer at Old Woman Creek NERR provided logistical support at that site. This research was funded by National Science Foundation Grant EAR-9316745.

ters were collected from a variety of locations, including some where the DOM has been well characterized (Thurman et al. 1982; Aiken et al. 1985, 1992). The objectives were to (1) isolate a molecular mass standard (1,800-Dalton polystyrene sulfonate) and the DOM source waters with a 1,000-Dalton-membrane TFUF unit; (2) use HPSEC to examine the molecular weight averages of the filtrate and retentate for each DOM sample and compare them with those measured for the whole water; (3) conduct characterization studies such as spectroscopic analyses for the whole water samples and the retentates; and (4) examine the role of chelated metals on the molecular weights of TFUF isolated DOM.

Methods

Sites and sampling protocol—Samples were collected from the Suwannee and Ogeechee Rivers, Georgia; the Great Dismal Swamp, Virginia; and Old Woman Creek Estuary near Huron, Ohio. All samples were collected in precleaned containers, filtered through glass-fiber filters (type A/E, Gelman Sciences), and stored at 4°C. Aliquots from each raw water sample were saved for HPSEC, spectroscopic, and total organic carbon (TOC) analysis, and the remainder was processed by TFUF. Fulvic acid from the Suwannee River was isolated from a different sampling event (same location) using XAD chromatography and stored in a lyophilized state. The TOC of each sample was measured using a Shimadzu 5000 TOC analyzer equipped with a platinum-on-alumina catalyst. Samples were acidified to pH 2.0 using HCl and were measured as nonpurgeable organic carbon. Ultraviolet (UV)–visible (VIS) measurements were taken using a Varian Cary 1 spectrophotometer, and the molar absorbance at 280 nm was recorded for each sample.

A polystyrene sulfonate standard (Polysciences; manufacturer's listed molecular mass = 1,800 Daltons) was used to examine the retention efficiency of the ultrafiltration membrane for its listed molecular mass cutoff. The standard was dissolved into 4 liters of Milli-Q (Millipore) water to yield a TOC concentration of 1.6 mg liter⁻¹. Three 1-liter aliquots were then taken, and the ionic strengths were adjusted to 0, 0.01, and 0.1 M with NaCl. Each standard was then concentrated, and samples of the preconcentrate, retentate, and filtrate were saved for analysis.

Ultrafiltration protocol—The ultrafiltration equipment used for this study was an Amicon CH2 concentrator equipped with a S1N1 spiral wound membrane cartridge. The membrane is composed of a polysulfone polymer and has a manufacturer-listed nominal molecular mass cutoff value of 1,000 Daltons. Prior to use, the membrane was washed by pumping NaOH (pH 11.5) through the system to remove the protective coating of glycerin. The unit was then rinsed by pumping Milli-Q water across the membrane until all traces of the NaOH were gone (as determined by conductivity), and the TOC of the filtrate was equal to that of the feed water (<50 μM carbon). Standards and DOM samples were then run through the system and concentrated by a factor of 10 at a back pressure of 6 psi. Aliquots of the retentates and filtrates were saved for analysis. Between processing runs, the system was cleaned as described above. When not in

use, the membrane cartridge was removed, filled with Milli-Q water, and stored at 4°C.

Cation-exchange analysis—Trace metals were removed from the DOM using a cation-exchange resin (hydrogen-form Bio-Rad AG 50W-X8 resin). The resin was rinsed five times with methanol and then five times with Milli-Q water (to remove any residual organic material) and exchanged with 1 N NaOH, which further cleans the resin and saturates the cation-exchange sites with sodium. The resin was then packed into a 25-ml column and rinsed with five column volumes of Milli-Q water to remove the NaOH. After rinsing, five column volumes of 10% high-purity HCl were poured through the column to resaturate the cation-exchange sites with protons. The resin was again rinsed with Milli-Q water until the TOC value of the eluent was equal to the TOC of the feed water (50–80 μM). The DOM samples were eluted through the freshly prepared resin column, and the eluent was collected in a reservoir at the bottom of the column. The proton-exchanged concentrates were subsequently placed into solid Teflon beakers, quick frozen using liquid nitrogen, lyophilized, and stored until further use. Solutions were made up from the freeze-dried samples, and their TOCs were measured as discussed previously. As a consequence, the final retentate working solution TOC concentrations may vary for different samples because it would be impossible to gravimetrically determine the amount of organic carbon present a priori.

HPSEC—All samples were analyzed with a Waters HPSEC connected to a variable wavelength ultraviolet (UV) light detector and controlled by the HPSEC software MAXIMA. Standards were made using polystyrene sulfonate (Polysciences) of different molecular weights (MW) (18,000, 8,000, 5,400, and 1,800) and acetone (MW = 54). The mobile phase was composed of 0.1 M NaCl, 0.002 M Na₂HPO₄, and 0.002 M KH₂PO₄ and degassed prior to use. Twenty-microliter volumes of each sample to be analyzed were injected and run through the HPSEC column at a rate of 1.0 ml min⁻¹. Number-averaged (M_n) and weight-averaged (M_w) molecular weights for the humic substances and colloids were determined using the following equations:

$$M_n = \frac{\sum_{i=1}^N h_i}{\left(\sum_{i=1}^N h_i/M_i \right)} \quad (1)$$

and

$$M_w = \frac{\sum_{i=1}^N h_i(M_i)}{\sum_{i=1}^N h_i} \quad (2)$$

where h_i is the height of the sample SEC curve eluted at molecular weight M_i (Chin et al. 1994). Corrections for band broadening were made using a polystyrene sulfonate (PSS) standard (4.6 K) and the techniques described by Yau and co-workers (1979). This method involved comparing the PSS polydispersities (i.e., M_w/M_n) measured by HPSEC to the chemical manufacturer's specification. Dispersion was insignificant in our system, and the chromatograms were evaluated without further need for correction factors. The

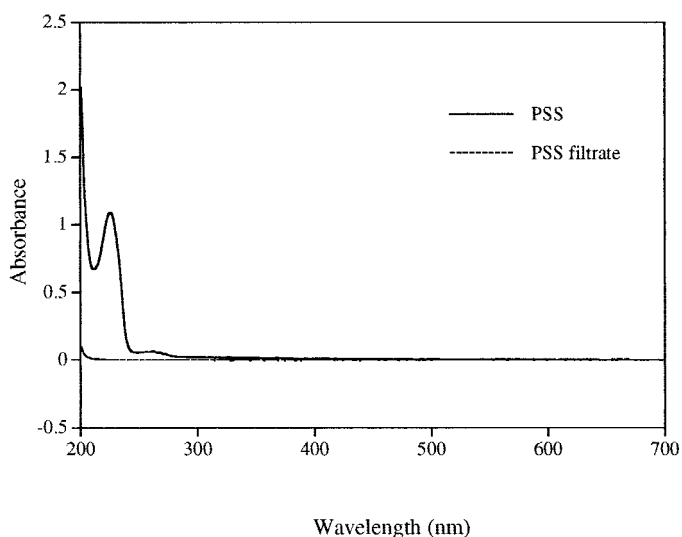


Fig. 1. Pre- and post-TFUF UV/VIS scan of the 1,800-Dalton polystyrene sulfonate standard used to determine the effectiveness of the S1N1 membrane. TOC analysis confirms observations made by absorbance spectroscopy (see Table 1).

MAXIMA software calculated the M_w , M_n , and polydispersity for each sample. Details regarding the selection of the molecular weight standards and mobile phase composition can be found elsewhere (Chin et al. 1994).

Results and Discussion

The retention efficiency of the polysulfone membrane was tested using an 1,800-Dalton PSS standard at different ionic strengths (concentration factor $\times 10$). This standard was chosen because its molecular weight and polydispersity are similar to values measured for aquatic DOM (by FFF, ultracentrifugation, HPSEC), and we wanted to independently determine the ultrafilter cutoffs as specified by the manufacturer. Based upon both TOC and its UV absorbance (at 226 nm), the standard was completely retained by the ultrafiltration membrane (Fig. 1). Moreover, adjustment of the solution phase ionic strength appeared to have no effect on the membrane rejection for this compound. Based upon previous studies (Ghosh and Schnitzer 1980; Aiken 1984; Beckett et al., 1987; Chin and Gschwend 1991; Kilduff and Weber 1992; Burdige and Gardner 1998), coiling of DOM

and PSS molecules coupled with ionic interactions at the membrane interface may affect the performance of ultrafiltration membranes. Ionic strength effects observed by some of these investigators (Beckett et al. 1987; Chin and Gschwend 1991; Kilduff and Weber 1992) include an increase in the analyte retention at lower ionic strengths even when significantly larger molecular mass membranes ($\geq 10,000$ Daltons as specified by the manufacturer) were used. This PSS polymer is capable of undergoing coiling as a function of both pH and ionic strength to a greater degree than aquatic fulvic acids (Chin and Gschwend 1991), but our results show that even at the highest ionic strength used in this study, the analyte molecules were still retained by this membrane. The ionic strength artifacts appear to be limited to the larger cutoff membranes ($> 3,000$ Daltons) where the configuration of the analytes (coiled vs. uncoiled) may play an important role. For our membrane, the pore sizes appear to be sufficiently small and are not as sensitive to analyte configurational changes.

The 1,000-Dalton S1N1 membrane retention coefficient (RC) is defined as

$$RC = 1 - (TOC_{perm}/TOC_{feed}) \quad (3)$$

where TOC_{perm} and TOC_{feed} are the respective permeate and raw water TOC values (Karger et al. 1973; Aiken 1984; Buessler et al. 1996). Generally, the RC values were largest for those samples with the highest initial TOCs, i.e., Great Dismal Swamp and Suwannee River water (Table 1). Our results appear to be consistent with the results reported by Gustaffson and co-workers (1996) for this specific type of membrane and a 3,000 dextran molecular weight standard. These and other studies, however, show much higher losses to similar membranes (Osmonics, Filtron, and Membrex), and in some cases breakthrough of standards that possess molecular weights much greater than the membrane cutoff occurred (Aiken 1984; Gustaffson et al. 1996). The later studies (all participants in the intercalibration "colloid-cook-out" exercise) were conducted in seawater, where the DOM concentrations are significantly smaller than those used in this study (10's to 100's of μM carbon vs. mM carbon). Moreover, the standards that were employed in these studies were made up at "realistic" marine DOM concentrations and employed larger volumes of solution (10's to 100's of liters) (Gustaffson et al. 1996). At these analyte levels, potential artifacts such as losses to the membranes become magnified because the permeate is of the same magnitude as

Table 1. Retention coefficients and mass balances for the S1N1 membrane and the waters samples used in this study.

Sample	TOC (mg liter ⁻¹)			Mass balance (%)	Retention coefficient
	Initial	Filtrate	Retentate		
Suwannee River	34.5	9.16	nd*	nd	73.4
Dismal Swamp	45.8	13.3	nd	nd	71.0
Ogeechee River	3.75	1.34	23.1	97%	64.3
Old Woman Creek	4.89	2.12	17.8	79%	56.6
1,800-Dalton PSS	1.6	sab†	12.9–15.8	98–80	100.0

* nd = not determined.

†sab = same as blank.

Table 2. Properties of DOM sample retentates, whole water, and filtrates. Suwannee River samples include the fulvic acid isolate. Unless noted, DOC of the retentates are reconstituted from freeze-dried samples.

Sample	TOC		Molar absorbance (liter mole carbon ⁻¹ cm ⁻¹)	Molecular weight		
	mg liter ⁻¹	mmol		Weight averaged (M_w)	Number averaged (M_n)	Polydispersity (M_w/M_n)
Suwannee River						
Whole water	34.5	2.88	390	2,200	1,440	1.53
Filtrate	9.16	0.76	191	1,060	826	1.28
Retentate	32.7	2.73	448	2,670	1,700	1.57
H ⁺ -saturated retentate	56.6	4.72	243	2,520	1,790	1.41
Fulvic acid	35.9	2.99	471	2,430	1,790	1.36
Ca ⁺⁺ -saturated fulvic acid*	5.43	0.45	912	2,660	1,530	1.74
Dismal Swamp						
Whole water	45.8	3.82	341	1,760	1,180	1.49
Filtrate	13.3	1.11	221	974	723	1.35
Retentate	38.8	3.23	430	2,190	1,630	1.34
H ⁺ -saturated retentate	64.6	5.38	315	2,050	1,610	1.27
Ogeechee River						
Whole water	3.75	0.31	554	1,350	1,120	1.21
Filtrate	1.34	0.11	145	795	697	1.14
Retentate†	23.1	1.93	526	1,570	1,020	1.54
H ⁺ -saturated retentate	24.6	2.05	291	1,450	963	1.51
Old Women Creek						
Whole water	4.89	0.41	187	784	645	1.22
Filtrate	2.12	0.18	184	557	548	1.02
Retentate†	17.8	1.48	237	1,270	947	1.34
H ⁺ -saturated retentate	15.5	1.29	203	1,220	865	1.41

† Unaltered retentate.

* Final concentration after elution from the cation-exchange column.

the initial DOM concentration. For example TFUF is only able to isolate approximately 20–30% of the total open ocean DOM pool (Benner et al. 1997). Although the TFUF RC values for waters with the lowest initial DOC (Ogeechee and Old Woman Creek) (Table 1) were generally lower than those measured for the Suwannee River and Great Dismal

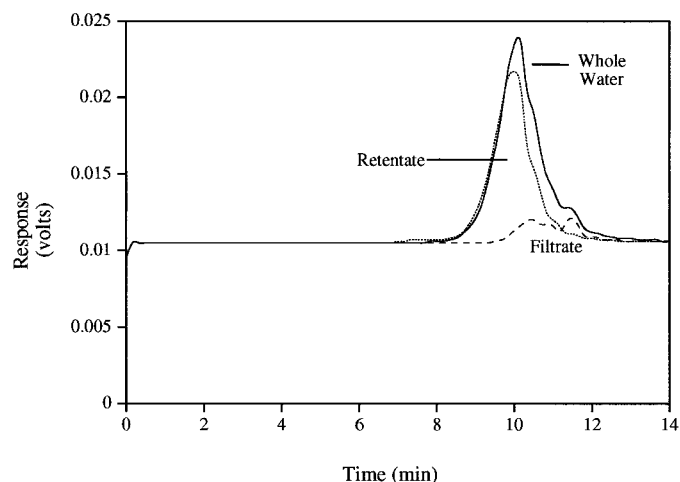


Fig. 2. Overlay of TFUF Great Dismal Swamp retentate, filtrate, and raw water HPSEC chromatograms, showing loss of low-molecular-weight moieties.

Swamp samples, we did not observe any strong correlation between initial DOC and the RC.

Mass balances based upon the retentate (corrected for the concentration factor) and filtrate concentrations were typically good (>79%) for the Old Woman Creek and Ogeechee River water samples and fell within the range observed for the PSS 1,800-Dalton standard (Table 1). We did not conduct mass balances on the other two samples, but we assumed that they were similar in magnitude.

The M_w values, as determined by HPSEC, were consistently larger for the retentates than for the whole waters (Table 2). The Great Dismal Swamp HPSEC chromatograms (Fig. 2) show clearly the early elution of the retentate peaks relative to the whole water peak. Conversely, we were barely able to detect the filtrate peaks but they possessed longer retention times (Fig. 2). All the remaining DOM samples and their TFUF retentates exhibited this behavior. This segregation of the whole water DOM pool into distinct fractions is also reflected in the molecular weight distributions of the raw water and the various TFUF-segregated fractions as quantified by the sample's polydispersity value (i.e., M_w/M_n). In all cases, polydispersity values for the filtrate DOM were lower than those for the whole water or retentate DOM. These results suggest that the DOM in the filtrate is comprised of relatively small molecules that reside in a narrow molecular weight distribution.

The M_w values of the filtrates were nearly identical to the

1,000-Dalton membrane cutoff, and the M_n values were smaller (Table 2). Because M_w and M_n values are the statistical averages for a range of molecules with different molecular weights rather than for discrete molecular weights, the listed cutoff value for the S1N1 membrane is reasonably accurate when defined within this context. This study is the first in which HPSEC was used to independently quantify the molecular weights of aquatic DOM segregated by ultrafiltration. These results, however, appear to be in some disagreement with results reported for the fractionation of DOM using other TFUF and stirred-cell systems (Aiken 1984; Buessler et al. 1996; Gustaffson et al. 1996). Many ultrafiltration-DOM studies incorporate a wide range of ultrafiltration membrane cutoffs and isolate the DOM in a cascade fashion, i.e., water samples are sequentially passed through membranes of diminishing pore sizes (e.g., Orem and Gaudette 1984, Chin and Gschwend 1991). Macromolecules (natural and synthetic) are capable of existing in variety of configurations that range from random coil substances such as PSS to globular proteins (Yau et al. 1979). As stated previously, much of the work in the literature that has reported UF artifacts with DOM segregation have been observed for these larger cutoff membranes. Typically, the large pore size ($\geq 10,000$ Daltons) ultrafiltration membranes are designed for biochemical applications and are commonly calibrated with proteins. Conversely, the smaller pore size membranes ($\leq 3,000$ Daltons) are calibrated to different standards (e.g., vitamin B₁₂, PSS) that do not possess globular configurations and may be more suitable for DOM segregation applications. Recently, Burdige and Gardner (1998) showed that nearly all the DOM present in marine sedimentary porewaters is capable of passing through a 3,000-Dalton ultrafilter. Their values suggest that much of the DOM pool is < 3000 Daltons, which corroborates molecular weight values measured by other methods (VPO, ultracentrifugation, HPSEC, and FFF) for humic substances and raw water DOM (Thurman et al. 1982; Aiken and Malcolm 1987; Beckett et al. 1987; Reid et al. 1990; Chin and Gschwend 1991; Chin et al. 1994). Our research suggests that the 1,000-Dalton S1N1 membrane is capable of roughly segregating DOM (at high enough initial concentrations) into fractions that are larger and smaller than the pore size as specified by the manufacturer.

The monomodal peaks seen in the chromatograms (Fig. 2) indicate that aquatic DOM is composed of a complex mixture that is not easily separated into individual components by HPSEC (which typically lacks the resolution of other forms of chromatography). Moreover, the polydispersity of these analytes is low (Table 2), which supports the theory that DOM molecules occupy a relatively narrow range of sizes (Aiken and Malcolm 1987; Chin et al. 1994). In an earlier study (Chin et al. 1994), we measured the molecular weights of purified fulvic acids by HPSEC, and the observed small polydispersities were attributed to the method by which they were fractionated from the other DOM components. Typically, fulvic acids are isolated by trapping the fractions on a hydrophobic resin packed in a column. In principal, all fulvic acids trapped in this manner possess the same capacity factor for that column, irrespective of the water source (Aiken et al. 1992). Thus, this method of isolation

constrains the fulvic acids with respect to polarity and possibly to molecular size. The results presented herein are surprising in that natural processes in aquatic systems appear to be constraining the size of DOM molecules. Evidence from studies of marine systems has shown that the high-molecular-weight (polysaccharides, proteins) and monomeric low-molecular-weight (e.g., fatty and amino acids) DOM components are capable of being biologically scavenged (Amon and Benner 1994, 1996; Guo et al. 1995; Burdige and Gardner 1998), leaving behind a nonlabile pool of relatively low-molecular-weight organic matter. Moreover, non-biological processes such as adsorption and precipitation may also account for the loss of the larger organic matter fractions (Gu et al. 1996; Wang et al. 1997; Chin et al. 1998). All these processes could be responsible for naturally constraining the molecular weight distribution of DOM.

In an earlier study, we observed a correlation between the molar absorptivity (ϵ , which is absorbance divided by the TOC with units of liters mol carbon⁻¹ cm⁻¹) at 280 nm for a number of humic substances and their weight-average molecular weights (Chin et al. 1994). Molar absorptivity can be used to estimate the degree of humic substance aromaticity (Gauthier et al. 1987; Traina et al. 1990), and larger humic substances typically possess a greater degree of aromaticity. For our fractionated DOM samples, ϵ values were largest for the retentates and smallest for the filtrates (Table 2). These results appear consistent with those observed by Mopper and co-workers (1996), who found that the coastal water permeate possessed organic materials that absorbed at shorter wavelengths. These investigators hypothesized that the 1,000-Dalton TFUF retentates were comprised of high-molecular-weight fractions containing chromophores capable of absorbing light at longer wavelengths. One exception to this trend was the Ogeechee River water sample, which possessed larger-than-expected molar absorptivities. We attributed this anomaly to the presence of metals in the DOM components. Treatment of all the sample TFUF retentates by cation-exchange chromatography resulted in decreases in their molar absorptivity, but the Ogeechee sample appeared to be most affected (Table 2). Thus, the presence of metals may influence the UV spectroscopy of unaltered DOM.

Our observations suggest that larger and more aromatic aquatic DOM components were preferentially retained by TFUF, whereas the smaller and presumably less aromatic constituents passed through the membrane. Surprisingly, the data also correlate well with the molar absorptivity- M_w relationship observed by Chin and co-workers (1994) for fulvic and humic acids isolated from various natural water sources (Fig. 3). Because molar absorptivity can be determined relatively quickly, this relationship may allow one to estimate an approximate DOM M_w value for those samples treated with a cation-exchange resin in lieu of an actual HPSEC measurement.

In addition to changes in molar absorptivity, the protonation-exchanged DOM retentates possessed slightly smaller M_w (Table 2). We hypothesized that the exchange of metals associated with the DOM retentates by protons may be the cause for this decrease. The magnitude of this change would in turn be dependent upon the type and nature of the complexes formed (i.e., alkali earth's vs. transition and posttran-

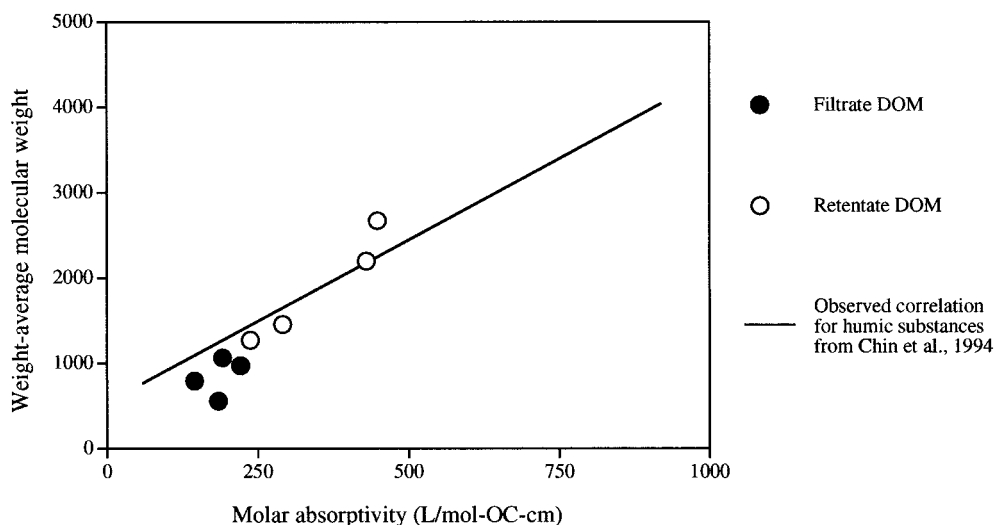


Fig. 3. Relationship between weight-average molecular weight and molar absorptivity (at 280 nm) of retentates (○) and filtrates (●). The line is the correlation observed for humic substances reported by Chin and co-workers (1994).

sition metals, inner-sphere vs. outer-sphere complexes). Nonetheless, metals associated with unaltered aquatic DOM apparently will influence their true molecular weights.

To test this hypothesis, we exchanged a proton-saturated Suwannee River fulvic acid (SRFA) isolate with calcium using a calcium cation-exchange resin. The resin was prepared by saturating the proton-exchange resin with a solution of calcium chloride. The fulvic acid sample was eluted from the resin column and assayed by HPSEC and UV/VIS spectroscopy. The concentration of the eluent was substantially lower than the initial concentration because of mobile phase dilution effects. We observed an increase in both the M_w and molar absorptivity of the calcium-saturated SRFA relative to its protonated form (Table 2). As stated earlier, we suspected that the presence of multivalent metals may have affected the molar absorptivity measurements of the Ogeechee River DOM. The changes in ϵ observed for the calcium-saturated SRFA are larger than expected, and we currently do not have an explanation for this phenomenon. Thus, unaltered DOM, in the presence of multivalent metals, would possess larger than expected molecular weights and ϵ .

Summary and conclusions

TFUF has been widely used by oceanographers and limnologists for the isolation of DOM from the bulk aqueous phase. The isolates in turn are used in characterization and reactivity studies, and it has been implicitly assumed that the membranes utilized are properly fractionating the DOM pool by size. An extensive intercomparison study has been conducted between different types of TFUF systems, but to date there has been no means of independently (by a separate analytical method) verifying the molecular weight fractions of TFUF retentates. In this study, we independently measured the molecular weights of unaltered DOM and their TFUF retentates and filtrates using high pressure exclusion chromatography. The 1,000-Dalton polysulfone membrane

employed in this study does appear to preferentially isolate the >1,000-Dalton DOM fraction based upon our HPSEC measurements of retentates and filtrates. Moreover, multivalent metals in the aqueous phase may play an important role in determining the molecular weight of DOM isolates. The process of proton-exchanging DOM and humic isolates brings about decreases in their molecular weights by exchanging out the heavier di- and trivalent metals.

In addition to preferentially isolating the high-molecular-weight fractions, TFUF also appears to fractionate aquatic DOM by chromophore composition. The molar absorptivity results indicate that the retentates possess a larger number of light-absorbing moieties than does the filtrate. The correlation between molar absorptivity and molecular weight for the retentates, filtrates, and unaltered DOM samples appears to be similar to that observed for humic materials isolated from various sources.

This particular membrane effectively fractionates DOM based upon molecular size. Moreover, the preferential accumulation of chromophoric moieties could also be exploited in studies that examine the photochemical behavior of DOM. Thus, properly used within the context of its limitations, this membrane technology provides a satisfactory means of isolating large amounts of aquatic DOM, based upon size, for natural water samples.

References

- AIKEN, G. R. 1984. Evaluation of ultrafiltration for determining the molecular weight of fulvic acid. *Environ. Sci. Technol.* **18**: 978-981.
- , AND MALCOLM R. L. 1987. Molecular weight of aquatic fulvic acids by vapor pressure osmometry. *Geochim. Cosmochim. Acta* **51**: 2177-2184.
- , D. M. MCKNIGHT, K. A. THORN, AND M. THURMAN. 1992. Isolation of hydrophilic organic acids from water using non-ionic macroporous resins. *Org. Geochem.* **18**: 567-573.

- , ———, R. L. WERSHAW, AND P. MCCARTHY. 1985. Humic substances in soil, sediment, and water. Wiley-Interscience.
- AMON, R. M. W., AND R. BENNER. 1994. Rapid cycling of high molecular-weight dissolved organic matter in the ocean. *Nature* **369**: 549–552.
- , AND ———. 1996. Bacterial utilization of different size classes of dissolved organic matter. *Limnol. Oceanogr.* **41**: 41–51.
- BACKHUS, D. A., AND P. M. GSCHWEND. 1990. Fluorescent polycyclic aromatic hydrocarbons as probes for studying the impact of colloids on pollutant transport in groundwater. *Environ. Sci. Technol.* **25**: 1214–1220.
- BECKETT, R., Z. JUE, AND J. C. GIDDINGS. 1987. Determination of molecular weight distributions of fulvic and humic acids using flow field flow fractionation. *Environ. Sci. Technol.* **21**: 289–294.
- BENNER, R., B. BIDDANA, B. BLACK, AND M. MCCARTHY. 1997. Abundance, size distribution, and stable carbon and nitrogen compositions of marine organic matter isolated by tangential flow ultrafiltration. *Mar. Chem.* **57**: 243–263.
- , J. D. PAKULSKI, M. MCCARTHY, J. I. HEDGES, AND P. G. HATCHER. 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* **255**: 1561–1564.
- BUSSLER, K. O., AND OTHERS. 1996. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: Overview and organic carbon results. *Mar. Chem.* **55**: 1–31.
- BURDIGE, D. J., AND K. G. GARDNER. 1998. Molecular weight distribution of dissolved organic carbon in marine porewater. *Mar. Chem.* **62**: 45–65.
- CHEN, R. F., J. L. BADA, AND Y. SUZUKI. 1993. The relationship between dissolved organic carbon and fluorescence in anoxic marine porewaters. *Geochim. Cosmochim. Acta* **57**: 2149–2153.
- CHIN, Y. P., G. R. AIKEN, AND E. O'LOUGHLIN. 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ. Sci. Technol.* **28**: 1853–1858.
- , AND P. M. GSCHWEND. 1991. The abundance, distribution, and configuration of porewater organic colloids in recent sediment. *Geochim. Cosmochim. Acta* **55**: 1309–1317.
- , S. J. TRAINA, C. R. SWANK, AND D. E. BACKHUS. 1998. Abundance and properties of natural organic matter in porewaters of a freshwater wetland. *Limnol. Oceanogr.* **43**: 1287–1296.
- DANIELSEN, K. M., Y. P. CHIN, J. S. BUTERBAUGH, T. L. GUSTAFSON, AND S. J. TRAINA. 1995. The solubility enhancement and fluorescence quenching of pyrene by humic substances. *Environ. Sci. Technol.* **29**: 2162–2165.
- DUNNIVANT, F. M., R. P. SCHWARZENBACH, AND D. L. MACALADY. 1992. Reduction of substituted nitrobenzenes in aqueous solutions containing natural organic matter. *Environ. Sci. Technol.* **26**: 2133–2141.
- GAUTHIER, T. D., W. R. SEITZ, AND C. L. GRANT. 1987. Effects of structural and compositional variations of dissolved humic materials on pyrene K_{oc} values. *Environ. Sci. Technol.* **21**: 243–248.
- GHOSH, R., AND M. SCHNITZER. 1980. Macromolecular structure of humic substances. *Soil Sci.* **129**: 266–276.
- GU, B., T. L. MELHORN, L. LIANG, AND J. F. MCCARTHY. 1996. Competitive adsorption: Displacement and transport of organic matter on iron oxide I. *Geochim. Cosmochim. Acta* **60**: 1943–1950.
- GUSTAFSSON, O., K. O. BUSSLER, AND P. M. GSCHWEND. 1996. On the integrity of cross-flow filtration for collecting marine colloids. *Mar. Chem.* **55**: 93–111.
- GUO, L., AND P. H. SANTSCHI. 1996. A critical evaluation of cross-flow ultrafiltration techniques for sampling colloidal organic carbon in seawater. *Mar. Chem.* **55**: 113–127.
- , ———, L. A. CIFUENTES, S. E. TRUMBORE, AND J. SOUTHON. 1996. Cycling of high molecular weight dissolved organic matter in the Middle Atlantic Bight as revealed by carbon isotopic signatures. *Limnol. Oceanogr.* **41**: 1242–1252.
- , ———, AND K. W. WARNKEN. 1995. Dynamics of dissolved organic carbon in oceanic environments. *Limnol. Oceanogr.* **40**: 1392–1403.
- KARGER, B., L. R. SNYDER, AND C. HORVATH. 1973. Introduction to separation science. Wiley and Sons.
- KILDUFF, J., AND W. J. WEBER, JR. 1992. Transport and separation of organic macromolecules in ultrafiltration processes. *Environ. Sci. Technol.* **26**: 569–577.
- MOPPER, K., Z. FENG, S. B. BENTJEN, AND R. F. CHEN. 1996. Effects of cross-flow filtration on the absorbance and fluorescence properties of seawater. *Mar. Chem.* **55**: 53–74.
- OREM, W. H., AND H. E. GAUDETTE. 1984. Organic matter in anoxic marine porewater: Oxidation effects. *Org. Geochem.* **5**: 175–181.
- , AND P. G. HATCHER. 1987. Solid-state ^{13}C -NMR studies of dissolved organic matter in porewaters from different depositional environments. *Org. Geochem.* **11**: 73–82.
- , ———, E. C. SPIKER, N. M. SZEVEKENYI, AND G. E. MACIEL. 1986. Dissolved organic matter in anoxic porewater from Mangrove Lake, Bermuda. *Geochim. Cosmochim. Acta* **50**: 609–618.
- REID, P. M., A. E. WILKINSON, E. TIPPING, AND N. M. JONES. 1990. Determination of molecular weights of humic substances by analytical UV scanning ultracentrifugation. *Geochim. Cosmochim. Acta* **54**: 131–138.
- THURMAN, E. M., R. L. WERSHAW, R. L. MALCOLM, AND D. J. PINCKNEY. 1982. Molecular size of aquatic humic substances. *Org. Geochem.* **4**: 27–37.
- TRAINA, S. J., J. NOVAK, AND N. E. SMECK. 1990. An ultraviolet absorbance method of estimating percent aromatic carbon content of humic acids. *J. Environ. Qual.* **19**: 151–154.
- WANG, L., Y. P. CHIN, AND S. J. TRAINA. 1997. Adsorption of polymaleic acid and an aquatic fulvic acid by goethite. *Geochim. Cosmochim. Acta* **61**: 5313–5324.
- WHITEHOUSE, B. G., G. PETRICK, AND P. M. STRAIN. 1990. Cross-flow filtration of colloids from aquatic environments. *Limnol. Oceanogr.* **35**: 1368–1375.
- YAU, W., J. J. KIRKLAND, AND D. D. BLY. 1979. Modern size-exclusion liquid chromatography. Wiley and Sons.

Received: 6 August 1998
 Accepted: 17 March 1999
 Amended: 14 April 1999