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Compositional Differences between Size Classes of Dissolved Organic Matter from Freshwater and Seawater Revealed by an HPLC-FTIR System

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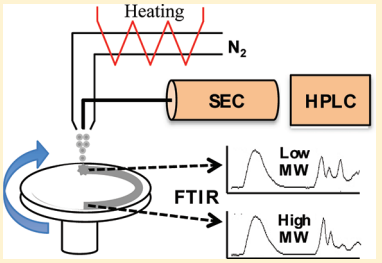
Supporting Information

ABSTRACT: The molecular complexity of dissolved organic matter (DOM) hinders its characterization. New approaches are thus needed for a better understanding of DOM reactivity and fate in aquatic systems. In this study, high-performance liquid chromatography (HPLC), using size-exclusion separation, was coupled with Fourier transform infrared spectroscopy (FTIR). A solvent-elimination interface was used to deposit DOM fractions onto a germanium disk that were then analyzed by FTIR. Samples included ultrafiltered DOM (UDOM) and fulvic acids from the St. Lawrence Estuary and its tributaries. Results showed significant compositional changes with molecular size and origin, especially in UDOM. Larger fractions of UDOM contained more carbohydrates, amides, aromatics/alkenes and aliphatics, while smaller fractions contained more carboxylate and OH groups. Small marine molecules (500−900 Da) were also enriched in sulfate groups that appeared bound to UDOM. Large marine molecules were the most amide-rich fractions. Fulvic acids were enriched in carboxylate and OH groups, showed little changes in composition, and appeared similar to small terrigenous (riverine) UDOM even in marine water. This work shows that an HPLC-FTIR system is a powerful and complementary tool in the characterization of DOM. The compositional changes observed may explain the reported contrasting reactivity and fate of DOM having different size and origin.



INTRODUCTION

Dissolved organic matter (DOM) found in natural waters is involved in many dynamic processes. Although most of the DOM produced is rapidly mineralized, some portions resist degradation and act as a long-term carbon sink.[1] In the oceans, this DOM represents more than 90% of the organic carbon and a reservoir similar in size to atmospheric CO_2.[2] Determining the fate of DOM is thus critical for understanding the global carbon cycle. However, the study of DOM is limited by the lack of information on its composition. While the vast majority of compounds in precursor organisms can be identified, more than 80% of the DOM found in natural waters remains uncharacterized at the molecular level.[3,4]

Recently, promising DOM characterization has been carried out by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS).[4−6] While this powerful technique is able to resolve and identify the elemental formulas of thousands of molecular ions having minuscule mass differences, it has limitations. For example, common ionization sources are selective or more sensitive to some components of DOM and structural information is not readily available.[5,6] Other analytical tools capable of providing structural information are thus needed. One analytical alternative would be to exploit the separation power of high-performance liquid chromatography (HPLC) prior to DOM characterization.[5] However, very few detection modes can provide information on the composition of chromatographic analytes and some of them, such as conventional mass spectrometers, are limited by the nature of the mobile phase and low resolution. Woods et al.[7] have recently coupled size-exclusion chromatography (SEC) with nuclear magnetic resonance (NMR) spectroscopy to quantify functional groups in freshwater DOM. Fourier transform infrared spectroscopy (FTIR) is another technique that can quantify different functional groups. NMR and FTIR exploit two very different properties of matter and can reveal different information. FTIR has been widely used to study organic matter,[8−10] but its coupling with HPLC is hindered by the strong absorption of common mobile phases. To avoid this problem, a solvent-elimination interface can be used prior to FTIR analysis.[11] Such systems are mostly used for polymer analysis.[12] Although HPLC-NMR and HPLC-FTIR provide complementary information, HPLC-FTIR can completely eliminate spectral interferences from the mobile phase, and is much cheaper.

The objective of the present work was to use, for the first time, a SEC-HPLC-FTIR system to characterize ten DOM samples from freshwater and seawater. To the best of our knowledge, the only time HPLC-FTIR has been used with DOM or in organic geochemistry was with a reversed-phase separation, based on polarity changes, and with soil-derived fulvic acids.[13] SEC is a popular tool because it separates DOM compounds of different molecular weights (MW), and MW can be estimated from a calibration.[14] Revealing compositional

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Table 1. Physicochemical Characteristics of Sampling Sites and Samples' Elemental Composition

	station	sampling depth (m)	salinity (g kg^{-1})	sample type[a]	C/N$_{(atom)}$	H/C$_{(atom)}$	O/C$_{(atom)}$	S/C$_{(atom)}$	% ash[b]
St. Lawrence	1[c]	2	<1	UDOM	26.4	1.24	0.70	0.017	23.4
	F-1[d]	2	19.0	UDOM	22.3	1.24	0.68	0.011	17.4
	25[e]	2	27.7	UDOM	17.4	1.35	0.78	0.035	15.7
	23[f]	2	28.1	UDOM	16.5	1.38	0.83	0.047	16.1
		300	33.3	UDOM	12.6	1.55	0.83	0.052	18.0
				FA	46.9	1.15	0.49	0.0032	1.27
tributaries	Sag 30[g]	2	6.0	UDOM	45.4	1.44	0.96	0.044	45.9
				FA	103.1	0.85	0.55	0.0027	0.81
	C[h]	<1	<1	UDOM	37.3	1.09	0.63	0.0029	23.5
	M[i]	<1	<1	UDOM	55.8	1.05	0.67	0.0017	13.5

[a]UDOM = ultrafiltered dissolved organic matter, FA = fulvic acid. [b]Ash content determined by 100 - %(C + N + H + O + S). [c]46°44′44″ N, 70°17′26″ W. [d]47°24′18″ N, 70°17′02″ W. [e]48°17′26″ N, 69°22′52″ W. [f]48°42′11″ N, 68°39′27″ W. [g]Saguenay, 48°21′40″ N, 70°23′35″ W. [h]Chaudière, 46°40′40″ N, 71°13′50″ W. [i]Manicouagan, 49°11′41″ N, 68°19′55″ W.

differences between DOM size classes is important. The size-reactivity continuum model indicates that, on average, smaller DOM molecules found in natural waters tend to be more refractory than larger ones.[15] The deep ocean contains the highest proportions of small molecules and the oldest DOM.[3,16] However, the chemical characteristics responsible for these differences in DOM reactivity remain mostly unknown.

EXPERIMENTAL SECTION

Environmental Setting and Sampling. The samples of this study were collected along the salinity gradient of the St. Lawrence Estuary (stations 1, F-1, 25, and 23) and in three of its major tributaries (Saguenay Fjord, Chaudière River, and Manicouagan River). Physicochemical characteristics of the sampling sites are presented in Table 1. A map is available in the Supporting Information (SI) section (Figure S1). The St. Lawrence system is the second largest river system in North America.[17] The St. Lawrence Estuary is divided into two sections at the mouth of the Saguenay Fjord: the Lower and Upper Estuaries. The upstream section of the Upper Estuary is shallow and well-mixed, whereas the downstream section of the Upper Estuary is deeper (~100 m).[18] The Lower Estuary has three main water layers: (1) a surface layer, less than ~50 m thick, having a salinity between 25 and 31; (2) an intermediate cold (−1 to 2 °C) layer, formed at the surface in winter and extending to ~150 m deep; (3) a warmer but saltier bottom layer. The bottom layer of the Lower Estuary is a mixture of the Labrador Current and North Atlantic waters. The Saguenay Fjord has a < 5 m thick freshwater layer that overlays a much saltier layer originating from the St. Lawrence. The pH of the water sampled in this study ranged from 6.80 (Manicouagan River) to 8.23 (station 23, 2 m). A more detailed description of these sites can be found in a previous study.[19]

Two types of DOM samples were analyzed: ultrafiltered DOM (UDOM) and fulvic acids (FA). The UDOM samples from the St. Lawrence Estuary and the Saguenay Fjord were extracted onboard the R/V Coriolis II in June 2009 and May 2007, respectively.[19] Water samples were collected using Niskin bottles. The two other tributaries, Chaudière and Manicouagan, were sampled using a bucket in May 2008. 50−144 L of prefiltered (combusted 0.5 μm glass microfiber filters, Pall) water samples were passed through tangential-flow ultrafiltration (TFF) cartridges (0.54 m^2 filtration area, code: PLAC, Millipore) having a cutoff of 1000 Da. After ultrafiltration, the retentate was desalted by diafiltration with 6−12 equivalent volumes of Milli-Q water, depending on water salinity. The final concentrate was kept frozen and freeze-dried. Dissolved organic carbon (DOC) measurements of acidified subsamples were performed on a Shimadzu TOC-5050 analyzer with a Pt catalyst at 680 °C. These measurements indicated that 20% (station 23, 300 m) to 79% (station 1) of total DOC was isolated as UDOM with a consistent decreasing trend with increasing salinity.

Two FA samples were analyzed: station 23 at 300 m and Sag 30 at 2 m. These FA were extracted in 2000 according to the method developed by Thurman and Malcolm[20] as described in a previous study.[21] Briefly, 210 L of prefiltered (combusted 0.5 μm glass microfiber filters) water samples were acidified to a pH of 2 prior to adsorption onto a nonionic macroreticular XAD-8 resin (Rohm & Haas Co.). The FA (and humic acid) fractions were then eluted with 0.1 M NaOH. Purification steps included acidification, centrifugation, elution on IR-120 cation-exchange resin (Rohm & Haas Co.), and freeze-drying. These FA represented 14% (station 23, 300 m) and 35% (Sag 30) of total DOC.

Elemental Analysis. The dry weight percentages of carbon (%C), nitrogen (%N), hydrogen (%H), sulfur (%S), and oxygen (%O) in the UDOM and FA samples were measured with an Elementar MICRO Cube CHNS/O elemental analyzer. Atomic elemental ratios were calculated (Table 1) after subtracting the quantity of H and O from water physically sorbed to the UDOM and FA samples. To do so, aliquots were weighted, heated at 70 °C for 2 h, and then immediately weighted a second time. Differences between the two masses represented 3.6% (Sag 30 FA) to 8.8% (Chaudière) of the sample mass.

ATR Spectra. FTIR spectra of whole UDOM and FA samples were taken with a Pike MIRacle attenuated total reflectance (ATR) accessory equipped with a single reflection diamond crystal. A Varian Scimitar 1000 FTIR spectrometer was used. ATR spectra were taken at a resolution of 4 cm^{-1} and were averaged from 200 coadded scans. A N_2 purge was done before and during spectra collection. To allow an easier comparison with the spectra collected after HPLC-FTIR (see below), the ATR spectra were corrected using the "ATR Correct" tool in Varian's Resolution 4.0 software to reduce under-estimation of signal at high wavenumbers.[22]

Size-Exclusion Chromatography. The UDOM and FA samples were prepared to a concentration of ~3.5 mg mL^{-1} in

a NH_3 aqueous solution (pH 9.5). The effect of NH_3 on sample composition was tested by drying the solution and collecting ATR spectra. No significant change compared to untreated samples was observed. An Agilent 1200 HPLC system equipped with a diode-array detector was used for SEC. The conditions chosen were those described by Reemtsma and These.[23] Samples were separated on a PL Aquagel−OH 30 column (250 × 4.6 mm, 8 μm, Varian) and a mobile phase composed of 20% methanol and 10 mM ammonium bicarbonate water solution heated to 40 °C. The addition of methanol slightly decreases retention times, probably by the reduction of hydrophobic interactions between DOM molecules and the stationary phase, while ammonium bicarbonate improves the chromatographic resolution compared to 100% water.[23] The use of a completely volatile mobile phase avoided spectral interference caused by buffer salt codeposition. 10 μL of sample solution were injected into the column. The flow rate was set to 0.3 mL min^{-1}. UV detection was carried out at a wavelength of 254 nm. Elemental analyses of sample solutions before and after SEC revealed a complete recovery of the DOM (or carbon) out of the SEC column.

The SEC column was calibrated using polystyrene sulfonate sodium salts (PSS) with MW weights of 1100, 3650, 6530, 14 900, and 32 000 Da (Polymer Standard Service). The 32 900 Da standard elutes in a volume close to the maximal limit (exclusion or void volume) of the resolving range specified by Varian. Acetone and salicylic acid were also used during calibration to include standards of lower MW. Acetone also serves as a probe for the permeation volume (here ∼3.6 mL).[24] PSS have been considered the most representative standards tested for DOM.[14,25] However, the basic shape, charge density, functional group distribution, and specific volume of PSS do not change with MW, unlike DOM samples. The calculated MW of UDOM and FA samples should thus be viewed as apparent MW. The calibration semilog plot (log MW vs retention time) obtained is shown in the SI section (Figure S2), and was almost identical to that published by Reemtsma and These.[23] A linear regression revealed two distinct curves, including one between 1100 and 58 Da having a flatter slope.

HPLC-FTIR Interface and Optics Module. The SEC eluent was deposited as "tracks" onto a rotating germanium (Ge) disk using the LC-Transform 400 (Lab Connections, Inc., Marlborough, MA), a solvent-elimination interface connected downstream of the diode-array detector. This interface used a pneumatic nebulizer with heated N_2. During the deposition, the N_2 pressure and temperature were respectively set at 32 psi and 108.3 °C to ensure narrow tracks and FTIR spectra with good signal-to-noise ratio. The disk rotation speed was set to 10° min^{-1}.

The Ge disk was then placed on the LC-3000 Automated Optics Module fitted inside the Varian Scimitar 1000 FTIR spectrometer. The height of the optic module was adjusted to maximize energy throughput obtained with epoxy deposited on a calibration disk provided by Lab Connections. The method of obtaining spectra from the LC-3000 is called "transflection".[26] The IR beam passes through the sample, is refracted through the Ge, and is reflected back by a thin layer of aluminum at the bottom of the disk. Then the beam passes once more through the sample on its route to the detector.

A background spectrum was taken at a pristine section of the Ge disk. Sample spectra were then collected at every degree of a track (i.e., 25−40 spectra per track). A spectrum of a blank spot of the track (no analyte) was subtracted from each sample spectra to eliminate noise. This was especially important at the beginning and end of a track, where the sample deposits were very faint. All spectra were collected using a resolution of 4 cm^{-1} and were averaged from 200 scans. A second derivative of the spectra was performed, but leads to inconclusive results, which is attributed to the relatively low signal-to-noise ratio of the transflection technique.

All FTIR peaks or group of peaks were integrated with a baseline passing from valley to valley. The absorbance was expressed as "relative absorbance (%)" by dividing the area of a certain peak by the total area of the spectra, and multiplying by 100. Because the total area is larger than the sum of the areas under the integrated peaks, the relative absorbance tends to be small and the sum of the relative absorbances does not give 100%.

RESULTS AND DISCUSSION

Bulk Characterization. Table 1 shows major differences in the samples' elemental composition, which are attributed to their contrasting origins or isolation methods. UDOM from marine waters (station 23, 300 m) was enriched in N, H, and S compared to terrigenous UDOM from rivers. These contrasting features are typical.[27] FA samples were more C-rich than their UDOM counterpart, as previously reported in other areas.[3,19,21] Solid-phase extraction techniques, like XAD extractions of FA, have been found to isolate less than 20% of S- and N-containing compounds in DOM.[28]

The SEC chromatograms, obtained with UV detection, of the samples collected at station 23 are displayed in Figure 1. The

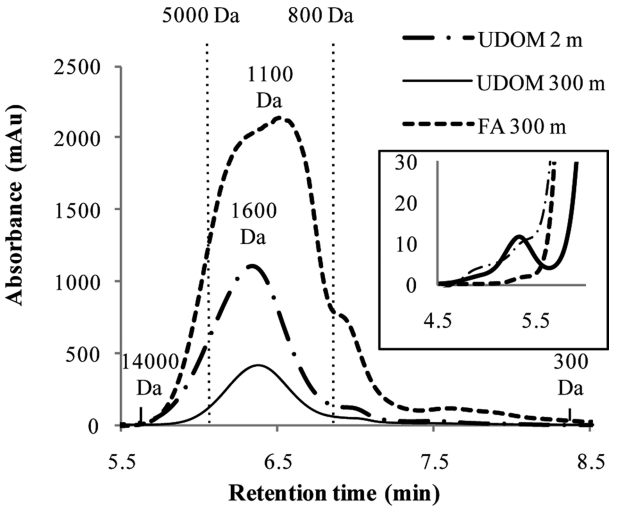

Figure 1. SEC chromatograms of the samples collected at station 23. The corresponding apparent molecular weight of peak maxima and limits are shown.

other chromatograms were similar, and are available in the SI section (Figure S3). These results indicated that the majority of the molecules (66−84%) have apparent MW between 800 and 5000 Da, with peak maxima between 1100 and 2500 Da. The UDOM samples showed a near-normal distribution around the peak maxima, except for small shoulders near 7 min, which were found in most UDOM samples, and near 5.3 min in UDOM from 23 300 m, M, and Sag 30. These features indicate a small proportion of very small (600−700 Da) and very large molecules (>25 000 Da), respectively. The presence of DOM with an apparent MW lower than the ultrafiltration membrane

cutoff (1000 Da) may be caused by different behaviors between PSS standards and DOM during separation (see Experimental Section) or by an ultrafiltration membrane that retained molecules smaller than 1000 Da by charge effects.[25] FA chromatograms showed broader and more resolved features, as well as greater proportions of smaller molecules compared to UDOM samples. The UDOM from marine waters exhibited lower UV absorption (mAu) than freshwater and FA samples (Figures 1 and S3). This is caused by a higher proportion of chromophores in terrigenous DOM.[29] This supports the idea that FA samples are mostly terrigenous and transported to station 23 almost conservatively.[21] In contrast, the UDOM samples at station 23 contain more marine DOM.[19] Photobleaching of DOM during its transit to the ocean can contribute to the lower UV absorbance of the DOM collected in marine waters.[30]

FTIR Band Assignment. The FTIR spectra obtained with bulk samples (ATR) or with HPLC-FTIR tracks showed an apparent simplicity, due to overlapping signals, that is typical of natural organic matter.[31] Examples from each type of samples (marine UDOM, riverine UDOM and FA) are shown in Figure 2. Spectra of the other samples are available in the SI section (Figure S4). Some absorption bands can be assigned to more than one functional group or structure. In these cases, correlations with elemental analysis data and/or shifts in peak maxima positions were used for greater accuracy (see below). Absorption band assignments were based on published information on natural organic matter and other complex molecules.[8,32,33] A table of common FTIR bands can be found in the SI section (Table S1).

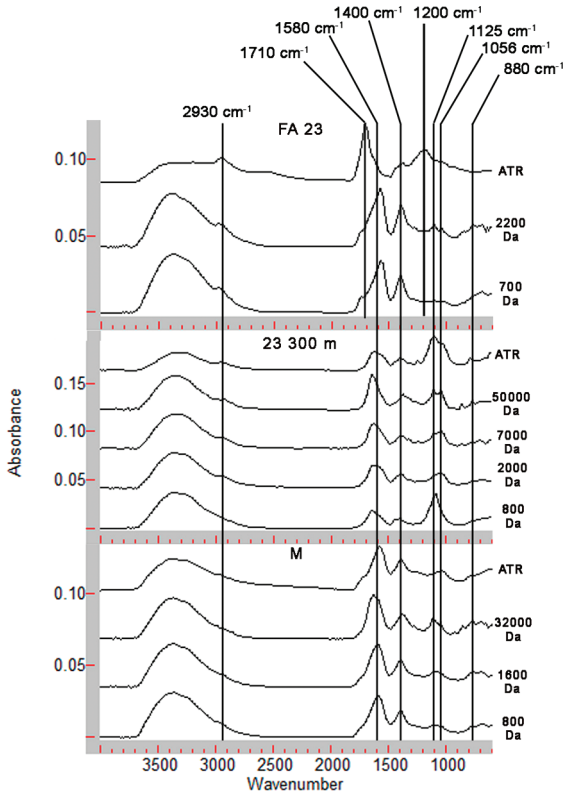

Figure 2. FTIR spectra collected at different positions on the HPLC-FTIR tracks and ATR spectrum (bulk sample) of samples FA 23 300 m, UDOM 23 300 m, and UDOM M. See Table 1 for a more detailed description of the samples.

ATR and track spectra were similar for all samples, except for FA samples (Figure 2). Compared to the other techniques, ATR tends to underestimate the absorbance at high wavenumbers.[22,26] The other differences between ATR and track spectra in FA samples can be attributed to the dominance of COOH (1710 and 1200 cm^{-1}) over COO^- (1580 and 1400 cm^{-1}) in bulk FA (ATR spectra). During their extractions, FA were protonated using a cation-exchange resin. However, the solution injected and mobile phase used in SEC were slightly basic; thus, most COOH groups were converted into COO^-.

Changes in FTIR track spectra were observed for the 2990−2820, 1710, 1660−1575, 1415−1375, 1200, 1125−1080, 1065−1045, and 885−860 cm^{-1} bands (Figure 2). The 880−860 cm^{-1} band is associated to substituted aromatics and alkenes. The bands between 2990 and 2820 cm^{-1} result from the CH stretching modes of CH_3, CH_2 and CH groups. These aliphatic bands overlap with the very broad band between 3600 and 2800 cm^{-1}, coming mostly from OH absorptions.

The 1660−1575 cm^{-1} band is caused by amides, COO^-, and aromatic groups. However, most amides absorb at higher wavenumbers than COO^-. Peak maxima position of different MW and different samples indicated that COO^- was mostly responsible for this band in FA and in the UDOM samples from rivers, especially at low MW (LMW) (Figure 3A). Amides were responsible of this band in marine UDOM (23 300 m) and in the high MW (HMW) fraction of most UDOM samples (23 2 m, 25, and F-1).

The N/C ratio of the ten samples was correlated with two FTIR bands (1125−1090 and 1065−1045 cm^{-1}) of the largest molecules (>7000 Da), but not as much with bands measured in bulk (ATR) samples (Table 2). This finding suggests that large molecules in DOM are responsible for changes in N content. In contrast, the average N content of the molecules smaller than 7000 Da was relatively constant in the ten samples. However, these correlations are most likely due to similar dynamics between N and functional groups devoid of N. The band at 1065−1045 cm^{-1} is typical of C−O and C−H absorptions of carbohydrates. N-containing structures, such as amino acids, and carbohydrates are reactive components of DOM and they generally follow each other.[3] The other band, at 1125−1090 cm^{-1}, can be attributed to numerous groups: OH and C−O−C of carbohydrates, aliphatic esters and ethers, sulfate (SO_4^{2-}), S−SO−S, N−SO−N, and sulfonic acids. Table 2 shows that this band was also correlated with S/C ratio (R^2 ~0.93), but only for the spectra of LMW fractions (500 − 900 Da). This finding suggests that LMW molecules in DOM are responsible for changes in S content. However, the fact that sulfate group absorbs at 1125−1090 cm^{-1} and the increase of S content with increasing marine conditions (Table 1) suggest that sulfate from seawater was responsible for the variation of this band. To verify this, sulfate was quantified in four samples (C, F-1, 25, 23 2 m) by a standard nephelometric method using sulfate precipitation with Ba^{2+} (see Supporting Information, S1).[34] Results indicated that more than 80% of the S was in the sulfate group in the more marine samples 25 and 23 2 m, while this proportion was about 40% in sample F-1 and less than 5% in sample C. Moreover, the ATR spectra of the $BaSO_4$ precipitate showed an intense absorbance near 1120 cm^{-1} that was greatly diminished in the spectra of the DOM remaining after $BaSO_4$ removal (not shown).

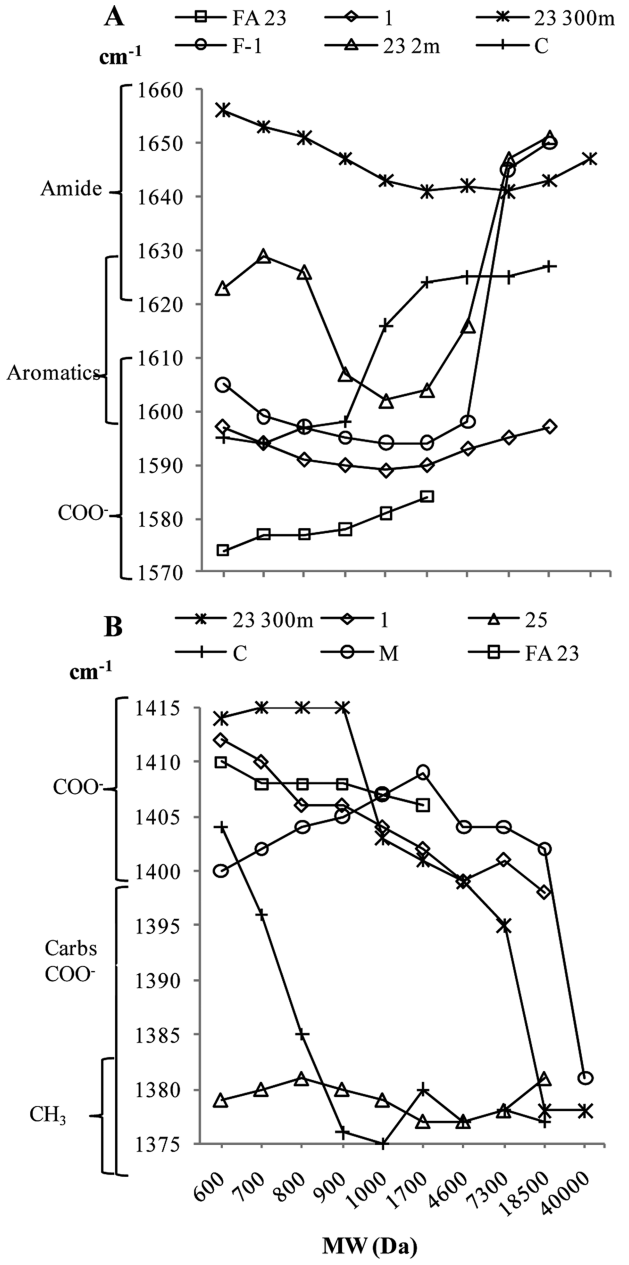

Figure 3. Peak maxima position versus apparent molecular weights (MW) for two spectral regions in representative samples. Fulvic acid is identified by FA, all the other samples are UDOM. The most probable functional groups and their absorbance ranges are shown beside the wavenumbers. See Table 1 for a more detailed description of the samples.

Small molecules (500−900 Da) in UDOM and FA, also seem to be responsible for the variability of H contents in the 10 samples, as indicated by the good negative correlation of H/C ratio and the 1415−1375 cm^{-1} band measured in LMW fractions (R^2 = −0.808 for 500−900 Da, Table 2). However, the negative correlation suggests that this band was not caused by aliphatic structures in LMW fractions. Figure 3B indicates that COO^- was mostly responsible for the 1415−1375 cm^{-1} band in LMW molecules. In larger molecules, this band comes mostly from carbohydrate (OH bending) and aliphatic (CH_3 umbrella bending) structures (Figure 3B).

The O/C ratio of the samples was correlated with the same band (1125−1090 cm^{-1}) that varied with the N content of HMW and with sulfate content in LMW. In this case, the best correlation was calculated with the midsize molecules (1000−1700 Da), but the R^2 was very close to the R^2 calculated with the bulk samples (0.872 vs 0.822, Table 2). This was expected, considering that a large proportion of the molecules are included in the 1000−1700 Da range. The enrichment in sulfate group in the LMW fractions of marine UDOM certainly contributed to the variations of the O/C ratio of bulk samples. However, midsize molecules contain other O-rich groups that also absorb at 1125−1090 cm^{-1}.

Compositional Changes with Molecular Weight and Origin. The information collected in the previous section allowed a more complete interpretation of the spectral changes observed. Absorbance trends according to MW for representative samples are presented in Figure 4. The same results, but plotted in function of sampling locations for representative MW and bulk samples (ATR) are available in SI (Figure S5).

The samples from the St. Lawrence Estuary showed the most spectral changes across the MW classes, the riverine samples showed moderate changes, and the FA samples showed little changes. Table 3 summarizes all the compositional changes observed according to MW, sample dominant origin, and extraction procedure.

Figure 4A shows that the absorbance from aliphatic groups increased with MW for all samples, although this trend was less marked for FA. Figure 4B confirmed the increasing contribution of amides with MW observed in Figure 3A. The LMW fraction of marine UDOM contained more amides than COO^- groups, while it was the opposite for terrigenous LMW UDOM. Thus, in terrigenous UDOM, the increase in the proportion of amides with MW was counterbalanced by a decrease in the proportion of COO^- groups, which explains why the 1600−1575 cm^{-1} band only slightly increased for these samples (Figure 4B). A similar situation occurred with the 1415−1375 cm^{-1} band (Figure 4C) that was attributed to COO^- in LMW and FA fractions, but to carbohydrate and

Table 2. Correlations between the Elemental Composition of Samples and the Relative Absorbance of Specific Absorption Bands[a]

	N/C		S/C	H/C	O/C
band (cm^{-1})	1125−1090	1065−1045	1125−1090	1415−1375	1125−1090
MW range (Da)	7000−40 000	7300−40 000	500−900	500−900	700−1700
Rel. abs. range (%)[b]	0.15−5.54	0.15−0.95	0.10−11.1	1.74−3.73	0.11−9.88
R^2 for MW range	0.676	0.820	0.927	−0.808	0.872
R^2 for ATR	−0.001	0.700	0.447	−0.080	0.822

[a]The relative absorbance of apparent molecular weight (MW) ranges and of the ATR spectra (bulk sample) were used to calculate two coefficients of determination of the linear regressions (R^2 for MW range and R^2 for ATR, respectively). [b]The relative absorbance range represents the lowest and highest averaged relative absorbance values used to calculate the correlation.

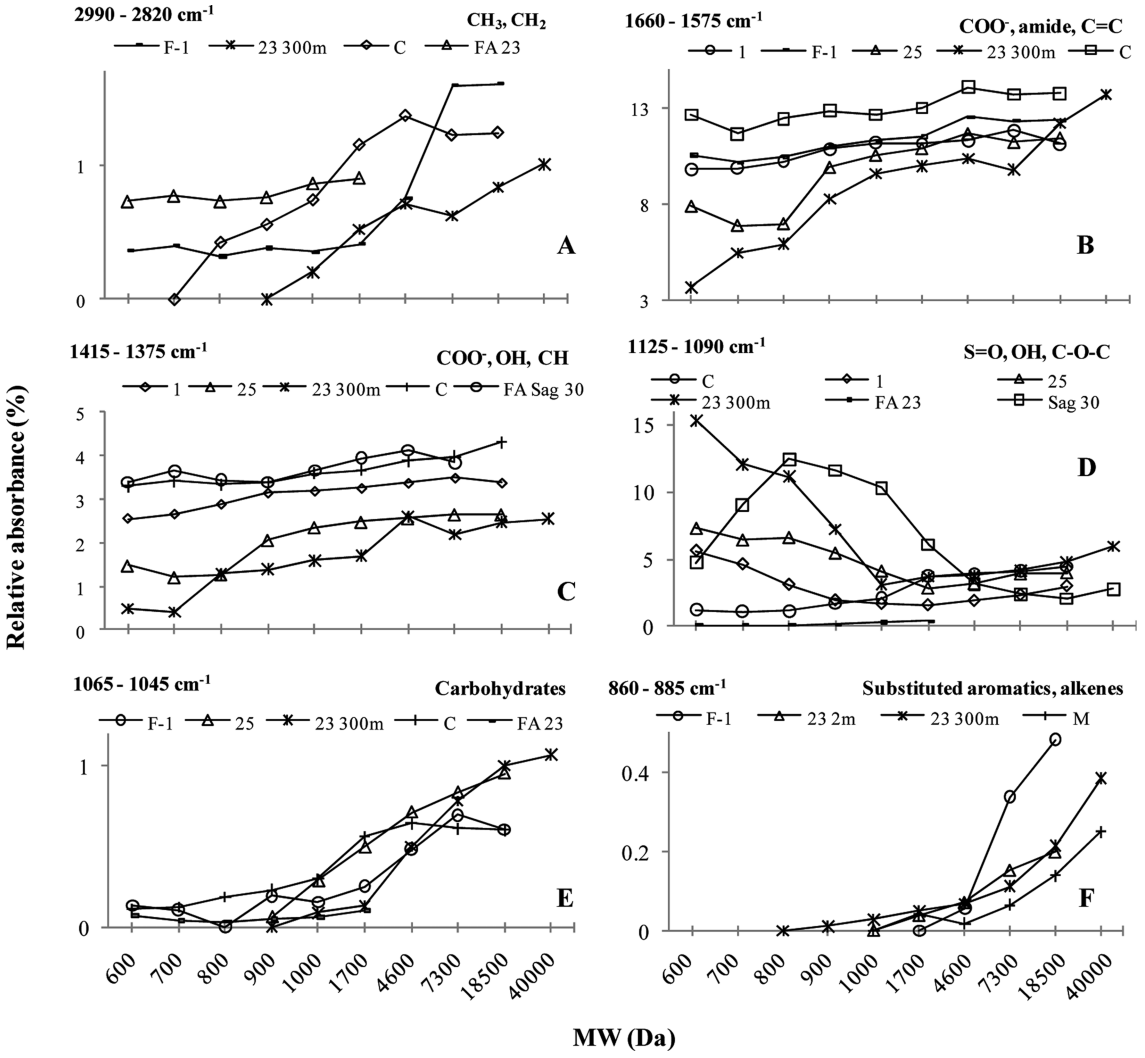

Figure 4. Relative absorbance versus apparent molecular weights in representative samples. Fulvic acid samples are identified by FA, all the other samples are UDOM. See Table 1 for a more detailed description of the samples.

Table 3. Summary of Functional Group Relative Proportions According to Sample Types and Size Classes[a]

	marine UDOM[b]		riverine UDOM		
functional group	LMW[d]	HMW[e]	LMW	HMW	FA[c]
carbohydrate		+		+	
amide	+	++		+	
sulfate	++				
$COOH/COO^-$	+		++		++
aromatic/alkene		+		+	
OH	+		+		+
aliphatic		+		+	

[a]+ indicates an enrichment in the given group. [b]UDOM = ultrafiltered dissolved organic matter. [c]FA = fulvic acid. [d]LMW = low molecular weight. [e]HMW = high molecular weight.

aliphatic structures in HMW fractions. Most of the COO^- in UDOM samples was found in the LMW fractions, with greater proportions in riverine and FA samples than in marine UDOM (Figure 4C). The spectra of LMW UDOM and FA samples were also dominated by the broad band from the OH groups (3600−2800 cm^{-1}, Figure 2). The increasing proportion of carbohydrates with MW was confirmed by the strong trend observed with the 1065−1045 cm^{-1} band for all the UDOM samples (Figure 4E). This trend was slightly more marked in marine UDOM.

Figure 4D shows the strong absorbance caused by sulfate groups in LMW fractions of the most marine UDOM (station 23) and of Sag 30 UDOM, the most S-rich samples (Table 1). This band (1125−1090 cm^{-1}) slightly increased with MW for the fractions depleted in sulfate and S (i.e., terrigenous samples and marine fractions >1000 Da). This trend can be attributed to carbohydrates (see above), esters, and ethers in these fractions.

Substituted aromatics and alkenes were also found to increase with MW (Figure 4F). The absorption at 885−860 cm^{-1} is caused by out-of-plane deformations of the hydrogen atoms remaining on the rings. Although we expect terrigenous samples to contain more aromatic structures than marine samples,[27] it was impossible to see this trend here.

Biogeochemical Implications and Perspectives. The observed compositional changes with MW certainly contribute to the contrasting reactivity of the different DOM size classes in natural waters. For instance, amides, mostly from proteins and peptides, and carbohydrates were found to be more important in the composition of HMW UDOM. This is consistent with previous studies based on other approaches.[7,8,35] The highest

proportion of amides was measured in HMW marine UDOM. These structures are produced in situ; thus, a high proportion suggests a fresher and more reactive material.[3,15]

In contrast, LMW UDOM and FA were enriched in COO^- and OH groups. Midsized UDOM molecules (1000−1700 Da) also appeared to contain O-rich functional groups. Moreover, molecules smaller than 1500 Da were poor in C−H structures, aliphatic or aromatic, and thus appeared to possess a relatively large proportion of their carbon attached to oxygen atoms. Such highly substituted compounds could comprise the carboxyl-rich alicyclic molecules (CRAM) proposed by Hertkorn et al.[36] CRAM is expected to be refractory and enriched in small to midsized fractions,[7] in agreement with the size-reactivity continuum model.[15] The proportion of carboxyl groups was especially important in terrigenous samples.

Another feature could contribute to the relative recalcitrance of LMW molecules in the oceans. The marine LMW UDOM analyzed here was enriched in sulfate groups. The presence of sulfur in sedimentary organic matter has been shown to increase its recalcitrance.[37] Several pieces of evidence suggest an association between this LMW DOM and sulfate. First, the presence of sulfate was not caused by an incomplete diafiltration, as indicated by the lack of correlation between S/C ratio and ash content (Table 1). Second, although it has been reported that charged chemical species smaller than 1000 Da can be retained by ultrafiltration cartridges,[25] free SO_4^{-2} appears too small to resist permeation into the pores of the membrane. Finally, sulfate eluted with DOM molecules having different sizes (mostly 500−1000 Da) during SEC, between 6.5 and 7.5 min. Inorganic salts usually elute immediately before total permeation time of the column (here ∼12 min) and are recognizable by a feature called "salt trough" in UV detection.[4,38] However, sulfate was not strongly bound to DOM since it was displaced by Ba^{2+} in the method used to quantify sulfate.[34] It is not known whether these interactions occur in nature or if they were promoted at high UDOM concentration during ultrafiltration.

It is important to note that only a fraction of bulk DOM (i.e., ∼20−80% for UDOM) was analyzed and that the extraction methods likely lead to more homogeneous samples and results. Although significant compositional differences among samples were observed here, larger differences are expected in bulk DOM. For instance, most of the marine DOM is of very small MW and was not analyzed here, although it may have unique structures. This homogeneity coming from the extraction methods may explain why there were more differences between UDOM and FA from the same marine location (i.e., 23 300 m) than between freshwater and marine UDOM samples. Ultrafiltration has been shown to preferentially isolate carbohydrates compared to solid-phase extractions.[39] FA showed little changes in composition and appeared similar to small terrigenous (riverine) UDOM even in marine water.

This study revealed the capability of an HPLC-FTIR system for the characterization of DOM fractions. This system provides new information that is complementary to other techniques such as HPLC-NMR or FT-ICR-MS. Moreover, HPLC-FTIR possesses some advantages over these techniques, including its lower cost. Although FT-ICR-MS analyses provide elemental formulas, the structures containing these elements and their biogenic origin remain generally unknown.[5] FT-ICR-MS analyses have detected S in small molecules and a relatively high O content in large molecules.[40,41] The present study revealed functional groups and structures that caused these differences in the studied samples.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional material including a map of the sampling sites, the calibration for SEC analysis, the SEC chromatograms and FTIR spectra of the samples not shown in the article, the plots of relative absorbance vs sampling locations for representative MW and bulk samples, the details of the nephelometric quantification of sulfate, and a table of FTIR band assignment. This material is available free of charge via the Internet at http://pubs.acs.org.

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