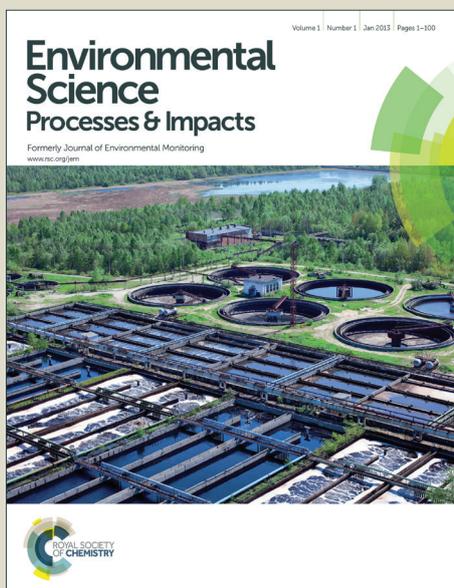


Environmental Science Processes & Impacts

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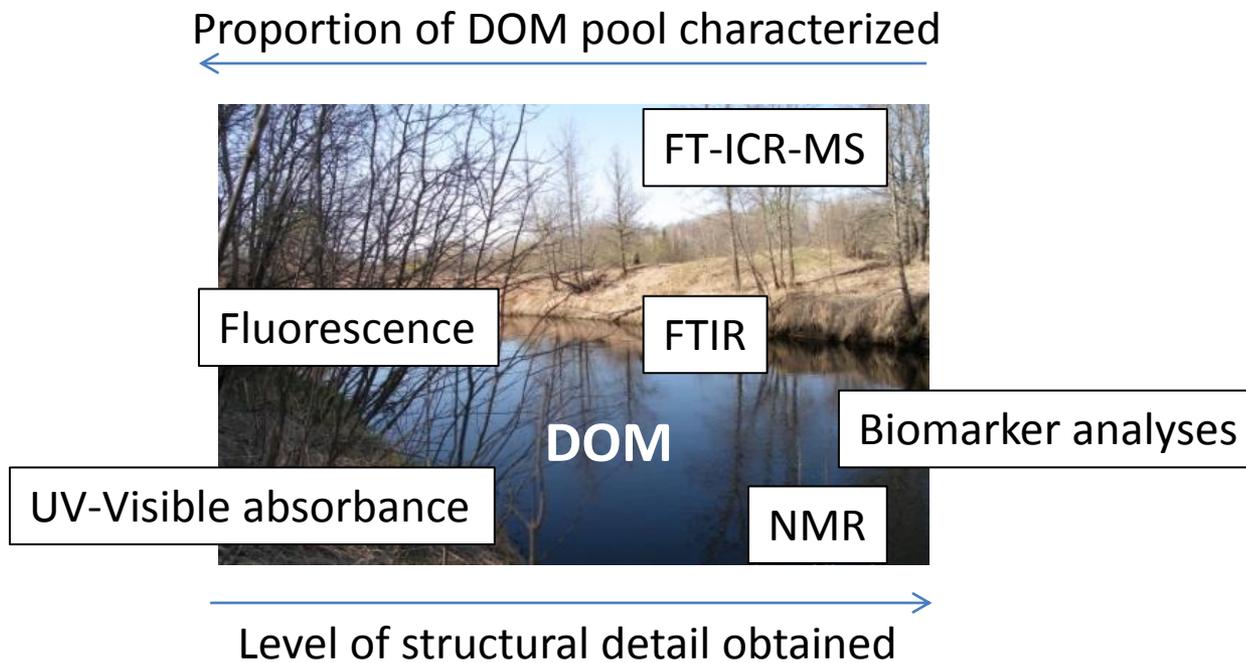


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Environmental impact statement:

Natural dissolved organic matter (DOM) is a food/nutrient source for aquatic organisms, absorbs light in surface waters, and interacts as a reactant, sorbent, and chelator with anthropogenic compounds. It can act as a photosensitizer or oxidation inhibitor in reactions with key pollutants. Specific DOM components are precursors to the formation of disinfection by-products. DOM has also been shown to affect the mobilization of contaminants from soils or sediments. To understand DOM's environmental roles and to predict their extent in a given aquatic system requires DOM structural characterization.

One sentence summary:

Current approaches to isolation and structural characterization of natural dissolved organic matter are presented and evaluated for strengths and weaknesses.

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CRITICAL REVIEW

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Structural characterization of dissolved organic matter: A review of current techniques for isolation and analysis

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Natural dissolved organic matter (DOM) in aquatic systems plays many environmental roles: providing building blocks and energy for aquatic biota, acting as a sunscreen in surface water, and interacting with anthropogenic compounds to affect their ultimate fate in the environment. Such interactions are a function of DOM composition, which is difficult to ascertain due to its heterogeneity and the co-occurring matrix effects in most aquatic samples. This review focuses on current approaches to the chemical structural characterization of DOM, ranging from those applicable to bulk samples and in situ analyses (UV-visible spectrophotometry and fluorescence spectroscopy) through the concentration/isolation of DOM followed by the application of one or more analytical techniques, to the detailed separation and analysis of individual compounds or compound classes. Also provided is a brief overview of the main techniques used to characterize isolated DOM: mass spectrometry (MS), nuclear magnetic resonance mass spectrometry (NMR) and Fourier transform infrared spectroscopy (FTIR).

Introduction

Identifying relationships between natural aquatic dissolved organic matter (DOM, see Appendix for a list of abbreviations) structure and reactivity is critical to fields as varied as climate change studies, ecology, and toxicology. Oceanic DOM is the largest reactive component of the global carbon cycle¹ and is believed to serve as a significant short-term sink for atmospheric carbon. DOM in inland regions is a major transport mechanism for C from the terrestrial environment (or landscape) into inland aquatic systems. This DOM may be transformed into atmospheric greenhouse gases (carbon dioxide or methane) or converted into particulate and sedimentary organic matter or transported downstream to the sea.^{2,3} The relative reactivity of various DOM structures affects the ultimate DOM fate (storage/sedimentation, transport, or release as CO₂ or CH₄) and DOM turnover time.

Ecologically, DOM acts as an important energy (food) source for heterotrophic aquatic organisms^{4,5} and as a nutrient source for autotrophs and heterotroph (e.g., Boyer et al.).⁶ However, the chemical structures and sizes of the DOM components may affect DOM bioavailability and nutritive value.⁷ Aquatic DOM in coastal bays and estuaries has been implicated as a causative factor in harmful algal blooms (e.g., Mulholland et al., 2002⁸ and references therein) by providing harmful algae with nutrients that are not readily available to the normal phytoplankton producers of the ecosystem. DOM also plays an ecological role as a sunscreen by attenuating both harmful ultraviolet (UV) irradiation⁹ and photosynthetically active radiation (PAR).¹⁰

DOM interacts with anthropogenic compounds in aquatic environments by acting as a reactant, chelator, and sorbent.

DOM can act as both a photosensitizer¹¹⁻¹³ and as an inhibitor¹⁴ in the photoalteration/degradation of such compounds. In riverine, estuarine, and coastal marine systems, DOM (a major component of NOM or natural organic matter) can alter the bioavailability¹⁵, mobility,¹⁶ and ultimate fate of associated (e.g., sorbed or chelated) hydrophobic organic contaminants and trace metals.¹⁷⁻²² In freshwater systems specific components of DOM have been shown to react with chlorine and ozone to produce harmful disinfection by-products in drinking water.²³⁻²⁵

Despite the importance of aquatic DOM and much work on DOM characterization, a comprehensive understanding of the general chemical structures of DOM and the variations in these structures under different environmental conditions remains elusive. One reason is that DOM is often present in low concentrations (1-20 mg/L) in water with a much higher concentration of inorganic compounds (seawater has approximately 35 g salt per liter), which can adversely affect chemical analyses. A second reason is the extreme heterogeneity of organic matter structures and molecular weights included within aquatic DOM (e.g., Hertkorn et al.²⁶), which complicates isolation and analysis approaches. The researcher tasked with characterizing aquatic DOM is faced with a continuum of characterization choices. Measurements of UV-visible absorption,^{27, 28} fluorescence characteristics,^{29, 30} and C/N and isotopic values^{31, 32} on bulk DOM samples provide information on a large portion of the DOM pool, but do not give detailed structural information. Approaches such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry (MS) can provide functional

group, compound class, and elemental formula information on DOM,³³⁻³⁵ but usually require an isolation and concentration step prior to use, and thus a fractionation of the DOM pool.^{35, 36} Actual molecular-level structural characterization, which requires

detailed extraction and separation protocols, often derivatization, and then analytical approaches such as tandem mass spectrometry and/or NMR, is limited to a few compounds and compound classes, and thus a very small portion of the total DOM pool.³⁷

In this paper we review this continuum of characterization

choices to help those tackling DOM studies to choose the approach best suited to their study questions.

Optical characterization of bulk DOM

Both UV-Visible absorption and fluorescence are commonly used to provide information on DOM concentrations and composition in aqueous systems.^{29, 38} Both measurement approaches, however, require absorption of UV-Visible light by DOM moieties and thus provide information on only the chromophoric DOM (or CDOM) rather than the total DOM pool. In most water systems CDOM is the main dissolved constituent absorbing light³⁹ and therefore bulk or filtered water can be analyzed without further sample processing to provide concentration and composition information.

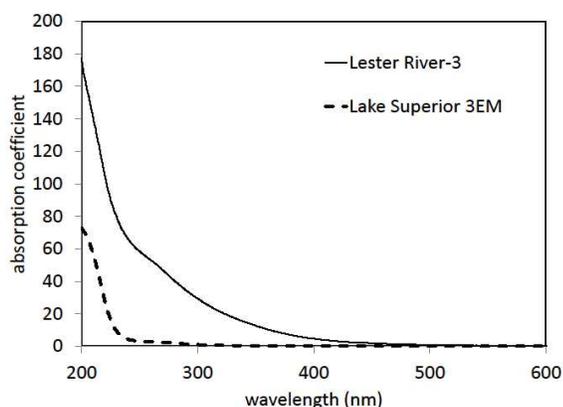


Figure 1. UV-Visible spectroscopic analysis of sterile-filtered water from the Lester River (Duluth, MN) and from Lake Superior (eastern basin, open lake).

In many freshwater systems, where terrestrially-derived organic matter is the main CDOM source, light absorption in the UV and blue range (e.g. at wavelengths 254, 350, and 440 nm) is linearly correlated with dissolved organic carbon (DOC) concentration, providing a mechanism for rapid and inexpensive in-lab or in-situ optical determinations of DOC concentrations or concentration differences.^{40, 41} The linear correlation of light absorption and DOC concentration breaks down, however, in systems with strong autochthonous or anthropogenic inputs of DOC or where DOC has been extensively photodegraded,⁴⁰ which includes most ocean waters where autochthonous and photodegraded DOC are both prevalent.

The absorption spectrum for CDOM generally appears as a relatively featureless (i.e., no discrete peaks) approximately exponential increase in absorption or absorption coefficient as wavelength decreases from the visible into the UV range (Fig. 1).

Data are often presented, especially in the oceanographic community, using the Napierian absorption coefficient for each wavelength after blank and backscatter correction.⁴² Decadic absorption coefficients are also common in the aquatic sciences literature, especially in freshwater studies.⁴³ The common use of these two absorption coefficient approaches has led to some confusion; for comparisons of absorption coefficients or proxies derived from them, one must check which approach is actually used in each study to ensure that the appropriate values are being presented and compared.

The absorption-coefficient data are then often fitted to the equation:

$$a_{\lambda} = a_{\lambda_{ref}} e^{-S(\lambda - \lambda_{ref})} \quad (1)$$

Where a_{λ} is the corrected Napierian absorption coefficient (in m^{-1}) at wavelength λ , λ_{ref} is a reference wavelength for the sample (usually 250 nm) and S is an empirically-fit term called the spectral slope.^{28, 44} S generally decreases with increasingly brown, terrestrially derived water, and often increases upon photodegradation;⁴⁵ however, it is dependent upon the wavelength range over which it is calculated and whether a linear least squares regression to $\ln(a_{\lambda})$ or the exponential equation (1) is used to determine S .^{28, 45} Thus a ratio of spectral slopes (S_R ; the ratio of S calculated from 275–295 nm to S calculated from 350–400 nm) is often used instead, showing more robust trends among samples and an inverse relationship with CDOM molecular-weight as well.²⁸

In addition to spectral slope, ratios of absorbance at specific wavelengths have also been used to provide insight into DOM composition. The two most popular absorption ratios are the $E_2:E_3$ ratio (the ratio of a sample's absorption at 250 nm relative to its absorption at 365 nm) and the $E_4:E_6$ ratio (absorption at 465 nm relative to 665 nm), although the latter ratio can be problematic due to low absorption above background levels at 665 nm.²⁸ The $E_2:E_3$ ratio has been shown to be inversely related to molecular weight and inversely proportional to the amount of aromatic material in the sample.⁴⁶⁻⁴⁹ The $E_4:E_6$ ratio has been considered inversely correlated to aromaticity from condensed aromatic rings but has also been shown to be related to general "humification" with an inverse relationship to molecular weight, and positive correlations with O:C atomic ratio, carboxyl content, and total acidity.⁵⁰

UV-visible absorption data are also combined with dissolved organic carbon concentration data to provide a specific UV absorption parameter (SUVA). The wavelength used for this parameter varies but is often 254 nm. Thus $SUVA_{254}$ is the ratio of the ultraviolet light absorption coefficient at wavelength 254 nm to the sample DOM concentration, in mg/L, and has been shown to correlate to the aromatic content per unit of organic carbon concentration.⁴³ $SUVA_{254}$ values vary depending upon whether the Napierian absorption coefficient or the decadic absorption coefficient is used, and both approaches appear in the literature.^{28, 43}

DOM fluorescence, like UV-Visible light absorption, has been used for both DOC quantification and characterization. For

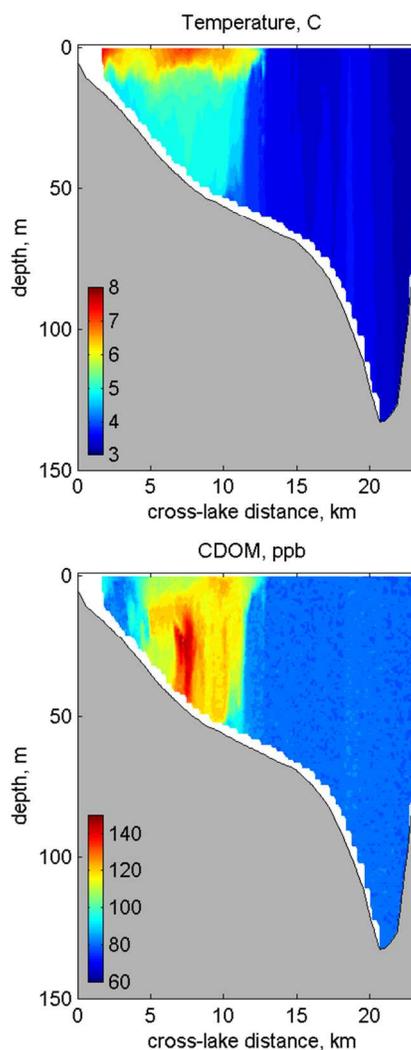


Fig.2. Maps of temperature and colored dissolved organic matter composition on a glider transect across the western arm of Lake Superior in May 2012, showing springtime trapping of CDOM in the near-shore thermal bar.

measurements of relative CDOM concentration, DOC fluorometers are often used to provide in situ water-column measurements from mobile platforms such as CTD rosettes and autonomous underwater vehicles (AUVs or gliders) as shown in Figure 2. CDOM fluorescence is generally determined using excitation in the UVA range (approximately 370 nm) and emission in the blue range (centered at 440 to 460 nm). Again, as with UV-Visible absorption, terrestrially-derived DOM, which consists primarily of CDOM, tends to give the largest fluorescence response, although there is some indication that there is in situ production of CDOM in marine waters as a function of microbial degradation of autochthonous DOC.⁵¹⁻⁵³

In addition to its use as a quantification tool, DOM fluorescence is also used to provide insights into the chemical composition of DOM. Such use ranges considerably in terms of data collected (limited excitation and/or emission wavelengths vs excitation-emission matrices or EEMS) and data analysis performed. Early EEMS work used fluorescence ratios of excitation maxima and minima to define peak types⁵⁴ and to develop a fluorescence index (the ratio of emission intensity at

450 nm vs 500 nm using excitation at 370 nm) to estimate microbial vs higher-land-plant contributions to the DOM pool.^{55, 56} These approaches are still used today (e.g. a modified fluorescence index⁵⁷ was used in a recent study to investigate DOM in peat pore waters)⁵⁸ and peak-picking and the use of the fluorescence index are the subject of a recent critical evaluation.⁵⁹ Over the past decade, multivariate analyses of excitation-emission matrices (EEMS) using principal component analysis and parallel factor (PARAFAC) analysis⁶⁰ have become the favored approach for investigating detailed fluorescence differences among samples.^{29, 55, 61} PARAFAC data, peak-type data from excitation maxima and minima, and fluorescence index data are often combined to link DOM characteristics from a particular study with literature data. Examples include recent work linking optical characteristics of DOM (river water DOM during storm events,⁶² and pine needle leachates²⁵) to its potential to form disinfection by-products.

There have been attempts to link data from EEMS analyses to chemical functional group information, such as that obtained by NMR⁵⁵ and lignin analyses⁶³ and to distributions of elemental formulae determined from FT-ICR-MS.⁶⁴ At present such studies are limited to isolated DOM fractions or specific biomarker extractions because compound-class or molecular-level characterization approaches generally require samples high in DOM concentration and low in salt concentration.

DOM isolation options

The concentration of DOM is generally done in one of four ways: 1. reverse osmosis coupled with electro dialysis (RO/ED), 2. ultrafiltration, 3. solid phase extraction (SPE), or 4. direct drying or freeze drying. Drying or freeze drying a sample concentrates salts as well as organic matter and thus can only be done with samples that have exceptionally low ionic strength if the sample is intended for NMR, MS, or elemental analysis. However, freeze drying is often coupled with the other three methods of DOM isolation after the samples have been de-salted and initially concentrated.

It is important to distinguish between the terms isolation, concentration, and extraction. Following the language of Koprivnjak,⁶⁵ concentration refers to decreasing the total volume of the solution so that the DOC concentration increases; isolation refers to separating DOC from inorganic compounds. Methods 1, 2, and 3 above both concentrate and isolate dissolved organic matter. Finally, extraction will be used here as a general term to refer to the combination of isolation and concentration. These methods will be compared by the recovery of DOC, which will be referred to as the efficiency of the method. Recovery is defined as in Eq. 2 or if blank data is available, Eq. 3.

$$RECOVERY = \frac{DOC_{ext} \times V_{ext}}{DOC_{sample} \times V_{sample}} \quad (2)$$

$$RECOVERY = \frac{(DOC_{ext} - DOC_{blank}) \times V_{ext}}{DOC_{sample} \times V_{sample}} \quad (3)$$

where V_{ext} is the volume of the extract; V_{sample} is the volume of the original sample; DOC_{ext} is the DOC concentration in the extract; $\text{DOC}_{\text{sample}}$ is the DOC concentration of the original sample; and $\text{DOC}_{\text{blank}}$ is the DOC concentration of a pure water or saltwater blank that has been run through the extraction process.

Reverse osmosis and ultrafiltration retain organic matter by a similar mechanism, namely, physical means by forcing water through a membrane, which is impermeable or semi-impermeable to most of the DOM. The solution remaining, called the retentate, has increased DOC concentration. RO/ED and ultrafiltration isolate DOM from inorganic salts by two different mechanisms. RO is coupled with electrodialysis (ED) which uses electrical potential across a combination of cation and anion permeable membranes to desalt samples. In ultrafiltration, samples are desalted by diafiltration, which is essentially repeated dilution and rinses of the retentate with ultrapure water and subsequent ultrafiltration, with more of the smaller molecular-weight salt moieties passing through the membrane with each dilution/filtration cycle.

Solid phase extraction retains organic matter by a very different mechanism than reverse osmosis or ultrafiltration. In SPE, molecules in a liquid sample are adsorbed onto a stationary phase and then eluted in a small volume of a solvent of suitable polarity. Formerly, Amberlite XAD resins were the most commonly used stationary phases for DOM extraction. These resins are composed of methyl methacrylate or styrene-divinylbenzene polymers,⁶⁶ and differ in the proportion of aliphatic vs aromatic nature that they contribute to reverse-phase chromatography. Using one or a combination of XAD resins is still recognized as the classic extraction approach. However, this approach is usually time-intensive, with samples exposed to extreme pH changes and multiple resin columns,⁶⁷⁻⁷⁰ which enhance the possibility for chemical changes to the native DOM and both contamination (due to much sample manipulation) and incomplete recovery (possible if there is bleed through or resin overloading).⁷¹ In addition, some of the XAD resin types are no longer manufactured. More recently other non-polar stationary phases based upon styrene divinylbenzene (e.g., XC resins from 3M, or Bond Elut PPL from Agilent) or hydrocarbons bonded to a silica matrix (e.g., C-18 from many manufacturers, where the hydrocarbon has 18 carbon atoms), are typically used. In this case, non-polar molecules are retained on the stationary phase as sample is passed through a cartridge and eluted with organic mobile phase. Samples are desalted as needed by rinsing the cartridges with ultrapure water and then eluted with organic mobile phase.

RO/ED, ultrafiltration, and SPE are further described and compared below.

RO/ED:

Reverse osmosis (RO) as a method for concentrating natural dissolved organic matter was explored by Serkiz and Perdue.⁷² While this method proved effective, with DOM recoveries >90%, RO alone concentrates both dissolved organic matter and inorganic constituents. As salt concentration increases, carbonates and sulfates precipitate and can foul membranes.⁷³ Like drying

and freeze drying alone, this method was limited to waters with exceptionally low salinity until recently. In the mid-2000s, reverse osmosis was coupled with electrodialysis (ED) which is a technique to remove salts from the sample.⁶⁵ Since then, a number of papers have been published on this method demonstrating the effectiveness of RO/ED with freshwater⁶⁵ and in seawater.⁷³⁻⁷⁵

For marine samples, these studies⁷³⁻⁷⁵ used generally the same method. RO/ED is done in three steps:⁷³ 1. ED is used alone to remove salts until the conductivity of the sample has decreased to $15 \mu\text{S cm}^{-1}$; 2. RO and ED are used in conjunction; RO removes water while the retentate is circulated through the ED stacks to keep the conductivity at $15 \mu\text{S cm}^{-1}$ which prevents salt precipitation on the membranes; 3. ED is again used alone to remove salts from the resulting concentrated sample. After the RO/ED process, the system is drained and rinsed with 0.01 M NaOH. Both the drained portion and the NaOH rinse are saved as the extracted sample. This method is typically done with 20 L for freshwater samples⁶⁵ and with 100-400 L for seawater samples;⁷⁴ the sea water samples are reduced to a final volume of <10 L. The time required by the entire process per sample is not stated, but the RO portion of the process, when the majority of the water is removed, can be performed at waste-stream flow rates of 1.3 L/min to 2.7 L/min depending on the salinity.

RO/ED has been shown to effectively remove salts from sea water. This was first demonstrated by Vetter et al.⁷⁵ where the final conductivity for isolated seawater was reduced to 9.5 mS cm^{-1} , thus improving the mass ratio of DOM to sea salts from roughly 1:17,500 to 1:180.⁷³ RO/ED was further refined by using pulsed electrical current in the final step of the ED phase; using this approach, the final salt concentrations in seawater were reduced to $< 0.1 \text{ mS cm}^{-1}$ ⁷³ a level that allowed samples to be analysed by NMR and FT-ICR-MS.⁷⁴

While RO/ED systems are at present more expensive than either an SPE or ultrafiltration apparatus, the proponents argue that it is able to yield much higher recoveries than other DOM extraction methods. RO/ED was able to recover an average of 75% of the dissolved organic matter from a set of 16 different seawater samples, and for some of the samples recovery greater than 90% of the DOC was reported.⁷⁴ In addition to retaining a higher amount of the dissolved organic carbon, Koprivnjak et al. argue that the DOC in RO/ED extracts are more representative of the DOC found in the initial sea water samples.⁷⁴ This conclusion is based on the fact that UV-Visible spectra and molar C/N ratios of extracted DOM resemble those of the initial seawater samples. Also, RO/ED is able to retain additional portions of the DOM pool as compared to SPE or ultrafiltration as shown by additional peaks in ¹³C-NMR and ¹H-NMR spectra as well as more alkyl carbon peaks found in FT-ICR-MS spectra.⁷⁴ One of the caveats with RO/ED is that it requires a chemically harsh 0.01 M NaOH (pH 12) rinse to remove organic matter from the RO membrane. This rinse may degrade molecules present in the DOM pool.

One of the limitations of RO/ED for DOM extraction is the time and cost required to run this system. As a result, blanks, carry-over issues, and loss of material have not been well characterized. Koprivnjak et al.⁷⁴ assumed that loss of material during the RO/ED process was by adsorption to the membrane because losses across the RO membrane could not account for the

lack of mass balance.⁷⁴ However, they did not quantify the extent to which carry-over occurred from one sample to the next. In addition, only a single blank sample, for which pre-extraction flushing may not have been complete, was run through the ship-board RO/ED system.⁷⁴ The amount of DOC ($\mu\text{mol/L}$) recovered in the procedural blank was on average 14.5% (range 2.2 and 23%) of the amount recovered in the seawater samples and was not subtracted to calculate recovery. If the method blank is subtracted from samples (which may be over-conservative due to the flushing issue), then the average recovery is 64% rather than 75% (calculated from Koprivnjak et al. data⁷⁴). To maximise RO/ED usefulness to the field of DOM analysis, possible carry-over from sample to sample, mass balance issues, and method blanks need to be better characterized.

Ultrafiltration:

Ultrafiltration has been widely used since it (in its cross-flow form) was popularized as a DOM extraction technique by Benner.⁷⁶ The fundamental difference between RO and ultrafiltration is in membrane permeability. RO membranes theoretically retain all sizes of organic matter as well as inorganic salts (and thus require coupling with ED for desalting) while ultrafiltration theoretically retains only the high molecular weight fraction (typically >1000 Da) of the DOM; small molecules and inorganic salts pass through the membrane. In ultrafiltration DOM is isolated by diafiltration with ultrapure water. The primary advantage of ultrafiltration over SPE is that it can generally recover a larger portion of the dissolved organic matter,^{36, 77} although few head-to-head comparisons have been done. Also, ultrafiltration does not require “harsh” chemical manipulations, i.e. acidification to pH 2 for SPE.⁷⁶ However, ultrafiltration is limited to extracting only the high molecular weight (HMW) portion of the dissolved organic matter and results are highly dependent on experimental procedures and equipment used.⁷⁸⁻⁸⁰

There are two main versions of ultrafiltration applied to DOM work: cross-flow ultrafiltration (CFF), also called tangential flow ultrafiltration, (as used in Benner et al.⁷⁶) and stirred cell ultrafiltration (as applied in Simjouw et al.³⁶). Both approaches are methods to reduce the amount of membrane sorption and fouling. They differ in the volume of sample that can be processed, with cross-flow approaches used on larger volume samples (10s to 1000s of liters) and stirred cell ultrafiltration generally used on samples of 5 liters or less.

A number of different commercial ultrafiltration systems are available. However, these different systems have varied responses and recoveries.⁷⁹ The most commonly used CFF ultrafiltration systems are an Amicon DC-10L or DC30 system^{76, 78, 81} (or custom-built equivalents of these as they have become hard to obtain) and Amicon 8400 for stirred cell ultrafiltration.^{36, 77} Both polysulfone filters (such as Amicon SIONI with a pore size of 1 nm and a molecular weight cutoff of 1,000 Daltons) and cellulose polymer membranes have been used. Regenerated cellulose polymer membranes have been shown to be less subject to DOC sorption than polysulfone membranes.⁸²

The method for DOM extraction by CFF was outlined in Benner et al.⁷⁶ and essentially the same procedure, with minor

changes, remains in use. The sample (often 100-200 L) is first sterile-filtered (usually through a 0.2 μm filter) to remove bacteria and other particles. The ultrafiltration system, which was thoroughly rinsed with ultrapure water immediately before use⁸⁰, is then conditioned with sample to reduce the loss of DOC by sorption to the membrane. As differences in pressure can change the amount of material that is recovered, the pressure should be held constant.⁷⁹ Filtrate is removed until a desired concentration factor is achieved (typically concentration factors of 10-30 are used for natural samples). Then, diafiltration with 6-9 volumes of Millipore® water is used to remove salts.⁷⁶ Stirred-cell ultrafiltration follows the same basic steps of rinsing, preconditioning, sample concentration and diafiltration for desalting, with the volumes scaled down proportionally relative to sample processed.³⁶

The limitations of ultrafiltration have been extensively characterized by an inter-laboratory comparison exercise in the oceanographic community (“the Colloid Cookout”⁷⁹). A series of studies done as part of this collaboration found significant differences in organic matter extraction when different ultrafiltration systems or operating conditions were used.⁷⁹ Additionally, studies were done to quantify the blanks, loss of material by sorption to the membrane, and the effective molecular weight cutoff of a 1,000 Da membrane.^{78, 80}

These studies found that good blanks can be achieved with large-volume ultrafiltration but only after extensive flushing of the system, i.e. >50 L.^{78, 80} Also, Gustaffson et al.⁸⁰ stress the importance that the system is flushed immediately before use.

Loss of material by sorption to the membrane is a significant problem. When various molecular weight standards were tested, initial loss of 80-100% occurred, presumably from sorption to the membrane. However, once the membrane was saturated with material an equilibrium condition arose such that molecules were both adsorbed and desorbed from the membrane and no further loss of material occurred.⁸⁰ For natural samples, loss to the membrane was prevented by conditioning the system with sample (5 L) before ultrafiltration. Still, DOC material was lost during the first few liters of extraction until equilibrium was established.¹⁹ These studies strongly recommend that the system is checked by mass balance where DOC is quantified in the initial sample, the filtrate, and the retentate every time that ultrafiltration is done.^{19, 80} Additionally, Gustaffson et al.⁸⁰ point out that even if 100% of the material is accounted for by mass balance, the system could still be functioning improperly because there are the competing processes of blank issues and loss of material by sorption to the membrane. While still subject to the same issues, regenerated cellulose polymers were later shown to be less subject to molecular sorption than other ultrafiltration membrane types.⁸²

For DOM extraction, the membranes typically used have a nominal molecular weight cutoff of 1,000 Da which corresponds to 1 nm. The effective molecular weight cutoff varies depending on experimental conditions. Effective molecular weight cutoff is defined as the molecular weight for which 90% of the molecules are actually retained by the membrane under experimental conditions. However, ultrafiltration systems are generally designed for industrial processes with highly concentrated solutions rather than dilute natural samples, for which the

effective molecular weight cutoff is highly dependent on the experimental conditions^{79, 80} and the concentration factor.^{78, 81} The effective molecular weight cutoff has generally been found to be higher than manufacturer specifications for dilute natural samples. For example, using artificial seawater samples, the effective molecular weight cutoff for a 1 kDa membrane was reported to be as high as 50 kDa,⁸⁰ though other studies report lower, but still elevated values (5-6 kDa) for the effective molecular weight cutoff.^{19, 81}

Concentration factor (Volume of initial sample/Volume of retentate) is an important factor determining effective molecular weight cutoff. Molecules of intermediate size are occasionally rejected by the membrane such that, at a given concentration factor, molecules of different sizes will be retained in different proportions.⁷⁸ As the concentration factor increases, the retained material then shifts toward higher molecular weight meaning that the total percentage recovery of DOM decreases as the concentration factor increases.⁸¹

DOC recoveries by ultrafiltration are generally higher for samples with terrestrially derived DOC and decrease for ocean samples.^{36, 78} The average molecular weight of DOC decreases as a result of photodegradation⁸³ and microbial degradation, so that open ocean systems have a smaller average molecular weight

than coastal or terrestrial systems. Also, the apparent molecular weight of a sample decreases as salinity increases which is presumably due to coiling of large molecules.⁷⁷ Further, ultrafiltration has a lower DOC recovery for deep ocean systems than for surface waters.^{76, 81} This result suggests a possible mechanism of a “diagenetic sequence from macromolecular material to small refractory molecules” with depth where macromolecules are produced by phytoplankton primary production and then the larger molecules are preferentially taken up and remineralized by ocean heterotrophic microbes.⁸¹ In summary, the typical DOC recoveries for open ocean samples range from 20-40%, and 50-70% or higher for coastal and freshwater samples (Table 1).

Ultrafiltration is highly effective at retaining large polysaccharides⁷⁶ and their degradation products as well as amino sugars.³⁶ Beyond that generality, the type of DOM recovered by ultrafiltration is highly dependent on the sample. For example, Kruger et al.⁷⁷ found elevated SUVA₂₅₄ values (relative to initial sterile-filtered water) in ultrafiltration extracts from fresh water river and lake samples which suggest a high portion of aromatics in the HMW fraction. Conversely, Simjouw et al.³⁶ found that chromophoric material was preferentially recovered with SPE rather than ultrafiltration for Chesapeake Bay Estuary Samples.

Table 1 This table summarizes the typical DOC recovery by ultrafiltration from a range of samples.

Study	% DOC Recovery	Sample type
Benner et al. 1992 ⁷⁶	33	North Pacific Surface
Benner et al. 1992 ⁷⁶	22	North Pacific 4000 m
Benner et al. 1997 ⁸¹	23-35	Surface Atlantic and Pacific (n = 9)
Benner et al. 1997 ⁸¹	20-24	Depth sample (2400-4000 m) Atlantic and Pacific (n = 4)
Guo and Santschi 1996 ⁸⁴	35	Surface Pacific
Guo and Santschi 1996 ⁸⁴	55	Coastal North Atlantic
Simjouw et al. 2005 ³⁶	50.8	Chesapeake Bay mouth
Simjouw et al. 2005 ³⁶	50.7	Elizabeth River, Chesapeake Bay Estuary, VA, USA
Kruger et al. 2011 ⁷⁷	64	Lake Superior, MN
Kruger et al. 2011 ⁷⁷	59	Lester River, Duluth, MN
Kruger et al. 2011 ⁷⁷	78	Brule River, Brule, WI

It is debatable if the DOM recovered by ultrafiltration is representative of the DOM in the initial samples. Benner et al. found that the C/N ratios for ultrafiltration extracts were similar to the C/N ratios of the initial sea water samples.⁷⁶ Similarly, the E₂/E₃ ratios of the ultrafiltration extracts are very close to the E₂/E₃ ratios in the initial fresh water samples.⁷⁷ However, when mass balance was quantified by both DOC concentrations and UV-Visible absorbance, recoveries were very different which suggests a bias in the material retained by ultrafiltration.⁷⁷ Regardless of how well ultrafiltration recovers a representative DOM extract, the DOM extracted by ultrafiltration is different

from that extracted by C-18 SPE as shown by FTIR and direct temperature-resolved mass spectrometry.³⁶

One of the major advantages of ultrafiltration compared to SPE or RO/ED is that ultrafiltration does not require chemical manipulations that may alter or degrade dissolved organic matter composition. In contrast, SPE attains the highest recoveries when the sample is acidified to pH 2, and RO/ED extract is rinsed from the membrane by pH 12 NaOH. Granted, all extraction methods could cause chemical changes to the dissolved organic matter; removing salts and concentrating the DOM alone would certainly change the chemical environment,⁷⁷ and adsorbing/desorbing

from the ultrafiltration membrane could cause some chemical changes as well. Still, ultrafiltration is chemically the gentlest of these extraction methods.

5 SPE:

Solid Phase Extraction (SPE) has been, and still is, the most widely used DOM extraction method. This is probably because SPE is the “easier and quicker technique”³⁶ compared to either RO/ED or ultrafiltration. Also, SPE is by far the cheapest of these three methods. The general principle of SPE is that molecules are retained on a solid phase as sample is pulled through a cartridge; molecules are then eluted by appropriate solvent. Typically, nonpolar materials are used as solid phases which retain hydrophobic molecules from aqueous solution. Hydrophobic molecules are then eluted by organic solvent such as methanol or acetonitrile. Before the mid-1990’s, XAD resins were the SPE sorbents of choice for DOM extraction but some of these are no longer commercially available. In many recent studies, XAD

20 resins have been replaced by C-18 sorbents or, more recently still, styrene divinylbenzene phases. Because C-18 and styrene divinylbenzene sorbents have been shown to be more effective at DOM extraction across a wide range of water types, this review will focus primarily on their use in SPE. Unlike the other methods of DOM extraction, the DOM material recovered by SPE is inherently biased in the types of molecules that are recovered because the method selects for specific chemical fractions based mainly upon polarity.

Sample processing by SPE is quite simple compared to ultrafiltration or RO/ED. SPE can be done with either disks or cartridges as discussed below. For SPE cartridges the method is described by Dittmar et al.⁸⁵ SPE cartridges are prepared by rinsing with organic mobile phase. After filtered samples are acidified to pH 2 -3 with 6 N HCl, samples are generally loaded at no more than 40 mL/min,⁸⁵ though some studies state that loading efficiency is independent of flow rate up to 150 mL/min.⁸⁶ Dittmar et al.⁸⁵ recommend that for every gram of sorbent no more than 2 mmol DOC or 10 L of sample be loaded.

Table 2 This table reviews the percent of DOC that can be recovered by SPE extraction from various studies. Clearly, composition of the dissolved carbon, which varies spatially and temporally, is the biggest factor in terms of the percent of material that can be recovered. This is seen by the fact that variability from different sites within the same studies is greater than variability across studies.

Study	SPE conditions	% Recovery	Sample
Thurman, 1985 ⁸⁷	XAD, pH 2	5-15	General review of XAD recoveries in ocean waters
Hedges et al. 1992 ¹	XAD-2	~10	North Pacific
Hedges et al 1992 ¹	XAD-8	~30	Amazon River
Mills et al. 1982 ⁸⁸	C-18 pH 2	38	Estuarine DOC
Lara and Thomas, 1994 ⁸⁹	XAD-2, pH 2	50	Cultures grown in Antarctic seawater, of the 50% retained only 30% could be eluted by standard means.
Simjouw et al. 2005 ³⁶	C-18 pH 2	44.9, 27.4	Two samples from the same location on the Elizabeth River (Norfolk, VA, USA) collected on different dates.
Simjouw et al. 2005 ³⁶	C-18 pH 2	38.8, 36.4	Two samples from the same location in the Chesapeake Bay Mouth, USA.
Dittmar et al. 2008 ⁸⁵	PPL pH 2	62 ± 6	North Brazil shelf and coastal zone
Dittmar et al. 2008 ⁸⁵	PPL pH 2	62 ± 6	Apalachicola River and tributaries, USA
Dittmar et al. 2008 ⁸⁵	PPL pH 2	65 ± 6	Apalachicola, salt marshes, USA
Dittmar et al. 2008 ⁸⁵	PPL pH 2	43 ± 2	Gulf of Mexico, deep sea
Dittmar et al. 2008 ⁸⁵	PPL pH 2	43 ± 5	Weddell Sea (surface to bottom)
Kruger et al. 2011 ⁷⁷	C-18 pH 2	63 ± 6	Lester River, Duluth, MN, USA
Kruger et al. 2011 ⁷⁷	C-18 pH 2	38 ± 11	Lake Superior, open lake, USA
Kruger et al. 2011 ⁷⁷	C-18 pH 2	25 ± 3	Brule River, MN, USA
Kim et al. 2003 ⁹⁰	C-18 pH 2	60%	Stream water from New Jersey, USA, black water stream and a pristine mountain stream in Costa Rica. Poor blanks
Dalzell et al. 2009 ⁴⁶	C-18 pH 2	44	Great Bridge, Elizabeth River (upper river), Virginia, USA
Dalzell et al. 2009 ⁴⁶	C-18 pH 2	54	Town Point, Elizabeth River (mid river), Virginia, USA
Dalzell et al. 2009 ⁴⁶	C-18 pH 2	24	Chesapeake Bay Mouth, USA

Cartridges are rinsed with 0.01 M HCl in Millipore® water to remove salts. Then, DOM is eluted from the stationary phase with organic mobile phase.⁸⁵ If sample storage space or preservation is an issue, samples can be loaded on cartridges in the field and

cartridges can be preserved by freezing or refrigeration and eluted later without detrimental effects on the recoveries.⁸⁶ For disks, the method is the same except for a few small changes. Disks are generally rinsed and eluted 3 times with organic mobile phase as

described by Kim et al.⁹⁰ and Simjouw et al.³⁶ If the mobile phase (often methanol or 90:10 methanol:water) is an appropriate solvent for the particular analysis technique, the extract can be used as is. If not (i.e. TOC analysis, RP-LC-MS, NMR), the extract can be dried down and made up in the appropriate solvent.

There is a small debate in the literature about the use of disks or cartridges for SPE extractions. Kim et al.⁹⁰ argue that the disks can be used with higher flow rates than cartridges and report a high recovery of ~60% of the DOC although in this case recovery is determined only by UV-Visible absorbance and not by DOC concentrations. However, the highest reported DOM recoveries for SPE extractions have been with styrene divinylbenzene cartridges.⁸⁵ Both cartridges and disks are commercially available and both methods seem to be robust. Increased recoveries using styrene-divinylbenzene based phases relative to C-18 based phases have been seen in both cartridge studies⁷⁸ and disk studies (see Fig. 3).

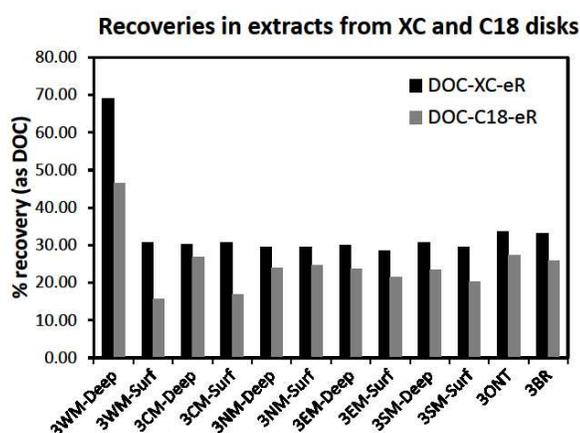


Figure 3. Recovery (as % DOC) in extracts (eR) via solid phase extraction with C-18 and XC (a styrene-divinylbenzene phase) disks (3M, Empore) in a suite of open-water (3WM, 3CM, 3NM, 3EM) and nearshore (3ONT, 3BR) Lake Superior samples. Open-water samples are from surface (5 m) and deep (hypolimnion) waters. Nearshore samples are surface-water.

In summarizing DOC recoveries by SPE (Table 2, Fig. 3) a few patterns in DOC recoveries are evident. First, XAD resins exhibited lower recoveries than C-18, PPL, or XC resins, especially in systems with more autochthonous DOC. XAD resins were able to recover only 5-15% of the DOC in ocean water.⁸⁷ Second, when the same method is used, intra-study variability between samples is greater than inter-study variability. These results suggest that sample to sample variability in DOM composition is the biggest factor in the amount of material that is recovered. Third, samples from similar aquatic systems (e.g., open-ocean vs estuarine vs riverine) have similar DOC recoveries even when they are analyzed by different studies. These results suggest that the method itself is fairly robust. Fourth, as with ultrafiltration, SPE recoveries are lower for ocean and large-lake samples than for more terrestrially-derived samples.

SPE is typically described as recovering lower amounts of material than ultrafiltration or RO/ED. Certainly, XAD resins had

lower recoveries than the other methods. However, to the authors' knowledge, only two studies have done a direct comparison quantifying recoveries in SPE and ultrafiltration using the same samples. Simjouw et al.³⁶ found that C-18 SPE had 10-15% lower recoveries than ultrafiltration, but drew these conclusions based on only 2 samples.³⁶ Kruger et al.⁷⁷ tested three fresh water samples and found higher recoveries by ultrafiltration for two of them and lower recovery for the third. Also, since this study was done, SPE recoveries have been improved by switching to styrene divinylbenzene sorbents such as XC (Fig. 3) and PPL.⁸⁵ Like ultrafiltration, SPE tends to have higher recoveries with fresh water and coastal samples than open ocean samples. Apparently, both overall molecular weight and hydrophobicity of the DOM is lower for open ocean samples. It is worth noting that a combination of SPE and ultrafiltration can be applied to achieve higher recoveries than either method alone because SPE and ultrafiltration retain different portions of the overall DOM.³⁶

Solid phase extraction has an inherent bias in the type of material recovered toward the more hydrophobic material. C-18 SPE enriches alkane/alkene structures as well as aromatic proteins and phenolic (lignin like) compounds.³⁶ Reviewing several XAD-based SPE studies, Benner et al.⁷⁶ concluded that the material recovered by SPE is invariant with depth and oceanic environment based on ¹H and ¹³C NMR. For Benner et al.,⁷⁶ this result suggested that SPE recovers primarily older, more refractory components of marine DOM. In comparing deep-water humic extracts and ultrafiltered material, Benner et al.,⁷⁶ pointed out that the humic extract had more unsubstituted alkyl carbon and less carbohydrate character than the ultrafiltered DOM. These observations are consistent with the current hypothesis that a large portion of the refractory dissolved organic matter in the oceans consists of carboxyl-rich alicyclic molecules (CRAM) with a structure of primarily fused alicyclic rings with carboxylic acid groups.⁹¹ Such material, upon sample acidification, should be retained well by hydrophobic phases. Consistent with Benner's observations of carbohydrate enrichment in ultrafiltered DOM relative to XAD-extracted DOM, we have observed that large polysaccharides are not well retained by C-18 or PPL phases (Swenson, unpublished data), and that ultrafiltered DOM is enriched in polysaccharide and aminosugar moieties relative to C-18 extracts.³⁶

One of the major criticisms of SPE is that SPE is chemically fairly harsh.^{76, 92} SPE with C-18 or styrene divinylbenzene sorbents is not as harsh as former SPE methods using XAD resins because XAD resins generally require several steps to elute molecules including treatment with strong base. Still, styrene divinylbenzene and C-18 phases retain the highest amount of DOM when the sample is first acidified to pH 2 with HCl.^{85, 88, 89} This step could alter the dissolved organic matter in the sample by hydrolyzing ester and peptide bonds as well as altering the higher order structure of large molecules.⁶⁵ While acidification does improve the recovery of DOM by SPE, it does not necessarily have to be part of the SPE process if one is concerned more with potential alterations of molecules rather than higher proportional recoveries of the bulk DOM.

Analytical approaches for functional group, compound class, and molecular formula determinations

5 NMR

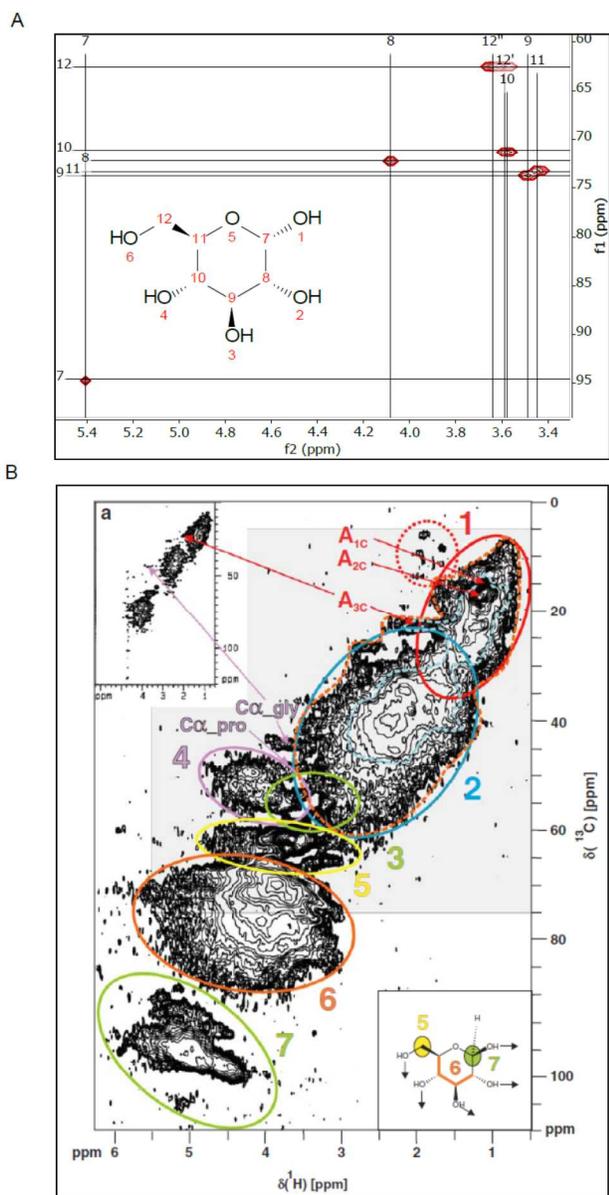


Fig. 4. (A) Predicted $^1\text{H},^{13}\text{C}$ HSQC NMR spectrum for D-Glucose (MestReNova Version 9.0). (B) Two dimensional NMR spectra of UDOM: $^1\text{H},^{13}\text{C}$ HSQC NMR spectra of surface UDOM with seven groups of major constituents and of deep UDOM (insert a) top left.⁸⁴ Figure (B) was taken from Herkorn et al.⁸⁴ with permission from Elsevier (License No. 3313160317544).

15 Nuclear magnetic resonance (NMR), in various experimental forms and often in conjunction with FT-ICR-MS, has been extensively applied to the study of DOM and related materials.^{33, 90, 91, 93-109} Hertkorn et al. have provided one of the most recent

and comprehensive reviews on the application of NMR to DOM,⁹⁴ while Simpson et al. have provided some earlier reviews.^{96, 100}

Reviews on the application of NMR to substances closely related to DOM have also appeared.^{93, 106, 107, 110-116} While solution-state NMR has most often been used to characterize DOM, solid-state NMR has been used as well.^{96, 98, 108, 116}

25 A brief description of basic NMR will be provided here. Details of the most important solution-phase experiments, including theory and interpretation, can be found elsewhere.^{117, 118}

In the presence of a magnetic field (e.g., in an NMR magnet), the nuclei of atoms such as ^1H , ^{13}C , ^{15}N , and ^{31}P may undergo transitions between spin states when subjected to radiation of specific radio frequencies, which depend upon the magnetic field strength, the particular nucleus (i.e., its magnetogyric ratio), and the atom's chemical environment. The NMR data (e.g., chemical shifts in ppm, scalar through-bond coupling constants in Hz, dipolar through-space interactions) generated in various types of NMR experiments can be used to determine the structures of molecules.

As an illustration, consider a two-dimensional $^1\text{H},^{13}\text{C}$ HSQC experiment. This type of experiment can provide the chemical shifts (i.e., resonance frequency versus a reference standard) for hydrogen atoms in a molecule and carbon atoms to which they are attached. Methylene hydrogens can be distinguished from methyl and methine hydrogens in versions of the HSQC experiment. The data are typically displayed in a two-dimensional map as shown in Figure 4A for a predicted HSQC spectrum of D-glucose. Here the x-coordinate "f2-axis" is the ^1H (hydrogen) chemical shift, while the y-coordinate "f1-axis" is the ^{13}C (carbon) chemical shift. Thus, each carbon-bound proton has a location in the HSQC chemical space that is defined by a set of H/C chemical shift coordinates. For example, the anomeric proton/carbon (number 7 in Figure 4A) appears in this (predicted) HSQC spectrum at 5.4 ppm (hydrogen) x 94 ppm (carbon). Ideally, molecular structure elucidations would be greatly facilitated if each proton and each carbon in the molecule(s) under study would have a unique discernable chemical shift. Unfortunately, in nearly all cases except very small molecules, there is an overlap of signals. Hence, investigators of complicated molecules tend to use a battery of two dimensional experiments or even three-dimensional experiments.

The reader is encouraged to compare the simple (predicted) HSQC spectrum of D-glucose in Figure 4A with a corresponding reported observed HSQC spectrum of DOM.⁹¹ (Figure 4B). The signal overlap in areas that are characteristic of carbohydrates (as shown in Figure 4A) are clearly evident. Thus, in a given NMR experiment, such as HSQC, if signals for a given type of molecule (e.g., a carbohydrate) are present in all of the appropriate areas of chemical space, then the presence of such a type of molecule might be possible but not proven. Additional NMR data or other analytical data would be required for proof. On the other hand, if one or more appropriate areas of chemical space are blank, then the absence of such a type of molecule could be concluded.

Typically, DOM material must be isolated and concentrated and inorganic ions need to be removed if NMR studies of DOM are to be performed.^{94, 108, 119-122} As in the case of FT-ICR-MS, chromatographic separations have been conducted and fractions

have subsequently been analyzed by NMR,^{99, 123} SEC¹²⁴ and HILIC⁹⁹ have been found to be very effective in fractionating DOM prior to NMR analysis. However, samples can be analyzed directly with appropriate water-suppression techniques.^{97, 102}

Zheng and Price have made hydrodynamic radius measurements on DOM in natural waters directly with diffusion NMR.⁹⁵

The solution NMR experiments that have been applied to DOM comprise one-, two-, and three-dimensional experiments, diffusion edited (DOSY) experiments, and NMR coupled on-line and off-line with HPLC and SEC. One-dimensional experiments include ¹H,^{94, 96, 97, 124} ¹³C,^{94, 15} ¹⁵N,³¹ ³¹P, and DEPT (for multiplicity determinations).⁹⁴ Two-dimensional homonuclear experiments include ¹H,¹H COSY (2-3 bond H-H scalar couplings),^{94, 99} ¹H,¹H TOCSY (2-5 bond H-H scalar couplings),^{94, 99} and ¹H,¹H NOESY (H-H dipolar couplings).¹⁰⁰ Two-dimensional heteronuclear experiments include ¹H,¹³C HSQC (one-bond C-H couplings)^{94, 96, 99} (also DEPT-HSQC, phase-edited ¹H,¹³C HSQC^{94, 99}), and ¹H,¹³C HMBC (2-4 bond C-H couplings).^{94, 99} Three dimensional experiments include ¹H,¹³C,¹H HSQC-TOCSY (2-5 bond H,H couplings and one-bond C-H couplings).^{93, 94, 99} Diffusion experiments such as DOSY, which can differentiate DOM components on the basis of diffusion coefficients, have also been applied to DOM studies.⁹⁵ ¹H,¹H COSY NMR, which observes protons that are coupled to each other through two or three bonds, may be one of the most significant experiments for DOM.⁹⁴ On-line NMR experiments such as SEC-NMR have also been conducted.¹⁰¹

Mass spectrometry

Three fundamental approaches have been most popular in recent applications of mass spectrometry to DOM: (1) high-performance liquid chromatography (HPLC) coupled on-line to a mass spectrometer,¹²⁵⁻¹²⁷ (2) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) without prior fractionation,^{26, 35, 90, 94, 104, 119, 128-140} and (3) FT-ICR-MS with prior chromatographic fractionation of DOM material.^{141, 142} In all these cases, the DOM material was first isolated from water by one of the approaches described above.^{90, 94, 121, 143, 144} In rare cases, DOM samples have been analyzed directly (after only filtration). For example, Sleighter and Hatcher reported the FT-ICR-MS analyses of filtered whole water from the Dismal Swamp.¹¹⁹

The selective derivatization of DOM components prior to FT-ICR-MS analysis and analysis by other techniques has also been pursued. For example, borohydride reductions of aldehyde and ketone groups have been used to identify DOM components with these groups by FT-ICR-MS.¹⁴⁵ O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) has been used to address specific low molecular weight aldehydes and ketones by gas chromatography-mass spectrometry (GC-MS).¹⁴⁶ Monobromo(trimethylammonio) bimanane has been used to address thiol-containing components by fluorescence.¹⁴⁷ Various methods have been used to determine carbohydrates.¹⁴⁸ CuO has been used to liberate phenols from lignin for analysis by GC-MS.¹⁴⁹ Navalon et al have applied multiple methods, including hydrolysis and silylation followed by GC-MS, HPLC-MS, and matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF-MS) to river

DOM.¹²⁷

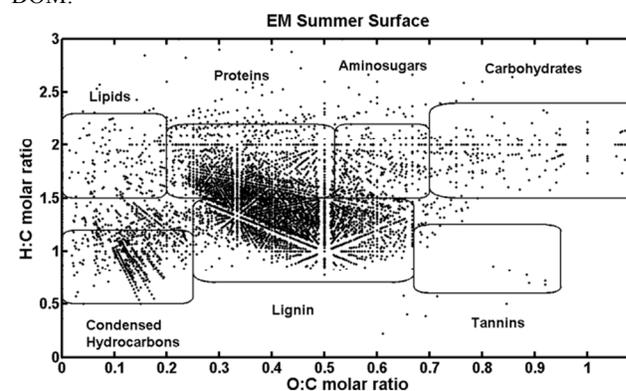


Figure 5. Van Krevelen diagram of the XC extract of a Lake Superior surface water sample collected in August 2010 from site EM (47°34'N, 86°39'W)

FT-ICR-MS is the currently the only analytical technique that can observe a majority of individual DOM components. NMR can observe some specific compounds in DOM, but only if they are present at a sufficiently high level.⁹⁹ The details of the application of FT-ICR-MS to DOM have been provided in a recent review¹¹⁹ and will only be summarized here. The key feature of FT-ICR-MS is that resolving power is sufficiently high to achieve errors in m/z values for individual DOM components that are less than 0.5 ppm for components of ~400 Da.¹¹⁹ No other commercial instruments can approach this level of resolution.¹⁵⁰ With the resolving power of FT-ICR-MS, empirical formula for the literally thousands of individual DOM components can be calculated from their corresponding m/z values (which typically range from 200-700 Da).¹¹⁹ Furthermore, this information can be used to further categorize the DOM components. For example, “van Krevelen” diagrams can use empirical formulae data (i.e., H/C and O/C ratios) to create plots showing how sample formulae overlap with those of general compound classes such as lipids, proteins, amino sugars, carbohydrates, lignin, tannins, condensed aromatics, and unsaturated hydrocarbons (Fig. 5).^{26, 119, 130, 135, 137} Furthermore, double-bond equivalents and aromaticity indices have also been used to characterize DOM components.^{129, 136} Additional structural information for individual DOM components can also be obtained from FT-ICR-MS-MS studies using collision induced dissociation (CID).¹³³

While mass spectrometry provides the advantages described above, this technique has a number of significant disadvantages. At best mass spectrometry can provide empirical formula (and associated information; see above) and molecular weight data and some information about structure via derivatization experiments and fragmentation (e.g., CID) experiments. However, this knowledge most often falls short of what is needed to assign complete structures to individual DOM components. Thus, mass spectrometry is typically used in conjunction with other techniques such as NMR.

Other significant disadvantages of mass spectrometry stem from the difficulty in transforming all of the analytes in a sample into ions that can be observed. Typical ionization sources include electrospray,^{26, 132, 134} atmospheric chemical ionization,²⁶ and atmospheric pressure photoionization^{26, 132} and both positive and

negative ions may be detected.¹⁰⁴ However, none of these ion sources provide universal ionization of all possible analytes and biases may be shown toward certain classes of compounds.¹¹⁹ Additional disadvantages of mass spectrometry include interference from contaminants,¹¹⁹ signal suppression by various co-analytes,¹⁵¹ and degradation of analytes in the ionization source.

FTIR

FTIR has been used for years to characterize humic substances¹⁵²⁻¹⁵⁴ and has been applied to bulk sediments as well.¹⁵⁵⁻¹⁵⁷ The basic premise of the technique is that infrared light absorption at specific wavelengths occurs due to molecular vibration (stretching, bending, scissoring). Specific functional groups absorb at specific wavelengths and the intensity of this absorption is a function of the change in dipole moment the molecule undergoes upon vibration and the concentration of the sample (i.e., the number of matching molecules available to absorb the light). FTIR therefore provides an overview of transiently polar covalent bonds within a sample, including organic functional groups, the C=O bonds in carbonate species, and the Si-O bonds in silicates. FTIR is somewhat forgiving in terms of sample preparation (for example NaCl does not yield an absorption peak), but is sensitive to differences in water absorption within a sample, as water yields a strong FTIR signal.

Sample interactions with infrared radiation (IR) are usually measured via transmittance or reflectance of the IR beam, and there are several different sample-handling mechanisms for mediating this interaction between the IR beam and sample material. For DOM work, the classic sample-handling approach is measuring transmittance of the IR beam through a pellet consisting of homogenized KBr, which acts as an optically-transparent diluent, and dried sample.^{33, 158} This approach provides good resolution and response from 600 to 4000 cm^{-1} but can be considered sample-destructive, as the sample is intimately mixed with KBr in sample preparation. Since KBr is hygroscopic care must be taken in keeping the sample-KBr mixture water-free (e.g., through use of oven-drying and desiccation). Another approach often used for DOM work is attenuated total reflectance (ATR) where the sample is pressed against a crystal of higher refractive index.¹⁵⁹ IR light penetrates through the crystal and into the sample, creating an evanescent wave in the sample; if the sample interacts with this wave, less light will end up being reflected back to the detector (as described by Koshhesab¹⁶⁰). Because the depth of penetration of a wave into a sample is wavelength-dependent, ATR spectra show less response at higher wavenumbers (especially noticeable above 2000 cm^{-1}) and algorithms are often used to convert the spectra into a form more comparable to transmission data; depending upon the sample type, wavelength dependent differences in the sample's refractive index may also need to be taken into account in this algorithm.¹⁶¹ ATR is non-destructive as the sample can be easily be recovered from the crystal surface after analysis. Dried DOM fractions or water-soluble soil organic matter can also be measured via diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy.^{162, 163} DRIFT requires a fine-particle sample that has relatively low absorbance, in order to maximize the diffuse reflectance relative to Fresnel or mirror reflectance; such a

sample is usually prepared by grinding a mixture of dried analyte with an optical diluent such as KBr¹⁶² or KCl.¹⁶⁴ The particle size must be controlled as it affects the amount of scattering and absorption that occurs, and thus the resulting signal response from the analyte.¹⁶⁴ DRIFT spectra also require application of an algorithm to convert them into spectra similar in appearance to transmittance spectra, and also to convert the instrument response into a function of analyte concentration (given in Kubelka-Munk units). Because of the variations in response with the physical nature of the sample, the DRIFT approach is better for a quick qualitative analysis rather than quantitation, although with appropriate care and use of standards and blanks, quantitation can be done.¹⁶⁴

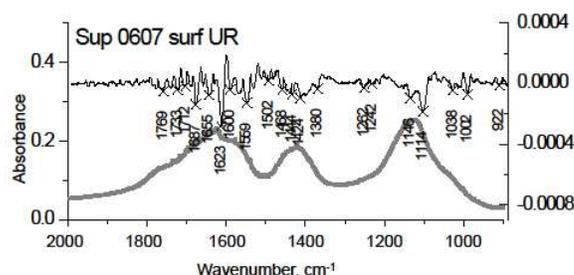


Fig.6. FTIR spectrum of the KBr pellet preparation of ultrafiltered DOM from Lake Superior surface water (originally published in Stephens and Minor¹⁵⁸), and its second derivative. The downward spikes in the second derivative show individual peak locations that overlap in the original spectrum.

FTIR analysis of an isolated DOM sample yields a simplified spectrum relative to that of pure compounds due to the overlap of many spectral bands from the extremely heterogeneous DOM. The resolution in such spectra can be enhanced by using the second derivative to identify individual peaks within the overlapping responses³³ (see Figure 6) or to use Fourier self-deconvolution.³³ An additional approach to further probe the information content of a set of FTIR analyses is to apply generalized perturbation-based two dimensional correlation spectroscopy calculations to the data set.^{33, 165, 166} In this approach the second dimension is a perturbation occurring through the suite of samples (e.g., a salinity gradient, a depth gradient, or time). In phase and out-of-phase correlations in the spectral intensities of peaks as a function of this perturbation are identified. As not all peaks will show the same across-perturbation (i.e., across-sample) trends, this approach can also help to deconvolute overlapping peaks.

95 Molecular-level structural analyses: Biomarker and compound class

Direct isolation and analysis of individual compounds and compound classes from filtered aqueous samples can provide useful information on DOM composition in a biomarker sense, but does not allow characterization of a quantitatively important portion of the marine DOM pool. Summing compound-class information from multiple techniques leads to characterization of <11% of total marine DOC from surface waters.³⁷ The three most

common biochemical classes analysed in this manner are hydrolysable carbohydrates, hydrolysable amino acids, and lignin derived phenols. Hydrolysable carbohydrates have been used to provide comparative source and diagenetic information in sample sets.^{167, 168} Hydrolysable amino acids are often used to provide insights into the potential bioavailability of DOM and the extent of degradation the DOM may have undergone^{169, 170} using degradation indices based upon multivariate analysis of amino acid distributions within a large suite of natural organic matter samples.^{171, 172} Lignin-derived phenols are used to indicate terrestrial inputs into DOM samples and the extent of degradation these terrestrial inputs have undergone.^{63, 173, 174}

The analysis of hydrolysable carbohydrate compositions, with quantitation of individual monomer units (usually neutral aldose sugars but sometimes amino-sugar moieties as well) involves acid hydrolysis of a sample, subsequent neutralization and desalting, and analysis using anion exchange chromatography with pulsed amperometric detection.^{81, 175, 176} Note that separate hydrolysis and chromatography parameters are needed for amino-sugar and neutral sugar determinations.¹⁶⁷

Compositional information on total hydrolysable amino acids is usually obtained by acid hydrolysis of the samples (with an additional aliquot alkaline-hydrolysed if tryptophan quantification is desired),¹⁷¹ derivatization of the hydrolyzates with o-phthalaldehyde, and subsequent reverse-phase HPLC analysis with fluorescence detection.^{177, 178}

Lignin in DOM samples is usually concentrated by C-18 extraction⁸⁶ with subsequent release and isolation of lignin-derived phenols using cupric oxide oxidation and extraction¹⁷⁹⁻¹⁸¹ and analysis of these phenols by gas chromatography^{179, 180} or gas chromatography-mass spectrometry.⁸⁶

In addition to these compound class (and monomer composition) determinations, individual biomarker compound/compound types are often targeted for specific studies, such as the use of β -hydroxy fatty acids to identify contributions of bacterial lipopolysaccharides to ultrafiltered dissolved organic matter.¹⁸² Such studies are beyond the scope of this review.

40 Summary and conclusions

Due to its inherent heterogeneity and its presence in relatively low concentrations within solutions that often have high concentrations of other constituents, DOM is, at present, impossible to characterize quantitatively to the level of full molecular structures. The ability to characterize DOM falls within a continuum, where relatively generalized approaches (such as carbon concentrations, isotopic composition, and optical parameters) can be applied to the bulk DOM pool, and as the chemical detail obtainable from a technique increases, the portion of the DOM pool investigated by that technique decreases, with compound-class and biomarker measurements providing the highest level of structural specificity but applied to a very small proportion of the total DOM.

Researchers interested in natural DOM composition and reactivity, either in an ecological or a geochemical sense, or due to DOM interactions with anthropogenic compounds, need to carefully consider the DOM characterization approaches

available. Each has its strengths, weaknesses, and biases, which must be remembered when the resulting data are interpreted.

60 Combining multiple structural characterization techniques can provide a more comprehensive view of DOM composition, filling in the “blind spots” of individual techniques.^{34, 183, 184} In addition, the coupling of structural information with stable C and N and/or radiocarbon isotopic information can provide key constraints on DOM sources and reactivity within a particular system.^{185, 186}

Abbreviations

ATR = Attenuated total reflectance

CDOM = Chromophoric or colored dissolved organic matter

CID = Collision induced dissociation

70 COSY = Correlation spectroscopy

CRAM = Carboxyl-rich alicyclic molecules

CFF = Cross-flow [ultra-]filtration

DEPT = Distortionless enhancement by polarization transfer

DOC = Dissolved organic carbon

75 DOM = Dissolved organic matter

DOSY = Diffusion-ordered spectroscopy

DRIFT = Diffuse reflectance infrared Fourier transform [spectroscopy]

80 E2:E3 = Ratio of UV-Visible light absorption at 250 nm relative to 365 nm

E4:E6 = Ratio of UV-Visible light absorption at 465 nm vs 665 nm

EEMS = Excitation-emission matrix [fluorescence] spectroscopy

85 FT-ICR-MS = Fourier transform ion cyclotron resonance mass spectrometry

FTIR = Fourier transform infrared spectroscopy

GC = Gas chromatography

HILIC = Hydrophilic interaction chromatography

90 HPLC or UHPLC = (ultra) high performance liquid chromatography

HSQC = Heteronuclear single-quantum correlation

HMBC = Heteronuclear multiple bond correlation

IHSS = International Humic Substances Society

95 MALDI-TOF-MS = Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

MDLT = Material derived from linear terpenoids

MS = Mass Spectrometry

NMR = Nuclear magnetic resonance spectroscopy

NOESY = Nuclear Overhauser effect spectroscopy

NOM = natural organic matter
 PAR=photosynthetically active radiation
 PARAFAC = Parallel factor analysis, often used on EEMS data
 RO/ED = Reverse osmosis/electrodialysis
 5 S = Spectral slope
 SEC = Size-exclusion chromatography
 SPE = solid phase extraction
 SS NMR = Solid-state nuclear magnetic resonance
 SUVA254 = Specific UV absorbance at 254 nm
 10 UV = Ultraviolet spectroscopy

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Notes and references

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