

A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater

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Abstract

A simple protocol is presented for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater using commercially prepacked cartridges. The method does not require major instrumentation and can be performed in the field. Modified styrene divinyl benzene polymer type sorbents (Varian PPL and ENV) and sorbents of a silica structure bonded with different hydrocarbon chains (Varian C8, C18, C18OH, and C18EWP) were considered. Except for C18OH, which heavily contaminated the samples, none of the sorbents leached significant amounts of dissolved organic carbon (DOC) or nitrogen (DON). Samples from the North Brazil shelf with strong mixing gradients of terrigenous and marine DOM were used to compare the various sorbents. PPL was the most efficient—on average, 62% of DOC was recovered as salt-free extracts. C18 was found to be most efficient among the silica-based sorbents, but it showed only two-thirds of the extraction efficiency of PPL. As indicated by [¹H]NMR, C/N, and $\delta^{13}\text{C}$ analyses, PPL extracted a more representative proportion of DOM than C18. Therefore, PPL was used for comparative studies in the Gulf of Mexico and Antarctica. From brackish marsh and river waters, 65% and 62% of total DOC, respectively, could be extracted. For purely marine DOM in Antarctica and the deep sea, the extraction efficiency was lower (43% on average). The efficiency of the new method to isolate marine DOM is better than or similar to highly laborious methods. A further advantage is the complete desalination of the sample. The isolation of a major DOM fraction, which is salt-free, offers many possibilities to further characterize DOM by advanced analytical techniques.

Introduction

Dissolved organic matter (DOM) in the ocean is one of the largest active organic carbon reservoirs on earth. The major fraction of DOM is still chemically uncharacterized, and little is known about sources and cycling in the marine environment (Hedges 2002). The very low concentration of organic molecules dissolved in seawater compared with the huge amounts of inorganic salts is a major challenge in analytical chemistry. Through isolation of DOM, highly concentrated organic samples with low salt content can be obtained, and different analytical techniques can then be performed on these concentrates.

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Solid-phase extraction (SPE), using XAD resins, was established in the late 1970s and has been used often (e.g., Aiken et al. 1979, Lara and Thomas 1994, Dittmar et al. 2001). A sequential combination of different XAD resins retains up to 65% of phytoplankton-derived DOM (Lara and Thomas 1994). Unfortunately, the XAD resins used for the extraction of marine DOM are not available anymore. More recently, ultrafiltration with a 1-kDa cutoff has become a widely recognized method for the extraction of DOM (ultrafiltered DOM, or UDOM), isolating up to 30% of marine DOM (e.g., Amon and Benner 1996, Benner et al. 1997). The sequential combination of reversed osmosis and electrodialysis was recently used for a more efficient isolation of marine DOM (Vetter et al. 2007). However, the isolates still contain several hundred times more salt than DOC, which precludes detailed molecular characterization. Prepacked cartridges and discs with silica-C18 sorbents were used for the extraction of DOM from freshwater systems (e.g., Kim et al. 2003) and for the isolation of lignin from seawater (Louchouart et al. 2000). A combination of ultrafiltration and SPE significantly increased the total extraction yield for estuarine DOC to 70% (Simjouw et al. 2005).

Table 1. Properties of the solid phase sorbents used in this study, according to the manufacturer's (Varian) information.

Sorbent	Structure	Pore size, Å	Retention properties
C18	Octadecyl bonded phase, silica-based	60	Retention of nonpolar compounds
C18EWP	Octadecyl bonded phase, silica-based	500	More efficient retention of large molecules, compared with C18
C18OH	Non-encapped octadecyl bonded phase, silica-based, with active silanol groups	150	Enhanced retention of basic compounds, compared with C18
C8	Octyl bonded phase, silica-based	60	Not as retentive for nonpolar compounds as C18
PPL	Styrene divinyl benzene polymer	150	Retention of highly polar to nonpolar substances from large volumes of water
ENV	Styrene divinyl benzene polymer	450	Similar to PPL, larger pore size

However, the combination of these two extraction methods is highly laborious and time-consuming. A drawback of silica-based hydrophobic sorbents is that they can undergo phase collapse in aqueous matrices with concomitant loss of retention, and the silica structure can decompose through acidic or basic hydrolysis.

A considerable number of new polymeric and silica-based materials for SPE of polar compounds is now available, as summarized by Fontanals et al. (2005). Highly polar resins could be particularly suited to extract DOM efficiently from seawater. Furthermore, these cartridges are easy to handle and may not require extensive precleaning, which would be a major advantage compared with XAD or ultrafiltration. The use of commercially prepacked cartridges with highly polar sorbent materials could offer an efficient method to isolate a considerable fraction of DOM from seawater in a simple and reproducible way and would enable us to obtain routinely compositional information on a large number of samples.

The objective of this study was to establish a robust and efficient method for the extraction of DOM from seawater. To differentiate bulk DOM from DOM that is extractable through SPE, we propose the term SPE-DOM. A suitable SPE method should be able to reproducibly extract DOM at high efficiency out of large volumes (many liters) of seawater. The extracts should not contain significant amounts of salts, which is particularly important for many molecular analyses. The sorbents should not contaminate the samples or require extensive precleaning.

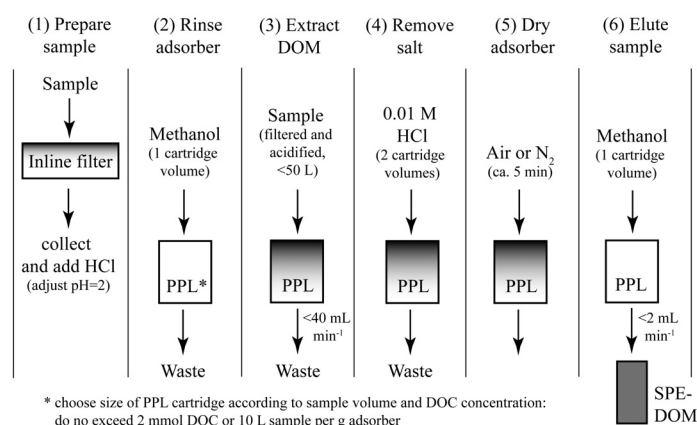
For this purpose, we examined polar to highly polar prepacked polymer and silica-based sorbents. Elemental (C/N), isotopic ($\delta^{13}\text{C}$), and molecular (^1H NMR) analyses were performed to examine differences in the compositional characteristics of extracted DOM by different sorbents. Contrasting marine environments in several climate zones were chosen to test the extraction efficiency of terrigenous and marine-dominated seawater samples from the North Brazil shelf, the Northern Gulf of Mexico, and the Southern Ocean (Antarctica).

Materials and methods

A suite of different Varian Bond Elut sorbents with varying hydrocarbon chains bonded to a silica structure (C8, C18, C18OH, C18EWP) and modified styrene divinyl benzene polymer type sorbents (PPL, ENV) were tested (Table 1). A scheme

of the extraction procedure is presented in Figure 1. The samples were filtered immediately after sampling (Figure 1, step 1). Large-volume samples (>10 L) were passed through thoroughly rinsed Nucleopore polycarbonate filter cartridges (sequentially through 3 μm , 1 μm , and 0.2 μm pore sizes) and smaller volumes (<10 L) through a sequence of Whatman GMF and GF/F inline glass fiber filters (precombusted at 450°C for 5 h) using a peristaltic pump and Bioprene peristaltic tubing. Samples were then acidified to pH 2 with HCl (32%, p.a.) to increase the extraction efficiency for organic acids and phenols (Figure 1, step 1). Precipitation of DOM due to acidification was not observed for the samples considered in this study. To avoid overloading and breakthrough, different cartridge sizes were chosen depending on sample volume and DOC concentration. Not more than 10 L sample or 2 mmol DOC was passed through 1 g of sorbent.

According to the manufacturers guidelines, the cartridges were rinsed with 1 cartridge filling of methanol (p.a.) immediately before use (Figure 1, step 2). For the DOM adsorption, the seawater samples were passed through the cartridges with the help of a peristaltic pump or gravity at flow rates not exceeding 40 mL min⁻¹ (Figure 1, step 3). The SPE cartridges were attached to the sample bottles with Teflon tubings (ID 2 mm) and Luer adaptors. Before elution of DOM, the cartridges were rinsed with at least 2 cartridge volumes (12–80 mL) of 0.01 M HCl for complete removal of salt (Figure 1, step 4). Sorbents

**Fig. 1.** Scheme for the isolation of SPE-DOM from seawater.

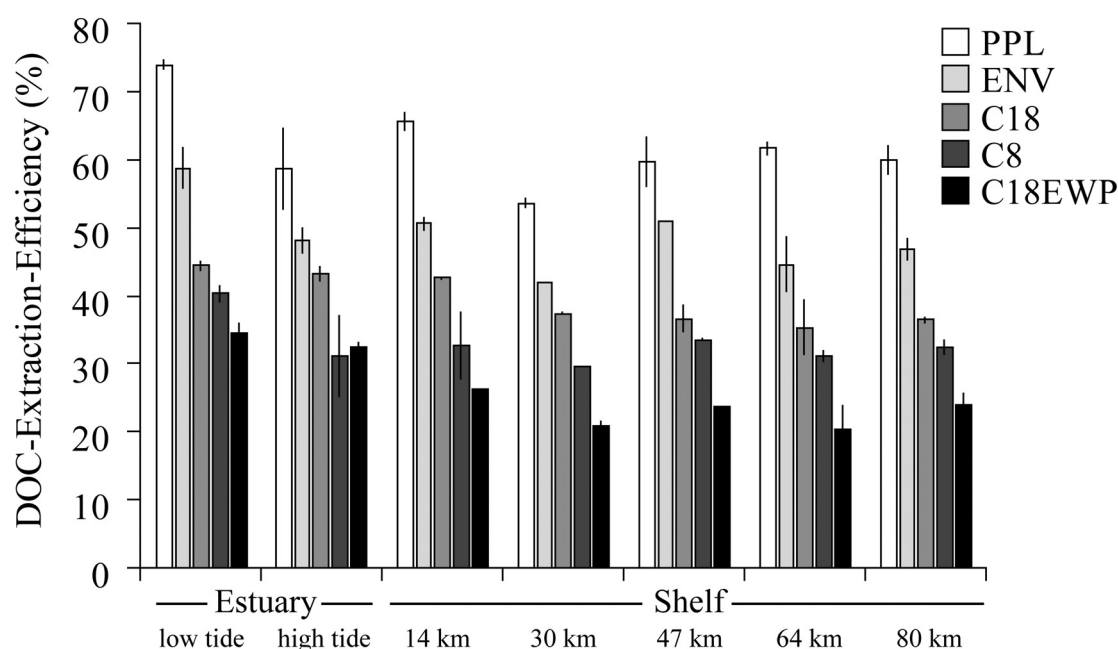


Fig. 2. Extraction efficiencies for the isolation of SPE-DOM at 1°S in the Maracaçumé Estuary (low and high tide) and on the adjacent North Brazil shelf (distance offshore, km) using different sorbents. Average values and standard deviation.

were dried (Figure 1, step 5), and DOM was immediately eluted with 1 cartridge volume (6–40 mL) methanol at a flow rate of $<2 \text{ mL min}^{-1}$ directly into muffled glass ampoules (Figure 1, step 6). Whereas silica-based sorbents were kept wet at all stages, the polymer-based sorbents were dried with nitrogen gas or air for about 5 min before elution, according to the manufacturer's guidelines. The elutes were stored at -20°C in the glass ampoules until further analyses.

Assessment

Blank determination—Blanks for each sorbent were obtained by extracting ultrapure water and artificial seawater (35 g NaCl L^{-1}) with the same procedures and volumes as for the samples. DOC and total dissolved nitrogen (TN) were determined by high-temperature catalytic oxidation (HTCO) with a Shimadzu TOC/TN analyzer and a modified MQ Scientific (DOC only, model MQ-1001; Peterson et al. 2003). The relative standard deviation of the method and each run was $<3.5\%$, and the detection limit was $5 \mu\text{M}$ DOC and $0.5 \mu\text{M}$ TN. The method was repeatedly checked with seawater consensus reference material (DOC-CRM; University of Miami). The procedural blanks of the various sorbents did not contain detectable amounts of DOC or TN, with the exception of C18OH elutes. This non-endcapped version of the silica-based C18 sorbent was very unstable at pH 2, and even blanks obtained from nonacidified water contained more DOC than seawater. This sorbent was thus disregarded for further analyses.

Extraction efficiencies of the different sorbents—All sorbents were tested with the 1-g cartridges on the North Brazil shelf off the Maracaçumé Estuary (1°S , 46°W). The small fishing cutter

(~10 m length), hired for this experiment, was ideal to test the applicability of the method in the field under rough sea conditions. Surface samples were collected in duplicate (20 L each) from 6 stations along a transect from the mangrove-fringed coast to 81 km offshore. Salinities along the transect ranged from 34.1 to 35.1. In the estuary, samples were taken at both high and low tide. Samples were filtered through Nucleopore filter cartridges and acidified. The filters were rinsed with at least 5 L sample before use. Aliquots were sealed into muffled glass ampoules and stored at -20°C for DOC analysis. DOC concentrations ranged from $163 \mu\text{M}$ in the estuary (low tide) to $73 \mu\text{M}$ offshore. Near-shore concentrations were elevated mainly because of the contribution of terrigenous DOM from mangroves (Dittmar et al. 2006).

For extraction, 0.5 L of each sample was filtered, acidified (pH 2), and extracted in duplicate. Selected samples were extracted without acid addition and at pH 12 (addition of NaOH), both of which caused a reduction of extraction efficiencies by about two-thirds compared with pH 2. Precipitated salts (at pH 12) were included in the extraction procedure. To determine the efficiency to extract SPE-DOM, DOC was quantified in the filtered water samples and dried solid-phase extracts (elutes), which were redissolved in ultrapure water. The elutes were dried either under N_2 at room temperature or in a freeze-drier. Extraction efficiencies were calculated as the DOC concentration in the methanol extract divided by the concentration factor and the DOC concentration in the corresponding seawater sample.

The extraction efficiencies varied considerably between the different sorbents (Figure 2). All silica-based sorbents were less

Table 2. Extraction efficiencies (average values and standard deviations) for the isolation of SPE-DOM using PPL cartridges in different marine environments, and relevant sample characteristics and extraction parameters.

Location	Number of samples	Efficiency, % of DOC	C/N of SPE-DOM	DOC in seawater, μM	Extraction volume, L	Elution volume, mL	Cartridge size, g
North Brazil shelf and coastal zone	14	62 ± 6	21 ± 1	86 ± 16	0.5	6	1
Apalachicola River and tributaries	3	62 ± 6	33 ± 2	212 ± 10	1	6	1
Apalachicola salt marshes	50	65 ± 6	32 ± 4	379 ± 111	1	6	1
Gulf of Mexico deep sea	4	43 ± 2	25 ± 0	49 ± 4	4	6	1
Weddell Sea (surface to bottom)	8	43 ± 5	27 ± 1	45 ± 5	50	40	5

efficient than the styrene divinyl benzene polymer sorbents. The C18EWP silica-based sorbent was the least efficient of all sorbents; on average $26 \pm 5\%$ of bulk DOC could be isolated by this type. The reason for this low efficiency is probably the wide pores of C18EWP (500 Å) compared with those of the other silica sorbents (60 Å). More than 70% of DOC is <1 kDa (Benner et al. 1997, Dittmar and Kattner 2003), and the predominantly small molecules in the ocean are presumably more efficiently retained by a sorbent with smaller pores. C18 was slightly more efficient ($39 \pm 4\%$) than C8 ($33 \pm 4\%$), which could be due to the higher carbon density and thus higher density of adsorber sites and enhanced hydrophobic character of C18 compared to C8.

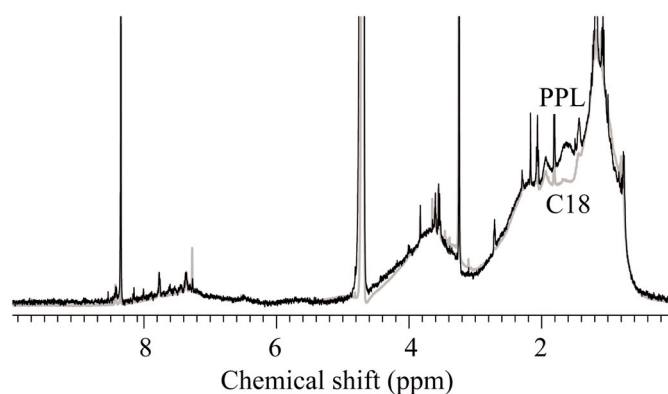
The highest extraction efficiencies of DOC were achieved with the styrene divinyl benzene type sorbents, ENV ($49 \pm 5\%$) and in particular PPL ($62 \pm 6\%$). These sorbents are made for the extraction of a wide range of highly polar to nonpolar substances from large volumes of water. PPL has a smaller pore size (150 Å) than the overall similar ENV sorbent (450 Å), which is probably the reason for the higher extraction efficiency of DOC by PPL. Extraction efficiencies were consistently high for PPL on the Brazilian shelf, and no significant trend was observed from near-shore to offshore (Figure 2).

In comparison, ultrafiltration isolated 23% of DOC at the outermost station, which is similar to values reported for the open ocean (e.g., Benner et al. 1997, Benner and Opsahl 2001). Ultrafiltration (1 kDa cutoff) was performed with a Millipore ProFlux M12 and an Amicon S3Y1-Module. The samples (40 L) were filtered (0.2 μm) before ultrafiltration and then concentrated to 350 mL. To remove salts, the concentrates were diafiltered 4 times with ultrapure water. The performance of the ultrafiltration was confirmed with polystyrene sulfonate polymer standards. Blanks were obtained from the filtration of ultrapure water and artificial seawater (see above). To test how much the UDOM and SPE-DOM fractions overlap, UDOM was redissolved in water and extracted with PPL. Forty-six percent of ultrafiltered DOC was extractable with PPL. Hence, by combining ultrafiltration with PPL extraction, the overall extraction efficiency for DOC out of seawater could be increased by approximately 10%.

Compositional characteristics of SPE-DOM extracted via PPL and C18—PPL was more efficient than C18 to extract organic

nitrogen components from seawater (North Brazil shelf and coastal zone). The molar C/N ratio of the PPL extracts (21 ± 1 on average; Table 2) was similar to bulk DOC/DON ratios (17 ± 3 on average). The C/N ratio of DOM extracted with C18 was significantly higher (37 ± 2 on average). C18 was more selective for terrigenous components than PPL. DOM on the North Brazilian shelf is a mixture of marine ($\delta^{13}\text{C}$ -21 to -22‰) and mangrove-derived ($\delta^{13}\text{C}$ -28 to -31‰) DOM (Dittmar et al. 2006), and the stable carbon isotope ratios ($\delta^{13}\text{C}$) of the PPL extracts were on average -23.4‰ , and of the C18 extracts, -24.8‰ . The selective extraction of terrigenous components by C18 is consistent with the higher C/N ratios compared with PPL. Stable carbon isotopes were determined in the solid-phase extracts after high-temperature flash combustion (Carlo Erba NC2500) on an isotope-ratio mass spectrometer (Finnigan Delta XL IRMS); for details see Dittmar et al. (2006).

Liquid state ^1H NMR spectra (Figure 3) revealed a remarkable similarity in molecular composition of the PPL and C18 extracts. NMR spectroscopy was performed with a Bruker DMX 500 spectrometer at 303 K in 0.1 N NaOD of CD_3OD (Dittmar et al. 2006, Hertkorn et al. 2006). Bulk chemical properties and detailed structural information of the 2 SPE-DOM spectra are very similar. For instance, the aromatic section of the proton chemical shift (>6 ppm), which is most indicative for terrigenous components, was present at similar proportions in both extracts. This overall similarity indicates

**Fig. 3.** ^1H NMR spectra of estuarine SPE-DOM from North Brazil isolated from seawater with C18 (gray line) and PPL (black line) sorbents.

that the fraction of DOM that is extractable only with PPL but not with C18 covers almost the entire range of compound classes. Major differences were observed only at a chemical shift range between 1 and 2 ppm, which comprises partially functionalized aliphatics. The higher N content of the PPL extracts compared with the C18 extracts may therefore be partially attributed to a higher proportion of amine functional groups rather than proteins or heterocycles.

Application of PPL in different marine environments—PPL proved to be the most efficient sorbent for the extraction of DOM from seawater as tested with samples from the North Brazil shelf. As indicated by the compositional analyses, C18 isolated a fraction of DOM that was also isolated by PPL. In addition, PPL isolated a nitrogen-rich group of molecules (probably amines of planktonic origin).

The performance of our method and the extraction efficiency of the PPL sorbent were further examined by using terrigenous and marine-dominated seawater samples from the Gulf of Mexico and Antarctica (Table 2). A total of 50 samples (1 L each) were taken at the surface and at 2 m water depths during hourly sampling over a tidal cycle in a creek in Apalachicola Bay salt marshes (Northern Gulf of Mexico). Three additional samples were collected from Apalachicola River and tributaries. In the creek, salinity ranged from 7 (low tide) to 19 (high tide), and DOC concentrations ranged from 227 μM (high tide) to 700 μM (low tide). In the rivers, salinity was zero, and DOC concentrations varied between 206 and 224 μM . SPE was performed through gravity on 1-g cartridges. From brackish marsh waters, $65 \pm 6\%$ of total DOC could be extracted, and from river waters, $62 \pm 6\%$ (Table 2). The different DOC concentrations and salinities did not significantly affect the extraction efficiency. The C/N ratio of SPE-DOM in the marsh and rivers was 33 on average, which is typical for terrigenous DOM.

Four deep-sea samples from the central Gulf of Mexico (DOC 46 μM) were taken at 600 m water depth on RV *Seward Johnson* with Niskin bottles mounted on a CTD. Four liters of each sample were filtered and extracted onboard. Four samples from the Weddell Sea were taken on RV *Polarstern* with Niskin samplers in vertical profiles covering the water column from the sea surface to the bottom (1600 m). DOC concentrations varied between 41 and 53 μM . The samples (200 L) were filtered onboard and each sample was extracted in parallel on four 5-g SPE cartridges (50 L sample per cartridge) using peristaltic pumps. The extraction efficiencies were uniformly $43 \pm 4\%$ independent of the sampling site. The C/N ratio was 26 on average, which is higher than reported open-ocean values for bulk DOM (Bronk 2002). No difference in extraction efficiency was found between surface and deep-water samples.

The significant and very consistent difference between coastal and open-ocean DOM indicates a higher extraction efficiency for terrigenous DOM than for marine DOM. Compared with ultrafiltration, the fractionation effect of SPE is relatively small. Benner and Opsahl (2001) reported UDOM

recoveries of 49% for the Mississippi and 22% for the adjacent Gulf of Mexico.

DOC recovery and overloading of PPL—To identify possible overloadings of the columns and breakthroughs, a second SPE cartridge was used in sequence for the extraction of deep-sea DOM in the Weddell Sea. Less than 1% of bulk DOC was retained on the second cartridge; thus the extraction capacity of the 5-g PPL cartridge had not been reached with 50 L seawater (equivalent to ~ 2.5 mmol DOC). A second elution step with acetone increased the overall extraction efficiency by $<3\%$, indicating that only a few compounds are retained on PPL which are not extractable with methanol. For specific research questions that focus on the more hydrophobic fraction of SPE-DOM, however, the small acetone fraction might be worth considering. For the Gulf of Mexico deep-sea samples, the DOC recovery was determined. The recovery was calculated as the sum of DOC concentrations in the methanol extract (divided by the concentration factor) and permeate (seawater sample that had passed through the SPE cartridge), divided by the concentration in the original seawater sample. The recovery was $98 \pm 1\%$, which confirms the previous observations of the absence of procedural contamination and the full recovery through methanol elution.

Discussion and outlook

The development of a very practical and robust method that is able to extract $>60\%$ of coastal and $>40\%$ of deep-sea DOC opens new possibilities in marine chemistry. The PPL method is applicable to extract SPE-DOM without major instrumentation and can be performed under rough field conditions without electric power. A large number of samples can be processed in parallel. Operational costs can be reduced by reusing the cartridges. After a sequential rinse with 1 cartridge volume of acetone and methanol, no blank increase or decrease in extraction efficiency was observed. The method is also suitable to be included in automated sampling devices, e.g., for the autonomous extraction on moorings. PPL retains a major fraction of N-containing compounds, enabling us to characterize in greater detail the almost unknown cycling of dissolved organic nitrogen in the ocean. Furthermore, sample preparation can be directly aligned with and optimized to the subsequent chemical analyses. For example, high-resolution ^1H NMR requires samples free of H_2O or organic solvents containing ^1H . Deuterated solvents can be used for SPE, and NMR can be performed directly on the elutes without additional sample preparation. Another major advance of this method is the complete desalting of the sample, which is not possible by ultrafiltration or electrodialysis. The sensitivity of high-resolution cryogenic NMR probes is maximal with samples of low conductivity and can therefore be considerably improved compared with previous studies. SPE-DOM extracted via PPL was already characterized through a variety of molecular techniques, including lignin-derived phenols (Dittmar, unpublished), black carbon (Dittmar 2008), ^1H NMR (this study), and

ultra-high-resolution mass spectrometry (Koch et al. 2007). For the latter, SPE-DOM was directly injected into a Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS) without additional procedural steps.

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