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Proposed guidelines for solid phase extraction of Suwannee River dissolved organic matter

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ABSTRACT

This paper proposes improved guidelines for dissolved organic matter (DOM) isolation by solid phase extraction (SPE) with a styrene–divinylbenzene copolymer (PPL) sorbent, which has become an established method for the isolation of DOM from natural waters due to its ease of application and appreciable carbon recovery. Suwannee River water was selected to systematically study the effects of critical SPE variables such as loading mass, concentration, flow rate, and up-scaling on the extraction selectivity of the PPL sorbent. High-field Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy were performed to interpret the DOM chemical space of eluates as well as permeates and wash liquids with molecular resolution. Up to 89% dissolved organic carbon (DOC) recovery was obtained with a DOC/PPL mass ratio of 1:800 at a DOC concentration of 20 mg/L. With the application of larger loading volumes, low quantities of highly oxygenated compounds were retained on the PPL sorbent. The effects of the flow rate on the extraction selectivity of the sorbent were marginal. Up-scaling had a limited effect on the extraction selectivity with the exception of increased self-esterification with a methanol solvent, resulting in methyl ester groups. Furthermore, the SPE/PPL extract exhibited highly authentic characteristics in comparison with original water and reverse osmosis samples. These findings will be useful for reproducibly isolating DOM with representative molecular compositions from various sources and concentrations and minimizing potential inconsistencies among interlaboratory comparative studies.

INTRODUCTION

Dissolved organic matter (DOM) is a complex mixture of organic molecules with ~ 50% carbon content and various proportions of heteroatoms such as oxygen, nitrogen, phosphorus and sulfur.[1] DOM is one of the most abundant contributors to the global carbon pool, and is actively involved in key aquatic ecosystem processes,[2,3,4] such as biodegradation,[5] heterotrophic transformation process,[6] complexation with trace elements[7] and modification of bacterial metabolism.[8] Due to its pivotal role in the environment, a growing number of biologists, ecologists, chemists, geologists and bioinformaticians have conducted DOM characterization studies to decipher the global biogeochemical carbon cycling. [1-3]

DOM isolation is essential as it directly affects both dissolved organic carbon (DOC) recovery and the selectivity of recovered molecular structures, upon which all consecutive steps such as organic structural spectroscopy and data evaluation have to exclusively rely.[9-11] In the case of DOM characterization, consequences of erroneous sampling always exceed those resulting from inattentive analysis. Meanwhile, DOM isolation should provide high yield for providing representative materials with limited bias to ensure authenticity.[12-14] In sharp contrast to even the most complex mixtures of biomolecules extracted from living organisms, polydisperse biogeochemical supermixtures[1] such as freshwater and marine DOM from water bodies cannot be resolved into individual molecules as a result of the huge number (~10^6) of diverse molecules present.[1] Therefore, it is highly desirable to develop a reproducible and standardized DOM isolation method which provides representative fractions enabling large scale studies of DOM while minimizing the inconsistencies among laboratories.[15]

Suitable DOM isolation methods encompass reverse osmosis/electrodialysis (RO/ED) and ultrafiltration, both of which isolate DOM through membrane passage with physical pressure, which may also concentrate salts.[16-19] Solid phase extraction (SPE) has become a widely applied method for DOM isolation,[10, 20] and employs sample-, sorbent-, and solvent dependent interactions to temporarily retain DOM, which is subsequently eluted in commonly concentrated solutions. To date, two main SPE methods for DOM isolation are commonly used: simultaneous enrichment of a wide range of DOM compounds by a single

sorbent and stepwise selective fractionation of DOM using different sorbents.[20-28] Due to its ease of operation and the limited diversity of the interactions involved, the first category has been the most widely used SPE method for DOM isolation.

Since the 1980s, scientists have investigated different types of SPE sorbents to improve DOC recovery.[20] The classical XAD and improved DAX resins were the first generation sorbents for DOM isolation, which enabled a recovery of hydrophobic acids in the range of 19-90%.[20-22] These sorbents have been supplemented by the second generation of silica-based sorbents as a result of extensive labors and commercial availability.[18, 19, 25] More recently, polymer-based sorbents have been widely utilized due to their stability over wide pH ranges and simple extraction procedures. In particular, the sorbent of functionalized styrene divinyl benzene polymer[28, 29] (PPL) capable of extracting hydrophobic and certain polar compounds such as phenols, showed both appreciable DOM recovery and adequate depiction of the intrinsic DOM molecular diversity inherent to specific sources such as river, ground, lake and sea water.[29] In comparison with classical XAD-2 resins, which are also styrene divinylbenzene polymers polymers, contemporary PPL resin features a larger specific surface area (600 versus 300 m^2/g), and proprietary functionalization for the improved retention of polar compounds such as phenols. Unlike the silica-based sorbent C18 and classical XAD-8 resin, PPL resin isolated representative DOM components with an abundance of aliphatic groups, and was recommended for DOM extraction from natural waters.[30] Furthermore, in contrast to DOM isolated by means of reversed osmosis/electrodialysis (RO/ED) process and SPE with XAD sorbents, PPL-based SPE allowed the isolation of marine DOM with beneficial properties for both high-field Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) and nuclear magnetic resonance (NMR) spectroscopy detection while showing appreciable DOC recovery.[13, 31]

Despite approval of its use for DOM isolation and the critical dependence of all science developed from isolated polydisperse SPE-DOM, the DOM isolation parameters of SPE with PPL sorbent have not yet been systematically investigated. In this study, Suwannee River water was chosen due to its widespread

utilization in many previous studies, limited interference from inorganic constituents and its availability at higher DOM concentration than other natural waters. The objective of this study was to develop an optimized SPE method for DOM isolation with PPL cartridges, to define standardized DOM isolation and analysis conditions for different DOM sources and laboratories. The influence of critical SPE variables such as loading mass, concentration, flow rate and up-scaling on DOM recovery, composition and structure were systematically investigated in eluates, permeates and wash liquids (Figure 1) by means of DOC analysis, ultra-high resolution FTICR mass spectrometry and NMR spectroscopy. The latter two, information-rich detection methods provide the most direct relationships between the acquired data and molecular parameters available for DOM characterization to date.[1, 11, 13] Moreover, the SPE/PPL extract was compared with the authentic Suwannee River water (Suw-water) and the RO isolate from the International Humic Substances Society (IHSS SR natural organic matter reference, 2R101N, Suw-RO).

EXPERIMENTAL SECTION

Sample Preparation.

Suwannee River water was collected in May 2012 from the headwaters of the Suwannee River (30º48'14"N, 82º25'03"W) as it flows out from the Okefenokee Swamp, as described by Green et al..[32] After sampling, the water was immediately filtered with 0.47 µm GF/F glass fiber (Whatman, precombusted at 450 °C) and stored at 4 °C in the dark using acid-washed polyethylene bottles. These samples were then transported by air at ambient temperature to Germany (~20 h), and consecutively stored for several months in the fridge at 4 °C. In the lab, this water was acidified to pH 2 with HCl and subjected to commonly 100 mg cartridges with PPL sorbent (Agilent Bond Elut PPL; in case of up-scaling, the cartridge size ranged from 100 mg to 5 g; cf. text). Blanks were used with acidified Milli-Q water (HCl, pH 2). The SPE procedure was performed in triplicate according to Dittmar et al.[29] Samples were loaded

on the cartridges in Teflon reservoirs (60 mL) connected by Luer adaptors at fixed flow rates with a peristaltic pump. After loading the samples, cartridges were rinsed with 1 mL of pH 2 water (at the same ratio for up-scaling experiments). After the washing step, the cartridges were dried with nitrogen gas for 10 min and were then eluted with 1 mL methanol (at the same ratio for up-scaling experiments). The samples collected in the loading and washing steps and the final eluting steps were termed permeates, wash liquids and eluates, respectively (Figure 1). Following SPE, the permeates and wash samples were kept at -4 °C in the dark and the methanolic eluates were kept at -25 °C prior to further analysis.[33] FTMS and NMR analysis of the DOM solutions was performed immediately after sample workup; the DOM used for NMR characterization was kept under dry conditions at -25°C until analysis. The ^{1}H NMR spectra did not change visibly after the CD_3OD solutions had been stored for several months at -25°C in the dark.

DOC measurement.

DOC concentrations were determined by high temperature catalytic oxidation using a Shimadzu TOC-VCPN analyzer, according to Flerus et al..[34] Samples (500 µL water solutions for the permeate and wash liquids, and 100 µL for the eluates) were evaporated and re-dissolved in 1 mL of ultra-pure water for analysis.

FTICR MS analysis.

High field FTICR mass spectra were acquired using a 12 T Bruker Solarix mass spectrometer (Bruker Daltonics, Bremen, Germany) and an Apollo II electrospray ionization (ESI) source in negative ionization mode. Samples were diluted in methanol to a concentration of ~5 µg/mL, and then were injected into an electrospray source at a flow rate of 120 µL/h with a nebulizer gas pressure of 138 kPa and a drying gas pressure of 103 kPa. Spectra were first externally calibrated based on clusters of arginine in methanol (5 µg/mL), and internal calibration was systematically performed in the presence of natural

organic matter, reaching accuracy values lower than 500 ppb. The spectra were acquired with a time domain of four megawords over a mass range of m/z 150-1000 amu, and 500 scans were accumulated for each mass spectrum. Elemental formulas were calculated for each peak in a batch mode by using software developed in-house.[35]

NMR analysis.

All ^{1}H NMR spectra were acquired with a Bruker Avance III 500 spectrometer (B_0 = 11.7 T) at 283 K from re-dissolved solids in CD_3OD (99.95% ^{2}H; Merck) with Bruker standard pulse sequences using 2-2.5 mm Bruker MATCH tubes. The reference ^{1}H NMR chemical shift of HD_2COD was 3.3 ppm. ^{1}H NMR spectra were recorded under solvent suppression with pre-saturation and 1 ms spin-lock (*noesypr1d)*, 5 s acquisition time, 5 s relaxation delay (d1), typically 1024 scans, and 1 Hz exponential line broadening. ^{1}H NMR section integrals were obtained by using the software AMIX at 0.01 ppm resolution.

Hierarchical clustering.

The intensities of the peaks measured by FTICR MS were logarithmically transformed (prior to transformation, zeros were substituted by ones) and the R package “pvclust” was used to apply Hierarchical Clustering with Multiscale Bootstrap Resampling. Clustering of the samples was based on the Euclidean distance and Ward’s linkage, with au/bp confidence levels (%) provided (au = approximately unbiased p-value (given in red in the figure) and bp = bootstrap probability value). Hierarchical clustering among Suw-water, Suw-RO and SPE/PPL eluate was performed on Hierarchical Clustering Explorer due to the limited number of samples.

RESULTS AND DISCUSSION

Effect of loading mass.

Although the effect of loading mass on the SPE extraction selectivity of DOM is significant, no systematic evaluation has been performed so far. The widely applied SPE method for DOM isolation developed by Dittmar et al. provided the advisable volume and amount of seawater DOM on 1 g PPL cartridges, but no detailed experiments about appropriate conditions for SPE of freshwater DOM have been performed to date.[29] Minor et al. loaded 7.2 mg Lake Superior DOM (freshwater) and 4.16 mg Lester River Mouth DOM (freshwater) on 500 mg PPL cartridges. They obtained large variability in DOC recovery (31±3% and 57±6%, respectively),[27] which might be attributed to the compositional or structural variance of DOM or effects of overloading. The effect of loading mass in this study was evaluated in the following three ways: (1) at identical DOC concentration with different volumes, (2) at the same volume with different concentrations, and (3) at equal mass with different concentrations and volumes. First, at the original concentration of 80 mg/L DOC, different sample volumes (1.25-125 mL, corresponding to 0.1-10 mg of DOC) were loaded on 100 mg PPL cartridges, and the DOC recovery was followed (Figure 2A). Here, the DOC mass of the eluates increased linearly with loading volumes ranging from 1.25 to 25 mL. However, further increase in the volume decreased the DOC recovery. This trend was quite different from that of a classical breakthrough curve, which is independent of loading volumes after the breakthrough is reached.[36] This disparity resulted mostly from the heterogeneity of DOM, which covered a wide range of compounds, from polar to non-polar characteristics. The interactions among the polydisperse and heterogeneous mixture of DOM molecules and the PPL sorbent which occurred during the loading step were much more diverse than those defining the classical breakthrough curves originating from single compounds or certain groups of related compounds. It is conceivable that DOC is fractionated progressively during loading onto PPL sorbent with the least strongly adsorbed polar molecules gradually being replaced on the PPL as more sample is loaded onto the PPL. The DOC recovery of the

eluates increased slightly up to a 25 mL loading volume, and then decreased. The DOC recovery of the permeates showed the opposite trend whereas that of the wash liquids remained quite low. DOC blanks measured from each step were found below the detection limit of the instrument, and no relevant mass peaks except random noise peaks were observed in FTICR mass spectra and associated van Krevelen diagrams (data not shown).

Depending on the loading volume, the negative electrospray ionization FTICR mass spectra of the eluates provided two distinct sets of DOM molecules, demonstrating the dependence of the eluate DOM molecular composition on the DOM/PPL mass ratio employed in SPE (Figure 3). At larger loading volumes, certain constituents of highly oxygenated molecules (O/C: 0.5-0.97) were gradually replaced by less oxygenated ones (O/C: 0.4-0.6) which also showed higher DBE values, indicative of higher molecular unsaturation (Figure 3B and 3C). ^{1}H NMR spectra showed increased aliphaticity (δ_H: 0.5-1.9 ppm) at the expense of carboxylic rich aromatic molecules (CRAM) (δ_H: 1.9-3.1 ppm), and carbohydrate and methoxy groups (δ_H: 3.4-4.9 ppm)[12,13] at higher loading volumes (Figure 4). These changes in molecular composition were in agreement with the decrease in DOC of eluates at higher loading volumes as shown above and indicated progressive displacement of weakly bonded DOC constituents such as carbohydrates and functionalized aliphatics by less oxidized (i.e. oxygen-deficient) DOM molecules of higher aliphatic and aromatic content. Concomitant molecular changes in the fractions of permeates and wash liquids were shown in Figures S1, S2 and S3. At small loading volumes, compounds with high O/C ratios (0.6-0.97) as well as small molecular weight (mainly 180-400 m/z) were detected in the permeates, indicating these molecules had poor retention on the cartridges (Figure S1). With increasing loading volumes, compounds with lower O/C ratios and higher molecular weight could also be found in the permeates. Interestingly, the compounds found in permeates at large volumes showed classical DOM-like ^{1}H NMR spectra with rather contiguous and broad NMR resonances, representative of aliphatics, CRAM, carbohydrates, olefins and aromatics, whereas those obtained from small-volume experiments exhibited mainly aliphatic peaks with superimposed small NMR resonances (Figure S2A). Similar

trends were observed in the wash liquids, but with larger proportions of aliphatics and CRAM observed in the ^{1}H NMR spectra at large loading volumes. Taking into account the DOC recoveries and relative uniformity of the DOM compositions, 6.25 mL of SR DOM (0.5 mg of DOC mass) was chosen as the optimal loading volume and used for further experiments. At this volume, the mass ratio employed for DOM and PPL sorbent was 1:200. At ~50% carbon content of DOM, the optimum mass ratio of DOC and PPL sorbent accounted to 1:100, which was in near accordance with the ratio of 1-5% proposed for the retention of more uniform mixtures.[36]

As the sample volume and DOC concentration might influence the SPE extraction selectivity, additional experiments were performed at the same DOC mass (0.5 mg) but with different loading volumes and DOC concentrations. Here, only marginal variance in the DOC recovery of permeates, wash liquids and eluates was detected (Figure S4A). A rather congruent presence of aliphatic NMR resonances was observed in the permeates and wash liquids (Figure S2B and S3B). Near 89% common mass peaks were observed in all six FTICR mass spectra for the eluates (Figure S5A), in line with the distinctive congruence observed in the ^{1}H NMR spectra (Figure S6A). This indicated near uniform molecular compositions and structures at identical DOC loading mass, independent of the volumes and concentrations used for SPE. This finding is important for the practical application in specific ecosystems in which DOC quality and concentrations may vary substantially.[37]

Hence, the results above showed that DOC recovery and structures were quite uniform up to a DOC/PPL mass ratio of 1: 200. We then have investigated the effects of variable DOC concentration at a fixed volume (6.25 mL) of SR DOM solution. The highest DOC recovery (~89%) was obtained at a concentration of 20 mg/L, corresponding to a DOC/PPL mass ratio of 1:800 (Figure 2B). The FTMS-derived cluster dendrogram (Figure 5A) clearly showed two clusters at a 96% significance level: the eluate molecules obtained at larger concentrations were statistically different from those obtained at small concentrations. A large number of CHNO compounds were exclusively detected at larger concentrations (Figure 5B). The common eluate molecules that were present in all six different concentrations

were less numerous than those found at larger concentrations, and contained only ~ 20% CHNO compounds (Figure 5C). ^{1}H NMR section integrals showed a rather congruent abundance of key substructures at higher concentrations (≥ 20 mg/L) whereas a very considerable increase of aliphatic units was observed at low concentrations at the expense of both functionalized aliphatics (XCC**H**) and oxygenated aliphatics (OC**H**) (Table 1). Superimposed low amplitude lipid-derived NMR resonances were observed in the permeates and wash liquids (Figures S2C and S3C). Thus, an optimal DOC concentration of 20 mg/L was chosen for further experiments. However, in natural environments, the concentration of DOM varied greatly, typically ranging from 5 to 20 mg/L in freshwater with the exception of Suwannee River water (~84 mg/L at the time of sampling) and other peat-draining waters at ~80 mg/L.[27, 38, 39] Based on our results, dilution is suggested in the case of highly concentrated DOM such as sewages, and use of larger volumes of freshwater is recommended at low DOC content within the capacity of the cartridge for meaningful SPE-based isolation of DOC. Samples with high contents of suspended solids and high ionic strength require centrifugation after filtration and dilution, respectively.

Effect of flow rate

A proper flow rate is important for desirable DOC recovery during the SPE process, especially during loading and eluting steps. While a fast flow rate diminishes DOC recovery as a result of insufficient absorption of analytes on sorbents, a too slow flow rate lowers the overall productivity.[36] In our study, different flow rates were chosen in the range of 0.5-5 mL/min. Here, variable flow rates had no significant effect on DOC recovery (Figure S4B), FTICR mass spectra (Figure S5B) and NMR spectra (Figures S2D, S3D and S6B). These findings are encouraging as independence of DOC recovery, composition and structure with respect to the flow rate, and will facilitate a meaningful DOM isolation by means of SPE/PPL under demanding field conditions. Hence, a flow rate of 0.5 mL/min (2-3 seconds per drop) was kept for all further experiments.

Effect of up-scaling

The up-scaling of SPE conducted under the optimized conditions (20 mg/L DOC and 0.5 mL/min flow rate) with cartridges of different sizes, ranging from 100 mg to 5 g of PPL sorbent material (i.e. 100, 200, 500, 1000, 5000 mg; Table 1), resulted in a near uniform DOC recovery (87-89%; Figure S4C). The NMR spectra of the permeates and wash liquids showed mainly aliphatics and rather sharp resonances of oxygenated carboxylic acids (Figure S2E and S3E). Approximately 94% common masses were present in the FTICR mass spectra of all five eluates (Figure S5C), demonstrating the high reproducibility of the SPE procedure. In contrast, ^{1}H NMR section integrals (Table 1) already suggested a clear division of the five SPE-DOM eluates into (1) a uniform group of small cartridges (100-500mg) with higher aliphaticity and lower content of OC**H** groups (Table 1) and a uniform group of large cartridges (1g and 5g) with the opposite distribution. Here, methoxy groups (OCH_3; δ_H: 3.4-4.0 ppm) showed higher abundance in the case of larger PPL cartridges used (Figure S6C). These newly observed compounds were likely methyl esters, which might have been formed at the low pH associated with the increased residence time during SPE at larger scale up. The exposure of humic substances to high concentrations of methanol and traces (catalytic amounts) of strong acid has been found to initiate self-esterification.[33, 40]

Comparison of original Suwannee River water, IHSS reverse osmosis isolate and SPE extract

In order to assess the relationships between three well available types of Suwannee river organic matter, ^{1}H NMR spectra were recorded from the dried and re-dissolved authentic Suw-water, Suw-RO [IHSS reference sample of Suwannee River NOM (2R101N)] and SPE/PPL used in our study, all of which were isolated at the same time in May 2012.[32] To ensure maximum compatibility and to avoid distortions from differential solubility,[41] these organic matter isolates were dissolved in mixtures of CD_3OD and D_2O (50/50, v/v) such that the ^{1}H NMR spectra were acquired under identical conditions.

In agreement with previous reports,[10, 31, 42] Suw-RO showed a slightly higher recovery (94.2%) [32] than the PPL extract (89 %). Despite the slight variation in DOC recovery, a satisfactory overall congruence

of all three Suwannee River organic matter isolates was observed with respect to NMR line shape distribution (Figure S7) and the ^{1}H NMR section integrals (Figure S8), and difference spectra were acquired to reveal the dissimilarities among these materials (Figure S7). Here, the SPE/PPL extract showed a relatively higher contribution of aliphatic NMR resonances (δ_H: 0.5 – 2.2 ppm) than Suw-water and Suw-RO, albeit at however variable selectivity (Figure S7). A rather sharp peak at δ_H ~ 1.22 ppm may consist of contributions from long chain aliphatic compounds in the SPE eluate, which are less abundant in the two other isolates. This putative selectivity of the PPL sorbent towards hydrophobic and low molecular weight molecules in DOM has been previously reported.[30,42] Comparing Suw-water with Suw-RO, aliphatics and olefins were depleted in Suw-RO whereas CRAM and in particular carbohydrates and some methoxy groups (OCH_3) were more abundant in Suw-RO. Interestingly, common aromatic molecules (δ_H: 6.7 – 7.8 ppm) were also more abundant in Suw-RO than in Suw-water, with no obvious discrimination of electron-donating (δ_H < 7 ppm), electron-neutral (δ_H: 7.0 – 7.3 ppm) and electron-withdrawing (δ_H > 7.3 ppm) substituents.[43] The abundance of certain small molecules such as acetic acid (δ_H ~ 1.89 ppm; noticeable in both Suw-water and Suw-RO), and formate (δ_H ~ 8.3 ppm, depending on intrinsic pH; abundant in both Suw-water and Suw-RO), as well as other sharp NMR resonances was sample specific, but not decisive in the assessment of sample properties. However, tiny NMR resonances in the carbohydrate region (δ_H: 3.5 – 4.2 ppm) were distinctively different; in particular, Suw-RO exhibited patterns indicative of carbohydrates whereas the SPE/PPL sample showed a remarkable small contribution of methoxy groups (shaded box in Figure S7).

Hierarchical cluster analysis (HCA) grouped original Suw-water together with Suw-RO (Figure S8), suggesting that Suw-RO was more representative of the original water than the SPE/PPL extract, in accordance with previous reports. However, recognition of residual chemical exchange in our ^{1}H NMR spectra (Figure S7) placed the SPE eluate rather close to the Suw-water composition. The common discrimination of PPL-based SPE against carbohydrates does not overly apply in this study due to the limited abundance of carbohydrates in Suw-water.[32]

The remarkably large difference in the relative NMR resonance amplitude in the vicinity of the residual HDO resonance at δ_H ~ 5.0 ppm was initially surprising, and these effects remained under several different NMR acquisition conditions, including acquisition in pure CD_3OD solution. We presently attribute this phenomenon to the 800 MHz high-Q cryogenic probehead with inverse detection (i.e. the ^1H NMR coil is close to the sample) at exceptional sensitivity and frequency spread (chemical shift differences $\Delta\delta_{ij}$ between exchanging nuclei i and j show a large spread in frequency $\Delta\nu_{ij}$), in which residual chemical exchange between capable functional groups rather than differential abundance of olefins may primarily affect this region of chemical shift. Hence, we do not attribute high significance to the NMR resonance amplitude variations at δ_H: 4.5 – 6.5 ppm observed in these three samples.

NMR section integrals provided the relative quantities of the key substructures (Figure S8) among the three samples: general (CCC**H**; δ_H: 0.5-1.95 ppm) and functionalized (XCC**H**, X: O >> N > S; δ_H: 1.95-3.1 ppm) aliphatics contributed more than 70% of the total components, consistent with the previous DOM analyses.[12,44] General OC**H** units, such as carbohydrates, ethers, esters and alcohols, accounted for ~20%, whereas aromatics accounted for 4-5% and olefins less than 2%. As expected from the NMR difference spectra, the individual section integrals also varied in-between samples as well. Here, the SPE/PPL extract contained the highest percentage of pure aliphatics and lowest proportions of functionalized aliphatics, such as carboxyl-rich alicyclic molecules (CRAM) as well as OC**H** units. In contrast, Suw-RO was enriched in CRAM as well as carbohydrates and depleted of aliphatics. Alterations in olefin abundance were convoluted with effects of residual chemical exchange; however, it is likely that olefin abundance followed the order SPE/PPL < Suw-RO ~ Suw-water). The abundance of aromatics was nearly equal in Suw-RO and SPE/PPL extract and remarkably higher in Suw-RO than in Suw-water (Figure S7).

The count of FTMS-derived assigned molecular formulas for the three samples followed the order: Suw-RO > Suw-water > SPE/PPL, and ~ 75% of shared masses among the three samples were observed (Figure S9). Suw-RO showed the highest number of unique masses, whereas the SPE/PPL extract

showed the lowest. The average molecular weight ranged in the order Suw-RO > SPE/PPL > Suw-water (Table 1), and the Suw-RO sample was the most distinct from the other two based on elemental compositions, which was probably caused by the presence of high mass (m/z > 500) molecules of average H/C ratio (H/C ~1.25). To put the FTMS-derived molecular compositions into perspective, the respective atomic ratios of Suw-RO were compared with the respective elemental analysis from the IHSS database (http://www.humicsubstances.org/elements.html). With the exception of N- and S-containing molecules which appreared attenuated in mass spectra, a very good concordance was observed (Table S1). The small discrepancies observed likely resulted from ionization selectivity[45], which probably favored detection of aliphatic carboxylic acids with low O/C ratios, thereby explaining the small gain in H/C ratios and tiny decrease in O/C ratios.

Noticeable discrepancies in CHO and CHNO compounds among the three samples plotted in van Krevelen diagrams were observed in mainly two regions (Figure S10). The first region was in the highly oxygenated area with O/C ratios >0.9, for which fewer masses were found in the SPE/PPL extract. This was in line with the results described above, namely, that molecules with high O/C ratios could be detected in permeates and wash liquids. The second area was located in the more saturated section of lipids (O/C ratios: 0.2-0.3, H/C ratios: 1.2-1.6) where masses were depleted in the SPE/PPL extract. The loss of compounds in the first and second regions could be explained by their rather hydrophobic characteristics which adhere strongly to the PPL sorbent. These results were in agreement with those of other authors, who reported that SPE discriminated against certain major groups: fatty acids with high H/C and low O/C ratios and tannin-like compounds.[19] Also noteworthy was the presence of numerous oxygenated CHOS compounds at H/C ~1.3 and O/C ratios of ~0.6-0.9 in Suw-RO and of hydrogen-deficient CHOS compounds at H/C ~0.6 and O/C ratios of ~0.2-0.45 in Suw-water. In addition, the main dissimilarity denoted in the mass-edited H/C ratios was obtained for Suw-RO, which showed abundant mass peaks at both low and high mass (m/z: 150-250 and 650-730). Overall, FTMS-based clustering

analysis demonstrated that the SPE/PPL extract better resembled to Suwannee River water (Suw-water) than the Suw-RO sample (Figure S10).

CONCLUSIONS

The PPL-based SPE of DOM relies on interactions between polydisperse organic molecules of considerable structural variance and a modified styrene divinyl benzene type stationary phase, and is therefore prone to chemical selectivity dependent on extraction conditions, such as loading masses, concentration, flow rate as well as up-scaling. The combination of FTICR mass spectrometry and NMR spectroscopy allowed the effects of these conditions on the molecular composition and structure of DOM to be monitored. A near-maximum DOC recovery of 89% was realized at a DOC concentration of 20 mg/L and flow rate of 0.5 mL/min with a DOC/sorbent mass ratio of 1:800. High loading mass discriminated against highly oxygenated compounds, CRAM, carbohydrates and methyl esters. No significant effect of flow rate was observed. The increased residence time of acidic, methanolic DOM solutions on PPL cartridges may lead to DOM self-esterification. Comparison of the SPE/PPL extract with the original water and an RO sample showed that the SPE/PPL extract was highly representative in terms of DOM characteristics. This protocol for DOM isolation based on SPE with PPL allows for large-scale studies of DOM isolation while minimizing potential the inconsistencies between interlaboratory studies.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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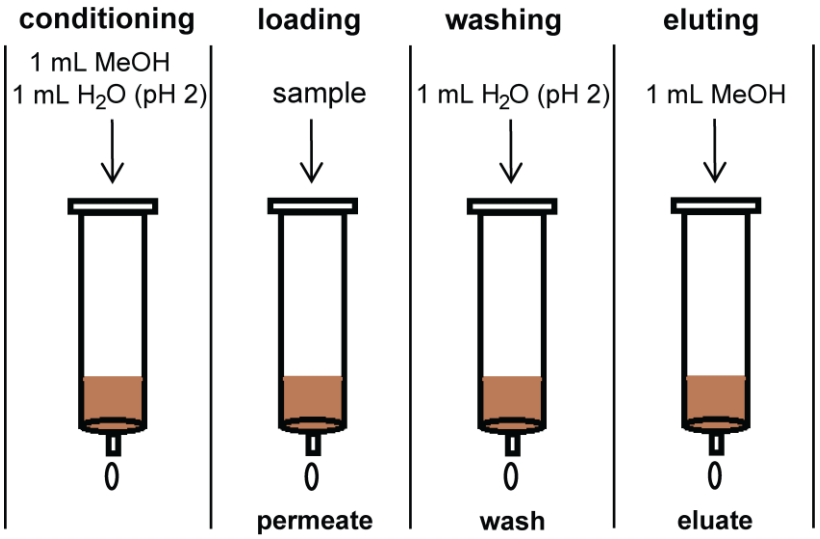

Figure 1. Scheme of the solid phase extraction of Suwannee River dissolved organic matter (SR DOM) with composition of investigated samples.

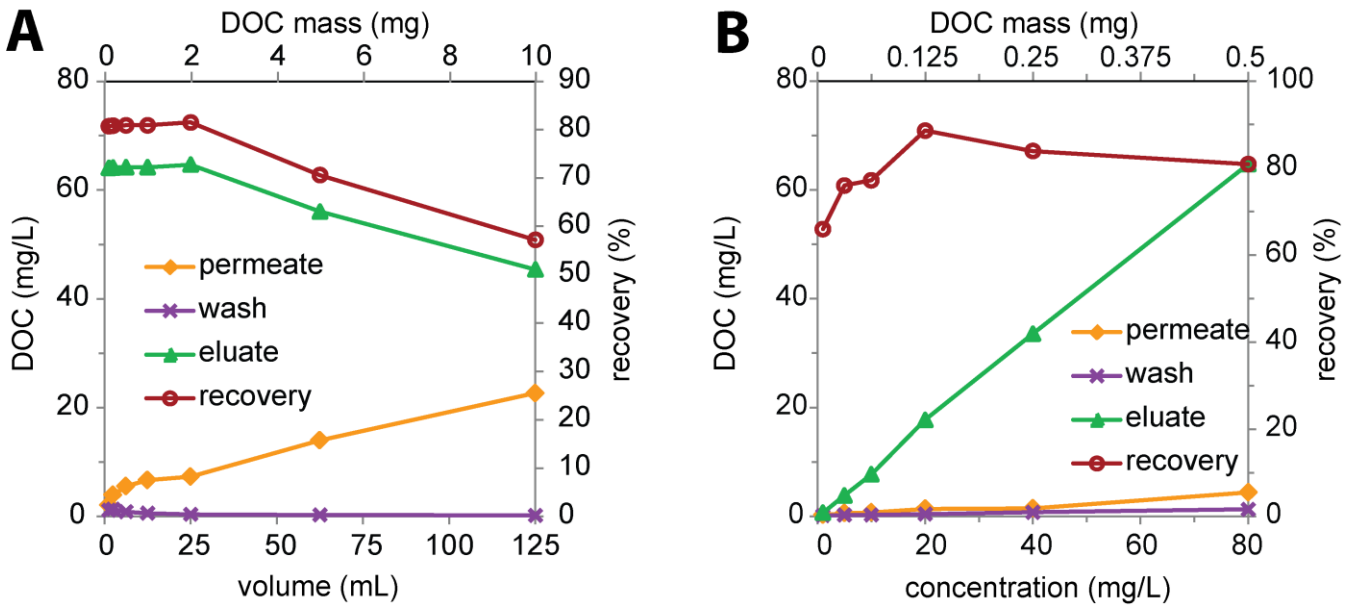

Figure 2. Values and recoveries of dissolved organic carbon (DOC) from Suwannee River SPE-DOM using 100 mg PPL cartridges with flow rate of 0.5 mL/min: (A) effect of loading volume at fixed DOC concentration (80 mg/L); and (B) effect of the DOC concentration at fixed loading volume (6.25 mL). The DOC values of the permeates, wash liquids and eluates are shown on the left y-axis, and the DOC recovery is presented on the right y-axis. The recovery was calculated according to the formulas: 100% × (DOC of eluate)/(original DOC).

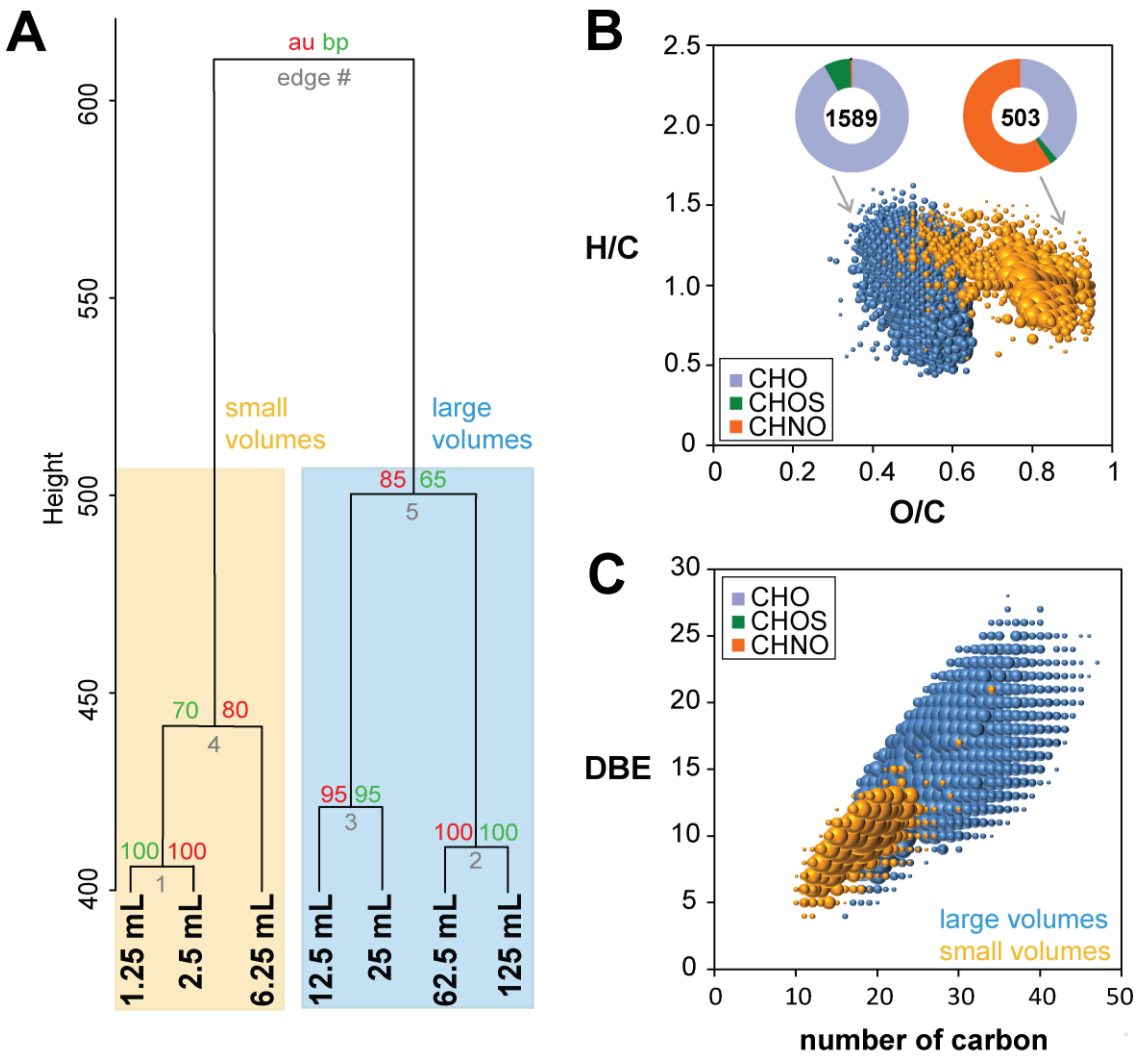

Figure 3. (A) Cluster dendrogram of eluates at different loading volumes (DOC: 80 mg/L; flow rate: 0.5 mL/min; 100 mg PPL cartridges), based on peak amplitudes from FTICR MS measurement. Boxes are drawn around the clusters with au ⩾ 80%; (B) Van Krevelen diagram of the masses uniquely detected at large and small loading volumes (< 10 mL; in orange). The two pie charts indicate the number of assigned molecular formulas; (C) Double bond equivalents (DBE) vs. number of carbon atoms. Identical color code corresponds to the shading in cluster dendrogram A. The bubble size corresponds to the intensities obtained by FTICR MS.

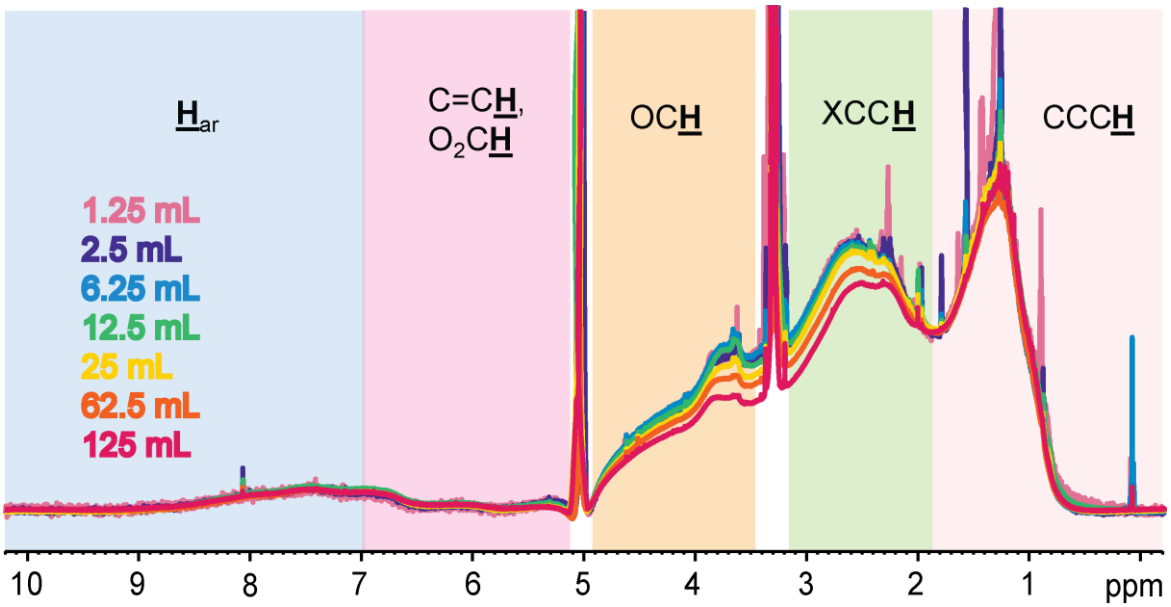

Figure 4. ^{1}H NMR spectra (500 MHz, CD_3OD) of the eluates obtained at different loading volumes (DOC: 80 mg/L; flow rate: 0.5 mL/min; 100 mg PPL cartridges). The spectra were normalized to identical total NMR integral.

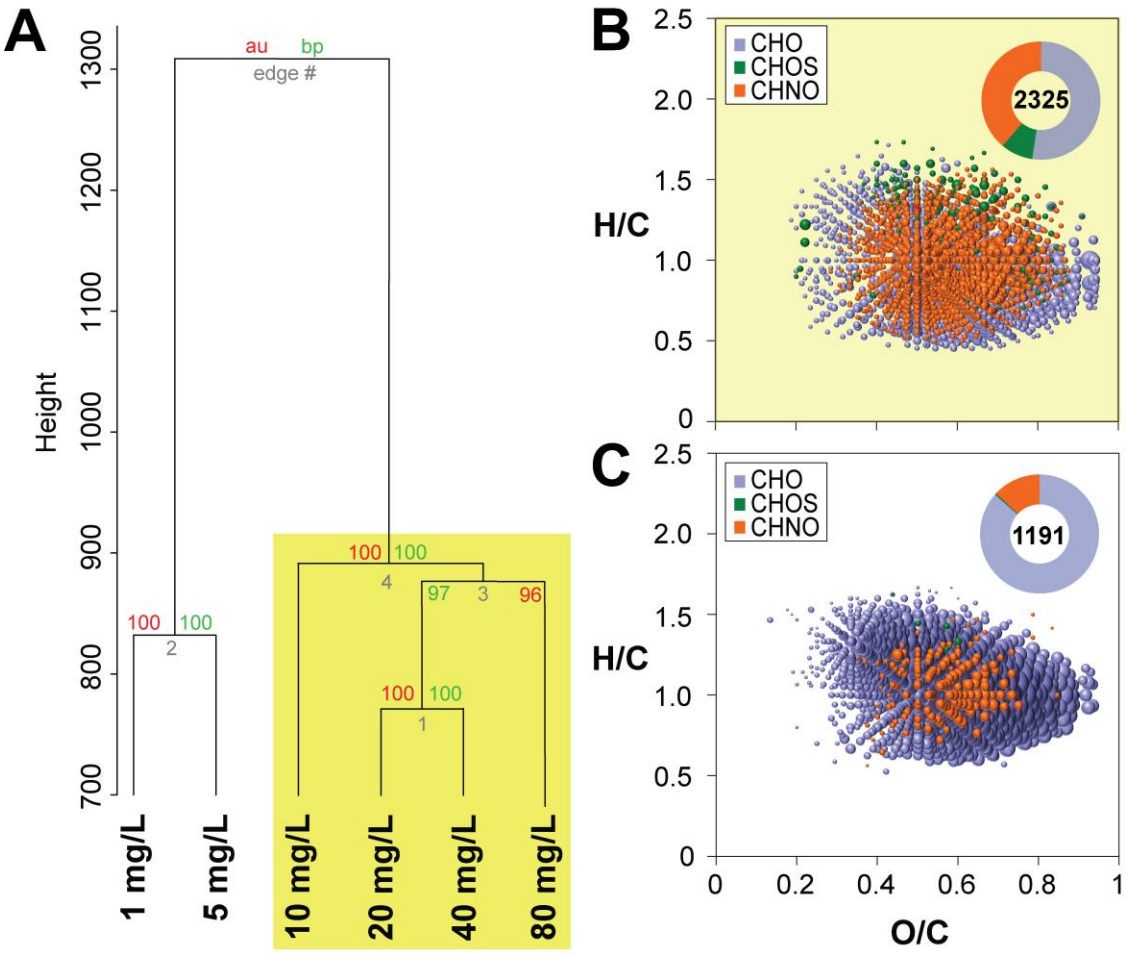

Figure 5. (A) Cluster dendrogram of eluates at different concentrations (volume: 6.25 mL; flow rate: 0.5 mL/min; 100 mg PPL cartridges), based on measured intensities from FTICR MS measurement. Boxes are drawn around the clusters with au $\geqslant$ 96%; (B) Van Krevelen diagrams of the masses uniquely detected at large concentrations (DOC: $\geq$ 10 mg/L), yellow. highlighted section in the cluster dendrogram A; (C) detected at all concentrations. Pie charts indicate the number of assigned formulas. The bubble size corresponds to the intensities obtained by FTICR MS.

Table 1. ^{1}H NMR section integrals for the key structures of eluates obtained under different different loading concentrations (volume: 6.25 mL; flow rate: 0.5 mL/min; 100 mg PPL cartridges) and different up-scaling (concentration: 20 mg/L; volume: 6.25 mL; flow rate: 0.5 mL/min). (*X: O, N, S)

	$\delta(^{1}H)$ ppm	9.5-7.0	7.0-5.3	4.9-3.6	3.1-1.9	1.9-0.5
	key structure	$\underline{H}_{ar}$	$\underline{H}C=C$, $H\underline{C}O_2$	$\underline{H}CO$	$\underline{H}CCX^{*}$	$\underline{H}CCC$
concentration (mg/L)	1	5.9	1.3	5.3	32.5	55.0
	5	5.5	1.1	4.7	35.1	53.6
	10	3.5	0.9	19.1	43.5	33.0
	20	3.8	0.4	17.9	41.4	36.5
	40	4.3	0.4	17.5	41.4	36.4
	80	4.5	0.2	17.3	41.5	36.4
up-scaling (mg)	100	3.8	0.4	18.0	41.3	36.5
	200	3.7	0.5	18.0	41.4	36.4
	500	3.7	0.5	18.0	41.3	36.5
	1000	3.7	0.5	22.4	40.9	32.5
	5000	3.7	0.5	22.5	41.0	32.3

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