Organic Geochemistry 41 (2010) 445–453

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Comparison of XAD with other dissolved lignin isolation techniques and a compilation of analytical improvements for the analysis of lignin in aquatic settings

Robert G.M. Spencer [a,b], George R. Aiken [c], Rachael Y. Dyda [b], Kenna D. Butler [c], Brian A. Bergamaschi [d], Peter J. Hernes [b,*]

[a] Department of Plant Sciences, University of California, Davis, One Shields Ave., CA 95616, USA
[b] Department of Land, Air and Water Resources, University of California, Davis, One Shields Ave., CA 95616, USA
[c] United States Geological Survey, 3215 Marine Street, Boulder, CO 80303, USA
[d] United States Geological Survey, 6000 J Street, Placer Hall, Sacramento, CA 95819, USA

ARTICLE INFO

Article history:
Received 10 October 2009
Received in revised form 23 January 2010
Accepted 8 February 2010
Available online 11 February 2010

ABSTRACT

This manuscript highlights numerous incremental improvements in dissolved lignin measurements over the nearly three decades since CuO oxidation of lignin phenols was first adapted for environmental samples. Intercomparison of the recovery efficiency of three common lignin phenol concentration and isolation techniques, namely XAD, C_{18} with both CH_3OH ($C_{18}M$) and CH_3CN ($C_{18}A$) used independently for priming and elution steps, and tangential flow filtration (TFF) for a range of aquatic samples including fresh, estuarine and marine waters, was undertaken. With freshwater samples XAD8-1, $C_{18}M$ and TFF were all observed to recover ca. 80–90% of the lignin phenols and showed no fractionation effects with respect to diagnostic lignin parameters. With estuarine and marine samples more lignin phenols were recovered with $C_{18}M$ and XAD8-1 than TFF because of the increased prevalence of low molecular weight lignin phenols in marine influenced samples. For marine systems, differences were also observed between diagnostic lignin parameters isolated via TFF vs. $C_{18}M$ and XAD8-1 as a result of the high molecular weight lignin phenols being less degraded than the bulk. Therefore, it is recommended for future studies of marine systems that only one technique is utilized for ease of intercomparison within studies. It is suggested that for studies solely aimed at recovering bulk dissolved lignin in marine environments that $C_{18}M$ and XAD8-1 appear to be more suitable than TFF as they recover more lignin. Our results highlight that, for freshwater samples, all three common lignin phenol concentration and isolation techniques are comparable to whole water concentrated by rotary evaporation (i.e. not isolated) but, that for marine systems, the choice of concentration and isolation techniques needs to be taken into consideration with respect to both lignin concentration and diagnostic parameters. Finally, as the study highlights XAD8-1 to be a suitable method for the isolation of dissolved lignin phenols from aquatic systems (statistically indistinguishable from $C_{18}M$, $P < 0.1$), lignin data representative of whole waters can be produced for IHSS reference materials or other XAD sample archives.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The analysis of lignin at the molecular level has been undertaken in a wide range of geochemical applications as a biomarker approach for investigating sources and biogeochemical processing or reactivity of organic matter (OM) in sediments and soils, and in limnological and oceanographic studies. Lignin phenols are abundant biopolymers that have emerged as important tracers of terrigenous dissolved organic matter (DOM) for several reasons. First, they are quantitatively significant in vascular plants and are unique markers for vascular plant DOM in aquatic systems (Opsahl and Benner, 1997; Hernes and Benner, 2006). Second, they have compositional characteristics that can be linked to important source information (Hedges and Mann, 1979; Opsahl et al., 1999). Finally, they contain compositional ratios that are sensitive to, and can record, biogeochemical processing (Hedges et al., 1988; Hernes and Benner, 2003; Spencer et al., 2009a).

Dissolved lignin has been examined in a broad range of aquatic settings with different aims. Studies focused on the distribution and cycling of terrigenous DOM in the oceans have frequently utilized lignin to investigate terrestrially derived DOM (Meyers-Schulte and Hedges, 1986; Opsahl and Benner, 1997; Hernes and Benner, 2006). Also, studies centered on the fate and reactivity of terrigenous DOM in the marine environment have employed lignin for process studies (Opsahl and Benner, 1998; Hernes and Benner, 2003) and to investigate the flux of terrigenous DOM to the oceans (Opsahl et al., 1999; Dittmar et al., 2001; Spencer et al., 2009b). In

* Corresponding author. Tel.: +1 530 752 7827; fax: +1 530 752 5262.
E-mail address: pjhernes@ucdavis.edu (P.J. Hernes).

0146-6380/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.orggeochem.2010.02.004

river systems, dissolved lignin measurements have been utilized to highlight changes in DOM sources vs. variation in hydrologic conditions (Dalzell et al., 2005; Hernes et al., 2008; Spencer et al., 2008). Furthermore, the influence of specific land cover contributions to riverine DOM and DOM source differences between tributaries have been investigated by using the unique features of lignin as a biomarker (Eckard et al., 2007; Kraus et al., 2008; Spencer et al., 2008).

Although the utility of lignin as a biomarker in geochemical studies investigating DOM has been widely demonstrated, the low concentrations of organic compounds typically found in DOM require the concentration and isolation of lignin prior to analysis. This is particularly true for marine samples, where concentrations of organic compounds are very low and inorganic salt contents are high. Two approaches have commonly been used to yield enough DOM for biochemical analysis, namely solid phase extraction (SPE) and ultrafiltration or tangential flow filtration (TFF). SPE techniques such as Amberlite XAD™ resins have been extensively used for the isolation of DOM since the 1970s from freshwater (Aiken et al., 1979, 1992) and marine environments (Stuermer and Harvey, 1974; Meyers-Schulte and Hedges, 1986). XAD resins are also used by the International Humic Substances Society (IHSS) to produce their widely used and well-studied aquatic standard reference materials (e.g. Suwannee River humic acid and fulvic acid). These IHSS reference materials are chemically characterized to a degree currently not possible with whole water samples and thus remain fundamental for controlled experiments relating DOM composition to its properties (Cory et al., 2007; Stubbins et al., 2008; Boyle et al., 2009). C_{18} silica-based SPE techniques have also been used for the isolation of DOM from fresh, estuarine and marine waters (Louchouarn et al., 2000; Hernes and Benner, 2003; Kim et al., 2003). SPE techniques are chemically selective (principally on the basis of hydrophobicity) for certain constituents of DOM, whereas ultrafiltration is selective, based on molecular size. TFF typically equipped with a 1 kDa cutoff has been used in a number of studies to isolate high molecular weight (HMW) DOM for the analysis of lignin in freshwater and marine samples (Opsahl and Benner, 1997; Mannino and Harvey, 2000; Benner and Opsahl, 2001; Dalzell et al., 2005).

Although TFF and C_{18} have largely been the preferred choice for concentrating dissolved lignin in recent studies, the widespread availability of archived XAD isolates represents a great potential resource for utilizing lignin biomarkers to further understanding of DOM cycling, provided XAD extraction efficiency for dissolved lignin approaches 100%. The aim of this study was to examine the recovery efficiency and possible fractionation effects on lignin phenols of the three common lignin concentration and isolation techniques (XAD, C_{18} and TFF) and to compare these with whole water samples concentrated by rotary evaporation (i.e. not isolated) for a range of freshwater samples. The three common lignin concentration and isolation techniques were also compared for an estuarine and a marine sample where rotary evaporation was not practical because of high concentrations of inorganic salts. It is important to examine any isolation-derived fractionation as this clearly could impact on comparison of data between studies utilizing different isolation and concentration methodologies, including those using IHSS reference materials isolated via XAD. Finally, we indicate numerous incremental improvements in making dissolved lignin measurements that have occurred since CuO oxidation of lignin phenols was first adapted for environmental samples (Hedges and Ertel, 1982).

2. Materials and methods

2.1. Water sample collection and processing

Water samples were collected at five locations with a range of DOM sources, composition and dissolved organic carbon (DOC) concentration. Samples were collected from organic rich blackwater in the Florida Everglades (two sites; FE1 and FE2), an organic rich river (Penobscot River, Maine – the 2nd largest river system in New England; PR), an estuarine site (Penobscot Bay, Maine; PB) and a marine site in the Gulf of Maine (GoM; Table 1). Large volume (27–230 l) water samples were filtered in the field with capsule filters (0.45 μm; Geotech Dispos-a-filter) pre-rinsed with at least 2 l of sample water. Samples were kept in the dark and shipped on ice to home laboratories where they were processed within 48 h.

2.2. DOC and $SUVA_{254}$ analyses

DOC measurements were performed with an OI Analytical Model 700 TOC analyzer (Aiken, 1992) for freshwater samples and with a Shimadzu Model TOC-V_{CPH} analyzer for marine and estuarine samples. For calculation of the specific UV absorbance at 254 nm ($SUVA_{254}$), UV–visible absorbance was measured with a Hewlett–Packard photodiode array spectrophotometer (model 8453) using a 10 mm quartz cell for all samples except the marine one, which required a 50 mm cell. $SUVA_{254}$ values were determined by dividing the UV absorbance at λ 254 nm by the DOC concentration and are reported in the units of l mg C^{-1} m^{-1} (Weishaar et al., 2003).

2.3. Isolation and extraction techniques

2.3.1. XAD

XAD isolation and concentration was conducted as described by Aiken et al. (1992). In brief, samples were acidified to pH 2 using HCl and passed first through a column of XAD8 resin, followed by a column of XAD4 resin. Volumes passed through XAD columns varied by sample, with 16 l for FE1 and FE2, 45 l for PR, 120 l for PB and 210 l for GoM. To remove chloride from the XAD resin, deionized water was passed through each column until the effluent specific conductance reached 750 μS/cm. Each column was then eluted with 0.1 N NaOH to obtain the XAD8-1 (frequently referred to as the hydrophobic organic acids or HPOAs) and XAD4-1 (frequently referred to as transphilic organic acids or TPIAs) fractions. Each eluate was desalted, hydrogen saturated and lyophilized. After elution with NaOH, a second elution was carried out with 0.01 N HCl through each column, followed by elution with CH_3CN to obtain the XAD8-2 (frequently referred to as the hydrophobic organic neutrals or HPONs) and XAD4-2 (frequently referred to

Table 1
Sample location, key and description.

Location	Key	Latitude	Longitude	Salinity	DOC (μM)	$SUVA_{254}$ (l mg C^{-1} m^{-1})
Florida Everglades 1	FE1	26.3596	−80.3705	Freshwater	3080.8	3.7
Florida Everglades 2	FE2	25.9742	−80.6689	Freshwater	1773.5	3.5
Penobscot River	PR	44.8266	−68.6966	Freshwater	1090.8	3.8
Penobscot Bay	PB	44.3583	−68.8417	27.2	183.2	2.9
Gulf of Maine	GoM	43.7408	−67.2800	33.1	83.3	1.2

as transphilic organic neutrals or TPINs) fractions (Fig. 1). Residual HCl was removed from the CH_3CN eluates by rotary evaporation to near dryness followed by addition of 300 ml CH_3CN and rotary evaporation to near dryness. This process was repeated (3×), after which sufficient distilled H_2O was added to re-dissolve the isolate; this solution was lyophilized.

2.3.2. C_{18}

C_{18} isolation and concentration utilized a modified version of that described by Louchouarn et al. (2000) and Hernes and Benner (2003). Two sizes of C_{18} cartridges were used, depending on initial sample DOC concentration. High-DOC freshwater samples (FE1, FE2 and PR) utilized HyperSep C_{18} cartridges (Thermo Scientific, 500 mg resin/3 ml cartridge volume) and low-DOC estuarine and marine samples (PB and GoM) utilized Mega BE C_{18} cartridges (Varian 10 g/60 ml). All samples were acidified to pH 2.5 with 12 N H_2SO_4 and an appropriate volume of CH_3OH was added, equal to 2% of the sample volume. Various manufacturers recommend CH_3OH concentration of 0.5–2% in samples to maintain C_{18} column conditioning, and the absence of this step in the original methods paper (Louchouarn et al., 2000) and previous studies (e.g. Hernes and Benner, 2002, 2003, 2006) may have led to decreased extraction efficiency. Subsequent to acid and CH_3OH addition, samples were passed (2×) through C_{18} cartridges (the second time to capture the 5–20% that passes through the first time (Hernes and Benner, 2006); using peristaltic pumps at a flow rate of ca. 5 ml min^{-1} for FE1, FE2 and PR and of ca. 75 ml min^{-1} for PB and GoM. Volumes passed through C_{18} cartridges varied by sample, with 250 ml for FE1 and FE2, 500 ml for PR and 10 l for PB and GoM. C_{18} cartridges are activated using polar solvents such as CH_3OH or CH_3CN, which were also used to elute sorbed DOM. Both CH_3OH and CH_3CN were examined and, immediately before extraction of water samples, The cartridges were cleaned and primed by rinsing with either CH_3OH or CH_3CN, followed by a small volume of acidified (pH 2.5 using 12 N H_2SO_4) Milli-Q water. The cartridges were then eluted with either CH_3OH or CH_3CN and the sample dried under vacuum centrifugation.

2.3.3. TFF

Ultrafiltration of DOM to obtain the HMW fraction was carried out with a tangential flow ultrafiltration system (Amicon DC-10 l) with a polysulfone membrane (S10Y1; 1 kDa nominal cut off). The transmembrane pressure was optimized at ca. 10 psi, which is similar to that used in a range of previous studies (Buesseler et al., 1996; Benner et al., 1997; Ahad et al., 2006) and was maintained in all experimental work. The membrane was thoroughly cleaned before use, and between all samples (Gueguen et al., 2002). Following cleaning, it was preconditioned with sample water as this has been shown to reduce contamination and minimize losses to the membrane as a result of sorption (Gueguen et al., 2002). For all samples, a constant concentration step (10 l filtered water to 1 l, i.e. concentration factor of 10) was used. The mass balance of fractionation of DOC was checked for every sample and ranged from 97.7% to 99.2% (mean 98.8%, $n = 5$), indicating little sorption to, or contamination from, the TFF system. Replicate mass balances conducted on duplicate riverine samples also showed consistency of fractionation with the TFF system. For the estuarine and marine samples (PB and GoM), after concentration the HMW fraction was diafiltered with Milli-Q to reduce the high inorganic salt content, which resulted in loss of ca. 3% of the HMW DOC. The desalted HMW samples and the freshwater HMW samples were then dried under vacuum centrifugation.

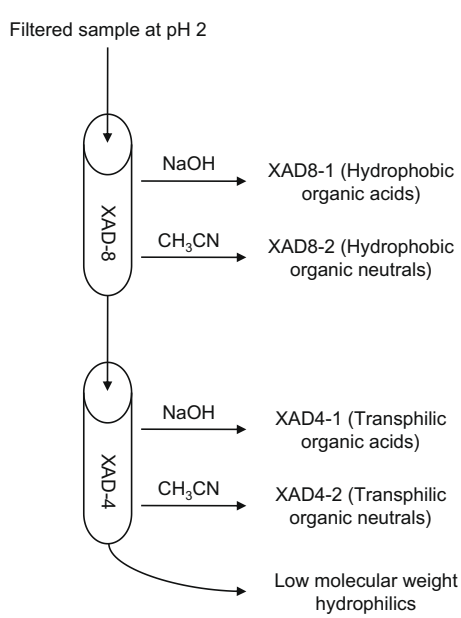

Fig. 1. Schematic diagram of isolation procedure using XAD resins.

2.3.4. Whole water samples

Whole water samples (250–500 ml) were acidified to pH 2 with 12 N HCl to minimize precipitation and then rotary evaporated to ca. 3 ml. The concentrates were transferred to Monel reaction vessels (Prime Focus, Inc.) and dried under vacuum centrifugation in preparation for lignin analysis. For all four isolation methods (XAD, C_{18}, TFF and rotary evaporation) replicate samples were run and mean sample deviation within each method was ⩽10% for marine samples and ⩽3% for freshwater samples. System blanks were also examined for all four isolation and extraction procedures and were below detection limits.

2.4. Lignin phenol analysis

Six lignin phenols were quantified for all samples and included three vanillyl phenols (vanillin, acetovanillone and vanillic acid) and three syringyl phenols (syringaldehyde, acetosyringone and syringic acid). Additionally, two cinnamyl phenols (*p*-coumaric acid and ferulic acid) were quantified solely for the freshwater samples (FE1, FE2 and PR). In marine samples, the cinnamyl phenol concentrations are not usually reported because of significant non-lignin sources (Hernes and Benner, 2002). Here, lignin concentrations of the three vanillyl phenols and the three syringyl phenols are abbreviated as Σ_6 and, where the two cinnamyl phenols are also included, lignin concentrations are abbreviated as Σ_8. Similarly, carbon-normalized yields (normalized to the total DOC of the sample, i.e. not the DOC of the isolated fraction) of six and eight phenols are abbreviated to Λ_6 and Λ_8 respectively, with units of mg (100 mg OC)$^{-1}$.

Lignin phenols were measured for all samples, utilizing the CuO oxidation method of Hedges and Ertel (1982), with improvements to the methodology as summarized in Table 2. In brief, samples were purged with Ar, oxidized for 3 h at 155 °C in 8% NaOH (>10 min sparging to remove O_2) with a stoichiometric excess of CuO and addition of glucose to minimize superoxidation effects. After the oxidation step, cinnamic acid was added as an internal standard for the extraction procedure and samples were acidified to pH 1 with 12 N H_2SO_4, extracted (3×) with ethyl acetate, passed through Na_2SO_4 drying columns and dried under a gentle stream of ultrapure N_2. Following redissolution in pyridine, lignin phenols were silylated with *N*/*O* bis-trimethylsilyltrifluoromethylacetamide (BSTFA) and quantified using gas chromatography–mass spectrometry (GC–MS) with an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector (MSD)

Table 2
Improvements in lignin phenol methodology from the original technique (Hedges and Ertel, 1982; N/A not applicable).

Original technique (Hedges and Ertel, 1982)	Modification	Reference for modification	Notes
N/A	Addition of glucose to reaction vessel	Louchouarn et al. (2000)	Minimizes superoxidation effects in low-carbon samples
Overnight purging of samples and reagents in glove box	Direct sparging of reagent bottles (10 min) and purging of reaction vessels in purge block	Louchouarn et al. (2000) and Eckard et al. (2007)	Faster, simpler technique with no measurable difference
^{14}C *p*-hydroxyacetophenone internal standard with ethyl vanillin as external standard	Ethyl vanillin as internal standard	Opsahl and Benner (1995)	Eliminates steps, eliminates radioactive and carcinogenic reagents with no measurable difference
Ethyl vanillin as quantification standard	Cinnamic acid as quantification (internal) standard	Opsahl et al. (1999) and Hernes and Benner (2002)	Significant ethyl vanillin can be lost during laboratory processing in samples with limited matrix
Oxidation at 170 °C (external reaction vessel temperature)	Oxidation at 155 °C (internal reaction vessel temperature)	Goni and Hedges (1992)	More accurate and representative temperature control
Ethyl ether extraction	Ethyl acetate extraction	Goni and Montgomery (2000)	Substitutes highly explosive reagent for safer reagent, eliminates distillation step, slightly higher extraction efficiency of syringyl phenols
GC with flame ionization detector	GC with MSD	Opsahl and Benner (1997)	Increased sensitivity and eliminates co-elution problems
N/A	Five-point calibration curve with matching internal standard concentrations in all sample and calibration vials	Hernes and Benner (2002)	Eliminates non-linearity issues with changing internal standard concentrations
N/A	Quantification by interpolation between two calibration curves	This study	Minimizes quantification error associated with changing column characteristics as subsequent samples "dirty" front end of column

and DB5-MS column; 30 m × 0.25 mm i.d., using cinnamic acid as an internal standard and a five point calibration scheme (Hernes and Benner, 2002). All calibration mixtures were prepared with an identical concentration of cinnamic acid and sample dilutions for injection were prepared with the goal of matching the cinnamic acid concentration in the calibration mixtures. This is necessary to ensure that only non-linear behavior of the unknown compounds comes into play in the calibration curves and is not compounded by non-linear behavior of the internal standard. All calibration curves were generated with quadratic fits to the calibration standard responses. Sensitivity analyses conducted on the calibration curves showed that optimal quantification was achieved with a vanillin to cinnamic acid ratio of 4:1.

Calibration curves were generated before and after every 12 injections, all samples being quantified using both calibration curves and final reported amounts were calculated by weighted interpolation between the calibration quantifications. We have noted differences in quantification of individual phenols between the two calibrations by as much as 16% (primarily the acids) because of changing GC column conditions when injecting environmental samples, and in particular SPE samples like XAD and C_{18}. Restoring the column to optimal conditions requires venting the GC-MSD instrument, trimming 10–20 cm off the injection end of the column, then pumping down overnight. This represents a significant limitation on throughput for SPE samples if the column is trimmed as soon as compound responses vary by >5% (i.e. every 3–4 samples). Our calibration "sandwich" allows reasonable throughput while accurately correcting for changing compound responses. One blank was run for every ten sample oxidations and all samples were blank corrected. Blank concentrations of lignin phenols were low (30–50 ng) and for freshwater samples never exceeded 1% and for estuarine and marine samples 5%, respectively, of the total lignin phenols in an extract.

2.5. Statistical treatment

XAD8-1 and C_{18}M sample sets across all five sites were compared non-parametrically using both the Kolmogorov–Smirnov Two Sample test and the Wilcoxon Two Sample test at the $P < 0.1$ level (Sokal and Rohlf, 1981). Because of the high degree of uncertainty in standard deviation for $n = 3$, we report sample mean deviation (SMD) for averages across the three freshwater sites.

3. Results and discussion

3.1. Total XAD recovery of dissolved lignin phenols

For the five samples isolated using XAD, the majority of lignin phenols (Σ_8: 85.6–92.6%; mean 88.4%; $n = 3$ and Σ_6: 79.8–92.7%; mean 86.3%; $n = 5$) were in the XAD8-1 fraction (Table 3). Recovery in the XAD8-2 (Σ_8: 3.2–9.8%; mean 6.2%; $n = 3$ and Σ_6: 3.2–13.8%; mean 6.7%; $n = 4$) and XAD4-1 fractions (Σ_8: 3.2–6.3%; mean 4.7%; $n = 3$ and Σ_6: 3.0–13.8%; mean 6.7%; $n = 5$) were small but similar, while only ca. 1% of XAD-recovered Σ_8 and Σ_6 was in the XAD4-2 fraction (Table 3). Previous studies have also found that the majority of lignin phenols recovered using XAD (ca. 90%) are in the XAD8-1 fraction and have typically focused solely on this fraction with respect to isolation of DOM for lignin analysis (Ertel et al., 1986; Kraus et al., 2008; Spencer et al., 2008). Therefore, for the purposes of this study, only the XAD8-1 fraction was compared with the other isolation and concentration techniques. However, to examine total XAD recovery, the sum of lignin phenols in all fractions was assessed in comparison with whole water concentrated using rotary evaporation for the freshwater samples (Table 4; Fig. 2). For both Σ_8 and $\Sigma_6 > 90\%$ recovery was observed for the total of the XAD isolates, highlighting excellent recovery of lignin phenols with XAD resin (Σ_8: 91.1–97.6%; mean 94.2%; $n = 3$ and Σ_6: 94.3–98.2%; mean 96.7%; $n = 3$). This is somewhat inconsistent with previous studies that have compared XAD extracts from a mixed column of XAD2, XAD4 and XAD7 resins to whole water samples in which lignin concentrations were three times greater than in the XAD isolates (Kattner et al., 1999; Lobbes et al., 2000). In this study, XAD4 post XAD8 extraction captured ca. 4–7% of the total lignin vs. rotary evaporated samples, so XAD8 recovered by far the majority of the lignin phenols. Our results highlight the importance of resin types for maximizing recovery and clearly XAD8 is the best choice of the resins examined here for recovering lignin phenols.

Table 3
Lignin phenol concentration and carbon-normalized yield for the five samples in the four XAD fractions.

Key	XAD fraction[a]	Σ_6 (μg l^{-1})	Σ_8 (μg l^{-1})	Λ_6 [mg (100 mg OC)$^{-1}$]	Λ_8 [mg (100 mg OC)$^{-1}$]
FE1	XAD8-1	56.72	69.75	0.153	0.188
FE1	XAD8-2	3.51	4.47	0.009	0.012
FE1	XAD4-1	3.92	5.06	0.011	0.014
FE1	XAD4-2	0.87	0.98	0.002	0.003
FE2	XAD8-1	31.28	38.20	0.147	0.179
FE2	XAD8-2	1.09	1.31	0.005	0.006
FE2	XAD4-1	1.02	1.31	0.005	0.006
FE2	XAD4-2	0.35	0.42	0.002	0.002
PR	XAD8-1	46.93	50.42	0.358	0.385
PR	XAD8-2	5.41	5.83	0.041	0.044
PR	XAD4-1	2.42	2.67	0.018	0.020
PB	XAD8-1	2.73	–	0.124	–
PB	XAD8-2	0.47	–	0.021	–
PB	XAD4-1	0.22	–	0.010	–
GoM	XAD8-1	0.23	–	0.023	–
GoM	XAD4-1	0.04	–	0.004	–

[a] XAD8-1, XAD8-2, XAD4-1 and XAD4-2 refer to the classically defined hydrophobic organic acid, hydrophobic organic neutral, transphilic organic acid and transphilic organic neutral fractions respectively.

Table 4
Sum of lignin phenols in all XAD fractions compared to whole water concentrated with rotary evaporation (REV) for freshwater samples.

Site	Σ_6 (μg l^{-1}) REV total	Σ_8 (μg l^{-1}) REV total	Σ_6 (μg l^{-1}) XAD total	Σ_8 (μg l^{-1}) XAD total	Σ_6 XAD total recovery (%)	Σ_8 XAD total recovery (%)
FE1	66.21	88.13	65.02	80.26	98.2	91.1
FE2	35.79	43.91	33.74	41.24	94.3	93.9
PR	56.04	60.34	54.76	58.92	97.7	97.6

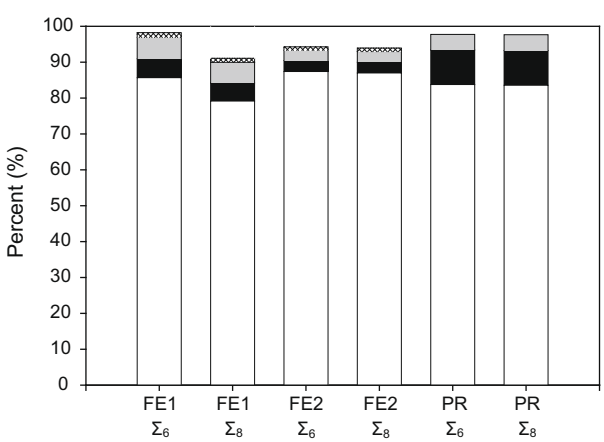

Fig. 2. Total XAD recovery of dissolved lignin phenols (Σ_6 and Σ_8) for freshwater sites vs. rotary evaporated sample. XAD8-1, fraction white; XAD8-2, fraction black; XAD4-1, light gray; XAD4-2, white with black crosshatch.

3.2. Recovery of dissolved lignin phenols with C_{18}, TFF and XAD8-1

For investigation of the recovery of lignin phenols with C_{18} SPE, two polar solvents (CH_3OH and CH_3CN) were tested for priming and elution of the cartridges. Cartridges primed and eluted with CH_3OH and CH_3CN are referred to here as C_{18}M and C_{18}A respectively. For the freshwater samples, recovery of lignin phenols was compared with whole water concentrated using rotary evaporation and, for C_{18}M, Σ_8 recovery was 76.2–91.1% (mean 86.0%; sample mean deviation, SMD 6.5%; $n = 3$) while Σ_6 recovery was 78.8–99.6% (mean 90.6%; SMD 7.9; $n = 3$; Tables 5 and 6; Fig. 3). Freshwater sample recovery with C_{18}A was considerably less efficient than with C_{18}M and ranged from 48.3% to 77.4% (mean 67.4%; SMD 12.7%; $n = 3$) and 48.7% to 82.8% (mean 70.3%; SMD 14.3; $n = 3$) for Σ_8 and Σ_6 respectively (Tables 5 and 6; Fig. 3). TFF Σ_8 recovery was 80.4–92.3% (mean 86.3%; SMD 4.0% $n = 3$) and for Σ_6 recovery was 80.0–93.8% (mean 88.8%; SMD 5.6%; $n = 3$) (Tables 5 and 6; Fig. 3). XAD8-1 recovery ranged from 79.1% to 87.0% (mean 83.2%; SMD 2.7%; $n = 3$) and 83.7% to 87.4% (mean 85.6%; SMD 1.3; $n = 3$) for Σ_8 and Σ_6 respectively. The results demonstrate very similar high recoveries of lignin phenols from freshwater samples with C_{18}M and TFF (Fig. 3). While XAD8-1 recovery of lignin phenols was slightly lower, SMDs for C_{18}M, TFF, and XAD8-1 all overlap (Table 6), indicating extraction efficiency is comparable. Furthermore, XAD8-1 recovery of lignin demonstrated the lowest variability of the four extraction methods, as evidenced by the low SMDs (Table 6). Considerably less lignin is recovered with C_{18}A than with any of the other methods, so it does not appear to be an appropriate technique for isolation of dissolved lignin phenols from freshwater.

The high inorganic salt contents of estuarine and marine samples preclude rotary evaporation as a concentration technique, so comparisons could only be made between the remaining techniques. Furthermore, non-lignin sources of cinnamyl phenols in marine DOM limits accurate measurement of lignin phenols to the six vanillyl and syringyl phenols (Hernes and Benner, 2002). The estuarine (PB) and marine samples (GoM) yielded similar dissolved lignin extraction efficiency, with XAD8-1 and C_{18}M recovering the most, and similar amounts of lignin in both samples (Table 5). In fact, across all five freshwater and marine sample sites, we found no significant difference between the distribution of the XAD8-1 vs. C_{18}M Σ_6 measurements ($P < 0.1$). As with the freshwater samples, C_{18}A showed the lowest recovery of lignin phenols from the marine samples, with only ca. 43–57% of the lignin recovered relative to XAD8-1 and C_{18}M. For PB and GoM samples, TFF recovered ca. 56–70% of the lignin vs. XAD8-1 and C_{18}M systems (Table 5). Reduced recovery of lignin from marine samples with TFF is unsurprising as LMW lignin phenols have been shown to make up a greater portion of the total dissolved lignin pool in mar-

Table 5
Isolation/extraction of dissolved lignin phenols with rotary evaporation (REV) $C_{18}M$, $C_{18}A$, TFF and XAD8-1.

Key	Isolation/extraction	Σ_6 (μg l^{-1})	Σ_8 (μg l^{-1})	Λ_6 [mg (100 mg OC)$^{-1}$]	Λ_8 [mg (100 mg OC)$^{-1}$]	C:V	S:V	$(Ad:Al)_v$	$(Ad:Al)_s$
FE1	REV	66.21	88.13	0.179	0.238	0.72	1.18	0.79	0.88
FE1	$C_{18}M$	65.96	80.25	0.178	0.217	0.70	1.21	0.77	0.86
FE1	$C_{18}A$	54.84	68.19	0.148	0.184	0.69	1.23	0.76	0.83
FE1	TFF	60.17	75.90	0.164	0.201	0.69	1.23	0.80	0.81
FE1	XAD8-1	56.72	69.75	0.153	0.188	0.68	1.19	0.82	0.82
FE2	REV	35.79	43.91	0.168	0.206	0.55	1.44	1.02	0.92
FE2	$C_{18}M$	28.22	33.45	0.132	0.157	0.53	1.45	1.05	0.98
FE2	$C_{18}A$	17.44	21.21	0.082	0.100	0.52	1.41	1.01	0.98
FE2	TFF	28.64	35.29	0.135	0.162	0.56	1.46	1.06	0.94
FE2	XAD8-1	31.28	38.20	0.147	0.179	0.54	1.44	1.07	0.92
PR	REV	56.04	60.34	0.428	0.461	0.11	0.41	1.29	1.03
PR	$C_{18}M$	52.35	54.76	0.400	0.418	0.09	0.38	1.30	0.99
PR	$C_{18}A$	44.45	46.22	0.339	0.353	0.10	0.37	1.33	1.00
PR	TFF	52.54	55.69	0.404	0.426	0.10	0.39	1.28	0.98
PR	XAD8-1	46.93	50.42	0.358	0.385	0.11	0.42	1.38	1.03
PB	REV	–	–	–	–	–	–	–	–
PB	$C_{18}M$	2.32	–	0.106	–	–	0.49	1.71	1.63
PB	$C_{18}A$	1.16	–	0.053	–	–	0.50	1.79	1.68
PB	TFF	1.54	–	0.073	–	–	0.47	1.65	1.53
PB	XAD8-1	2.73	–	0.124	–	–	0.47	1.73	1.67
GoM	REV	–	–	–	–	–	–	–	–
GoM	$C_{18}M$	0.23	–	0.023	–	–	0.37	2.01	1.88
GoM	$C_{18}A$	0.13	–	0.013	–	–	0.40	1.91	1.79
GoM	TFF	0.16	–	0.016	–	–	0.29	1.73	1.61
GoM	XAD8-1	0.23	–	0.023	–	–	0.42	1.92	1.82

Table 6
Recovery of lignin phenols from freshwater with $C_{18}M$, $C_{18}A$, TFF and XAD8-1 vs. rotary evaporated sample (sample mean deviation in parentheses).

Site	Isolation/extraction	Σ_6 % recovery	Σ_8 % recovery
FE1	$C_{18}M$	99.6	91.1
FE1	$C_{18}A$	82.8	77.4
FE1	TFF	90.9	86.1
FE1	XAD8-1	85.7	79.1
FE2	$C_{18}M$	78.8	76.2
FE2	$C_{18}A$	48.7	48.3
FE2	TFF	80.0	80.4
FE2	XAD8-1	87.4	87.0
PR	$C_{18}M$	93.4	90.8
PR	$C_{18}A$	79.3	76.6
PR	TFF	93.8	92.3
PR	XAD8-1	83.7	83.6
Mean	$C_{18}M$	90.6 (7.9)	86.0 (6.5)
Mean	$C_{18}A$	70.3 (14.3)	67.4 (12.7)
Mean	TFF	88.8 (5.6)	86.3 (4.0)
Mean	XAD8-1	85.6 (1.3)	83.2 (2.7)

ine systems vs. riverine samples (Hernes and Benner, 2003). Therefore, it is evident that size-based TFF will isolate progressively less lignin as salinity increases and the proportion of lignin in the HMW fraction decreases. Thus, for studies concerned with bulk lignin phenol concentration for marine systems, $C_{18}M$ and XAD8-1 are generally more appropriate techniques. However, utilizing TFF and $C_{18}M$ in tandem to measure both HMW and LMW lignin has proven very informative in understanding the relationship between size and reactivity for terrigenous DOM in estuarine and coastal environments (Hernes and Benner, 2002, 2003; Benner et al., 2005), highlighting the importance of matching up the method with the scientific questions of interest. Comparisons involving TFF are not always straightforward because of the micro-environmental conditions that alter membrane retention efficiency, such as ionic strength, (Kilduff and Weber, 1992; Cheryan, 1998) and the concentration factors used (Buesseler et al., 1996; Guo and Santschi, 1996). However, recent work has shown that ionic strength and variable DOC content have little impact on 1 kDa TFF membranes (Gueguen et al., 2002), so concentration factor seems a more likely potential source of any disparity between studies using TFF.

3.3. Fractionation of dissolved lignin phenols

Potential fractionation of lignin phenols with varying concentrations and isolation techniques impacts on how lignin data are interpreted for aquatic samples. Therefore, fractionation effects for freshwater samples were evaluated by comparing rotary evaporated samples to $C_{18}M$, $C_{18}A$, TFF and XAD8-1 samples for a range of commonly examined lignin parameters (Table 5; Fig. 4). Syringyl phenols (S) are exclusively found in angiosperms and cinnamyl phenols (C) are solely found in non-woody tissue, so ratio values of these phenols to ubiquitous vanillyl phenols (V) can differentiate OM sources from angiosperm and gymnosperm plant types (S:V) and non-woody and woody tissues (C:V; Hedges and Mann, 1979). The two samples from the Florida Everglades (FE1 and FE2) show C:V and S:V values consistent with traditionally defined angiosperm non-woody tissues. The Penobscot River (PR) C:V and S:V values show a mixture of traditionally defined angiosperm non-woody tissues and gymnosperm woody sources, reflecting the large gymnosperm forested area within the Penobscot catchment and input of anthropogenic lignin sources such as paper mill effluent. Regardless of the concentration or isolation technique, the two samples from the Florida Everglades (FE1 and FE2) are clearly distinct from one another and the sample from the Penobscot River (PR) based on C:V and S:V signatures. Furthermore, C:V and S:V values across all techniques for each sample type are within typical analytical reproducibility of one another (Table 5; Fig. 4). Therefore, we conclude that C:V and S:V values for freshwater samples undergo little technique-dependent fractionation and should be comparable. This is perhaps not surprising as the primary techniques used in environmental studies ($C_{18}A$ is not commonly used) were all seen to recover similar amounts of lignin phenols from freshwater. However, even with the lower recovery for total lignin, C:V and S:V values derived from $C_{18}A$ extraction were still compa-

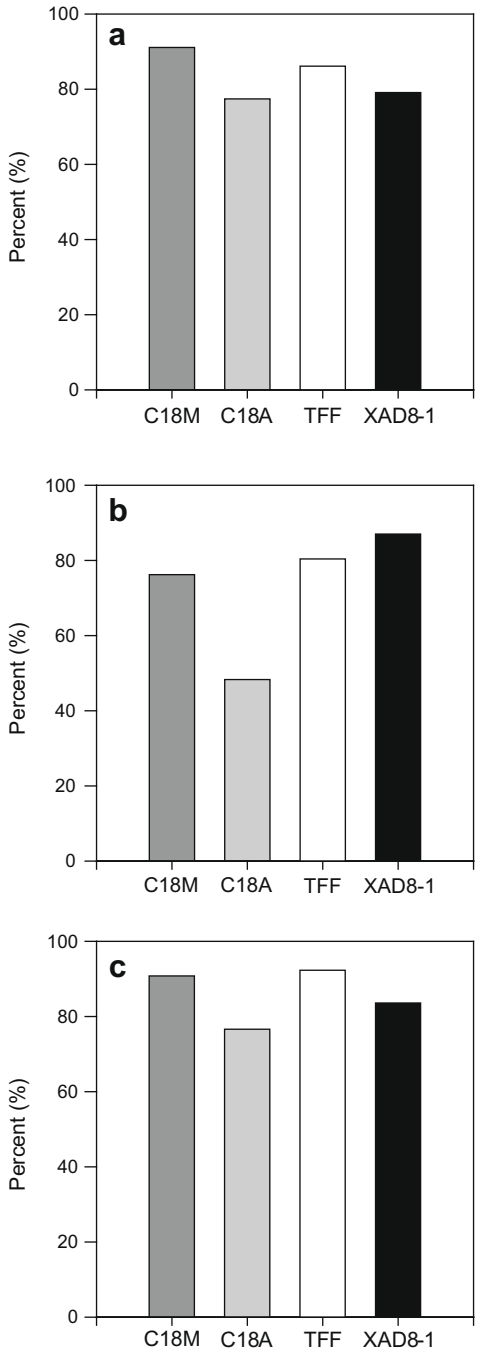

Fig. 3. Recovery (%) of dissolved lignin phenols (Σ_8) for freshwater sites with C_{18}M, C_{18}A, TFF and XAD8-1 vs. rotary evaporated sample. (a) FE1; (b) FE2 and (c) PR.

rable to those from other techniques. For saline samples, C:V values cannot be reliably calculated, but intercomparison of S:V values for the various techniques reveals consistency between techniques for the estuarine sample, PB (Table 5). However, the GoM sample had slightly lower S:V values for the TFF sample than for the samples isolated via C_{18} and XAD8-1 (Table 5). S:V values have been shown to behave differently between the HMW and LMW fractions (Hernes and Benner, 2003). The lower S:V value for the TFF GoM sample in comparison to the other methods is characteristic of the HMW fraction and does not necessarily represent the S:V value of the bulk lignin pool as shown by lignin photochemical degradation experiments on whole water (Spencer et al., 2009a) and combined examination of the HMW retentate via TFF and LMW permeate via C_{18} (Hernes and Benner, 2003).

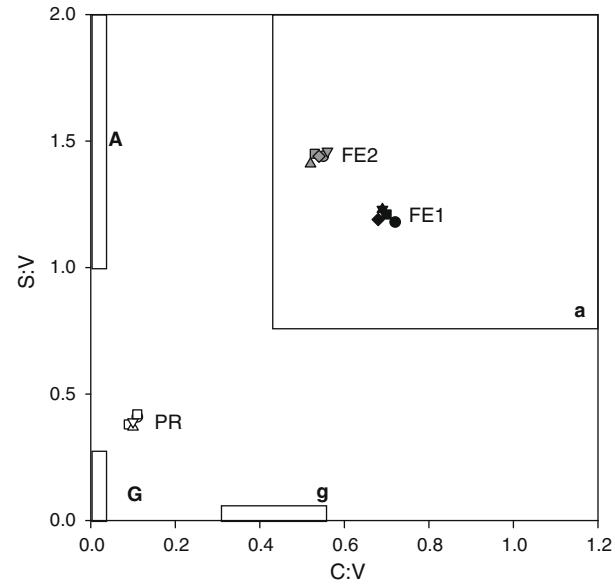

Fig. 4. S:V vs. C:V plot for freshwater sites FE1 (black); FE2 (gray) and PR (white). Rotary evaporated, C_{18}M, C_{18}A, TFF and XAD8-1 samples are represented by circles, squares, triangles up, triangles down and diamonds respectively for each site. The boxes and lettering indicate traditionally defined source signatures (Hedges and Mann, 1979). A, Angiosperm woody tissue; a, angiosperm non-woody tissue; G, gymnosperm woody tissue; g, gymnosperm non-woody tissue.

A number of studies have shown that increased biogeochemical processing of OM results in increased quantities of oxidized lignin phenols (Hedges et al., 1988; Hernes and Benner, 2003; Spencer et al., 2009a). This increase leads to a higher yield of acidic phenols during CuO oxidation, so, with certain caveats (Hernes et al., 2007), acid:aldehyde ratios of lignin phenols can be used to investigate the relative degree of degradation of OM. Values of vanillic acid to vanillin $(Ad{:}Al)_v$ for the three freshwater samples were all comparable for the isolation and concentration techniques, within typical analytical precision (Table 5); $(Ad{:}Al)_v$ ratio values for the estuarine and marine samples were higher than for the freshwater samples, indicative of the more degraded nature of lignin in marine systems (Hernes and Benner, 2002, 2003). For samples PB and GoM, C_{18} and XAD8-1 were comparable with respect to $(Ad{:}Al)_v$ values within typical analytical precision; however, the TFF samples showed more variability. The estuarine and marine TFF $(Ad{:}Al)_v$ values were slightly lower than those obtained with C_{18} and XAD8-1. The lower $(Ad{:}Al)_v$ values obtained via TFF are likely due to the less degraded nature of the HMW lignin fraction in comparison with bulk lignin captured by the other techniques, which includes more degraded LMW material. Values of syringic acid to syringaldehyde, $(Ad/Al)_s$, were also comparable for the three freshwater samples, independent of isolation/concentration technique (Table 5), showing little fractionation. Like $(Ad{:}Al)_v$ values, $(Ad/Al)_s$ values were higher for estuarine and marine samples, highlighting the more degraded nature of the vascular plant derived DOM (Table 5). As observed for $(Ad{:}Al)_v$, the $(Ad/Al)_s$ ratios for samples PB and GoM isolated with C_{18}M and XAD8-1 are comparable and again TFF samples gave lower values because of the less degraded nature of the HMW fraction vs. bulk DOM in the marine environment. The increased variability in diagnostic lignin parameters recovered using TFF in the marine environment could also be a result of decreased analytical precision, related to lower concentrations of lignin phenols analyzed in comparison to C_{18} and XAD8-1. It should be noted that most studies investigating lignin phenols with TFF in the marine environment utilize volumes (>100 l) that are much greater than those in this study, so analytical precision is likely improved over this comparison study.

4. Conclusions

This study provides the first intercomparison of XAD isolation and concentration methodology with other common isolation and concentration procedures for the measurement of dissolved lignin phenols. Our results demonstrate that both $C_{18}M$ and XAD8-1 are useful techniques for isolating lignin phenols across a range of aquatic settings, with typical lignin recovery of ca. 80–90% from freshwater compared to rotary evaporation, while yielding comparable lignin concentrations within marine systems. Across all five sites, $C_{18}M$ and XAD8-1 measurements were statistically indistinguishable from each other ($P < 0.1$). TFF was found to be an appropriate technique for concentrating lignin phenols from freshwater, with lignin recovery of ca. 80–90%. However, for marine systems, because of the prevalence of LMW lignin phenols, TFF does not recover lignin as well as $C_{18}M$ or XAD8-1, so the latter techniques are recommended for studies aimed at determining bulk dissolved lignin phenol concentrations from marine systems. Thus, for examination of lignin phenol concentrations in freshwater settings, it appears valid to compare measurements from different studies utilizing $C_{18}M$, TFF or XAD8-1. For the marine environment, investigation and comparison of lignin phenol concentrations seems appropriate for studies using $C_{18}M$ and XAD8-1; however, TFF clearly recovers less lignin and could result in an underestimate of fluxes of terrigenous carbon to the oceans. Nevertheless, in combination with solid phase extraction, TFF can still provide unique and valuable size information toward the understanding of terrigenous DOM dynamics in the oceans (Hernes and Benner, 2002, 2003).

No fractionation effects were observed for fresh waters between samples isolated or concentrated using $C_{18}M$, XAD8-1 and TFF for some commonly examined lignin parameters [C:V, S:V, $(Ad/Al)_v$ and $(Ad/Al)_s$] vs. rotary evaporated whole waters. This clearly demonstrates that recovery with all these techniques is based primarily on bulk chemical properties, as opposed to interactions at the functional group scale. Thus, intercomparison of diagnostic lignin parameters between various studies utilizing these isolation and concentration methods appears valid for freshwaters. Within marine systems differences were observed between diagnostic lignin parameters isolated via different techniques, so it is recommended for future studies of marine systems or across estuarine gradients that one technique be used for ease of intercomparison within studies. $C_{18}M$ or XAD8-1, because of their high recovery efficiency for lignin phenols from marine settings, are natural choices for future studies focused on bulk dissolved lignin in the marine environment. The results should facilitate comparison of existing lignin data sets where appropriate and assist future studies in choosing appropriate isolation and concentration methods for lignin phenols in aquatic settings. Finally, as the results highlight, XAD8 is a suitable method for the isolation of dissolved lignin phenols from aquatic systems, so lignin data representative of whole water can be produced for IHSS reference materials (e.g. Suwannee River humic and fulvic acid) or other XAD sample archives. Thus, interpretable lignin phenol data may be added to the database of chemical property information available on IHSS reference materials (http://ihss.gatech.edu/ihss2/) and tied into future studies linking DOM composition to its fundamental properties.

Acknowledgements

The study was supported by the USGS National Research Project (http://water.usgs.gov/nrp), and by NASA Grant NNH04AA62I. The use of brand names in this report is for identification purposes only and does not imply endorsement by the US Geological Survey. We would like to thank the Aqueous Organic Geochemistry Group at UC Davis and two anonymous reviewers for constructive comments.

Associate Editor—**G.D. Abbott**

References

Ahad, J.M.E., Ganeshram, R.S., Spencer, R.G.M., Uher, G., Gulliver, P., Bryant, C.L., 2006. Evidence for anthropogenic ^{14}C-enrichment in estuarine waters adjacent to the North Sea. Geophysical Research Letters 33, L08608. doi:10.1029/2006GL025991.

Aiken, G.R., 1992. Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method. Environmental Science and Technology 26, 2435–2439.

Aiken, G.R., Thurman, E.M., Malcolm, R.L., Walton, H.F., 1979. Comparison of XAD macroporous resins for the concentration of fulvic-acid from aqueous solution. Analytical Chemistry 51, 1799–1803.

Aiken, G.R., McKnight, D.M., Thorn, K.A., Thurman, E.M., 1992. Isolation of hydrophobic organic-acids from water using nonionic macroporous resins. Organic Geochemistry 18, 567–573.

Benner, R., Opsahl, S., 2001. Molecular indicators of the sources and transformations of dissolved organic matter in the Mississippi river plume. Organic Geochemistry 32, 597–611.

Benner, R., Biddanda, B., Black, B., McCarthy, M., 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. Marine Chemistry 57, 243–263.

Benner, R., Louchouarn, P., Amon, R.M.W., 2005. Terrigenous dissolved organic matter in the Arctic Ocean and its transport to surface and deep waters of the North Atlantic. Global Biogeochemical Cycles 19, GB2025. doi:10.1029/2004GB002398.

Boyle, E.S., Guerriero, N., Thiallet, A., Del Vecchio, R., Blough, N.V., 2009. Optical properties of humic substances and CDOM: relation to structure. Environmental Science and Technology 43, 2262–2268.

Buesseler, K.O., Bauer, J.E., Chen, R.F., Eglinton, T.I., Gustafsson, O., Landing, W., Mopper, K., Moran, S.B., Santschi, P.H., VernonClark, R., Wells, M.L., 1996. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: overview and organic carbon results. Marine Chemistry 55, 1–31.

Cheryan, M., 1998. Ultrafiltration and Microfiltration Handbook. Technomic Publishing. Co., Lancaster, PA.

Cory, R.M., McKnight, D.M., Chin, Y.P., Miller, P., Jaros, C.L., 2007. Chemical characteristics of fulvic acids from Arctic surface waters: microbial contributions and photochemical transformations. Journal of Geophysical Research – Biogeosciences 112, G04S51. doi:10.1029/2006JG000343.

Dalzell, B.J., Filley, T.R., Harbor, J.M., 2005. Flood pulse influences on terrestrial organic matter export from an agricultural watershed. Journal of Geophysical Research – Biogeosciences 110, G02011.

Dittmar, T., Lara, R.J., Kattner, G., 2001. River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. Marine Chemistry 73, 253–271.

Eckard, R.S., Hernes, P.J., Bergamaschi, B.A., Stepanauskas, R., Kendall, C., 2007. Landscape scale controls on the vascular plant component of dissolved organic carbon across a freshwater delta. Geochimica et Cosmochimica Acta 71, 5968–5984.

Ertel, J.R., Hedges, J.I., Devol, A.H., Richey, J.E., Ribeiro, M.D.G., 1986. Dissolved humic substances of the Amazon River system. Limnology and Oceanography 31, 739–754.

Goni, M.A., Hedges, J.I., 1992. Lignin dimmers: structures, distribution, and potential geochemical applications. Geochimica et Cosmochimica Acta 56, 4025–4043.

Goni, M.A., Montgomery, S., 2000. Alkaline CuO Oxidation with a microwave digestion system: lignin analyses of geochemical samples. Analytical Chemistry 72, 3116–3121.

Gueguen, C., Belin, C., Dominik, J., 2002. Organic colloid separation in contrasting aquatic environments with tangential flow filtration. Water Research 36, 1677–1684.

Guo, L., Santschi, P.H., 1996. A critical evaluation of the cross-flow ultrafiltration technique for sampling colloidal organic carbon in seawater. Marine Chemistry 55, 113–127.

Hedges, J.I., Ertel, J.R., 1982. Characterization of lignin by capillary gas chromatography of cupric oxide oxidation products. Analytical Chemistry 54, 174–178.

Hedges, J.I., Mann, D.C., 1979. Characterization of plant tissues by their lignin oxidation products. Geochimica et Cosmochimica Acta 43, 1803–1807.

Hedges, J.I., Blanchette, R.A., Weliky, K., Devol, A.H., 1988. Effects of fungal degradation on the CuO oxidation products of lignin: a controlled laboratory study. Geochimica et Cosmochimica Acta 52, 2717–2726.

Hernes, P.J., Benner, R., 2002. Transport and diagenesis of dissolved and particulate terrigenous organic matter in the North Pacific Ocean. Deep Sea Research Part I 49, 2119–2132.

Hernes, P.J., Benner, R., 2003. Photochemical and microbial degradation of dissolved lignin phenols: implications for the fate of terrigenous dissolved organic matter in marine environments. Journal of Geophysical Research – Oceans 108, 3291. doi:10.1029/2002JC001421.

Hernes, P.J., Benner, R., 2006. Terrigenous organic matter sources and reactivity in the North Atlantic Ocean and a comparison to the Arctic and Pacific oceans. Marine Chemistry 100, 66–79.

Hernes, P.J., Robinson, A.C., Aufdenkampe, A.K., 2007. Fractionation of lignin during leaching and sorption and implications for organic matter "freshness". Geophysical Research Letters 34, L17401. doi:10.1029/2007GL031017.

Hernes, P.J., Spencer, R.G.M., Dyda, R.Y., Pellerin, B.A., Bachand, P.A.M., Bergamaschi, B.A., 2008. The role of hydrologic regimes on dissolved organic carbon composition in an agricultural watershed. Geochimica et Cosmochimica Acta 72, 5266–5277.

Kattner, G., Lobbes, J.M., Fitznar, H.P., Engbrodt, R., Nöthig, E.-M., Lara, R.J., 1999. Tracing dissolved organic substances and nutrients from the Lena River through the Laptev Sea (Arctic). Marine Chemistry 65, 25–39.

Kilduff, J., Weber, W.J., 1992. Transport and separation of organic macromolecules in ultrafiltration processes. Environmental Science and Technology 26, 569–577.

Kim, S., Simpson, A.J., Kujawinski, E.B., Freitas, M.A., Hatcher, P.G., 2003. High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C-18 solid phase disk. Organic Geochemistry 34, 1325–1335.

Kraus, T.E.C., Bergamaschi, B.A., Hernes, P.J., Spencer, R.G.M., Stepanauskas, R., Kendall, C., Losee, R.F., Fujii, R., 2008. Assessing the contribution of wetlands and subsided islands to dissolved organic matter and disinfection byproduct precursors in the Sacramento–San Joaquin River Delta: a geochemical approach. Organic Geochemistry 39, 1302–1318.

Lobbes, J.M., Fitznar, H.P., Kattner, G., 2000. Biogeochemical characteristics of dissolved and particulate organic matter in Russian rivers entering the Arctic Ocean. Geochimica et Cosmochimica Acta 64, 2973–2983.

Louchouarn, P., Opsahl, S., Benner, R., 2000. Isolation and quantification of dissolved lignin from natural waters using solid-phase extraction and GC/MS. Analytical Chemistry 72, 2780–2787.

Mannino, A., Harvey, H.R., 2000. Terrigenous dissolved organic matter along an estuarine gradient and its flux to the coastal ocean. Organic Geochemistry 31, 1611–1625.

Meyers-Schulte, K.J., Hedges, J.I., 1986. Molecular evidence for a terrestrial component of organic matter dissolved in ocean water. Nature 321, 61–63.

Opsahl, S., Benner, R., 1995. Early diagenesis of vascular plant tissues, lignin and cutin decomposition and biogeochemical implications. Geochimica et Cosmochimica Acta 59, 4889–4904.

Opsahl, S., Benner, R., 1997. Distribution and cycling of terrigenous dissolved organic matter in the ocean. Nature 386, 480–482.

Opsahl, S., Benner, R., 1998. , Photochemical reactivity of dissolved lignin in river and ocean waters. Limnology and Oceanography 43, 1297–1304.

Opsahl, S., Benner, R., Amon, R.M.W., 1999. Major flux of terrigenous dissolved organic matter through the Arctic Ocean. Limnology and Oceanography 44, 2017–2023.

Sokal, R.R., Rohlf, F.J., 1981. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman, San Francisco. 859 pp.

Spencer, R.G.M., Aiken, G.R., Wickland, K.P., Striegl, R.G., Hernes, P.J., 2008. Seasonal and spatial variability in dissolved organic matter quantity and composition from the Yukon River basin, Alaska. Global Biogeochemical Cycles 22, GB4002. doi:10.1029/2008GB003231.

Spencer, R.G.M., Stubbins, A., Hernes, P.J., Baker, A., Mopper, K., Aufdenkampe, A.K., Dyda, R.Y., Mwamba, V.L., Mangangu, A.M., Wabakanghanzi, J.N., Six, J., 2009a. Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River. Journal of Geophysical Research – Biogeosciences 114, G03010. doi:10.1029/2009JG000968.

Spencer, R.G.M., Aiken, G.R., Butler, K.D., Dornblaser, M.M., Striegl, R.G., Hernes, P.J., 2009b. Utilizing chromophoric dissolved organic matter measurements to derive export and reactivity of dissolved organic carbon to the Arctic Ocean: a case study of the Yukon River, Alaska. Geophysical Research Letters 36, L06401. doi:10.1029/2008GL036831.

Stubbins, A., Hubbard, V., Uher, G., Law, C.S., Upstill-Goddard, R.C., Aiken, G.R., Mopper, K., 2008. Relating carbon monoxide photoproduction to dissolved organic matter functionality. Environmental Science and Technology 42, 3271–3276.

Stuermer, D.H., Harvey, G.R., 1974. Humic substances from seawater. Nature 250, 480–481.

Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fuji, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science and Technology 37, 4702–4708.