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A mini-electrodialysis system for desalting small volume saline samples for Fourier transform ion cyclotron resonance mass spectrometry

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Abstract

An affordable, commercially available mini-electrodialysis (mini-ED) system has been evaluated for the efficient desalting of small volume samples of seawater before analysis by electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS). Mini-ED FT-ICR mass spectra were compared with spectra for samples that were treated by C_{18} solid phase extraction, a commonly used method for rapid sample preparation for this type of analysis. In this comparison, it is clear that mini-ED provides more representative molecular information, compared with C_{18} isolation, and recovers the overwhelming majority of peaks from salt-free samples, indicating that it adequately represents the DOM that can be ionized and analyzed by ESI FT-ICR MS. The ED system produces a significant carbon blank. However, the substances contributing to this blank are not detectable by ESI FT-ICR MS. Based on these findings mini-ED is recommended as a promising method for the desalting of aqueous environmental samples before analysis by ESI FT-ICR MS.

Dissolved organic matter (DOM) in the ocean is one of the largest pools of dynamic carbon on earth (Hedges 1992). Major advances are being made in the chemical characterization of DOM, delivering greater understanding of its origin, structure, and function in the global carbon cycle (Mopper et al. 2007). One of the most powerful techniques currently in use for the analysis of DOM is electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), which has been applied to offer new insight into the nature and reactivity of DOM (Kim et al. 2003; Stenson et al. 2003; Kujawinski et al. 2004, 2009; Koch et al. 2005, 2008; Grannas et al. 2006; Hertkorn et al. 2006; Dittmar et al. 2007; Reemtsma et al. 2008; Sleighter and Hatcher 2008; Bhatia et al. 2010; D'Andrilli et al. 2010; Stubbins et al. 2010). However, salty samples are not conducive to ESI and need to be desalted before analysis, ideally without altering or fractionating the DOM. This has commonly been accomplished by solid-phase extraction (SPE) using C_{18}, XAD, and PPL resins to isolate, recover, and concentrate the DOM without concentrating the salts (Aiken et al. 1979; Lara and Thomas 1994; Dittmar et al. 2001; Kim et al. 2003; see Mopper et al. 2007 and Dittmar et al. 2008 for reviews). SPE techniques are readily implemented for inexpensive and rapid shipboard isolation of DOM. They typically require acidification of the sample to pH 2 before loading onto the resin and generally recover less than 50% of the DOM from open ocean waters (Dittmar et al. 2008), excluding substances that are either ionic at pH 2 or are so hydrophilic that they escape sorption (Sleighter and Hatcher 2008). Another salt-removal approach is ultrafiltration, which isolates DOM on the basis of size and typically recovers 10-40% of DOM from open ocean samples (Santschi et al. 1995; Buesseler et al. 1996; Guo and Santschi 1996; Benner and Opsahl 2001; Simjouw et al. 2005; Hernes and Benner 2006).

The most promising non-discriminating technique for DOM isolation from saline waters is reverse osmosis coupled with electrodialysis (RO/ED), which can isolate up to 95% of marine DOM (Vetter et al. 2007; Koprivnjak et al. 2009). However, because the dead volumes for previously employed RO/ED systems are about 3 L, they demand large sample volumes typically exceeding 5 L. Obtaining requisite water sample volumes for these RO/ED systems places some constraints on sampling, and, in some cases, obtaining large volume sam-

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ples is not feasible. Also, the equipment is rather expensive and requires trained operators for implementation. To overcome these limitations a novel method for desalting small volumes of seawater samples for subsequent analysis by ESI FT-ICR MS was developed, evaluated, and is reported herein. This method couples small scale electrodialysis (mini-ED) with rotary evaporation. Naturally saline samples and freshwater samples amended with salt were desalted and subsequently analyzed using ESI FT-ICR MS to determine the level of molecular information retrievable. Blanks were evaluated to determine if contamination was a problem. The most pressing concern was to evaluate whether the mini-ED was able to recover all ESI FT-ICR MS peaks in a freshwater DOM sample from the Great Dismal Swamp, VA, USA, to which artificial sea-salts were added. The mini-ED isolates of two samples collected from locations in the Congo River and estuary were compared, one is freshwater and the other having a measurable salt content.

Materials and procedures

Sampling

Samples used to test the mini-ED system were taken from the Great Dismal Swamp (Suffolk, Virginia, USA), and the Congo River and estuary (near Kinshasa, Democratic Republic of Congo; Spencer et al. 2009). All samples were filtered through a 0.2 µm filter (Whatman, Polycap TC) to remove particulates and bacteria.

Sample amendment

Artificial seawater was made with combusted (450°C, 5 h) NaCl and $MgSO_4$ (Fisher Sci., certified ACS grade) mixed with ultra quality MilliQ water (Millipore). The salts were added to MilliQ water with a mass ratio of 64:7 for NaCl:$MgSO_4$. Freshwater samples amended with different amounts of artificial seawater were used to test the mini-ED system. The salinity of each sample before desalting was measured by a calibrated conductivity meter.

Mini-electrodialysis system

The mini-ED system (Harvard Apparatus; Fig. 1) used here was designed for the rapid purification of proteins, nucleic acids, carbohydrates, and other biomolecules (see www.harvardapparatus.com for further details). The system comes along with a small power supply (maximum 200Vdc, 100mA). The sample chamber is made of Teflon, a completely inert material suited for high sample recovery and low sample contamination, which is important when dealing with low dissolved organic carbon (DOC) seawater samples. The membranes of the mini-ED system are commercially available at molecular weight cut-offs (MWCO) ranging from 100 Da to 300,000 Da and constructed of three materials—polycarbonate, regenerated cellulose, and cellulose acetate (see www.harvardapparatus.com for further details). However, only cellulose acetate membranes are available for molecular weight cutoffs below 1000 Da. Cellulose acetate 100 and 500 Da membranes were chosen for the current study as they allow passage of the major inorganic ions present in seawater, whereas still retaining more DOM molecules than conventional ultrafiltration systems (1000 Da). Membranes from Harvard Apparatus arrived pre-cut and stored in a 0.05% sodium azide solution. Before use, membranes were soaked in MilliQ water. The mini-ED system was stored and operated at room temperature (~25°C) in a dedicated chemical fume hood to maintain a relatively clean environment.

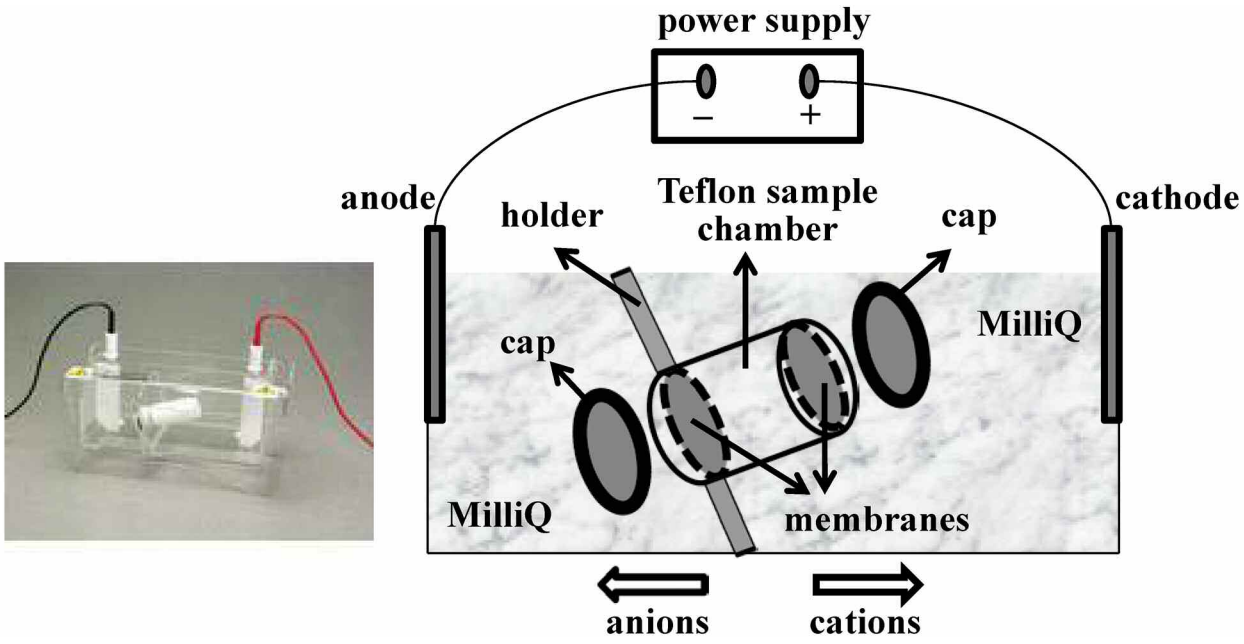

Fig. 1. Left: The mini-ED system from Harvard Apparatus; Right: A diagram showing the various components of the system.

DOM isolation by mini-ED

A pre-rinsed dialysis membrane was placed over one end of the Teflon sample chamber and the Teflon end cap was installed to hold it in place. The chamber was then inverted and 1.5 mL sample was added using a precombusted glass Pasteur pipette. Once filled, a second membrane was placed on the top and secured in place using the second end cap, making sure that no bubbles were trapped in the chamber. The chamber was then placed in the holder at the center of the mini-ED bath (Fig. 1). The chamber holder supports the chamber and orientates it in the plane of the electrodes. The holder also provides a physical barrier between the anode and cathode ends of the bath forcing any current applied to flow through the sample chamber. MilliQ water was placed in the bath surrounding the chamber and the power supply was connected to the electrodes. To begin desalting a voltage of 200 V was applied to the system. In theory, mobile charged ions (e.g., Na^+, Cl^-, SO_4^{2-}) move from the sample chamber to the bath driven by the applied electrical field, whereas neutral and less mobile molecules are preferentially retained in the chamber. These conditions promote the loss of inorganic salts and the retention of DOM. However, dissolved organic molecules may be lost if they are charged and have a dynamic radius that is smaller than the membrane cutoff.

At the beginning of desalting, the low conductivity of MilliQ in the mini-ED bath external to the sample chamber prohibited a high current through the system and desalting occurred slowly. Over time, salts moved from the sample chamber into the surrounding MilliQ water, increasing the conductivity and the electrical current. After few hours, the exact timing depending upon initial sample salinity, the electrical current began to decrease along with the electrolyte concentration differential between the sample in the chamber and the surrounding water. At this point, the movement of ions from the chamber to the bath slowed. When the conductivity in the surrounding water stabilized, the voltage was turned off and the surrounding water was partly replaced with new MilliQ water. Thus, renewing the salt gradient between the sample and the surrounding water, while retaining some salts in the surrounding water to facilitate an electrical current. Desalting was recommenced at 200 V and the conductivity of the surrounding water was monitored until it leveled off again. At this point the bath water was re-diluted with MilliQ. By repeating these procedures several times, the samples were desalted until their salinity was below 0.2 practical salinity units (PSU), which was low enough to run FT-ICR MS. For the estuarine Congo DOM sample (salinity 0.2 PSU; DOC 10 mgC L^{-1}) desalting to an even lower salinity (<0.01 PSU) was accomplished and completed within 24 h. Because of its reasonably high DOC concentration, this sample did not require further treatment prior to ESI FT-ICR MS analysis.

For typical seawater (salinity ~35 PSU and DOC < 1 mgC L^{-1}), approximately 5 h was required to accomplish the first step of the process, during which the samples were desalted to a salinity of ~5 PSU. For these low DOC seawater samples both concentrating and desalting was required to obtain appropriate samples for ESI FT-ICR MS analysis. Therefore, numerous (10 to 15) subsamples were desalted as described above and then combined and concentrated to ~2 mL by rotary evaporation. The exact number of subsamples required for this process depended upon the concentration factor required to bring the final DOC concentration to > 6 mgC L^{-1}, which is sufficient to obtain high quality ESI FT-ICR MS spectra (personal observation). As evaporation concentrated both the DOM and salts in the sample, the concentrated sample was returned to the mini-ED and the desalting process was repeated. The entire process, including the original desalting of multiple subsamples, required 3~7 d. To minimize the adsorption of DOM onto the membranes, the chamber was shaken for few minutes before the desalted sample was transferred out of the chamber.

Solid phase extraction of DOM using C_{18}

DOM was also isolated with C_{18} solid phase extraction disks (3M, Empore) using previously established protocols (Kim et al. 2003) to compare with the results obtained from the mini-ED method.

Dissolved organic carbon measurement

DOC concentrations for filtered samples were determined as non-purgeable organic carbon (NPOC) by high temperature combustion (720°C) on a Shimadzu TOC-5000V analyzer equipped with a high sensitivity platinum catalyst (Shimadzu Scientific Instruments). Reference materials for low carbon water and deep seawater obtained from the Consensus Reference Materials Project, Hansell Laboratory, University of Miami, were used to correct and monitor the performance of the instrument (Doval and Hansell 2000).

ESI FT-ICR MS data acquisition

Immediately before ESI FT-ICR MS analysis, samples were diluted 50:50 (v/v) with LC-MS grade methanol (contains 0.1%, v/v, NH_4OH to improve DOM ionization efficiency. This dilution was conducted less than half an hour before analyzing the samples to minimize potential esterification of the DOM by methanol (Bateman et al. 2008; Flerus et al. 2011). Samples were analyzed under negative ion mode without further treatment using a Bruker Daltonics 12 Tesla Apex Qe ESI FT-ICR MS housed in the College of Sciences Major Instrumentation Cluster (COSMIC) at Old Dominion University (Virginia, USA). Instrument blanks were analyzed with 50:50 (v/v) H_2O: MeOH (contains 0.1%, v/v, NH_4OH) before and after running the samples. Blanks and samples were infused into the ESI ion source using a syringe pump with an infusion rate of 120 µL h^{-1}. Ions were accumulated in the hexapole for 2.0 s before being transferred to the ICR cell. Exactly 300 transients were co-added for each sample and digitized with a 4M Word data acquisition size. Fourier transformation and magnitude calculation of the free induction decay signal (FID) was accomplished by the Bruker Daltonics Data Analysis software.

ESI FT-ICR MS data analysis

Mass spectra were internally calibrated using naturally present fatty acids (Sleighter et al. 2008). Data lists of m/z values and peak height were copied out with a signal-to-noise ratio (S/N) above 4 and m/z range from 200 to700. Each sample's m/z list was aligned with the instrumental blanks' m/z lists in MATLAB allowing the maximum m/z difference to be the m/z divided by 400,000, which is the average resolving power at m/z 400 (where resolving power is defined as $m/\Delta m_{50\%}$ where $\Delta m_{50\%}$ is the peak width at half-height of peak m). Following alignment, all peaks found in instrumental blanks were removed from the sample's peak list. These blank-corrected peak lists were imported to a molecular formula calculator (Molecular Formula Calc v.1.0 ©NHMFL, 1998) to obtain molecular formulas using ^{12}C (number range: 1 to 50), ^{13}C (0 to 1), ^{1}H (1 to 100), ^{14}N (0 to 6), ^{16}O (0 to 30), ^{34}S (0 to 2), ^{31}P (0 to 2) and ^{35}Cl (0 to 1). We allow the difference between the exact mass of calculated formula and the measured m/z to be less than 1 ppm. We only retain those formulas that possibly occur within natural DOM by the following criteria of the atomic ratio in each formula: (i) $2 \le H \le (2C + 2)$, $0 \le O \le (C + 2)$, $O/C < 1.2$, $0.3 < H/C < 2.25$, $N/C < 0.5$, $S/C < 0.2$, $P/C < 0.1$, $(S + P)/C < 0.2$ (Stubbins et al. 2010); (ii) Formulas containing an odd number of N have odd nominal mass, whereas those containing even numbers of N have even nominal mass (McClafferty and Turecek 1993); (iii) Double-bond equivalents (DBE) ≥ 0, and must be a whole number (McClafferty and Turecek 1993), where $DBE = 1 + 1/2(2C - H + N + P + Cl)$. For some of the peaks, they were removed as salt peaks from the final dataset because they can only be calculated as formulas containing Cl. The ^{13}C isotopic peaks were identified as those meeting both the following criteria: (i) if there are matched ^{12}C formula and ^{13}C formula; (ii) if the ^{13}C formula peak height is lower than the ^{12}C formula peak height. Once identified, these ^{13}C isotope peaks were removed from the dataset.

Assessment

Initial results comparing the 100 and 500 MWCO Da membranes indicate that the 100 Da membranes were not able to desalt samples efficiently, most likely due to their retention of anions with large ionic radii (e.g., SO_4^{2-}). In contrast, the 500 Da MWCO membranes resulted in efficient and fairly rapid desalting of samples. Based upon these findings, the performance of the 500 Da membranes was further explored. Compared with the commonly used isolation method of ultrafiltration, which generally employs a 1000 Da membrane, mini-ED is expected to retain lower molecular weight DOM and achieve higher total recoveries. In addition, ultrafiltration relies upon the passive diffusion of salts across the membrane. Under these conditions if DOM is small enough to cross the membrane at higher concentration in the sample then it will be lost to the waste. In ED, an electrical current causes the active transport of charged ions across the membrane. Under these conditions, DOM would have to be both small (<500 Da) and charged to be actively removed to waste with similar efficiency as the salts. Thus, the mini-ED should retain DOM in the range from 500 to 1000 Da as well as DOM < 500 Da that is neutral or poorly charged and therefore not actively transported across the membrane. Compared with solid phase extraction techniques (SPE), the mini-ED does not require the samples to be acidified to pH 2, which can potentially affect the molecular characteristics of DOM. Further, it does not rely on chemical affinities between the SPE substrate and the DOM. Thus, the mini-ED is expected to recover a large, minimally fractionated proportion of the DOM, ensuring that the recovered DOM is as representative of the original sample as is possible at present for small volume samples.

Blank test

Collecting, processing, and analyzing low DOC water requires extreme care to avoid contamination. Thus, to assess potential contamination from the mini-ED, a system blank was determined. First, MilliQ water was used to fill the mini-ED bath, as per normal operating procedure (See "*DOM isolated by mini-ED*"). Approximately 31.5 PSU artificial seawater was then placed inside the sample chamber, and the system was operated for 48 h as described for seawater samples. Artificial seawater DOC increased from an initial ~0.1 mgC L^{-1} to ~2 mgC L^{-1} in the desalted sample indicating contamination.

Although the artificial seawater DOC increased by 2 mgC L^{-1} after desalting, the ESI FT-ICR MS for the blank contained mainly peaks displaying mass defects between 0.7 and 0.8 Da. These peaks were assigned as salt-derived peaks based upon their mass defects. Mass defect is the distance a peak is displaced from the closest exact nominal mass (i.e., whole number). The displacement between the nominal mass and a peak is determined by the atoms contained in the analyte molecule and is the basis of formula assignment using FT-ICR MS. Even before calculating the molecular formula for each peak in the FT-ICR mass spectra, it is possible to classify the peaks by elemental composition based upon their mass defects. The ultrahigh resolution of FT-ICR MS enables mass defects to be calculated at least to the fifth decimal place. The atomic masses of isotopes ^{1}H, ^{12}C, and ^{16}O are 1.00783 Da, 12.00000 Da, and 15.99491Da, respectively. Normally DOM molecules are observed with mass defect range from 0.0 to 0.4, although some long-chain fatty acids appear at mass defects of 0.4 to 0.6 (Sleighter et al. 2008). When DOM-chloride adducts are present, they have mass defects of 0.7 to 0.8, because the atomic mass of the isotope ^{35}Cl is 33.96787 Da.

In the ESI FT-ICR MS for the blank, we also observed few peaks where normal DOM molecules are generally observed (mass defects 0.0 to 0.4) but they were consistent with peaks found commonly in the instrument blank run on the FT-ICR MS at ODU. This instrument blank was obtained from a mixture of 50:50 (v/v) MilliQ water and LC-MS grade methanol (containing 0.1%, v/v, NH_4OH). These results indicate that DOC contamination from the mini-ED did not affect the mass spectral data. Apparently, the contaminating organic mole-

cules were not charged during negative ESI or were smaller or greater in mass than the limits of mass spectral window detected by ESI FT-ICR MS as configured in the experiments conducted. Given that the ED membranes used were cellulose acetate, it is likely that the DOC blank from the mini-ED was also cellulosic and, as such, would be expected to have very low ionization efficiencies in negative ion mode ESI (Shen and Perreault 1998). The salt-derived peaks in the blank were clearly separated in each nominal mass window from those of the analyte DOM, and as such, were readily discarded and eliminated from consideration in the dataset. Besides removing the blank peaks by the way we mentioned earlier in the data analysis section, we also discarded all peaks with a mass defect ≥ 0.7 in all samples.

Molecular information retrieved by the mini-ED system for low salinity samples

To evaluate the effectiveness of the mini-ED system for recovery of DOM molecular information, mass spectra were compared for samples before and after desalting. This was accomplished by analyzing samples that contained no salt, and samples with low enough levels of salts that they could be analyzed directly by FT-ICR MS. Here, we used two closely spaced samples from the lower Congo River system: Congo River site (salinity 0 PSU) and Congo estuary site (salinity 0.2 PSU). The first of these has been well characterized and is representative of DOM from major tropical rivers (Spencer et al. 2009; Stubbins et al. 2010). Both samples were amenable to direct analysis on the FT-ICR MS, yielding well-resolved mass spectra (Fig. 2a and 2c). The mass spectrum of the river sample shows typical characteristics for DOM derived from terrestrial biomass (e.g., clusters of peaks between 0.0 and 0.4 Da at each nominal mass extending m/z range from 200 to 600 Da; Sleighter and Hatcher 2008). Molecular formula information obtained from the FT-ICR MS data covered all the typical compound classes in the van Krevelen diagram (Fig. 3a). In the expanded mass spectral region of the estuary sample (Fig. 2d, f, and h), most peaks were common to the river water sample (Fig. 2b). However, the estuary sample contained additional peaks with mass defects approximately from 0.7 to 0.8 displayed over the whole m/z range from 200 to 600 (Fig. 2d; Table 1), which were likely due to the presence of salts in the estuarine sample. The number of peaks with low mass defects, i.e., between 0.00 and 0.06, was significantly lower for the estuary sample than the river sample (compare Fig. 2b and 2d, this pattern was common at nominal masses throughout the spectrum, also see Table 1). These low-mass-defect peaks should have been present in both samples, due to their proximity in the Congo River. The absence of these peaks in the spectrum of the estuary sample is probably because nonvolatile salts such as NaCl interfere with spray formation and suppress ionization by forming salt-solvent adducts (Brown and Rice 2000). The presumption that the presence of salt suppressed the ionization of DOM causing the absence of low-mass-defect peaks was made with the expectation that the two water samples were identical except in salinity. This may not be the case and the observation of diminished signals in the low-mass-defect region may have arisen from inherent differences in the DOM at the two sites.

Desalting the estuarine sample by both C_{18} extraction and mini-ED allowed these two methods of salt removal to be evaluated and the molecular information they retain to be compared with whole water spectra where salt removal was not employed. The mass spectra obtained for these two sample-processing methods are shown in Fig. 2e and Fig. 2g. Salt peaks were significantly removed by both desalting methods (Fig. 2f and 2h; Table 1). A number of peaks with mass defects between 0.00 and 0.06 appeared in the FT-ICR MS spectra of the estuary sample following mini-ED desalting (boxed-in area, in Fig. 2; Table 1). These peaks also occurred in the Congo River sample as mentioned above, but they were not observed following C_{18} extraction, suggesting that the SPE method discriminates against these molecules. We can exclude the possibility that these low-mass-defect peaks were from the mini-ED blank because we did not observe them in the blank test. These peaks represent compounds either with low H/C ratios or with high O/C ratios such as tannin-like compounds (Fig. 3). A similar discrimination was observed with DOM isolation by C_{18} (Sleighter and Hatcher 2008) and was attributed to the fact that these molecules are hydrophilic and not readily extracted by the resin.

Molecular information retrieved by the mini-ED system for higher salinity samples

The salinity for seawater is normally above 35 PSU. So it was important to evaluate the mini-ED system using samples with salinities greater than those for the Congo estuary sample. However, FT-ICR mass spectra for high salinity samples are not attainable without salt removal. This makes the comparison of FT-ICR MS data before and after mini-ED impossible for seawater. Instead artificial salts were added to the Dismal Swamp freshwater DOM (DOC ~40 mgC L^{-1}). Prior to salts addition, the mass spectrum of diluted Dismal Swamp sample (DOC ~10 mgC L^{-1}) was obtained. The Dismal Swamp sample was then mixed with high salinity MilliQ water to yield samples with a DOC of 10 mgC L^{-1} and salinities of 15 PSU and 35 PSU. The resultant samples were then desalted using the mini-ED system. A further aliquot of diluted Dismal Swamp water (DOC ~10 mgC L^{-1}) without addition of any salts was also processed using the mini-ED system. The resultant samples were analyzed by FT-ICR MS. The addition of salts resulted in residual salt peaks with mass defects of 0.7 to 0.8 that could not be completely removed. However, other than peaks assigned to salts, the peak distribution within each nominal mass window was well preserved (Fig. 4). Other differences between the treatments' mass spectra were minor. The mass spectra for the two desalted samples were near-identical, indicating the combined mini-ED / FT-ICR MS technique delivered reproducible results as found for replicate analyses of untreated Dismal Swamp samples (Sleighter et al. 2010).

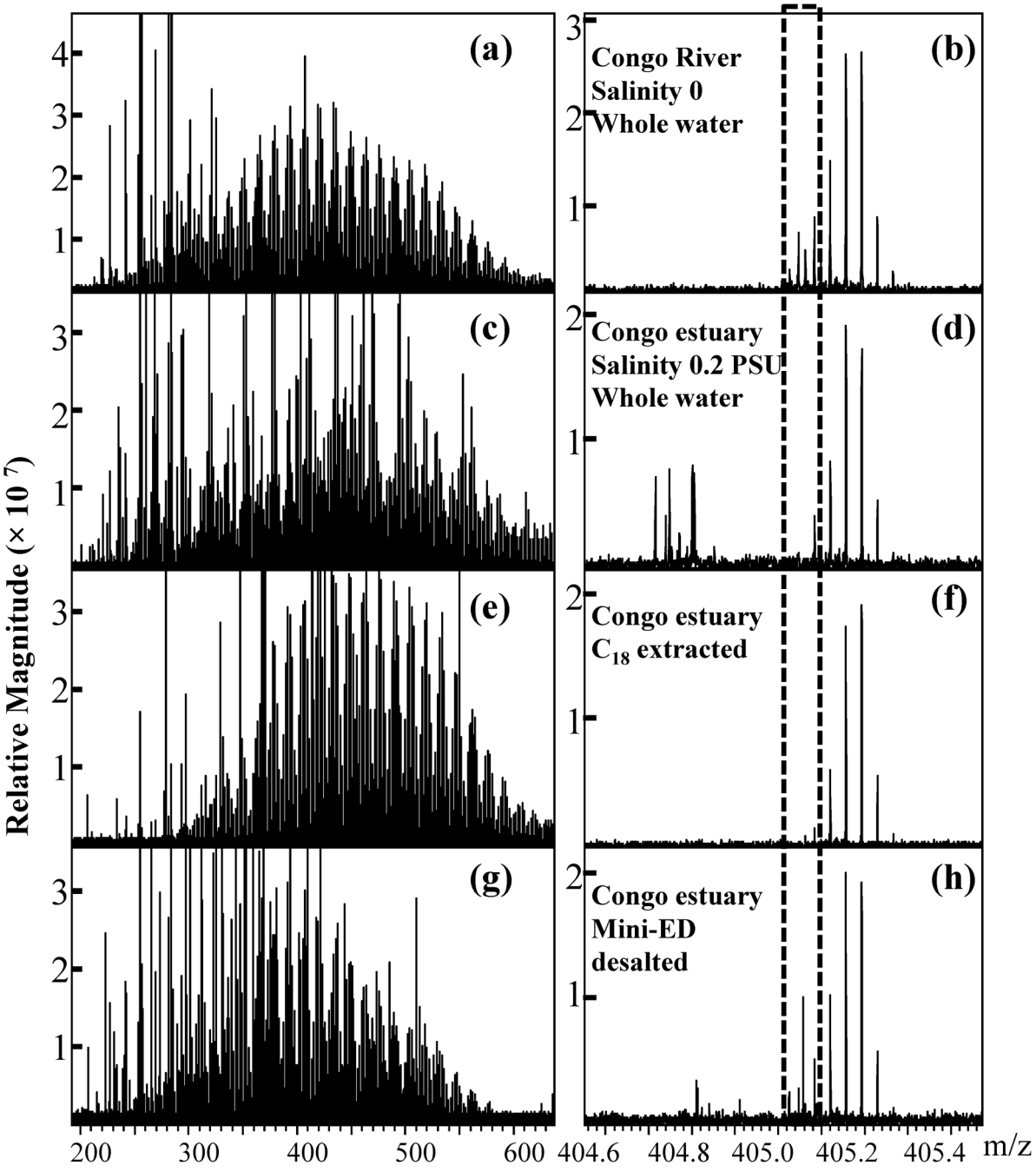

Fig. 2. Negative ESI FT-ICR mass spectra of DOM from the Congo River and estuary. The spectra of untreated water (a, b, c, and d), C_{18}-extracted water (e and f), and water desalted by the mini-ED (g and h) are shown. On the left, full spectra are displayed, whereas on the right, each expanded region m/z between 404.50 and 405.50 is shown. The boxed-in area represents peaks with mass defects between 0.00 and 0.06.

To further examine the molecular signatures retrieved using mini-ED, molecular formulas for peaks in the desalted and original Dismal Swamp samples were compared. Above 80% of peaks in each treatment were successfully assigned formulas, which is promising for comparison. Table 2 summarizes the information of peaks and assigned molecular formulas retrieved in each treatment. The number averaged percentage (%a in Table 2) and magnitude-weighted percentage (%b in Table 2) were calculated with the same equation formulated by Sleighter et al. (2009).

Number averaged% = (Number of each formula type / Total number of formulas) × 100

Magnitude-weighted% = (Sum of peak magnitudes of each formula type/Summed total peak magnitude) ×100

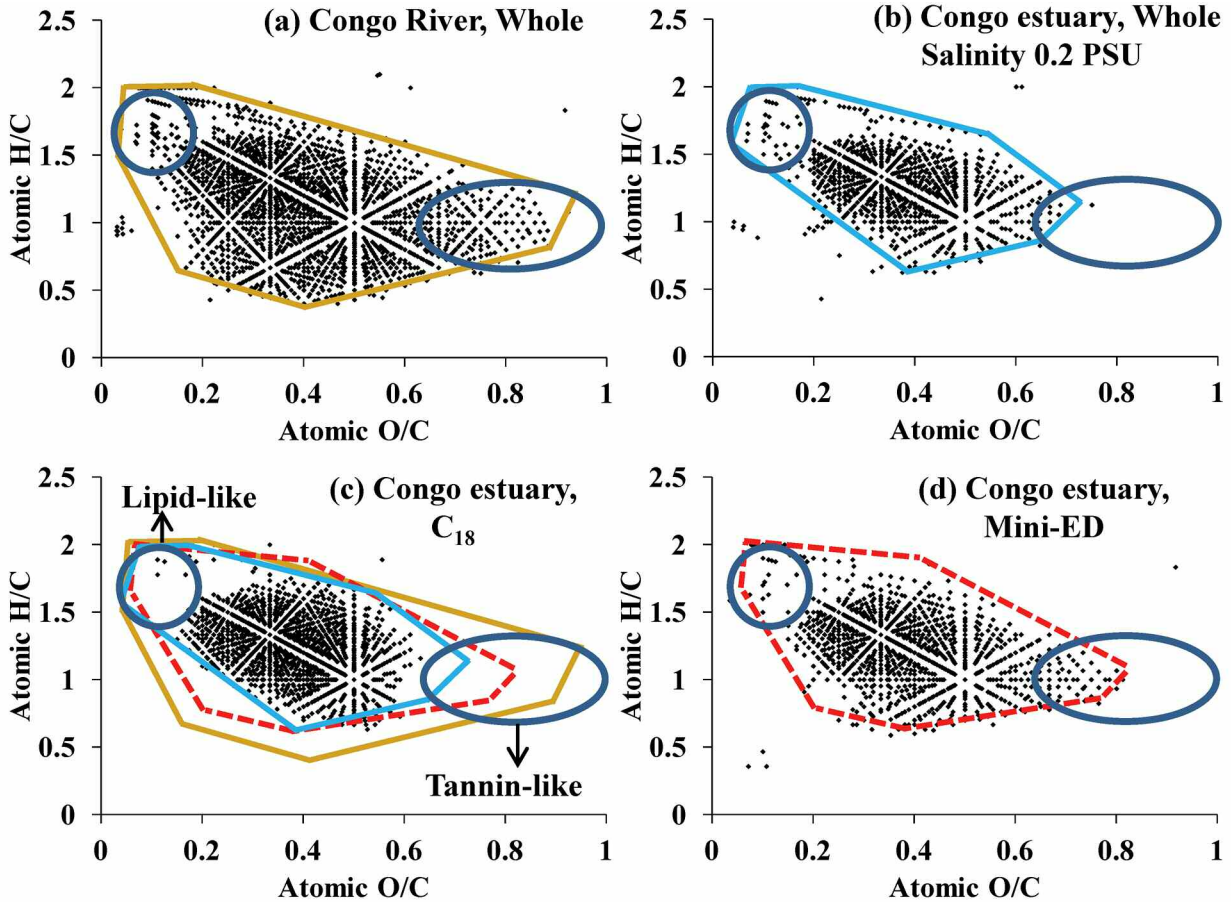

Fig. 3. Van Krevelen diagrams for the DOM, obtained from the assigned formulas (CHO-only) in each sample. Angular colored boxes represent the regions of main data coverage for each sample. Regions defined in diagrams (a), (b), and (d) are also plotted in diagram (c) to show the relative coverage compared with the sample extracted by C_{18}. Circled areas represent regions in which tannin-like and lipid-like compounds are normally observed.

Table 1. The number of peaks in the Congo River and estuary water DOM (S/N above 4).

Sample	Total	Mass defect ≥ 0.7	Mass defect 0.00 to 0.06
Congo River, Whole water	3215	62	421
Congo estuary, Whole water	2808	986	41
Congo estuary, C_{18} extracted	2232	139	19
Congo estuary, mini-ED desalted	2021	505	173

After mini-ED the percentage of peaks assigned a molecular formula decreased from above 95% in the fresh Dismal Swamp DOM to below 90% in the mini-ED desalted DOM. This was attributed to the samples after mini-ED containing more heteratomic molecular formulas, which are more difficult to assign molecular formula than are CHO-only formulas. The total number of formulas in each of the four Dismal Swamp treatments are close to each other and the percentages are in the same order as the CHO-only formulas dominate in each treatment, indicating that the DOM after mini-ED is similar to the fresh Dismal Swamp DOM. The desalted samples provide similar mass spectral data, suggesting the mini-ED method isolates a reproducible fraction of the DOM. The major difference is that in the desalted Dismal Swamp (35 PSU) DOM, there is a higher percentage of CHON formulas and more formulas with atomic N/C ratios above 0.1 (Table 3). N/C ratios of 0.0 to 0.1 suggest long-chain alkyl amines, whereas N/C ratios of 0.1 to 0.4 suggest peptides and proteins (Sleighter and Hatcher 2007). The occurrence of more peptides or proteins in the desalted 35 PSU Dismal Swamp DOM than in the 15 PSU treatment is not readily explained and further work is required to address this potential mini-ED fractionation effect.

In each treatment, the majority of peaks were assigned as CHO-only formulas. Van Krevelen diagrams for the CHO-only

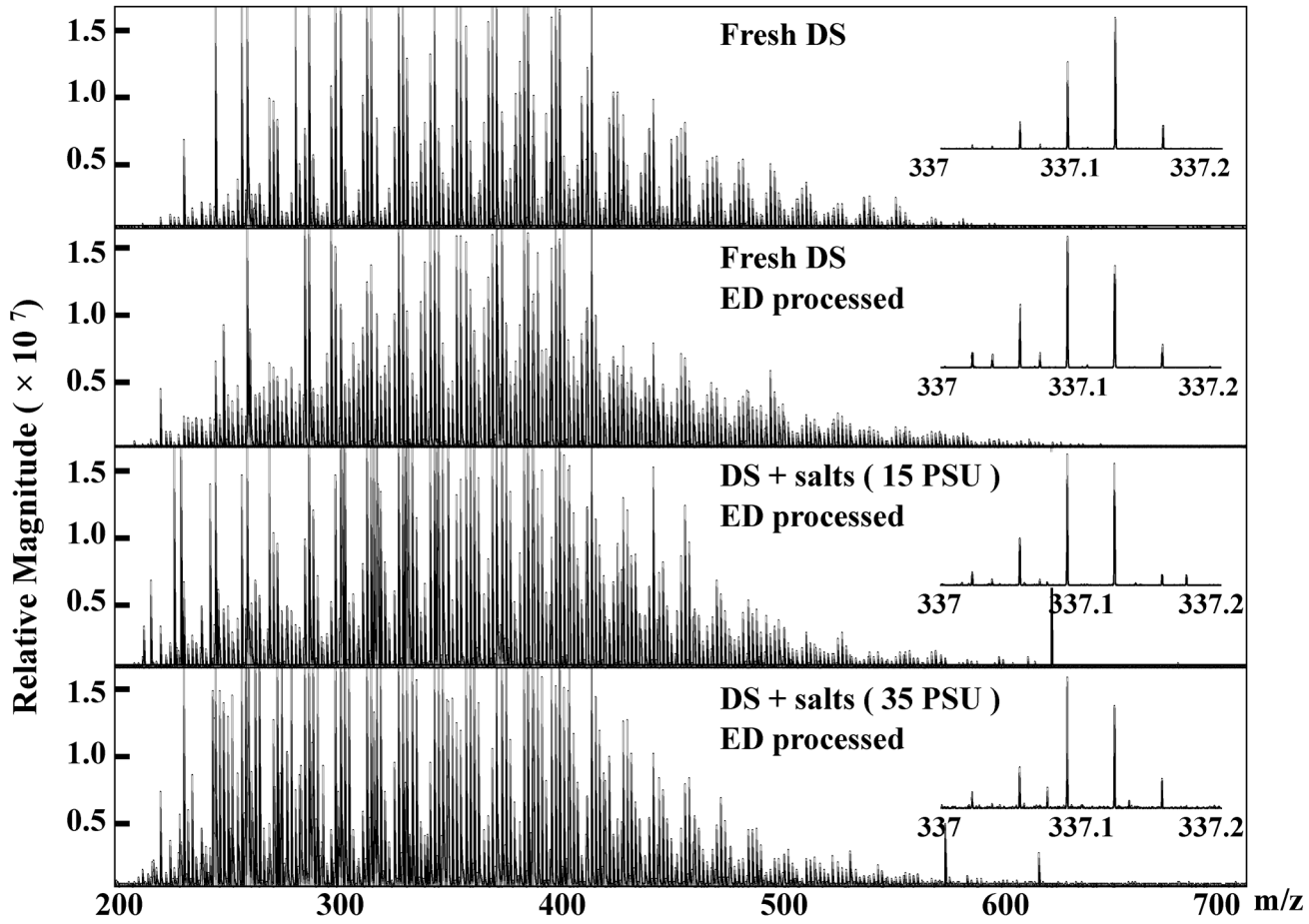

Fig. 4. Negative ESI FT-ICR mass spectra of fresh Dismal Swamp (DS) DOM and mini-ED processed Dismal Swamp samples from different salinity level. The inset shows the region m/z between 337.0 and 337.2 as an example of the similarity among spectra.

Table 2. The percentage of peaks assigned a molecular formula, the total number of formulas, the number averaged percentage (%a) and magnitude-weighted percentage (%b) of each type of molecular formula present in each sample, excluding the instrumental blank peaks and peaks due to ^{13}C isotope. CHONSP includes CHOS, CHOP, CHONS, CHONP, CHOSP, and CHONSP formulas.

Sample	% of Peaks assigned formulas	# Formulas	CHO		CHON		CHONSP	
			%a	%b	%a	%b	%a	%b
DS	96.4	1488	94.4	97.9	3.7	1.4	1.9	0.8
DS, ED processed	81.2	1572	86.2	92.9	7.1	2.9	6.7	4.2
DS + salts (15 PSU), ED processed	84.7	1379	81.2	87.1	8.8	4.1	10.0	8.8
DS + salts (35 PSU), ED processed	88.9	1387	72.6	79.9	18.2	12.1	9.2	8.0

Table 3. The number averaged percentage (%a) and magnitude-weighted percentage (%b) of each type of CHON formulas identified in each sample.

Sample	N/C < 0.1		0.1 < N/C < 0.4	
	%a	%b	%a	%b
DS	96.4	96.4	3.6	3.6
DS, ED processed	92.0	91.4	8.0	8.6
DS + salts (15 PSU), ED processed	92.6	89.7	7.4	10.3
DS + salts (35 PSU), ED processed	79.1	81.7	20.9	18.3

formulas show the four treatments to have considerable overlap (figure not shown). There is no specific area in the diagrams showing any unique formulas from one treatment to the other. As no systematic differences were apparent from visual inspection of the van Krevelen diagrams of the CHO-only formulas that were assigned to various compound classes following Stubbins et al. (2010). Carboxylic-rich alicyclic molecules (CRAM) are a class of molecules hypothesized to dominate the refractory DOM pool of the oceans and are defined as having DBE/C = 0.30 to 0.68; DBE/H = 0.20 to 0.95; DBE/O = 0.77 to 1.75 (Hertkorn et al. 2006). Aliphatics are defined as having DBE/C < 0.3 and H/C 1.0 to 3.0 (Perdue 1984). Aromatics are defined by having high aromaticity index (AI; Koch and Dittmar 2006). AI is calculated from molecular formula as: AI = (1 + C – O – 0.5H) / (C – O). When AI > 0.5, the corresponding molecular formulas are determined to be aromatic. Tannin-like formulas are defined as those having atomic O/C ratios between 0.60 and 0.95 and atomic H/C ratios between 0.55 and 1.40. This is inferred from Sleighter and Hatcher (2007). The percentages of each compound class in each sample are similar (Table 4), suggesting that the mini-ED method recovers DOM representative of the whole initial DOM. The major difference here is that in the mini-ED desalted samples, the percentage of CRAM-like formulas decreased but the percentage of tannin-like formulas increased. This suggests that the mini-ED retains tannins-like compounds preferentially to CRAM-like compounds. In oceanic DOM samples, which do not contain much tannin-like material, this problem may be avoided.

DOC recovery

DOC recovery experiments were initially planned to be conducted together with each sample desalting process before FT-ICR MS analysis. However, the desalted sample volume (1.5 mL) was not enough to fulfill both FT-ICR MS analysis and DOC measurement. Therefore, DOC recovery was determined in a separate set of test. Freshwater from the Dismal Swamp, which had an original DOC of 40 mgC L^{-1}, was 1:3 (v:v) diluted with artificial seawater to a DOC concentration of 10 mgC L^{-1}. Two different salinity levels of artificial seawaters were used and the salinity was adjusted accordingly to ~15 PSU and ~35 PSU. The saline samples were then desalted using mini-ED as all the real samples were treated. The test with an adjusted salinity of ~15 PSU gave a DOC recovery of ~57%. The test with an adjusted salinity of ~35 PSU gave a DOC recovery of ~55%. Note that the DOC recovery calculation here was corrected by excluding the contamination DOC (~2 mgC L^{-1}) mentioned above (See "*Blank test*"). These recoveries are somewhat similar to recoveries of terrestrial DOM obtained using the C_{18} extraction methodology (Kim et al. 2003; Simjouw et al. 2005; Mopper et al. 2007) or ultrafiltration (Buesseler et al. 1996; Guo and Santschi 1996; Benner and Opsahl 2001; Hernes and Benner 2006). However, when compared with the large-scale RO/ED methodology that typically shows recoveries of better than 75% (Vetter et al. 2007; Koprivnjak et al. 2009), the mini-ED system is not as effective at retaining DOM. This may be due to the use of a 500 Da mini-ED membrane rather than the 300 Da membrane that is commonly used in the larger RO/ED system (Vetter et al. 2007; membrane cutoff information obtained from personal communication with Dr. Mike Perdue at Georgia Institute of Technology). Much of the lost DOC (~45% of the initial DOC), may end up in the surrounding MilliQ water in the mini-ED system. Future work should determine carbon mass balances for the system and the molecular classes of compounds lost to the surrounding MilliQ bath water. Whereas recoveries are lower than the large-scale RO/ED system, the mini-ED does not show fractionation as observed in C_{18} extraction approaches. The FT-ICR MS spectra are representative of the whole DOM offering a method for the isolation of DOM from saline waters without the loss of DOM molecular information.

Comments and recommendations

Mini-ED is an efficient method for desalting small volume (0.5 to 10 mL) samples before analysis by ESI FT-ICR MS. More representative molecular information can be expected from this method when compared with C_{18} extraction. Contamination from the mini-ED system does not significantly affect ESI FT-ICR mass spectra. If mini-ED is applied to desalt samples before other analyses, e.g., HPLC or NMR, analysis-specific blanks and recoveries should be assessed. In this regard, future study should attempt to find a source of other membrane and bath materials to reduce contamination, thereby enabling coupling of mini-ED to other analytical techniques. Significantly, the desalting approach described here can be done quite easily on small amounts of sample either aboard ship or in the laboratory. The small sample size requirement makes

Table 4. The number averaged percentage (%a) and magnitude-weighted percentage (%b) of each type of CHO-only formulas identified in each sample.

Sample	CRAM-like		Aliphatics		Aromatics		Tannin-like		Others	
	%a	%b	%a	%b	%a	%b	%a	%b	%a	%b
DS	59.1	71.6	4.5	3.9	8.2	4.4	19.6	15.8	8.6	4.3
DS, ED processed	49.8	60.4	2.1	1.8	13.8	9.0	24.7	26.9	9.5	1.9
DS + salts (15 PSU), ED processed	51.5	56.7	4.8	3.6	7.1	4.1	30.3	33.2	6.3	2.4
DS + salts (35 PSU), ED processed	47.0	47.6	8.5	9.1	4.8	2.9	33.1	33.5	6.7	7.0

this technique amenable to studies of porewaters and other environmental samples and experimental designs where large water volumes are not feasible. There are still a lot of tests to be completed to optimize this method, such as looking for bigger chambers to minimize the processing time. An important consideration when coupling to other analytical techniques will be the DOC mass balance. It is also unknown how the DOC recovery will change under different initial DOC concentrations and qualities. Testing the system with real oceanic DOM samples, which are both much lower in DOC concentration and chemically distinct from those samples studied herein, is a major priority. Finally, as part of this oceanic sample isolation, the mini-ED performance should be systematically compared with currently available DOM isolation techniques such as PPL extraction, ultrafiltration, and the larger volume RO/ED.

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