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Received November 23, 2015

Revised January 11, 2016

Accepted January 12, 2016

Research Article

Determination of size-dependent metal distribution in dissolved organic matter by SEC-UV/VIS-ICP-MS with special focus on changes in seawater

Iron is an essential micronutrient for all marine organisms, but it is also a growth limiting factor as the iron concentrations in the open ocean are below 1 nmol/L in sea water iron is almost entirely bound to organic ligands of the dissolved organic matter fraction, which are mostly of unknown structure. The input from rivers was traditionally considered as less important due to estuarine sedimentation processes of the mainly colloidal iron particles. However, recent studies have shown that this removal is not complete and riverine input may represent an important iron source in the open ocean. In this context, iron transport by land-derived natural organic matter (NOM), and dissolved organic matter (DOM) have been identified as carrier mechanisms for riverine iron. The aim of this work is to characterize complexes containing iron and other metals in waters simulating estuarine conditions in order to help understand which role iron-DOM compounds play in the open ocean. A method based on size-exclusion chromatography (SEC) with sequential UV/VIS and ICP-MS detection was developed for investigation of DOM size distribution and for assessment of the size-dependent metal distribution in NOM-rich surface water. Furthermore, sample matrix experiments were also performed revealing a dependence of DOM size distribution upon seawater concentration and different compounds present in seawater. Finally, efforts toward determination of DOM size with standardization with typical SEC standards indicate that only relative comparisons are possible with this approach, and that the sample matrix composition strongly influences obtained results.

Keywords:

Humic substances / Iron / Natural organic matter / Ocean / SEC-UV/VIS-ICP-MS
DOI 10.1002/elps.201500538

1 Introduction

Iron is an important micronutrient for marine organisms [1], [2] as a significant proportion of biological electron transport systems are based on Fe(II)/Fe(III) redox reactions. In the late 1980s, iron deficiency was identified as a major factor for the formation of high-nutrient, low-chlorophyll (HNLC) regions, which are rich in macronutrients such as nitrogen and phosphorus, but also where phytoplankton growth in the euphotic zone remains low [3]. Approximately 30% of the world's oceans are considered as HNLC areas [4]. For example, in the northeast Pacific, iron surface concentrations of approximately

0.05 nmol/L were reported by Martin *et al.* in 1989 [5]. The pool of iron in the ocean consists of particulate, colloidal, and

dissolved species, where the dissolved fraction is considered to be mostly bioavailable [6]. Dissolved iron was originally defined as filterable over 0.2 or 0.45 μm membranes [7], but it is now known that this size fraction includes colloids as well [8], [9].

Iron deficiency in the ocean is due to a number of factors. The solubility of iron(III) in seawater is very low, while the lifetime of the more soluble iron(II) is rather short due to oxidation or biouptake [10]. Despite the low solubility of inorganic iron(III) in seawater of between 0.08 and 0.2 nmol/L [11], [12], almost constant deep water concentrations of around 0.7 nmol/L have been measured in various ocean basins [13]. This is attributed to the fact that iron is almost entirely (i.e. >99%) coordinated to organic ligands [14] and therefore the actual concentrations can exceed the solubility product of dissolved inorganic iron in seawater. The ligands found in the upper ocean are assumed to be largely derived from bacterial siderophores [15], but rather little is

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Table 1. Instrumental operation conditions for size exclusion chromatography and UV/VIS detection

Chromatographic separation	Ultimate 3000 × 2 Dual Titanium
Column	BioBasic SEC 120, 5 μm, 120 Å
Flow rate [mL/min]	0.300
Mobile phase	15 mM ammonium acetate, pH 6.9, 25% MeOH
Injection volume [μL]	50
UV/VIS wavelength [nm]	205, 220, 254, and 320

currently known about the chemical structure of these ligands. Nevertheless, several marine siderophores have been characterized on a molecular level, e.g. the hydroxamates Ferrioxamine E and G [16] or Amphibactin D and E [17].

The source of external iron in marine environments is still not fully understood and a number of factors should be considered. Atmospheric dust input plays a very important role over wide areas of the ocean [3, 5] while the re-dissolving of sediments [18] and the riverine input [19] lead to higher concentrations of iron in coastal and estuarine mixing zones in comparison to offshore regions [20]. Riverine iron mainly consists of colloidal particles of iron oxides and iron bound to natural organic matter (NOM), of which humic substances (HS) are a significant part [21]. It is well accepted that a large proportion of the incoming iron is removed due to salting out [22], [23], but this elimination is not necessarily complete [24].

Newer research has indicated that peat bog-derived HS can extend further out into the open sea than previously assumed and are apparently able to transport riverine iron into offshore regions [25], [26]. NOM is a highly complex mixture of all kind of degradation products and despite the crucial role NOM plays in biogeochemical processes, the size, molecular structure, and compositions remain poorly understood [27]. Traditionally, NOM was considered to primarily contain components with molecular weights from 20 to 200 kDa. These results were obtained by gel- and ultrafiltration (i.e. by noncolligative methods) [28], [29], [30]. Conversely, estimations based on colligative methods propose much lower average molecular weights. For example, Pavlik *et al.* investigated NOM taken freshly from the Suwannee River by Vapor Pressure Osmometry and determined an average molecular weight of 614 g/mol [31]. This suggests that NOM consists of relatively small molecules which form supramolecular assemblies held together by hydrophobic effects and hydrogen bonds; a view that several newer studies also support [32], [33]. The formation of these aggregates may be influenced by concentration, pH, ionic strength, and the salts present. All of those parameters are subject to changes in the estuarine mixing zone upon the dilution in seawater. The finding that land-derived NOM can transport iron and other trace metals to the open sea is still relatively new and requires further studies.

Cathode stripping voltammetry (CSV) studies of iron and HS in seawater and coastal water showed some covariations [34] and therefore proved that HS can reach out further into the open ocean and play a more important role in iron transport than previously assumed. Electrochemical experiments may provide insight into the behavior of a species in solution regarding, for example, reaction kinetics, mechanism, and concentration [35], where the low LODs for metals (sub-μg/L) are a special advantage of CSV. While insight into the redox chemistry of metal-NOM assemblies can deliver valuable information, such methods cannot sufficiently characterize them as information about the size, shape, and chemical composition the coordination compounds is not easily derived.

Field-flow fractionation based separation and classification is another widespread technique to characterize NOM and its metal binding capabilities. For example, it has been shown by asymmetric flow field-flow fractionation (AsFFF) coupled to ICP-MS and optical emission spectroscopy that iron bound to NOM is almost completely resistant to salting out, whereas colloidal FeO_x particles precipitate almost entirely at only several ‰ of seawater [26], [36]. By covering a size range from about 1 to 10⁹ kDa, the working range of FFF techniques is very large and overlaps to some degree with size exclusion chromatography (SEC). In FFF, unspecific interactions with the stationary phase are of less concern [37], but on the other hand, the technique does not provide insight for the characterization of low molecular weight compounds below < 1 kDa where SEC remains the method of choice. As a matter of fact the major DOM fractions are heavier than 1 kDa; however this size range is interesting to investigate the presence of small complexing moieties, which are not amenable to FFF.

The size-distribution of NOM can be studied by SEC-UV/VIS and also SEC-ICP-MS, though size determination via comparison with typical standards (e.g. polystyrene sulfonate) as frequently performed in previous studies [38], [39], [40], [41] should be treated with caution as the results can vary widely depending on, for example, sample matrix or mobile phase composition. NOM has been intensively studied using SEC [42], [43], [44] but the influence of mobile phase composition (especially regarding pH and ionic strength) and adsorption of material to the stationary phase are known to be problematic [45]. Not only the behavior on SEC materials, but also the formation of supramolecular assemblies is potentially altered.

In the present study, an SEC separation method was developed and combined on-line with UV/VIS and ICP-MS detection. The affinity of different metals for different size fractions was investigated and compared to results from total quantification of relevant metals obtained via ICP-SFMS. Changes occurring in the estuarine mixing zone were simulated by varying the sample matrix in order to support future studies aiming at the investigation of the fate of iron during distribution into offshore regions. Compared to previous studies a special focus is placed on the investigation of the low molecular weight fraction aiming at the detection

of putative ligands originating from land derived DOM and on size distribution in artificial seawater or ionic seawater components.

2 Materials and methods

2.1 Materials

Artificial seawater was prepared with subboiled water in a cleanroom (EN ISO 146441–5, Laminar flow) according to the procedure proposed by Kester *et al.* [46]. Sodium chloride ($\geq 99.5\%$) was purchased from Merck KGaA, Darmstadt, Germany. Magnesium chloride hexahydrate (99%), calcium chloride dihydrate, sodium sulfate ($\geq 99.5\%$), potassium chloride ($\geq 99.5\%$), sodium hydrogencarbonate ($\geq 99.5\%$), potassium bromide (99%), boric acid ($\geq 99.5\%$), strontium chloride hexahydrate (99.995%), and sodium fluoride (99.99%) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The pH was adjusted to 8.2 using Na_2CO_3 and measured with a PHM92 pH-Meter (Radiometer, Copenhagen, Denmark) with a micro electrode (inLab Micro, Mettler, Toledo, USA).

Water was purified employing an Ultra Clear basic reverse osmosis system (SG Wasseraufbereitung und Regenierstation GmbH, Barsbuettel, Germany) and subsequently subboiled. Methanol LC-MS Grade was obtained from Thermo Fisher Scientific (Loughborough, UK). Buffer stocks were prepared with acetic acid (glacial, suprapure) from Merck and ammonium hydroxide from Sigma-Aldrich ($> 25\%$ in water, for trace analysis). Nitric acid (analytical reagent grade) was purchased from Merck and doubly subboiled before use. Elemental standards for total quantification (ICP multi-element standard solution VI CertiPUR) and internal standardization (Indium ICP standard, CertiPUR) were obtained from Merck. The accuracy of results from total quantification was confirmed by measuring the certified reference material Tm27.3 lot 0510 from the Canada Centre for Inland Waters National Laboratory for Environmental Testing (Burlington, Ontario, Canada). Ferrioxamine E (from *Streptomyces* antibioticus, $\geq 95\%$ (HPLC)) was obtained from Sigma-Aldrich. Sodium hydroxide monohydrate (suprapure, 99.99%) for pH adjustments was purchased from Merck.

2.2 Instrumentation

An Ultimate 3000 \times 2 Dual Titanium HPLC system from Thermo Fisher controlled by Chromeleon 6.8 software was used for all SEC experiments. The eluents and samples were in contact with titanium and PEEK surfaces only. HPLC operation conditions are listed in Table 1.

The HPLC-system was coupled to an Elan 6100 dynamic reaction cell II quadrupole ICP-MS from Perkin-Elmer-Sciex (Ontario, Canada). For minimization of spectral interferences (especially $^{40}\text{Ar}^{16}\text{O}$, which is interfering with ^{56}Fe), the dynamic reaction cell with CH_4 as reaction gas was utilized. For

total quantification of the monitored metals in Suwannee River NOM, an Element 2 high resolution sector field ICP-MS instrument (ICP-SFMS) from Thermo Fisher (Bremen, Germany) was used in medium resolution mode. Measurements were performed in an EN ISO 146441–4 cleanroom.

Full details of ICP-MS operation conditions are listed in Tables 1 and 2.

2.3 Samples

Suwannee River NOM 2R101N (SR NOM) was purchased from the International Humic Substance Society (St. Paul, Minnesota, USA). The freeze-dried samples were dissolved in several exact v/v concentrations of artificial seawater (ranging from 0 to 100%) or in various salt solutions representative of those present in seawater in order to investigate their influence on the size distribution. Unless stated otherwise, 0.5 g/L of material were dissolved, vortexed for at least 60 s and filtered over 0.45 μm syringe filters.

All experiments were performed in triplicate and averaged in the depicted Figures. Matrix blanks (meaning seawater blanks for the samples dissolved in seawater and water blanks for the samples in water) were subtracted from all shown chromatograms and Ferrioxamine E standards were measured between the samples to control stability of retention time and sensitivity. For better comparability, some of the presented chromatograms were normalized to the highest signal.

For the reference material Suwannee River NOM, total concentrations for the monitored elements were determined using ICP-SFMS. 1 mg sample was suspended in 1 mL water or 50% artificial seawater, vortexed for 1 min, filtered over 0.45 μm , diluted 1:10 with 2% subboiled HNO_3 and spiked with 1 $\mu\text{g/L}$ indium for internal standardization.

3 Results and discussion

3.1 Total quantification of Suwannee River NOM

In a first step, total quantification of the monitored metals was performed to quantify the distribution of the different metals over the size fractions obtained after NOM speciation via SEC-ICP-MS. After the isolation of NOM according to the protocols of the International Humic Substance Society [47], [48], which includes reverse osmosis followed by a cation exchange, it can be assumed that free salts and metal cations are mostly removed and the remaining metal ions are bound to NOM (free iron would precipitate in only a few per mil of seawater [36]). The absence of free iron is confirmed by the fact that only 8% less iron were found the sample dissolved in 50% artificial seawater compared to the sample dissolved in pure water (see Table 3). This observation is further supported by the SEC-ICP-MS results presented below.

To compare our data with the concentrations reported by Kuhn *et al.* [49] in Suwannee River NOM after reverse

Table 2. Instrumental operation conditions ICP-MS and SEC-ICP-MS methods

ICP- MS	Thermo Element 2 (total quantification)	Elan DRC II (combined with Ultimate 3000)
Nebulizer	PFA	PFA
Spray chamber	Cyclonic	Cyclonic
RF power [W]	1350	1250
Nebulizer gas flow [L/min]	1.05	0.9
Auxiliary gas flow [L/min]	0.8	1.3
Plasma gas flow [L/min]	16	16
Cell gas flow [mL/min]	–	1.0 CH ₄
rpq	–	0.8
Resolution	4000 (FWHM 5%)	Nominal
Monitored ions	⁵⁵ Mn, ⁵⁶ Fe, ⁶⁰ Ni, ⁶⁵ Cu, ⁶⁶ Zn	⁵⁵ Mn, ⁵⁶ Fe, ⁶⁰ Ni, ⁶⁵ Cu, ⁶⁶ Zn

Table 3. Total metal concentration [$\mu\text{g/L}$] measured via ICP-SFMS in a Suwannee River DOM solution ($n = 3$). Nondissolved particles were removed by syringe filtration

[$\mu\text{g/L}$] metal in	⁵⁶ Fe	⁶⁵ Cu	⁶⁶ Zn	⁵⁵ Mn	⁶⁰ Ni
1 mg/mL Suwannee River NOM					
H ₂ O	983	47.3	80.8	6.1	25.6
50% seawater	905	31.4	67.7	4.8	13.5
Percent RSD	2.8	2.0	2.1	3.3	0.93

Table 4. Metal concentration in Suwannee River DOM in nM mg/C compared to the concentrations published by Kuhn *et al.* [44]

nmol per mg carbon	Fe	Cu	Zn
Suwannee River NOM in H ₂ O	50.3 \pm 1.41	2.13 \pm 0.04	3.54 \pm 0.07
Kuhn <i>et al.</i>	41.3	0.825	1.36
Difference %	+21.8	+158	+160

osmosis, the concentrations are given in nmol/mg of carbon using the carbon content reported by the IHSS for the purchased batch [50] and taking into account that 0.69 mg were actually dissolved. The suspended fraction was determined by differential weighing after syringe filtration and removal of the solvent. It can be seen in Table 4 that the results for the different metals differ to some extent, which can be explained by different sampling time points, but also by varying degrees of contamination during the isolation procedures applied.

3.2 Size-dependent metal distribution of Suwannee River NOM in 50% artificial seawater

The investigation of NOM in (artificial) seawater was the main focus of this study aiming to support research on the question of which constituents/fractions of NOM may be able to transport iron and other biologically relevant trace metals to the open ocean. UV/VIS and ICP-QMS chromatograms of

Suwannee River NOM (0.5 mg/mL) depicted in Fig. 1 were recorded by applying the SEC method described in the experimental section. The precision regarding retention time and peak area of the ICP-MS signals obtained with the applied method was assessed by measurement of 10 μM Ferrioxamine E. For 12 replicate measurements distributed over an 8-h sequence, a retention time precision of 1.2% RSD and a peak area precision of 5.3% RSD were obtained. The determination of LODs and recoveries for the DOM constituents obtained after SEC-ICP-MS analysis is not feasible as the identity of the fractions is unknown and no standards are available. The total column recovery for DOM bound iron was approximately 30% for the Suwannee River samples. LODs were determined for the Ferrioxamine E standard as the 3-fold standard deviation of the baseline signal and yielded a value of 70 nM.

Based on these results, interpretation on the affinities of the individual metals for the organic fractions can be made. Iron is predominately found in the largest (earliest eluting) fraction. It can be assumed that the higher molecular weight fractions appear to be more abundant at 254 nm than they actually are due to their higher molar extinction coefficients [51]. The affinity of iron to those fractions is therefore presumably even higher than the presented chromatograms indicate. The preferred binding of iron to DOM (dissolved organic matter) constituents, which either show a tendency to form voluminous aggregates (possibly bridged by iron [52]) or are actually large on their own, has previously been reported in literature. For example, Kuhn *et al.* made these observations for reverse osmosis-isolated Suwannee River water samples [49] and Krachler *et al.* for river water samples taken at unpolluted sites in North Scotland (UK) [26] both based on results from ICP-AsFIFFF. In the present study, we are able to resolve the DOM size region further and can confirm these observations. Overall the UV/VIS chromatograms recorded at 254 nm and the Ni and Cu chromatograms were found to be similar. The Zn chromatogram resembles the UV/VIS chromatogram at 205 nm. Similar results concerning the size distribution of Fe, Ni, Cu, and Zn were obtained by Bolea *et al.*, who investigated compost leachate comparing AsFIFFF, SEC, and UF [45].

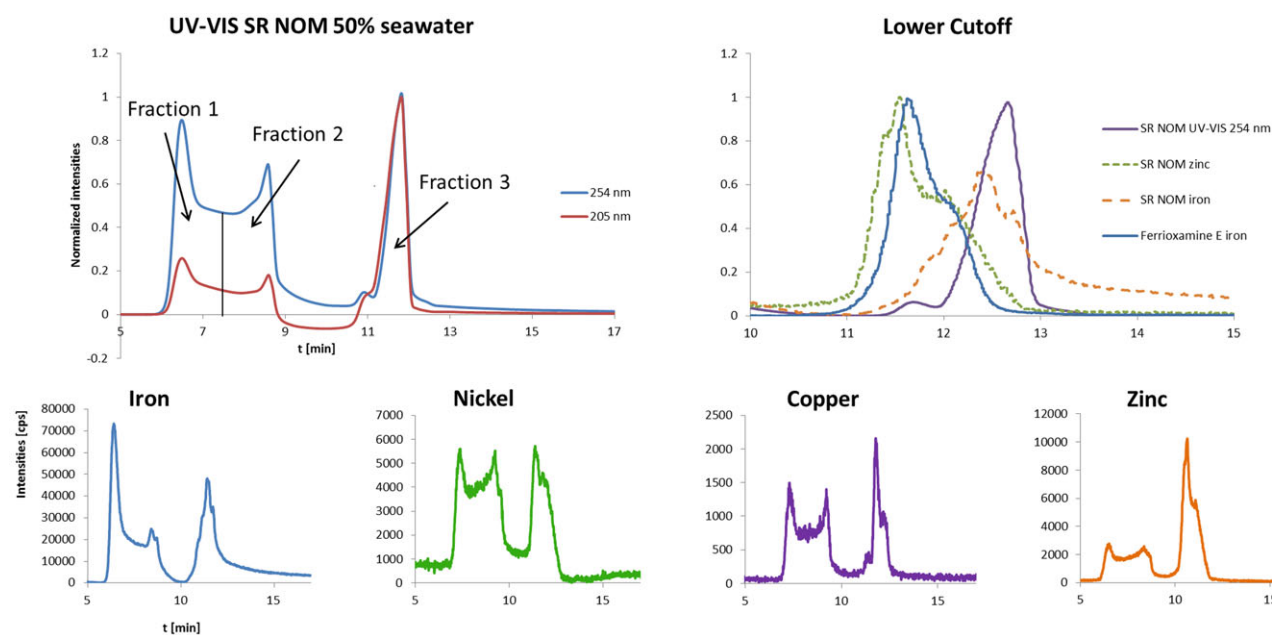


Figure 1. Chromatograms of Suwannee River NOM in 50% artificial seawater (conditions given in Tables 1 and 2). Three DOM fractions can be distinguished in the SEC-UV/VIS chromatogram. The chromatograms obtained by SEC-ICP-MS signals clearly show that iron is predominantly bound to the largest size fraction, whereas zinc has a high affinity to the low molecular weight fraction (fraction 3).

The region of the low molecular weight cutoff is depicted in Fig. 1. The iron, zinc, and UV/VIS (254 nm) chromatograms of Suwannee River NOM in 50% artificial seawater are overlaid with the Fe chromatogram of Ferrioxamine E ($M = 653.53$ g/mol). The last Zn fraction and Ferrioxamine E almost co-elute as do the third Fe fraction and fraction 3 in the UV/VIS. This shows that iron also binds to the lowest molecular size fraction and this fraction may be of special interest concerning its ability to transport iron to the open sea, as it is more likely to consist of covalently-bound units or complexes with high stability constants. It should be noted that at the peak apex of the Zn fraction, a small shoulder peak in the UV/VIS chromatogram can be seen, which is presumably corresponding to the Zn fraction, indicating a Zn-containing complex. Generally speaking, the affinity of the investigated metals to different size fractions points to substantial chemical differences among the size fractions. Moreover, it can be unambiguously concluded that in the smallest size fractions Fe, Cu, Ni, and Zn are associated to low molecular weight entities or ligands.

To investigate the influence of NOM concentration on the metal distribution, concentrations of Suwannee River NOM between 0.05 and 1 g/L were analyzed, but no significant concentration-dependent changes in the size exclusion chromatograms and distribution of metals were observed.

Moreover, native samples (just preconcentrated by evaporation and subsequently diluted in 50% artificial seawater) from the Craggie Burn Creek in Scotland were investigated with the presented method to assess the differences among the sampling sites and the influence of the isolation proce-

dures on Suwannee River NOM. The obtained results hardly differed from the presented data.

3.3 Investigation of the influence of ionic seawater components

Only one size fraction was obtained if Suwannee River NOM was dissolved in subboiled water instead of seawater. This raised the question if it is possible to determine which constituents of seawater are primarily responsible for the NOM size distribution described in Chapter 3.2. It is well-known that the behavior of NOM strongly depends on solution chemistry [53], such as ionic strength or sample matrix, as the change of the zeta potential and the electrostatic forces in the sample lead to a different conformation and an altered hydrodynamic radius of the sample components.

In Fig. 2, UV/VIS chromatograms of Suwannee River NOM dissolved in various salts recorded at

254 nm are shown. The sodium carbonate solution was adjusted to pH 7.8. Suwannee River NOM has a pH of 3.9 if dissolved in water and, if dissolved in 50% artificial seawater with pH 7.8, the pH increases to only 4.0. Therefore, the pH of all tested solutions varied to a very low extent. Solutions with 0.25 mol/L salt concentration according to the overall salt concentration in artificial seawater were prepared and the separation was conducted as described earlier. The chromatogram obtained from the sample dissolved in boric acid is the most similar to the one obtained in pure water.

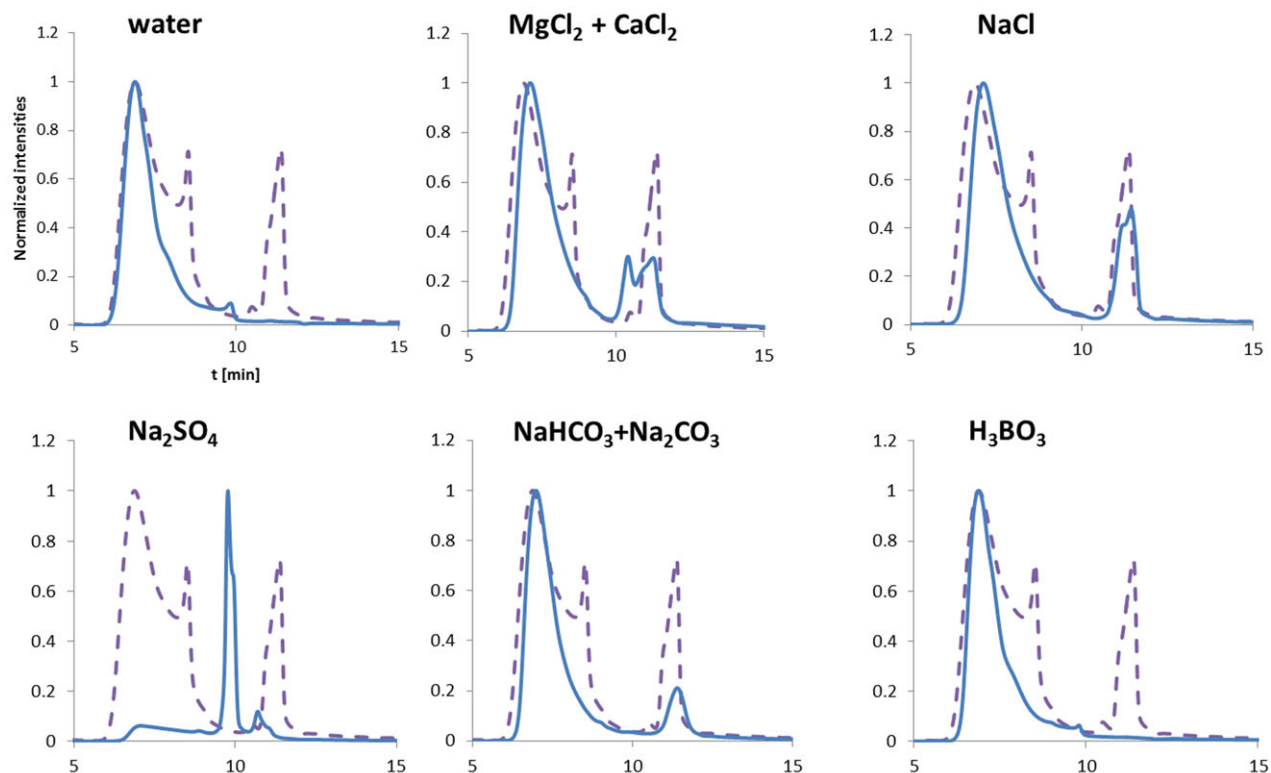


Figure 2. Influence of selected seawater constituents on DOM size distribution. The depicted UV/VIS chromatograms indicate that the conformation of DOM is severely altered in dependence of salt added. Solid line: selected seawater constituent, dashed line: Suwannee River DOM in 50% seawater.

The chloride salts seem to be responsible for the last peak representing the smallest NOM fraction, whereas in sodium sulfate only one rather sharp peak lying between the size fractions in 50% seawater was obtained. An explanation of this behavior has not yet been fully elucidated, but it is likely to be associated with the ionic strength in the sample matrix. This assumption is supported by the similarity of the chromatogram in boric acid (which is a very weak acid) to the chromatogram in water.

Seawater concentrations of 0, 25, 50, 75, and 100% were tested. Fraction 2 became more abundant with rising seawater concentration; fraction 3 did not exhibit further changes when more than 20% seawater were used. Finally, the influence of the pH on the separation was investigated; Suwannee River NOM (0.5 g/L in 50% artificial seawater) was adjusted to pH 5.03, 7.07, 7.93, and 9.08 with dilute NaOH. The UV/VIS chromatogram remained largely unchanged, though more large components were dissolved with increasing pH and the total areas of fraction 1 rose by approximately 26%, whereas the areas of fraction 2 and 3 remained almost unchanged. The increase in solubility of humic substances at basic pH is well known, which seems to be a good explanation for these observations.

3.4 Comparison of river NOM to polystyrene sulfonates (PSS) via SEC

To gain insight into apparent size of the Suwannee River DOM, sample fraction comparisons to a size ladder measured with PSS with 1, 4, 6, and 11 kDa were performed. The standards were dissolved in the same solvent as the sample to obtain valid results. It was shown by Perminova *et al.* that PSS has a charge density of 5.4 mmol/g [38], which is similar to that of dissolved humic material [39] and charge density is considered to be an important factor in molecular weight determination via SEC. Therefore, PSS should be well suited for this type of analysis. However, PSS can ultimately only give a rough orientation for size determination, which is why no calibration function was sought for the present study.

As depicted in Fig. 3, the first Suwannee River NOM fraction elutes before the 11 kDa PSS and the last fraction after the 1 kDa standard in 50% seawater. In 50% seawater it is therefore apparent that the sample constituents cover a wide molecular size range. In pure water neither the PSS from 11 to 4 kDa nor Suwannee River NOM are separated, however the 4 and the 1 kDa standards are separated. That hydrodynamic radii of the sample constituents depend on ionic strength has

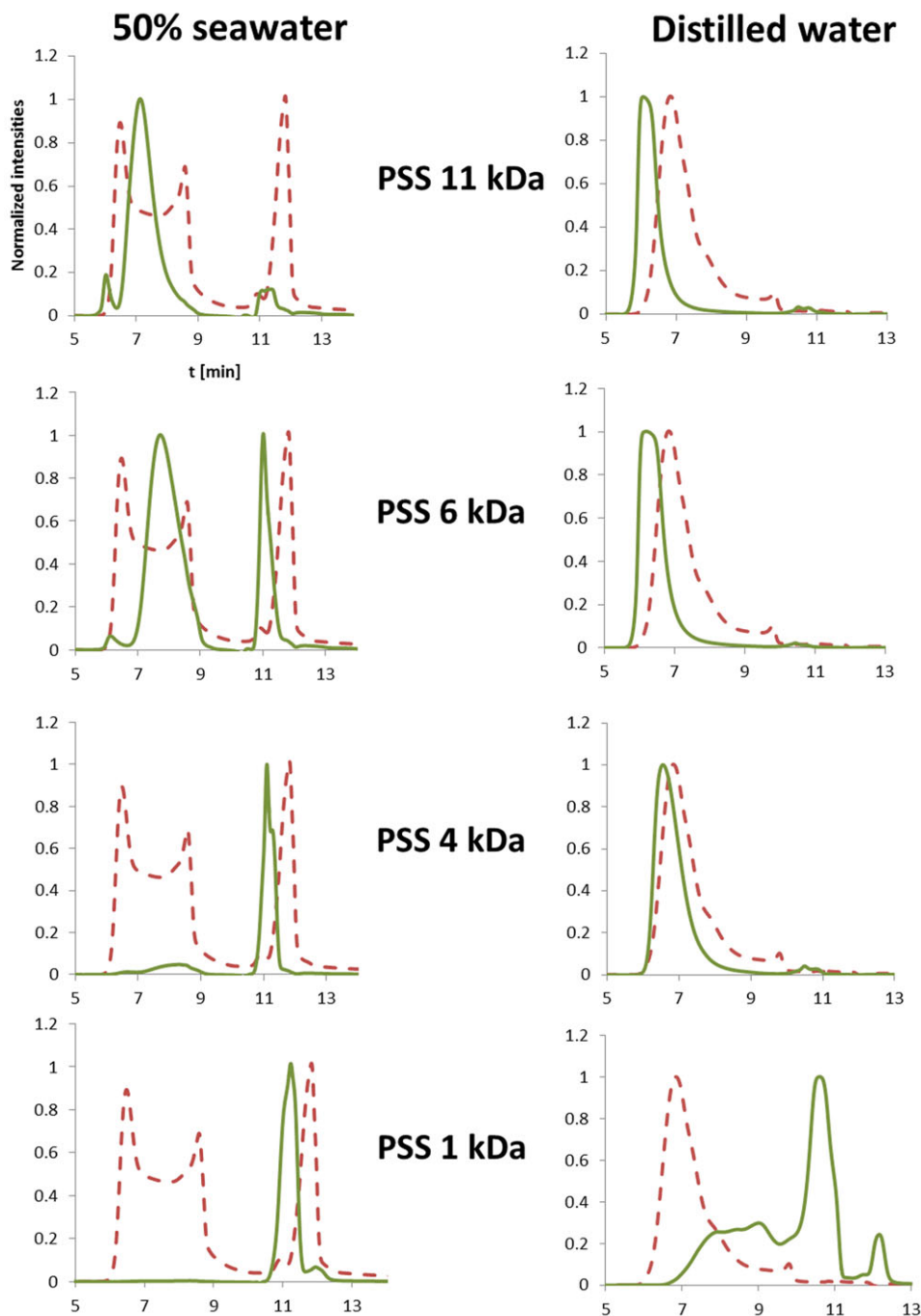


Figure 3. Comparison of Suwannee River DOM to polystyrene sulfonate (PSS) standards. The results obtained in the aqueous phase (distilled water) suggest aggregate formation due to hydrophobic effects of both DOM and PSS molecules leading to larger molecular structures. The chromatograms obtained for both DOM and PSS dissolved in 50% seawater indicate a wide size range. Dashed line: Suwannee River DOM, solid line: PSS.

already been addressed in Section 3.3. The chromatograms obtained from samples dissolved in distilled water suggest aggregate formation of the PSS and Suwannee River NOM leading to significantly increased hydrodynamic radii. These findings indicate that size determination and calibration via SEC utilizing common standards has to be critically assessed regarding solvent polarity and ionic strength. Nevertheless, the chromatograms obtained for both DOM and PSS dissolved in 50% seawater allow the estimation of a size range of between two and three orders of magnitude in the lower

kDa range. Especially the finding that a low molecular weight fraction is present in DOM supports the hypothesis that this fraction may play a role in iron transport into the ocean.

4 Concluding remarks

It was shown that the total iron content of low molecular weight NOM in a size range of approximately 1–11 kDa decreases very little upon dilution in seawater. This strongly

supports the theory that this NOM fraction is apparently able to keep iron in solution in the estuarine mixing zone, which goes in line with current hypotheses. In a next step, mixing zone samples from the river Halladale in Scotland will be investigated with our SEC-ICP-MS method. They contain about 40% seawater and still 2.5 mg/L Fe, as the river flows through a natural organic matter rich area.

Our measurements showed that Suwannee River NOM undergoes a diversification in size distribution upon dilution in seawater. Iron is predominantly bound to the large NOM fraction, but a significant proportion is also found in the low molecular weight fraction and consequently the focus of further investigations will concern the identification of low molecular weight iron-binding ligands using high-resolution molecular mass spectrometry. For copper, nickel, and zinc, findings previously made in compost leachate concerning the distribution among the size fractions could be reinforced in a different environment. This corroborates the idea that while absolute size determination of DOM using SEC must always be considered with caution, valid relative statements can be made based on such measurements.

The authors thank the Austrian Science Fund for the financial support (FWF, projects P19629-N19 and P 25849-N19).

The authors have declared no conflict of interest.

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