https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

Ocean Science
Discussions
Open Access
EGU

A protocol for quantifying mono- and polysaccharides in seawater and related saline matrices by electro-dialysis (ED) – combined to HPAEC-PAD

Sebastian Zeppenfeld[1], Manuela van Pinxteren[1], Anja Engel[2], Hartmut Herrmann[1,*]

1 Atmospheric Chemistry Department (ACD), Leibniz-Institute for Tropospheric Research (TROPOS), Leipzig, Germany

2 GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

**Correspondence to*: Hartmut Herrmann (herrmann@tropos.de)

Abstract

An optimized method is presented to determine free (DFCHO) and combined monosaccharides (CCHO) in saline matrices, such as oceanic seawater, Arctic ice core samples or brine using a combination between desalination with electro-dialysis and high performance anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD). Free neutral sugars, such as glucose and galactose, were found with 95-98% recovery rates. Free amino sugars and uronic acids were strongly depleted during electro-dialysis at pH=8, but an adjustment of the pH could result in higher recoveries (58-59% for amino sugars at pH=11; 45-49% for uronic acids at pH=1.5). The applicability of this method for the analysis of CCHO was evaluated with standard solution and real seawater samples compared with another established desalination method using membrane dialysis. DFCHO in real field samples from different regions on earth ranged between 11-118 nM and CCHO between 260-1410 nM. This novel method potentially contributes to a better understanding of biogeochemical processes in the oceans and sea-air transfer processes of organic matter into the atmosphere during further research studies.

Introduction

The majority of organic matter (OM) in oceanic seawater can be assigned to the chemical classes of proteinogenic amino acids, carbohydrates and lipids (Benner and Kaiser, 2003; Kaiser and Benner, 2009; Kuznetsova and Lee, 2002; Marty et al., 1979; Skoog et al., 1999; Wakeham et al., 1997). Previous studies uncovered that combined amino acids are the most abundant organic substances in fresh particles sinking

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020


Ocean Science
Discussions
Open Access
EGU

within the water column (Wakeham et al., 1997), although more recent studies indicate that carbohydrates may be equally abundant (Cisternas-Novoa et al., 2019), while hydrolysable carbohydrates dominate the chemical composition of dissolved organic matter (DOM) (Kaiser and Benner, 2009). Marine carbohydrates also appear in high concentrations in other related saline matrices, such as ice cores, brine and melt ponds in the Arctic (Ewert and Deming, 2013; Underwood et al., 2013; Zeppenfeld et al., 2019). Hence, a reliable analysis of carbohydrates is essential for understanding biogeochemical processes in the (Arctic) ocean and their impact on Earth's atmosphere.

Most marine carbohydrates exist as polysaccharides or combined sugars (CCHO), which are linear or branched chains of monosaccharides, including deoxy sugars, amino sugars and uronic acids. In living marine microorganisms including prokaryotes, polysaccharides assume their functions as structural compounds or as energy storage (Skoog and Benner, 1997). Storage carbohydrates mainly consist of glucose, such as laminarans and other glucans, while structural heteropolysaccharides (e.g. galactans) such as occurring in algal cell walls can contain a lot of galactose, mannose and rhamnose (McCarthy et al., 1996). Furthermore, an elevated release of polysaccharides by phytoplankton, mostly of gelatinous nature, has been associated to stress situations, such as a deficiency of nutrients, freezing or fluctuating water potential (Berman-Frank et al., 2007; Bianchi and Canuel, 2011; Borchard and Engel, 2012, 2015; Ittekkot et al., 1981; Krembs et al., 2002; Krembs and Deming, 2008). These exuded polysaccharides are relatively depleted in glucose and galactose and mainly contain acidic sugars, fucose, rhamnose and arabinose in their chemical structure (Borchard and Engel, 2012; Passow, 2002). Even though polysaccharides are ubiquitous in nature, a latest study revealed that the individual sugar pattern is different between algae and terrestrial plants (Hepp et al., 2016) and may allow a source apportionment of carbohydrates in seawater.

Dissolved free monosaccharides (DFCHO) have been found to form another fraction of marine carbohydrates (Engel and Händel, 2011; Ittekkot et al., 1981; Kirchman et al., 2001). DFCHO are considered to be either directly released by phytoplankton cells or be the product of enzymatic degradation of polysaccharides (Pakulski and Benner, 1994). In most studies, DFCHO are found in lower concentrations than CCHO, since marine microbes utilize them with high turnover rates (Sakugawa and Handa, 1985; Thornton et al., 2016). From the concentrations of DFCHO, or rather the ratio between CCHO and DFCHO, information about in situ activities of local phytoplankton and bacteria in the seawater can be obtained (Pakulski and Benner, 1994; Sakugawa and Handa, 1985). Recently, correlations between the

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

concentrations of free glucose in Arctic surface water samples and their ice nucleating activity (INA) suggested a potential link between the formation of INA and marine carbohydrates (Zeppenfeld et al., 2019).

At the ocean surface, wind and wave interactions lead to bubble bursting. The emitted sea spray aerosol contains marine carbohydrates, including hydrogels, which contribute to the chemical and physical properties of these particles (Bigg and Leck, 2008; Frossard et al., 2014; Hawkins and Russell, 2010; Rosenørn et al., 2006). They have been detected in particles at different maritime regions on earth, including the North Atlantic, the Arctic and Antarctica (Barbaro et al., 2015; Frossard et al., 2014; Gao et al., 2011, 2012; Leck et al., 2013; Russell et al., 2010). However, understanding the quantitative fluxes of marine carbohydrates from the ocean to the atmosphere is still challenging, since chemical analysis of sugars in seawater strongly suffers from matrix effects, especially caused by sea salt.

The concentrations of individual monosaccharides in seawater, related saline matrices and aerosol particles can be determined with different kind of chromatographic methods, such as high performance liquid chromatography and gas chromatograph. These methods require a quite difficult sample preparation, including a labor-intensive derivatization step (Panagiotopoulos and Sempéré, 2005). In the last decades, high performance anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) has been established as a reliable alternative, since it facilitates a sensitive quantification of sugar compounds both in seawater and in airborne particles without a prior derivatization (Iinuma et al., 2009; Panagiotopoulos and Sempéré, 2005; van Pinxteren et al., 2012; Skoog and Benner, 1997). However, the presence of sea salt in seawater samples strongly affects the chromatographic performance of the HPAEC-PAD and needs to be removed before analysis.

Several procedures are available for the desalination of seawater. The desalination using anion exchange resins AG2-X8 and the cation exchange resin AG50W-X8 exhibits strong drawbacks such as the complete loss of charged sugars (amino sugars, uronic acids) and quite low recovery rate of neutral sugars between 20-80% depending on the individual monosaccharide (Borch and Kirchman, 1997; Mopper et al., 1992; Rich et al., 1996). The use of silver cartridges (Dionex OnGuard II Ag/H Cartridges) is faster and easier, but requires very expensive consumables and the capacity of removable sea salt per cartridge is strongly limited (Mopper et al., 1992; Panagiotopoulos and Sempéré, 2005). The desalination applying dialysis membranes achieves reproducible and very high recovery rates of hydrolysable polysaccharides (> 90%). However, this method does not allow the analysis of DFCHO, since these small molecules pass the membrane during dialysis (Engel and Händel, 2011).

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

93

94 Electro-dialysis is a fast way to remove ions by applying an electrical field. The use of two different chemo-
95 selective ion exchange membranes allows the exclusive removal of small anions, or cations respectively.
96 Hence, uncharged small substances (neutral DFCHO) and macromolecules (CCHO) can, in principle, be
97 recovered in high quantities. Electro-dialysis is being used for the desalination of salty water to generate
98 potable water, the denitrification of wastewater and soil remediation (Gain et al., 2002; Ottosen et al.,
99 2000; Sadrzadeh and Mohammadi, 2008; Tsiakis and Papageorgiou, 2005; Wisniewski et al., 2001). For
100 analytical sample preparation, electro-dialysis has been reported as a powerful desalination, e.g. for the
101 analysis of DOM and marine neutral DFCHO (Josefsson, 1970; Koprivnjak et al., 2009; Mopper et al., 1980;
102 Vetter et al., 2007; Wirth et al., 2019). However, biases, which have hitherto not been discussed in this
103 analytical context, can occur during the application of electro-dialysis due to the osmotic and electro-
104 osmotic loss of water, the migration and diffusion of monosaccharides and the appearance of sudden pH
105 changes.
106
107 Within the present study, a novel protocol for the desalination of seawater samples and related saline
108 samples, applying electro-dialysis and HPAEC-PAD is presented, accounting for the described biases. This
109 method with a low need of consumables allows the analysis of individual monosaccharides with (CCHO)
110 and without hydrolysis (DFCHO). This developed technique was applied to analyze a diverse set of
111 carbohydrates in different kinds of ambient seawater samples.
112

113 # 2. Experimental

114 ## 2.1 Chemicals and materials

115 Prior to the analysis of carbohydrates in seawater, all used laboratory glassware had been washed with
116 ultrapure water (conductivity >18.2 MΩ·cm) thoroughly and pre-heated in a muffle furnace at 550°C for 4
117 h. All plastic equipment was washed in 10% HCl solution and washed with ultrapure water three times.
118 For calibrating the HPAEC-PAD and determining the recovery of individual monosaccharides, a mixed stock
119 solution was prepared from fucose (Roth, 95%), galactosamine (Sigma, 99%), rhamnose (Sigma, 99%),
120 arabinose (Sigma, 99%), glucosamine (Fluka), galactose (Fluka, 99%), glucose (99%), xylose (Fluka, 99%),
121 mannose (Fluka,99%), fructose (Aldrich, 99%), ribose (Aldrich, 98%), muramic acid (Sigma, 95%),
122 galacturonic acid (Sigma-Aldrich, 97%), glucuronic acid (Sigma, 97%), mannuronic acid (Sigma, 90%).

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

Synthetic seawater samples were made of commercially available sea salts (Sigma). The salinity in practical salinity units (PSU) and the pH of water aliquots was measured by using a conductivity meter (pH/Cond 3320, WTW).

2.2 Field samples

Seven different real seawater samples, one ice core from Arctic sea ice and two brines collected within Arctic ice cores (**Table 1**) were measured and used for evaluating recovery rates and comparability of the method presented here. These saline samples were collected during different campaigns of our department and kept stored at -20°C. All sampling bottles had been rinsed with dilute hydrochloric acid (10% v/v) prior to the campaign. Field blanks (ultra-pure water filled up in sampling bottles during the campaign) were collected during each campaign and treated in the same way as the samples.

Table 1 Sampling details of discussed saline samples including seawater samples (SWS), ice cores (IC) and brine (B). SML stands for surface microlayer *(Engel et al., 2017)*. **(Wendisch et al., 2019)*

Saline sample (SS)	Location	Sampling date	Campaign	Latitude	Longitude	Depth (m)
SWS 1	Tropical Atlantic	13.11.2011	Cape Verde	16.935°N	024.915°W	0 (SML)
SWS 2	Tropical Atlantic	13.11.2011	Cape Verde	16.935°N	024.915°W	2
SWS 3	Raunefjorden	16.05.2011	Raunefjorden	60.274°N	005.181°E	2
SWS 4	North Atlantic	07.05.2012	ANT-XXVIII-5	33.3°N	013.5°W	0 (SML)
SWS 5	Arctic Ocean	13.07.2017	PS 106*	81.229°N	018.744°E	1
SWS 6	Arctic Ocean	14.07.2017	PS 106*	81.015°N	026.883°E	1
SWS 7	North Sea	25.05.2017	PS 106*	57.288°N	005.213°E	1
IC 1	Arctic Ocean	12.06.2017	PS 106*	81.824°N	011.571°E	0-0.8
B 1	Arctic Ocean	12.06.2017	PS 106*	81.824°N	011.571°E	0-0.8
B 2	Arctic Ocean	12.06.2017	PS 106*	81.824°N	011.571°E	0-1.5

2.3 The electrodialysis system

The centerpiece of the PCCell Micro Bench Electrodialysis system for small sample volumes consisted of three separated compartments (**Figure 1**): The sample compartment was an open chamber that was filled up with 9 ml of the standard solution or seawater sample. The functionalized anion exchange membrane (quaternary ammonium aliphatic polyether) and cation exchange membrane (sulfonated aromatic

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

polyether) bordered this compartment on both sides. Depending on their chemical properties, the membranes allowed exclusively the migration of either positively or negatively charged ions. The contact surface with the sample was 7.8 cm^2 for each membrane. For maintaining the conductivity within the system and receiving the sea salt from the sample, the next compartment contained the concentration circuit, a 16 $g \cdot L^{-1}$ NaCl solution (Merck). This solution was circulated at a rate of 60 $ml \cdot mL^{-1}$.Two end membranes on each side divided the concentration circuit from the third department including the electrodes. The mixed metal oxide (MMO) anode was made of a titanium base body coated by RuO_2, IrO_2 and TiO_2. The MMO cathode was based on stainless steel. The electrodes were permanently surrounded by a circulating 0.25 M Na_2SO_4 (Fluka) electrolyte circuit for avoiding unwanted redox reactions, e.g. the generation of corrosive elemental chlorine from chloride. Spacers were inserted between each membrane for keeping the electrolyte and concentration circuits well mixed. The sample solution was homogenized with a pipette during each desalination. The electrolyte and the concentration solutions were regularly renewed. The maximal electrical current I_{max} within the ED cell was adjusted by an automatic online

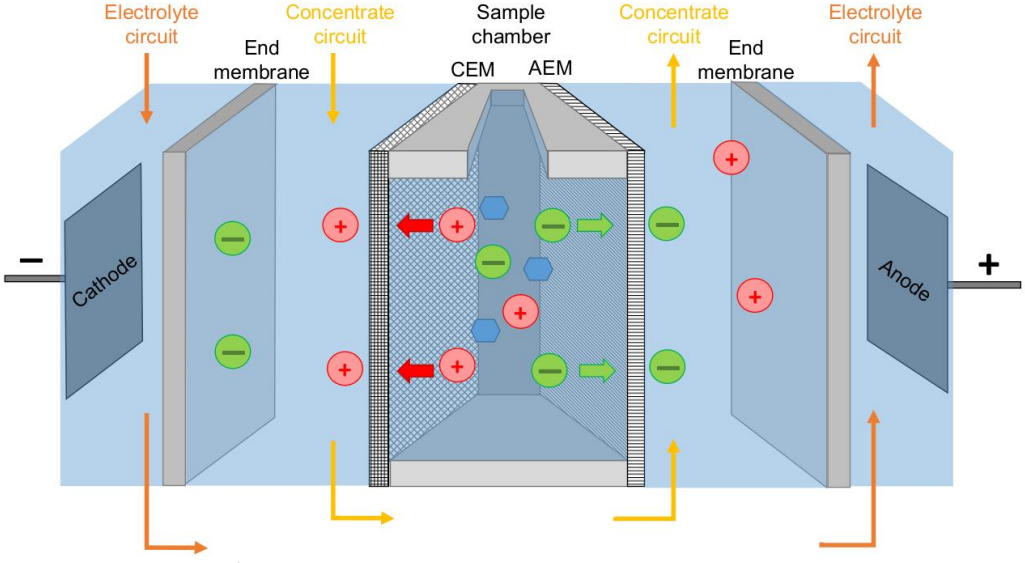

Figure 1 *Schematic setup of the used ED cell. Red circles represent cations, green circles anions, and blue hexagons are carbohydrates. CEM and AEM stand for cation exchange membrane and anion exchange membrane respectively.*

adaption of the voltage, which never exceeded 25 V. The desalination was stopped when the electric current dropped to a value of 0.20 A.

The used ion-exchange membranes have a quite long lifetime as long as they are not damaged mechanically. However, very high attention needs to be given to remove residues of previous desalinated

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020


Ocean Science
Discussions
Open Access
EGU

samples in order to avoid carry-over phenomena and obtain good reproducibility. Hence, every time before a new sample was desalinated, the sample chamber was always first exposed to ultrapure water for ten minutes and then flushed once with an aliquot of the new sample, which was disposed after.

2.4 HPAEC-PAD system

HPAEC-PAD was applied for the analysis of marine carbohydrates in seawater samples. Here, we used an Dionex ICS-3000 ion chromatography system coupled to an autosampler AS-1 as it has been already described for the analysis of saccharidic biomass burning markers in atmospheric particles (Iinuma et al., 2009). Several neutral monosaccharides, amino sugars and uronic acids were separated on a Dionex CarboPac 20 analytical column (3x150mm) combined with a Dionex CarboPac PA20 guard column (3x30mm), which was permanently temperature conditioned at 30°C. The separation of these saccharides was conducted by applying the gradient profile shown in **Table 2**, which was an adaption to the elution by (Meyer et al., 2008). Neutral and amino sugars eluted within the first 19 min at 4 mM NaOH. By adding sodium acetate, sugar acids eluted and organic and inorganic contaminants were flushed from the column. After the removal of remaining acetate by 250 mM NaOH, the system was equilibrated at 4 mM NaOH for the next sample injection. The flow rate of the eluent was 0.5 $mL \cdot min^{-1}$. The retention times, peak widths and resolution factors of the measured monosaccharides are shown in **Table 3**. For the injection of a sample aliquot, a 25 µL loop was used. Each sample was measured as a duplicate and each standard as a triplicate. Limits of detection (LOD) of individual monosaccharides were ranging between 2-12 nM, which is in good agreement with literature (Engel and Händel, 2011; Panagiotopoulos and Sempéré, 2005).

For the preparation of eluents A-D, filtered ultra-pure water (conductivity >18.2 MΩ·cm) was degassed with helium for 20 min. Eluents A and B were made by adding a defined volume of low-carbonate NaOH solution (Fisher Chemical, 50% w/w) to the degassed water. Eluent C was prepared by dissolving sodium acetate (Thermo scientific, anhydrous) in ultra-pure water, filtering it through a nylon membrane (0.2 µm, Thermo Scientific), degassing the solution with helium for 20 min and adding the corresponding volume of NaOH solution.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

193 ***Table 2*** *Gradient profile applied on the CarboPac PA20 column during HPAEC-PAD analysis*

Time (min)	%A (250 mM NaOH)	%B (20 mM NaOH)	%C (1 M Na-acetate/ 250 mM NaOH)	%D (H_2O)
0	0	20	0	80
19	0	20	0	80
20	5	0	15	80
35	5	0	15	80
38	20	0	40	40
39	100	0	0	0
44	100	0	0	0
45	0	20	0	80
78	0	20	0	80

194

195 ***Table 3*** *Averaged peak characteristics of monosaccharide standards separated by the presented elution averaged over 24 runs.*
196 *Resolution factors are calculated between peak and following peak in chromatogram.*

	Retention time (min)	Peak width (min)	Resolution factor
Fucose	3.79±0.01	0.27	10.5
Galactosamine	7.28±0.01	0.40	1.0
Rhamnose	7.70±0.02	0.42	1.1
Arabinose	8.15±0.02	0.42	2.2
Glucosamine	9.23±0.02	0.56	2.1
Galactose	10.33±0.02	0.51	2.8
Glucose	11.81±0.03	0.56	3.4
Xylose	13.89±0.03	0.65	0.8
Mannose	14.43±0.04	0.77	2.8
Fructose	16.52±0.05	0.75	2.0
Ribose	18.20±0.05	0.93	13.2
Muramic acid	25.84±0.01	0.23	9.0
Galacturonic acid	29.27±0.02	0.53	4.6
Glucuronic acid	31.49±0.02	0.45	2.8
Mannuronic acid	32.83±0.02	0.49	

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020


Ocean Science
Discussions
Open Access
EGU

2.5 Protocol for the analysis of DFCHO and CCHO in seawater and other saline samples

Stored frozen samples were defrosted in a fridge at 4°C. 9 ml of the filtered sample (0.2 µm, Millex, PTFE) was desalinated with electrodialysis as described above. In the end of each desalination, electro-(osmotic) water loss was replenished with ultra-pure water and mixed thoroughly. In order to analyze free amino sugars or uronic acids, pH could be adapted with concentrated HCl or 1 M NaOH.

A concentration step using a vacuum concentrator (MiVac) at 55°C allowed the detection of low concentrated DFCHO, as it occurs in most seawater samples. For this purpose, a round-bottom glass vial was filled with an aliquot of 6 ml desalted sample, which was weighted empty and filled. After reaching a remaining volume of less than approximately 600 µl, the glass vial was weighted again (in order to calculate the concentration factor) and the concentrated aliquot was pipetted in the autosampler vial for HPAEC-PAD analysis. This step allowed a decrease of LOD by a factor of 10. Each sample was prepared and measured as duplicate.

In order to measure CCHO, marine polysaccharides need to be cleaved into their monomeric compounds by acid hydrolysis. We applied the optimized conditions described by Engel and Händel (2011) with slide modifications. An aliquot of 1 ml desalted sample was hydrolyzed with hydrochloric acid (HCl concentration in sample= 0.8 M) in pre-heated (550°C, 4 h) glass ampules for 20 h at 100°C. Neutralization was performed by evaporating all liquid under vacuum at 55°C until dryness. The dry residue was dissolved in 700 µl ultra-pure water, treated with a vortex homogenizer (IKA MS 3 basic) and filled in the autosampler vial for HPAEC-PAD analysis. Each sample was prepared and measured as duplicate.

2.6 Parameter optimization and assessment of method

Impact of osmosis and electro-osmosis during ED desalination

For quantifying the loss of water in the sample due to osmosis and electro-osmosis, a synthetic sea salt solution was pipetted into the desalination chamber, which was desalinated for 0, 5, 10, 15, 20 and 25 min with a voltage of 25 V and a maximal current of 0.6 A. After the lapse of time, the total remaining volume was pipetted quantitatively into a glass vial and weighted (Mettler Toledo, XS105 DualRange). These measurements were repeated for four different sea salt solution (10, 20, 30 and 40 PSU) and as triplicate for each time. The recovery of the sample mass was calculated as the ratio between the mass after the corresponding desalination time and the averaged mass after 0 min.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

226 Recovery of DFCHO within the ED membrane system

227 Standard addition experiments with real seawater were performed, for quantifying the recovery of
228 monosaccharides due to diffusion and migration under consideration of all matrix effects. For that reason,
229 sample 7 was filtered (0.2 µm, Millex, PTFE) and spiked with a sugar standard mix (neutral sugars, amino
230 sugars, uronic acids) resulting in a concentration increase of 10 µg L^{-1} and 100 µg L^{-1}.These samples were
231 desalinated using electrodialysis (I_{max}=0.6 A, stop at 0.2 A). In the end of each run, (electro-) osmotic water
232 loss was either replenished or not, and the sample directly measured with the HPAEC-PAD. These
233 measurements were repeated as triplicates for each concentration. In order to account for possible
234 wasting phenomena, repetitions were performed with new membranes, as well with membranes, which
235 already had been used for some time before. Given recovery rates for neutral monosaccharides are the
236 average of the results for 10 µg $L^{-1}$and 100 µg L^{-1}. For sugar acids and amino sugars, only the averaged
237 recovery rates for 100 µg L^{-1} are given for avoiding determinations close to the LOD.

238 In order to investigate the influence of pH on the migration of charged monosaccharides, this experiment
239 was repeated for three different pH values: At pH=8 (natural pH of seawater), pH of 1.5 (acidified with
240 concentrated HCl) and pH of 11 (addition of 1 M NaOH). Since high pH in seawater leads to precipitation
241 of hydroxides of alkaline earth metals, an additional filtering (0.2 µm) was performed for these runs.

242

243 Recovery of CCHO within the ED membrane system

244 Recovery experiments were performed with solutions and a suspension of the polysaccharide standards
245 sodium alginate (Aldrich), laminarin from Laminaria digitate (Sigma) and cellulose powder from spruce
246 (Fluka) at natural pH. Stock solutions were added to filtered sample SWS 7 resulting in concentrations of
247 10 mg L^{-1}. Aliquots of 1 ml with and without desalinations were hydrolyzed (HCl 0.8 M, 100°C, 20 h) and
248 neutralized by evaporation with the vacuum concentrator (55°C) until dryness. The residue was
249 reconstituted in 700 µL, treated with a vortex homogenizer (IKA MS 3 basic) and filled in the autosampler
250 vial for HPAEC-PAD analysis. Recovery rates were calculated as a ratio between the determined
251 monosaccharide concentrations after hydrolysis of the standard solutions with and without desalination.

252 In order to compare our method on the recovery of CCHO with another established method, aliquots of
253 four seawater samples were treated following the electro-dialysis protocol presented here and the
254 protocol by Engel and Händel (2011) using membrane desalination, an acid hydrolysis with HCl (0.8 M,

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020


Ocean Science
Discussions
Open Access
EGU

100°C, 20h), neutralization by evaporation (nitrogen, 50°C) and an elution on a Dionex CarboPac PA10 column.

3. RESULTS AND DISCUSSION

A reproducible quantification of carbohydrates in seawater samples using HPAEC-PAD requires a prior removal of disturbing sea salt. Here, we present electrodialysis as a reliable desalination method, its parameter optimization and the discussion of arising phenomena resulting in a protocol for the analysis of marine carbohydrates.

3.1 Kinetics and efficiency of desalination

During the desalination of seawater by electrodialysis, anions and cations migrate through an electrical field and pass chemo-selective membranes. Depending on their electrical charge, they move either to the positively charged anode or to the negatively charged cathode. In this process, the salt flux through the membranes j_S (mol·m^{-2}·s^{-1}), which determines the desalination time, is proportional to the applied electrical current *I* (Han et al., 2017; Vanoppen et al., 2015). **Figure 2** shows the current within the used ED system and the salinity of the seawater sample during a typical desalination of an artificial seawater sample (40 PSU) for two different applied maximal currents I_{max} within the system. For almost the entire desalination run, the current *I* was maintained at I_{max} due to automatic adjustment of the voltage. During this time, the salt flux was approximately constant. Towards the end of the desalination, when almost all salt ions were removed, the current dropped down and the salt flux became lower. Since a direct salinity measurement was not possible in the sample chamber without contaminating the sample, the end of each desalination was defined, when the current *I* reached a value of 0.2 A. At this point, the salinity of the sample typically ranged between 0.2 and 0.4 PSU, which was found to be sufficiently low for the carbohydrate analysis at the HPAEC-PAD. This reduction in salinity represents an overall desalination of more than 99% of the initial salt concentration. A desalination reaching a salinity below 0.1 PSU was possible, but was not necessary for this application and would have resulted in longer desalination times. Consequently, for minimizing the required desalination time, a high I_{max} is favorable.

However, it was observed that the application of an I_{max} of more than 0.8 A during the desalination, resulted in a strong rise of the pH and a white precipitation in the (synthetic) seawater solution, apparently

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

284 due to the formation of hydroxides of alkaline earth metals. This uncontrolled precipitation strongly
285 disturbed the efficiency of the desalination and the reproducibility of the carbohydrate measurements and
286 caused a scaling of the membranes. Previous studies explained these unfavorable changes of pH by a
287 strong concentration polarization at the membranes surface leading to water splitting to H^+ and OH^- ions,
288 when a certain limiting current is exceeded. This phenomenon has been preferably observed at anion
289 exchange membranes with quaternary amino groups in the presence of divalent cations, such as Mg^{2+} and
290 Ca^{2+} (Cowan, 1962; Martí-Calatayud et al., 2018; Ottosen et al., 2000). The described phenomena
291 exclusively occurred when (synthetic) seawater was desalinated and not during the desalination of NaCl
292 standard solutions. This finding shows the importance of performing parameter optimization tests with

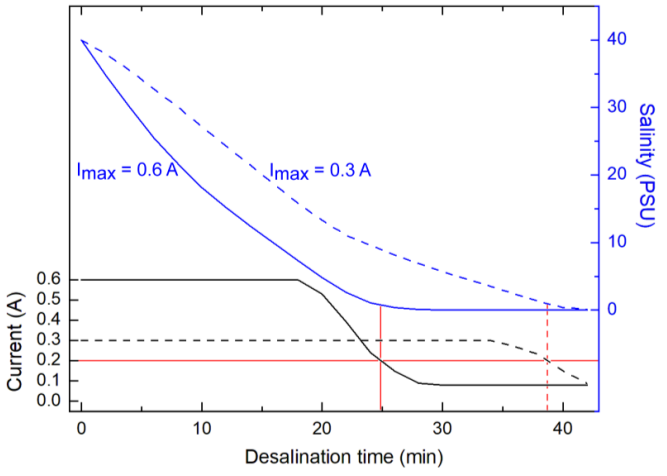

Figure 2 *Measured current within the ED system and salinity of a synthetic seawater sample (40 PSU) versus desalination time with two different maximal applied currents I_{max} (0.6 A solid line, 0.3 A dashed line). The red line represents the current and the corresponding salinity, when desalination was stopped.*

293 synthetic seawater standards that include all important seawater constituents such as divalent cations. In
294 summary, the optimum maximal current I_{max} of 0.6 A was found for the used ED system for avoiding scaling
295 effects and performing desalination as fast as possible.

296

297 ## 3.2 Possible biases during the application of electrodialysis

298 During the application of ion exchange membranes, the passive transport of water (*osmosis*) and solutes
299 with a low molecular weight (*diffusion*), such as dissolved monosaccharides, can occur triggered by a
300 concentration gradient between the sample and concentration channels (Galama et al., 2014; Galier et al.,
301 2012). By operating an electrical field, the active transport of charged molecules (*migration*) and hydrated

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020


Ocean Science
Discussions
Open Access
EGU

water (*electro-osmosis*) takes place (Galama et al., 2014; Galier et al., 2012). While osmosis and electro-osmosis induce an unavoidable loss of water and hence of the total volume of the sample, diffusion and migration of the analytes result in a loss of analyzable molecules. All these phenomena might falsify the determined concentration of the analytes in the sample and need to be characterized for an accurate sample preparation for the analysis of marine carbohydrates.

Osmotic and electro-osmotic transport of water

Osmosis describes the passive transport of free water molecules through a partially permeable membrane caused by large differences of the osmotic pressures between the concentrate circuit and the sample solution (Sata, 2007). The direction and the quantity of the water transport depends on the residence time t_R of the sample solution within the membrane system, the difference between the concentrations of solutes in the sample solution and the concentration circuit $(c_s - c_c)$ and membrane specific parameters, such as the osmotic water transfer coefficient the membrane area and the membrane thickness (Galama et al., 2014). The quantitative effect of osmosis can be reduced by minimizing t_R and $(c_s - c_c)$. Hence, c_c was set at 16 g NaCl L^{-1}, which is approximately in the middle between the concentrations of a typical seawater sample before (30-39 PSU) and after the desalination (0.2-0.4 PSU) for balancing the positive and negative contribution of osmosis on the total sample volume during a typical desalination. Under these conditions, a maximum loss of 3% sample volume was observed in the described ED system due to osmosis.

In aqueous solutions, water molecules form a hydration shell around ions (Ohtaki and Radnai, 1993). Whenever ions pass through membranes during electrodialysis, a cotransport of these hydrating water molecules occurs, known as electro-osmosis (Galama et al., 2014). The electroosmotic water transfer j_W ($m^3 \cdot m^{-2} \cdot s^{-1}$) is proportional to the salt flux j_S in the system and can be expressed by formula (Eq-I) with the molar volume of water V_M ($1.8 \cdot 10^{-5} \cdot m^3 \cdot mol^{-1}$) and the salt hydration number n_H (mol water·mol^{-1} salt) (Galier and Balmann, 2015; Han et al., 2015).

$$j_W = n_H \cdot V_M \cdot j_S \qquad \text{(Eq-1)}$$

The salt hydration number of NaCl, as the major compound of sea salt, has been reported with values between 11 and 14 (Han et al., 2015; Rutgers and Hendrikx, 1962; Singlande et al., 2006; Walker et al.,

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

329 2014). Assuming a NaCl concentration of 30 g·L^{-1} and n_H to be 14, a maximal reduction of the sample
330 volume onto 87 % due to electro-osmosis is expected, additionally to osmosis.

331 The recovery of the sample volume due to electro-osmosis and osmosis during the desalination was
332 characterized for four different salinities for the used ED system (**Figure 3**). During the active removal of
333 sea salt, electro-osmosis is the dominating force causing the water loss in the sample. The electroosmotic
334 water loss is continual as long as the salt flux stays constant. However, in the final stages of each
335 desalination, the salt flux decreases and consequently the electroosmotic water transfer decreases, too.

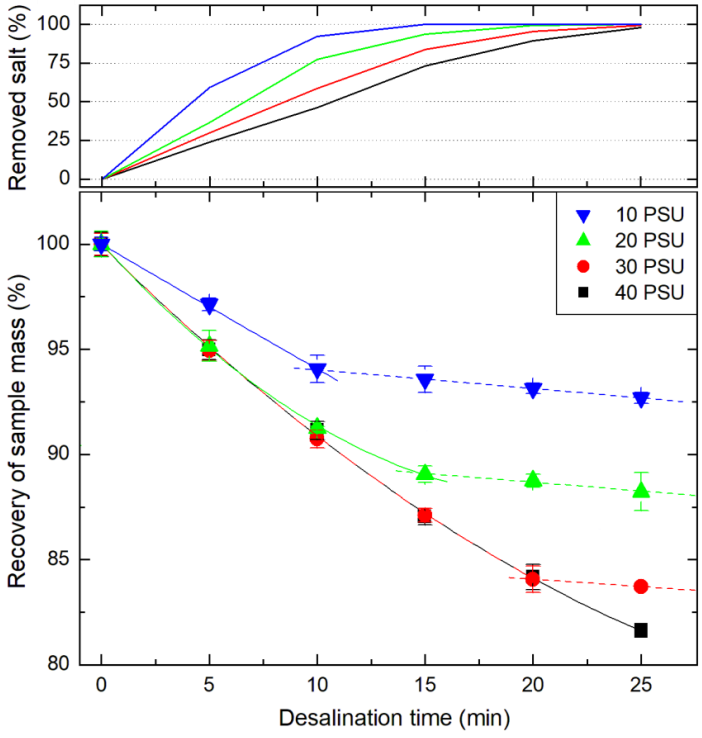

Figure 3 *Combined effect of electro-osmosis and osmosis (solid lines) and osmosis (dashed lines) on the recovery of sample mass as a function of the desalination time within the described membrane system (I_{max}=0.6 A, c_c=16 g NaCl L^{-1}) for artificial sea salt solutions with four different initial salinities.*

336 For a synthetic seawater sample with a salinity of 30 PSU, 84% of sample mass was recovered. This is in
337 good agreement with the estimation mentioned above considering the additional contemporaneous
338 contribution of osmosis of about 2-3%. Once the sea salt is removed, osmotic water transfer remains at
339 constant rate of approximately 0.1%·min^{-1}.

340 The overall water loss resulting from osmosis and electro-osmosis needs to be taken into account since it
341 falsifies the determined concentrations of marine carbohydrates. For its compensation, the chamber was

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

342 replenished with ultra-pure water in the end of each desalination until the initial sample volume was
343 reached. This procedure was performed with a maximal overall error of 0.5%.

344

345 Analysis and recovery of DFCHO in seawater samples

346 The recovery of neutral monosaccharides during electro-dialysis is impacted by diffusion and convection
347 processes (Galier and Balmann, 2015). Additionally, free amino sugars and uronic acids migrate through
348 an electrical field due to their charge and pass the ion exchange membranes. Recovery tests were
349 performed with standard solutions spiked to a real seawater sampled, which have been typically reported
350 for seawater samples (Kirchman et al., 2001; Mopper et al., 1980; Skoog et al., 1999; Zeppenfeld et al.,
351 2019). Recovery rates of neutral sugars (Glc, Man, Xyl, Gal, Ara, Fuc, Rha, Fru) ranged between 95-98% at
352 the natural pH of seawater (approx. pH=8) (**Table 4**). Hence, the overall impact of diffusion and convection
353 on the recovery of monosaccharides is quite low for the short contact time with these membranes.
354 However, a higher loss of neutral monosaccharides due to diffusion was observed, when the sample
355 solution remained within the membrane system for a longer period of time, which calls for a fast
356 desalination. An overestimation of the determined concentrations was avoided by performing a correction
357 of the water loss in the end of each desalination. Charged monosaccharides were found with much lower
358 recoveries of 25-31% for uronic acids and 16-19% for amino sugars at pH=8. This is due to their weak
359 acidic/basic properties (pK_a (amino sugars) = 7.6-8.5 (Bichsel and von Gunten, 2000; Sinnott, 2007), pK_a
360 (uronic acids) = 3.3-3.5 (Kohn and Kovác, 1978)) and hence their partially ionic state, which makes them
361 migrate through the electrical field. However, a low pH can protonate the carboxylic group of uronic acids
362 and a high pH deprotonates the amino group of amino sugars for reducing this effect. Here, we found that
363 an initial pH of 1.5 before desalination could increase the recovery of free uronic acids up to 45-49%, while
364 a high pH of 11 resulted into a higher recovery of free amino sugars up to 58-59%. The recovery of neutral
365 sugars seemed to be quite unaffected within the range of the tested pH, with the exception of fructose,
366 which was recovered with 89% at pH 1.5, certainly due to its instability within acid conditions. To our
367 knowledge, here we report for the first time a method, which allows a possible determination of free
368 amino sugars and uronic acids in saline matrices, such as seawater or the brine from Arctic sea ice.
369
370
371
372

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

373 ***Table 4*** *Recovery of individual free monosaccharides (neutral, amino sugars, uronic acids) after desalination with electro-dialysis*
374 *including correction of (electro-) osmotic water loss. n.d.= not determined*

Monosaccharide	Recovery rate (%)		
	pH_{Start} = 1.5	pH_{Start}= 8 (seawater)	pH_{Start}= 11
Galactose	97.6±1.0	96.0±0.9	97.2±1.4
Fucose	97.2±2.4	97.9±1.6	97.0±1.0
Glucose	96.9±1.5	97.2±1.4	97.1±2.1
Mannose	95.8±1.8	95.8±1.3	95.8±2.1
Xylose	95.6±2.3	95.9±2.2	95.2±2.0
Rhamnose	93.5±1.6	94.2±1.6	95.1±1.4
Arabinose	93.4±2.3	95.1±1.1	94.5±1.9
Fructose	89.3±3.4	94.6±2.7	94.1 ±2.2
Glucuronic acid	48.7±2.7	31±1.9	n.d.
Mannuronic acid	44.9±0.6	25±1.9	n.d.
Muramic acid	24.7±1.7	n.d.	n.d.
Glucosamine	1.5±0.4	18.9±1.2	59±3.2
Galactosamine	1.2±0.3	15.9±1.5	58±3.4

375

376 Analysis and recovery of CCHO in seawater samples with standard polysaccharides

377 Recovery experiments with standard solutions of common polysaccharides were performed with and
378 without desalination by electrodialysis. The neutral, water-soluble polysaccharide laminarin was
379 recovered with 91.0±5.4%. The acidic polysaccharide alginic acid could be recovered with 93.2±5.3%. Even
380 though alginic acid might move within the electrical field due to its acidic molecular structure, its molecular
381 weight does not allow passing the membrane and does not leave the sample solution. Standard
382 desalination experiments with a suspension of the water insoluble cellulose, which could represent the
383 fraction of particulate polysaccharides, resulted in much worse recoveries of 48±19%. The reason for this
384 high, less reproducible loss of polysaccharide was likely caused by sedimentation within the sample
385 chamber. Engel and Händel (2011) described adsorption processes during the desalination with dialysis
386 membranes and tackled this problem with sonification of the membranes. However, sonification could not
387 be applied in our gadget. In this study, flushing the chamber several times with a defined volume of ultra-
388 pure water after desalination and reuniting the washing water with the desalinated sample could increase
389 the yield of cellulose up to 85.2±6.9% under consideration of dilution factors. This procedure was not

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

found to be feasible, since a dilution of a natural sample reduced the sensitivity of low concentrated sugars in seawater in the analysis by HPAEC-PAD. Rather, we recommend electro-dialysis only for the application at filtered samples (dissolved compounds), while particulate organic matter might be better analyzed from filters after filtration.

Comparison of electro-dialysis and dialysis method for the determination of CCHO in seawater samples

In order to evaluate the presented procedure for the analysis of CCHO, comparison studies have been performed measuring four ambient seawater samples (SWS 1-4) with the established membrane dialysis protocol after Engel and Händel (2011) and with the here presented method. **Figure 4** shows the results of the individual monosaccharides after hydrolysis with HCl. Major concentrated sugars, such as glucose, galactose and xylose/mannose, were determined at similar concentrations. Furthermore, a good agreement was observed for minor concentrated sugars, such as fucose and galactosamine. Mild discrepancies were found for rhamnose and arabinose, which appeared in higher concentrations after the electro-dialysis method, and glucosamine, which was determined at lower concentrations. These variations might be explained by statistical uncertainties or co-elution of unknown substances. In summary, the here presented method using electro-dialysis has shown to be in good agreement with the established membrane dialysis method regarding the analysis of CCHO. In addition, the electro-dialysis

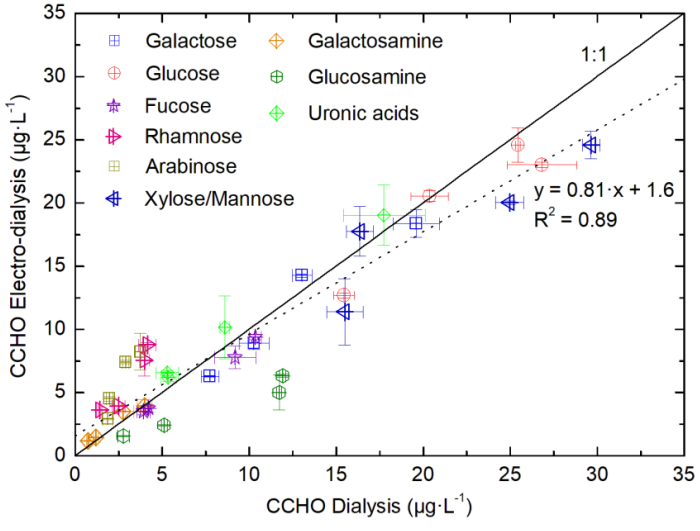

Figure 4 Determined monosaccharide concentrations in four seawater samples (SWS 1-4) after hydrolysis (CCHO) comparing the desalination by dialysis (Engel&Händel, 2011) and electro-dialysis (presented in this study).

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

offers the major advantage of analysing the full spectrum of DFCHO as well - which comprise a group of hardly investigated but potentially important marine compounds.

3.3 Chromatographic performance with HPAEC PAD after desalination.

Several kinds of saline samples were desalinated with electro-dialysis and analyzed on the CarboPac PA20 column. **Figure 5** shows some examples for DFCHO and CCHO chromatograms in a brine and a seawater sample after desalination with electro-dialysis. The insufficient chromatographic separation of mannose and xylose using previous kinds of analytical columns has been frequently described in literature (Borch and Kirchman, 1997; Engbrodt, 2001; Engel and Händel, 2011; Kirchman et al., 2001). Therefore, xylose and mannose have frequently given only as sum concentrations. The elution of the sugars on a CarboPac20 column, applied in the present study, strongly improved the separation between the both sugars mannose and xylose (resolution factor=0.8), and allowed the individual determination of these two sugars. However, most of the analyzed samples showed high concentrations of xylose, which strongly overlapped the smaller peak of mannose. For these cases, we kept reporting a sum value for Xyl/Man.

3.4 DFCHO and CCHO in saline field samples from different regions.

Several real samples were analyzed on DFCHO and CCHO (**Table 5** and **6**). In both sugar fractions, glucose was the most abundant monosaccharide, as it has been reported before (Panagiotopoulos and Sempéré, 2005). In some of the samples, free fructose could be determined reaching concentrations comparable to glucose. However, fructose cannot be determined in CCHO, since hydrolysis leads to complete destruction of this sugar. High DFCHO was found in the samples from the Arctic including brine and ice core samples reaching up to 118 nM in comparison to seawater samples from the Atlantic SWS 1-4 (11-15 nM). However, a clear regional trend could not be identified for CCHO with concentrations, ranging between 260-1410 nM. Traces of free amino sugars and uronic acids were found after neutral desalinations. However, a stronger enrichment is required in order to determine them quantitatively and will be the focus of further studies.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

Ocean Science
Discussions
Open Access
EGU

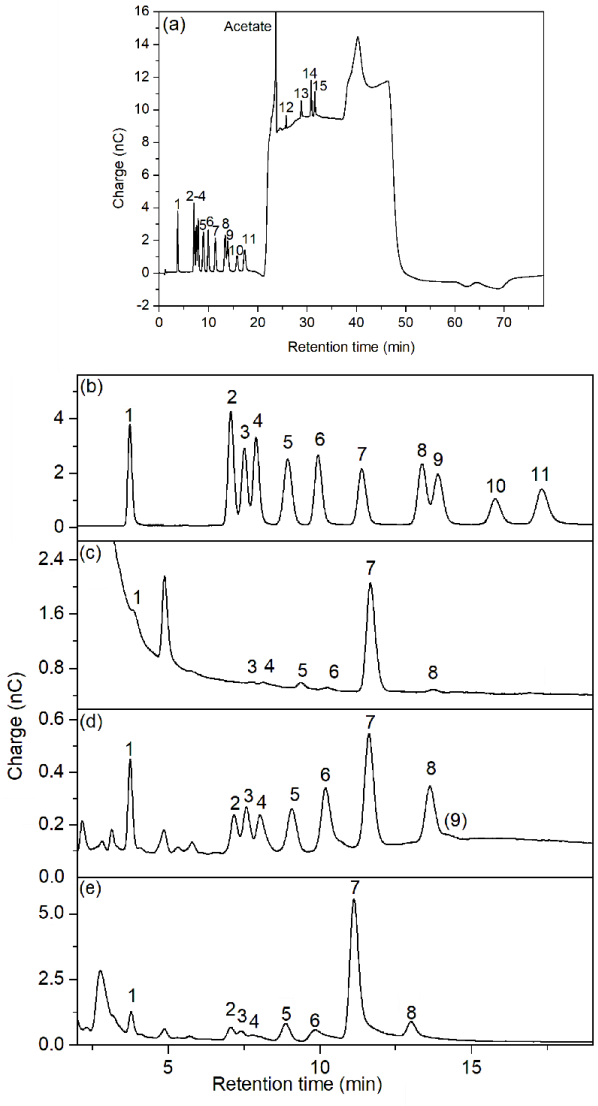

Figure 5 Chromatograms of a) full chromatogram of standard solution 100 µg·L^{-1}; b-e) neutral sugars and amino sugars of b) standard solution 100 µg·L^{-1}; c) DFCHO in brine (B2) desalinated at natural pH; d) CCHO in a seawater sample (SWS 3); e) CCHO in Arctic brine (B2). 1 fructose, 2 galactosamine, 3 rhamnose, 4 arabinose, 5 glucosamine, 6 galactose, 7 glucose, 8 xylose, 9 mannose, 10 fructose, 11 ribose, 12 muramic acid, 13 galacturonic acid, 14 glucuronic acid, 15 mannuronic acid.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

460 **Table 5** Mol percentages of individual neutral monosaccharides within DFCHO in seawater, Arctic brine and ice core samples.
461 <LOD stands for below detection limit.

	Glc	Gal	Xyl/Man	Rha	Fuc	Ara	Fru	Total DFCHO
	mol%	mol%	mol%	mol%	mol%	mol%	mol%	nM
SWS 1	57	8	<LOD	<LOD	7	8	20	15
SWS 2	52	<LOD	<LOD	<LOD	<LOD	<LOD	48	14
SWS 3	80	<LOD	15	<LOD	<LOD	5	<LOD	11
SWS 4	87	<LOD	5	<LOD	<LOD	8	<LOD	14
SWS 5	89	3	3	<LOD	<LOD	4	<LOD	35
SWS 6	16	8	22	<LOD	34	20	<LOD	27
IC 1	50	6	2	3	15	<LOD	25	118
B 1	23	27	13	8	29	<LOD	<LOD	15
B 2	85	2	3	2	6	1	<LOD	53

462

463

464 **Table 6** Mol percentages of individual monosaccharides within CCHO in seawater, Arctic brine and ice core samples. <LOD
465 stands for below detection limit.

	Glc	Gal	Xyl/Man	Rha	Fuc	Ara	GalN	GluN	Gal-ac	Gluc-ac	Total CCHO
	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	nM
SWS 1	20	15	22	7	7	8	3	4	12	3	680
SWS 2	25	12	24	8	7	7	2	3	11	1	290
SWS 3	20	14	21	9	10	9	3	6	7	2	580
SWS 4	31	12	26	5	6	7	2	3	6	2	410
SWS 5	84	3	2	2	2	2	1	1	<LOD	3	1410
SWS 6	48	7	10	4	2	<LOD	2	4	15	7	260
IC 1	54	9	11	1	<LOD	1	2	1	10	10	330
B 1	47	11	13	4	4	3	3	5	<LOD	10	420
B 2	65	8	10	3	4	1	2	3	<LOD	3	640

466

467

468 5. Summary and conclusion

469 In this study, a novel protocol was presented for the analysis of both DFCHO and CCHO in saline aqueous
470 samples by applying HPACE-PAD with prior desalination by electro-dialysis. Recovery rates for neutral
471 monosaccharides ranged between 95-98%. By adjusting pH, charged monosaccharides such as free amino
472 sugars and uronic acids could be recovered with 58-59% at pH = 11 and 45-49% at pH = 1.5, respectively.
473 Dissolved polysaccharide standards, such as laminarin and alginic acid showed good recovery rates of 91-
474 93%, while a suspension of insoluble cellulose was quite difficult to recover reproducibly. Hence, electro-
475 dialysis for carbohydrate analysis is recommended to be used for filtered samples or for samples with low

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

amount of particulate matter. In this study, the osmotic and electro-osmotic loss of water was considered in order to avoid an overestimation of the determined concentrations. In real seawater from different locations, Arctic brine and sea ice core samples, CCHO was found in concentrations between 260 and 1410 nM. DFCHO ranged in much lower concentrations with 11-118 nM. Within both, DFCHO and CCHO, the most dominant monosaccharide was glucose, followed by other neutral sugars.

In this study, the successful application of electro-dialysis in combination with HPAEC-PAD for the analysis of marine carbohydrates (both free and combined) in marine matrices, such as seawater, ice cores and brine could be demonstrated. The application of electro-dialysis for other more salt sensitive analyses should be the focus of further researches, e.g. the reported interference of suspended sea spray aerosol in Arctic snow samples during the quantification of insoluble light absorbing impurities such as black carbon and dust performed via nebulization. Hence, this developed method has the potential to contribute strongly in further research studies understanding biogeochemical processes in the oceans and related saline matrices and sea-air exchange processes, especially for studying hot spot regions of climate change, such as the Arctic.

Acknowledgements

We gratefully acknowledge the funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project-ID 268020496 – TRR 172, within the Transregional Collaborative Research Center "ArctiC Amplification: Climate Relevant Atmospheric and SurfaCe Processes, and Feedback Mechanisms (AC)³"in sub-projects B04. Additional support through the Leibniz Association SAW funding of the project "Marine biological production, organic aerosol particles and marine clouds: a Process Chain (MarParCloud)", (SAW-2016-TROPOS-2) is also gratefully acknowledged. We thank Svantje Pöge for supporting work in the laboratory. We thank Jon Roa for CHO analysis following the Engel & Händel (2011) protocol. We thank Susanne Fuchs, Kristin Recklies and Christian Weller of Thermo Fisher in his in-house collaborations for fruitful discussions. We thank Patrick Altmeier and Philip Kalkhoff from PCCell GmbH (Heusweiler, Germany) for guidance and technical support for helping to put electro-dialysis to operation in our laboratory.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

505 *Data availability.* The data will be available through the World Data Center PANGAEA
506 (https://www.pangaea.de/) in the near future.

507

508 *Author contributions.* SZ wrote the manuscript with contributions from MvP, HH and AE. SZ and MvP
509 collected seawater samples during different field campaigns. SZ optimized the presented method and
510 performed the chemical measurements. AE performed CCHO analysis with supplied samples following her
511 published protocol for evaluation purposes. All co-authors proofread and commented the manuscript.

512

513 *Competing interest.* The authors declare that they have no conflict of interest.

514

515 References

516 Barbaro, E., Kirchgeorg, T., Zangrando, R., Vecchiato, M., Piazza, R., Barbante, C. and Gambaro, A.: Sugars
517 in Antarctic aerosol, Atmospheric Environment, 118, 135–144, doi:10.1016/j.atmosenv.2015.07.047,
518 2015.

519 Benner, R. and Kaiser, K.: Abundance of amino sugars and peptidoglycan in marine particulate and
520 dissolved organic matter, Limnology and Oceanography, 48(1), 118–128, doi:10.4319/lo.2003.48.1.0118,
521 2003.

522 Berman-Frank, I., Rosenberg, G., Levitan, O., Haramaty, L. and Mari, X.: Coupling between autocatalytic
523 cell death and transparent exopolymeric particle production in the marine cyanobacterium
524 Trichodesmium, Environmental Microbiology, 9(6), 1415–1422, doi:10.1111/j.1462-2920.2007.01257.x,
525 2007.

526 Bianchi, T. S. and Canuel, E. A.: Chemical biomarkers in aquatic ecosystems, Princeton University Press.,
527 2011.

528 Bichsel, Y. and von Gunten, U.: Formation of Iodo-Trihalomethanes during Disinfection and Oxidation of
529 Iodide-Containing Waters, Environ. Sci. Technol., 34(13), 2784–2791, doi:10.1021/es9914590, 2000.

530 Bigg, E. K. and Leck, C.: The composition of fragments of bubbles bursting at the ocean surface, Journal of
531 Geophysical Research: Atmospheres, 113(D11), doi:10.1029/2007JD009078, 2008.

532 Borch, N. H. and Kirchman, D. L.: Concentration and composition of dissolved combined neutral sugars
533 (polysaccharides) in seawater determined by HPLC-PAD, Marine Chemistry, 57(1), 85–95,
534 doi:10.1016/S0304-4203(97)00002-9, 1997.

535 Borchard, C. and Engel, A.: Organic matter exudation by <I>Emiliania huxleyi</I> under
536 simulated future ocean conditions, Biogeosciences, 9(8), 3405–3423, doi:10.5194/bg-9-3405-2012, 2012.

537 Borchard, C. and Engel, A.: Size-fractionated dissolved primary production and carbohydrate composition
538 of the coccolithophore *Emiliania huxleyi*, Biogeosciences, 12(4), 1271–1284, doi:10.5194/bg-12-1271-
539 2015, 2015.

540 Cisternas-Novoa, C., Le Moigne, F. A. C. and Engel, A.: Composition and vertical flux of particulate organic
541 matter to the oxygen minimum zone of the central Baltic Sea: impact of a sporadic North Sea inflow,
542 Biogeosciences, 16(4), 927–947, doi:10.5194/bg-16-927-2019, 2019.

543 Cowan, D. A.: Research in the problem of scaling of electrodialsis demineralizers, Texas Water Comm.
544 Bull., 6206, 29, 1962.

545 Engbrodt, R.: Biogeochemistry of dissolved carbohydrates in the Arctic, Berichte zur Polar-und
546 Meeresforschung (Reports on Polar and Marine Research), 396, 106pp, 2001.

547 Engel, A. and Händel, N.: A novel protocol for determining the concentration and composition of sugars
548 in particulate and in high molecular weight dissolved organic matter (HMW-DOM) in seawater, Marine
549 Chemistry, 127(1), 180–191, doi:10.1016/j.marchem.2011.09.004, 2011.

550 Engel, A., Bange, H. W., Cunliffe, M., Burrows, S. M., Friedrichs, G., Galgani, L., Herrmann, H., Hertkorn,
551 N., Johnson, M., Liss, P. S., Quinn, P. K., Schartau, M., Soloviev, A., Stolle, C., Upstill-Goddard, R. C., van
552 Pinxteren, M. and Zäncker, B.: The Ocean's Vital Skin: Toward an Integrated Understanding of the Sea
553 Surface Microlayer, Front. Mar. Sci., 4, 165, doi:10.3389/fmars.2017.00165, 2017.

554 Ewert, M. and Deming, J. W.: Sea Ice Microorganisms: Environmental Constraints and Extracellular
555 Responses, Biology, 2(2), 603–628, doi:10.3390/biology2020603, 2013.

556 Frossard, A. A., Russell, L. M., Burrows, S. M., Elliott, S. M., Bates, T. S. and Quinn, P. K.: Sources and
557 composition of submicron organic mass in marine aerosol particles, Journal of Geophysical Research:
558 Atmospheres, 119(22), 12,977-13,003, doi:10.1002/2014JD021913, 2014.

559 Gain, E., Laborie, S., Viers, P., Rakib, M., Durand, G. and Hartmann, D.: Ammonium nitrate wastewater
560 treatment by coupled membrane electrolysis and electrodialysis, Journal of Applied Electrochemistry,
561 32(9), 969–975, 2002.

562 Galama, A. H., Saakes, M., Bruning, H., Rijnaarts, H. H. M. and Post, J. W.: Seawater predesalination with
563 electrodialysis, Desalination, 342, 61–69, doi:10.1016/j.desal.2013.07.012, 2014.

564 Galier, S. and Balmann, H. R.: Demineralization of glucose solutions by electrodialysis: Influence of the
565 ionic composition on the mass transfer and process performances, The Canadian Journal of Chemical
566 Engineering, 93(2), 378–385, doi:10.1002/cjce.22076, 2015.

567 Galier, S., Courtin, M. and Balmann, H. R.: Influence of the Ionic Composition on the Demineralisation of
568 Saccharide Solutions by Electrodialysis, Procedia Engineering, 44, 826–829,
569 doi:10.1016/j.proeng.2012.08.587, 2012.

570 Gao, Q., Nilsson, U., Ilag, L. L. and Leck, C.: Monosaccharide compositional analysis of marine
571 polysaccharides by hydrophilic interaction liquid chromatography-tandem mass spectrometry, Anal
572 Bioanal Chem, 399(7), 2517–2529, doi:10.1007/s00216-010-4638-z, 2011.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

573 Gao, Q., Leck, C., Rauschenberg, C. and Matrai, P. A.: On the chemical dynamics of extracellular
574 polysaccharides in the high Arctic surface microlayer, Ocean Science, 8(4), 401–418, 2012.

575 Han, L., Galier, S. and Roux-De Balmann, H.: Ion hydration number and electroosmosis during
576 electrodialysis of mixed salt solution, Desalination, 373, 38–46, doi:10.1016/j.desal.2015.06.023, 2015.

577 Han, L., Galier, S. and Roux-De Balmann, H.: A phenomenological model to evaluate the performances of
578 electrodialysis for the desalination of saline water containing organic solutes, Desalination, 422, 17–24,
579 doi:10.1016/j.desal.2017.08.008, 2017.

580 Hawkins, L. N. and Russell, L. M.: Polysaccharides, Proteins, and Phytoplankton Fragments: Four
581 Chemically Distinct Types of Marine Primary Organic Aerosol Classified by Single Particle
582 Spectromicroscopy, Advances in Meteorology, doi:10.1155/2010/612132, 2010.

583 Hepp, J., Rabus, M., Anhäuser, T., Bromm, T., Laforsch, C., Sirocko, F., Glaser, B. and Zech, M.: A sugar
584 biomarker proxy for assessing terrestrial versus aquatic sedimentary input, Organic Geochemistry, 98,
585 98–104, doi:10.1016/j.orggeochem.2016.05.012, 2016.

586 Iinuma, Y., Engling, G., Puxbaum, H. and Herrmann, H.: A highly resolved anion-exchange
587 chromatographic method for determination of saccharidic tracers for biomass combustion and primary
588 bio-particles in atmospheric aerosol, Atmospheric Environment, 43(6), 1367–1371,
589 doi:10.1016/j.atmosenv.2008.11.020, 2009.

590 Ittekkot, V., Brockmann, U., Michaelis, W. and Degens, E. T.: Dissolved free and combined carbohydrates
591 during a phytoplankton bloom in the northern North Sea, Marine Ecology Progress Series, 4, 299–305,
592 1981.

593 Josefsson, B. O.: Determination of soluble carbohydrates in sea water by partition chromatography after
594 desalting by ion-exchange membrane electrodialysis, Analytica Chimica Acta, 52(1), 65–73,
595 doi:10.1016/S0003-2670(01)80042-8, 1970.

596 Kaiser, K. and Benner, R.: Biochemical composition and size distribution of organic matter at the Pacific
597 and Atlantic time-series stations, Marine Chemistry, 113(1–2), 63–77,
598 doi:10.1016/j.marchem.2008.12.004, 2009.

599 Kirchman, D. L., Meon, B., Ducklow, H. W., Carlson, C. A., Hansell, D. A. and Steward, G. F.: Glucose fluxes
600 and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar
601 Front Zone, Antarctica, Deep Sea Research Part II: Topical Studies in Oceanography, 48(19–20), 4179–
602 4197, doi:10.1016/S0967-0645(01)00085-6, 2001.

603 Kohn, R. and Kovác, P.: Dissociation constants of D-galacturonic and D-glucuronic acid and their O-methyl
604 derivatives, Chem. zvesti, 32(4), 478–485, 1978.

605 Koprivnjak, J.-F., Pfromm, P. H., Ingall, E., Vetter, T. A., Schmitt-Kopplin, P., Hertkorn, N., Frommberger,
606 M., Knicker, H. and Perdue, E. M.: Chemical and spectroscopic characterization of marine dissolved
607 organic matter isolated using coupled reverse osmosis–electrodialysis, Geochimica et Cosmochimica
608 Acta, 73(14), 4215–4231, doi:10.1016/j.gca.2009.04.010, 2009.

609 Krembs, C. and Deming, J. W.: The role of exopolymers in microbial adaptation to sea ice, in
610 Psychrophiles: from biodiversity to biotechnology, pp. 247–264, Springer., 2008.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

611 Krembs, C., Eicken, H., Junge, K. and Deming, J. W.: High concentrations of exopolymeric substances in
612 Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms, Deep
613 Sea Research Part I: Oceanographic Research Papers, 49(12), 2163–2181, doi:10.1016/S0967-
614 0637(02)00122-X, 2002.

615 Kuznetsova, M. and Lee, C.: Dissolved free and combined amino acids in nearshore seawater, sea surface
616 microlayers and foams: Influence of extracellular hydrolysis, Aquatic sciences, 64(3), 252–268, 2002.

617 Leck, C., Gao, Q., Mashayekhy Rad, F. and Nilsson, U.: Size-resolved atmospheric particulate
618 polysaccharides in the high summer Arctic, Atmospheric Chemistry and Physics, 13(24), 12573–12588,
619 doi:https://doi.org/10.5194/acp-13-12573-2013, 2013.

620 Martí-Calatayud, M. C., García-Gabaldón, M. and Pérez-Herranz, V.: Mass Transfer Phenomena during
621 Electrodialysis of Multivalent Ions: Chemical Equilibria and Overlimiting Currents, Applied Sciences, 8(9),
622 1566, doi:10.3390/app8091566, 2018.

623 Marty, J. C., Saliot, A., Buat-Ménard, P., Chesselet, R. and Hunter, K. A.: Relationship between the lipid
624 compositions of marine aerosols, the sea surface microlayer, and subsurface water, J. Geophys. Res.,
625 84(C9), 5707, doi:10.1029/JC084iC09p05707, 1979.

626 McCarthy, M., Hedges, J. and Benner, R.: Major biochemical composition of dissolved high molecular
627 weight organic matter in seawater, Marine Chemistry, 55(3), 281–297, doi:10.1016/S0304-
628 4203(96)00041-2, 1996.

629 Meyer, A., Fischer, H., Kuzyakov, Y. and Fischer, K.: Improved RP-HPLC and anion-exchange
630 chromatography methods for the determination of amino acids and carbohydrates in soil solutions, J.
631 Plant Nutr. Soil Sci., 171(6), 917–926, doi:10.1002/jpln.200700235, 2008.

632 Mopper, K., Dawson, R., Liebezeit, G. and Ittekkot, V.: The monosaccharide spectra of natural waters,
633 Marine Chemistry, 10(1), 55–66, doi:10.1016/0304-4203(80)90058-4, 1980.

634 Mopper, K., Schultz, C. A., Chevolot, L., Germain, C., Revuelta, R. and Dawson, R.: Determination of
635 sugars in unconcentrated seawater and other natural waters by liquid chromatography and pulsed
636 amperometric detection., Environ. Sci. Technol., 26(1), 133–138, doi:10.1021/es00025a014, 1992.

637 Ohtaki, Hitoshi. and Radnai, Tamas.: Structure and dynamics of hydrated ions, Chem. Rev., 93(3), 1157–
638 1204, doi:10.1021/cr00019a014, 1993.

639 Ottosen, L. M., Hansen, H. K. and Hansen, C. B.: Water splitting at ion-exchange membranes and
640 potential differences in soil during electrodialytic soil remediation, Journal of Applied Electrochemistry,
641 30(11), 1199–1207, doi:10.1023/A:1026557830268, 2000.

642 Pakulski, J. D. and Benner, R.: Abundance and distribution of carbohydrates in the ocean, Limnology and
643 Oceanography, 39(4), 930–940, 1994.

644 Panagiotopoulos, C. and Sempéré, R.: Analytical methods for the determination of sugars in marine
645 samples: A historical perspective and future directions, Limnology and Oceanography: Methods, 3(10),
646 419–454, doi:10.4319/lom.2005.3.419, 2005.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

647 Passow, U.: Transparent exopolymer particles (TEP) in aquatic environments, Progress in Oceanography,
648 55(3), 287–333, doi:10.1016/S0079-6611(02)00138-6, 2002.

649 van Pinxteren, M., Müller, C., Iinuma, Y., Stolle, C. and Herrmann, H.: Chemical Characterization of
650 Dissolved Organic Compounds from Coastal Sea Surface Microlayers (Baltic Sea, Germany), Environ. Sci.
651 Technol., 46(19), 10455–10462, doi:10.1021/es204492b, 2012.

652 Rich, J. H., Ducklow, H. W. and Kirchman, D. L.: Concentrations and uptake of neutral monosaccharides
653 along 14°W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the
654 DOM flux, Limnology and Oceanography, 41(4), 595–604, doi:10.4319/lo.1996.41.4.0595, 1996.

655 Rosenørn, T., Kiss, G. and Bilde, M.: Cloud droplet activation of saccharides and levoglucosan particles,
656 Atmospheric Environment, 40(10), 1794–1802, doi:10.1016/j.atmosenv.2005.11.024, 2006.

657 Russell, L. M., Hawkins, L. N., Frossard, A. A., Quinn, P. K. and Bates, T. S.: Carbohydrate-like composition
658 of submicron atmospheric particles and their production from ocean bubble bursting, Proc. Natl. Acad.
659 Sci. U.S.A., 107(15), 6652–6657, doi:10.1073/pnas.0908905107, 2010.

660 Rutgers, A. J. and Hendrikx, Y.: IONIC HYDRATION, Transactions of the Faraday Society (England)
661 Superseded by J. Chem. Soc., Faraday Trans., I and II, Vol: 58, doi:10.1039/tf9625802184, 1962.

662 Sadrzadeh, M. and Mohammadi, T.: Sea water desalination using electrodialysis, Desalination, 221(1–3),
663 440–447, 2008.

664 Sakugawa, H. and Handa, N.: Chemical studies on dissolved carbohydrates in the water samples collected
665 from the North Pacific and Bering Sea, Oceanologica acta, 8(2), 185–196, 1985.

666 Sata, T.: Ion Exchange Membranes: Preparation, Characterization, Modification and Application, Royal
667 Society of Chemistry., 2007.

668 Singlande, E., Roux-de Balmann, H., Lefevbre, X. and Sperandio, M.: Improvement of the treatment of
669 salted liquid waste by integrated electrodialysis upstream biological treatment, Desalination, 199(1), 64–
670 66, doi:10.1016/j.desal.2006.03.020, 2006.

671 Sinnott, M.: Carbohydrate Chemistry and Biochemistry: Structure and Mechanism, Royal Society of
672 Chemistry., 2007.

673 Skoog, A. and Benner, R.: Aldoses in various size fractions of marine organic matter: Implications for
674 carbon cycling, Limnology and Oceanography, 42(8), 1803–1813, doi:10.4319/lo.1997.42.8.1803, 1997.

675 Skoog, A., Biddanda, B. and Benner, R.: Bacterial utilization of dissolved glucose in the upper water
676 column of the Gulf of Mexico, Limnology and Oceanography, 44(7), 1625–1633,
677 doi:10.4319/lo.1999.44.7.1625, 1999.

678 Thornton, D. C. O., Brooks, S. D. and Chen, J.: Protein and Carbohydrate Exopolymer Particles in the Sea
679 Surface Microlayer (SML), Front. Mar. Sci., 3, 135–143, doi:10.3389/fmars.2016.00135, 2016.

680 Tsiakis, P. and Papageorgiou, L. G.: Optimal design of an electrodialysis brackish water desalination plant,
681 Desalination, 173(2), 173–186, 2005.

https://doi.org/10.5194/os-2020-2
Preprint. Discussion started: 17 January 2020

682 Underwood, G. J. C., Aslam, S. N., Michel, C., Niemi, A., Norman, L., Meiners, K. M., Laybourn-Parry, J.,
683 Paterson, H. and Thomas, D. N.: Broad-scale predictability of carbohydrates and exopolymers in Antarctic
684 and Arctic sea ice, PNAS, 110(39), 15734–15739, doi:10.1073/pnas.1302870110, 2013.

685 Vanoppen, M., Bakelants, A. F. A. M., Gaublomme, D., Schoutteten, K. V. K. M., Bussche, J. V.,
686 Vanhaecke, L. and Verliefde, A. R. D.: Properties Governing the Transport of Trace Organic Contaminants
687 through Ion-Exchange Membranes, Environ. Sci. Technol., 49(1), 489–497, doi:10.1021/es504389q, 2015.

688 Vetter, T. A., Perdue, E. M., Ingall, E., Koprivnjak, J.-F. and Pfromm, P. H.: Combining reverse osmosis and
689 electrodialysis for more complete recovery of dissolved organic matter from seawater, Separation and
690 Purification Technology, 56(3), 383–387, doi:10.1016/j.seppur.2007.04.012, 2007.

691 Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J. and Peterson, M. J.: Molecular indicators of diagenetic
692 status in marine organic matter, Geochimica et Cosmochimica Acta, 61, 5363–5369, doi:10.1016/S0016-
693 7037(97)00312-8, 1997.

694 Walker, W. S., Kim, Y. and Lawler, D. F.: Treatment of model inland brackish groundwater reverse
695 osmosis concentrate with electrodialysis — Part II: Sensitivity to voltage application and membranes,
696 Desalination, 345, 128–135, doi:10.1016/j.desal.2014.04.026, 2014.

697 Wendisch, M., Macke, A., Ehrlich, A., Lüpkes, C., Mech, M., Chechin, D., Dethloff, K., Velasco, C. B.,
698 Bozem, H., Brückner, M., Clemen, H.-C., Crewell, S., Donth, T., Dupuy, R., Ebell, K., Egerer, U., Engelmann,
699 R., Engler, C., Eppers, O., Gehrmann, M., Gong, X., Gottschalk, M., Gourbeyre, C., Griesche, H., Hartmann,
700 J., Hartmann, M., Heinold, B., Herber, A., Herrmann, H., Heygster, G., Hoor, P., Jafariserajehlou, S., Jäkel,
701 E., Järvinen, E., Jourdan, O., Kästner, U., Kecorius, S., Knudsen, E. M., Köllner, F., Kretzschmar, J., Lelli, L.,
702 Leroy, D., Maturilli, M., Mei, L., Mertes, S., Mioche, G., Neuber, R., Nicolaus, M., Nomokonova, T.,
703 Notholt, J., Palm, M., van Pinxteren, M., Quaas, J., Richter, P., Ruiz-Donoso, E., Schäfer, M., Schmieder,
704 K., Schnaiter, M., Schneider, J., Schwarzenböck, A., Seifert, P., Shupe, M. D., Siebert, H., Spreen, G., Stapf,
705 J., Stratmann, F., Vogl, T., Welti, A., Wex, H., Wiedensohler, A., Zanatta, M. and Zeppenfeld, S.: The Arctic
706 Cloud Puzzle: Using ACLOUD/PASCAL Multiplatform Observations to Unravel the Role of Clouds and
707 Aerosol Particles in Arctic Amplification, Bull. Amer. Meteor. Soc., 100(5), 841–871, doi:10.1175/BAMS-
708 D-18-0072.1, 2019.

709 Wirth, M. A., Sievers, M., Habedank, F., Kragl, U., Schulz-Bull, D. E. and Kanwischer, M.: Electrodialysis as
710 a sample processing tool for bulk organic matter and target pollutant analysis of seawater, Marine
711 Chemistry, 217, 103719, doi:10.1016/j.marchem.2019.103719, 2019.

712 Wisniewski, C., Persin, F., Cherif, T., Sandeaux, R., Grasmick, A. and Gavach, C.: Denitrification of drinking
713 water by the association of an electrodialysis process and a membrane bioreactor: feasibility and
714 application, Desalination, 139(1–3), 199–205, 2001.

715 Zeppenfeld, S., van Pinxteren, M., Hartmann, M., Bracher, A., Stratmann, F. and Herrmann, H.: Glucose
716 as a Potential Chemical Marker for Ice Nucleating Activity in Arctic Seawater and Melt Pond Samples,
717 Environ. Sci. Technol., 53(15), 8747–8756, doi:10.1021/acs.est.9b01469, 2019.

718

719