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

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Submitted to Ocean Science, ACP/OS special issue: 'Marine organic matter: from biological production in the ocean to organic aerosol particles and marine clouds'

Abstract

An optimized method is presented to determine dissolved free (DFCHO) and dissolved combined carbohydrates (DCCHO) in saline matrices, such as oceanic seawater, Arctic ice core samples or brine using a combination of a desalination with electro-dialysis (ED) 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 free uronic acids were strongly depleted during ED 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 DCCHO was evaluated with standard solutions and seawater samples compared with another established desalination method using membrane dialysis. DFCHO in field samples from different regions on earth ranged between 11-118 nM and DCCHO 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

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; McCarthy et al., 1996). 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 ocean and their impact on Earth's atmosphere.

Most marine carbohydrates exist as polysaccharides or combined carbohydrates (CCHO), which are linear or branched chains of monosaccharides, including hexoses, pentoses, deoxy sugars, amino sugars and uronic acids. In aquatic environments, CCHO either appear in a particulate (PCCHO) or dissolved form (DCCHO). 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 with 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 recent 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 carbohydrates (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 CCHO (Pakulski and Benner, 1994). DFCHO are mostly found in lower concentrations than CCHO, since marine microbes utilize them with high turnover rates (Engbrodt, 2001; Engel and Händel, 2011; Ittekkot et al., 1981; Thornton et al., 2016) as it has been reported for amino acids analogously as well (Kuznetsova and Lee, 2002). 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 concentrations of free glucose in Arctic

63   surface water samples and their ice nucleating activity (INA) suggested a potential link between the

64   formation of INA and marine carbohydrates (Zeppenfeld et al., 2019).

65   At the ocean surface, wind and wave interactions lead to bubble bursting. The emitted sea spray aerosol

66   contains marine carbohydrates, including hydrogels, which contribute to the chemical and physical

67   properties of these particles (Bigg and Leck, 2008; Frossard et al., 2014; Hawkins and Russell, 2010;

68   Rosenørn et al., 2006). They have been detected in particles at different marine environments on earth,

69   including the North Atlantic, the Arctic and Antarctica (Barbaro et al., 2015; Frossard et al., 2014; Gao et

70   al., 2011, 2012; Leck et al., 2013; Russell et al., 2010). However, understanding the quantitative fluxes of

71   marine carbohydrates from the ocean to the atmosphere is still challenging, since chemical analysis of

72   sugars in seawater strongly suffers from matrix effects, especially caused by sea salt.

73   The concentrations of individual monosaccharides in seawater, related saline matrices and aerosol

74   particles can be determined with different kinds of chromatographic methods, such as high performance

75   liquid chromatography and gas chromatography. These methods require a prior derivatization in order to

76   enable the chromatographic separation and detectability of these carbohydrates (Panagiotopoulos and

77   Sempéré, 2005). In the last decades, high performance anion exchange chromatography coupled to pulsed

78   amperometric detection (HPAEC-PAD) has been established as a reliable alternative, since it facilitates a

79   sensitive quantification of sugar compounds both in seawater and in airborne particles without a prior

80   derivatization (Iinuma et al., 2009; Panagiotopoulos and Sempéré, 2005; van Pinxteren et al., 2012; Skoog

81   and Benner, 1997). However, the high ionic content in seawater samples strongly affects the

82   chromatographic performance of the HPAEC-PAD and needs to be removed before analysis.

83   Several procedures are available for the desalination of seawater. The desalination using anion exchange

84   resins AG2-X8 and the cation exchange resin AG50W-X8 exhibits strong drawbacks such as the complete

85   loss of charged sugars (amino sugars, uronic acids) and quite low recovery rate of neutral sugars between

86   20-80% depending on the individual monosaccharide (Borch and Kirchman, 1997; Mopper et al., 1992;

87   Rich et al., 1996). The use of silver cartridges (Dionex OnGuard II Ag/H Cartridges) is faster and easier, but

88   requires very expensive consumables and the capacity of removable sea salt per cartridge is strongly

89   limited (Mopper et al., 1992; Panagiotopoulos and Sempéré, 2005). The desalination applying dialysis

90   membranes achieves reproducible and very high recovery rates of hydrolysable polysaccharides (> 90%).

91   However, this method does not allow the analysis of DFCHO, since these small molecules pass the

92   membrane during dialysis (Engel and Händel, 2011).

93

94   Electro-dialysis (ED) is a fast way to remove ions by applying an electrical field. The use of two different
95   chemo-selective ion exchange membranes allows the exclusive removal of small anions, or cations
96   respectively. Hence, uncharged small substances (neutral DFCHO) and macromolecules (CCHO) can, in
97   principle, be recovered in high quantities. Amongst others, ED is being used for the desalination of salty
98   water to generate potable water and the denitrification of wastewater and soil remediation (Gain et al.,
99   2002; Ottosen et al., 2000; Sadrzadeh and Mohammadi, 2008; Tsiakis and Papageorgiou, 2005; Wisniewski
100   et al., 2001). For analytical sample preparation, ED has been reported as a powerful desalination, e.g. for
101   the analysis of DOM and marine neutral DFCHO (Josefsson, 1970; Koprivnjak et al., 2009; Mopper et al.,
102   1980; Vetter et al., 2007; Wirth et al., 2019). However, following biases, which have hitherto not been
103   discussed in this analytical context, can occur during the application of ED and might falsify the determined
104   concentration of the analytes in the sample. In contact with ion exchange membranes, the passive
105   transport of water (*osmosis*) and solutes with a low molecular weight (*diffusion*), such as DFCHO, can occur
106   triggered by a concentration gradient between the sample and concentration channels (Galama et al.,
107   2014; Galier et al., 2012). Additionally, the active transport of charged molecules (*migration*) and water
108   bound to ions in their hydration sphere (*electro-osmosis*) takes place by operating an electrical field
109   (Galama et al., 2014; Han et al., 2015, 2017). While osmosis and electro-osmosis induce an unavoidable
110   loss of water and hence of the total volume of the sample, diffusion and migration of the analytes result
111   in a loss of analyzable molecules. Furthermore, water splitting and associated pH fluctuations have been
112   reported, when a limiting current is exceeded during an ED desalination (Cowan, 1962; Martí-Calatayud et
113   al., 2018; Ottosen et al., 2000; Vetter et al., 2007).

114

115   Within the present study, a novel protocol for the desalination of seawater samples and other saline
116   samples (e.g. ice cores and brine from Arctic sea ice), applying ED and HPAEC-PAD is presented, accounting
117   for the described biases. This method with a low need of consumables allows the analysis of
118   monosaccharides with (DCCHO) and without hydrolysis (DFCHO), including the possible determination of
119   free amino sugars and free uronic acids. This developed technique was applied to analyze a diverse set of
120   carbohydrates in different kinds of ambient seawater samples.

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2. Experimental

2.1 Chemicals and materials

Prior to the analysis of carbohydrates in seawater, all used laboratory glassware had been washed with ultrapure water (resistivity >18.2 MΩ·cm) thoroughly and pre-heated in a muffle furnace at 550 °C for 4 h. All plastic equipment was first rinsed with 10% HCl solution for three times and then washed with ultrapure water another three times. For calibrating the HPAEC-PAD and determining the recovery of individual monosaccharides, a mixed stock solution was prepared from fucose (Roth, 95%), galactosamine (Sigma, 99%), rhamnose (Sigma, 99%), arabinose (Sigma, 99%), glucosamine (Fluka), galactose (Fluka, 99%), glucose (99%), xylose (Fluka, 99%), mannose (Fluka,99%), fructose (Aldrich, 99%), ribose (Aldrich, 98%), muramic acid (Sigma, 95%), galacturonic acid (Sigma-Aldrich, 97%), glucuronic acid (Sigma, 97%), mannuronic acid (Sigma, 90%). Synthetic seawater samples were made from commercially available sea salts (Sigma) achieving four solutions with salinities of 10, 20, 30 and 40 practical salinity units (PSU). The salinity and the pH of water aliquots was measured by using a conductivity meter (pH/Cond 3320, WTW).

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

144 ## 2.2 Field samples

145 Seven different seawater samples, one ice core from Arctic sea ice and two brines collected within Arctic

146 ice cores (**Table 1**) were measured and used for evaluating recovery rates and comparability of the method

147 presented here. These saline samples were collected during different sampling campaigns and stored at -

148 20 °C. All sampling bottles had been rinsed with dilute hydrochloric acid (10% v/v) prior to the campaign.

149 Field blanks (ultra-pure water filled up in sampling bottles during the campaign) were collected during

150 each campaign and treated in the same way as the samples.

151

152 ## 2.3 The ED system

153 The centerpiece of the PCCell Micro Bench ED system for small sample volumes consisted of three

154 separated compartments (**Figure 1**): The sample compartment was an open chamber that was filled up

155 with 9 mL of the standard solution or seawater sample. The functionalized anion exchange membrane

156 (quaternary ammonium aliphatic polyether) and cation exchange membrane (sulfonated aromatic

157 polyether) bordered this compartment on both sides. Depending on their chemical properties, the

158 membranes allowed exclusively the migration of either positively or negatively charged ions. For that

159 matter, the anion exchange membrane bordering the sample chamber was oriented to the anode and the

160 cation exchange membrane to the cathode. The contact surface with the sample was 7.8 cm^2 for each

161 membrane. For maintaining the conductivity within the system and receiving the sea salt from the sample,

162 the next compartment contained the concentration circuit, a 16 g·L^{-1} NaCl solution (Merck). This

163 concentration was chosen in order to minimize the osmotic water transfer as discussed below. This

164 solution was circulated at a rate of 60 mL·min^{-1}. Two end membranes on each side divided the

165 concentration circuit from the third compartment containing the electrodes. The end membranes were

166 cation exchange membranes with an increased chemical durability and an additional reinforcement in

167 order to withstand the strong differential pressure within the ED system. The mixed metal oxide (MMO)

168 anode was made of a titanium base body coated by RuO_2, IrO_2 and TiO_2. The MMO cathode was made of

169 stainless steel. The electrodes were permanently surrounded by a circulating 0.25 M Na_2SO_4 (Fluka)

170 electrolyte circuit for avoiding unwanted redox reactions (e.g. the generation of corrosive elemental

171 chlorine from chloride). Spacers were inserted between each membrane for keeping the electrolyte and

172 concentration circuits well mixed. The sample solution was homogenized during each desalination by

173 drawing some liquid into a Pasteur pipette and draining it immediately back to the sample compartment.

174 The electrolyte and the concentration solutions were renewed after every tenth desalination. The maximal

175 electrical current I_{max} within the ED cell was adjusted by an automatic online adaption of the voltage, which

176 never exceeded 25 V. The maximal current was set on 0.6 A in order to perform a fast desalination, but

177 also to avoid a scaling of the membranes due to water splitting and is discussed more in detail below. The

178 desalination was stopped when the electric current dropped to a value of 0.20 A.

179 The used ion-exchange membranes have a quite long lifetime as long as they are not damaged

180 mechanically. However, very high attention needs to be given to remove residues of previous desalinated

181 samples in order to avoid carry-over phenomena and obtain good reproducibility. Hence, every time

182 before a new sample was desalinated, the sample chamber was always first filled with ultrapure water for

183 ten minutes and then flushed once with an aliquot of the new sample, which was disposed after.
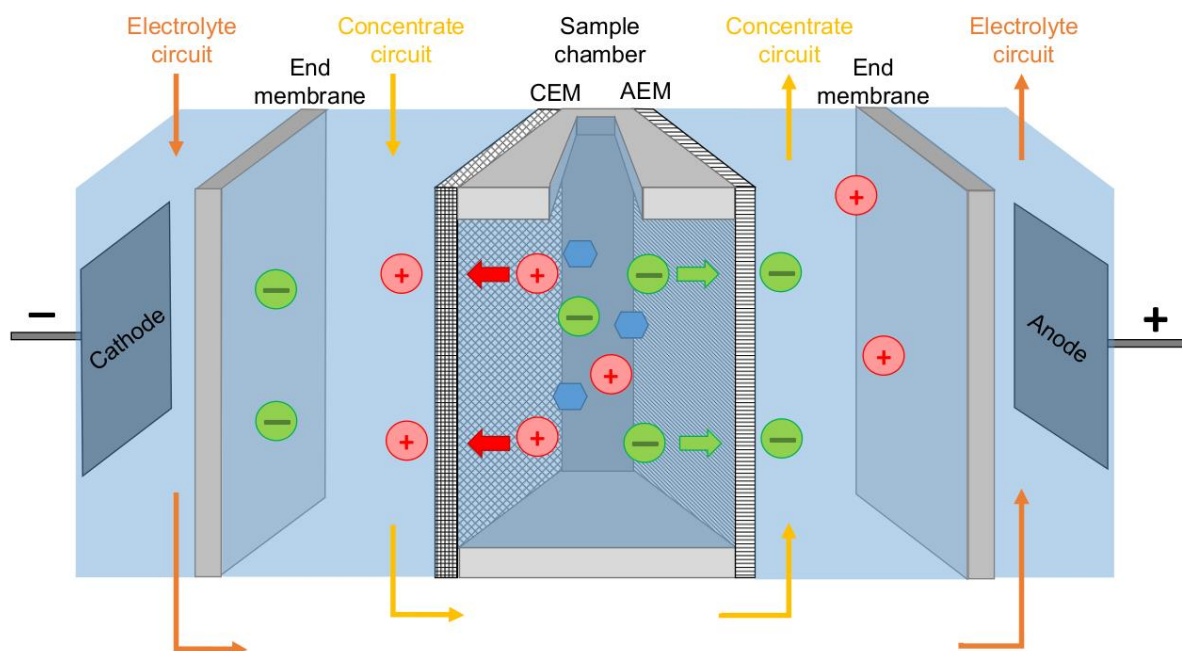
Figure 1 *Schematic setup of the used ED cell. Red circles represent cations, green circles anions, and blue hexagons are carbohydrates. CEM and AEM stand for cation exchange membrane and anion exchange membrane respectively.*

184

185 2.4 HPAEC-PAD system

186 HPAEC-PAD was applied for the analysis of marine carbohydrates in seawater samples. Here, we used an

187 Dionex ICS-3000 ion chromatography system coupled to an autosampler AS-1 as it has been already

188 described for the analysis of saccharidic biomass burning markers in atmospheric particles (Iinuma et al.,

189 2009). Several neutral monosaccharides, amino sugars and uronic acids were separated on a Dionex

190 CarboPac PA20 analytical column (3x150mm) combined with a Dionex CarboPac PA20 guard column

191 (3x30mm). The column oven temperature was maintained at 30 °C. The separation of these saccharides

192 was conducted by applying the gradient profile shown in **Table 2**, which was an adaption of Meyer et al.

193 (2008). Neutral and amino sugars were eluted in 4 mM NaOH within the first 19 min. By adding sodium

194 acetate, sugar acids eluted. At the same time, organic and inorganic contaminants were flushed from the

195 column. After the removal of the remaining acetate by 250 mM NaOH, the system was equilibrated with

196 4 mM NaOH for the next sample injection. The flow rate of the eluent was 0.5 mL·min^{-1}. The retention

197 times, peak widths and resolution factors of the measured monosaccharides are shown in **Table 3**. For the

198 injection of a sample aliquot, a 25 μL loop was used. Each sample was measured in duplicate and each

199 standard in triplicate. Limits of detection (LOD) of individual monosaccharides ranged from 2-12 nM, which

200 is in good agreement with reported data (Engel and Händel, 2011; Panagiotopoulos and Sempéré, 2005).

201

202 For the preparation of eluents A-D, filtered ultra-pure water (resistivity >18.2 MΩ·cm) was degassed with

203 helium for 20 min. Eluents A and B were made by adding a defined volume of low-carbonate NaOH solution

204 (Fisher Chemical, 50% w/w) to the degassed water. Eluent C was prepared by dissolving sodium acetate

205 (Thermo scientific, anhydrous) in ultra-pure water, filtering it through a nylon membrane (0.2 μm, Thermo

206 Scientific), degassing the solution with helium for 20 min and adding the corresponding volume of NaOH

207 solution.

208

209 *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$_2$O)
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

210

211 ***Table 3*** *Averaged peak characteristics of monosaccharide standards separated by the presented elution averaged over 24 runs.*
212 *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	

213

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

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

219 A concentration step using a vacuum concentrator (MiVac) at 55 °C allowed the detection of expected
220 DFCHO concentrations in seawater. For this purpose, a round-bottom glass vial was filled with an aliquot
221 of 6 mL desalted sample, which was weighed empty and filled. After reaching a volume of less than
222 approximately 600 μL, the glass vial was weighed again (in order to calculate the concentration factor) and
223 the concentrated aliquot was pipetted in the autosampler vial for HPAEC-PAD analysis. This step allowed
224 a decrease of LOD by a factor of 10. Each sample was prepared and measured in duplicate.

225 In order to measure DCCHO, marine polysaccharides need to be cleaved into their monomeric compounds

226 by acid hydrolysis. We applied the optimized conditions described by Engel and Händel (2011) with slight

227 modifications. An aliquot of 1 mL desalted sample was hydrolyzed with hydrochloric acid (HCl

228 concentration in sample= 0.8 M) in pre-heated (550 °C, 4 h) glass ampules for 20 h at 100 °C. Neutralization

229 was performed by evaporating all liquid under vacuum at 55 °C until dryness. The dry residue was dissolved

230 in 700 µL ultra-pure water, treated with a vortex homogenizer (IKA MS 3 basic) and filled in the

231 autosampler vial for HPAEC-PAD analysis. Each sample was prepared and measured in duplicate.

232

233 ## 2.6 Parameter optimization and assessment of method

234 ### Impact of osmosis and electro-osmosis during ED desalination

235 For quantifying the loss of water in the sample due to osmosis and electro-osmosis, a synthetic sea salt

236 solution was pipetted into the desalination chamber, which was desalinated for 0, 5, 10, 15, 20 and 25 min

237 with a maximal voltage of 25 V and a maximal current of 0.6 A. After the lapse of time, the total remaining

238 volume was pipetted quantitatively into a glass vial and weighed (Mettler Toledo, XS105 DualRange).

239 These measurements were repeated in triplicate for four different sea salt solutions (10, 20, 30 and

240 40 PSU). The recovery of the sample mass was calculated as the ratio between the mass after the

241 corresponding desalination time and the averaged mass after 0 min.

242 ### Recovery of DFCHO within the ED membrane system

243 Standard addition experiments with seawater samples were performed, for quantifying the recovery of

244 monosaccharides due to diffusion and migration under consideration of all matrix effects. For that reason,

245 sample 7 was filtered (0.2 µm, Millex, PTFE) and spiked with a sugar standard mix (neutral sugars, amino

246 sugars, uronic acids) resulting in a concentration increase of 10 µg L^{-1} and 100 µg L^{-1}.These samples were

247 desalinated using ED (I_{max}=0.6 A, stop at 0.2 A). At the end of each run, (electro-) osmotic water loss was

248 either replenished or not, and the sample directly measured with the HPAEC-PAD. These measurements

249 were repeated in triplicate for each concentration. In order to account for possible wasting phenomena,

250 repetitions were performed with new membranes and membranes which already had been used for some

251 time before. Given recovery rates for neutral monosaccharides are the average of the results for 10 µg

252 L^{-1}and 100 µg L^{-1}. For sugar acids and amino sugars, only the averaged recovery rates for 100 µg L^{-1} are

253 given for avoiding determinations close to the LOD.

254   In order to investigate the influence of pH on the migration of charged monosaccharides, this experiment

255   was repeated for three different pH values: At pH=8 (natural pH of seawater), pH of 1.5 (acidified with

256   concentrated HCl) and pH of 11 (addition of 1 M NaOH). Since high pH in seawater leads to precipitation

257   of hydroxides of alkaline earth metals, an additional filtering (0.2 µm) was performed for these runs.

258   Recovery of CCHO within the ED membrane system

259   Recovery experiments were performed with solutions and a suspension of the polysaccharide standards

260   sodium alginate (Aldrich), laminarin from Laminaria digitate (Sigma) and cellulose powder from spruce

261   (Fluka) at natural pH. Stock solutions were added to filtered sample SWS 7 resulting in concentrations of

262   10 mg L^{-1}. Aliquots of 1 mL with and without desalinations were hydrolyzed (HCl 0.8 M, 100 °C, 20 h) and

263   neutralized by evaporation of the volatile liquid with the vacuum concentrator (55 °C) until dryness. The

264   residue was reconstituted in 700 µL, treated with a vortex homogenizer (IKA MS 3 basic) and filled in the

265   autosampler vial for HPAEC-PAD analysis. Recovery rates were calculated as a ratio between the

266   determined monosaccharide concentrations after hydrolysis of the standard solutions with and without

267   desalination.

268   In order to compare our method on the recovery of DCCHO with another established method, aliquots of

269   four filtered (0.2 µm, Millex, PTFE) seawater samples were treated following the ED protocol presented

270   here and the protocol by Engel and Händel (2011) using membrane desalination, an acid hydrolysis with

271   HCl (0.8 M, 100 °C, 20h), neutralization by evaporation (nitrogen, 50 °C) and an elution on a Dionex

272   CarboPac PA10 column.

273

274   3. RESULTS AND DISCUSSION

275   A reproducible quantification of carbohydrates in seawater samples using HPAEC-PAD requires prior

276   removal of sea salt. Here, we present ED as a reliable desalination method, its parameter optimization and

277   the discussion of arising phenomena resulting in a protocol for the analysis of marine carbohydrates.

278

279   3.1 Kinetics and efficiency of desalination

280   During the desalination of seawater by ED, anions and cations migrate through an electrical field and pass

281   chemo-selective membranes. Depending on their electrical charge, they move either to the positively

282   charged anode or to the negatively charged cathode. In this process, the salt flux through the membranes

283   j_S (mol·m^{-2}·s^{-1}), which determines the desalination time, is proportional to the applied electrical current I

284   (Han et al., 2017; Vanoppen et al., 2015). **Figure 2** shows the current within the used ED system and the

285   salinity of the seawater sample during a typical desalination of an artificial seawater sample (40 PSU) for

286   two different applied maximal currents I_{max} within the system. For almost the entire desalination run, the

287   current I was maintained at I_{max} due to automatic adjustment of the voltage. During this time, the salt flux

288   was approximately constant. Towards the end of the desalination, when almost all salt ions were removed,

289   the current dropped down and the salt flux became lower. Since a direct salinity measurement was not

290   possible in the sample chamber without contaminating the sample, the end of each desalination was

291   defined, when the current I reached a value of 0.2 A. At this point, the salinity of the sample typically

292   ranged between 0.2 and 0.4 PSU, which was found to be sufficiently low for the carbohydrate analysis at

293   the HPAEC-PAD. This reduction in salinity represents an overall desalination of more than 99% of the initial

294   salt concentration. A desalination reaching a salinity below 0.1 PSU was possible, but was not necessary

295   for this application and would have resulted in longer desalination times. Consequently, for minimizing the

296   required desalination time, a high I_{max} is favorable.

297   However, it was observed that the application of an I_{max} of more than 0.8 A during the desalination,

298   resulted in a large pH increase and a white precipitation in the (synthetic) seawater solution, apparently

299   due to the formation of hydroxides of alkaline earth metals. This uncontrolled precipitation strongly

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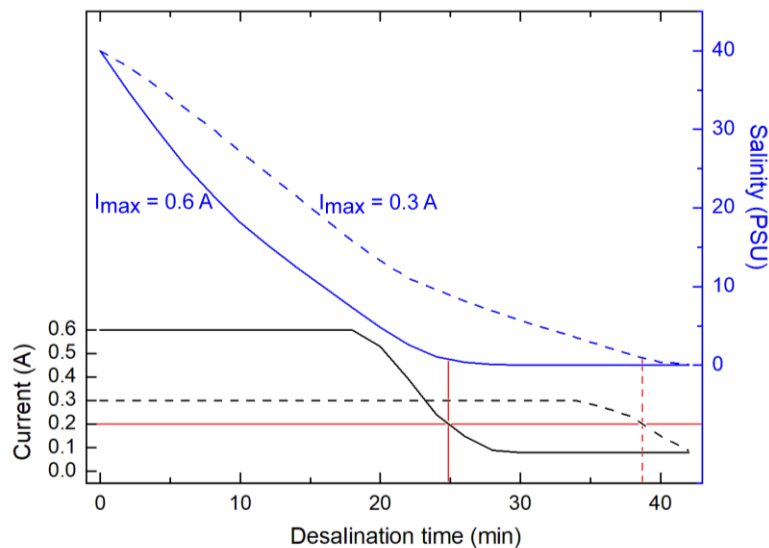
Figure 2 *Measured current within the ED system and salinity of a synthetic seawater sample (40 PSU) versus desalination time with two different maximal applied currents I_{max} (0.6 A solid line, 0.3 A dashed line). The red line represents the current and the corresponding salinity, when desalination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

301   caused a scaling of the membranes. Previous studies explained these unfavorable changes of pH by a

302   strong concentration polarization at the membranes surface leading to water splitting to H^+ and OH^- ions,

303   when a certain limiting current is exceeded. This phenomenon has been preferably observed at anion

304   exchange membranes with quaternary amino groups in the presence of divalent cations, such as Mg^{2+} and

305   Ca^{2+} (Cowan, 1962; Martí-Calatayud et al., 2018; Ottosen et al., 2000). The precipitation exclusively

306   occurred when (synthetic) seawater was desalinated and not during the desalination of NaCl standard

307   solutions. This finding shows the importance of performing parameter optimization tests with synthetic

308   seawater standards that include all important seawater constituents such as divalent cations. In summary,

309   the optimum maximal current I_{max} of 0.6 A was found for the used ED system for avoiding scaling effects

310   and performing desalination as fast as possible.

311

312   ### 3.2 Possible biases during the application of ED

313   Osmotic and electro-osmotic transport of water

314   Osmosis describes the passive transport of free water molecules through a partially permeable membrane

315   caused by large differences of the osmotic pressures between the concentrate circuit and the sample

316   solution (Sata, 2007). The direction and the quantity of the water transport depends on the residence time

317   t_R of the sample solution within the membrane system, the difference between the concentrations of

318   solutes in the sample solution and the concentration circuit $(c_s - c_c)$ and membrane specific parameters,

319   such as the osmotic water transfer coefficient the membrane area and the membrane thickness (Galama

320   et al., 2014). The quantitative effect of osmosis can be reduced by minimizing t_R and $(c_s - c_c)$. Hence, c_c was

321   set at 16 g NaCl L^{-1}, which is approximately in the middle between the concentrations of a typical seawater

322   sample before (30-39 PSU) and after the desalination (0.2-0.4 PSU) for balancing the positive and negative

323   contribution of osmosis on the total sample volume during a typical desalination.

324   In aqueous solutions, water molecules form a hydration shell around ions (Ohtaki and Radnai, 1993).

325   Whenever ions pass through membranes during ED, a cotransport of these hydrating water molecules

326   occurs, known as electro-osmosis (Galama et al., 2014). The electroosmotic water transfer j_W ($m^3 \cdot m^{-2} \cdot s^{-1}$)

327   is proportional to the salt flux j_S in the system and can be expressed by formula (Eq-I) with the molar

328   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

329   and Balmann, 2015; Han et al., 2015).

330     $j_W = n_H \cdot V_M \cdot j_S$ (Eq-1)

331   The salt hydration number of NaCl, as the major compound of sea salt, has been reported with values

332   between 11 and 14 (Han et al., 2015; Rutgers and Hendrikx, 1962; Singlande et al., 2006; Walker et al.,

333   2014). Assuming a NaCl concentration of 30 g·L^{-1} and n_H to be 14, a maximal reduction of the sample

334   volume by 13 % due to electro-osmosis was expected, additionally to osmosis.

335   The recovery of the sample volume due to electro-osmosis and osmosis during the desalination was

336   characterized for four different salinities for the used ED system (**Figure 3**). During the active removal of

337   sea salt, electro-osmosis is the dominating force causing the water loss in the sample. The electroosmotic

338   water loss is continual as long as the salt flux stays constant. However, in the final stages of each

339   desalination, the salt flux decreases and consequently the electroosmotic water transfer decreases, too.

340   For a synthetic seawater sample with a salinity of 30 PSU, 84% of sample mass was recovered. This is in
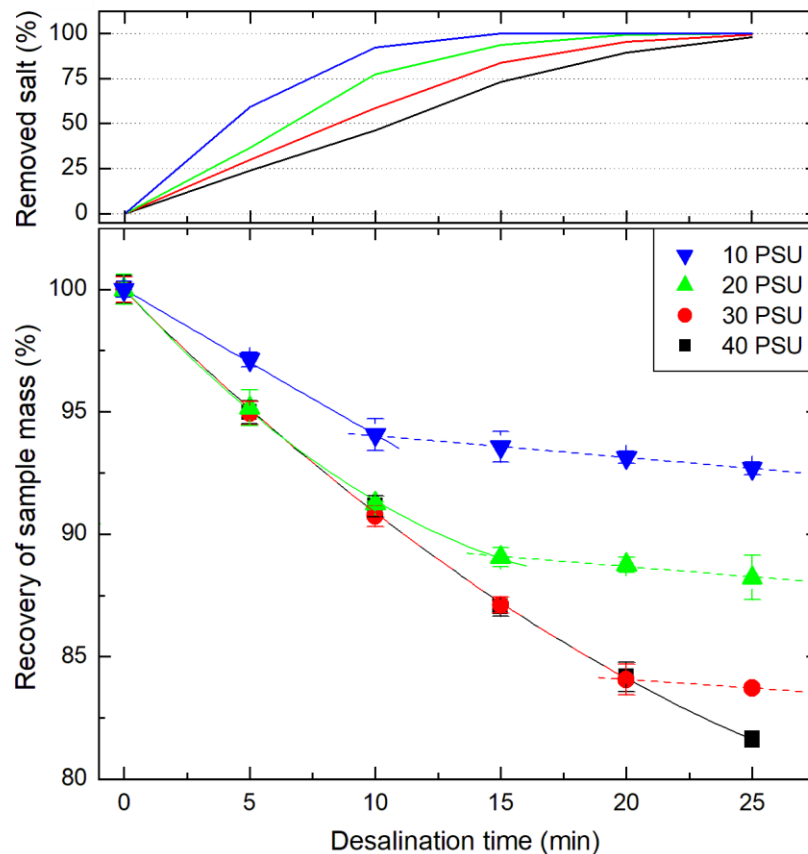
Figure 3 *Combined effect of electro-osmosis and osmosis (solid lines) and osmosis (dashed lines) on the recovery of sample mass as a function of the desalination time within the described membrane system (I_{max}=0.6 A, c_c=16 g NaCl L^{-1}) for artificial sea salt solutions with four different initial salinities.*

341   good agreement with the estimation mentioned above considering a small additional contemporaneous

342   contribution of osmosis. Once the sea salt is removed, osmotic water transfer remained at a constant rate

343   of approximately 0.1%·min^{-1}.

344   The overall water loss resulting from osmosis and electro-osmosis needs to be taken into account since it

345   falsifies the determined concentrations of marine carbohydrates. For its compensation, the chamber was

346   replenished with ultra-pure water at the end of each desalination until the initial sample volume was

347   reached. This procedure was performed with a maximal overall error of 0.5%.

348

349   Analysis and recovery of DFCHO in seawater samples

350   The recovery of neutral monosaccharides during ED is impacted by diffusion and convection processes

351   (Galier and Balmann, 2015). Additionally, free amino sugars and uronic acids migrate through an electrical

352   field due to their charge and pass the ion exchange membranes. Recovery tests were performed with

353   standard solutions spiked to a seawater samples with concentrations, which have been typically reported

354   for seawater samples before (Kirchman et al., 2001; Mopper et al., 1980; Skoog et al., 1999; Zeppenfeld et

355   al., 2019). Recovery rates of neutral sugars (Glc, Man, Xyl, Gal, Ara, Fuc, Rha, Fru) ranged between 95-98%

356   at the natural pH of seawater (approx. pH=8) (**Table 4**). Hence, the overall impact of diffusion and

357   convection on the recovery of monosaccharides seems to be quite low for the short contact time with

358   these membranes. However, a higher loss of neutral monosaccharides due to diffusion was observed,

359   when the sample solution remained within the membrane system for a longer period of time, which calls

360   for a fast desalination. This is in agreement with Vanoppen et al. (2015) who concluded that diffusion and

361   affinity for the membrane are the main drivers for losses of uncharged, low-molecular organics during a

362   ED desalination. An overestimation of the determined concentrations was avoided by performing a

363   correction of the water loss in the end of each desalination. Charged monosaccharides were found with

364   much lower recoveries of 25-31% for uronic acids and 16-19% for amino sugars at pH=8. This is due to

365   their weak acidic/basic properties (pK$_a$ (amino sugars) = 7.6-8.5 (Bichsel and von Gunten, 2000; Sinnott,

366   2007), pK$_a$ (uronic acids) = 3.3-3.5 (Kohn and Kovác, 1978)) and hence their partially ionic state, which

367   makes them migrate through the electrical field. However, a low pH can protonate the carboxylic group of

368   uronic acids and a high pH deprotonates the amino group of amino sugars for reducing this effect. Here,

369   we found that an initial pH of 1.5 before desalination could increase the recovery of free uronic acids up

370   to 45-49%, while a high pH of 11 resulted into a higher recovery of free amino sugars up to 58-59%. The

371   recovery of neutral sugars seemed to be quite unaffected within the range of the tested pH, with the

372 exception of fructose, which was 89% recovered at pH 1.5, certainly due to its instability within acid

373 conditions.

374 **Table 4** *Recovery of individual free monosaccharides (neutral, amino sugars, uronic acids) after desalination with ED including*
375 *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

376

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

378 Recovery experiments with standard solutions of common polysaccharides were performed with and

379 without desalination by ED. The neutral, water-soluble polysaccharide laminarin was recovered with

380 91.0±5.4%. The acidic polysaccharide alginic acid could be recovered with 93.2±5.3%. Even though alginic

381 acid might move within the electrical field due to its acidic molecular structure, its molecular weight does

382 not allow passing the membrane and it does not leave the sample solution. Standard desalination

383 experiments with a suspension of the water insoluble cellulose, which could represent PCCHO, resulted in

384 much lower recoveries of 48±19%. The reason for this high, less reproducible loss of polysaccharides was

385 likely caused by sedimentation within the sample chamber. Engel and Händel (2011) described adsorption

386 processes during the desalination with dialysis membranes and tackled this problem with sonification of

387 the membranes. However, sonification could not be applied in our apparatus. In this study, flushing the

388 chamber several times with a defined volume of ultra-pure water after desalination and reuniting the

389   washing water with the desalinated sample could increase the yield of cellulose up to 85.2±6.9% under

390   consideration of dilution factors. This procedure was not found to be feasible, since a dilution of a natural

391   sample reduced the sensitivity of low concentrated sugars in seawater in the analysis by HPAEC-PAD.

392   Rather, we recommend ED only for the application to filtered samples (DCCHO), while PCCHO might be

393   better analyzed from filters after filtration.

394

395   Comparison of ED and dialysis method for the determination of DCCHO in seawater samples

396   In order to evaluate the presented procedure for the analysis of DCCHO, comparison studies were

397   performed measuring four ambient seawater samples (SWS 1-4) with the established membrane dialysis

398   protocol after Engel and Händel (2011) and with the method presented here. **Figure 4** shows the results

399   of the individual monosaccharides after hydrolysis with HCl. The similarity of the equality line and the

400   regression line (R^2=0.89) using all sugar data indicate a good overall agreement between both methods.

401   Major concentrated sugars, such as glucose, galactose and xylose/mannose, were determined at similar

402   concentrations. Furthermore, a good agreement was observed for minor concentrated sugars, such as

403   fucose and galactosamine. Discrepancies were found for rhamnose and arabinose, which was

404   overestimated by the ED method, and glucosamine, which was determined at lower concentrations. These

405   variations might be explained by statistical uncertainties or co-elution of unknown substances. In
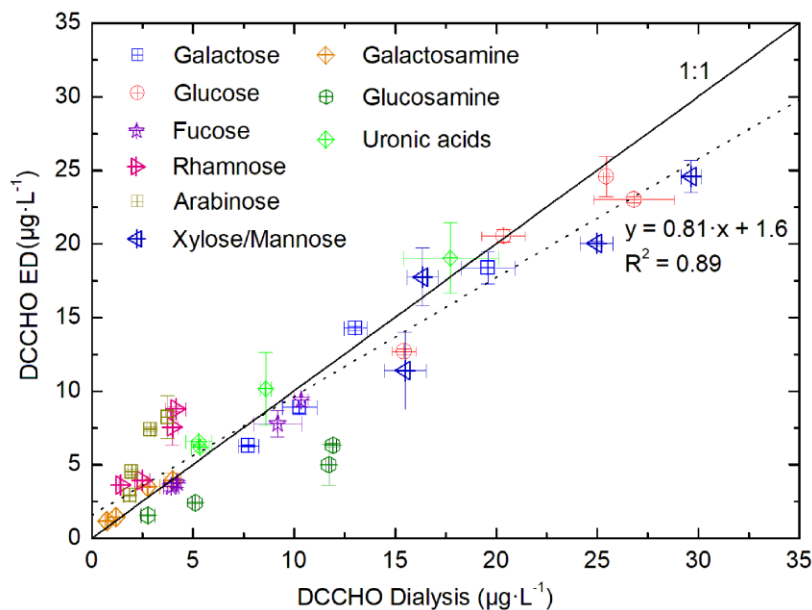
Figure 4 Determined monosaccharide concentrations in four seawater samples (SWS 1-4) after hydrolysis (DCCHO)
comparing the desalination by dialysis (Engel&Händel, 2011) and ED (presented in this study).The full line represents
the line of equality. The dotted line represents the regression line between the data of both methods.

406   summary, the here presented method using ED has shown to be in good agreement with the established

407   membrane dialysis method regarding the analysis of CCCHO. In addition, the ED offers the major

408   advantage of analysing the full spectrum of DFCHO as well - which comprise a group of hardly investigated

409   but potentially important marine compounds.

410

411   3.3 Chromatographic performance with HPAEC PAD after desalination.

412   Several kinds of saline samples were desalinated with ED and analyzed on the CarboPac PA20 column.

413   **Figure 5** shows some examples for DFCHO and DCCHO chromatograms in a brine and a seawater sample

414   after desalination with ED. The insufficient chromatographic separation of mannose and xylose using

415   previous kinds of analytical columns has been frequently described in literature (Borch and Kirchman,

416   1997; Engbrodt, 2001; Engel and Händel, 2011; Kirchman et al., 2001). Therefore, xylose and mannose

417   have been reported as sum concentrations frequently. The elution of the sugars on a CarboPac20 column,

418   applied in the present study, strongly improved the separation between the both sugars mannose and

419   xylose (resolution factor=0.8), and allowed the individual determination of these two sugars. However,

420   most of the analyzed samples showed high concentrations of xylose, which strongly overlapped the

421   smaller peak of mannose. For these cases, we kept reporting a sum value for Xyl/Man.

422

423   3.4 DFCHO and DCCHO in saline field samples from different regions.

424   Several samples were analyzed on DFCHO and DCCHO (**Table 5** and **6**). In both sugar fractions, glucose was

425   the most abundant monosaccharide, as it has been reported before (Panagiotopoulos and Sempéré,

426   2005). In some of the samples, free fructose could be determined reaching concentrations comparable to

427   glucose. However, fructose cannot be determined in DCCHO, since hydrolysis leads to complete

428   destruction of this sugar. High DFCHO was found in the samples from the Arctic including brine and ice

429   core samples reaching up to 118 nM in comparison to seawater samples from the Atlantic SWS 1-4 (11-

430   15 nM). However, a clear regional trend could not be identified for DCCHO with concentrations, ranging

431   between 260-1410 nM. Traces of free amino sugars and uronic acids were found after neutral

432   desalinations. However, a stronger enrichment is required in order to determine them quantitatively and

433   will be the focus of further studies.

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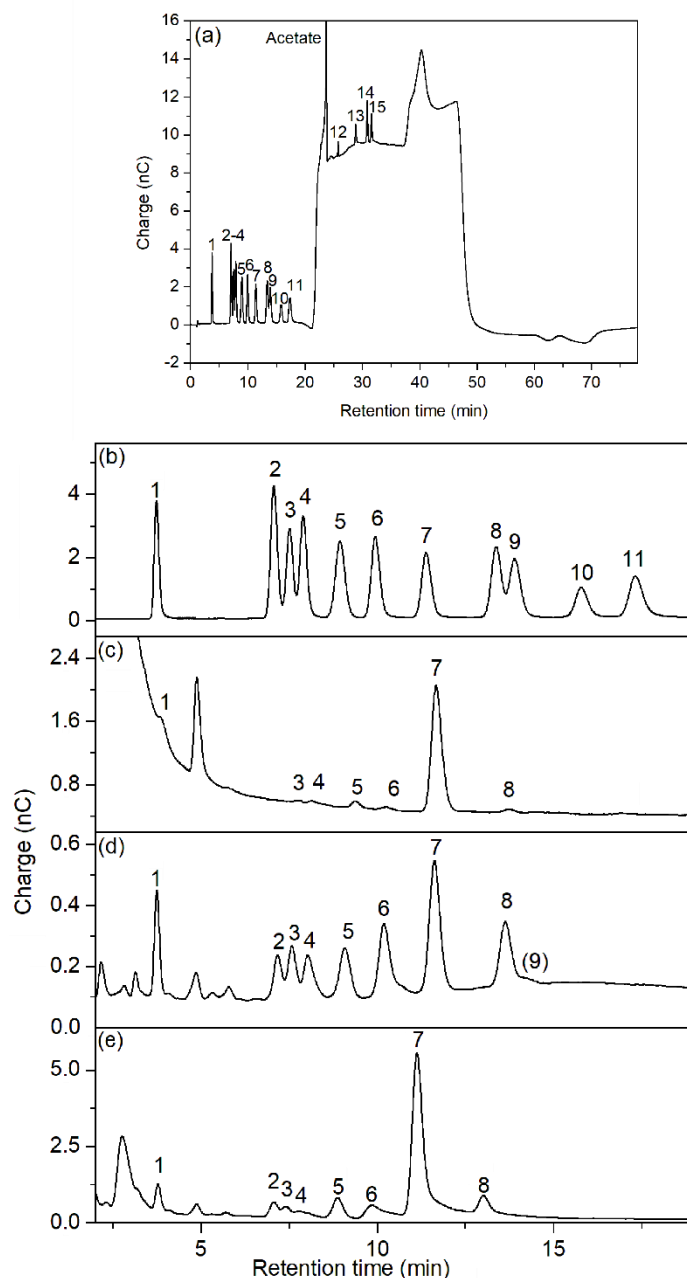
Figure 5 Chromatograms of a) full chromatogram of standard solution 100 µg·L⁻¹; b-e) neutral sugars and amino sugars of b) standard solution 100 µg·L⁻¹; c) DFCHO in brine (B2) desalinated at natural pH; d) DCCHO in a seawater sample (SWS 3); e) DCCHO in Arctic brine (B2). 1 fructose, 2 galactosamine, 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.

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

	Glc mol%	Gal mol%	Xyl/Man mol%	Rha mol%	Fuc mol%	Ara mol%	Fru mol%	Total DFCHO 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

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

	Glc mol%	Gal mol%	Xyl/Man mol%	Rha mol%	Fuc mol%	Ara mol%	GalN mol%	GluN mol%	Gal-ac mol%	Gluc-ac mol%	Total DCCHO 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

5. Summary and conclusion

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

477 avoid an overestimation of the determined concentrations. In seawater from different locations, Arctic

478 brine and sea ice core samples, DCCHO was found in concentrations between 260 and 1410 nM. DFCHO

479 ranged in much lower concentrations of 11-118 nM. Within both, DFCHO and DCCHO, the most dominant

480 monosaccharide was glucose, followed by other neutral sugars.

481 In this study, the successful application of ED in combination with HPAEC-PAD for the analysis of marine

482 carbohydrates (both free and combined) in marine matrices, such as seawater, ice cores and brine could

483 be demonstrated. The application of ED for other more salt sensitive analyses should be the focus of

484 further research, e.g. the reported interference of suspended sea spray aerosol in Arctic snow samples

485 during the quantification of insoluble light absorbing impurities such as black carbon and dust performed

486 via nebulization. Hence, this developed method has the potential to contribute strongly in further research

487 studies understanding biogeochemical processes in the oceans and related saline matrices and sea-air

488 exchange processes, especially for studying hot spot regions of climate change, such as the Arctic.

489

490 ## Acknowledgements

491

492 We gratefully acknowledge the funding by the Deutsche Forschungsgemeinschaft (DFG, German Research

493 Foundation) – Project-ID 268020496 – TRR 172, within the Transregional Collaborative Research Center

494 "ArctiC Amplification: Climate Relevant Atmospheric and SurfaCe Processes, and Feedback Mechanisms

495 (AC)³"in sub-projects B04. Additional support through the Leibniz Association SAW funding of the project

496 "Marine biological production, organic aerosol particles and marine clouds: a Process Chain

497 (MarParCloud)", (SAW-2016-TROPOS-2) is also gratefully acknowledged. We thank for the opportunities

498 to use aqueous samples from various sampling campaigns in order to develop the method presented here.

499 We thank Swantje Pöge for supporting work in the laboratory. We thank Jon Roa for DCCHO analysis

500 following the Engel & Händel (2011) protocol. We thank Susanne Fuchs, Kristin Recklies and Christian

501 Weller of Thermo Fisher in his in-house collaborations for fruitful discussions. We thank Patrick Altmeier

502 and Philip Kalkhoff from PCCell GmbH (Heusweiler, Germany) for guidance and technical support for

503 helping to put ED to operation in our laboratory.

504

505 *Data availability.* All data are available on the public repository PANGAEA;
506 https://doi.pangaea.de/10.1594/PANGAEA.910575.

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

511

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

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