

1 **Answers to the Referees' comments regarding the manuscript:**

2

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

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13 the ocean to organic aerosol particles and marine clouds'

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18 We thank both reviewers for the evaluation of our manuscript. In this document, all of their constructive
19 comments were answered thoroughly. The referees' comments are marked blue and our replies black.
20 The given line numbers of changed sentences are referring to the new lines in the revised manuscript.

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32 **Reviewer: 2, 28 Apr 2020**

33 **This is a detailed and thorough analytical development paper applied to a number of matrices and tested**
34 **using marine samples. The authors have managed to achieve sensitive detection limits for a challenging**
35 **analysis and the paper is suitable for publication with minor revision. I have detailed the changes needed**
36 **below:**

37 Authors: Thank you. Based on your very useful comments, we performed following changes in the
38 manuscript.

39

40 **DFCHO and CCHO are not obvious abbreviations; are these accepted forms?**

41 Authors: We agree that DFCHO and CCHO are not obvious abbreviations. We assume that CHO is originally
42 derived from the aldehyde group as an important structural element of carbohydrates. However, among
43 others, these abbreviations are frequently used within the marine chemistry community (e.g. in Borcard
44 and Engel, 2015; Engel and Händel, 2011; Jugnia et al., 2006; Richardot et al., 1999; Tranvik and Jørgensen,
45 1995). Therefore, we prefer to keep these abbreviations.

46

47 **L13. 'dissolved free'; should also be DCCHO in that case.**

48 Authors: We replaced 'free (DFCHO) and combined monosaccharides (CCHO)' with 'dissolved free (DFCHO)
49 and dissolved combined carbohydrates (DCCHO)' (new lines 13-14). Furthermore, we replaced 'CCHO' with
50 'DCCHO' throughout the manuscript. Additionally, we added the sentence, which reads: 'In aquatic
51 environments, CCHO either appear in a particulate (PCCHO) or dissolved form (DCCHO).' (new lines 40-41)
52 Furthermore, we replaced 'Rather, we recommend ED only for the application to filtered samples
53 (dissolved compounds), while particulate organic matter might be better analyzed from filters after
54 filtration.' with 'Rather, we recommend ED only for the application to filtered samples (DCCHO), while
55 PCCHO might be better analyzed from filters after filtration.' (new lines 392-393)

56

57 **L20. Delete 'real'.**

58 Authors: We deleted 'real'. The new sentence now reads: 'The applicability of this method for the analysis
59 of DCCHO was evaluated with standard solution and seawater samples compared with another established
60 desalination method using membrane dialysis.' (new lines 19-21)

61

62 **L45. 'with' not 'to'.**

63 Authors: The word 'to' was replaced with 'with'. The new sentence now reads: 'Furthermore, an elevated
64 release of polysaccharides by phytoplankton, mostly of gelatinous nature, has been associated with stress
65 situations, such as a deficiency of nutrients, freezing or fluctuating water potential....' (new lines 45-47)

66 **L50. 'recent' not 'latest'.**

67 Authors: We replaced 'a latest study' with 'a recent study'.

68

69 **L57. Analogous to DFCC and DCAA?**

70 Authors: We believe that the reviewer was referring to DFAA (dissolved free amino acids) and DCAA
71 (dissolved combined amino acids). It is true that DFAA and DFCHO in seawater are mostly found in lower
72 concentrations than their macromolecular equivalents (DCAA/DCCHO). Previous publication explained this
73 finding with marine microbes processing these free sugars and amino acids with a very high turnover rate.
74 We added this information to our manuscript, which now reads: 'DFCHO are mostly found in lower
75 concentrations than DCCHO, since marine microbes utilize them with high turnover rates (Engbrodt, 2001;
76 Engel and Händel, 2011; Ittekkot et al., 1981; Thornton et al., 2016) as it has been reported for amino acids
77 analogously as well (Kuznetsova and Lee, 2002).' (new lines 57-59)

78 **L68. 'oceanic environments' is more appropriate.**

79 Authors: We replaced 'maritime regions' with 'marine environments'. (new line 68)

80

81 **L74. kinds**

82 Authors: We replaced 'with different kind of chromatographic methods' with 'with 'different kinds of
83 chromatographic methods'. (new line 74)

84

85 **L75. gas chromatography**

86 Authors: We replaced 'gas chromatograph' with 'gas chromatography'. (new line 75)

87

88 **L76. How is it labour intensive; give brief details?**

89 Authors: There are several ways to derivatize sugars depending on the applied chromatographic analysis,
90 requiring the use of toxic chemicals, robust lab parameters and internal standards. Derivatization is not
91 needed when HPAEC-PAD is applied. However, we came to the conclusion that our use of the word 'labour
92 intensive' is our subjective opinion and possibly misleading. Since this word is not important for
93 understanding the text, we decided to delete it and rephrase the sentence. We replaced 'These methods
94 require a quite difficult sample preparation, including a labor intensive derivatization step' with 'These
95 methods require a prior derivatization in order to enable the chromatographic separation and detectability
96 of these carbohydrates (Panagiotopoulos and Sempéré, 2005)'. (new lines 75-77)

97

98 **L81. The 'high ionic strength/content of seawater samples' is better.**

99 Authors: We replaced 'the presence of sea salt in seawater samples' with 'the high ionic content in
100 seawater samples' (new line 81)

101

102

103 **L107. Related saline samples; what are they?**

104 Authors: We agree that the term 'related saline samples' is not precise. For being more concrete, we added
105 the examples ice cores and brine from Arctic sea ice. The new sentence now reads: 'Within the present
106 study, a novel protocol for the desalination of seawater samples and other saline samples (e.g. ice cores
107 and brine from Arctic sea ice), applying electro-dialysis and HPAEC-PAD is presented, accounting for the
108 described biases.' (new line 115-117)

109 **L116. Resistivity,not conductivity.**

110 Authors: We replaced 'conductivity' with 'resistivity'. (new line 127)

111

112 **L117. How long were items soaked in 10 % HCl?**

113 Authors: The plastic items were rinsed with 10% HCl three times. We added this information to the main
114 text, which now reads: 'All plastic equipment was first rinsed with 10% HCl solution for three times and
115 then washed with ultrapure water another three times.' (new lines 128-129)

116

117 **L123. 'from' not 'to'**

118 Authors: We replaced 'Synthetic seawater samples were made of commercially available sea salts (Sigma)'
119 with 'Synthetic seawater samples were made from commercially available sea salts (Sigma)'. (new lines
120 134-135)

121

122 **L129. Delete 'real'**

123 Authors: Done. We applied this change throughout the manuscript.

124

125 **L131. Add 'sampling campaigns; delete'of our department'**

126 Authors: We changed this sentence, which now reads: 'These saline samples were collected during
127 different sampling campaigns and stored at -20 °C.' (new lines 147-148)

128 **and addany details to acknowledgements.**

129 Authors: Additional details about the sampling campaigns, such as locations and dates, are given in Table 1.
130 Furthermore, we added a sentence to the acknowledgments: 'We thank for the opportunities to use
131 aqueous samples from various sampling campaigns in order to develop the method presented here.' (new
132 lines 497-498)

133

134 **L132. Delete 'kept'.**

135 Authors: Done. The new sentence now reads: 'These saline samples were collected during different
136 sampling campaigns and stored at -20 °C' (new lines 147-148)

137 **L143. mL ; change throughout.**

138 Authors: We replaced 'ml' with 'mL' throughout the manuscript. Furthermore, we replaced 'μl' with 'μL'
139 throughout the manuscript.

140

141 **L149. I presume this is 60 mL.min-1 ; space before 'Two'**

142 Authors: Yes, thank you. This was a typing mistake. The new sentence now reads: 'This solution was
143 circulated at a rate of 60 mL·min⁻¹. Two end...'(new lines 163-164)

144

145 **L150. 'compartment' or 'section' 'containing' the electrodes.**

146 Authors: We replaced 'the third department including the electrodes' with 'the third compartment
147 containing the electrodes'. (new line 165)

148

149 **L152. 'made of' stainless steel.**

150 Authors: We replaced the word 'based on' with 'made of'. The new sentence reads: 'The MMO cathode
151 was made of stainless steel.' (new lines 168-169)

152 **L153. (e.g. to end of sentence)**

153 We replaced 'for avoiding unwanted redox reactions, e.g. the generation of corrosive elemental chlorine
154 from chloride.' with 'for avoiding unwanted redox reactions (e.g. the generation of corrosive elemental
155 chlorine from chloride).' (new lines 170-171)

156 **L155-156. Explain more clearly how homogenisation was achieved.**

157 Authors: In order to describe more clearly how homogenization was achieved, we rephrased the sentence,
158 which now reads: 'The sample solution was homogenized during each desalination by drawing some liquid
159 into a Pasteur pipette and draining it immediately back to the sample compartment.' (new lines 172-173)

160 **L156. Renewed how often (based on number of samples)?**

161 Authors: We renewed these solutions after every tenth desalination. The new text now reads: 'The
162 electrolyte and the concentration solutions were renewed after every tenth desalination.' (new line 174)

163

164 **L163. 'filled with' or similar**

165 Authors: We replaced 'exposed to' with 'filled with'. (new line 182)

166

167 **L171. Did the guard and analytical columns have the same packing (different codes given)?**

168 Authors: To our knowledge, the guard and analytical column do have the same packing. The only difference
169 between these both columns is their length. Therefore, the given code for both columns is almost identical

170 with 'Dionex CarboPac PA20 analytical column (3x150mm)' and 'Dionex CarboPac PA20 guard column
171 (3x30mm)'. However, we missed writing 'PA' in 'Dionex CarboPac PA20 analytical column (3x150mm)'.
172 This was corrected now.

173 **L172. What was maintained at 30 oC, and how?**

174 Authors: The analytical column and guard column were permanently maintained at 30 °C by keeping them
175 in a column oven. In order to make this clearer to the reader, we rephrased the sentence, which now
176 reads: 'Several neutral monosaccharides, amino sugars and uronic acids were separated on a Dionex
177 CarboPac PA20 analytical column (3x150mm) combined with a Dionex CarboPac PA20 guard column
178 (3x30mm). The column oven temperature was maintained at 30 °C.' (new lines 189-191)

179

180 **L173. Adaptation of Meyer et al. (2008)**

181 Authors: We replaced 'an adaption to the elution by (Meyer et al., 2008).' with 'an adaption of Meyer et
182 al. (2008).' (new lines 192-193)

183 **L174. 'were eluted in 4 nM NaOH solution'.**

184 Authors: We rephrased the sentence, which now reads: 'Neutral and amino sugars were eluted in 4 mM
185 NaOH within the first 19 min.' (new line 193)

186

187 **L175. Were they contaminants?**

188 Authors: Sugar acids are not contaminants, but interesting analytes that we want to quantify. These sugar
189 acids elute from the analytical column when sodium acetate is added to the eluent, since they interact
190 strongly with the stationary phase. At the same time, contaminants are flushed from the column as well,
191 when sodium acetate is added. In order to improve the understandability to the reader, we rephrased the
192 sentence, which now reads: 'By adding sodium acetate, sugar acids eluted. At the same time, organic and
193 inorganic contaminants were flushed from the column.' (new lines 193-195)

194

195 **L176. 'the remaining.... Equilibrated with 4 mM NaOH solution.**

196 Authors: We added the word 'the', and replaced 'at' with 'with'. The sentence now reads: 'After the
197 removal of the remaining acetate by 250 mM NaOH, the system was equilibrated with 4 mM NaOH for the
198 next sample injection.' (new lines 195-196)

199

200 **L179. 'in' not 'asa'**

201 Authors: 'As a duplicate' was replaced with 'in duplicate'. Furthermore, we replaced 'as triplicate' with 'in
202 triplicate' throughout the manuscript.

203

204

205 **L180. 'ranged from 2-12 nM**

206 Authors: We replaced 'were ranging between 2-12 nM' with 'ranged from 2-12 nM'. (new line 199)

207

208 **L181. with reported data (refs)**

209 Authors: We replaced 'in good agreement with literature' with 'in good agreement with reported data'.
210 (new line 200)

211

212 **L183. resistivity <18.2....**

213 Authors: We changed 'conductivity' to 'resistivity'. (new line 202) Thank you for pointing on this oversight.

214

215 **L193. Do you know how the pH changed with each change in the gradientprofile?**

216 Authors: An integrated pH reference electrode measures the pH, which is displayed online. We observed
217 a constant pH of 12.0 from 0 min to 19 min. By adding eluent C from 19 min to 35 min, the pH continuously
218 raised until reaching a pH=13. Setting eluent A on 100% from 35 min to 44 min resulted into a permanent
219 increase of pH until 13.5. Setting all eluents on their initial concentrations caused a slow adaption to
220 pH=12.0 from 44 min to 78 min for the next injection. However, we did not add this information to the
221 manuscript, since we don't believe that it has an important significance for the paper

222 **L198. 4 oC; insert space between numbers and units through the paper.**

223 Authors: We inserted a space between numbers and '°C' throughout the manuscript.

224

225 **L199. 'at the end'.**

226 Authors: We changed 'in the end' to 'at the end' throughout the manuscript.

227

228 **L202. 'of expected DFCHO concentrations in seawater'.**

229 Authors: We replaced 'A concentration step using a vacuum concentrator (MiVac) at 55 °C allowed the
230 detection of low concentrated DFCHO, as it occurs in most seawater samples.' with 'A concentration step
231 using a vacuum concentrator (MiVac) at 55 °C allowed the detection of expected DFCHO concentrations
232 in seawater.' (new lines 219-220)

233 **L204. Weighed; change throughout.**

234 Authors: 'Weighted' was replaced with 'weighed' throughout the manuscript.

235

236

237 **L205. Delete 'remaining'.**

238 Authors: Done. The changed sentence now reads: 'After reaching a volume of less than approximately
239 600 µl,...'.(new lines 221-222)

240

241 **L208. 'in' duplicate**

242 Authors: 'as duplicate' was replaced with 'in duplicate' throughout the manuscript.

243

244 **L223.solutions**

245 Authors: 'solution' was replaced with 'solutions'.(new line 239)

246

247 **L224. 'repeated in triplicate for four.....'; delete 'and as triplicate for eachtime'.**

248 Authors: We rephrased this sentence, which now reads: 'These measurements were repeated in triplicate
249 for four different sea salt solutions (10, 20, 30 and 40 PSU).(new lines 239-240)

250

251 **L234. Replace 'as well with' by 'and'; rmove comma after membranes.**

252 Authors: We replaced 'as well with' with 'and'. We removed the comma after 'membranes. The revised
253 sentence now reads: 'In order to account for possible wasting phenomena, repetitions were performed
254 with new membranes and membranes which already had been used for some time before.' (new lines
255 249-251)

256

257 **L248.The samples can't be neutralised by evaporation; clarify this text.**

258 Authors: One crucial step for the sample treatment is the neutralization of the sample after acid hydrolysis.
259 However, the neutralization of acids by the addition of a base (e.g. NaOH) will introduce new ions to the
260 sample, which disturb the analysis at HPAEC-PAD. Hence, a neutralization using a base appears quite
261 pointless after a prior desalination.

262 The advantage of using hydrochloric acid is the volatility of HCl, when the contained water molecules
263 evaporate at the same time. By removing HCl from the system by evaporation, a neutralization can actually
264 be achieved. Amongst other references, this procedure has been already described by Engel and Händel
265 (2011) and Panagiotopoulos and Sempéré (2005).

266 In order to make this approach clearer to the reader, we rephrased the sentence, which now reads:
267 'Aliquots of 1 mL with and without desalinations were hydrolyzed (HCl 0.8 M, 100 °C, 20 h) and neutralized
268 by evaporation of the volatile liquid..' (new lines 262-263)

269

270

271 **L259-260. 'requires prior removal of sea salt'**

272 Authors: We removed 'requires a prior removal of disturbing sea salt.' with 'requires prior removal of sea
273 salt.' (new lines 275-276)

274

275 **L283. 'Large pH increase'**

276 Authors: We replaced 'strong rise of the pH' with 'large pH increase'. (new line 298)

277

278 **L301. What is hydrated water; is it the hydronium ion?**

279 Authors: We actually meant neutral water, which is bound to ions in their hydration sphere. We corrected
280 the sentence, which now reads: 'By operating an electrical field, the active transport of charged molecules
281 (*migration*) and water bound to ions in a hydration sphere (*electro-osmosis*) takes place...' (new lines 107-
282 108)

283

284 **L330. 'of 87 %'**

285 Authors: We agree that the used preposition 'onto 87 %' is wrong. In order to give a unmistakable phrasing,
286 we changed the sentence which now reads: 'a maximal reduction of the sample volume by 13 % due to
287 electro-osmosis was expected' (new lines 333-334)

288

289 **L339. 'a constant rate'**

290 Authors: We added the word 'a'. The new sentence now reads: 'Once the sea salt is removed, osmotic
291 water transfer remains at a constant rate of approximately $0.1\% \cdot \text{min}^{-1}$ '. (new lines 342-343)

292

293 **L342. 'at the end'**

294 Authors: We replaced 'in the end' with 'at the end'. (new line 346)

295

296 **L366. '89 % recovered at pH 1.5'**

297 Authors: We replaced '...with the exception of fructose, which was recovered with 89% at pH 1.5,...' with
298 '...with the exception of fructose, which was 89% recovered at pH 1.5,...'. (new lines 371-372)

299

300 **L381. 'it does not leave'**

301 Authors: We added the word 'it'. The new sentence now reads: '...and it does not leave the sample
302 solution'. (new line 382)

303

304 **L383. Replace 'worse' with 'lower'.**

305 Authors: We replaced 'in much worse recoveries' with 'in much lower recoveries'. (new line 384)

306

307 **L387. Replace 'gadget' with 'system' or 'apparatus'.**

308 Authors: We replaced 'gadget' with 'apparatus'. (new line 387)

309

310 **L392. 'to filtered samples'**

311 Authors: We replaced 'at filtered samples' with 'to filtered samples'. (new line 392)

312

313 **L396. 'were performed'**

314 Authors: We replaced 'studies have been performed' with 'studies were performed'. (new lines 396-397)

315

316 **L398. 'method presented here'**

317 Authors: We replaced 'the here presented method' with 'the method presented here'. (new line 398)

318

319 **L416. 'been reported'; delete 'givenonly'**

320 Authors: We deleted the word 'given only' and added 'been reported'. The changed sentence now reads:
321 'Therefore, xylose and mannose have been reported as sum concentrations frequently.' (new line 416-
322 417)

323

324 **L479. 'of' not 'with'**

325 Authors: We replaced 'lower concentrations with 11-118 nM' with 'lower concentrations of 11-118 nM'.
326 (new line 479)

327

328 **L484. research**

329 Authors: We replaced 'further researches' with 'further research'. (new line 486)

330

331 **Additional changes**

332 We replaced 'combined to' with 'combined with' (title).

333 We added 'hexoses, pentoses' to line 39.

334 **Cited references:**

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