

Reply to the comments of Anonymous Referee #1 (received on 17 Jan 2020)

First, we thank the Anonymous Referee for the evaluation of our manuscript and constructive suggestions and comments.

We answer each comment point-by-point in the following text. All manuscript changes based on these comments can be found in the supplements. Furthermore, all lines named in the following answers refer to the supplemented changed manuscript.

Comment #1:

Firstly, the paper justifies the effort on the assumption that impurities in BCG indicator impact spectrophotometric total alkalinity measurements. It seems to be good paper. Three things were true that led to spectrophotometric pH requiring BCG purification are the impurities in dyes, the impurities cause drift in total alkalinity for the system used for the novel autonomous analyser CONTROS HydroFIAr TA and lastly no two sets of dye had the same impurities.

On a similar note, the authors claim that BCG with impurity quantities higher than 6% provided AT values, which failed fundamental quality requirements but still conclude that to gain optimal AT measurements, an Indicator purification is not necessarily required as long as the purchased dye has a purity level of at least 98 % and they are able to provide quality measurements to avoid identified issues. I don't see how this is true. Purification of dye is expensive but then it is not strictly recommended by the author to carryout high quality measurements. I guess with high quality measurements nothing should be compromised.

Answer #1:

You are totally right by saying “with high quality measurements nothing should be compromised”. But we also think that there must be a reasonable balance between the effort to achieve high-quality measurements and their costs. Most users of spectrophotometric A_T analyzers (no matter if they work at research institutes, industry, or somewhere else) are dependent on a certain budget for their measurements. Additionally, they are maybe not able to perform indicator purifications because they do not have the necessary facilities at hand. Consequently, they have to use unpurified BCG as long as there is no commercial provider for purified BCG. Our results show that the quality A_T measurements based on unpurified BCG is fully comparable to those made with purified BCG as long as the impurity quantity does not exceed a certain level (see Table 4). We also show that there is at least one provider of BCG who can routinely fulfill this requirement (there could be others, but during our study we were not able to figure them out). These are the reasons why we do not strictly recommend a purification of BCG to gain high-quality measurements. At the same time, however, we want the reader to be aware of the fact that the impurity level of their purchased BCG is not unimportant at all. We changed the abstract and parts of the manuscript in order to better address this (see supplement).

Comment #2:

I think there are benefits to this approach, but the authors need to be clearer and accurately spell out what they are as stated in abstract (line 6- 7) that impurities and quality of impurities do impact the drift behaviour of the analyser.

Answer #2:

Yes, there are benefits to this approach which are stated in lines 298 - 299 of the supplemented manuscript. But in comparison to “high-purity” BCG purchased from TCI, which can be used without any extra work, the gained benefits are not significant enough to justify the need and corresponding effort of indicator purification (it does not measurably improve the precision and accuracy of the measurements). In our opinion, there is no reason why a smaller linear drift is principally better than a somewhat larger **linear** drift as both of them are easily correctable by regular reference measurements. The only thing to avoided is a non-linear drift behavior which we found for the less pure commercial BCG products, hence no need to inform the scientific community about this issue. We changed the cost-benefit analysis (lines 299 - 300) to better address this statement.

Of course, in comparison to “low-purity” BCG, purification is beneficial. But we think that the readers of this manuscript would prefer to buy a slightly more expensive unpurified BCG than have extra work with the purification (providing they have the facilities to purify BCG).

In addition, we added a hint that the drift pattern should be assessed for each batch of BCG from the same supplier. This procedure ensures that batch to batch variability in purity is monitored (see lines 311 - 313).

Comment #3:

So my question is that how accurate are these total alkalinity measurements using the analyser and are they taken into account when the total alkalinity is determined.

Answer #3:

Our previous work with this analyzer (Seelmann et al., 2019) revealed a relative analyzer uncertainty of 0.08 % under laboratory conditions. This was determined by the same standard addition experiment as described in this work (see Sect. 2.2.3). And yes, the uncertainty of the A_T measurements was taken into account (manuscript was changed to address this, see lines 195 - 196).

Comment #4:

Second, assessment: I have concerns that characterisation of the pure BCG and impure BCG would results in separate values for the extinction coefficients. I don't see any section in paper that shows the characterisation of pure BCG was conducted.

Answer #4:

A full characterization of the purified BCG was beyond the scope of the study and therefore, not conducted. Of course, it is beyond a doubt that purified BCG might have different extinction coefficients than impure BCG (similar to mCP). In our study, all A_T values were calculated using the coefficients reported by Breland and Byrne (1993). However, Li et al. (2013) investigated the influence of different BCG constants and coefficients on the

measured A_T value and concluded that they are insignificant (also with regard to indicator impurities). The reason for that is the calibration of the system with CRM as it was also done during our measurements (the measurement principle of the CONTROS HydroFIA[®] TA follows a similar procedure like it is described by Li et al. (2013)). Therefore, any uncertainties regarding the coefficients are taken into account for subsequent measurements. And the measurement quality both with purified and unpurified “high-purity” BCG is entirely sufficient for a spectrophotometric analyzer also without a characterization. However, by using “low-purity” BCG, this calibration seems to reach its limits as the measurement uncertainty impairs. That is why we added the statement “the usage of “low-purity” BCG is not recommended” (see line 265). Otherwise a characterization of “low-purity” BCG instead of purified BCG would be necessary. And this procedure seems to be senseless, because there is no need of using “low-purity” BCG as “high-purity” BCG is available.

Finally, we concluded that a characterization was not necessary. We included the section “BCG characterization” in the “Cost-benefit analysis” section (line 314 - 327) to justify our decision.

Comment #5:

If the paper is accepted for publication, I hope the authors could make their points clear so the reader could make proper decision for their research needs.

Answer #5:

We hope that we could clarified all unclear points named by Referee with our changes.

Comment #6:

There are typos in the manuscript which I feel needs to be restructured. Specifically Line 3 influences from impurities. I believe it should read as influences of impurities.

Answer #6:

Changed (see line 3)

Comment #7:

Line 8: Could you please specify the kind of drift. Whether there is change in total alkalinity or how the drift is caused by the impurities.

Answer #7:

Added this information (see lines 10 – 12 and 184 - 187)

Comment #8:

Lines 40 describe to described

Answer #8:

Changed (see line 45)

Comment #9:

Section 2.2 It is stated that all analysis were carried out in air conditioned labs. My question is based on the temperature range for the instrument and sample what was the approximate temperature conditions. As I believe that most of the indicators have a temperature range where they are most effective and work the best.

Answer #9:

The CONTROS HydroFIA® TA has an internal sample temperature control where the measured sample is constantly temperature controlled to 25.0 ± 0.1 °C which is the ideal temperature for BCG (the used characteristics by Breland and Byrne (1993) were carried out at 25°C). The temperature control is realized by a Peltier element and temperature measurement directly behind the cuvette. Therefore, the temperature of the seawater sample is independent of the room temperature. This information has now been added to the manuscript (see line 104).

Comment #10:

Line 106-107 was the purified BCG prepared using the sodium salt in order to make sure that samples and indicators are of similar ionic strength?

Answer #10:

Yes, we prepared the purified BCG solution in deionized water and added a certain amount of sodium hydroxide solution to transfer the BCG into the sodium salt (in solution). Information to this were added in the manuscript (lines 123 - 129).

Comment #11:

Line 120 Equation 2 shows how the precision was calculated. It would be nice to show in the form of an equation how the total alkalinity for the samples was calculated as well.

Answer #11:

Equation 2 (now Equ. 4) does not show how the precision is calculated. It shows how the accuracy is calculated. See lines 142 – 145 (“The root mean square error (RMSE) ... gave us information about the measuring accuracy. It was calculated by...”). The precision was calculated by the standard deviation. However, we decided against an equation for the standard deviation as this is a common statistical tool.

General calculation equations for A_T were added to the manuscript (see lines 105 - 114).

Comment #12:

Line 144 additionally could be changed to ‘in addition to’

Answer #12:

Changed (see lines 166)

Comment #13:

Line 178 reset to ‘resets’.

Answer #13:

Changed (see lines 203)

Comment #14:

What is the frequency of cleaning the analyser? Could you specify please. And are standard runs or CRM used in between runs to maintain the calibration.

Answer #14:

The frequency of cleanings depends on the application of the analyzer and cannot be stated in detail. For example: the more measurements are carried out with the analyzer the higher is the frequency of cleanings. That of course differs from user to user. Furthermore, there is a dependency on the measured water matrix as well, e.g. high turbidity coastal water requires more often cleanings. We made a recommendation based on our experiences (see lines 204 - 210)

Comment #15:

Line 189 to 191. There is something wrong and it is difficult to understand. Probably reword or restructure the sentences so that it is easy to understand. I don't understand how the characters the author is mentioning in this section.

Answer #15:

Reworded the sentences for a hopefully better understanding (see lines 223 - 226)

Comment #16:

Was pure BCG characterised?

Answer #16:

No (please see above)

Comment #17:

Line 199 Figure 5 appears on page 12. Could it be moved closer to where it is mentioned in the text for easier referral?

Answer #17:

Unfortunately, the placement of the figures is automatically performed by LaTeX, which was used for preparing the manuscript (using the Copernicus LaTeX template). We tried to move it, but it was not possible. Unfortunately, the movement of the figure is shown as a change in the manuscript by using the tool "latexdiff" (see caption of Fig. 5 and lines 266 – 269). Please ignore these changes.

However, we think that the placement of the figures will be improved during the typesetting progress.

Comment #18:

Line 204 Author refers to paper by Seelmann et al., 2019 and refers to accuracies I when compared to CRM. It would be nice to at least state some values here so that it is easier for the readers to follow though.

Answer #18:

Added (see lines 239 – 240 and 243). We also added the requirements and the typical performance of the analyzer in Table 4 for a better comparison.

Comment #19:

Lines 215 reword the sentence probably.

Answer #19:

Reworded the sentence (see lines 249 - 252)

Comment #20:

Line 217- 229 how the total alkalinity measurement deteriorates. This section is a bit confusing as the author tries to show total alkalinity and with that talks about the precision and accuracy. Could the author be more specific? Probably with the help of equation or something how their system compares to other studies.

Answer #20:

Please see Table 4 (page 13) in the changed manuscript (changed because of a previous comment). There is a comparison of the measurement uncertainties for purified, “high-purity” and “low-purity” BCG. Additionally, the fundamental A_T quality requirements reported by Dickson et al. (2007) can be found there in addition to the typical performance of the analyzer found during our previous study (Seelmann et al., 2019). It is obvious that the measurement quality (especially accuracy) deteriorates with increasing impurity level.

Comment #21:

Line 245 what does FC refer to in this section.

Answer #21:

FC means flash chromatography. This abbreviation was firstly explained in the Introduction section. But we added an additional explanation there (see line 287)

Comment #22:

Line 250 with BCG i.e. can be changed to ‘using BCG’

Answer #22:

Changed (see line 293)

Comment #23:

Lines 257 delete ‘be’

Answer #23:

Deleted (see line 301)

Reply to the comments of Truls Johannessen (Referee #2) (received on 06 Feb 2020)

First, we thank Truls Johannessen for the evaluation of our manuscript and his highly supportive comments.

We answer each comment point-by-point in the following text.

Comment #1:

This paper is general well written and the presentation of results are appropriate and comprehensive, and their arguments that indicator purification increase the quality of the alkalinity measurements are well justified and summarized in figure 4 and 5.

Answer #1:

Thank you for this supportive evaluation of our manuscript. We appreciate it.

Comment #2:

The important issue now is: To what degree can their results be justified and used by other groups using the same instrument set up as Seelmann et al did?

The cost assessment seems to give an additional dimension to the paper. Of course, we should always strive towards simplification and better cost efficiency as long as this don't cause a compromise to the precision and accuracy of measurements required to address scientific questions pursued. The benefit here must always be balanced by the general costs of the fieldwork campaign and costs related to manpower in use. These costs often greatly exceed the costs in performing the analytical work and then it will be better to make sure that measurements are performed the best way there is. There is a clear recommendation and the end of the paper claiming the following lines 260 and 268:

“To achieve the best long-term measurement experience with the analyzer it is not necessary to use purified BCG, as the purest available indicator (e.g. BCG from TCI) generate fully satisfying quality results. Users of the CONTROS HydroFIA TA should take the consequences of indicator impurities into account when choosing their BCG supplier. From this perspective, it would be beneficial to invest into higher purity indicator avoiding the issues described above. If applicable, an HPLC analysis of the used indicator following the here described analytical method can show any types and quantities of impurities. However, if there is no HPLC available, long-term laboratory measurements as described here can help to evaluate whether the purchased indicator is suitable or not by evaluating the drift behaviour.”

This paper is a valuable contribution to the scientific community dealing with delicate measurements, in this case of the carbon system variable alkalinity. It stimulated discussions related to the use of different dye(s) and their purity. This is convincingly stated in lines 255-257:

“Finally, if we compare the purified BCG with "high-purity" BCG like from TCI, the only benefit gained from the purification is a reduced drift per AT measurement. There is no improvement in the measurement quality (precision and accuracy) as long as the impurity level is 2 % or below.”

Answer #2:

Thank you for this supportive conclusion of our paper. It shows us that we will contribute beneficial information to the scientific community by publishing our results.

Comment #3:

My conclusion is that this paper can be published with minor revision (typos).

Answer #3:

We highly appreciate this evaluation of our manuscript. Thank you! We will do our best to correct all typos before publication.

Impact of impurities in bromocresol green indicator dye on spectrophotometric total alkalinity measurements

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Abstract. Due to its accurate and precise character, the spectrophotometric pH detection is a common technique applied in measurement methods for carbonate system parameters. However, impurities in the used pH indicator dyes can influence the measurements quality. The work described here focuses on ~~influences from~~ impacts of impurities in the pH indicator dye bromocresol green (BCG) on spectrophotometric seawater total alkalinity (A_T) measurements. In order to evaluate the extent of such influences, purified BCG served as a reference. First, a high-performance liquid chromatography (HPLC) purification method for BCG was developed as such a method does not exist at the time of this study. A subsequent analysis of BCG dye from four different vendors with this method revealed different types and quantities of impurities. After successful purification, A_T measurements with purified and unpurified BCG were carried out using the novel autonomous analyzer CONTROS HydroFIA[®] TA. Long-term measurements in the laboratory revealed a direct influence of impurity types and quantities on the drift behavior of the analyzer. The purer the BCG, the smaller was the ~~drift increment~~ A_T increase per measurement. The observed drift is generally caused by deposits in the optical pathway mainly generated by the impurities. However, it could not be fully overcome. Furthermore, we could show that a certain impurity type in some indicator dyes changed the drift pattern from linear to non-linear, which can impair ~~the A_T measurements during a~~ long-term ~~deployment~~ deployments of the system. Consequently, such indicators are impractical for these applications. Laboratory performance characterization experiments revealed no improvement of the measurement quality (precision and accuracy) by using purified BCG as long as the impurities of the unpurified dye do not exceed a quantity of 2 % (relationship of peak areas in the chromatogram). However, BCG with impurity quantities higher than 6 % provided A_T values, which failed fundamental quality requirements. Concluding, to gain optimal A_T measurements especially during long-term deployments, an indicator purification is not necessarily required as long as the purchased dye has a purity level of at least 98 % ~~-and is free of the previously named impurity type.~~ Consequently, high-quality A_T measurements do not require pure but the purest BCG, which is purchasable.

1 Introduction

Global observations of the marine carbonate system are of high importance to understand not only the biogeochemical processes but also the uptake, transport and accumulation of anthropogenic CO_2 in the ocean. The measurable key parameters characterizing the ocean carbon cycle are pH, total alkalinity (A_T), $p\text{CO}_2$, and total dissolved inorganic carbon (C_T). Due

25 to their corresponding thermodynamic relationships, it is only necessary to measure two of these four parameters for a full
characterization of the marine carbonate system (Millero, 2007). Traditionally, A_T and C_T were the preferred parameters
for this purpose when measuring discrete samples. However, more recently, pH measurements have become more common
within the oceanographic communities. During centuries of ocean carbon observations, several analytical methods have been
established, ranging from manual bench top systems for laboratory work via at-sea flow-through analyzers to in situ sensors.
30 Among all these available methods, spectrophotometric pH determination techniques using sulphonephthalein indicator dyes
are described as simple, fast, and precise (e.g. Clayton and Byrne, 1993; Tapp et al., 2000; Bellerby et al., 2002; Aßmann et al.,
2011). They have been utilized in marine research especially for ocean carbon observations since the late 1980's (Robert-Baldo
et al., 1985; Byrne, 1987; Byrne and Breland, 1989; King and Kester, 1989). Since Breland and Byrne (1993) showed that the
sulphonephthalein indicator dye bromocresol green (BCG) is suitable for seawater pH determination in the pH range 3.4 to 4.6,
35 it has been used in several spectrophotometric A_T measurement systems with comparable precision and accuracy as traditional
methods (Yao and Byrne, 1998; Li et al., 2013; Seelmann et al., 2019).

Investigations of Yao et al. (2007) on seawater pH measurements with the most common indicator dye, meta-cresol purple
(mCP) from different vendors reveals different types and quantities of light-absorbing impurities. These impurities can con-
tribute to pH offsets of up to 0.01 pH units. To overcome the uncertainties caused by indicator impurities, Liu et al. (2011)
40 developed a preparative high-performance liquid chromatography (HPLC) method to purify mCP and characterized this puri-
fied dye. Furthermore, to produce large batches of purified mCP, Patsavas et al. (2013a) developed a flash chromatography (FC)
method resulting in a 3.5 times increased yield per run. However, not all users of spectrophotometric seawater pH measure-
ment systems are able to purify or to purchase purified mCP. Therefore, Douglas and Byrne (2017) published a mathematical
correction for accurate pH measurements using unpurified mCP.

45 In order to apply these findings to spectrophotometric A_T measurements, Nand and Ellwood (2018) ~~describe~~described
a simple colorimetric method for determining seawater A_T using purified bromophenol blue (BPB) as pH indicator dye.
However, at the time of this study, there are no comparable detailed investigations on how indicator impurities in BCG may
influence spectrophotometric seawater A_T measurements.

Since our previous work dealt with an open-cell single-point titration analyzer with spectrophotometric pH determination
50 using BCG as indicator dye (Seelmann et al., 2019), we investigated the influences of any impurities in BCG from different
vendors in comparison to purified BCG as reference. Hence, the first step of this study was to develop a purification method for
BCG. Due to similarity in the chemical structure of BCG and mCP (see Fig. 1) and the available facilities in our laboratory, we
decided to develop an HPLC analysis and purification method for BCG based on the mCP purification method published by
Liu et al. (2011). Once the developed method was sufficient for BCG purification, a small batch of purified BCG was produced.
55 Following this, comparative experiments were carried out with a novel autonomous analyzer for seawater A_T using purified
and unpurified BCG in order to evaluate the influence of impurities in the indicator dye.

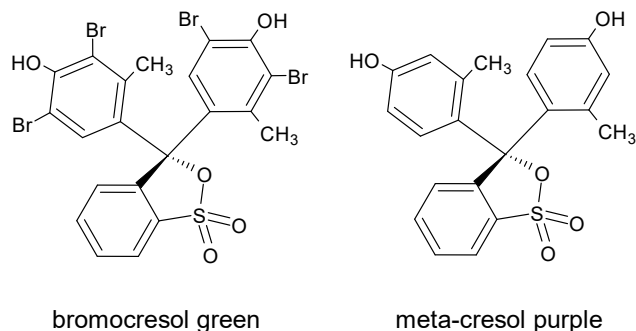


Figure 1. Chemical structure of bromocresol green and meta-cresol purple

2 Materials and methods

2.1 HPLC method

2.1.1 Reagents and instrumentation

60 The BCG indicator (as sodium salt) was obtained from the following vendors: Acros Organics, Alfa Aesar, Carl Roth, and TCI. The solvents used in the HPLC purification were water (H₂O), acetonitrile (ACN), and trifluoroacetic acid (TFA). The ACN (HPLC grade) and the TFA (Purity: $\geq 99.9\%$) were obtained from Fisher Scientific and Carl Roth, respectively.

A Shimadzu LC system performed both the analytical and preparative chromatography. This system included an auto-sampler (SIL-10ADvp) (only for analytical mode), a isocratic preparative LC pump (LC-8A), an isocratic analytical HPLC
65 pump (LC-10ADvp), a column oven (CTO-10ASvp), a single channel UV-VIS detector (SPD-10Avp), and an LC controller (SCL-10Avp).

The Primesep B2 HPLC columns were obtained from SIELC Technologies. This is a reverse-phase column with embedded basic ion-pairing groups that retains analytes by reverse-phase and ion-exchange mechanisms. For developing the purification method, an analytical Primesep B2 column (4.6 x 250 mm, particle size: 5 μm) was chosen. The purification was performed
70 by a preparative Primesep B2 column (21.2 x 250 mm, particle size: 5 μm). Analytical separations were performed at 25 °C, but preparative chromatography was undertaken at room temperature.

2.1.2 Method development

The method development included the optimization of the mobile phase composition for BCG separation on the chosen column and was performed in analytical mode. For this purpose, a 10 mg mL⁻¹ BCG solution of each vendor was prepared in the
75 mobile phase and 20 μL were injected. One HPLC run with a flow-rate of 1.5 mL min⁻¹ took 60 min and was monitored using the UV-VIS detector at 280 nm. The optimal mobile phase composition was determined by systematically changing the concentrations of the solvents starting from the conditions described by Liu et al. (2011). There, the mobile phase composition

was 70:30 ACN:H₂O (volume:volume) with 0.05 % of TFA. Afterwards, the ACN and TFA concentration were increased by 5 % and 0.05 % increments, respectively, until the mobile phase consisted of 85 % ACN and 0.2 % TFA. One BCG injection was done per mobile phase composition each followed by a blank run. Blank runs were carried out by injecting the mobile phase as sample.

2.1.3 Comparison of BCG from different vendors

Once the optimal mobile phase composition was found, we tested BCG from different vendors for impurity types and quantities. For that, a BCG solution of each vendor was prepared and analyzed as described in Sect. 2.1.2 with the optimal mobile phase composition. To quantitatively compare the purity of each dye, we defined the relative purity of BCG at 280 nm wavelength (P_{BCG}), which was calculated as follows:

$$P_{BCG} = \frac{A_{BCG}}{\sum_{i=1}^n A_i} \times 100\% \quad (1)$$

where A_{BCG} is the area of the BCG peak, n is the number of peaks, and A_i is the area of the i^{th} peak.

2.1.4 Purification of BCG

The purification was performed by the LC system in preparative mode. A 7.5 mg mL⁻¹ BCG solution was prepared in the mobile phase and 10 mL were injected onto the preparative column. Impurities were separated by isocratic flow (flow rate 31.2 mL min⁻¹) with 75:25:0.1 ACN:H₂O:TFA as mobile phase. The pure BCG was collected manually in a round bottom flask at its retention time of about 52 min. Approximately 90 % of the mobile phase was removed from the BCG eluate using a rotary evaporator, with the final 10 % left to evaporate in a dark open box at room temperature. The pure crystalline dye was transferred to a brown flask for further experiments.

In order to verify the success of the purification, the purified BCG was analyzed by the analytical HPLC procedure as described in Sect. 2.1.2.

2.2 Total alkalinity measurements

2.2.1 Reagents and instrumentation

Total alkalinity measurements were performed using the novel autonomous analyzer CONTROS HydroFIA[®] TA (Kongsberg Maritime Contros GmbH, Kiel, Germany). Its measurement principle is based on a single-point open-cell titration of the seawater sample with subsequent spectrophotometric pH detection using BCG as indicator (Brelund and Byrne, 1993; Yao and Byrne, 1998; Li et al., 2013; Seelmann et al., 2019). The seawater sample was titrated with 0.1 mol kg⁻¹ hydrochloric acid (HCl) obtained from Carl Roth and constantly temperature controlled to 25.0 ± 0.1 °C by the systems internal heat exchanger.

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The A_T value of the sample was calculated by the following general equation:

$$\frac{-V_{sw} \times \rho_{sw} \times A_T + V_t \times \rho_t \times C_t}{V_{sw} \times \rho_{sw} + V_t \times \rho_t} = [\text{H}^+]_F + [\text{HF}] + [\text{HSO}_4^-] + [\text{HI}^-] \quad (2)$$

110 where V_{sw} and V_t are the volumes of the seawater sample and the added titrant (HCl and BCG solutions), respectively, and ρ_{sw} and ρ_t are the densities of the seawater sample and the added titrant, respectively. C_t is the acid concentration in the combined titrant solution. $[\text{H}^+]_F$ is the free concentration of hydrogen ions, and $[\text{HI}^-]$ is the concentration of the protonated (i. e. acidic) form of BCG. $[\text{HF}]$ and $[\text{HSO}_4^-]$ are the concentrations of hydrogen fluoride and the bisulfate ion in the seawater sample. $[\text{H}^+]_F$, or pH_F , in the sample-titrant mixture is measured spectrophotometrically. Following Breland and Byrne (1993) and Yao and Byrne (1998), pH_F is described by

$$\text{pH}_F = 4.4166 + 0.0005946 \times (35 - S_{\text{mix}}) + \log \left(\frac{R - 0.0013}{2.3148 - R \times 0.1299} \right) \quad (3)$$

115 where S_{mix} is the salinity of the sample-titrant mixture, and R is the ratio between the absorbances at 444 and 616 nm.

Certified reference material, CRM, (batch 160, $A_{T,\text{reference}} = 2212.44 \pm 0.67 \mu\text{mol kg}^{-1}$) was obtained from A. G. Dickson at the Scripps Institution of Oceanography of the University of California, San Diego. The seawater for the experiments was prepared by diluting concentrated seawater solutions called "Absolute Ocean" obtained from ATI Aquaristik. Its absolute A_T value was not important as it was only for mimicking semi-continuous measurement conditions between the references. All total alkalinity measurements were carried out in an air-conditioned laboratory and after the system was "calibrated" with a freshly opened CRM.

2.2.2 Long-term measurements

For the long-term measurements, $0.002 \text{ mol kg}^{-1}$ BCG solutions ~~in deionized water~~ were prepared from unpurified BCG (from different vendors) and purified BCG and used as indicator dye in the analyzer. The unpurified dyes (sodium salts) were dissolved in deionized water (DI-water). The purified dye was dissolved in DI-water with sodium hydroxide (NaOH) as additive. The exact amount of NaOH was calculated by the molar ratios and molar masses of BCG and NaOH. This transferred the pure BCG to its sodium salt and improved its solubility. For both unpurified and purified BCG solutions the ionic strength was kept very low (only created by the dissolved BCG sodium salt itself) in order to realize high concentrations of BCG stock solution. However, the dilution of the sample seawater by the added BCG and HCl solution was considered in the A_T calculation procedure.

130 The prepared seawater sample ($\approx 25 \text{ L}$) was measured more than 300 times with a measuring interval of 15 min, which took about four days. For monitoring the drift, a freshly opened CRM was measured at the beginning and at the end of this experiment, as well as daily in between. Each of these CRM measurements consisted of five consecutive single measurements.

2.2.3 Standard addition experiment

135 In order to evaluate the impact of impurities on the measuring performance of the system, we carried out a standard addition
experiment with each unpurified and the purified BCG. This experiment is the standard validation procedure for evaluating the
performance of the analyzer under laboratory conditions. Therefore, a seawater sample (with relatively high A_T) was titrated
with an HCl solution (0.1 mol kg^{-1}) to lower its A_T in five steps. The titration was carried out by adding different precisely
known volumes of HCl to a known volume of seawater resulting in five seawater samples with stepwise decreased A_T . The
140 theoretical A_T ($A_{T,\text{titrated}}$) was calculated from the volumes of added acid and seawater, the concentration of the acid, and
the original A_T of the seawater. To determine the practical A_T ($A_{T,\text{measured}}$), each of these samples was repeatedly measured
with the analyzer ($n = 5$).

The precision was determined by averaging the standard deviation (σ) of each sample measurement. The root mean square
error (RMSE) of the linear regression after plotting $A_{T,\text{measured}}$ vs. $A_{T,\text{titrated}}$ gave us information about the measuring
145 accuracy. It was calculated by

$$\text{RMSE} = \pm \sqrt{\frac{1}{n} \times \sum_{i=1}^n (A_{T,\text{fitted},i} - A_{T,\text{measured},i})^2} \quad (4)$$

where n is the number of samples, $A_{T,\text{fitted},i}$ is the i^{th} A_T value calculated with the linear regression equation with $A_{T,\text{titrated},i}$
as x variable, where $A_{T,\text{measured}}$ is the average of the five repeatedly measured A_T values of each titrated seawater sample.
Slope and intercept of this regression were important for the evaluation of linearity and sensitivity. Within the standard val-
150 idation procedure of the analyzer, these terms must fulfill within their uncertainties the following requirements: Slope = 1;
intercept = 0.

3 Results and discussion

3.1 HPLC separation and purification of BCG

3.1.1 Method development

155 Table 1 summarizes the influence of the different mobile phase compositions on the BCG separation. For saving solvents
and time, it is important to keep the time of each HPLC run under 60 min, but, at the same time, with an optimal separation
of BCG from its impurities. Hence, the optimal separation of BCG was achieved with 75:25:0.1 ACN:H₂O:TFA as mobile
phase. There, the pure BCG was eluted from the column as fast as possible (retention time: 52 min) with the best dye-impurity
separation.

160 3.1.2 Comparison of BCG from different vendors

Figure 2 shows the resulting analytical HPLC chromatograms. There, BCG from different vendors shows different types and
quantities of impurities. However, the retention time of the pure BCG was 52 min in all chromatograms. Another similarity

Table 1. Mobile phase compositions and their impact on the BCG separation

Mobile phase composition:			BCG separation:	
ACN (%)	H ₂ O (%)	TFA (%)	BCG peak (min)	Sufficient separation of impurities
70	30	0.05	no elution ^a	-
70	30	0.10	60	no ^b
70	30	0.15	no elution ^a	-
70	30	0.20	no elution ^a	-
75	25	0.10	52	yes
80	20	0.10	56	yes
85	15	0.10	60	no ^b

^aWithin 60 min run time^bImpurities found in subsequent blank run**Table 2.** Summary of analytical HPLC of unpurified BCG from different vendors

	Acros Organics	Alfa Aesar	Carl Roth	TCI
Number of peaks	3	7	3	5
P_{BCG} (%)	93.2	85.4	92.5	98.1

between all chromatograms was the cluster of several peaks around 3-5 min. Only the peak areas of these peaks strongly differed. As there are no peaks at these retention times in the blank chromatogram, this peak cluster had to be caused by the indicator and not by the solvent. BCG from Acros Organics, Alfa Aesar and Carl Roth showed an intensive peak around 58 min, which is not present in the BCG from TCI. However, BCG from TCI showed three other small peaks around 26 min, 29 min, and 42 min. Alfa Aesar BCG also showed the 42 min peak additionally-in addition to small peaks around 7 min, 10 min, and 35 min. These various quantities of total absorbance at 280 nm resulted in different P_{BCG} . The calculated P_{BCG} for each vendor (following Equ. 1) are summarized in Table 2. It has to be taken into account that these quantities are only valid when using an UV detector. Other detectors may result in different purity levels.

3.1.3 Purification of BCG

In order to test the effectiveness of the purification method, we decided to purify the least pure BCG from Alfa Aesar. Furthermore, to produce the most pure dye, also BCG from TCI was chosen for purification. The obtained yields were between 60 % and 70 % for both BCGs with around 50 mg purified BCG recovered per injection.

Figure 3 shows the analytical HPLC chromatograms of purified TCI BCG, and Alfa Aesar BCG, respectively. Both chromatograms still show the peak cluster around 3-5 min, but with much smaller areas, especially with purified Alfa Aesar BCG.

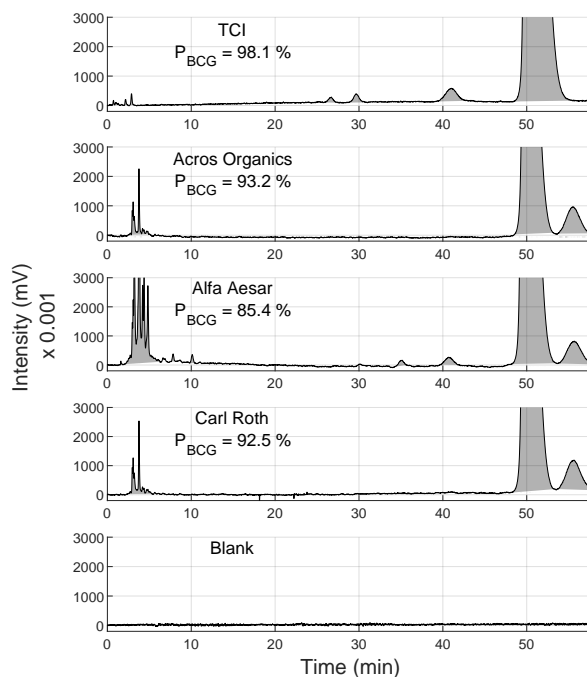


Figure 2. Analytical HPLC chromatograms of unpurified BCG from different vendors with their P_{BCG} and a chromatogram from a solvent injection without BCG (Blank). All peaks are highlighted with gray background color.

Table 3. Summary of analytical HPLC of purified BCG

	Purified from	
	TCI	Alfa Aesar
Number of peaks	2	4
P_{BCG} (%)	99.6	99.3

Furthermore, the 42 min and 58 min peaks of Alfa Aesar BCG could not be totally removed. However, the purity of TCI BCG, and Alfa Aesar BCG improved to 99.6 %, and 99.3 %, respectively. The results are summarized in Table 3.

180 Since the relative purity of Alfa Aesar BCG was improved from 85.4 % to 99.3 %, the success of the purification was proven. Hence, the HPLC purification method developed here is considered sufficient for the nearly full removal of impurities from BCG.

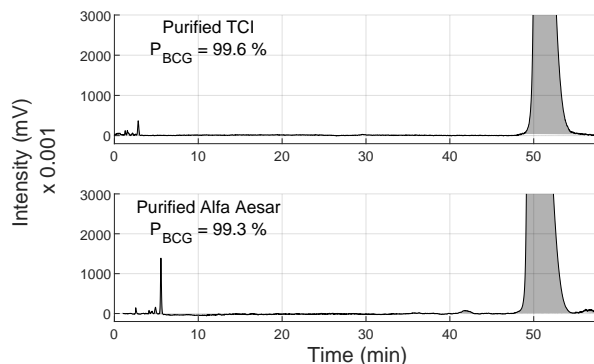


Figure 3. Analytical HPLC chromatograms of purified BCG from TCI, and Alfa Aesar with their P_{BCG} . All peaks are highlighted with gray background color.

3.2 Total alkalinity measurements

3.2.1 Long-term measurements

During previous studies with the CONTROS HydroFIA[®] TA analyzer, we found that a linear drift to higher A_{T} values appears to be the typical behavior of the system (Seelmann et al., 2019). We also found out that the drift is caused by deposits in the optical pathway. As a result, the light intensity decreases and therefore the absorbances at 444 and 616 nm (wavelengths where the acid and base form of BCG have their absorbance maxima) changes in a certain ratio so that the A_{T} values increases per measurement. In the present study we wanted to examine the impact of BCG impurities and the usage of purified BCG, respectively, on the drift behavior of the system.

In order to evaluate the drift of the system supposedly caused by impurities of the BCG indicator dye, the bias between the measured A_{T} value and the reference A_{T} value of the CRM ($\Delta A_{\text{T}} = A_{\text{T,measured}} - A_{\text{T,reference}}$) was plotted vs. the measurement counter. Figure 4 shows the results for A_{T} measurements with purified and unpurified TCI BCG, as well as unpurified BCG from Alfa Aesar and Acros Organics. Measurements with purified and unpurified TCI BCG resulted in a linear drift to higher values with the regression equation $y = (0.0193 \pm 0.0009) \times x + (-0.18 \pm 0.16)$, and $y = (0.0317 \pm 0.0004) \times x + (-0.16 \pm 0.10)$, respectively. However, unpurified Acros Organics and Alfa Aesar BCG showed a non-linear drift to higher values. All A_{T} measurements took the analyzers relative uncertainty determined with 0.08 % (Seelmann et al., 2019) into account. However, Fig. 4 does not show these uncertainties as they are too small for the scaling of the y-axis.

One important outcome of this experiment is, that the magnitude and shape of the drift directly depends on the purity of the used BCG. The drift caused by purified TCI BCG is reduced by $0.0124 \mu\text{mol kg}^{-1}$ per measurement with respect to unpurified TCI BCG. This indicates that the drift of the system must be primarily caused by impurities of the BCG indicator and not by the indicator itself as hypothesized in our previous study (Seelmann et al., 2019). However, there is still a remaining small drift component even with the most pure TCI BCG. Hence, BCG purification appears to significantly reduce but not completely

eliminate the observed system drift. However, a flush with ethanol or isopropyl alcohol removes any impurity deposits in the optical pathway caused by the indicator dye and, therefore, ~~reset-resets~~ the drift. The frequency of these cleanings during long-term deployments can be reduced by using ~~pure dye-purer dye~~. But finally, the user of the CONTROS HydroFIA® TA analyzer decides the cleaning interval as its frequency depends on the certain application of the system and how often measurements are conducted. Furthermore, there is a dependency on the measured water matrix as well, e.g. high turbidity coastal water requires more often cleanings than open ocean water. We can only make recommendations based on our experiences with the analyzer. During our field deployments of the analyzer (not part of this study), we ran a cleaning procedure using ethanol right before a new "calibration" of the system with CRM. As our analyzer measured around 1000 A_T values per month, we carried out an ethanol flush with a subsequent calibration on monthly bases.

Another important outcome is that the shape of the drift differed with the amount of impurities. Below a certain purity grade (between 93.2 % and 98.1 %), the drift behavior appears to change from linear to non-linear. However, for unattended long-term installations of the CONTROS HydroFIA® TA analyzer it is highly preferable to have a linear drift. Under this condition, the correction during the post-processing of the data is easier and the necessary reference measurements can be reduced to a pre- and post-deployment measurement. Furthermore, the upper limit of the analyzer's working range will be reached faster with a non-linear increase of the A_T values per measurement. Hence, there is the risk, that the measured A_T values are rendered useless towards the end of a long-term deployment.

Due to the nearly similar drift behavior of Acros Organics and Alfa Aesar BCG, we also hypothesize that the observed non-linear behavior was mainly caused by the impurity with the retention time around 58 min, which is only present in BCG from Acros Organics, Carl Roth, and Alfa Aesar. Additional tests with the Carl Roth indicator supported the hypothesis (results not shown). This certain impurity might be a molecule with a higher adsorption tendency to the glass wall of the cuvette compared to other impurities. ~~The more often the analyzer measures, the higher is the resulting increase of the A_T values. As a consequence, if the~~ If the used indicator dye contains this impurity ~~type~~, the magnitude and shape of the drift ~~does not only depend on the relative purity grade but also on its character.~~ is mainly driven by the presence of this molecule than by the BCG purity itself. As a consequence, the usage of BCG indicators containing this impurity should be avoided especially during long-term deployments.

Additional to the impacts on the drift, we also experienced, that the frequency of system cleanings has to be increased when using BCG with low purity. For unattended long-term deployments, this must be taken into account.

3.2.2 Standard addition experiment

For better presentation, the results and discussions for the various BCG types are divided into two groups: 1) For "high-purity" BCG ($P_{BCG} > 98\%$), and 2) "low-purity" BCG ($P_{BCG} < 94\%$). At the time of this study, we cannot say anything about the behavior of BCG with $98\% > P_{BCG} > 94\%$, because none of the tested dyes fall in this range.

The results of the standard addition experiment carried out with purified and unpurified TCI BCG ("high-purity" BCG) are shown in Fig. 5. By plotting $A_{T,measured}$ vs. $A_{T,titrated}$, purified and unpurified TCI BCG show a linear equation of $y = (0.996 \pm 0.013) \times x + (11 \pm 29)$, and $y = (0.997 \pm 0.012) \times x + (7 \pm 26)$, respectively. Both correlations satisfy the quality

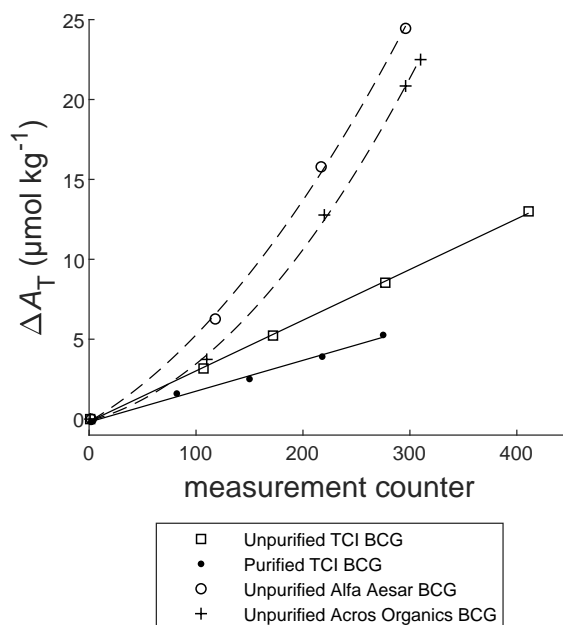


Figure 4. Bias (ΔA_T) between measured A_T and reference value of the CRM as a function of the measurement counter of the CONTROS HydroFIA[®] TA analyzer, where filled circles, open squares, crosses, and open circles represent the average of five repeated measurements made with purified BCG (TCI), unpurified TCI BCG, unpurified Acros Organics BCG, and unpurified Alfa Aesar BCG, respectively. The solid lines are the linear regressions of the associated measurement points. The dashed lines represent a non-linear regression.

requirements (slope = 1, intercept = 0) within their uncertainty, and they were statistically indistinguishable. Hence, the sensitivity and linearity of these measurements are considered satisfactory. The evaluation of precision and accuracy, which is summarized in Table 4 revealed no significant differences between measurements. Furthermore, both accuracies were in full agreement with previous laboratory performance characterizations of the system (Seelmann et al., 2019) (Seelmann et al. (2019): $\pm 1.0 \mu\text{mol kg}^{-1}$) and with the requirements of Dickson et al. (2007) for standard open-cell A_T titrators for which an accuracy of $\pm 2 \mu\text{mol kg}^{-1}$ is required. However, the requirement for precision (standard open-cell titrator: $\pm 1 \mu\text{mol kg}^{-1}$) were not fully achieved, but both results are entirely comparable to our previous laboratory performance characteristic (Seelmann et al., 2019) (Seelmann et al. (2019): $\pm 1.5 \mu\text{mol kg}^{-1}$). Consequently, above a relative purity grade of 98 % no negative influence of indicator impurities on the measurement performance of the analyzer could be identified.

In contrast, "low-purity" indicators behaved totally different. The results of the standard addition experiment carried out with unpurified BCG from Acros Organics and Alfa Aesar are shown in Fig. 5 with their linear equations of $y = (1.097 \pm 0.013) \times x + (-228 \pm 29)$, and $y = (1.147 \pm 0.036) \times x + (-352 \pm 82)$, respectively. Clearly, these correlations were not satisfactory, and statistically different to the correlation of purified BCG. Hence, these "low-purity" dyes do not show the sensi-

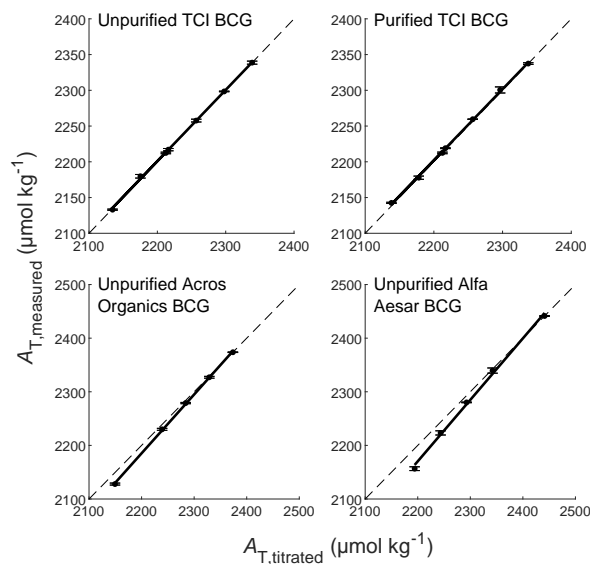


Figure 5. $A_{T,measured}$ as a function of $A_{T,titrated}$ of each titration step measured with purified and unpurified TCI BCG as well as unpurified BCG from Acros Organics and Alfa Aesar. The black filled circles represent the average of five repeated measurements for each sample with their standard deviations (σ) as errorbar. The black solid lines indicate the linear fit of the data points. The black dashed lines indicate the 1-to-1 line of these plots.

250 tivity and linearity behavior that is required for most accurate measurements with the analyzer. Furthermore, the evaluation of precision and accuracy (Table 4) revealed shows that measurements with Alfa Aesar BCG cannot meet the quality requirements whereas Acros Organics BCG still falls ($P_{BCG} = 93.2\%$) still fell within acceptable ranges regarding precision and accuracy requirements. However, measurements using Alfa Aesar BCG ($P_{BCG} = 85.4\%$) did not meet the quality requirements.

Summing up, we can state that the uncertainty of A_T measurements only deteriorates significantly for a BCG purity grade below 94 %. Indicator dyes with $P_{BCG} > 98\%$ provide A_T measurements with a quality comparable to these measured with purified BCG. These findings partially support other studies dealing with different purified pH indicators for spectrophotometric pH (Yao et al., 2007; Liu et al., 2011; DeGrandpre et al., 2014; Lai et al., 2016) and A_T measurements (Nand and Ellwood, 2018). There, indicator purification always led to an improvement in measurement precision. Under the scope of this study, we proved that purification of BCG is not necessary to improve the quality of the A_T measurements with the CONTROS
 260 HydroFIA[®] TA analyzer as long as the used BCG is purer than 98 %. The reason for the deviation to other studies lies in the measuring principle of the system. Before starting measurements with newly prepared solutions, it is obligatory to "calibrate" the system by measuring a CRM. During this "calibration", the exact volume of the analyzers internal seawater sample loop, V_{SW} is determined being the only unknown variable within this method. Hence, all inevitable uncertainties (including impurities of the indicator) are combined in V_{SW} and thereby taken into account for subsequent A_T measurements.

Table 4. Precision and accuracy of unpurified and purified BCG

	"High-purity" BCG:		"Low-purity" BCG:		<u>Requirements for standard open-cell titrators^a</u>	<u>Typical performance of the analyzer^b</u>
	unpurified TCI	purified TCI	unpurified Acros Organics	unpurified Alfa Aesar		
Precision σ ($\mu\text{mol kg}^{-1}$)	± 1.6	± 1.5	± 1.4	± 2.7	<u>± 1</u>	<u>± 1.5</u>
Accuracy RMSE ($\mu\text{mol kg}^{-1}$)	± 1.2	± 1.1	± 1.7	± 5.3	<u>± 2</u>	<u>± 1.0</u>

^aDickson et al. (2007); ^bSeelmann et al. (2019)

265 The present results prove that this "calibration" procedure is able to compensate any influences of indicator impurities on the precision and accuracy up to an impurity level of 2 %. Consequently, the usage of "low-purity" BCG is not recommended.

~~$A_{T,\text{measured}}$ as a function of $A_{T,\text{titrated}}$ of each titration step measured with purified and unpurified TCI BCG as well as unpurified BCG from Acros Organics and Alfa Aesar. The black filled circles represent the average of five repeated measurements for each sample with their standard deviations (σ) as errorbar. The black solid lines indicate the linear fit of the data points. The black dashed lines indicate the 1-to-1 line of these plots.~~

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4 Cost-benefit analysis

4.1 Measurements with purified vs. unpurified BCG

5 **Cost-benefit analysis for BCG purification**

This study proves that an HPLC purification of BCG is entirely feasible. But is the purification of BCG worths the efforts and costs involved? To answer this question we compare the costs incurred and the benefits gained for A_T measurements with the CONTROS HydroFIA[®] TA analyzer. Due to the relatively long HPLC run time of 60 min and a flow rate of 31.2 mL min⁻¹, the purification method needs about 1.5 L of ACN per run (including pre- and post-flushes). To carry out the long-term and standard addition experiment for this study (around 500 measurements), approximately 144 mg of purified BCG were needed. Hence, with a yield of around 50 mg pure BCG per purification run, a minimum of three injections was necessary. However, for long-term measurement campaigns with the analyzer, the typical volume of BCG solution is 500 mL, which is sufficient for at least 2300 measurements. This would need 700 mg of purified BCG, which corresponds to a minimum of 14 purification runs and 21 L of ACN. ACN with HPLC grade is a relatively expensive chemical, and it must be probably disposed. This causes additional costs. Furthermore, the whole purification process takes about a full working day per run.

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Rough calculations on the actual costs per measurement with the CONTROS HydroFIA[®] TA analyzer revealed that indicator purification would approximately double measurement costs. The calculation for measurements with unpurified indicator are

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based on ready-to-use 500 mL cartridges for both chemicals (HCl and BCG) ordered from Kongsberg Maritime Contros GmbH without any preparation effort for the user.

To overcome this high increase in measurement costs, there could be the possibility to develop a FC-flash chromatography (FC) purification method for BCG as described for mCP by Patsavas et al. (2013a) to increase the yield of purified dye per run. According to the method description in this publication (Patsavas et al., 2013a), the solvent consumption of both methods (FC and HPLC) per purification run is approximately the same. Provided the FC method would increase the yield 3.5 times as it was described for mCP, only 4 injections will be necessary to produce enough purified BCG for a long-term deployment with at least 2300 measurements. The estimated measurement costs for such a FC method would be approximately a third of these for measurements with-using BCG purified by HPLC. Hence, if BCG purification would be necessary, the FC method would be the more cost-effective choice. However, it has to be taken into account that the calculations for these measurement costs (especially for the FC method) are just theoretically estimated and may differ from reality depending on availability of resources and equipment. Furthermore, a FC purification method for BCG is so far not developed and validated, which means additional costs and working time.

Finally, if we compare the purified BCG with "high-purity" BCG like from TCI, the only benefit gained from the purification is a reduced drift per A_T measurement. There is-However, as long as the drift pattern is linear, its actual slope is irrelevant as it can be easily corrected by regular reference measurements. Furthermore, there is no improvement in the measurement quality (precision and accuracy) as long as the impurity level is 2 % or below. Since the drift behavior can-not-be-cannot fully overcome, it seems not worth the effort to purify BCG for A_T measurements with the CONTROS HydroFIA® TA analyzer.

However, the types and quantities of impurities can have a strong influence on measurement quality in unattended long-term applications of the system as it was shown before (e.g. change of the drift behavior, non fulfillment of the quality requirements). Hence, the purity of the used BCG is not unimportant at all. To achieve the best long-term measurement experience with the analyzer it is not necessary to use purified BCG, as the purest available indicator (e.g. BCG from TCI) generate fully satisfying quality results. Users of the CONTROS HydroFIA® TA should take the consequences of indicator impurities into account when choosing their BCG supplier. From this perspective, it would be beneficial to invest into higher purity indicator avoiding the issues described above. If applicable, an HPLC analysis of the used indicator following the here described analytical method can show any types and quantities of impurities. However, if there is no HPLC available, long-term laboratory measurements as described here can help to evaluate whether the purchased indicator is suitable or not by evaluating the drift behavior. As there could be batch to batch variability in purity, the drift pattern should be also assessed for each batch of BCG provided by the same supplier.

315 **4.1 BCG characterization**

Most of the studies dealing with purification of indicator dyes for spectrophotometric seawater pH measurements conducted a subsequent characterization of the purified indicator (e.g. Liu et al., 2015; Patsavas et al., 2013b; Nand and Ellwood, 2018). Due to impurity impacts, coefficients and constants of purified indicators may be different to these of unpurified dyes. During our work with purified BCG, we decided to forgo of an indicator characterization. There were two reasons for this decision:

- 320 1. Li et al. (2013) investigated the impact of different BCG characteristics found in the literature on spectrophotometric A_T measurements and concluded that the influences are insignificant also with regard to possible impurities. They justified this conclusion with the calibration of the system using CRM. The CONTROS HydroFIA[®] TA analyzer follows a similar measurement principle as the analyzer described by Li et al. (2013) and also conducted a calibration routine. Therefore, any uncertainties regarding the coefficients are taken into account for subsequent measurements.
- 325 2. The measurement quality using both purified and unpurified "high-purity" BCG were fully satisfying and met the quality requirements for A_T measurements. Furthermore, both uncertainties did not significantly differ from each other.

Finally, we concluded that a characterization of purified BCG would not improve the measurement quality at all and therefore decided to not conduct it.

5 Conclusions

330 In this study, we successfully developed an HPLC purification method for BCG and subsequently tested the impact of using the purified and unpurified dye on measurements with a novel autonomous analyzer for seawater A_T , the CONTROS HydroFIA[®] TA.

HPLC analyses revealed that types and quantities of the impurities differed for each vendor. We tested indicator dyes from four different vendors with a resulting BCG purity ranging from 85.4 % to 98.1 %. After HPLC purification, the purity was
335 improved to between 99.3 % and 99.6 %, depending on BCG vendor.

Long-term measurements with the total alkalinity analyzer in the laboratory revealed that the systems drift behavior was strongly related to the purity of the used indicator and the type of containing impurities. The purer the BCG, the smaller was the A_T drift per measurement. However, the drift behavior cannot be overcome by purifying the used indicator dye. Furthermore, BCG containing a certain impurity type with a retention time of around 58 min showed a non-linear drift behavior, which can
340 cause several problems during long-term measurement campaigns by e.g. exceeding the upper working range limit, difficult data correction during post-processing, higher cleaning frequency or total loss of measuring capacity during long campaigns. Therefore, indicators containing this impurity should not be used for long-term deployments of the analyzer.

Laboratory performance characterization experiments with purified and unpurified BCG that only contains up to 2 % impurities revealed no significant influence of the purification on either linearity and sensitivity or the measurement quality (precision
345 and accuracy) of the system. The obligatory "calibration" routine of the analyzer after each change of indicator dye solution was sufficient to compensate any impurity effects. If the dye contains more than 6 % impurities, the measurements do not fulfill the linearity and sensitivity requirements. With even higher amounts of impurities, the analyzer cannot meet the quality requirements as they are demanded within the oceanographic communities (Dickson et al., 2007; Seelmann et al., 2019).

Taking all these results into account, a cost-benefit analysis revealed that a purification of BCG is not strictly recommended
350 to carry out high-quality measurements with the used analyzer. But the usage of "high-purity" BCG (e.g. from TCI) is highly recommended to avoid the previously identified issues. Furthermore, no characterization of the purified BCG was carried out.

Author contributions. KS: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization; MG: Methodology, Resources, Writing - Review & Editing; SA: Conceptualization, Methodology, Writing - Review & Editing; AK: Conceptualization, Resources, Writing - Review & Editing, Supervision, Funding acquisition

355 *Competing interests.* At the time of this study the co-author S. Aßmann was an employee of Kongsberg Maritime Contros GmbH (Kiel, Germany), which commercialized the used analyzer. However, its measurement principle follows conceptual design and dedicated studies performed during his doctorate in our working group. Apart from this there are no conflicts of interests to declare.

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360 **References**

- Aßmann, S., Frank, C., and Körtzinger, A.: Spectrophotometric high-precision seawater pH determination for use in underway measuring systems, *Ocean Sci.*, 7, 597–607, <https://doi.org/10.5194/os-7-597-2011>, 2011.
- Bellerby, R. G., Olsen, A., Johannessen, T., and Croot, P.: A high precision spectrophotometric method for on-line shipboard seawater pH measurements: the automated marine pH sensor (AMpS), *Talanta*, 56, 61–69, [https://doi.org/10.1016/S0039-9140\(01\)00541-0](https://doi.org/10.1016/S0039-9140(01)00541-0), 2002.
- 365 Breland, J. A. and Byrne, R. H.: Spectrophotometric procedures for determination of sea water alkalinity using bromocresol green, *Deep Sea Res. Part I Oceanogr. Res. Pap.*, 40, 629–641, [https://doi.org/10.1016/0967-0637\(93\)90149-W](https://doi.org/10.1016/0967-0637(93)90149-W), 1993.
- Byrne, R. H.: Standardization of standard buffers by visible spectrometry, *Anal. Chem.*, 59, 1479–1481, <https://doi.org/10.1021/ac00137a025>, 1987.
- Byrne, R. H. and Breland, J. A.: High precision multiwavelength pH determinations in seawater using cresol red, *Deep Sea Res. Part A. Oceanogr. Res. Pap.*, 36, 803–810, [https://doi.org/10.1016/0198-0149\(89\)90152-0](https://doi.org/10.1016/0198-0149(89)90152-0), 1989.
- 370 Clayton, T. D. and Byrne, R. H.: Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results, *Deep Sea Res. Part I Oceanogr. Res. Pap.*, 40, 2115–2129, [https://doi.org/10.1016/0967-0637\(93\)90048-8](https://doi.org/10.1016/0967-0637(93)90048-8), 1993.
- DeGrandpre, M. D., Spaulding, R. S., Newton, J. O., Jaqueth, E. J., Hamblock, S. E., Umansky, A. A., and Harris, K. E.: Considerations for the measurement of spectrophotometric pH for ocean acidification and other studies, *Limnol. Oceanogr. Methods*, 12, 830–839, <https://doi.org/10.4319/lom.2014.12.830>, 2014.
- 375 Dickson, A. G., Sabine, C. L., and Christian, J. R.: Guide to best practices for ocean CO₂ measurements, PICES Special Publications 3, 2007.
- Douglas, N. K. and Byrne, R. H.: Achieving accurate spectrophotometric pH measurements using unpurified meta-cresol purple, *Mar. Chem.*, 190, 66–72, <https://doi.org/10.1016/j.marchem.2017.02.004>, 2017.
- 380 King, D. and Kester, D. R.: Determination of seawater pH from 1.5 to 8.5 using colorimetric indicators, *Mar. Chem.*, 26, 5–20, [https://doi.org/10.1016/0304-4203\(89\)90061-3](https://doi.org/10.1016/0304-4203(89)90061-3), 1989.
- Lai, C.-Z., DeGrandpre, M. D., Wasser, B. D., Brandon, T. A., Clucas, D. S., Jaqueth, E. J., Benson, Z. D., Beatty, C. M., and Spaulding, R. S.: Spectrophotometric measurement of freshwater pH with purified meta-cresol purple and phenol red, *Limnol. Oceanogr. Methods*, 14, 864–873, <https://doi.org/10.1002/lom3.10137>, 2016.
- 385 Li, Q., Wang, F., Wang, Z. A., Yuan, D., Dai, M., Chen, J., Dai, J., and Hoering, K. A.: Automated Spectrophotometric Analyzer for Rapid Single-Point Titration of Seawater Total Alkalinity, *Environ. Sci. Technol.*, 47, 11 139–11 146, <https://doi.org/10.1021/es402421a>, 2013.
- Liu, X., Patsavas, M. C., and Byrne, R. H.: Purification and characterization of meta-cresol purple for spectrophotometric seawater pH measurements, *Environ. Sci. Technol.*, 45, 4862–4868, <https://doi.org/10.1021/es200665d>, 2011.
- 390 [Liu, X., Byrne, R. H., Lindemuth, M., Easley, R., and Mathis, J. T.: An automated procedure for laboratory and shipboard spectrophotometric measurements of seawater alkalinity: Continuously monitored single-step acid additions, *Mar. Chem.*, 174, 141–146, <https://doi.org/10.1016/J.MARCHEM.2015.06.008>, 2015.](https://doi.org/10.1016/J.MARCHEM.2015.06.008)
- Millero, F. J.: The Marine Inorganic Carbon Cycle, *Chem. Rev.*, 107, 308–341, <https://doi.org/10.1021/cr0503557>, 2007.
- Nand, V. and Ellwood, M. J.: A simple colorimetric method for determining seawater alkalinity using bromophenol blue, *Limnol. Oceanogr. Methods*, 16, 401–410, <https://doi.org/10.1002/lom3.10253>, 2018.
- 395

- Patsavas, M. C., Byrne, R. H., and Liu, X.: Purification of meta-cresol purple and cresol red by flash chromatography: Procedures for ensuring accurate spectrophotometric seawater pH measurements, *Mar. Chem.*, 150, 19–24, <https://doi.org/10.1016/j.marchem.2013.01.004>, ~~2013~~, [2013a](https://doi.org/10.1016/j.marchem.2013.01.004).
- [Patsavas, M. C., Byrne, R. H., and Liu, X.: Physical–chemical characterization of purified cresol red for spectrophotometric pH measurements in seawater, *Mar. Chem.*, 155, 158–164, <https://doi.org/10.1016/j.marchem.2013.06.007>, 2013b.](https://doi.org/10.1016/j.marchem.2013.06.007)
- 400 Robert-Baldo, G. L., Morris, M. J., and Byrne, R. H.: Spectrophotometric determination of seawater pH using phenol red, *Anal. Chem.*, 57, 2564–2567, <https://doi.org/10.1021/ac00290a030>, 1985.
- Seelmann, K., Aßmann, S., and Körtzinger, A.: Characterization of a novel autonomous analyzer for seawater total alkalinity: Results from laboratory and field tests, *Limnol. Oceanogr. Methods*, 17, 515–532, <https://doi.org/10.1002/lom3.10329>, 2019.
- 405 Tapp, M., Hunter, K., Currie, K., and Mackaskill, B.: Apparatus for continuous-flow underway spectrophotometric measurement of surface water pH, *Mar. Chem.*, 72, 193–202, [https://doi.org/10.1016/S0304-4203\(00\)00081-5](https://doi.org/10.1016/S0304-4203(00)00081-5), 2000.
- Yao, W. and Byrne, R. H.: Simplified seawater alkalinity analysis: Use of linear array spectrometers, *Deep Sea Res., Part I*, 45, 1383–1392, [https://doi.org/10.1016/S0967-0637\(98\)00018-1](https://doi.org/10.1016/S0967-0637(98)00018-1), 1998.
- 410 Yao, W., Liu, X., and Byrne, R. H.: Impurities in indicators used for spectrophotometric seawater pH measurements: Assessment and remedies, *Mar. Chem.*, 107, 167–172, <https://doi.org/10.1016/j.marchem.2007.06.012>, 2007.