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

Katharina Seelmann[1], Martha Gledhill[1], Steffen Aßmann[2], and Arne Körtzinger[1,3]

[1]GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany
[2]Kongsberg Maritime Contros GmbH, Kiel, Germany
[3]Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Correspondence: Katharina Seelmann (kseelmann@geomar.de)

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 impurities in the pH indicator dye bromocresol green (BCG) on spectrophotometric seawater total alkalinity (A_T) measurements. First, a high-performance liquid chromatog-
5   raphy (HPLC) purification method for BCG was developed. 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 per measurement. Furthermore, we could show that a certain impurity
10   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 of the system. 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, an
15   indicator purification is not necessarily required as long as the purchased dye has a purity level of at least 98 %.

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), pCO_2, and total dissolved inorganic carbon (C_T). Due
20   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.

25 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,

30 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 contribute to pH offsets of up to 0.01 pH units. To overcome the uncertainties caused by indicator impurities, Liu et al. (2011)

35 developed a preparative high-performance liquid chromatography (HPLC) method to purify mCP and characterized this purified dye. Furthermore, to produce large batches of purified mCP, Patsavas et al. (2013) developed a flash chromatography (FC) method resulting in a 3.5 times increased yield per run. However, not all users of spectrophotometric seawater pH measurement 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.

40 In order to apply these findings to spectrophotometric A_T measurements, Nand and Ellwood (2018) describe 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 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

45 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.

50 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.
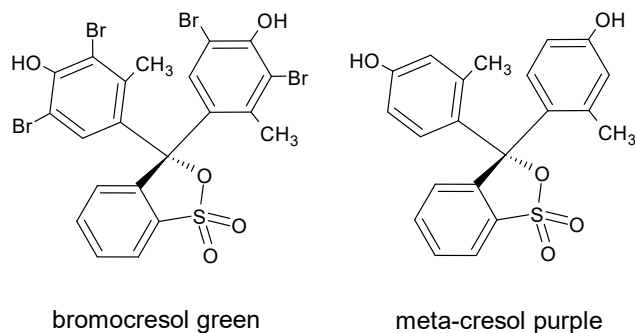
Figure 1. Chemical structure of bromocresol green and meta-cresol purple

2 Materials and methods

2.1 HPLC method

2.1.1 Reagents and instrumentation

55 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 (H_2O), 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

60 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

65 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 $10\ \mathrm{mg\,mL^{-1}}$ BCG solution of each vendor was prepared in the

70 mobile phase and 20 μL were injected. One HPLC run with a flow-rate of $1.5\ \mathrm{mL\,min^{-1}}$ took $60\ \mathrm{min}$ and was monitored using the UV-VIS detector at $280\ \mathrm{nm}$. 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:H$_2$O (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
75 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
80 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:

$$P_{\mathrm{BCG}} = \frac{A_{\mathrm{BCG}}}{\sum_{\mathrm{i}=1}^{n} A_{\mathrm{i}}} \times 100\% \tag{1}$$

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$^{\mathrm{th}}$ peak.

2.1.4 Purification of BCG

85 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:H$_2$O: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
90 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

95 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 (Breland 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. Certified reference material, CRM, (batch 160, $A_{\mathrm{T,reference}}$ = 2212.44 ± 0.67 µmol kg^{-1})
100 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

105

For the long-term measurements, $0.002 \, \mathrm{mol\,kg^{-1}}$ 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 prepared seawater sample (≈ 25 L) was measured more than 300 times with a measuring interval of $15 \, \mathrm{min}$, 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.

110

2.2.3 Standard addition experiment

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 ($0.1 \, \mathrm{mol\,kg^{-1}}$) 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 theoretical A_T ($A_\mathrm{T,titrated}$) 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 ($A_\mathrm{T,measured}$), each of these samples was repeatedly measured with the analyzer ($n = 5$).

115

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 $A_\mathrm{T,measured}$ vs. $A_\mathrm{T,titrated}$ gave us information about the measuring accuracy. It was calculated by

120

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

where n is the number of samples, $A_\mathrm{T,fitted,i}$ is the ith A_T value calculated with the linear regression equation with $A_\mathrm{T,titrated,i}$ as x variable, where $A_\mathrm{T,measured}$ 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 validation procedure of the analyzer, these terms must fulfill within their uncertainties the following requirements: Slope = 1; intercept = 0.

125

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]

[a] Within 60 min run time

[b] Impurities found in subsequent blank run

3 Results and discussion

3.1 HPLC separation and purification of BCG

3.1.1 Method development

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:H$_2$O: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.

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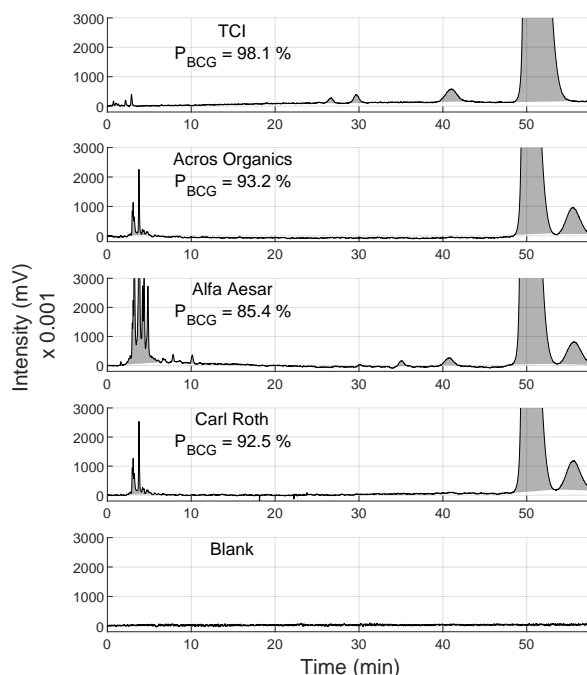
Figure 2. Analytical HPLC chromatograms of unpurified BCG from different vendors with their P_{BCG} and a chromatogram from a solvent injection without BCG (Blank). All peaks are highlighted with gray background color.

Table 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

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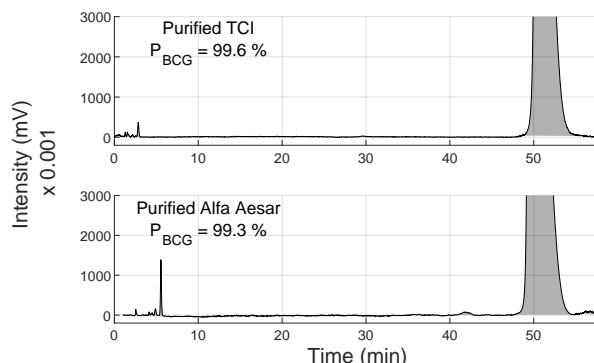
Figure 3. Analytical HPLC chromatograms of purified BCG from TCI, and Alfa Aesar with their P_{BCG}. All peaks are highlighted with gray background color.

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

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. 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.

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.

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 values appears to be the typical behavior of the system (Seelmann et al., 2019). 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 ($\Delta A_T = A_{T,measured} - A_{T,reference}$) 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

170   drift to higher values.

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 $0.0124\,\mu\mathrm{mol\,kg}^{-1}$ 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

175   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 the drift. The frequency of these cleanings during long-term deployments can be reduced by using pure dye.

Another important outcome is that the shape of the drift differed with the amount of impurities. Below a certain purity grade

180   (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

185   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

190   the resulting increase of the A_T values. As a consequence, if the used indicator dye contains this impurity, the magnitude and shape of the drift does not only depend on the relative purity grade but also on its character.

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

195   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
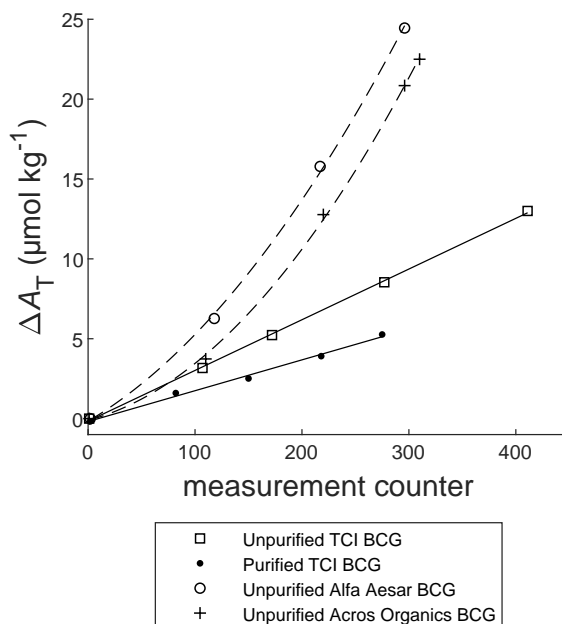
Figure 4. Bias (ΔA_T) between measured A_T and reference value of the CRM as a function of the measurement counter of the CONTROS HydroFIA® TA analyzer, where filled circles, open squares, crosses, and open circles represent the average of five repeated measurements made with purified BCG (TCI), unpurified 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.

200   $y = (0.996 \pm 0.013) \times x + (11 \pm 29)$, and $y = (0.997 \pm 0.012) \times x + (7 \pm 26)$, respectively. Both correlations satisfy the quality 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) and with the re-

205   quirements of Dickson et al. (2007) for standard open-cell A_T titrators for which an accuracy of ± 2 μmol kg^{-1} is required. However, the requirement for precision (standard open-cell titrator: ± 1 μmol kg^{-1}) were not fully achieved, but both results are entirely comparable to our previous laboratory performance characteristic (Seelmann et al., 2019). Consequently, above a relative purity grade of 98 % no negative influence of indicator impurities on the measurement performance of the analyzer could be identified.

210   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,

Table 4. Precision and accuracy of unpurified and purified BCG

	"High-purity" BCG:		"Low-purity" BCG:	
	unpurified	purified	unpurified	unpurified
	TCI	TCI	Acros Organics	Alfa Aesar
Precision σ ($\mu mol\,kg^{-1}$)	$\pm\,1.6$	$\pm\,1.5$	$\pm\,1.4$	$\pm\,2.7$
Accuracy RMSE ($\mu mol\,kg^{-1}$)	$\pm\,1.2$	$\pm\,1.1$	$\pm\,1.7$	$\pm\,5.3$

and statistically different to the correlation of purified BCG. Hence, these "low-purity" dyes do not show the sensitivity and linearity behavior that is required for most accurate measurements with the analyzer. Furthermore, the evaluation of precision and accuracy (Table 4) revealed that measurements with Alfa Aesar BCG cannot meet the quality requirements whereas Acros Organics BCG still falls within acceptable ranges.

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 $P_\mathrm{BCG} > 98$ % 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 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. 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 %.

4 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.
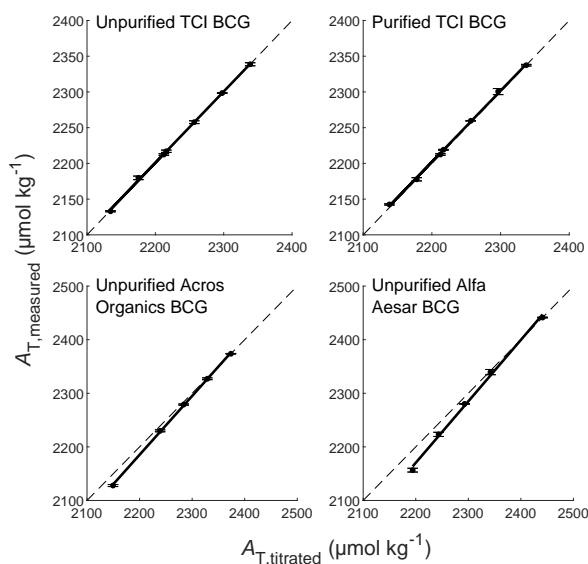
Figure 5. $A_{\mathrm{T,measured}}$ as a function of $A_{\mathrm{T,titrated}}$ of each titration step measured with purified and unpurified TCI BCG as well as unpurified BCG from Acros Organics and Alfa Aesar. The black filled circles represent the average of five repeated measurements for each sample 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.

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

240 additional costs. Furthermore, the whole purification process takes about a full working day per run.

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 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.

245 To overcome this high increase in measurement costs, there could be the possibility to develop a FC purification method for BCG as described for mCP by Patsavas et al. (2013) to increase the yield of purified dye per run. According to the method description in this publication (Patsavas et al., 2013), 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.

250 The estimated measurement costs for such a FC method would be approximately a third of these for measurements with 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.

255   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 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 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.

260   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

265   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.

5   Conclusions

In this study, we successfully developed an HPLC purification method for BCG and subsequently tested the impact of using the

270   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 improved to between 99.3 % and 99.6 %, depending on BCG vendor.

275   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. Furthermore, BCG containing a certain impurity with a retention time of around 58 min showed a non-linear drift behavior, which can 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

280   capacity during long campaigns.

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 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
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.

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

Competing interests. The co-author S. Aßmann is an employee of Kongsberg Maritime Contros GmbH (Kiel, Germany), which commercializes the analyzer used in this study. 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.

Acknowledgements. This work was supported by the European Union's Horizon 2020 Research and Innovation Programme (AtlantOS) (grant number: 633211).

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, 2002.

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, 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, 1989.

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,
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.

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.

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, 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.

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.

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.

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.

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.

335 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.

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, 2000.

Yao, W. and Byrne, R. H.: Simplified seawater alkalinity analysis: Use of linear array spectrometers, Deep Sea Res., Part I, 45, 1383–1392,

340 https://doi.org/10.1016/S0967-0637(98)00018-1, 1998.

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.