LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 10, 2012, 832–839

Reversed phase high performance liquid chromatographic determination of dissolved aluminium in open ocean seawater

Tomas Remenyi[1,2,3], Pavel Nesterenko[1], Andrew Bowie[2,3], Edward Butler[1], and Paul Haddad[1]*

[1]University of Tasmania, Australian Centre for Research of Separation Science, [2]University of Tasmania, Antarctic Climate and Ecosystems CRC

[3]University of Tasmania, Institute of Marine and Antarctic Studies

Abstract

Popular shipboard techniques to determine dissolved aluminium in open ocean seawater have two major disadvantages: relative to HPLC techniques, they require large sample volumes; and, in practice they are are only semi-automated. To address these two issues, the application of a reversed-phase high performance liquid chromatographic (RP-HPLC) method for the determination of the aluminium in open ocean seawater was developed. With this method, dissolved aluminium in the sample was complexed with lumogallion at pH 5.5 by the addition of a reagent, producing the fluorescent aluminium–lumogallion complex. This complex was concentrated onto a Chromolith® RP-18e Guard (10 × 4.6 mm) column and eluted with 95% methanol into a fluorescence detector (λ_{ex} = 505 nm, λ_{em} = 574 nm). Sample pre-treatment required 10 h (>100 samples can be prepared simultaneously) and sample analysis required 375 μL of sample per vial analysed, with 10 μL and 2.7 min per injection. Total required sample volume (including rinses) was 1.5 mL. Limit of detection was 0.13±0.05 nM, with precision of 2.7% at 1 nM on SAFe reference samples. Agreement with SAFe reference samples was within 4.6±4.6% (n = 13). The technique is robust, inherently automated and could easily be applied shipboard for open ocean analyses. It provides improvements in sample efficiency, operational robustness and automation relative to popular shipboard techniques, especially when applied to samples with dissolved aluminium concentrations that approach the limit of detection. This project was a contribution to the GEOTRACES program.

The international GEOTRACES program has successfully coordinated many of the world's biogeochemical oceanographic research programs, resulting in large increases in the number of samples to be analyzed, as well as the number of analytes regularly targeted per sample. The application of existing chemical analysis methods from various disciplines (e.g., medical, environmental, industrial) to the open ocean environment is of recent interest to the chemical oceanographic community as improvements in analytical efficiencies are sought to match the improvements in sampling technologies and expanded concentration ranges observed (e.g., de Baar et al. 2008; Middag et al. 2011a, 2011b, 2011c, 2012).

Aluminum is a target element of the international GEOTRACES program. It is an element that is readily scavenged and has relatively short residence times in the surface ocean. This makes it useful as a biogeochemical tracer of water masses to indicate the level of interaction the target water mass has had with terrestrial sources of trace nutrients (such as windblown dust depositing into the ocean [Measures and Edmond 1989], although this relationship is limited to surface waters due to sedimentary resuspension [Middag et al. 2009; Moran and Moore 1991]). Aluminum is ≈8% of the earth's crust (Taylor 1964), yet is present in natural waters at trace levels, with reported values ranging from ≈2.5 μM in rivers (Upadhyay et al. 2002) to < 1 nM in the open ocean (Measures and Edmond 1990; Middag 2010; Orians and Bruland 1985). The extremely low levels in the open ocean are due to the very low solubility of aluminum in the highly complex seawater matrix and geographic isolation from continental sources of alu-

*Corresponding author: E-mail: pavel.nesterenko@utas.edu.au

Acknowledgments

This study was supported by the Australian Centre for Research on Separation Science; Australian Government's Cooperative Research Centre program through the Antarctic Climate & Ecosystems Co-operative Research Centre; the Institute for Marine and Antarctic Studies; IRGS 100097 grant of the University of Tasmania; a joint CSIRO-UTAS PhD scholarship in Quantitative Marine Science (QMS) and a top-up CSIRO PhD stipend (through the Marine and Atmospheric Research division). The authors would like to thank the developers of LATEX, R [R Development Core Team 2010], JabRef [JabRef-Development-Team 2010], and Ocean Data View [Schlitzer 2002].

DOI 10.4319/lom.2012.10.832

minum. As a GEOTRACES target element, the number of samples per oceanographic cast and cruise have dramatically increased since 2005, requiring greater efficiency of analytical methods.

Current popular shipboard techniques are known to be labor intensive, as they are not robust enough to be completely automated and unattended (R. Middag pers. comm.). These techniques also require relatively large sample volumes (mL) relative to HPLC techniques (µL).

To reduce sample volume requirements and improve the capacity for automation, the method presented by Remenyi et al. (2011) was adapted to determine dissolved aluminum at trace levels in seawater. This development provided increased efficiencies in required sample volume, automation, and robustness compared with the most popular techniques currently used.

Methods and procedures

Apparatus

Sample and reagent were stirred using a Fisher Brand Whirlimixer. pH was measured with a labCHEM–pH meter (TPS Pty Ltd.). Sample management was achieved with an Alliance 2690 HPLC system, dwell volume = 1.2 mL. Analyte concentration and separation was achieved on a reversed phase, C_{18}, end-capped Chromolith RP-18e Guard (10 × 4.6 mm) column (Merck KGaA). The aluminum–lumogallion (Al–lumogallion) complex was detected using a scanning fluorescence detector model 474 (Waters), gain = 100, λ_{ex} = 505 nm bandwidth = 18 nm, λ_{em} = 574 nm bandwidth = 18 nm, with control and signal processing achieved using Empower software (Waters).

Sample and reagent bottles were Nalgene low-density polyethylene (LDPE) and analysis vials were Dionex polypropylene (PP). All were cleaned with trace metal protocols described in Cutter et al. (2010).

Reagents

All ultra-high purity water was from a Milli-Q Elix and Gradient coupled system (Millipore).

The solvent was ultra-high purity water and Supragradient HPLC grade methanol (Scharlau Chemie S.A.).

Lumogallion (97%, powdered, Pfaltz & Bauer Inc.) was made up to 5×10^{-3} M in ultra-high purity water (0.0517 g/30 mL).

Solid MES hydrate 99.5%, also known as 2-(N-morpholino)ethanesulfonic acid (Sigma-Aldrich), was made up to 1 M in ultra-high purity water (19.523 g /100 mL, this solution has a pH < 3). It was then purified with respect to transition elements by passing it through a 200×4.6 mm I.D. column packed with Diphonix resin, particle size 75-150 µm (Eichrom Technologies Inc.) at 0.2 mL min^{-1} (Tria et al. 2008). MES solutions at higher concentrations than 1 M created precipitates when stored below 4°C.

The reagent was 2.0×10^{-3} M lumogallion – 0.9 M MES at pH 6.1 ± 0.1. Lumogallion is a ligand that forms a fluorescent complex with aluminum. MES is a buffer, used to adjust the pH of the sample-reagent mixture to optimize complex yield (the target was pH 5.3 [Brown and Bruland 2008; Resing and Measures 1994; Tria et al. 2008]). pH was measured on subsamples so as to not contaminate the working reagent.

Adjustment of pH was done using 16 M SEASTAR Baseline nitric acid, and SEASTAR Baseline ammonia solution (ammonium hydroxide), Seastar Chemicals.

All standards were made up using 1000 $mg.L^{-1}$ (0.371 M) Al^{+3} in 0.5 M nitric acid (Merck KGaA).

All stock solutions were stored in the dark at ≈4°C.

Sample collection and analysis protocols

Samples were collected and analyzed using trace metal clean GEOTRACES protocols at every stage of sampling and analysis (with special emphasis on those specific for aluminum), as suggested in Bowie and Lohan (2009) and Cutter et al. (2010).

Sample analysis

Synthesis of the lumogallion-aluminum complex

The ratio of reagent to sample was 1:3. In practice, this was a 125 µL aliquot of reagent with a 375 µL aliquot of sample/standard. These were added to a 600 µL polypropylene vial, stirred using a Fisher Brand Whirlimixer for 60 s and then loaded into the auto-sampler. The solutions were then left overnight (>10 h) at room temperature, after which time the derivative concentration stabilized, and remained stable for at least 3 d.

Preparation of 100 samples required ≈120 min.

Analysis of the prepared sample

A schematic of the sample analysis system is presented in Fig. 1. Two eluents were applied for sample analysis in a stepped-gradient elution program, visualized as the dashed line in Fig. 2. Eluent A was a 5/95 (v/v) methanol/water mixture, applied to load the analyte onto the column, whereas simultaneously eluting the saline matrix off the column. Eluent B was a 90/10 (v/v) methanol/water mixture, applied to remove the analyte from the column and pass it into the detector. More details of this system can be found in Remenyi et al. (2011).

After complex formation was complete, prepared samples were analyzed. 10 µL of prepared sample was injected onto the column. At a constant flow rate of 2 mL min^{-1}, the stepped-gradient elution program was applied, with a matrix elimination step (Eluent A for 60 s), followed by an isocratic elution step (Eluent B for 60 s), followed by column preparation for the next injection (Eluent A for 30 s). Each vial was analyzed in triplicate. A six-point calibration (0, 1, 2, 4, 8, 16 nM) and twelve-sample station required 202 min (11.2 min per triplicate analysis).

Preliminary work (Remenyi et al. 2011) proved the co-elution of the seawater matrix with the analyte interfered with fluorescence of the Al–lumogallion complex. Although this is not a problem at higher concentrations (see Giesbrecht 2007), we suspected it may be an issue < 1 nM and therefore included the matrix elimination step.

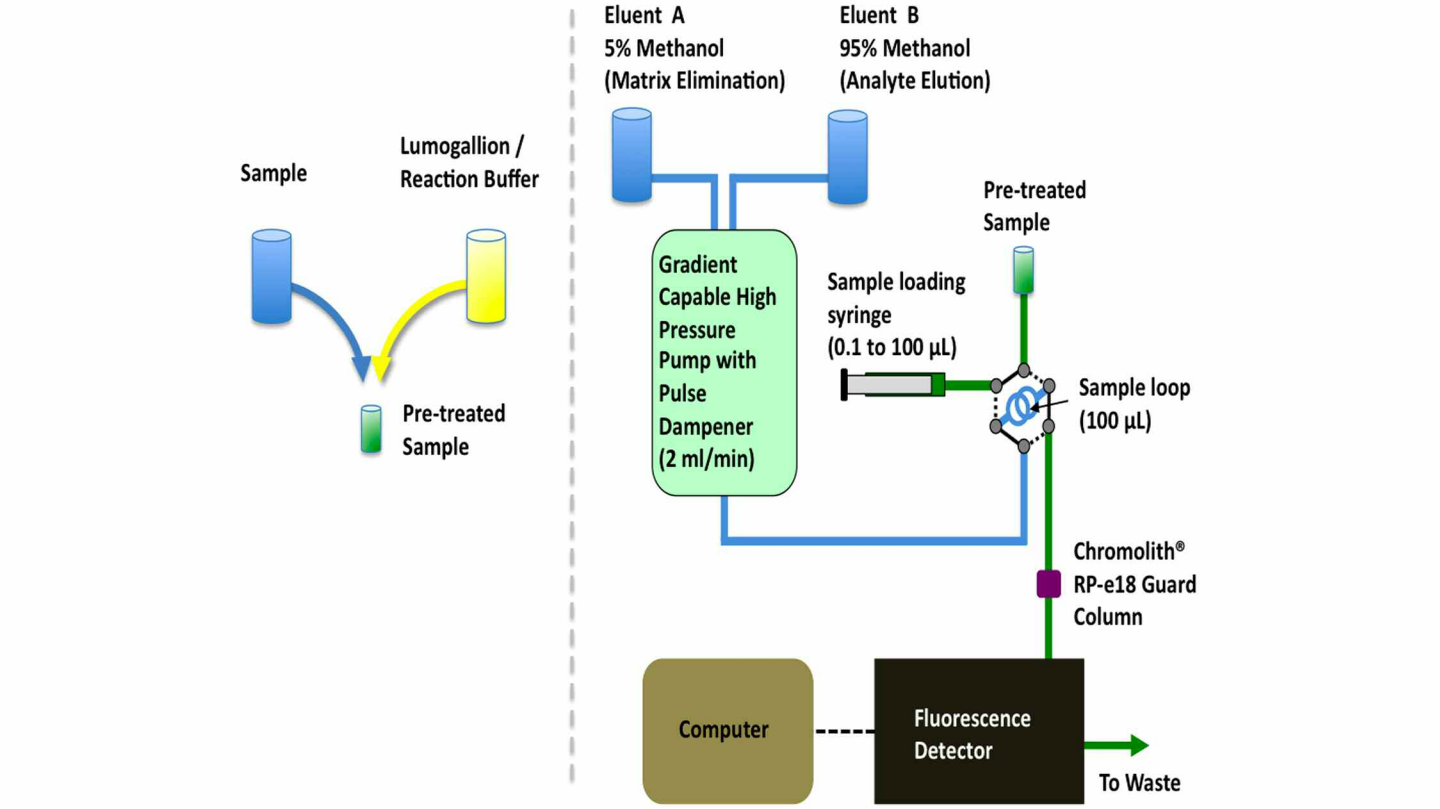

Fig. 1. Schematic of the sample analysis procedure. Reaction of the sample with the reagent (lumogallion/reaction buffer solution) is performed before injection of 10 µL of this mixture into the RP-HPLC method. During injection (column loading and matrix elimination) 'Eluent A' is used. During elution of the Al-lumogallion complex to the detector 'Eluent B' is used. Flow rate is maintained at 2 mL min^{-1} throughout. Fluorescence detector settings: λ_{ex} = 505 nm (bandwidth = 18nm), λ_{em} = 574 nm (bandwidth = 18 nm).

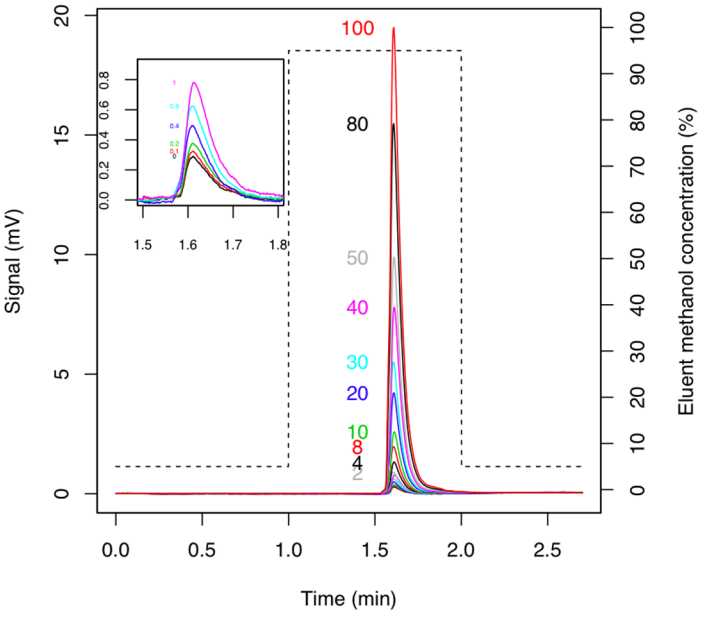

Fig. 2. Chromatograms of a calibration of the SAFe reference sample. A representative collection to demonstrate signal-to-noise and peak shape over four orders of magnitude, from 0.1-100 nM. The dashed line is the methanol concentration during the stepped-gradient elution program.

Assessment

Blank estimation

Blank estimation was achieved by injecting 2.5 µL of the reagent only. This is the volume of reagent when injecting 10 µL prepared samples, given the 1:3 ratio of reagent:sample. The absence of a fluorescent ligand in the eluent means the baseline signal is almost zero, making it much less significant than in a FIA method. The baseline for this method is the sum of the composition and flow rate of the eluent changing the refraction of stray light, altering the intensity entering the detector, the presence of fluorescent contaminants in the eluent emitting light at the target wavelength, and electronic noise. There is virtually no background signal until the analyte plug (containing the reagent contribution and the sample contribution of the Al–lumogallion complex measured) passes through the detector. Therefore, quantifying the blank is even more crucial than in an FIA method, where the bulk of the blank is accounted for in the baseline. The blank is the concentration of Al–lumogallion complex in the reagent only, along with any leaching of Al from the HPLC system during analysis. An injection of the same volume of reagent normally injected (1/4 of the prepared sample volume injected) is therefore the best estimation the blank. This is expected to be an

overestimate, since the fluorescence yield of the Al–lumogallion complex is higher in the reagent matrix (MES buffer and DIW) than when additional interferences are present within the seawater matrix, quenching the fluorescence signal.

Calibration and limit of detection

Ultra-high purity water could not be used for calibration or blank estimation. The fluorescent response of the Al–lumogallion complex was quenched by seawater compared to ultra-high purity water. The matrix effect of seawater is significant, and therefore must be used for calibration.

Calibration linearity range was tested from 0.1 to 100 nM, see Figs. 2 and 3. Above 100 nM, the lumogallion concentration in the reagent should be increased to maintain linearity. The upper concentration tested was 500 ppm, column capacity had not yet been reached but the Al–lumogallion complex became unstable for analysis (forming what appeared to be a gel). Standards made up at < 1 nM were highly unstable (changing within minutes of production) and required immediate analysis to achieve linearity (this was also found using the FIA method). To eliminate this error, calibration standards > 1 nM were used. This instability is expected to be interferring ions within the seawater matrix altering the equilibration chemistry of Al^{+3} when at the ultra-trace levels < 1 nM. At concentrations > 1 nM, Al^{+3} appeared to dominate effects by these other ions. Samples were stable, probably because they were equilibrated well before they were removed from the sea. It is also likely the stable species of Al available for analysis is a weakly bound complex, rather than the Al^{+3} standard used for calibration. This problem would be applicable to all methods that calibrate with a Al^{+3} standard.

Limit of detection was assessed as: 3 × σ blank / calibration slope
(n = 30 in triplicate)
Limit of detection was 0.13 ± 0.05 nM.

Accuracy and precision

Accuracy and precision of the method was evaluated by analyzing the SAFe reference standard and comparing the results to the Agreed Values presented by Bruland et al. (2012). Results of the 13 SAFe sub-samples analyzed are presented in Table 1. Agreement with SAFe reference samples was within 4.6 ± 4.6% (n = 13). All analyses were within 15% and most analyses within 5%. This is within similar tolerances to those presented by Brown and Bruland (2008) and Sohrin et al. (2008). The highest percentage deviations were associated with the lowest concentration sub-samples (SAFe–D1). Given the high precision on each set of replicates, it is expected the subsamples are slightly contaminated. Further investigation was not possible due to a limited supply of SAFe–D1 sub-samples.

Precision was calculated on SAFe–D2: mean value of 1.08 nM ± 0.3 nM (2.7%). Mean precision of SAFe analyses was 1.7 ± 0.8%, with all analyses within 3.5% relative standard deviation.

SAFe–D2-128 was analyzed five times in triplicate to investigate the influence of cleaning/handling/analysis protocols

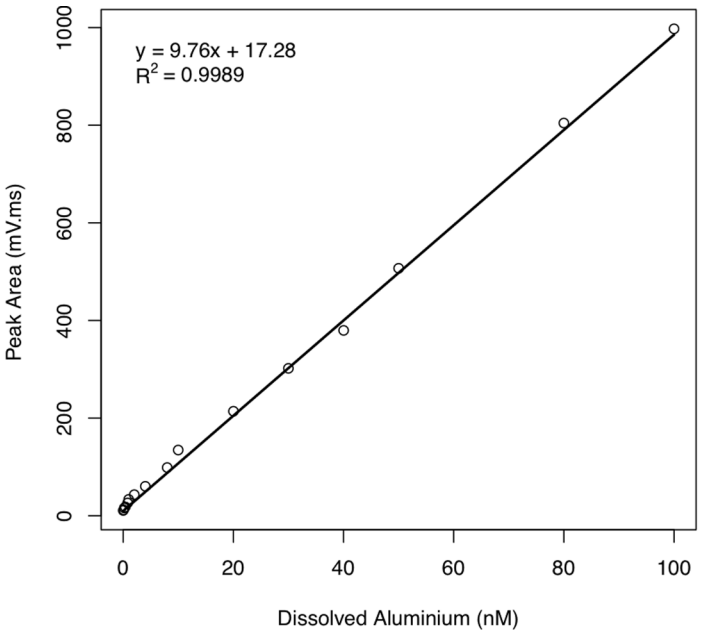

Fig. 3. Representative 16-point calibration curve of dissolved aluminium (nM). Sample = SAFe - S-441.

(including the use of polypropylene analysis vials) on the final concentration determined. The influence of cleaning/handling/analysis protocols was found to be minimal. Four of the vials had excellent agreement (mean = 1.06 nM; rsd% = 1.2), but one of the vials was 0.06 nM higher than the average. This presents a potential error from protocols of ≈0.06 nM. This accounts for the bulk of the disagreement with the SAFe agreed values. This is considered acceptable by the authors, as to be environmentally relevant as a biogeochemical tracer (beyond indicating the absence of recent dust deposition events to the region of interest), dissolved aluminum concentrations should be at least an order of magnitude larger than this error.

Synthesis of the lumogallion-aluminum complex

pH to achieve optimal derivative yield

Optimum yield of the Al–lumogallion complex is achieved at pH 5.0–5.5 (Brown and Bruland 2008; Lee et al. 1996; Resing and Measures 1994; Wu et al. 1995). Therefore, the reagent pH must be adjusted to account for the pH of the sample of interest. In this study, all samples were collected and then stored at pH 1.8, as prescribed in Cutter et al. (2010). The reagent mixture needed to be adjusted to pH 6.1 to achieve a pH of 5.3 when mixed with the acidified sample.

Discussion

A comparison of various performance parameters of the RP-HPLC method with methods also used to determine dissolved aluminum concentration in the SAFe reference samples is presented in Table 2.

The RP-HPLC technique is very similar in chemistry and application to the FIA method, which is also a single analyte,

Table 1. Analysis of the various SAFe sub-samples using the RP-HPLC. Agreed values described by Bruland et al. (2012). All values reported in nM (except where specified as %).

SAFe sub-sample	rep. 1	rep. 2	rep. 3	rsd%	mean	agreed value	error%
SAFe – S-559	1.72	1.72	1.73	0.3	1.72	1.71	0.6
SAFe – S-441	1.77	1.75	1.79	1	1.77	1.71	3.5
SAFe – S-260	1.86	1.8	1.84	1.6	1.83	1.71	7.0
SAFe – S-129	1.68	1.72	1.71	1.2	1.7	1.71	–0.6
SAFe – D2-128-1	1.09	1.07	1.09	1.3	1.08	1.06	1.9
SAFe – D2-128-2	1.05	1.1	1.03	3.5	1.06	1.06	0.0
SAFe – D2-128-3	1.13	1.12	1.1	1.3	1.12	1.06	5.7
SAFe – D2-128-4	1.07	1.02	1.05	2.5	1.05	1.06	–0.9
SAFe – D2-128-5	1.09	1.05	1.06	1.8	1.06	1.06	0.0
SAFe – D2-174	1.09	1.12	1.1	1.6	1.11	1.06	4.7
SAFe – D2-426	0.96	0.95	0.98	1.2	0.96	1.06	–9.4
SAFe – D1-381	0.75	0.73	0.72	2.4	0.73	0.64	14.1
SAFe – D1-165	0.71	0.73	0.7	2.3	0.71	0.64	10.9

Table 2. Comparison of various performance parameters of three methods used for determining dissolved aluminium in seawater.

Parameter	RP-HPLC*	FIA†	ICP-MS‡
LOD (nM)	0.13	0.10	0.27
Precision (%)	2.7 at 1 nM	2.5 at 5 nM	< 9.0 at 1 nM§
Pre-treatment time per 100 samples (h)	10	0	200
Analysis time per replicate (min)	2.7	7.8	3.3
Operator time per 100 samples (h)	3	39‖	8¶
Sample volume (mL)#	1.5	36	120

*This paper.
†Brown and Bruland [2008]
‡Sohrin et al. [2008]
§Interpreted from the statement:
Our data showed excellent agreement with the certied values, except that there was no certied value for Al. The RSD of our data were less than 9%. where samples analyzed were: SAFe–S1, SAFe–D2, NASS-5, CASS-4.
‖Operator must be at least partially attentive, if not fully occupied by the analysis
Time calculation: 100*samples* × 7.8*min* × 3*replicates*
¶Assuming the use of an auto-sampler during pre-concentration.
#Per triplicate analysis, including all rinses.

shipboard capable technique using the fluorescence reaction of the Al–lumogallion complex. As such, in practice the RP-HPLC technique could by used as an alternative method to the FIA technique. Major differences are the sample treatment. The FIA method's major advantage is standardizing the sample interaction time with the reagent before detection. This is achieved by performing all sample manipulations in-line, after sample injection. This limits contamination, standardizes sample pretreatment (as it is all automated) and allows the use of heat to reduce the time required for Al–lumogallion complex formation (Brown and Bruland 2008). This major advantage is also the major disadvantage, as the in-line sample treatment complicates the system. The preconcentration mechanism and the detection mechanism are intricately linked, limiting the options available for each process. Isolating parameter effects is difficult as one parameter has flow on effects to others, making optimization and trouble-shooting difficult and requiring a high level of experience to operate efficiently. With regard to this, pH stability of all reagents and maintainence of flow ratios are critical for maintaining system stability.

The disadvantages of the FIA method were key to selecting the RP-HPLC method. The chemistry employed in the FIA method was clearly superior to alternatives (Ahmed and Hossan 1995; Mulon et al. 2005 and references there in), however, the analytical set-up had many limitations. Changing the in-line sample treatment (as employed in the FIA method) with a pre-column (batch) reagent mixing step, separated the detection mechanism (formation of the Al–lumogallion complex) from the concentration mechanism (the focusing of the Al–lumogallion complex onto the C_{18} guard column), allowing independent optimization. Changing the buffer used from the toxic and volatile ammonium-acetate to the nontoxic and

nonvolatile MES, improved reagent pH stability over time. Replacing a peristaltic pump with a high pressure pump, removed potential back pressure issues, reduced baseline variability, decreased dead volumes, and improved the range of column types and flow rates that could be applied. Removing lumogallion from the eluent (and therefore the baseline) allowed greater capacity to quantify the blank, as it was injected as a discrete sample.

The RP-HPLC method achieved similar LOD and precision to the FIA method. This is expected as the chemistry is very similar, and blanks of both systems are dominated by reagent contamination levels. Total analysis times were similar, although required operator times were much lower for the RP-HPLC method, due to the capacity for reliable automation, attributed to robustness (discussed further in section 5.1).

Comparison of the RP-HPLC method to the ICP-MS method in Table 2 was for illustrative purposes only, as it was also used for analyzing the SAFe reference sample. However, they cannot be considered as direct alternatives to each other. The RP-HPLC technique does not have multiple analyte capacity, and the ICP-MS method is an impractical method to take to sea. A similarity of both methods is the use of off-line sample pretreatment steps that require significant periods of time (although not labor intensive). This approach decouples chemical mechanisms used for concentration and detection, allowing greater control over each mechanism. However, the ICP-MS method must optimize for multiple elements, which can decrease precision at low concentrations for difficult low atomic mass elements like Al. The RP-HPLC method had improved LOD, precision, and total analysis times relative to the ICP-MS method. However, required operator time is similar for both methods.

The RP-HPLC method had relatively similar performance to the existing methods. LOD and precision were similar across all methods. Pretreatment time per 100 samples was highly varied. The ICP-MS method required substantial sample preparation before injection into the ICP-MS, however this is offset by the multiple element capability. The RP-HPLC method requires 120 min labor, followed by 8 h equilibration time before the start of the analysis sequence; this is much less time than the ICP-MS method and 10 h more than required for the FIA method. Analysis time per replicate were of the same order of magnitude. However, if these values are converted into operator time per 100 samples, the values are very different.

For the RP-HPLC method, the operator prepares a sample set of 100, loads them into the autosampler, and enters sample details into the program's run-sheet (total time about 3 h). The operator can now leave and come back in 13.5 h when the analysis is finished.

For the FIA method, the operator needs to watch the instrument regularly, as it is not robust enough to leave unattended for long periods of time. This requires at least partial attention during every run, and hence requires ≈39 h operator time per 100 samples.

For the ICP-MS system, operator requirements for the ICP-MS system were not available through first-hand accounts (as they were for FIA and RP-HPLC methods). An estimate is made assuming an autosampler was used for preconcentration (requiring 2 h), with an operator required to be in attendance (or close at hand) during analysis with an ICP-MS (requiring 6 h).

These differences in operator time are substantial and highlight the advantages of using robust chemical techniques over less robust systems.

Robustness and automation

Method robustness was defined "as the sensitivity of the method to small changes in influencing factors." The advantages of applying robust instrumentation to existing, proven chemistry is demonstrated with the RP-HPLC method presented here. Small changes in sample pH, buffer concentration, system back-pressure, fluorescence settings, and laboratory temperature made little difference to signal intensity. As there are no mixing points, the only variable between samples was time since reagent was added, with the impact insignificant between calibrations. Changing over columns had no significant effect. The blank was significantly affected by lumogallion concentration (which is expected), and therefore, was minimized.

The authors experience with the FIA method (Brown and Bruland 2008) was that it exhibited high sensitivity to buffer concentration and pH, pump tubing integrity, system back pressure, column used (as they were made in-house as opposed to commercially produced products), mixing coil temperature stability, and sample pH. Limited robustness resulted in a requirement for regular (if not constant) attention and significant training to operate efficiently. This lack of robustness is a common acknowledgment by users of FIA methods (not limited to those discussed here).

The RP-HPLC method was very robust and easily automated. It was used to successfully analyze ≈300 samples within 1 week (inclusive of troubleshooting, which was mostly attributed to operator error). The system could be left unattended until completion once the automated analysis program was initiated. Operation and trouble-shooting assistance during development was available through free-access online communities.

An example depth profile is presented in Fig. 4 (GEOTRACES cruise GIPY6 along the SR3 meridional transect, station N20, 144°E, 49.3°S, within the Polar Frontal Zone of the Southern Ocean). Some values were close to the detection limit (displayed as the feint dotted line) but maintained oceanographic consistency and excellent precision. Oceanographic features include enrichment at the surface due to supply from aeolian dust deposition (albeit limited) or advection of waters entrained with DAl; depletion with depth due to scavenging and mixing throughout the water column; enrichment approaching the ocean floor due to supply of DAl from sediments (although this is quite limited). Concentrations are extremely low in this region relative to the global range due to

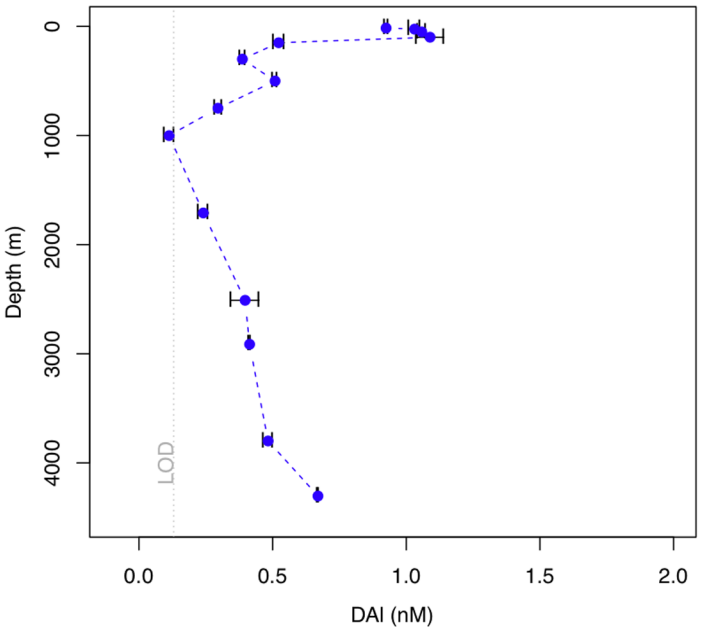

Fig. 4. Example depth profile of dissolved aluminium (DAl) from the SR3 transect (Station N20, 144°E, 49.3°S, within the Polar Frontal Zone of the Southern Ocean, sampled in April 2008 as part of the GEOTRACES program). Features include enrichment at the surface due to supply from aeolian dust deposition (albeit limited) or advection of waters entrained with DAl; depletion with depth as this is scavenged throughout the water column; enrichment approaching the ocean floor due to supply of DAl from sediments, although this is quite limited. Concentrations are extremely low in this region relative to the global range due to geographic and atmospheric isolation from continental sources coupled with diatom dominated communities at the surface.

geographic and atmospheric isolation from continental sources coupled with diatom dominated communities in surface polar waters.

Required sample volume

Relative to previously published methods, applying this RP-HPLC technique reduces the required sample volume significantly (as can be seen in Table 2), by ≈20-fold and ≈60-fold in the case of FIA and ICP-MS, respectively. This is due to the combination of the extremely sensitive fluorescence detection system and the advantages of HPLC, such as low system dead volumes, excellent analyte focusing on highly efficient columns, and little band broadening. However, as discussed earlier, comparison with ICP-MS in this case is not fair, as that is a multi-element technique.

Comments and recommendations

A major limitation of this technique is the time required for sample pretreatment. This is a function of the slow reaction kinetics of aluminum (Eigen 1963). Resing and Measures (1994) and Brown and Bruland (2008) have shown how the heating of the reagent-sample mixture would increase the kinetics of the Al–lumogallion complex formation. However, it also increases the rate of complex decomposition. Therefore, increasing the rate of kinetics requires in-line systems that can standardize the time between mixing with the reagent and detection (such as those used by Brown and Bruland 2008). The HPLC system available to the authors at the time of development was not capable of automating the addition of the reagent to the sample, hence a more conservative approach to sample pretreatment was adopted, focusing on the long-term stability of the Al–lumogallion complex.

The use of an automated sample dosing and injection system would reduce the time required for complex formation, while maintaining the large gains in robustness and automation. The authors would also expect an increase in sensitivity, as the time of maximum complex yield could be targeted.

The RP-HPLC method is an excellent technique to use for the determination of dissolved aluminum in open ocean seawater. It employs similar chemistry to the FIA method and has good agreement with agreed values for the SAFe standard refence material. Therefore, open ocean dissolved aluminum data produced with RP-HPLC are compatible for comparison with the existing global data set. The RP-HPLC method is recommended for future work for those determining aluminum in seawater due its robustness, sample volume requirements, and automation capacity, while achieving comparable LOD, precision, and accuracy to existing methods.

References

Ahmed, M. J., and J. Hossan. 1995. Spectrophotometric determination of aluminium by morin. Talanta 42:1135-1142. URL [doi:10.1016/0039-9140(95)01554-O].

Bowie, A. R., and M. C. Lohan. 2009. Analysis of iron in seawater, p. 235-257. *In* O. Wurl [ed.], Practical guidelines for the analysis of seawater. CRC Press.

Brown, M. T., and K. W. Bruland. 2008. An improved flow-injection analysis method for the determination of dissolved aluminum in seawater. Limnol. Oceanogr. Methods 6:87-95 [doi:10.4319/lom.2008.6.87].

Bruland, K., M. Brown, R. Middag, H. de Baar, Y. Sohrin, J. Ren, J. Cullen, and T. Giesbrecht. 2012. Dissolved aluminum values in nmol/kg, consensus values (1 std. dev.) for SAFe Reference Samples as of November 2011. <http://es.ucsc.edu/kbruland/GeotracesSaFe/2012GeotracesSAFeValues/SAFe\s\do4(R)ef\s\do4(A)l.pdf>

Cutter, G., P. Anderson, L. Codispoti, P. Croot, R. Francois, M. Lohan, H. Obata, and M. Rutgers van der Loeff. 2010. Sampling and sample-handling protocols for GEOTRACES Cruises. Tech. rep., GEOTRACES.

de Baar, H. J. W., and others. 2008. Titan: A new facility for ultraclean sampling of trace elements and isotopes in the deep oceans in the international Geotraces program. Mar. Chem. 111:4-21 [doi:10.1016/j.marchem.2007.07.009].

Eigen, M. 1963. Fast elementary steps in chemical reaction mechanisms. Pure. Appl. Chem. 6:97-116 [doi:10.1351/pac196306010097].

Giesbrecht, T. 2007. The distribution of aluminum in Beaufort Sea and the development of a sequential injection method for the determination of aluminum in natural waters. Master's thesis, Univ. of Victoria <http://hdl.handle.net/1828/2680>

JabRef-Development-Team. 2010. JabRef. JabRef Development Team. <http://jabref.sourceforge.net>

Lee, B. L., L. H. Chua, H. Y. Ong, H. G. Yang, J. F. Wu, and C. N. Ong. 1996. Determination of serum and urinary aluminum by HPLC with fluorometric detection of Al-lumogallion complex. Clin. Chem. 42:1405-1411. <http://www.clinchem.org/cgi/content/abstract/42/9/1405>

Measures, C. I., and J. M. Edmond. 1989. Shipboard determination of aluminum in seawater at the nanomolar level by electron capture detection gas chromatography. Anal. Chem. 61:544-547 [doi:10.1021/ac00181a009].

———, and ———. 1990. Aluminum in the South-Atlantic—steady-state distribution of a short residence time element. J. Geophys. Res. Oceans 95:5331–5340 [doi:10.1029/JC095iC04p05331].

Middag, R. 2010. Dissolved aluminium and manganese in the polar oceans. Doctorate of philosophy, Royal Netherlands Institute for Sea Research. <http://www.es.ucsc.edu/kbruland/People/Middag/Rob\s\do4(M)iddag\s\do4(P)roefschrift\s\do4(d)igital\s\do4(v)ersion\s\do4(l)ow\s\do4(r)esolution.pdf>

———, H. J. W. de Baar, P. Laan, and K. Bakker. 2009. Dissolved aluminium and the silicon cycle in the Arctic Ocean. Mar. Chem. 115:176-195 [doi:10.1016/j.marchem.2009.08.002].

———, H. de Baar, P. Laan, P. Cai, and J. van Ooijen. 2011a. Dissolved manganese in the Atlantic sector of the Southern Ocean. Deep-Sea. Res. II 58:2661-2677 [doi:10.1016/j.dsr2.2010.10.043].

———, H. J. W. de Baar, P. Laan, and M. B. Klunder. 2011b. Fluvial and hydrothermal input of manganese into the Arctic Ocean. Geochim. Cosmochim. Acta 75:2393-2408 [doi:10.1016/j.gca.2011.02.011].

———, C. van Slooten, H. de Baar, and P. Laan. 2011c. Dissolved aluminium in the Southern Ocean. Deep-Sea. Res. II. 58:2647-2660.

———, H. J. W. de Baar, P. Laan, and O. Huhn. 2012. The effects of continental margins and water mass circulation on the distribution of dissolved aluminum and manganese in Drake Passage. J. Geophys. Res. Oceans. 117:C01019 [doi:10.1029/2011JC007434].

Moran, S., and R. Moore. 1991. The potential source of dissolved aluminum from resuspended sediments to the North-Atlantic Deep-Water. Geochim. Cosmochim. Acta 55:2745-2751 [doi:10.1016/0016-7037(91)90441-7].

Mulon, J.-B., È. Destandau, V. Alain, and È. Bardez. 2005. How can aluminium(III) generate fluorescence? J. Inorg. Biochem. 99:1749-1755 [doi:10.1016/j.jinorgbio.2005.06.005].

Orians, K. J., and K. W. Bruland. 1985. Dissolved aluminum in the Central North Pacific. Nature 316:427-429 [doi:10.1038/316427a0].

R Development Core Team. 2010. R: A language and environment for statistical computing. <http://www.r-project.org>

Remenyi, T. A., P. N. Nesterenko, A. R. Bowie, E. C. V. Butler, and P. R. Haddad. 2011. Fast and sensitive determination of aluminium with RP-HPLC using an ultra-short monolithic column. Anal. Method. 3:2488-2494 [doi:10.1039/c1ay05192j].

Resing, J. A., and C. I. Measures. 1994. Fluorometric determination of Al in seawater by flow injection analysis with in-line preconcentration. Anal. Chem. 66:4105–4111 [doi:10.1021/ac00094a039].

Schlitzer, R. 2002. Interactive analysis and visualization of geoscience data with Ocean Data View. Comput. Geosci. 28:1211-1218 [doi:10.1016/S0098-3004(02)00040-7].

Sohrin, Y., and others. 2008. Multielemental determination of GEOTRACES key trace metals in seawater by ICPMS after preconcentration using an ethylenediaminetriacetic acid chelating resin. Anal. Chem. 80:6267-6273 [doi:10.1021/ac800500f].

Taylor, S. R. 1964. Abundance of chemical elements in the continental crust: a new table. Geochim. Cosmochim. Acta 28:1273-1285 [doi:10.1016/0016-7037(64)90129-2].

Tria, J., P. R. Haddad, and P. N. Nesterenko. 2008. Potential applicability of a high performance chelation ion chromtographic method to the determination of aluminium in Antarctic surface seawater. Chem. Listy. 102:s319-s323.

Upadhyay, S., P. S. Liss, and T. D. Jickells. 2002. Sorption model for dissolved aluminium in freshwaters. Aquat. Geochem. 8:255-275 [doi:10.1023/B:AQUA.0000003822.15914.1f].

Wu, J., C. Y. Zhou, H. Chi, M. K. Wong, H. K. Lee, H. Y. Ong, and C. N. Ong. 1995. Determination of serum aluminum using an ion-pair reversed-phase high-performance liquid chromatographic-fluorimetirc system with lumogallion. J. Chromatogr. B 663:247-253 [doi:10.1016/0378-4347(94)00458-H].

Submitted 18 April 2012
Revised 17 July 2012
Accepted 24 July 2012