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Fully-Automated Fluorimetric Determination of Aluminum in Seawater by In-Syringe Dispersive Liquid−Liquid Microextraction Using Lumogallion

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Supporting Information

ABSTRACT: A sensitive and selective automated in-syringe dispersive liquid−liquid microextraction (DLLME) method is presented. It was successfully applied to the determination of aluminum in coastal seawater samples. The complete analytical procedure including sampling, buffering, reaction of the analyte with fluorescence reagent lumogallion (LMG), extraction, phase separation, and quantification was completely automized and carried out within 4 min. DLLME was done using *n*-hexanol as an extracting solvent and ethanol as a dispersing solvent in a 1:8 v/v percent mixture. The Al−LMG complex was extracted by an organic solvent and separated from the aqueous phase within the syringe of an automated syringe pump. Two devices were specially developed for this work. These were (a) the fluorescence detector and accompanying flow cell for the organic phase enriched with the reaction product and (b) a heating device integrated into the holding coil to accelerate the slow reaction kinetics. The limits of detection (3σ) and quantification (10σ) were 8.0 ± 0.5 nmol L^{-1} and 26.7 ± 1.6 nmol L^{-1}, respectively. The relative standard deviation for eight replicate determinations of 200 nmol L^{-1} Al^{3+} was <1.5%. The calibration graph using the preconcentration system was linear up to 1000 nmol L^{-1} with a correlation coefficient of 0.999. Ambient concentrations of samples were quantifiable with found concentrations ranging from 43 to 142 nmol L^{-1}. Standard additions gave analyte recoveries from 97% to 113% proving the general applicability and adequateness of the analyzer system to real sample analysis.

Aluminum is the third element in order of abundance in both the pedosphere- and lithosphere-forming part of minerals, rocks, and clays.[1] Consequently, it is present in all natural waters. The Al^{3+} cation does not have any biological function, and Al^{3+} salts show a low solubility in water throughout, resulting in a low natural concentration level down to a few nanomoles per liter for open ocean seawater.

In oceanography, Al^{3+} concentration data can be used to trace atmospheric dust deposition and thus to estimate the entry and deposition of other essential elements such as silica or iron, which do have biological functions but are readily uptaken. Further Al^{3+} sources are river effluents and anthropogenic emissions.

Besides, there has been increased interest during the last several decades in the toxicity of Al^{3+} and its effect on plants,[2] aquatic ecosystems,[3,4] and humans.[5,6] Consequently, knowledge of Al^{3+} concentration levels in both biological and environmental media is of current interest. As a result, the development of novel, simple, robust, and transportable analytical instrumentation for fast, sensitive, and environmentally friendly methods is of high interest.

Lumogallion (LMG), 4-chloro-6-(2,4-dihydroxyphenylazo)-1-phenol-2-sulfonic acid, a tetradentate ligand that coordinates with Al^{3+} was first introduced as a selectivity fluorescence reagent for Al^{3+} complexation in the 1970s.[7] During the following years, the analysis of Al^{3+} with LMG was gaining widespread acceptance.[8−13] The Al−LMG complex offers excellent sensitivity with minimal interference. Consequently, it has been successfully used for the determination of Al^{3+}, even in complex and high-salt matrices such as body fluids[14] and seawater.[15] LMG further presents an important advantage over morin, another often-used fluorescence reagent for aluminum. It has lower hydrophilicity, which allows liquid−liquid extraction of the Al−LMG complex for analyte enrichment.[10]

Flow techniques (FT), divided in respect of their operation scheme, instrumentation, and flow pattern, have proven to be excellent tools for the automation of laboratory procedures. Outstanding advantages over batch-wise robotic automation are the performance of the complete procedure in a closed compartment (i.e., tubing manifold) and a self-cleaning process of this system by a continuous or semicontinuous flush with the

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carrier. This and a general gain in reproducibility and sample frequency are excellent conditions for analysis of trace concentrations of abundant elements such as aluminum. Unsurprisingly, the Al–LMG reaction has been automated using FT and successfully applied to field monitoring.[4,9]

Due to the low concentrations of Al^{3+} found in environmental water samples, analyte enrichment techniques have to generally be used such as liquid–liquid extraction (LLE)[16] or solid phase extraction (SPE).[17−19] While SPE is often praised for its avoidance of solvents and is less environmentally harmful than LLE, the quantity of crude required for cartridge fabrication is often forgotten.

Micro-LLE techniques overcome typical problems of SPE such as potential clogging, long extraction times, and higher costs while minimizing the environmental impact. Reported micro-LLE techniques used for the determination of Al^{3+} include single drop microextraction (SDME),[20] cloud point extraction (CPE),[21] and most recently, dispersive liquid–liquid microextraction (DLLME).[22,23]

The DLLME is a novel extraction technique, first reported in 2006[24], of rapidly increasing interest. Its simplicity and fastness are probably the most attractive benefits of this technique. It is based on the rapid injection of a solvent mixture into the aqueous sample by which one component of the solvent dissolves nearly instantaneously (i.e., the dispersion solvent) while the second component (i.e., the extraction solvent) remains and is disrupted into a cloud of fine droplets. The simultaneous enormous increase of the interaction surface with the sample enables efficient mass transfer of the analyte into the extraction solvent droplets. After collection of the droplets, generally done by centrifugation, the extraction solvent can be used for further analysis.

Since DLLME does not require solid supports for the extraction solvent such as hollow membranes or capillary tubes, its automation using FT is straightforward. It has been coupled to FT using one of the following operation modes: (1) Injection of the solvent mixture in a sample flow and collection of the extraction solvent droplets on a hydrophobic column with later elution.[25−27](2) Injection of the solvent mixture into a sample-filled reaction chamber with passive phase separation due to an extraction solvent density >1 g cm^{-3} (i.e., sedimentation of the droplets).[28] (3) Filling a fraction of a syringe pump with the solvent mixture and fast injection of the watery phase with passive phase separation due to an extraction solvent density <1 g cm^{-3} (i.e., floating of the droplets at the top of the syringe).[29−31]

The last operation mode has the advantage that it can be performed relatively fast since fewer steps are cleaning and no elution steps are required. Thus, this technique was used for the complete automation of DLLME-based determination of Al^{3+} in seawater.

EXPERIMENTAL SECTION

Reagents and Solutions. All solutions were of analytical-reagent grade and doubly distilled water provided by a Milli-Q Direct-8 purification system (resistivity >18 MΩ cm, Millipore Iberica S.A.U., Spain) was used throughout. All glassware and polyethylene were previously soaked in 10% (v/v) HNO_3 and rinsed with Milli-Q water prior to use. The aluminum stock solution of 13.5 mg L^{-1} was prepared by diluting a commercial 1000 mg L^{-1} $Al(NO_3)_3 \cdot 9H_2O$ atomic absorption standard (Scharlab, Barcelona, Spain) in 0.5 mol L^{-1} HNO_3. For calibration purposes, Al^{3+} standard working solutions were prepared by appropriate dilution of the stock solution with Milli-Q water or artificial seawater acidified with HCl to pH 3.

Reagent 1 was an ammonium acetate buffer of 2 mol L^{-1} (pH 5.1) prepared by adding 3.3 mL of glacial acetic acid to 10.8 g of ammonium acetate and then diluted to 100 mL. Reagent 2 was a LMG stock solution of 1.5 mmol L^{-1}, prepared by dissolving 103 mg of LMG in 200 mL of Milli-Q water. *n*-Hexanol was used as an extraction solvent with ethanol used as a dispersing solvent. The extractant solution was prepared by mixing *n*-hexanol with ethanol in a 1:8 volumetric ratio, if not otherwise indicated. A reference material of trace elements in wastewater (SPS-WW2 Batch 106, Spectrapure Standards AS, Oslo, Norway) was also analyzed for evaluation of the accuracy of the developed method, as recommended by the National Institute of Standards and Technology. For interference studies, a standard solution of 10 mmol L^{-1} NaF (Panreac, Barcelona, Spain) and a standard solution of 25 μmol L^{-1} of $Fe(NO_3)_3 \cdot 9H_2O$ (Scharlab, Barcelona, Spain) were used.

For masking of interfering fluoride and ferric ions, a Be^{2+} solution of 2.5 mmol L^{-1} was prepared by the dilution of a commercial beryllium nitrate solution 35% w/w in H_2O (Sigma Aldrich, Spain) and a solution of *o*-phenanthroline (Acros organics, Geel, Belgium) of 15 μmol L^{-1}. All reagent solutions were stored in polyethylene bottles at 4 °C in the dark.

Synthetic seawater was used for most optimization experiments and preparation of the calibration standards. It was prepared according to standard recipe[32] by dissolving in Milli-Q water the following reagents to the final concentrations given in mg L^{-1}: 20 ($SrCl_2 \cdot 6H_2O$), 30 (H_3BO_3), 100 (KBr), 700 (KCl), 1470 ($CaCl_2 \cdot 2H_2O$), 4000 (Na_2SO_4), 10780 ($MgCl_2 \cdot 6H_2O$), 23500 (NaCl), 20 ($Na_2SiO_3 \cdot 9H_2O$), and 200 ($NaHCO_3$).

Sample Collection and Preparation. Different coastal seawater samples were collected in polyethylene flasks from different beaches from Mallorca collected in March of 2012. A map of locations can be found in the Supporting Information. The required amount of 1 mol L^{-1} HCl to reach a final pH of 3 was added immediately (approximately 1 mL/L). The samples were measured in the proposed analyzer system without any other previous treatment but sedimentation of coarse particles.

Manifold Configuration. The sequential injection analysis (SIA) system used in this work is schematically illustrated in Figure 1. It comprised a 5000-step syringe pump (SP) from Crison Instruments SA (Alella, Barcelona, Spain), a rotary 8-port multiposition valve (MPV) purchased from Sciware SL (Palma de Mallorca, Spain), and a homemade fluorescence detector described in detail in Detection Cell and Equipment. All tubing connecting the different components of the flow system was of polytetrafluoroethylene (PTFE) with either 0.8 mm or 1.5 mm inner diameter (i.d.).

The SP was equipped with a 5 mL syringe (S1) from Hamilton Bonaduz (Bonaduz, Switzerland). A three-way solenoid head valve (V1) allowed the connection of S1 to either the central port of the MPV (position ON) or the detection flow cell and further to waste (position OFF). The MPV was used for the handling of solutions required for DLLME and the cleaning procedures. Lateral ports were connected to waste (position 1) and reservoirs of a blank standard (2), H_2O (3), a standard or sample (4), reagent 1 (5), reagent 2 (6), and the extraction solvent mixture (8). At port 7, a dilution chamber (DC) was realized with a 5 mL pipet tip. For real-sample measurements, a 45-position rotary autosam-

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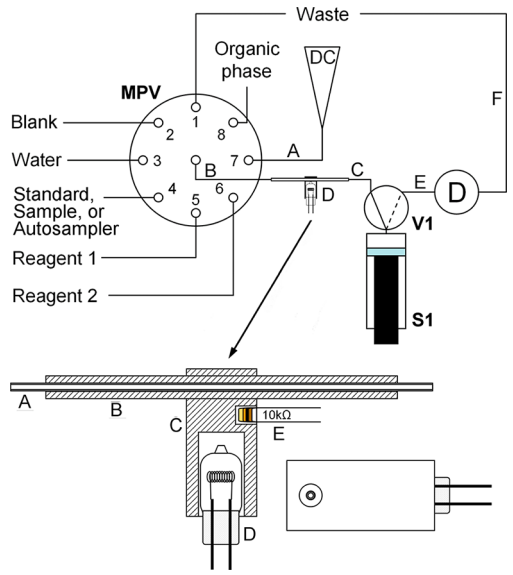

Figure 1. Sequential injection analysis (SIA) manifold used for DLLME of aluminum. Top: dilution chamber (DC), syringe pump (S1), MPV, 3-way solenoid valve (V1), connection tube A: 2 cm × 1.5 mm i.d., B and C: 15 cm × 0.8 mm i.d., D: heating device, E: 6 cm × 1.5 mm i.d., and F: 30 cm × 0.8 mm i.d. Bottom: A detailed representation of heating device as both a cross-sectional top view and side view. Elements include a glass capillary (A), a 10 cm × 4 mm o.d. × 2 mm i.d. brass tube (B), a 4 cm × 2 cm o.d. brass cylinder (C), a halogen light bulb (D), and an NTC resistance used as a sensor (E).

pler (AS) from Crison SA was connected to position 4 instead of the generally used supply of PTFE tube.

The central port of the MPV was connected to the head valve position ON of S1 by a holding coil (HC) consisting of two 10 cm PTFE tubes (0.8 mm i.d.) connected by a homemade heating device to accelerate the reaction. The heating device is shown in Figure 1. It consisted of a brass support for the tight insertion of a chemically inert 12 cm long glass capillary [1.5 mm i.d. × 2 mm outer diameter (o.d.)] used as a flow channel and a commercial halogen light bulb (12 V, 20 W) used as a heat source. Temperature control and bulb powering was done via a negative temperature coefficient (NTC) thermistor probe and a thermostat control circuit from CEBEK–Fadisel SL (Barcelona, Spain ref I-81), respectively. A temperature hysteresis of less than 1 K was achieved by increasing the value of the original feedback resistor on the operational amplifier of the thermostat circuit to 2 MΩ.

Detection Cell and Equipment. For fluorescence measurements, a specially made detection cell was used and is schematically shown in the Supporting Information. Shortly, it comprised a glass tube of 3 mm i.d. used as a detection cell flow channel. A bright green light-emitting diode (LED) of an emission wavelength of 500 nm powered by a mobile phone charger was used as an excitation light source and placed on top of a glass tube used as a detection flow cell (3, 3.5 cm × 5 mm o.d. × 3 mm i.d.) with a metal film bandpass interference filter between. The emission light was filtered by a long-path filter and detected with a photomultiplier tube. The photomultiplier tube (PMT) from Hamamatsu Phototonics K.K. (Hamamatsu, Japan, ref HS5784-04) was used for the detection of fluorescence emission and was mounted in a perpendicular position onto the glass tube.

An interference bandpass filter of 500 ± 10 nm (ref NT62-091) and a long-pass glass filter with a 580 nm cutoff wavelength (ref NT66-042) were purchased from Edmund Optics (Barrington, NJ, USA) and placed between the LED and glass tube and the glass tube and PMT, respectively. A control unit from Sciware SL was used for PMT supply and data readout. Spectra of the LED and filters used are given in the Supporting Information.

Software Control and Data Handling. The entire instrumentation used to perform the DLLME was controlled by AutoAnalysis 5.0 (Sciware SL) running on a commercial personal computer achieving complete automation of the analytical protocol (see Analytical Protocol and Flow Method).

The distinctive characteristic of this software is the possibility of using a single and versatile programming platform without further modification for whatever instrumentation, detection system, and data acquisition needed. Communication to the instrumentation assembly is based on individually loadable dynamic link libraries. The program is written in Delphi and C++ and allows the definition and execution of instruction protocols including the use of variables, loops, waiting steps, and operational procedures on a windows-based user interface. Detailed description can be found in the following papers.[33,34]

Analytical Protocol and Flow Method. The analytical procedure is given as Supporting Information. The instrument is initialized and the syringe is cleaned by the 5-fold aspiration of 0.2 mL of sample from the MPV and subsequent discharging to waste in V1 position OFF. Afterward, the sample followed by the buffer (R1) and LMG reagent (R2) were aspirated into the syringe and expulsed rapidly to the DC at MPV partition 7. For improved mixing, the content of the DC is aspirated again into S1 and then dispensed to the DC at a reduced flow rate to prolong the contact time between the liquid and the walls of the heating device, thus achieving efficient heating of the mixture and an enhanced reaction rate.

After a reaction time of 15 s, a small volume of the extraction solvent mixture is aspirated into S1 from the MPV position 8 followed by aspiration of the reaction mixture at the highest speed possible (30 mL min^{-1}). At this step, disruption of the organic solvent into small droplets of the extraction solvent is achieved at the rapid dissolution of the dispersing solvent into the aqueous sample.

After a waiting time for phase separation by the flotation and aggregation of the extraction solvent droplets at the top of the syringe, the syringe is emptied through the detection flow cell to the waste. This is done in two steps; the first step involves the slow passing of the organic phase containing the enriched reaction product for measurement with high time resolution, and the second step is the complete syringe evacuation at a flow rate of 15 mL min^{-1}.

RESULTS AND DISCUSSION

System Design and Preliminary Remarks. A simple system design was the aim. To minimize both dead volume and operation steps for saving time, the detection flow cell was mounted directly at the off position of V1. Syringe refilling was usually done in the head valve position; the off position was not required.

The detection flow cell was designed as a compromise between low dead volume in the range of the extraction solvent volume from former works (i.e., ca. 100 μL[29−31]) and detection sensitivity [i.e., maximum visible area for the PMT (65 mm^2)]. Using the described filters, a baseline of less than 2.5% of the working range was achieved. Due to the dependency of the

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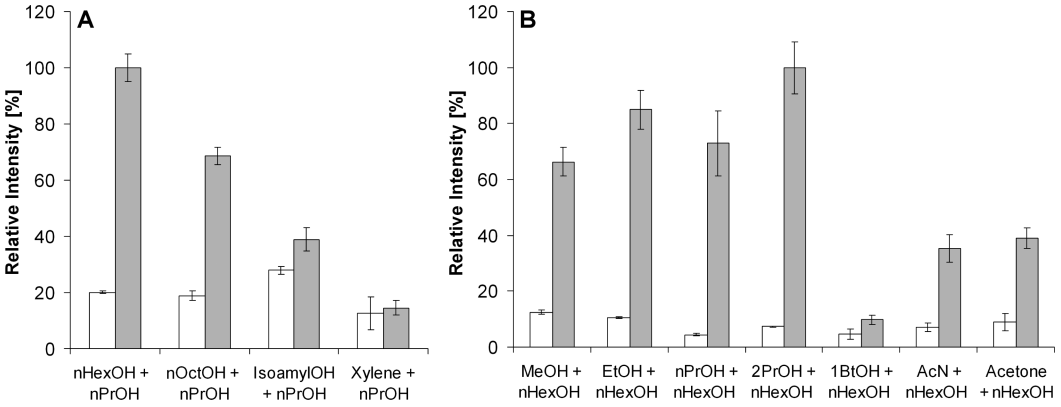

Figure 2. Study of the (A) extraction solvent using *n*-propanol as a dispersing solvent and (B) dispersing solvent using *n*-hexanol as an extraction solvent with the signal height obtained from the Milli-Q water blank samples and 500 nmol L^{-1} standard samples indicated. The standard deviation ($n = 3$) is indicated by error bars. Conditions and final concentrations: 15 μmol L^{-1} LMG, ammonium acetate 200 μmol L^{-1} (pH 5), 1 mL of solvent mixtures, a 1:10 ratio, and $T = 20$ °C.

sensitivity on both the PMT gain and the LED intensity, most results are given in the following as relative responses.

Excitation and emission spectra of the Al−LMG complex in *n*-hexanol as given elsewhere[10] have maxima at 500 and 580 nm, respectively. Thus, a LED with an emission spectrum of 500 nm was the optimal choice as the excitation light source. A higher sensitivity could have been achieved using an excitation bandpass filter of larger bandwidth and an emission filter of a shorter cutoff wavelength but to the cost of higher baseline level and noise.

Since the viscosity of *n*-hexanol used as an extraction solvent was sufficiently low, dilution after the extraction step as done in prior work was not required,[30] but measurement of the extraction solvent could be done directly after phase separation.

Sample heating was required due to the slow reaction kinetics between lumogallion and Al^{3+} as previously reported.[4] To avoid any contact with metals and due to the poor heat conductance of PTFE, a thin-walled glass capillary inserted in a heated brass support was used as a liquid guide. However, the heat transfer was inefficient at flow rates beyond 5 mL min^{-1} or a contact time of less than 5 s, respectively. Therefore, the heating step prior to the reaction was found to perform best at a flow rate of 5 mL min^{-1}.

All studies were done with both blank and standard solutions prepared with artificial seawater acidified to a pH of 3. The studies of the extraction and dispersion solvent type as well as the reaction time were performed with Milli-Q water.

Selection of Extraction and Dispersing Solvents. The main requirement of in-syringe DLLME is for an extraction solvent to be immiscible with water and of significantly lower density to allow efficient phase separation by floatation. Another characteristic of convenience is low viscosity and surface tension of the extraction solvent, since droplet fusion cannot be achieved by centrifugation but has to proceed spontaneously. Sticking to hydrophobic surfaces, such as the syringe piston head, is less pronounced for a less viscous solvent.

n-Hexanol, *n*-octanol, isoamyl alcohol, and a xylene isomeric mixture were tested as extraction solvents in 1:10 v/v percent mixtures with *n*-propanol. The results of repeated extractions of blank and standard solutions are shown in Figure 2A.

No significant extraction capacity of the complex was found for xylene. Since LMG is only moderately hydrophobic given the presence of hydroxyl groups and one sulfonate group of the molecule, the complex shows a higher affinity to slightly polar organic solvents than to nonpolar solvents.[10] Isoamyl alcohol gave an unacceptably high blank signal, on the order of the signal obtained with the standard solution. An additional problem is the high solubility of isoamyl alcohol in water.

n-Hexanol and *n*-octanol gave good extraction results with *n*-hexanol being superior to *n*-octanol with respect to both solvent characteristics discussed above and the standard signal, while blank signals did not differ significantly. Thus, *n*-hexanol was chosen for all further work.

In the next step, different dispersing solvents were tested in 1:10 v/v percent mixtures of *n*-hexanol with methanol, ethanol, *n*-propanol, 2-propanol, *n*-butanol, acetonitrile, and acetone. The results obtained from repeated extraction of Milli-Q water and standard samples are shown in Figure 2B. During the highly turbulent mixture of the organic and aqueous phases, the rapid dissolution of the dispersing solvent causes the disruption of the organic phase into small droplets of the extraction solvent, causing a nearly instantaneous multiplication of the effective extraction surface.

The relation between the standard and blank signals was found to decrease in the order *n*-propanol < 2-propanol < ethanol < methanol < acetonitrile < acetone < *n*-butanol. Hence, 2-propanol was chosen as the dispersing solvent for the study of reaction time and temperature. Although ethanol showed lower standard and higher blank signals, ethanol was chosen later since it did show much better performance when the aqueous-phase salinity was increased. This problem is explained in detail in Influence of Salinity.

Reaction Time and Temperature. It has been previously reported[4,8] that the chelating reaction of LMG with Al^{3+} is highly temperature and time dependent. Therefore, the effects of the temperature of the heating device and the reaction time in the dilution chamber on blank and standard signal heights were studied. Three different temperatures and four different reaction times were tested ranging from 20 to 42 °C and from 15 to 120 s, respectively, given as the final temperature of the reaction mixture in the dilution chamber. The results are given in the Supporting Information.

It was found that the blank signals did not show a significant dependency with time but increased slightly with temperature, which was probably due to a more efficient droplet formation at higher temperature because of a lower viscosity of the solvent. For the standard solution, strong dependency on the reaction

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temperature was found. While at 20 °C, the standard signal was about twice the blank signal and increasing slightly with time; a significant gain in sensitivity and a strong time dependency was found at a reaction temperature of 35 °C leading to stabilization for reaction times >60 s. For a reaction temperature of 42 °C, a further but less pronounced increase in sensitivity was found with no significant time dependency, indicating the steady state reaction conditions after only 15 s. Therefore, 15 s at 42 °C was chosen as the working conditions. Higher temperatures were not tested to avoid possible precipitation or incrustation in the heating device originating from seawater samples.

Influence of Salinity. Since this work was focused on the analysis of coastal seawater samples, the influence of sample salinity was studied using solutions prepared with NaCl in the range of 0−1 mol L^{-1}. A comparison between the use of ethanol and *n*-propanol as a dispersion solvent was performed with the results shown in the Figure 3.

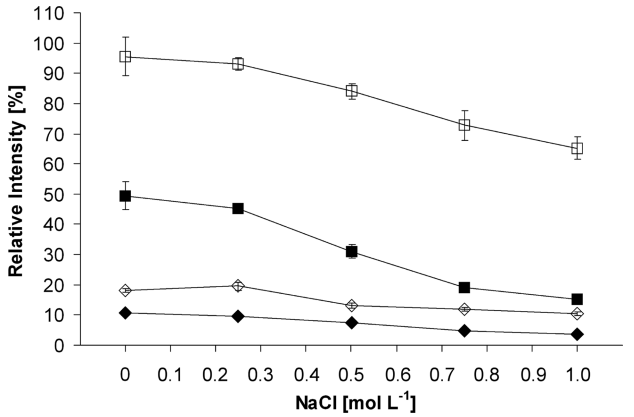

Figure 3. Influence of sample salinity on signal height of Milli-Q water blank sample (diamonds) and 250 nmol L^{-1} standard samples (squares) with the standard deviation (n = 3) indicated. Conditions as given in Figure 2, T = 42 °C, blank symbols for dispersion solvent *n*-propanol, and white symbols for dispersion solvent ethanol.

It was found that the signal decreased for both blank and standard solutions with increasing salinity with the major change observed from 0.25 mol L^{-1} to 0.75 mol L^{-1}. With ethanol used as the dispersion solvent, the signal decreased about 30% over the studied range, while *n*-propanol as the dispersion solvent made the signal decrease about 70%. The signal decrease was mainly related to the lower solubility of the dispersion solvent at a higher ionic strength of the aqueous phase, leading to less effective droplet formation and a higher content of dispersion solvent in the organic phase after extraction (i.e., dilution of the organic phase). Since the affinity of ethanol to water is much higher than it is for *n*-propanol, less dependency of the extraction efficiency on the salinity was found. For reasons of method robustness, ethanol was, therefore, chosen as the dispersion solvent, and artificial seawater was used for all further experiments.

Ratio and Volume of Extraction. Both the volumetric ratio of extraction and dispersing solvents and the absolute volume of the solvent mixture are known to affect the efficiency of DLLME.[22] Simultaneous optimization of both parameters was carried out following a 32 factorial design, with the levels of the volumetric ratio being 1:12, 1:8, and 1:6 and the levels of the solvent mixture volume being 0.6, 1.1, and 1.6 mL. Both the difference and the ratio of the standard and blank signals were used as response functions with an overall desirability ranging from 1 to 0.15 mL for the solvent mixture volume and 1 to 0.8 for the volumetric ratio (data not shown). The optima were found at a volumetric ratio of 1:8 and a volume of 0.95 mL. High robustness was achieved since a variation of 10% of both parameters did not change the overall desirability significantly.

Concentration of Lumogallion. The influence of the LMG concentration was studied in the range of 4−150 μmol L^{-1} given as the final concentration of the reaction mixture with the results given in Figure 4. It was found that the standard

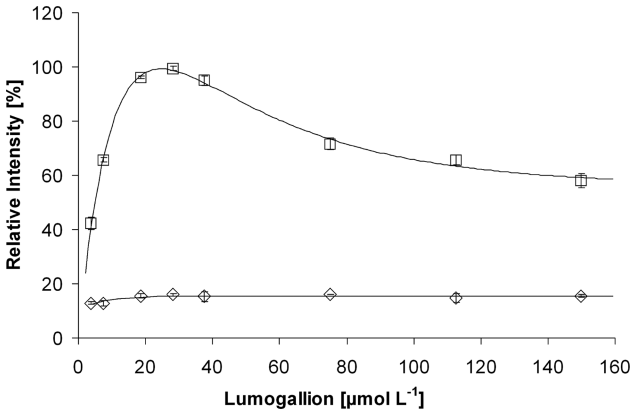

Figure 4. Influence of the concentration of LMG in the reaction mixture on the signal heights of artificial seawater blank samples (◇) and 250 nmol L^{-1} standard samples (□) with the standard deviations (n = 3) indicated. Conditions and final concentrations: 200 μmol L^{-1} buffer (pH 5.0), 0.95 mL of 1:8 ethanol/*n*-hexanol solvent mixture, and T = 42 °C.

signal increased drastically between 4 and 28 μmol L^{-1} and decreased for higher concentrations, stabilizing at a signal of half the maximum signal, found beyond 100 μmol L^{-1}. The bank signal maintained stable beyond 28 μmol L^{-1}.

While increasing the LMG concentration leads to enhanced reaction kinetics and thus an increase in complex formation, at higher LMG concentrations than 28 μmol L^{-1}, the capacity of extraction of the extraction solvent is overcome, indicated by the stabilization of the blank signal and decrease in the extraction efficiency of the Al−LMG complex.

pH and Concentration of Buffer Solution. The solubility of LMG, Al^{3+}, and its complex all depend on the pH of the aqueous phase. Since hydroxide formation of Al^{3+} is insignificant at a pH < 3.5, all samples and standard solutions were acidified to pH 3. However, this required the addition of an ammonium acetate buffer to guarantee optimal pH, which was reported to be pH 5.[9,10] Sample acidification of a pH < 3 was not done, so that the required amount of buffer could be minimized, and thus the volume of sample for the extraction procedure could be maximized. In order to avoid excessive dilution of the sample with the buffer addition, a buffer concentration of 5 mol L^{-1} was chosen.

The pH was studied in the range between 3.9 and 5.8. Sensitivity decreased significantly below pH 4.5 or above pH 5.5 with an optimum at pH 5.0 as expected, which was used further. Likewise, the final buffer concentration in the dilution chamber was studied in the range of 20−375 mmol L^{-1} acetate, with results shown in Figure 5. It was found that the sensitivity increased drastically from 20 to 150 mmol L^{-1} with a slow decrease beyond 150 mmol L^{-1}. Thus, 150 mmol L^{-1} (i.e., 300 μL) of a 2 mol L^{-1} ammonium acetate buffer was used further.

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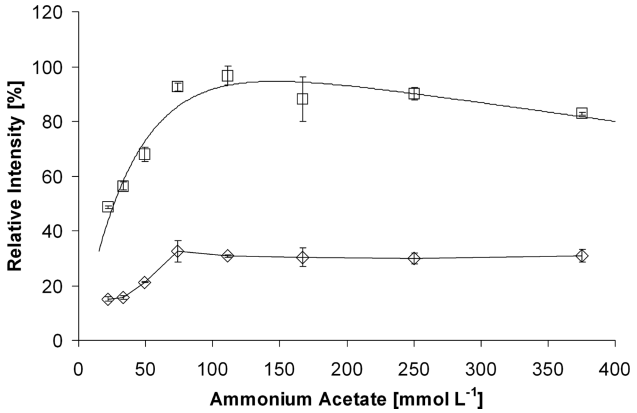

Figure 5. Influence of the ammonium acetate concentration in the final reaction mixture on the signal heights of artificial seawater blank samples (◇) and 250 nmol L^{-1} standard samples (□) with the standard deviations (n = 3) indicated. Conditions and final concentrations: 15 μmol L^{-1} LMG, 200 μmol L^{-1} buffer (pH 5.1), 0.95 mL of 1:8 ethanol/n-hexanol solvent mixture, and T = 42 °C.

At this time, the buffer solution and lumogallion were joined to one single reagent solution. It was proven that this did not affect the sensitivity or blank height of the method.

Chemical Interferences. Taking into account the results from prior works,[11,22] significant interferences of the given reaction were only found for the fluoride anion and ferric cation at typical concentrations in environmental water samples. The interference of fluoride is due to the fact that it forms a strong and poorly soluble complex with Al^{3+}, while Fe^{3+} competes with Al^{3+} over complex formation with LMG. Here, the interference of fluoride was most important due to its significant concentration in seawater in the range of 1.4 ppm.[35] The influence of fluoride can be reduced by masking it with a beryllium cation. The required Be^{2+} addition was studied by measuring a fluoride-free artificial seawater blank, standard, and standard with an addition of 2 ppm fluoride. From results shown in Figure 6, it became clear that an addition of 350 μmol L^{-1} Be^{2+} sufficiently compensates for about two-thirds of the fluoride interference but at the cost of a higher blank value, which increased slightly with a higher concentration of Be^{2+}.

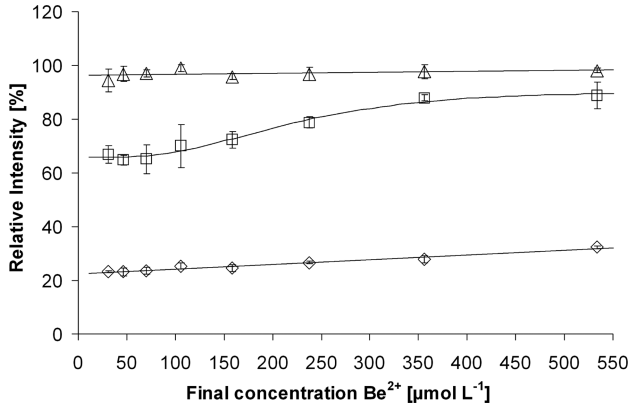

Figure 6. Influence of Be^{2+} on the signal height of artificial seawater blank samples (◇), 250 nmol L^{-1} standard samples (△), and 250 nmol L^{-1} standard samples with 2 ppm fluoride (□). The standard deviations (n = 3) are indicated. Conditions and final concentrations: 11 μmol L^{-1} LMG, 600 μmol L^{-1} ammonium acetate (pH 5.1), 0.95 mL of 1:8 ethanol/n-hexanol solvent mixture, and T = 42 °C.

The concentration of Fe^{3+} reported in coastal seawater ranges from 1 to 10 nmol L^{-1}.[36] In a former work, the addition of o-phenanthroline was used to suppress ferric cation interference in surface waters.[12] Signal heights obtained from artificial seawater without and with an addition of 11 nmol L^{-1} Fe^{3+} were compared; no significant difference was observed, and the addition of o-phenanthroline showed no significant effects. Thus, the reagent was modified only by the addition of beryllium to reach a final concentration of 350 μmol L^{-1} in the reaction mixture.

Method Performance. The proposed and optimized method was characterized by repeated calibrations proving a linear behavior of the signal height with increasing concentration up to 1000 nmol L^{-1}. A calibration example is given as Supporting Information. The calibration curve function, evaluated on 5 subsequent days, followed the equation: peak height = 1.76 ± 0.02 [L $nmol^{-1}$] · c [nmol L^{-1}] + 256 ± 8.7 (R^2= 0.999).

The system proved to be stable and robust over at least one week of operation, indicated by the low standard deviations of the blank and calibration curve slopes as well as the baseline stability, mainly influenced by fluctuations of the LED emission intensity and PMT gain. Limits of detection and quantification (LOD, LOQ) were calculated as the concentration yielding a peak height over the blank signal by a 3- and 10-fold standard deviation, respectively. An LOD of 8.0 ± 0.5 nmol L^{-1} and an LOQ of 26.7 ± 1.6 nmol L^{-1} were obtained, allowing determination of Al^{3+} in surface and coastal seawater samples. The LOD and LOQ were calculated according to the IUPAC recommendation.[37,38] The relative standard deviation (RSD) of repeated measurement was generally below 5% of the peak height. The RSD for eight replicate determinations of 200 nmol L^{-1} Al^{3+} was <1.5%.

In comparison with the former works employing lumogallion,[9,12] a lower sensitivity was achieved. The main reasons for this are most likely longer reaction times, higher incubation temperatures, more sensitive detection equipment, and additional purification of all used reagents in these studies.

The entire analytical procedure lasted about 262 s, enabling a measuring frequency of 13 h^{-1}. For each analysis, about 5 mL of sample including the required volume for syringe cleaning, 930 ng of LMG, and only 120 μL of n-hexanol and 830 μL of ethanol were required.

Validation and Real Sample Analysis. Coastal seawater samples were analyzed for evaluation of the applicability of the proposed analyzer system. For this, a rotary autosampler unit from Crison Instruments was connected to the MPV position 4 to analyze the samples rapidly one after another. The samples were acidified to pH 3 and not measured prior to at least 3 h to guarantee the total dissociation of the Al^{3+} without further treatment, sedimentation of particulate matter, and preservation at 4 °C until analysis. Sample spiking was done to evaluate the analyte recovery and matrix effects. The results are given in Table 1.

As can be seen, all samples showed concentrations in the range of 5-fold the LOQ, thus proving the suitability of the linear working range for coastal seawater samples. Standard addition gave analyte recoveries in the range from 97% to 113%, proving general applicability and adequateness of the analyzer system to real sample analysis. The results were in good agreement with the reported values for the surface seawater concentration of aluminum in the Mediterranean sea.[39] The trueness of the analytical method was proven by the

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Table 1. Results from Coastal Seawater Sample Analysis

sample	added (nmol L^{-1})	found[a] (nmol L^{-1})	recovery (%)	t_{exp}[b]
S-1	0	106 ± 12		
	100	219 ± 16	112.6	0.8
	200	318 ± 12	106.1	1.7
S-2	0	93 ± 16		
	100	197 ± 3	103.9	2.3
	200	297 ± 32	101.9	0.2
S-3	0	120 ± 20		
	100	232 ± 4	112.1	0.4
	200	322 ± 12	100.6	0.2
S-4	0	80 ± 9		
	100	192 ± 11	111.3	3.1
	200	274 ± 17	97.1	0.4
S-5	0	96 ± 12		
	100	200 ± 6	103.9	1.1
	200	292 ± 11	98.1	0.6
S-6	0	142 ± 11		
	100	245 ± 2	102.6	2.9
	200	346 ± 6	102.1	1.1
S-7	0	43 ± 6		
	100	146 ± 7	103.5	1.2
	200	239 ± 25	98.3	0.2

[a]Results are expressed as the mean value ± standard deviation (n = 3). [b]t_{crit} = 4.3.

student t test. The overall calculated values of t were ≤3.1 and given a critical value of 4.3 at the confidence level of 95%, the results did not show any significant differences from the expected concentration values.

To the best of our knowledge, no adequate reference material or commercial international seawater standards for aluminum are available. Thus, for testing the robustness and trueness of the proposed method, a dilution of the wastewater reference material SPS-WW2 was analyzed. The reference material was diluted 2000-fold to allow for the concentration to fit within the linear range of the method. The results are given in Table 2. *o*-Phenanthroline solution with a final concentration

Table 2. Results from the Measurement of Reference Material

sample	proposed method (μmol L^{-1})	certificated value (μmol L^{-1})	recovery (%)
SPS-WW2-B	254 ± 25	371 ± 2	68.6
SPS-WW2-A*	379 ± 15	371 ± 2	102.3

*To mask iron, 8 μmol L^{-1} *o*-phenanthroline was added.

of 8 μmol L^{-1} was added, as recommended elsewhere[10], to mask the considerable concentration of iron. The final concentration value calculated from the measured results was 379 ± 15 μmol L^{-1} and was compared using the student t-test to the given reference concentration value of 371 ± 2 μmol L^{-1}, with no significant difference found at a confidence level of 95%.

All calculations of the recovery percentages were made according to IUPAC.[40]

CONCLUSIONS

A novel method for the fully automated determination of aluminum in seawater using in-syringe DLLME of the Al–LMG complex and fluorimetric detection was developed. All implied chemical and physical parameters were thoroughly optimized, and the analyzer system was successfully applied to the determination of coastal seawater samples. The obtained analytical performance including limit of detection, reproducibility, repeatability, time of analysis, and data from the add-recovery test were well suited for field work application.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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