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In-syringe-stirring: A novel approach for magnetic stirring-assisted dispersive liquid–liquid microextraction

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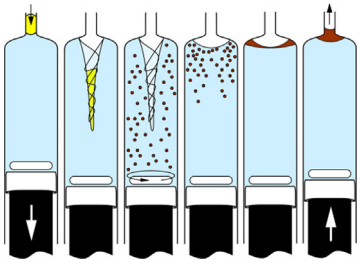
HIGHLIGHTS

- We propose a new automatic magnetic stirring assisted dispersive liquid–liquid microextraction.
- It allows the extraction of aluminum from seawater and freshwater samples within less than 4 min.
- The method was applicable to the natural samples.

GRAPHICAL ABSTRACT



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ABSTRACT

For the first time, the use of a magnetic stirrer within the syringe of an automated syringe pump and the resulting possible analytical applications are described. A simple instrumentation following roughly the one from sequential injection analyzer systems is used in combination with an adaptor, which is placed onto the barrel of a glass syringe. Swirling around the longitudinal axis of the syringe and holding two strong neodymium magnets, it causes a rotating magnetic field and serves as driver for a magnetic stirring bar placed inside of the syringe.

In a first study it was shown that this approach leads to a sealed but also automatically adaptable reaction vessel, the syringe, in which rapid and homogeneous mixing of sample with the required reagents within short time can be carried out.

In a second study in-a-syringe magnetic stirring-assisted dispersive liquid–liquid microextraction (MSA-DLLME) was demonstrated by the application of the analyzer system to fluorimetric determination of aluminum in seawater samples using lumogallion.

A linear working range up to 1.1 μmol L^{-1} and a limit of detection of 6.1 nmol L^{-1} were found. An average recovery of 106.0% was achieved for coastal seawaters with a reproducibility of 4.4%. The procedure lasted 210 s including syringe cleaning and only 150 μL of hexanol and 4.1 mL of sample were required.

1. Introduction

Dispersive liquid–liquid microextraction (DLLME) has drawn a major interest from scientists from different analytical disciplines since its first description by Rezaee et al. in 2006 [1]. This is most likely due to the possibility of high extraction efficiencies and large enrichment factors with a simple and rapid procedure.

DLLME is based on the dispersion of the extraction solvent into fine droplets, which multiplies enormously its contact surface with the aqueous sample and by this, the extraction efficiency for the analyte of interest.

The original methodology requires a dispersion solvent as major component of the organic phase, which dissolves preferably in the aqueous phase at the rapid injection of the solvent mixture into the aqueous phase. Thus, a very small amount of extraction solvent is

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effectively dispersed into droplets, which afterwards are forced to coalesce by a centrifugation step. The organic phase is then transferred into the detector or used for chromatographic separation.

However, the dispersion solvent assisted DLLME has a few inconveniences. The additional solvent leads to increase waste production. The method requires additional optimization effort (dispersion solvent quantity and kind) and, the most important, the dispersion solvent increases the solubility of the analyte in the aqueous phase. Furthermore, the distribution of the dispersion solvent between both phases and by this, the final volume of the organic phase depends on the sample salinity.

Consequently, alternative DLLME methodologies have been developed, where extraction solvent dispersion is achieved by kinetic energy. Depending on the mode of achieving droplet formation or stabilization of the droplets in the aqueous phase, ultrasound-assisted DLLME [2], air-assisted DLLME [3], vortex-assisted DLLME [4], magnetic stirring-assisted DLLME (MSA-DLLME) [5], and surfactant assisted DLLME [6] can be distinguished among others. For details, the reader is referred to recent and extensive review articles on this topic [7–10]).

In spite of the high interest in the development and application of DLLME techniques, the potential of their automation using analytical flow techniques (FT) [11,12] such as Sequential Injection Analysis (SIA) [13–15] has been widely disregarded. Direct coupling with the intended detection technique, higher reproducibility, higher sample throughput, and automated cleaning of the extraction vessel are possible benefits of FT-based automation. These have been demonstrated successfully. However, only by three distinct automation modalities so far:

1. Extraction in flow by confluence of the aqueous sample and an organic solvent mixture with droplet collection on a hydrophobic material and subsequent elution to a detection flow cell requiring additional solvent [16–18].
2. Use of an extraction vessel as a batch approach of automation of the manual DLLME protocol. The solution handling is accomplished by two separate SIA systems [19].
3. In-syringe DLLME by aspiration of the organic solvent mixture followed by sample aspiration at very high flow rate that leads to DLLME. After floating and coalescing of the solvent droplets, the organic phase is expelled into the detection flow cell [20–24].

Up to date, there are hardly any works on FT-based automation of DLLME, in which the dispersion solvent was omitted leaving alone an automation approach of air-assisted DLLME [25]. To the best of our knowledge, the present work reports the first FT-based automation of MSA-DLLME. It is based on a novel approach using a magnetic stirring bar within the syringe pump of a SIA system. Hence, a sealed but adaptable reaction vessel is obtained, in which all solutions can be aspirated with high precision and mixed homogeneously and nearly instantaneous. If air and an extraction solvent lighter than water are used, vortex formation will allow the contact of the extraction solvent with the turning stirring bar and hereby, the dispersion of the solvent into fine droplets. Stopping the stirring allows then droplet floatation, coalescence, and expulsion of the extraction solvent into a detection flow cell.

The system was used for the extraction of aluminum (Al^{3+}) as lumogallion (LMG) complex from seawater samples. This also allowed a critical comparison with a similar application but based on in-syringe dispersion solvent-assisted DLLME, which was reported recently [23]. In both works, LMG was chosen as a very selective fluorescence reagent for aluminum [26]. In contrast to the often-used morin, the LMG-Al complex is extractable into moderately hydrophobic organic solvents. It further shows low interference from sample matrix or other cations and has therefore been successfully used in oceanographic research over about three decades [27–32].

Although aluminum is a non-essential element, its determination in seawater is of interest as concentration data allow the calculation of atmospheric deposition of dust particles on the ocean surface due to its presence in numerous minerals. Then, these calculations allow the estimation of the entry of essential trace nutrients such as iron originating from the dissolution of the dust particles and which are limitation factors for growth of algae.

Herein, in-syringe MSA-DLLME is presented. The improvement of existing analyzer systems for aluminum as well as the demonstration and application of a novel extraction technique was intended with the critical discussion of its shortcomings and potential benefits for future works.

2. Methods and materials

2.1. Reagents

All chemicals were of reagent grade for analysis and ultrapure water (resistivity >18 MΩ cm Millipore Iberica S.A.U., Madrid, Spain) was used throughout. All glassware and polyethylene bottles were previously soaked in 10% (v/v) HNO_3 and rinsed with ultrapure water prior to use. All working solutions were stored in polyethylene bottles at 4 °C in the dark when not used.

An 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. Synthetic seawater (SSW) prepared according to standard recipe as given elsewhere [33] was used for most optimization experiments and for standard preparation. To eliminate aluminum contamination of the SSW, the formed $Al(OH)_3$ at the slightly alkaline pH of the SSW (pH 8) was removed by filtration through a 0.45 μm membrane filter.

Acidification was done to avoid Al^{3+} hydrolysis and loss of Al^{3+} availability for the complex formation with LMG. Adjustment to a lower pH was impractical due to the later required adjustment to the optimal reaction pH of 5.0.

A reagent solution of 1.5 mmol L^{-1} lumogallion (4-chloro-6-(2,4-dihydroxyphenylazo)-1 phenol-2-sulfonic acid) and a buffer solution of 5 mol L^{-1} of ammonium acetate (NH_4Ac) buffer, adjusted with glacial acetic acid to pH 5.4, were prepared. For extraction, n-hexanol was used throughout.

For measurement of aluminum in seawater, the interference of fluoride anion has to be taken into consideration since aluminum fluoride formation competes with the formation of the LMG-complex. In a previous work, this interference was considerably reduced at the addition of beryllium. Therefore, 25 mmol L^{-1} of beryllium nitrate were added to the LMG reagent solution to yield a final concentration of 350 μmol L^{-1} Be^{2+}, which had been found to be the optimal value in our previous work [23].

A 1 mg L^{-1} rhodamine B solution was used for studies of in-syringe homogenization by stirred-assisted mixing.

2.2. Sample collection and preparation

Coastal seawater samples were collected at different bays of the island Mallorca to evaluate the methods applicability to surface seawater analysis. The samples were acidified to pH 3 at the time of collection. The samples were measured with the proposed analyzer system without any other previous treatment but allowing only the grouse particles to sediment. Likewise, two pond water samples were collected on different places on the Mallorca Island, acidified to pH 3, and measured under the same conditions. After acidification and before measurement, the samples were allowed

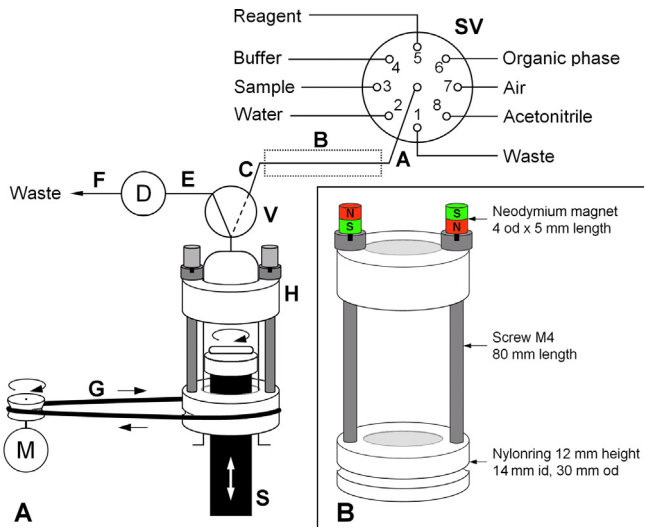

Fig. 1. (A) Analyzer manifold with selection valve (SV), syringe pump (S), solenoid 3-way head valve (V), detection flow cell (D), heating device integrated into the HC (B) and the magnetic stirring bar driver (H) placed onto the syringe barrel. A motor (M) is used to drive it via a rubber band (G). PTFE tubing (0.8 mm i.d.) of 15 cm (A, C), 10 cm (E), and 40 cm (F): 10 cm. (B) The magnetic stirring bar driver placed onto the syringe glass barrel shown in detail consisting of two nylon rings, two long iron screws and two neodymium magnets.

to stand for at least 3 h, both for sedimentation but also to ensure the dissolution of aluminum hydroxides.

2.3. Manifold configuration

The MSA-DLLME manifold is depicted in Fig. 1A with all tubing dimensions indicated. Polytetrafluoroethylene (PTFE) tubing of 0.8 mm inner diameter (id) was used for the entire manifold.

The computer controlled flow setup comprised a 5000-step multisyringe pump (Crison SL, Alella, Barcelona) and the rotary 8-port selection valve (SV, Crison SL, Alella, Barcelona) for liquid handling and distribution. The multisyringe pump was equipped with a sole glass syringe (S) of 5 mL purchased from Hamilton Bonaduz AG (Bonaduz, GR, Switzerland). A three-way solenoid head valve (V) on-top of the syringe enabled the connection to either the central port of the SV (position ON, activated) or to the detection cell and downstream located waste for quantification of the extracted analyte as well as for discharge during syringe cleaning (position OFF, deactivated).

Peripheral ports of SV were connected to reservoirs of waste (1), water (2), sample (3), buffer (4), lumogallion reagent (5), n-hexanol (6), air (7), and acetonitrile (8). Water and acetonitrile were used for cleaning of the detection flow cell or the syringe, which was routinely done daily.

The connection between the central port of the SV and the syringe head valve was done by a short holding coil (HC) consisting of two PTFE tubes of 15 cm in length holding a prior described heating device [23] in the middle. Heating was done to favor the slow reaction between LMG and Al^{3+}. Briefly, it consisted of a 12 cm long, 1.5 mm id glass tube inserted into a brass support, which was heated using a commercial halogen light bulb (12 V, 20 W). Temperature control with a hysteresis of <1 K was achieved using a control circuit from CEBEK Fadisel SL (Barcelona, Spain Ref. I-81).

2.4. Magnetic stirring bar driver

The entire analytical procedure was carried out in the syringe including sample mixing with reagents and extraction. To achieve homogeneous and rapid mixing without an additional mixing chamber as generally done [21,23,24], a magnetic Micro stirring bar (10 mm length, 3 mm diameter) was used within the syringe. This arrangement was done to the best of our knowledge for the very first time. The top position of the syringe piston was adjusted in such a way, that a gap less about 0.5 mm was left at emptying the syringe to avoid any damage.

To drive the stirring bar in the syringe, a commercial magnetic laboratory stirrer was impractical. Therefore, a rotating magnetic field was achieved by the use of a specially developed magnet driver, shown in Fig. 1B. Two rings made of nylon were used as bearings, which could be placed easily onto the syringe, with the bottom ring sliding on the flange of the syringe barrel. Two M4 steel screws of 80 mm in length were used as spacers and connection between both nylon rings. The so-obtained assembly could freely rotate around the syringe longitudinal axis.

By placing two neodymium magnets (5 mm × 4 mm o.d.) on top of the screws, the screws were magnetized and thus, a magnetic field in the syringe along its whole length was obtained. This magnetic force was sufficient to attract and, at turning the device to force the rotation of the stirring bar inside the syringe independently from the position of the syringe piston.

The bottom ring showed further a groove for the placement of a rubber band, which allowed propelling the driver with a low-cost DC motor. The DC motor was activated using a home-made relay and regulation circuit board by an auxiliary supply port of the multisyringe module. The circuit is given in Supplement material 1.

2.5. Detection equipment

A specially made detection cell was used for fluorescence measurements. A detailed description of the cell design can be found elsewhere [23]. Shortly, it comprised a glass tube of 3 mm id is used as detection cell flow channel. A bright green LED with an emission wavelength of 500 nm, powered by a mobile phone charger, was used as excitation light source and aligned with the glass tube. A photomultiplier tube (PMT) from Hamamatsu Phototonics K.K. (Hamamatsu, Japan, Ref.: HS5784-04) was used for detection of fluorescence emission and was mounted in perpendicular position onto the glass tube.

An interference band-pass filter of 500 ± 10 nm (Ref.: NT62-091) and a long-pass glass filter of 580 nm cut-off wavelength (Ref.: NT66-042) from Edmund Optics (Barrington, NJ, USA) were placed between LED and glass tube and glass tube and PMT, respectively. Spectra of the used LED and filters can be found in a previous work [23].

In addition, a polycarbonate collector lens (F 22 mm, Ø 22 mm) was placed onto the PMT to achieve higher sensitivity. A control unit from Sciware Systems, S.L. (Palma de Mallorca, Spain) was used for PMT supply and data readout. A gain of 18% was chosen for the PMT.

2.6. Software control and data handling

The software AutoAnalysis 5.0 (Sciware Systems, S.L., Palma de Mallorca, Spain) was used for operational control of the flow instrumentation as well as data acquisition from the detection equipment and data evaluation.

The program, written in Delphi and C++, allows the definition and execution of instruction protocols, including the use of variables, loops, waiting steps, and procedures on windows based user surface. Detailed descriptions of the software structure and features are given elsewhere [34].

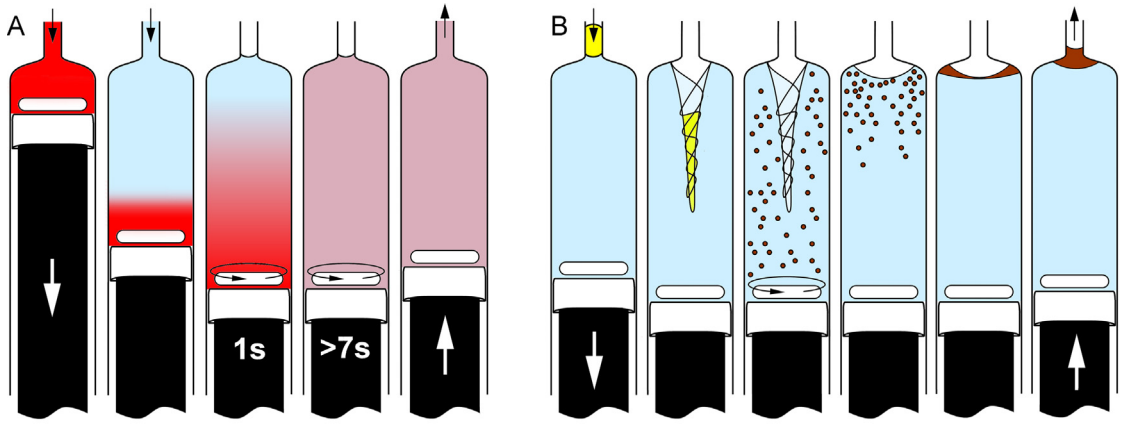

Fig. 2. Schemes of the both operation schemes tested in this work. (A) Mixing and homogenization of rhodamine B solution and water and (B) MSA-DLLME of LMG-Al complex with n-hexanol.

2.7. Analytical protocols and methods

The operation methods for testing in-syringe dilution and homogenization as well as MSA-DLLME are given schematically in Fig. 2. The operation method for MSA-DLLME is further available in Supplement material 2.

All analytical procedures required the cleaning of the syringe due to the unavoidable dead volume caused by the stirring bar. It was given by the syringe inner diameter of 10.5 mm and the height of the magnetic stirring bar of 3 mm minus its proper volumetric displacement of about 70 μL. However, the cleaning could be performed efficiently because the stirring allowed instantaneous homogenization of the dead volume in the syringe with the cleaning solution. So, three-fold aspiration of 0.8 mL of water (V in position ON, stirring activated) and discharge to waste (V in position OFF) was sufficient and allowed syringe cleaning in less than 30 s. In addition, procedures for cleaning of supply tubes on the SV and the detection cell were established.

In-syringe dilution and homogenization was studied using 1 mg L^{-1} rhodamine B solution and fluorimetric detection. Subsequently, 1 mL of rhodamine solution, 3 mL of ultrapure water, and 200 μL of air were aspirated into the syringe omitting stirring. Then, the syringe content was mixed by activation of the stirring for a defined time. Afterwards, the stirring was stopped and the syringe content was dispensed through the detection cell for the evaluation of the achieved homogenization.

MSA-DLLME was started by the aspiration of 240 μL buffer, 60 μL of LMG reagent, and 4.1 mL of sample into the syringe. Sample aspiration was done at a reduced flow rate of 4 mL min^{-1} to increase the heat transfer from the heating device to the sample and during repeated activation of the in-syringe stirring. Then, the stirring was deactivated and during a reaction time of 15 s, 150 μL of n-hexanol were aspirated into the HC to heating it up.

Afterwards, the stirring was started again and 400 μL of air were aspirated so that the n-hexanol in the HC and also part of the air could enter the syringe. The air allowed the formation of a vortex in the syringe (see Section 3.2.3). At contact of the organic phase with the stirring bar, it was dispersed into small droplets. The stirring was kept activated for 40 s to perform MSA-DLLME. The stirring speed was 2000 min^{-1}.

Afterwards, the stirring was stopped, which allowed the formed n-hexanol droplets to float and coalesce during 30 s at the brim of the concave liquid meniscus formed by the aqueous phase in the syringe. To improve droplet aggregation, the liquid surface was put in movement by a short movement of the piston (approx. 1 mm) by the instruction of complete filling just before the next step. The method was finalized by pushing the organic solvent, enriched with the LMG-Al complex, slowly through the detection cell to waste under continuous data evaluation. Finally, the remaining liquid was rapidly discharged from the syringe to waste.

3. Results and discussion

3.1. Study of in-syringe mixing

To evaluate the potential and characteristics of in-syringe magnetic stirring, the required mixing time for complete homogenization was studied for 1, 3, 7, 12, and 18 s. The experiment was done with an aqueous dye solution as well as with a dye solution prepared in a 20% (w/V) glycerol mixture. The later solution was used to simulate a sample of approximately twice the viscosity of water (about 12% higher after homogenization). The experiment was carried out in triplicate in order to evaluate the reproducibility of the mixing process. The operation scheme of this experiment is represented in Fig. 2A. The experimental conditions and the average measurements at each moment during syringe content expulsion and respective standard deviations are represented in Fig. 3.

For a mixing time of 1 s, the difference between using aqueous or 20% (w/V) glycerol dye solution was easily discernible while for 3 s, the behavior was similar. Besides, the mixing pattern after 3 s could be described as reproducible as the standard deviation decreased considerably. After 7 s of stirring, the dye gradient in the syringe was less than 5% and after 12 s, complete homogenization was achieved for both dye solutions.

It should be pointed out that later experiments showed that homogenization can be achieved even faster if a small volume of air is aspirated into the syringe permitting the formation of a vortex or evidently, if the aspiration is already initiated during aspiration of the solutions. In conclusion, in-syringe stirring permits homogeneous mixing of large volumes within a few seconds and, within the studied range, independently from the viscosity of the sample, which is in contrast to non-segmented FT.

3.2. In-syringe magnetic stirring assisted DLLME

3.2.1. Preliminary remarks

The main challenge was the study and optimization of the physical parameters related to the extraction. For these first experiments, the same reagent as optimized during a previous work was used, which seemed justified since it was based on the same chemical reaction and detection technique but on another different extraction technique [23].

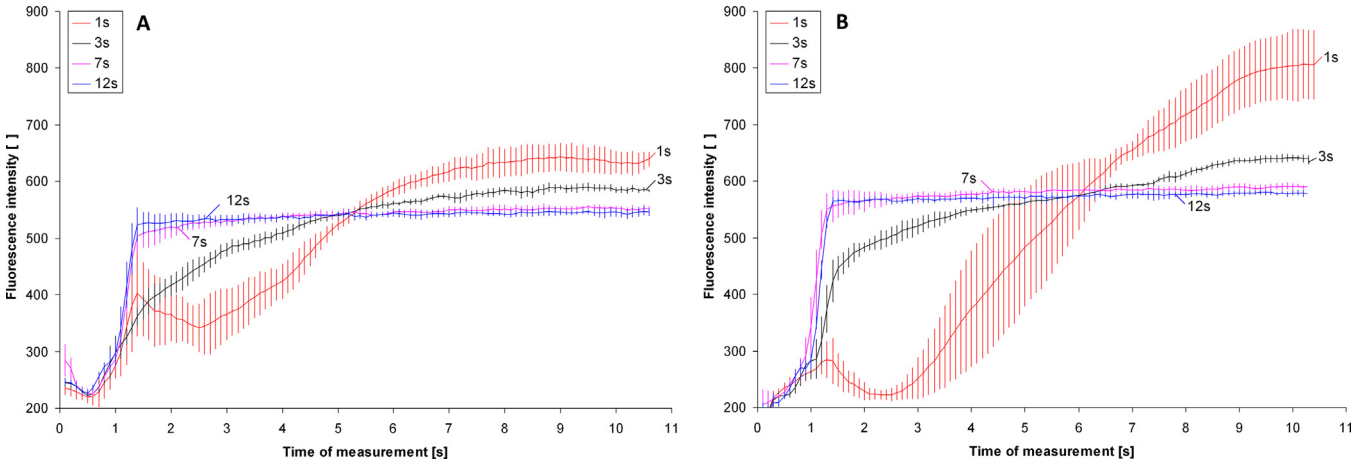

Fig. 3. Results from the study on the required time for homogenization of 1 mL of 1 mg L^{-1} rhodamine B solution with 3 mL of ultrapure water. (A) Dye solution prepared with water and (B) dye solution prepared with 20% (w/V) glycerol–water.

Likewise, n-hexanol was used for the extraction of the LMG-Al complex since it has fulfilled all requirements in the previous work. These were a lower density than water, a low solubility in water, and the best extraction capacity found of all tested solvents for the LMG-Al complex [23,29].

Therefore, only n-octanol was tested as alternative extraction solvent but discarded due to the observed sticking to the hydrophobic surfaces of the piston head and stirring bar, slow coalescence of the droplets after DLLME, and lower signal reproducibility. Other typical extraction solvents of lower polarity or lighter alcohols showed to be impractical.

3.2.2. Voltage of motor and stirring rotation speed

The stirring during the aspiration of n-hexanol into the syringe causes the disruption of the solvent into fine droplets and thus favors the mass transfer of the LMG-Al complex over a larger boundary surface increases the extraction efficiency. The influence of the voltage of the motor used for driving the stirring bar driver was studied in the range of 3.6 (ca. 1500 rpm) to 6.3 V (ca. 2600 rpm). The results are given in Supplement material 3A. The signal height escalated by factor of 2 between voltages of 4.6 V and 5.2 V. For lower voltages than 4.6 V or higher voltage than 5.2 V, the effect of voltage change on the signal height was little. A voltage of 5 V corresponded to a revolution speed of the stirrer in the syringe of about 2000 rpm. Only for higher voltages than 5 V, i.e. higher rotation speeds than 2000 rpm, a pronounced vortex was formed in the syringe and only then, the n-hexanol was drawn efficiently into the vortex and disrupted into small droplets. Therefore, a voltage of 5.5 V was chosen for all further experiments corresponding to a rotation speed of approximately 2300 rpm.

3.2.3. Volume of air

After the aspiration of n-hexanol, a volume of air was aspirated into the HC to aspirate the n-hexanol volume completely into the syringe. Vortex formation causing n-hexanol dispersion as well as droplet recombination after MSA-DLLME was improved if the volume of this air was larger than the HC inner volume (approximately 200 μL), i.e. when the head space of the syringe was partly filled with air.

The first observation is due to the fact that a vortex can be formed only on an open liquid surface in the syringe and the work required for the formation of the vortex is smaller if the surface is larger. Without air in the syringe, the extraction mode would mimic single drop extraction. However, preliminary experiments showed that this mode requires considerably longer extraction times to yield similar efficiencies, i.e. several minutes. This is in well accordance to the extraction times reported for non-automated single drop extractions [35]. The second observation is due to the formed meniscus of the aqueous phase and resulting prominence of the liquid surface on the syringe walls. Consequently, the floating n-hexanol droplets are forced to accumulate in this limited area achieving faster droplet coalescence.

The influence of the air volume on the signal height was studied in a range of 300–500 μL with conditions and results given in Supplement material 3B. It was observed, that the signal increased about 12% with a larger volume of air and also the reproducibility of measurement improved slightly from 6.6% to 2.3% RSD. On the other hand, a plus of air in the syringe also reduced the usable liquid volume in the syringe and by this the amount of sample, which could be used for the extraction procedure. Taking this into account, the effective signal increase is only 7%. Thus, a volume of 400 μL air was finally chosen.

3.2.4. Reaction and extraction times

The reaction time t_R and the extraction time t_E were optimized following a central composite experimental design. The studied range, experimental conditions, and results are given as Supplement material 4 and 5. Here, t_R is defined as the time between in-syringe mixing of sample and reagents and the addition of n-hexanol. The parameter t_E is defined as the stirring time in the presence of n-hexanol.

Two approaches were tested. In the first, n-hexanol and air are aspirated after t_R, i.e. the n-hexanol is only in short contact time with the heating device and enters the syringe nearly at ambient temperature. In the second, n-hexanol is aspirated during t_R into the HC while the air is aspirated after t_R, i.e. the solvent is allowed to heat up during t_R in the HC.

From the results it becomes clear that n-hexanol heating was highly favorable. This was due to the lower viscosity of hexanol at higher temperature and thus disruption into smaller droplets and improved extraction efficiency. Using the first approach, i.e. n-hexanol at ambient temperature, the best results were predicted for a minimal t_R, most likely because the mixture of sample and reagents in the syringe is at its maximal temperature, which decreases during t_R.

Using the second approach, i.e. pre-heated hexanol, signal heights were doubled. Longer t_R were advantageous due to proceeded reaction yield with a predicted maximum at 20 s. Simultaneously, shorter t_E were required than in the first approach where maximization of t_E was predicted as optimal in order to

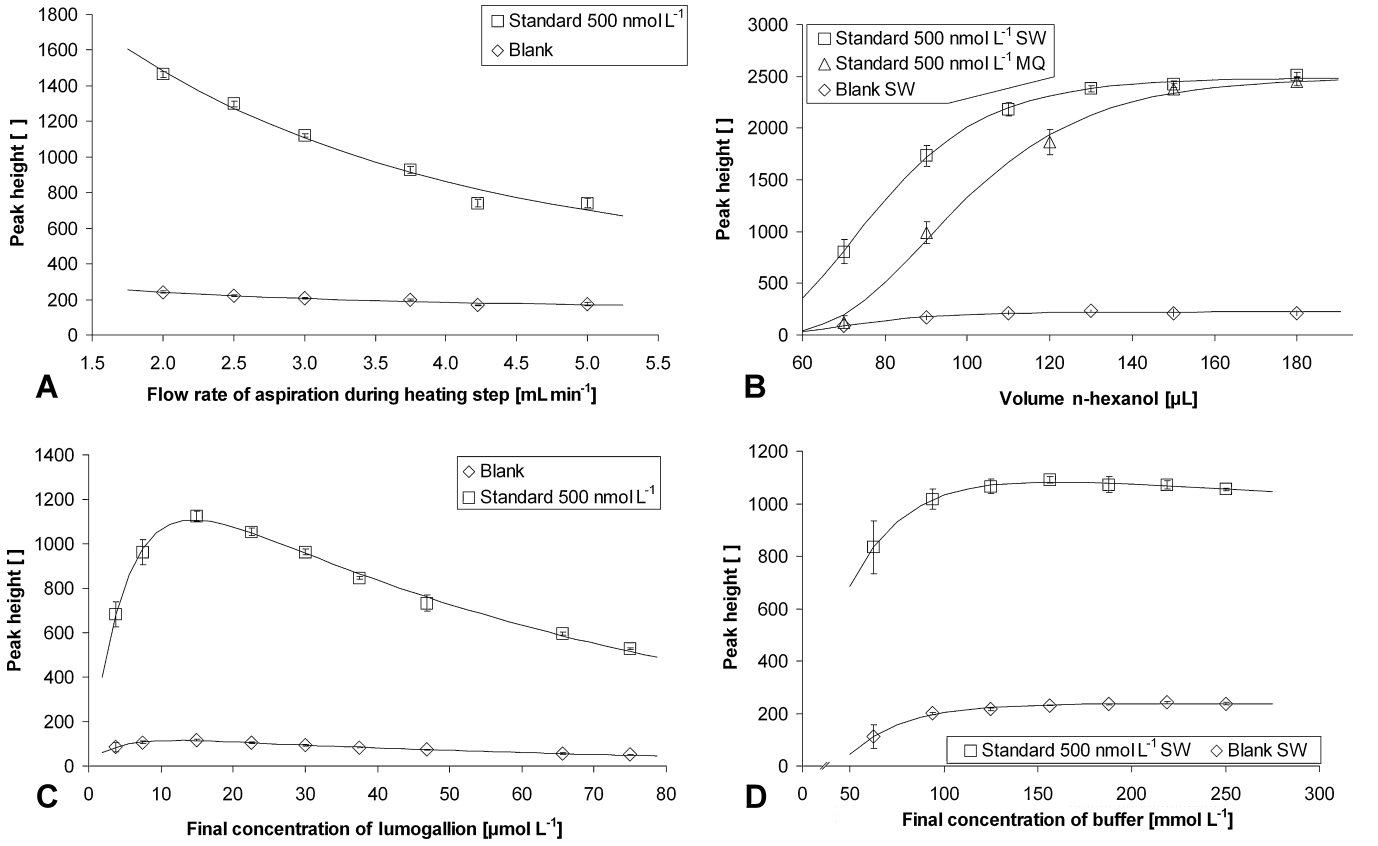

Fig. 4. Influence of aspiration flow rate (A), the volume of n-hexanol (B), the concentration of lumogallion (C), and NH4Ac buffer (D) in the final mixture. Conditions 500 nmol L^{-1} aluminum ultrapure standard (triangles), SSW standard (squares) and SSW blank (diamonds), motor voltage 5.5 V, 400 µL air, reaction temperature 45 °C, 15 s reaction time, 40 s extraction time. Further: (A) with 150 µL n-hexanol, 120 µL of 365 µmol L^{-1} LMG and 5 mol L^{-1} NH_4Ac pH 5.1. (B) as (A) with aspiration flow rate 4 mL min^{-1}, (C) as (B) with 150 µL n-hexanol, (D) as (C) with 60 µL 1.5 mmol L^{-1} LMG reagent.

compensate the shorter reaction time. On the base of the found results and optimization, 20 s of t_R and t_E of 40 s applying the second approach of heated n-hexanol were used further.

3.2.5. Volume of the extraction solvent

The volume of n-hexanol was studied in the range of 70–180 µL for blank SSW and for two 500 nmol L^{-1} Al^{3+} standards, one prepared with SSW, the other one prepared with ultrapure water. The results and experimental conditions are given in Fig. 4A.

It was found that for the SSW blank and SSW standard, the signals increased up to 130 µL reaching a stable level beyond. A similar behavior was observed for the signal obtained with the ultrapure standard. Here, the same signal level was reached but an about 20 µL larger volume of n-hexanol was required to achieve comparable results, being the result of the higher solubility of n-hexanol in ultrapure water compared to saltwater.

Visual inspection and the obtained results allowed the following conclusions. First, a small portion of the n-hexanol was not disrupted into droplets but remains floating at the surface explaining why the DLLME was less efficient at n-hexanol volumes lower than 100 µL. Second, the ionic strength of the sample did not affect the signal height as long as the solubility of n-hexanol in the sample [<6 µL mL^{-1}] is taken into account. The signal heights obtained with ultrapure and SSW standards with 150 µL of n-hexanol did not differ significantly (3% found), i.e. the dependency of the signal height on the ion strength of the sample solution was minimal. As consequence, a volume of 150 µL of n-hexanol was chosen for all further work. Increasing the n-hexanol volume beyond the studied range would probably have led to decreasing signal heights due to dilution of the extracted LMG-Al complex in the solvent.

3.2.6. Flow rate for sample aspiration

The flow rate for sample aspiration into the syringe was of high interest since it determined the contact time of the sample with the heating device integrated in the HC. A lower flow rate would favor a faster reaction rate and higher yield as reported elsewhere [23,28,29] but prolong the method execution time. A higher sample temperature could further improve the extraction efficiency due to lower viscosity, while the antagonistic effect of a higher solubility of n-hexanol and the LMG-Al complex in the aqueous phase has to be considered.

The effect of the flow rate on the signal height was studied in the range of 2–5 mL min^{-1}. The experimental conditions and results are represented in Fig. 4B. It was found that the signals of both SSW blank and standard followed an exponential decrease with higher flow rates. As a compromise between sensitivity and time of analysis, a flow rate of 4 mL min^{-1} was chosen.

At the adjusted temperature of the heating device of 65 °C, the sample entered the syringe at the chosen flow rate with about 45 °C. A higher heating device temperature was discarded to avoid bubble formation.

3.2.7. Concentration of lumogallion

After optimization of the physical parameters, the reagent composition, chosen from the previous work [23], was re-evaluated. First, the influence of LMG quantity in the aqueous phase before extraction was studied in the range of 3.8–75 µmol L^{-1} final concentration using a 500 nmol L^{-1} SSW standard and SSW blank. The results and experimental conditions are given in Fig. 4C.

It was observed that both the standard and blank signals increased rapidly up to a maximum at 15 µmol L^{-1} with a less

Table 1
Results from the analysis of costal seawater samples and natural water samples and with one spiked concentration. Conditions as in Fig. 4 D.

Type	Added concentration [nmol L^{-1}]	Signal ($n = 3$)	Found concentration [nmol L^{-1}]	Recovery
Seawater 1	0	504.2 ± 73.1	104.5	
	50	598.1 ± 20.2	153.1	97.3%
Seawater 2	0	1070.1 ± 51.5	398.8	
	100	1281.5 ± 105.1	508.7	109.9%
Seawater 3	0	496.0 ± 15.9	100.5	
	100	701.7 ± 27.3	207.4	106.8%
Seawater 4	0	378.2 ± 11.5	39.3	
	200	838.0 ± 39.1	278.2	119.4%
Seawater 5	0	475.3 ± 1.5	89.7	
	100	674.9 ± 8.7	193.4	103.7%
Pond water 1	0	581.1 ± 42.8	144.7	
	200	962.6 ± 59.2	342.9	99.1%
Pond water 2	0	400.3 ± 9.7	50.8	
	200	838.8 ± 39.4	262.9	106.1%

pronounced decrease for higher concentrations. This behavior was most likely due to the moderate solubility of the LMG-Al complex in water and thus retention of aluminum in the aqueous phase at high LMG concentration. Therefore, a LMG concentration of 15 μmol L^{-1} was chosen yielding the maximal signals.

3.2.8. Concentration of buffer

NH_4Ac buffer was used to adjust the optimal reaction pH and to increase the ionic strength of the aqueous phase to improve the extraction efficiency and to decrease its solubility for n-hexanol. A larger volume of buffer favors the method's robustness in respect of the pH and ionic strength of the proper sample. However, a larger volume of buffer also implies a smaller volume available for sample in the syringe and so, a lower possible preconcentration factor.

The effect of the final buffer concentration in the aqueous phase was studied in the range of 60–250 mmol L^{-1}. The NH_4Ac buffer solution was prepared highly concentrated (5 mol L^{-1}). At this concentration, the measurement of the pH value with a commercial pH meter is not reliable, so that the buffer was adjusted to pH 5.4, which yielded the reported optimal reaction pH value of 5.0 [23,29] at a 50-fold dilution with ultrapure water. Results and experimental conditions are given in Fig. 4D.

While the blank signal increased with the buffer concentration up to 150 mmol L^{-1} and remained stable beyond, a clear signal maximum was found at 150 mmol L^{-1} NH_4Ac for a 500 nmol L^{-1} acidified SSW standard. Therefore, this concentration was chosen as optimal.

3.2.9. Phase separation time

The time of phase separation by droplet flotation and aggregation was tested for 20, 30, and 40 s using a 500 nmol L^{-1} aluminum SSW standard. Average signal heights of 3 subsequent extractions yielded 1771 ± 82, 1915 ± 33, and 1949 ± 13 units for 20, 30, and 40 s, respectively. As expected, the signal height increased and the signal reproducibility improved with longer times but less pronounced from 30 to 40 s than from 20 to 30 s. To minimize the execution time, a phase separation time of 30 s was chosen for all further work.

3.2.10. Real sample analysis and analytical performance

Calibration was done with standards up from to 1.9 μmol L^{-1} and found to be linear up to at least 1.1 μmol L^{-1}. At 1.4 μmol L^{-1}, the deviation from the extrapolated calibration curve was still only about 6%. The calibration curve followed the function signal height = 1.925 [L $nmol^{-1}$] · c [nmol L^{-1}] + 302.6, r^2 = 0.998. The limit of detection and the limit of quantification were calculated from the triple and 10-fold standard deviation of the blank and the calibration curve slope yielding 6.1 nmol L^{-1} and 20.2 nmol L^{-1}, respectively.

The preconcentration factor was calculated from the used sample volume and the final volume of hexanol (ca. 125 μL) to be about 33. The extraction efficiency can be estimated to be higher than 95% since the baseline found for the aqueous phase after the extraction was negligible.

To evaluate the applicability of the proposed automated MSA-DLLME method, five coastal surface seawater samples and two pond-water samples were measured with the described analyzer system. All samples were further spiked with aluminum standards in a similar concentration range as the natural concentration. The same calibration prepared with synthetic seawater water was used for the evaluation of all samples. The results are summarized in Table 1.

The sample concentrations were all within the linear working range. An average recovery of 106.0% ± 7.3% was achieved, 102.6% ± 5.4% omitting seawater sample 4, for which the recovery values exceeded the acceptable range for unknown reason.

The method showed to be applicable to the determination of aluminum in various water samples. The positive deviation of most recovery values was most likely due to the fact that spiking was done directly before analysis without aging time. Since in this work, similar conditions of the chemical parameters have been applied as in our previous work, and the reaction is well-known to be highly selective, a study of single components as potential interferences was not repeated.

An average repeatability of 3.3% ($n = 4$) was found for calibrations standards while for real samples, the average value was slightly higher with 4.4% ($n = 4$). The entire analytical procedure including initial syringe cleaning took 210 s, allowing a measuring frequency of 17 h^{-1}. In contrast to MSA-DLLME based on manual operation [4], the proposed system achieved efficient extraction in 40 s instead of several minutes and under fully automated conditions. Thus, the analytical performance was adequate for the determination of aluminum in all tested matrices.

In comparison with a former work using the same reaction and instrumentation but based on dispersion solvent assisted DLLME [23], about 25% lower LOD and LOQ values, a 20% shorter time of analysis and an 8% higher sensitivity were achieved by simultaneous reduction of organic solvents from 950 μL to 150 μL. The repeatability and linear working range were comparable.

Another improvement over the former work was a higher robustness in respect of the sample salinity due to the omission of the dispersion solvent under the optimized conditions, i.e. a n-hexanol volume of 150 μL as discussed in Section 3.2.5. This was also demonstrated by the fact that the same calibration with standards prepared with SSW was adequate for both freshwater and seawater samples and the recovery values found both sample types were comparable.

Table 2
Comparison with prior methods for the determination aluminum using solvent emulsification or DLLME, respectively.

Extraction solvent [μL]	RSD [%]	LOD [ppb]	ULR [ppb]	Sample [mL]	Time [min]	Extraction	Detection	Ref.
600	1.7	0.05	–15	25	<10	IL-DLLME	FL	[40]
132	4.5	0.8	–250	20	>8	DLLME-SFO	ICP-OES	[44]
48	2.6–5.3	0.6–0.9	–1000	10	>11	US-DLLME	ICP-OES	[42]
75	3.2	1.7	n.g.	10	>15	USILDLLME	UV–vis	[43]
98	1.87	0.13	–1000	10	>10	US-DLLME	ICP-OES	[41]
950	<5	0.22	–27	3.9	4.4	In-syringe DSA-DLLME	FL	[23]
150	3.3–4.4	0.16	–33	4.1	3.5	In-syringe MSA-DLLME	FL	This work

Abbreviations: DSA, dispersion solvent assisted; DLLME, dispersive liquid–liquid mixroextraction; FL, fluorescence; IL, ionic-liquid based; IS-MSA, in-syringe magnetic stirring assisted; n.g., not given; SE, surfactant enhanced; SFO, solidification of organic drop; US, ultrasound assisted; ULR, upper linear working range limit; UV–vis, spectrophotometry.

These improvements are considered to be related to the possibility to perform the whole procedure including reagent and sample mixing in the syringe, i.e. faster, and due to the omission of dispersion solvent, which permitted the use of a larger volume of sample (4.1 mL), and a high and reliable extraction efficiency, which is not affected by the mixture of dispersion solvent and aqueous phase.

3.2.11. Comparison and outlook on further potential and applications

A comparison with prior reported methods based on emulsification of extraction solvent and sample or dispersive liquid–liquid microextraction for the determination of aluminum is given in Table 2. Apart from this and our former work [23], all other works reported manual procedures. The proposed work was found comparable or better in respect of most characteristics to the prior reported applications whereas the preconcentration factor and the linear working range were smaller. Nevertheless, the working range could be extended by in-syringe dilution of the sample with ultrapure water. In respect to the time of analysis, the presented work was clearly superior. However, it has to be taken into account that manual procedures allow treating several samples in parallel and consequently allow increasing the effective sample frequency.

The presented work is the first application of in-syringe MSA-DLLME and was done with the intention to demonstrate the potential of in-syringe stirring in comparison with dispersion solvent assisted DLLME [23]. The usefulness of in-syringe stirring when dealing with samples of distinct viscosities was further proven.

As a disadvantage, the dead volume produced by the stirring bar has to be addressed. However, syringe cleaning can be performed fast and, due to the stirring, very efficient. It should be pointed out, that no memory effect was observed and cleaning with water was sufficient to avoid cross-over of analyte at the change of sample or standard solution.

On the other side, the use of external extraction chambers elsewhere proposed [19] implies even more time for cleaning since the entire extraction chamber needs to be filled with the cleaning solution and then emptied. If only one pump is used, this takes an additional step to re-aspirate the cleaning solution from the extraction chamber before discharging it to waste. Using in-syringe extraction, the inner walls of the "extraction chamber" (the syringe) are wiped by the syringe piston and the required volume to clean is reduced to the small dead volume, so that not the entire syringe has to be cleaned.

Temperature turned out to be one of the most important parameters of this work due to its influence on both the liquid viscosity and the reaction rate. A more efficient heating device could therefore improve the achieved performance. Another improvement might be possible by fluorimetric measurement of the organic phase directly in the syringe following the recently proposed methodology of Lab-In-A-Syringe [22]. Such combination could also enable in-syringe titrations with the interesting characteristic that the titration vessel can be adapted in volume without the interference of air.

The proposed instrumentation could further be used to carry out classical analytical protocols, i.e. the step-wise addition and mixing of small volumes of reagents to a large volume of sample such as done in "batch automation" [36–38]. Finally, the proposed system could be coupled to liquid or gas chromatography to carry out sample clean-up and analyte pre-concentration.

In comparison with former in-syringe automation in syringe extraction but posterior derivatization in a reaction vial [39]. Here, both, the reaction and the extraction were carried out in-syringe, since the stirring action enabled complete mixing of all solutions. Nevertheless, fully automation could only be achieved, if standard preparation and sample provision using an autosampler would be enabled.

4. Conclusions

A stirring bar placed into the syringe of a computer controlled syringe pump was used for the first time for magnetic stirring-assisted dispersive liquid–liquid microextraction (DLLME). The optimized method enabled efficient DLLME within a comparably short time and is based on the disruption of the extraction solvent by the kinetic energy of the swirling stirring bar. Better or similar analytical performance than in previous works based on DLLME was achieved and the method's applicability to the determination of aluminum in surface seawater and freshwater samples was proven. Dependency of the analytical performance on the sample salinity and viscosity was demonstrated to be widely overcome. In-syringe stirring can enable novel protocols for sample preparation, analyte pre-concentration, and complex analytical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2013.05.049.

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