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In-syringe magnetic stirring-assisted dispersive liquid–liquid microextraction for automation and downscaling of methylene blue active substances assay

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ABSTRACT

A simple and rapid method for the determination of the methylene blue active substances assay based on in-syringe automation of magnetic stirring-assisted dispersive liquid–liquid microextraction was developed. The proposed method proved to be valid for the determination of anionic surfactant in waste, pond, well, tap, and drinking water samples.

Sample mixing with reagents, extraction and phase separation were performed within the syringe of an automated syringe pump containing a magnetic stirring bar for homogenization and solvent dispersion. The syringe module was used upside-down to enable the use of chloroform as an extraction solvent of higher density than water.

The calibration was found to be linear up to 0.3 mg/L using only 200 μL of solvent and 4 mL of sample. The limits of detection (3σ) and quantification (10σ) were 7.0 μg/L and 22 μg/L, respectively. The relative standard deviation for 10 replicate determinations of 0.1 mg/L SBDS was below 3%. Concentrations of anionic surfactants in natural water samples were in the range of 0.032–0.213 mg/L and no significant differences towards the standard method were found. Standard additions gave analyte recoveries between 95% and 106% proving the general applicability and adequateness of the system to MBSA index determination. Compared to the tedious standard method requiring up to 50 mL of chloroform, the entire procedure took only 345 s using 250-times less solvent.

1. Introduction

Anionic surfactants (AS) [1] are the most common surfactant group used in industrial detergent formulation, cosmetics, and household cleaners [2] and their consumption of AS is steadily increasing due to the raise of population. Although AS are biodegradable [3] it is well known that high concentrations of anionic surfactants in water can harm aquatic organisms [4,5]. Because of the quantity originated from wastewater treatments plants effluents and untreated urban wastewater discharges [6] is high, many aquatic ecosystems receive large quantities of AS. So that AS can also be found in surface and groundwater endangering the quality of drinking water. Hence, determining AS is of interest for environmental and health studies [7,8] as well as quality and safety control. The European environmental regulations established a maximum tolerated limit of 0.2 mg/L for AS in water supplies for human consumption [9].

The most commonly reference method used to determine AS as sum parameter in water is the methylene blue active substance index (MBAS) [10]. This method consists in the formation of ion-pairs between AS and the cationic dye methylene blue (MB) followed by their extraction into chloroform and determination of the extracted complexes by spectrophotometry. However, the reference method is not only long and tedious but also presents a series of drawbacks such as consumption of large volumes of sample and chloroform being a toxic organic solvent. To address these drawbacks, a number of studies were focused on the development of miniaturized and environmentally benign methods based on liquid–liquid extraction (LLE) automated using analytical flow techniques (FT). In Table 1, an overview and comparison of these methods is given. FT-based LLE was first proposed by Karlberg and Thelander [22] and Bergamin et al. [23] who demonstrated minimization of sample and reagent consumption, risk of sample contamination, and operator's intervention as well as enhanced sampling throughput. The determination of AS based on the coupling of LLE and FT was reported for the first time by Kawase et al. [11] in 1978. Analytical procedures used for the determination of AS are reviewed elsewhere [24]. In 2006, a new

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Table 1
Comparison of various flow methods for determination of MBAS index in water samples with developed method.

Flow technique	Extraction technique	Solvent volume (μL)	Dynamic range (mg/L)	LOD (mg/L)	RSD %	DR (h^{-1})	Refs.
FIA	MLLE	1770	< 360	4	1.5	80	[11]
FIA	LLE	490	0.1–4	–	3.0	50	[12]
FIA	LLE		0.04–3.5	0.04	1.2	20	[13]
FIA	LLE	200	0.1–1	0.07	6.7	20	[14]
FIA	DBALLME	2	< 5.0	0.4	5.0	15	[15]
FIA	MLLE	–	0.02–5	–	–	–	[16]
FIA	–	500	< 6	–	4.6	10	[17]
FIA	MMLLE	–	70–700	35	1.8	50	[18]
SIA	LLE	300	1–10	0.5	5.0	5	[19]
MCFA	MM	700	0.2–1.7	0.008	5.9	20	[1]
FIA	LLME	50	0.03–0.3	0.02	2.4	240	[20]
MCFA	LLME	44	0.05–2.0	0.02	1.5	18	[21]
SIA	MSA-DLLME	220	0.025–0.3	0.007	3	10	This work

Abbreviations: DBALLME, drop-based automated liquid–liquid extraction; DLLME, dispersive liquid–liquid microextraction; DR, determination rate; FIA, flow injection analysis; MCFA, multicommuted flow analysis; LLE, liquid–liquid extraction; LLME, liquid–liquid microextraction; LOD, limit of detection; MLLE, membrane liquid–liquid extraction; MM, multicommutated; MMLLE, microporous membrane liquid–liquid extraction; MSA-DLLME, Magnetic stirring-assisted dispersive liquid–liquid microextraction; RSD, Relative standard deviation; SIA, sequential injection analysis.

concept of miniaturization of LLE was proposed by Rezaee et al. [25] denoted dispersive liquid–liquid microextraction (DLLME). A mixture of an extraction solvent and a dispersion solvent with high miscibility in water is rapidly injected into an aqueous sample to form a cloudy component emulsion. By centrifugation, the extraction solvent containing the enriched analytes can be separated and then injected into an appropriated analytical instrument. The advantages of DLLME are its simplicity of operation, rapidity, low cost, high-recovery, high enrichment factor, and minimal waste generation [26]. However, the distribution coefficient of the analyte between organic and aqueous phase could be altered by the dispersion solvent making a comparison with standard protocols based on classical LLE difficult. Besides, method optimization requires finding a suitable dispersion solvent as well as an optimal mixing ratio with the extraction solvent. The alternative to tackle these problems was the replacement of the dispersion solvent by kinetic energy leading to air-assisted [27], vortex-assisted [28], ultrasound-assisted [29], magnetic-stirring-assisted (MSA) dispersion [30]. More recently, the concepts of DLLME and FT automation were combined [31–33]. Here, in-syringe DLLME has demonstrated to be a specially promising technique for automated DLLME, [34–37] with the late report of automated in-syringe MSA-DLLME [38,39] due to its simplicity and versatility. The aim of the present work was to develop a simplification of the MBAS method based on in-syringe MSA-DLLME with the novel modification that the syringe was used upside down in order to use chloroform as extraction solvent to achieve comparability towards the standard procedure for MBSA determination.

2. Material and methods

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals from Scharlab SA (Barcelona, Spain) unless otherwise indicated and bi-distilled quality water provided by a Milli-Q Direct-8 purification system (resistivity > 18 MΩ cm, Millipore Iberica S.A.U., Spain) was used throughout. All material were previously soaked for at least 24 h in 10% (v/v) HNO_3 and rinsed with water before used. A stock solution of 10 mg/L sodium dodecyl benzene sulphonate (SDBS) (Sigma Aldrich, Steinheim, Germany) was used as standard solutions of anionic surfactants. For calibration, SDBS standard working solutions were prepared daily by appropriate dilution. A stock solution of 700 mg/L methylene blue (MB) (Panreac SA, Barcelona, Spain) was prepared by dissolution of an appropriate amount of the reagent in Milli-Q water. A solution of 127 mmol/L sodium hydrogen phosphate and 100 mmol/L H_2SO_4 were used for in-syringe buffer preparation. To accelerate phase separation, a 648 mmol/L Na_2SO_4 solution was used as additional reagent. Chloroform was used as extraction solvent without any previous treatment. All reagent solutions were kept in glass bottles at 4 °C.

For the reference procedure, the following solutions were used as recommended [10]: MB solution: 30 mg/L MB in sulfuric acid–sodium phosphate buffer (concentrations 0.123 mol/L and 0.362 mol/L, respectively) and washing solution being the same buffer but without MB.

Solutions used in interference studies were prepared from $CaCl_2$, $MgCl_2 \cdot 2H_2O$, NH_4Cl, $AlCl_3 \cdot 6H_2O$, $Pb(NO_3)_2$, $CuSO_4 \cdot 5H_2O$, $FeCl_3 \cdot H_2O$, $NaNO_3$, $NaNO_2$, NaCl,$NaHCO_3$, Triton X-100, humic acid and CTAB. The substances were chosen in agreement with former interference studies [20,21]. In order to study the influence of water hardness on the extraction process, artificial freshwaters of different hardness grades were prepared according to standard recipes for “very hard water”, “hard water” and “moderately hard water” [10].

2.2. Sample collection and preparation

Different natural water samples were collected and analyzed: drinking water, pond water, well water, and tap water from different places on Mallorca and wastewater from entrance and effluent of a local biological treatment plant. Samples were collected in polyethylene bottles and stored at 4 °C until analysis. Wastewater samples and pond water were paper-filtered to remove suspended particles.

2.3. Manifold configuration

The system used in this work is depicted in Fig. 1 and follows a prior designs [38,39]. It comprised a 5000-step syringe pump (SP) from Crison SL (Alella, Barcelona, Spain) with a 5 mL glass syringe (S) and a rotary 8-port multiposition valve (MPV) from Sciware System SL (Palma de Mallorca, Spain). PTFE tubing of 0.8 mm inner diameter (id) was used for the entire manifold. A short PTFE tube was placed into the syringe inlet to minimize the dead volume. A three-way solenoid head-valve (V) on top of the syringe enabled the connection to either the central port of the MPV (position ON, activated) or to a detection cell and downstream located waste for quantification of the extracted analyte and discharge during syringe cleaning (position OFF, deactivated). Peripheral ports of the MPV were connected to reservoirs of waste (1), water (2), sample (3), MB (4), NaH_2PO_4 (5), chloroform (6), air (7), H_2SO_4 (8), and Na_2SO_4 (9). The connection between the common port of the MPV and the syringe head-valve was done by a holding coil (HC) of 26 cm in length. For sample measurements, a 15-position rotary autosampler from Crison SA was used. For dispersion of the extraction solvent, a magnetic stirring bar (10 mm × 3 mm in diameter) was placed inside the syringe.

In this work, given the fact that the extraction solvent had a higher density than water and thus accumulated at the bottom, the syringe module was used upside-down.

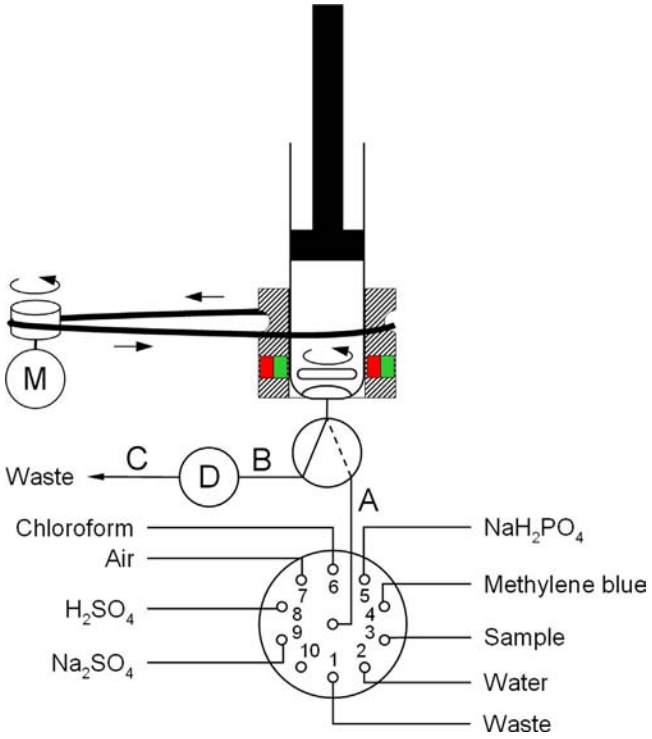

Fig. 1. Schematic manifold used for in-syringe magnetic stirring-assisted dispersive liquid–liquid microextraction (MSA-DLLME) MBAS determination. The manifold was composed of a multiposition valve (MPV), syringe pump (S) with a magnetic stirring bar inside, solenoid 3-way head valve (V), detection flow cell (D), and a DC motor (M), which is used to drive it via a rubber band. PFTE tubing were in length: 26 cm ((A), HC), 5 cm (B), and 50 cm (C).

As detection system, a USB 2000 CCD spectrometer, a deuterium-halogen light source (DH-2000-BAL), and optical fiber of 400 μm core diameter (all purchased from Ocean Optics, Dunedin, FL, USA) were used. A flow cuvette of 1 cm optical path length and 1.5 mm flow channel diameter from Hellma Analytics (Müllheim, Germany) and a fiber-optics cuvette support from Ocean Optics was used throughout. The cell was connected via a 10 cm long PTFE tube of 0.8 mm id to the OFF position of V. Furthermore, to improve the wettability of the cuvette for the organic phase, one-time silanization was done by flushing the cuvette subsequently with piranha solution (3:1 mixture of concentrated H_2SO_4 and 30% hydrogen peroxide), 2 mol/L of NaOH, water-free methanol, and water-free toluene. Then, the cuvette was blown dry by nitrogen flow and a 1:10 mixture of dichlorodimethylsilane in water-free toluene was let react with the free hydroxyl-groups of the wall surface for 10 min. Finally, the cuvette was flushed with methanol. It should be pointed out that preparation and handling of the solutions for silanization should be done with great care, under fume hood, and in the minimum amount possible (here <5 mL). Piranha solution is an extremely strong oxidizing and unstable reagent tending to decompose at the presence of smallest amounts of catalysts.

2.4. Magnetic stirring bar driver

The prior described magnetic stirring device to generate a rotating magnetic field [38] was simplified since the stirring bar remains at same position at the bottom of the syringe. It was made of a Deldrin® tube of 20 mm × 25 mm in diameter, which fitted snugly over the syringe glass barrel (14 mm inner diameter). It held two small neodymium magnets (5 mm × 4 mm in diameter), which were strong enough to levitate the stirring bar inside the syringe. Turning the driver device by a DC motor connected via a rubber band forced the stirring bar to rotate at equal speed. The motor was activated using a homemade relay and regulation circuit board enabling two different stirring speeds by employing two auxiliary supply ports of the syringe pump. The stirring speeds were approximately 1000 rpm and 2000 rpm for, slow mixing and solvent dispersion for DLLME, respectively. The circuit to control the motor is given elsewhere [38].

2.5. Data acquisition and evaluation

Absorbance measurements of the chloroform phase were done at 656 nm, corrected at a wavelength of 710 nm where MB did not show any significant absorbance, allowing the correction of analyte unspecific intensity variations. The instrumentation was controlled by the software package AutoAnalysis 5.0 (Sciware Systems SL) achieving complete automation of the analytical protocol (see Section 2.6) as well as data acquisition and processing [40,41]. Design of experiments and result evaluation were done with the software package STATISTICA 8.0. The difference of the absorbance between standard and blank signal was used as analytical response.

2.6. Analytical protocol and flow method

The method for MSA-DLLME is given as Supplementary material S-1. Additionally, the analytical protocol is given schematically in Fig. 2. First, the syringe was cleaned by three-fold aspiration of 0.5 mL of water (stirring activated) and discharge to waste. Then, the following solutions (for concentrations see Section 2.1.) were subsequently aspirated into the syringe: 200 μL of $NaSO_4$, 130 μL of H_2SO_4, 200 μL of NaH_2PO_4, 100 μL of MB, and 3.7 mL of sample., under low-speed stirring for mixing the syringe content. Then, 200 μL of chloroform were aspirated followed by 350 μL of air to drive all chloroform into the syringe. During air-aspiration and the following 80 s, rapid-speed stirring was activated. At contact of the chloroform with the stirring bar, the solvent was dispersed into small droplets, thus enabling DLLME. During the last five seconds, the stirring speed was decreased, which favored the coalescence of the fine chloroform droplets. Afterwards, during a phase separation time of 30 s, the enriched droplets were accumulated at the bottom of the syringe. In the following, the organic phase enriched with the analyte-MB ion-pairs was slowly propelled through the flow cuvette under

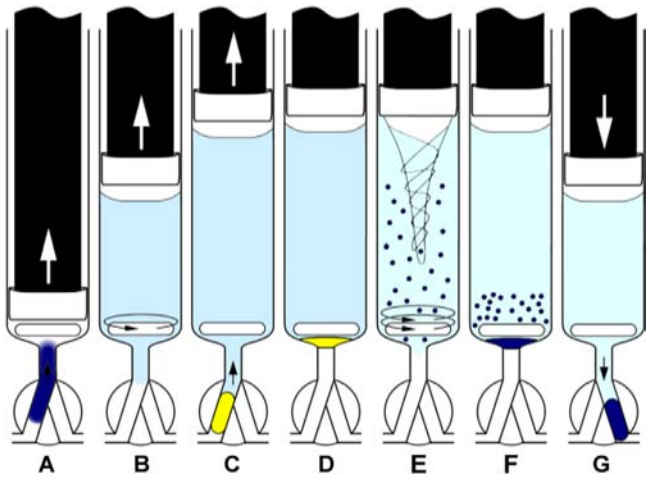

Fig. 2. Performed operation scheme for in-syringe MSA-DLLME of anionic surfactants into chloroform as MB ion-pairs. (A) Aspiration of MB, (B) Aspiration of sample and mixture with reagents, (C+D) Aspiration of chloroform, (E) DLLME, (F) Phase separation, (G) Propulsion of enriched organic phase to detector.

continuous data evaluation. All experiments were performed in triplicate.

2.7. Reference method

In order to evaluate the accuracy of the developed MSA-DLLME method, results were compared with those obtained by a simplified reference method derived from APHA 5540C [10]. 25 mL of sample were transferred into a separating funnel and containing 2.5 mL of MB solution (see Section 2.1) and 10 mL of chloroform were added. After extraction for 30 min, phases were let separate. Then, the organic layer was collected and the extraction was repeated twice with additional 10 mL of chloroform each. The extracts were combined and aliquot of 10 mL was washed twice with 50 mL of the recommended washing solution. Finally, the absorbance of washed extract was measured at 652 nm.

3. Results and discussion

3.1. Preliminary remarks

To achieve that the droplets would accumulate at the inlet of the syringe, the syringe pump was used up-side down implying several particular changes in the operation, here firstly described. Most importantly, it was unavoidable that air bubbles would accumulate in the syringe causing that each solution displacement required additional time. For example, dispensing caused the compression of the air cushion before the liquid in the HC would start to move. Likewise, the stored air pressure still caused liquid displacement over a few seconds while the syringe operation already had stopped. So, operation steps which required high reproducibility were followed by a waiting time of 2 s.

Due to the fact that any air, which surpasses the remaining dead volume inside the syringe, was expulsed towards the detection cell and waste at emptying the syringe during cleaning, the volume of air inside the syringe was reproducible and equal to the syringe dead volume. Consequently, all liquid was expulsed apart from adhered liquid films on the surfaces at emptying the syringe, so cleaning was more efficient than in the previous works [38,39]. An additional advantage was that the stirring bar was not displaced by the syringe piston, so that a much simpler device as described in Section 2.4 could be used for the creation of the rotating magnetic field.

The configuration allowed to use chloroform as denser solvent than water. An initial attempt to use *n*-hexanol as less harmful solvent and the prior system configuration, i.e. normal syringe orientation, was rejected due to the blank values resulted unacceptably high since solubility of MB itself in *n*-hexanol is significant.

Despite of the recommendation from the standard procedure of doing a washing step to eliminate some interferences by back-extraction, in this work a simple extraction was carried out. Several applications of direct extractions have demonstrated that the interference level at this simplified mode is equally low and the comparability with the standard method is given [13,21]. Therefore, it was opted for the simple extraction to minimize the sample manipulation and analysis time.

3.2. Phase separation time

First of all, the required time for the separation of both phases, chloroform and aqueous sample, was studied in the range of 5 to 40 s using both a blank (water) and 0.4 mg/L SBDS standard. While the blank signal remained constant over the studied range, the standard signal increased rapidly up to a maximum at 30 s with constant signals for longer times (data are not shown). Therefore, 30 s of phase separation time were applied further on.

3.3. Multivariate optimization of experimental conditions

A two-level fractional factorial design (2^{6-2}) was selected to screen the relevance of the the concentrations of H_2SO_4 (A), of MB (B), of Na_2SO_4 (C), and of NaH_2PO_4 (D) as well as the volume of chloroform (E) and the extraction time (F) in the method.

Triplicate measurements of the centre point were also added to evaluate the potential curvature and the significance of the result variability, using standard solutions (0.4 mg/L). The range of variables (data are presented in Supplementary material S-2) affecting the extraction and the results were obtained with variance (ANOVA) with 95% probably. The data are presented in Supplementary material S-3 and S-4. According to ANOVA table and Pareto chart results, the most significant factors were the extraction time (positive dependency) the volume of chloroform (negative dependency). Moreover, the interaction between H_2SO_4 and NaH_2PO_4 was statistically significant, while the concentration of Na_2SO_4 had no significant impact on the extraction recovery and thus was fixed.

Based on the screening study results, a face-centered central composite design (CCD) with a total number of 27 experiments was made to estimate the critical values of the variables to be significant plus the concentration of MB to achieve minimal consumption while the volume of chloroform was fixed to 200 μL for CCD and studied posteriori. Taking into account the results of screening, the ranges of four variables (A, B, D and F) were modified to achieve the highest extraction efficiency (data are presented in Supplementary material S-5). The quality of the fit of the linear-quadratic model was explained by the coefficient of determination and the lack of fit value ($p > 0.05$). A regression coefficient of $r^2 = 0.980$ (adjusted $r^2 = 0.957$) indicated a good relationship between the experimental data and the fitted model. The histogram of residuals and predicted vs. observed values showed satisfactory distributions. Therefore, critical values were obtained using the desirability function (data are presented in Supplementary material S-6). Thus, optimum conditions are used in all further experiments: 200 μL of 648 mmol/L $NaSO_4$, 130 μL of 100 mmol/L H_2SO_4, 200 μL of 127 mmol/L NaH_2PO_4 and 100 μL of 700 mg/L MB, and 30 s of separation time.

3.4. Volume of the extraction solvent

According to the reference method, chloroform was chosen as extraction solvent. The volume of chloroform used in the procedure is highly important since a larger volume could yield higher extraction efficiency while a smaller volume could yield a higher concentration factor and thus a higher sensitivity and minimize the environmental impact and costs of the method. This study was performed by the comparison of the sensitivity of four different calibration curves in a range from 100 to 250 μL of chloroform (data are not shown). Using 100 μL and 150 μL of chloroform, the repeatability was not acceptable with RSD values higher than 10%. For volumes larger than 200 μL, the signal height was reducing hence losing sensibility. Thus, 200 μL was chosen as best value to establish a compromise between the sensitivity and repeatability.

3.5. Extraction time

In LLE, the aim is to transfer a maximum amount of the analyte from one liquid donor phase to an immiscible acceptor phase. The extraction rate will decrease as the system approaches the steady-state expressed by the partition coefficient. Furthermore, DLLME and related techniques such as used in this work require very short extraction times, as the contact surface between both phases

is enormously increased by droplet dispersion. The effect of the stirring time on the absorbance was studied in the range of 50 to 110 s for a blank solution and for a 0.2 mg/L SBDS standard. It was observed that the absorbance of the blank remained constant over time while the standard signals increased nearly linearly with the extraction time up to 80 s, reaching a stable level and RSD values about 2% beyond. Thus, a pre-concentration time of 80 s was chosen for the method in order to minimize the analysis time.

3.6. Study of possible interferences

As only moderately soluble in water, MB can form extractable ion-pairs with other anions, which then act as positive interferences of the procedure. On the other hand, especially organic and large cations can compete with MB, leading to negative interference. The effect of potentially species on the proposed procedure in concentrations similar or higher than reported for surface water [9]. Standards of 0.100 mg/L of SBDS including the potentially interfering compounds were prepared from stock solutions (see Section 2.1) and assessed with the developed MSA-DLLME method. The percentage of found interference of each ion is given as Supplementary material S-7. Mostly, the interference level was well-below 10%, even for even higher concentration as normally found in natural waters.

Slight negative interferences were observed from aluminium and CTAB. However, it should be pointed out that these interferences are common for the MBAS method and were former reported to similar or even higher extent [20,21].

Further it was found that the observed effects of chloride and nitrate as typical interfering anions of the MBAS assay [10] were very low even at the studied concentration exceeding typical concentrations in surface waters. Moreover, similar observance was made by other researchers [13–21]. Thus, it was decided to omit the step of extraction washing.

On the other side, it was noted that the method could not be applied to seawater since the signals of both blank and standard solutions increased linear with the chloride concentration for concentrations beyond 600 mg/L chloride.

The effect of water hardness in the extraction process was also studied owing to the high concentration of carbonate in freshwaters on Mallorca Island. Five calibrations using SBDS standard prepared with Milli-Q water and artificial “moderately hard water”, “hard water”, and “very hard water” (see Section 2.1) were measured and compared. The results showed that there was no significant effect of the water hardness on the method sensitivity with 95% confidence intervals for no hardness added and the maximum hardness value. The results led to the conclusion that the selectivity of the method was appropriate for MBAS determination.

3.7. Method performance

Under the optimized experimental conditions, the proposed method was characterized by repeated calibrations proving a linear behavior of the signal height up to 0.300 mg/L. The calibration curve, evaluated on 5 subsequent days, followed the equation: peak height$=(2.9\pm0.04)$ [SBDS mg/L]$+(0.042\pm0.04)$ ($R^2=0.994$). Limits of detection and quantification (LOD, LOQ) were calculated as the concentration yielding a peak height passing the blank signal by it triple and ten-fold standard deviation, respectively. A LOD of 7 μg/L and a LOQ of 22 μg/L were obtained for SBDS in water samples. The relative standard deviation (RSD) of repeated measurement was generally below 4% of peak height. The RSD value for ten-fold determinations of a 0.100 mg/L SBDS standard was $<3\%$.

In contrast to MSA-DLLME based on manual operation [10], the entire procedure (i.e. mixing of sample and reagents, extraction, phase separation, measurement, and system cleaning) took about 345 s allowing a measuring frequency of 10 h^{-1}. In addition, using an autosampler, the proposed system operated completely automated.

The pre-concentration factor can be estimated from the ratio between the volume of the sample (3.69 mL) and the volume of the solvent (200 μL) to be 18.5.

3.8. Method validation and application to water samples

For sample analysis, a rotary autosampler unit was connected to MPV position 3 so as to analyze the samples rapidly one-after-one and overnight. In order to assess the accuracy, water samples and spiked water samples were analyzed by the reference procedure, MBAS reference method (C_{LLE}) and with the proposed MSA-DLLME method ($C_{MSA\text{-}DLLME}$) and results obtained were compared. The found linear relationship followed the equation $C_{MSA\text{-}DLLME}=1.059\ (\pm0.135)\times C_{LLE}-0.005\ (\pm0.018)$ where the values in parenthesis are 95% confidence limits. Since the estimated slope and intercept did not differ statistically from values 1 and 0, no evidence of systematic differences between the two sets of results was given.

In order to evaluate the applicability of the proposed automated MSA-DLLME method, seven water samples were measured

Table 2
Analysis of SBDS in different water samples including the results of addition-recovery tests: G, conductance; SD, standard deviation.

Sample	pH	G	Added	Found[a]			Recovery			t_{exp}[b]
		(mS/cm)	(mg/L)	(mg/L)		SD	%		SD	
Waste water T3	7.9	3	0.00	0.113	±	0.002				
			0.05	0.160	±	0.003	97.4	±	2.9	0.5
Waste water T2	8.1	3	0.00	0.144	±	0.010				
			0.05	0.201	±	0.003	105	±	2	0.4
Well water 1	7.2	0.9	0.00	0.153	±	0.013				
			0.05	0.234	±	0.009	105	±	5	2.2
Well water 2	7.5	0.7	0.00	0.178	±	0.008				
			0.05	0.240	±	0.008	107	±	4	0.8
Tap water	7.0	0.5	0.00	0.058	±	0.005				
			0.05	0.105	±	0.005	93.8	±	9.2	0.4
Pond water	8.5	1.4	0.00	0.213	±	0.007				
			0.05	0.254	±	0.014	95.8	±	6.5	0.4
Drinking water	6.3	0.1	0.00	0.032	±	0.004				
			0.05	0.081	±	0.002	99.5	±	6.4	0.3

G: Conductivity.

[a] Results are expressed as the mean value ± standard deviation ($n=3$).

[b] t_{crit}: 4.3.

with the proposed analyzer system. All samples were further spiked with SBDS standard to evaluate the analyte recovery and matrix effects. The results are given in Table 2. All samples showed natural concentrations of MBAS in the range of 0.032–0.213 mg/L, thus proving the suitability of the linear working range for samples. Standard addition of SDBS gave analyte recoveries in the range from 95% to 113% proving the general applicability and adequateness of the analyzer system to real sample analysis. The trueness of the analytical method was proven by student *t*-test. The overall calculated values of t were ≤ 1.55 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.

3.9. Discussion on system performance and operation

In this work, we firstly used in-syringe magnetic stirring-assisted DLLME in combination with a solvent denser than water, i.e. chloroform. This had led to the requirement to use the syringe upside down to facilitate droplet coalescence at the conical part of the syringe inlet and to allow the heavier organic phase or "the analytical fraction" to be pushed out completely from the syringe and through the detection cell before the sample.

The possibility to use halogenated solvents in-syringe in combination with stirring allows the direct transfer of standard extraction procedures, which employ these solvents, with the possibility of using only a fraction of these solvents in the future and achieving environmental friendlier methods.

The proposed configuration included also the possibility to expel practically all liquid from the syringe by the cushion and lower therefore the dead volume to be cleaned. This feature can be of high advantage when the organic content should be kept for a second in-syringe operation but with prior and complete elimination of the rest of sample. By example, it would allow repeated sample preparation with following extractions into the same volume of solvent to increase the method's sensitivity. By this work, we therefore hope to widen the versatility and applicability of this recent technique for automation of liquid–liquid extraction and sample preparation.

4. Conclusions

In this work, a novel method for the determination of the MBAS index based on in-syringe magnetic stirring-assisted dispersive liquid–liquid microextraction was presented. For the first time, a solvent denser than water was used in combination with this technique. Using multivariate optimization strategy enabled successful determination of the optimum conditions for the main experimental parameters taken into consideration during DLLME. Moreover, the developed system showed to be a robust and reliable alternative to existing methods for the spectrophotometric determination of anionic surfactants as sum parameter. The method proved to be selective with very low interference in spite of simplification of direct extraction from the acidified sample was done. A better sensitivity than in former works was achieved. The proposed method was successfully applied to the analysis of the MBAS index in a variety of water samples.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.06.063.

References

[1] E. Ródenas-Torralba, B.F. Reis, Á. Morales-Rubio, M. de la Guardia, Talanta 66 (2005) 591–599.
[2] J.L. Burguera, M. Burguera, Talanta 64 (2004) 1099–1108.
[3] World Health Organization (WHO), Environment Health Criteria 169, 1996.
[4] Y.-Y. Hu, Y.-Z. He, L.-L. Qian, L. Wang, Anal. Chim. Acta 536 (2005) 251–257.
[5] G. Könnecker, J. Regelmann, S. Belanger, K. Gamon, R. Sedlak, Ecotoxicol. Environ. Saf. 74 (2011) 1445–1460.
[6] P.A. Lara-Martín, A. Gómez-Parra, E. González-Mazo, J. Chromatogr. A 1114 (2006) 205–210.
[7] T. Cserháti, E. Forgács, G. Oros, Environ. Int. 28 (2002) 337–348.
[8] V. Gómez, L. Ferreres, E. Pocurull, F. Borrull, Talanta 84 (2011) 859–866.
[9] Council Directive 98/83/CE of November 1998 Relating to the Quality o Water Intended for Human Consumption.
[10] APHA, AWWA, WEF, L.S. Clesceri, A.D. Eaton, A.E. Greenberg, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington DC, 1998 (20th ed.).
[11] J. Kawase, A. Nakae, M. Yamanaka, Anal. Chem. 51 (1979) 1640–1643.
[12] F. Cañete, A. Rios, M.D. Luque de Castro, M. Valcarcel, Anal. Chem. 60 (1988) 2354–2357.
[13] M. del Valle, J. Alonso, J. Bartroli, I. Marti, Analyst 113 (1988) 1677–1681.
[14] M. Agudo, A. Rios, M. Valcarcel, Analyst 119 (1994) 2097–2100.
[15] H. Liu, P.K. Dasgupta, Anal. Chem. 68 (1996) 1817–1821.
[16] L.N. Moskvin, J. Simon, P. Löffler, N.V. Michailova, D.N. Nicolaevna, Talanta 43 (1996) 819–824.
[17] S. Fan, Z. Fang, J. Fresen, Anal. Chem. 357 (1997) 416–419.
[18] L. Jing-fu, J. Gui-bin, Microchem. J. 68 (2001) 29–33.
[19] R. Burakham, S. Lapanantnoppakhun, J. Jakmunee, K. Grudpan, Talanta 68 (2005) 416–421.
[20] T. Aydan, M. Takeuchi, H. Tanaka, J. Flow, Injection Anal. 26 (2009) 133–137.
[21] A.C. Pereira, F.R.P. Rocha, Anal. Methods 5 (2013) 2104–2109.
[22] B. Karlberg, S. Thelander, Anal. Chim. Acta 98 (1978) 1–7.
[23] F.H. Bergamin, B.F. Reis, E.A.G. Zagatto, Anal. Chim. Acta 97 (1978) 427–431.
[24] E. Olkowska, Z. Polkowska, J. Namiesnik, Talanta 88 (2012) 1–13.
[25] M. Rezaee, Y. Assadi, M.-R.M. Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1–9.
[26] L. Kocúrová, I.S. Balogh, J. Sklenářová, V. Andruch, Microchem. J. 102 (2012) 11–17.
[27] M.A. Farajzadeh, M.R.A. Mogaddam, Anal. Chim. Acta 728 (2012) 31–38.
[28] N.-B. Abu-Bakar, A. Makahleh, B. Saad, Talanta 120 (2014) 47–54.
[29] A. Saleh, Y. Yamini, M. Faraji, M. Rezaee, M. Ghambarian, J. Chromatogr. A 1216 (2009) 6673–6679.
[30] P.-P. Zhang, Z.-G. Shi, Q.-W. Yu, Y.-Q. Feng, Talanta 83 (2011) 1711–1715.
[31] A.N. Anthemidis, K.-I.G. Ioannou, Talanta 79 (2009) 86–91.
[32] F. Maya, B. Horstkotte, J.M. Estela, V. Cerdà, Anal. Bioanal. Chem. 404 (2012) 909–917.
[33] V. Andruch, C.C. Acebal, J. Škrlíková, H. Sklenářová, P. Solich, I.S. Balogh, F. Billes, L. Kocúrová, Microchem. J. 100 (2012) 77–82.
[34] B. Horstkotte, F. Maya, C.M. Duarte, V. Cerdà, Microchim. Acta 179 (2012) 91–98.
[35] F. Maya, J.M. Estela, V. Cerdà, Anal. Bioanal. Chem. 402 (2012) 1383–1388.
[36] B. Horstkotte, M. Alexovič, F. Maya, C.M. Duarte, V. Andruch, V. Cerdà, Talanta 99 (2012) 349–356.
[37] R. Suárez, B. Horstkotte, C.M. Duarte, V. Cerdà, Anal. Chem. 84 (2012) 9462–9469.
[38] B. Horstkotte, R. Suárez, P. Solich, V. Cerdà, Anal. Chim. Acta 788 (2013) 52–60.
[39] C. Henríquez, B. Horstkotte, P. Solich, V. Cerdà, Anal. Bioanal. Chem. 405 (2013) 6761–6769.
[40] E. Becerra, A. Cladera, V. Cerdà, Lab. Rob. Autom. 11 (1999) 131–140.
[41] B. Horstkotte, O. Elsholz, V. Cerdà, J. Flow, Injection Anal. 22 (2005) 99–109.