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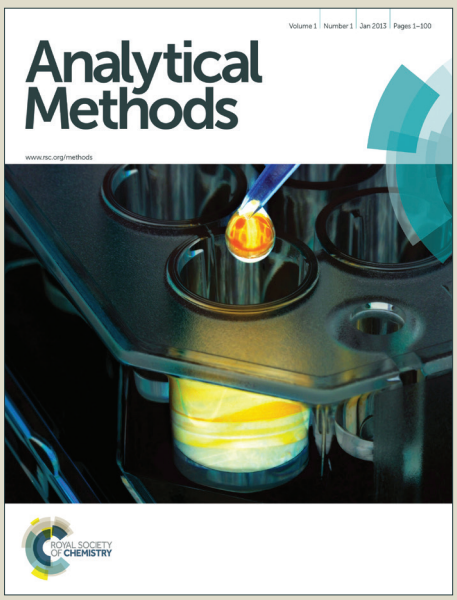

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ARTICLE TYPE

In-syringe magnetic stirring assisted dispersive liquid-liquid micro-extraction with solvent washing for fully automated determination of cationic surfactants

Burkhard Horstkotte[a] Ruth Suárez[b] Petr Solich, and Víctor Cerdà[b,]*

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An automated simple analyzer system for the extraction of cationic surfactants as an ion-pair with disulfine blue dye is described based on the technique in-syringe magnetic stirring-assisted dispersive liquid-liquid micro-extraction.

The use of chloroform as an extraction solvent denser than water required to operate the syringe pump upside-down. The remaining air cushion inside the syringe allowed emptying the syringe completely and reducing the dead volume significantly compared to prior works. Since the stirring bar placed inside the syringe to obtain a closed yet size-adaptable mixing chamber remains at the same position, the former magnetic stirring bar driver was simplified. The new system configuration further enabled automated in-syringe washing of the organic phase with water and barium acetate solution to minimize interferences.

High signal repeatability with < 5 % RSD was achieved both for extraction as well as for double organic phase washing. Only 220 µL of extraction solvent and 4 mL of sample were required for simple extraction achieving a detection limit below 30 nmol L^{-1} and a linear response up to 1 µmol L^{-1} of cetyltriamethylammonium bromide. The time of analysis was 240 s for simple extraction. Considerable reduction of interferences was achieved by extract washing requiring up to 545 s. Analyte recovery in real water samples was 95.6 ± 7.0 % applying extract washing.

Keywords: In-Syringe Analysis, Magnetic Stirring-Assisted Liquid-Liquid Micro-Extraction, Ion-Pair, Cationic Surfactants, Disulfine Blue Active Substances, Extract Washing

1. Introduction

Flow techniques (FT) comprise different methodologies of sample treatment in flow in a tubing manifold and, unlike chromatography, without gradual separation. FT differ in the way of sample introduction and flow patterns as well as in the configuration and operation of the specific analyzers, but have in common the automation of classical laboratory procedures including sample metering (aspiration or injection), handling (transport, splitting, etc.), modification (dilution, filtration, clean-up, concentration), performing of chemical reactions (reproducible mixing with reagent, heating), and measurement.

FT are powerful tools to achieve minimization of solution consumption and to improve the reproducibility of analytical procedures. In contrast to other automation approaches (e.g. robotic systems), FT are self-cleaning, i.e. the manifold is flushed by a carrier flow, which allows stand-alone operation while on the other hand, analysis are performed sequentially.

In 1990, the flow technique Sequential Injection Analysis (SIA)[1] originated from the idea of performing different flow procedures in one universal analyzer, which does not require manual re-configuration but which enables computer-controlled choice of the operation parameters such as timing, mixing patterns, and used volumes of sample and reagents.

The basic operation is a sequential aspiration of sample and further required solutions from the ports of a selection valve (SV) into a tube, denoted holding coil (HC), which connects the central common valve port to a bidirectional pump, generally of syringe type. Then, the flow is reversed and the stacked solutions are pushed through one lateral port of the SV to a detection flow cell. The reaction product is formed where the sample and reagent solutions penetrate each other by dispersion during aspiration and flow reversal. Since the procedure is exactly reproduced, quantification is possible even prior to reaching reaction steady-state.

Up-to-date, hundreds of reported SIA applications have demonstrated the great potential of this technique and scientists' appreciation of its prominent features, such as simplicity of

instrumentation, versatility of operation, and robustness. Comprehensive reviews and technical treatises on SIA can be found elsewhere[2-5].

In SIA, the only solution ever allowed to enter the syringe pump is the carrier solution, generally water. Consequently, the HC has to be long enough to avoid syringe contamination by any solution aspirated from the SV. Otherwise, pump cleaning after each analysis would be required with an unacceptable share of the whole time of the procedure.

However, mixing large with small volumes of solutions in a HC of typically 0.8 to 1.5 mm inner diameter (id) is limited by the small contact area and imperfect penetration of solutions. Hence, when large volume ratios are favorable, such as to perform dilutions or liquid-liquid extractions (LLE), a mixing chamber connected to one lateral port of the SV is often used.[6-8] Nevertheless, cleaning of such chamber also requires considerable time. First, the chamber has to be emptied, then completely filled with a cleaning solution, followed by the re-aspiration of the chamber's content, and its final discharge.

An ingenious approach from GlobalFIA company (Fox Island, WA, USA, www.globalfia.com) is a mixing chamber, which is shaken by a computer-controlled motor. Only one fraction of cleaning solution is required and standard extraction procedures can be patterned exactly while sped-up and miniaturized.

In the last two years, the idea of using a syringe as mixing and reaction vessel for SIA has been revisited. In 2012, Maya et al.[9] demonstrated in-syringe dispersive liquid-liquid microextraction (DLLME [10]) of benzo(a)pyren from water sample on a multi-syringe flow system. For this, a mixture of 1:9 parts octanol and acetonitrile was aspirated into the syringe followed by rapid aspiration of sample, which causes the disruption of the solvent mixture into fine droplets with later coalescence of the enriched octanol at the top of the syringe.

The special feature of a syringe as reaction and extraction vessel is its size-adaptability facilitating the separation of organic and aqueous phases as well as posterior cleaning, since only a part of the syringe has to be filled with cleaning solution.

In following works analytical reactions prior to in-syringe DLLME were included.[11-13] However, to achieve the mixing of the large volume of sample with reagents, an additional external mixing chamber had still to be used. Therefore, using a magnetic stirrer inside of the syringe [14,15] was therefore a break-through for the "Lab-In-A-Syringe" technique since homogeneous and, more importantly, reproducible mixing is achieved within seconds.[16] The kinetic energy from the stirrer further enables efficient in-syringe stirring-assisted DLLME.[17] Detailed synopsis of DLLME and related techniques can be found elsewhere.[18,19]

An important drawback of this approach is the dead volume inside the syringe (to allow rotation of the magnetic stirring bar) and the HC, which therefore is made as short as possible. Besides, straightforward automation of standard extraction protocols should also allow using of typical extraction solvents denser than water such as chloroform ($CHCl_3$) to improve comparability of methods. $CHCl_3$ has an over ten-times lower viscosity compared to previously used octanol and hexanol [9,11-16] and a greater difference in density towards water, which bears the potential of faster phase separation and droplet coalescence in DLLME.

In this work, we demonstrate the use of $CHCl_3$ for in-syringe stirring assisted DLLME for the determination of disulfine blue active substances (DBAS). Hereby, the syringe pump had to be used up-side down, which implied that air will accumulate in the syringe.

This resulted in the welcome benefit that all liquid could by expulsed from the syringe, which in turn facilitated automated of secondary procedure steps such as washing of the extraction solvent.

The DBAS index is the standard procedure for evaluation of the concentrations of quaternary ammonium cations (quats), which can be extracted as ion pair with disulfine blue (DSB) into $CHCl_3$.[21] Quats are widely used as disinfectants, cationic surfactants (CS), or softeners and show in part microorganism toxicity.[22,23] Environmental accumulation can be due to adsorption on negatively-charged surfaces such as clay particles. Control of waste-water effluents and better understanding of their environmental behavior has driven over decades the development of new analytical procedure for their determination.

As sum parameter, quats are mostly measured as ion-pair with acidic dyes after LLE, where the DBSA index seems the most accepted one but with the costs of a large consumption of harmful $CHCl_3$.[22] Using FT, either LLE downscaling including the use of alternative anionic dyes to DSB [24,25] or alternative procedures even omitting LLE have been proposed, among these taking advantage from complex formations and absorbance enhancement during ion-pair formation. [26-28]

A relevant problem is the presence of anionic species, especially anionic surfactants (AS), which compete in on-pair formation and lead to analytical underestimations. Combination with or sole use of solid phase extraction has therefore been reported as useful to suppress this interference and is also part of the sample preparation of the DBSA index.[21,28] Titration and membrane-based extraction protocols have been proposed further.[29-31] A synopsis about of determination of surfactants on HPLC but including a comprehensive section about sample pretreatment is further given elsewhere.[32]

In this work, we studied extract washing to decrease the overall interference of the procedure. Compared to the standard procedure, miniaturization and considerable reduction of the required volumes of solvent and sample in combination with a large pre-concentration factor was demonstrated.

2. Methods and Materials

2.1 Reagents

All reagents were of "pro analysis" grade and bidestilled quality water (resistivity >18 MΩ·cm) was used throughout for solutions preparation. All glassware and polyethylene bottles used were rinsed with water prior to use.

Stock solutions of 2 mmol L^{-1} cetyltriamethylammonium bromide (CTAB) in 20 mmol L^{-1} NaOH and 5 mmol L^{-1} sodium dodecylsulfonate (SDS) in water prepared. Working standards were prepared daily by appropriate dilution. A sodium acetate buffer of 2 mol L^{-1} was prepared and adjusted with acetic acid to pH 5.0 and used as reagent 1. A stock solution of 10 mmol L^{-1} DSB (acid blue I) was prepared in 50 %v/v ethanol. A 1:10

dilution was then used as reagent 2 for all experiments.

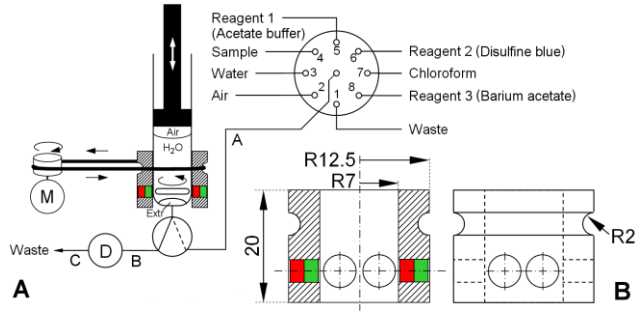

Figure 1. A: Analyzer manifold with selection valve (SV), syringe (S), solenoid 3-way head valve (V), detection flow cell (D), and DC motor (M). PTFE tubing (0.8 mm id) A: 35 cm, B: 10 cm, and C: 40 cm. B: The magnetic stirring bar driver design given in detail consisting of a Deldrin® tube and two neodymium magnets.

A barium acetate solution of 200 mmol L^{-1} was used as reagent 3 to decrease the interference of AS. Stock solutions of 200 µmol L^{-1} of other quaternary ammonium compounds were prepared for comparative studies given in Table 1. Didodecyl dimethyl ammonium bromide, tetradecyl trimethyl ammonium bromide, tetradecyl trimethyl ammonium bromide, tetraethyl ammonium iodide, tetrabutyl ammonium hydroxide, tetramethyl ammonium iodide, and N-dodecyl-N-methylephedrinium bromide were purchased from Sigma Aldrich (Prague, Czech Republic). Carbethopendecinium bromide was purchased from Dr. Kulich Phrama (Hradec Králové, Czech Republic). Dodecylisochinolinium bromide and dodecylpyridinium bromide were products from synthesis as described elsewhere.[33,34]

The following compounds were used for interference studies with concentrations given in Table 2 being NaCl, KCl, $MgCl_2 \cdot 6\,H_2O$, $CaCl_2 \cdot 2\,H_2O$, $FeCl_3 \cdot 6\,H_2O$, $Pb(NO_3)_2$, $AlCl_3$, $CuSO4 \cdot 7\,H_2O$, $MnCl_2$, $ZnCl_2$, NaH_2PO_4, NH_4NO_3, $NaHCO_3$, and Na_2SO_4.

Water-free methanol, toluene, and dichlorodimethylsilane were used for silanization of the detection flow cell described in section 2.4. A mixture of 5 %v/v n-hexanol in $CHCl_3$ was used as extraction solvent unless not stated otherwise.

For method characterization, well, tap, mineral, and lixiviate water samples were collected in 1 L polyethylene flasks. Particles were let to sediment before aliquots were taken for analysis.

2.2 Manifold configuration

The manifold is depicted in Figure 1a with tubing dimensions indicated. PTFE tubing of 0.8 mm inner diameter (id) was used for the entire manifold.

The computer controlled flow setup comprised a 16.000-step multisyringe pump (MS) and the rotary 8-port SV (Sciware Systems SL, Palma de Mallorca, Spain) for liquid handling and distribution. For sample measurement and interference studies, a rotary autosampler from the same company was used. The MS was equipped with one glass syringe of 5 mL purchased from Hamilton Bonaduz AG (Bonaduz, GR, Switzerland, Model 1005 TLL-SAL SYR). 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), air (2), water (3), sample (4), reagent 1 (5), reagent 2 (6), $CHCl_3$ (7), and reagent 3 (8). A HC of 35 cm connected the central port of the SV to the syringe head valve in position ON.

A magnetic stirring bar (10 mm x 3 mm) was placed inside the syringe allowing homogeneous solution mixing and dispersion of the extraction solvent. The position of the syringe piston was adjusted to leave a gap of 4 mm at complete emptying, so that the stirring bar could freely rotate.

The syringe module was used upside-down to use an extraction solvent of higher density than water. This implied the advantage that an air cushion would remain inside the syringe, which displaced all liquid from the syringe at emptying and by this reduced the dead volume to be cleaned between two analyses.

2.3. Stirring bar driver

Due to the fact that the stirring bar would remain at the same position inside the syringe, i.e. just above the inlet, the magnetic stirring bar driver used in previous works could be simplified.[14,15] It consisted in a tube turned of Deldrin® of 20 mm in height, 25 mm outer diameter (od), and 14 mm id, which was placed over the syringe glass barrel and could rotate freely around the syringe longitudinal axis. Additional holes permitted the observation of the stirring bar inside the syringe.

As shown in Figure 1b, the device held two oppositely faced neodymium magnets (5 mm x 4 mm od) and a groove for an elastic rubber band to impel the driver with a direct current (DC) motor (see Figure 1a). The magnets were strong enough to levitate the stirring bar inside the syringe, so that friction force was low, and to assure that even at high rotation speeds, the stirring bar would not gambol.

The DC motor was supplied via a homemade relay and regulation circuit board (supplement material 1). It enabled the choice of two different stirring speeds by activation of either two auxiliary analog supply ports (control voltages U1 and U2) of the MS module. The lower stirring velocity (U1 and U2 in ON) was adjusted to allow homogenization of the liquid content inside the syringe without vortex formation (ca. 1000 rpm). The higher speed (U1 in ON, U2 in OFF) was applied for DLLME to disrupt the organic solvent into fine droplets (ca. 3000 rpm).

2.4. Detection equipment and parameters

The software AutoAnalysis 5.0 (Sciware Systems SL) was used for operation control of the flow instrumentation as well as data acquisition from both detection equipments and later data treatment. 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. A detailed description of the software structure and features is given elsewhere.[35]

A flow cuvette of 1 cm optical path length and 1.5 mm flow channel diameter from Hellma Analytics (Müllheim, Germany) was used throughout. The cell was connected via a 10 cm long PTFE tube of 0.8 mm id to the syringe head valve in position OFF. Downstream, a 50 cm long PTFE tube allowed solution

discharge to waste.

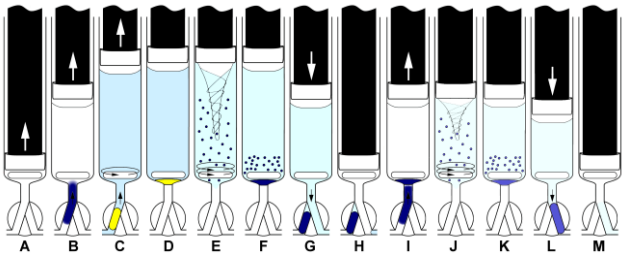

Figure 2. Operation scheme of extraction with simple extract washing. Aspiration of sample, buffer, and DSB (a & b), Mixing (c) and aspiration of ExtrS and Air (d), MSA-DLLME (e), sedimentation of ExtrS (f), saving ExtrS in HC and discharge of aqueous phase to waste (g & h), aspiration of DSB, barium acetate, and water (i), washing of ExtrS by MSA-DLLME (j), sedimentation of ExtrS (k), propelling ExtrS to detector (l), syringe content discharge to waste (m).

The flow cuvette was placed in a CUV-UV fiber optic cuvette holder including collimating lenses and connected directly to a miniature USB2000 spectrometer, both from OceanOptics (Dunedin, FL, USA). A Vio High Power White LED from GE Lighting was used as stable light source of wide emission spectrum (400 nm to 700 nm), and supplied by a constant current source (Sciware Systems SL).[36]

Absorbance measurements were performed at an analytical wavelength of 638 nm and corrected against the absorbance value measured at a reference wavelength of 550 nm on which DSB does not show any significant absorbance.

To improve the wettability of the cuvette walls by the organic phase and by this to obtain low baseline noise, silanization of the cuvette was done. For this, methanol and toluene were dried by the addition of water-free Na2SO4. The cuvette was cleaned with Piranha solution and then let stand filled with 2 mol of NaOH during 1 h. Following, the solution was flushed subsequently with water, methanol, and toluene. Then, the cuvette was blown dry by nitrogen flow and a 1:10 mixture of dichlorodimethylsilane and dried toluene was let to react with the surface hydroxyl-groups for 10 min. Finally, the cuvette was flushed with methanol.

2.5. Analytical protocols and methods

Two different procedures were used. Firstly, for direct analyte extraction (procedure 1) and secondly including extract washing with water and subsequently with barium acetate and DSB (procedure 2). The procedures are given as Electronic Supplementary Information (ESI) 2 and 3. An operation scheme and photo documentation are further given in Figure 2 and ESI 4, respectively. Both procedures started with the cleaning of the syringe by threefold aspiration of 0.6 mL of sample or the respective standard solution from SV under high speed stirring and dispense through the head valve position OFF to waste.

Then, buffer, DSB solution, and sample were aspirated into the syringe under low speed stirring for homogenization. Then, the required volume of the organic phase was aspirated followed by a volume of air being large enough to fill the HC, so that the organic phase entered the syringe completely. High speed stirring was done for 35 s for DLLME. Here, it was found advantageous to start and end with 5 s of stirring at lower speed to overcome the inertia of the solution at starting and to improve posterior droplet coalescence, respectively.

After phase separation and droplet coalescence, either the organic phase was pushed slowly through the detection cell followed by emptying the syringe completely at high speed (procedure 1) or, for extract washing, the organic phase was pushed into the HC, and then, the remaining liquid was dispensed through the detection cell to waste (procedure 2).

In procedure 2, the extract was re-aspirated into the syringe together with water, barium acetate, and DSB solution, followed by another DLLME step, phase separation, and then measurement. An additional washing step with pure water was done equally before performing the extraction step with barium acetate. A 40 µL larger volume of organic solvent was required for procedure 2 since a part of the organic phase would dissolve in the aqueous sample and washing solutions.

3. Results and Discussion

3.1. Preliminary considerations on system design and extraction solvent

In contrast to the first applications,[14,15] in the present work, in-syringe magnetic stirring assisted dispersive liquid-liquid microextraction (IS-MSA-DLLME) was studied with the syringe placed up-side down. This approach is similar to recently described piston-propelled flow-batch but uses the commercially available and instrumentation of a simple SIA system, i.e. a syringe pump and SV.[37,38] The approach implied several changes in the operation characteristics but also offers new potentials and possible applications.

First, trapping of air bubbles in the syringe had to be taken into account. To keep this process reproducible, the remaining dead volume when the syringe piston is in down position, given by the space required for free rotation of the magnetic stirring bar, was allowed to be air.

Consequently, the syringe could be emptied nearly completely, leaving alone any adhered liquid film on the surfaces. So, syringe and HC cleaning required less than half time and sample volume than in the previous works.[14,15] On the other hand, any solution handing required a posterior waiting time of 2 s due to the compressibility of the air inside the syringe and consequently delayed solution moving.

Second, the stirring bar is always located in the same position in the syringe, as it is not moved by the syringe piston. This fact allowed using a much simpler stirring bar driver than in the previous works and only two small neodymium magnets were sufficient to levitate the stirring bar inside the syringe, minimizing any friction.[14,15]

Third, the chosen syringe orientation required the use of a halogenated solvent of density higher than water. While solvents lighter than water as prior used are less toxic than halogenated ones,[10,12-16] $CHCl_3$ shows some important advantages. For one, $CHCl_3$ is used as extraction solvent in many standard procedures as well as for DBAS, so is likely to yield good comparability. Secondly, the present automated procedure allows reducing the required volume of $CHCl_3$ greatly and by this the environmental impact compared to standard procedures. Finally, $CHCl_3$ has an ten-times lower viscosity than prior used 1-hexanol,[12-15] while the relative density difference to water is larger than for 1-hexanol,

accelerating phase separation and solvent droplet coalescence after DLLME.

3.2. Preliminary experiments

Using pure $CHCl_3$ as extraction solvent, the signals were irregular and did not show the expected rectangular shape. It was proven, that this was not due to inhomogeneity of the organic phase after droplet coalescence but due to an insufficient wetting of the flow cell inner walls with the organic solvent. Therefore, cell silanization was done to yield higher hydrophobicity (see section 2.4.).

Since signal improvement was not sufficient, the addition of n-hexanol to the $CHCl_3$ was tested as a "sticky" additive. It was found, that a plateau-like signal shape was obtained for hexanol concentrations between 2.5 %v/v and 10 %v/v with best reproducibility found for 5 %v/v, which was used as additive further on.

By stepwise increasing the volume of solvent it was found that a volume of 220 µL was required for efficient droplet formation. Also, for smaller volumes, signal reproducibility decreased and especially droplet coalescence was incomplete, so that a small amount of the organic phase could remain in the syringe. A 40 µL larger volume was required when organic phase washing was done as about 20 µL were lost by dissolution in the aqueous phase in each washing step. A larger volume of organic phase would have required a larger holding coil (undesired increase of the system's dead volume) and have led to a signal decrease (dilution of the organic phase).

A typical peak sequence under optimised conditions is given for both procedures as ESI 5. It can be seen, that with higher analyte concentration, the signal plateau shows more and more inclination. This is due to the fact that a small volume of water remains in the cuvette from the initial syringe cleaning, which causes that the signal is initially lower until the water is pushed out by the solvent.

The phase separation time was tested over the range of 15 to 35 s using a 500 nmmol L^{-1} CTAB standard. The signal did not change significantly but the reproducibility was significantly better for 35 s compared to shorter times (data not shown). Therefore, 35 s for phase separation was used in all following experiments.

3.3. Optimization of simple extraction (procedure 1)

A Box-Behnken experimental design was chosen for the optimisation of the volumes of the sodium acetate buffer and DSB stock solution as well as the extraction time in the ranges of 50 – 250 µL (40 to 190 mmol L^{-1} acetate), 50 – 250 µL (12.5 to 62.5 µmol L^{-1}), and 15 to 45 s, respectively. A 1 µmol L^{-1} CTAB solution (4.1 mL) with the addition of 0.2 µmol L^{-1} SDS was used to favour conditions under which the selectivity against the interference of AS would be improved. As desirability, the reproducibility and the signal difference to water as blank solution were used. The results and conditions are given as ESI 6. A positive dependency was found for all parameters, but most pronounced for the extraction time.

In the following, univariant studies were done for all parameters, starting with the extraction time as the parameter of highest less effect and the adapted concentrations of buffer and DSB. The results and experimental conditions for each study are given in ESI 7 a-c.

First, it was found, that the extraction time had no significant effect on the blank signal while the signal for the standard increased from 15 to 50 s but following a saturation behavior and did not change significantly for times longer than 40 s. As compromise between time of analysis and signal height, 35 s were chosen for further work.

For the final buffer concentration, the signal height increased for low concentrations but did not change significantly beyond 200 µL, while the blank value decreased slightly and in approximation linearly with higher buffer concentrations. A volume of 250 µL corresponding to a concentration of 190 mmol L^{-1} was therefore chosen for further work.

Finally, the blank signal showed to increase linearly with higher concentrations of DSB while for the standard, a clear maximum was found. A stock solution volume of 150 corresponding to 36.6 µmol L^{-1} DSB in the final mixture was therefore chosen. To sum up, the univariant studies confirmed the results from the prior experimental design.

3.4. Optimization of extraction with extract washing (procedure 2)

The standard procedure for DBAS demands for AS and anion separation on an anionic exchange resin with subsequent elution of potentially retained CS with methanol, elute reduction by evaporation, and final carrying out the ion-pair extraction with DSB.[21] In this work, washing of the organic phase was done to reduce the interference level. Ba^{2+} was tested as promising cation to complex interfering anions and to decrease their interaction with the analyte. For this, the syringe was emptied with the organic phase stored in the HC, and then washed inside the syringe with a mixture of barium acetate and DSB solution. For extract washing, certain volumes of the DSB and the barium acetate stock solutions were mixed with 2 mL inside the syringe, denoted washing mixture in the following.

For the optimisation of the volume of barium acetate and DSB stock solutions, again a Box-Behnken design was chosen in the ranges of 30 – 150 µL (2.8 to 13 mmol L^{-1}) and 50 to 250 µL^{-1} (23.5 to 107 µmol L^{-1}), respectively. The results and experimental conditions are given as ESI 8.

For this and later univariant studies of the parameters, a standard of 500 nmol L^{-1} CTAB plus 250 nmol L^{-1} SDS was used and the height of the signal for this solution was taken as desirability. For both parameters, optima were found within the working domain, which were then used for univariant studies.

The experimental conditions and results of the univariant studies for the procedure of organic solvent washing are given in ESI 9 a-c. A linear signal increase for a standard of 500 nmol L^{-1} CTAB plus 250 nmol L^{-1} SDS with the washing time was found, while the influence on the blank signal was insignificant. This proves that a longer extraction time decreases, while only slightly, the SDS interference. As a compromise between time of analysis and signal height, 50 s were chosen for organic phase washing.

For the amount of DSB, a linear signal increase was found for the blank while a saturation curve was found for the standard

signal. For volumes below 250 µL, the standard signal increase was larger than for the blank, indicating that influence not using DSB would have let to loss of analyte. Therefore, a volume of 200 µL corresponding to a final DSB concentration of 88 µmol L^{-1} was used in the following. The effect of barium acetate on the blank signal was not significant, thus, extraction of an ion-pair between Ba^{2+} and DSB did not occur. However, addition of barium acetate to the washing mixture yielded an up to 33 % increase of the standard signal with slight signal decrease for concentrations beyond 13 mmol L^{-1}. Hence, this concentration, i.e. 150 µL of the stock solution, was chosen for future work.

Although the system configuration allowed to empty the syringe completely, it was noticed, that a minimum amount of sample would remain as liquid film on the surfaces. To avoid carry-over of sulfates or carbonates, which could led to precipitation with Ba^{2+} and interfere the determination, an additional washing step of the syringe with water but with low speed stirring was included.

3.5. Response to other quats and interference study

For characterization of the method's response to different quaternary ammonium compounds, other quats, mostly CS, were tested. Solutions of 600 nmol L^{-1} was prepared for each single compound with ultrapure water and their respective extraction efficiency evaluated by comparing the responses with the one obtained with a CTAB standard solution of equal concentration. The results are given in Table 1.

Most compounds gave less signal than CTAB and in tendency, the extraction efficiency decreased, as expected, with for shorter alkyl-chain length. In a former work, equal molar responses were achieved for different CS but careful adjustment of methanol as an additive to the aqueous phase had to be made, which also would be a significant variation from the standard procedure. [24]

Table 1: Relative response of different quaternary ammonium compounds compared to CTAB at a concentration level of 600 nmol L^{-1} using procedure 1. All solutions were prepared with ultrapure water.

Compound	Rel. response to CTAB [%]
Didodecyldimethylammonium bromide	55.1 ± 2.9
Tetradecyltrimethylammoniumbromide	110 ± 3.4
Tetrabutylammoniumhydroxide	3.39 ± 0.3
Tetraethylammonium iodide	1.55 ± 0.1
Tetramethylammonium iodide	3.56 ± 0.3
Carbethopendecinium bromide	3.95 ± 0.1
N-Dodecyl-N-methylephedrinium bromide	134 ± 4.5
Dodecylisochinolinium bromide	59.0 ± 0.6
Dodecylpyridinium bromide	58.4 ± 1.1

To study the interferences, the two procedures were tested on standard solutions including compounds in concentrations equal or higher than found in natural water samples. The results are given in Table 2. It can be seen, that using procedure 1 pattering the DBAS standard procedure, i.e. simple extraction, most tested compounds showed a strong interference while applying extract washing, the interference level was considerably reduced. The most notable interference was still observed from SDS, which suppressed the signal significantly by competing in the ion-pair formation with DSB. However, a considerable improvement, i.e. a signal increase, of about 60 % was achieved by extract washing with water and barium acetate.

As expected, the interference from larger and higher charged cations and especially the transition metal cations - well-known to form stable complexes with many organic reagents - was significantly larger even at lower concentration level than for the well-soluble alkali halogen salts NaCl and KCl. Extract washing with barium acetate solution especially decreased the interference of hydrogen phosphate and hydrogen-carbonate most-likely due to formation of insoluble precipitates, while for the cations the washing effect or "leaching" of the extraction solvent by the washing solution is supposed to be cause interference decrease.

A possible approach to improve the method could be the use of a less hydrophilic dye and thus stronger ion pairing reagent such as Erythrosine B. [31]

Recently we found in a work using in-syringe DLLME for the determination of AS based on ion-pairing with methylene blue that the relationship between NaCl concentration and blank signal was linear. It is therefore reasonable to assume that for lower concentrations than the used ones in this study, a proportional decrease of the interference level would be observed. [20]

3.6. Analytical performance and sample analysis

The finally chosen parameters and evaluated analytical performance are summarized in Table 3. Important benefits of the proposed system and method were the complete automation and miniaturization of the extraction procedure adopted from the DBAS protocol. Only 220 µL of the solvent mixture and 4 mL of sample were required for the simple extraction procedure, while the standard procedure requires several tens of milliliters of chloroform. In addition, using an automated system, open handling of harmful chloroform, sample transfer, or cleaning of glass material are avoided.

Table 2: Results of study of interferences. To a CTAB standard of 1.2 µmol L^{-1}, the listed compounds at the given concentration level were added. Procedure 1 refers to simple extraction, procedure 2 refers to extraction plus organic solvent washing with water and subsequent with barium acetate and DSB. Relative response values compared to a CTAB standard prepared with ultrapure water of equal concentration are given.

Compound	Concentration [mmol L^{-1}]	Rel. response (procedure 1)	Rel. response (procedure 2)
NaCl	100	139 %	106 %
KCl	50	133 %	105 %
$MgCl_2$	5	147 %	119 %
$CaCl_2$	2	142 %	109 %
Fe^{3+}, Pb^{2+}, Al^{3+}, Cu^{2+}, Mn^{2+}, Zn^{2+}	each $50 \cdot 10^{-3}$	421 %	136 %
NaH_2PO_4, NH_4NO_3	each 0.1	121 %	101 %
$NaHCO_3$	10	91 %	103 %
SDS	$0.6 \cdot 10^{-3}$	14 %	23 %
Na_2SO_4	10	97 %	98 %

Performing organic solvent washing, the method towards the

sample matrix was considerably improved although to the cost of a prolonged time of analysis, a 40 µL larger volume of chloroform, and about 20 % lower sensitivity (calculated from calibration slopes).

The method was highly sensitive with limits of detection below 20 nmol L^{-1} for both procedures. The procedure repeatability was 4 % and a linear working range up to 800 nmol L^{-1} was achieved. An extension is straightforward by simply using a smaller volume of sample and carrying out in-syringe sample dilution with water.

The results of the analysis are given in Table 4. It can be seen, that the DBAS index expressed as concentration of CTAB surfactant in the untreated samples was generally in the range of the LOQ. Using both procedures, the blank values decreased with organic solvent washing while for samples spiked with a CTAB standard, the signal and analyte recovery increased throughout.

The analyte recovery with procedure 2 was generally within acceptable limits, i.e. 90 – 104 %, however, a recovery value of 85 % was found for the lixiviate. Lower recovery values were most-likely related to analyte adsorption to particulate organic matter, clay particles, or due to interference of present AS.

An extraction efficiency of > 95 % and a preconcentration factor of 22.7 for 4 mL sample (17 for 3 mL) were achieved. The final solvent volume (175 µL) was calculated from the flow rate during the measurement step, peak width (7 s), the sensitivity (slope), the used volume of sample, and the molar extinction coefficient of DSB of about 47,000 AU L mol^{-1}.[39]

In comparison with prior indicated applications using FT for the determination of CS, the excellent sensitivity and low detection limit of 12 nmol L^{-1} (4.4 ppb) should be pointed out, which were found be superior to the former works. On the other hand, one analysis required a significantly longer time due to batch-wise operation and employing both analyte extraction and extract washing.

Table 3: Optimized conditions and analytical performance of the proposed procedures for the determination of DBAS. Organic solvent composition was 5 v/v% n-hexanol in chloroform.

Parameter	Procedure 1	Procedure 2
Organic solvent consumption	220 µL	260 µL
Sample volume *	4 mL	4 mL
Sodium acetate (3.1 mol L^{-1})	250 µL	250 µL
Disulfine blue (1 mmol L^{-1})	150 µL	150 µL + 250 µL
Barium acetate (200 mmol L^{-1})	-	150 µL
Time of analysis	240 s	545 s
Sample frequency	15 h^{-1}	6.6 h^{-1}
Average repeatability	3.3 % RSD	3.5 % RSD
Limit of detection	16 nmol L^{-1}	12 nmol L^{-1}
Limit of quantification	52 nmol L^{-1}	41 nmol L^{-1}
Linear working range *	up to 0.8 µmol L^{-1}	
Calibration function (3 mL sample)	750 mAU L $µmol^{-1}$ · c + 47.5 mAU	622 mAU L $µmol^{-1}$ · c + 91.3 mAU

* Due to in-syringe stirring, in-system sample dilution with water can be carried out to extend the linear working range. For this, the possible 4 mL are put together from sample and water.

Table 4: Results from sample analysis using simple extraction (procedure 1) and extraction with organic solvent washing with water and subsequent with barium acetate (procedure 2) under the optimized conditions given in table 3.

P	Sample	Addition CTAB [µmol L^{-1}]	Found CTAB [µmol L^{-1}]	Rel. Recovery [%]
1	Well water 1	-	0.028	
		0.500	0.396	73.6%
2	Well water 1	-	0.036	
		0.500	0.496	92.1%
1	Well water 2	-	0.094	
		0.600	0.587	82.2 %
2	Well water 2	0.600	0.077	
			0.662	97.6 %
2	Well water 3	-	0.034	
		0.500	0.529	99.1%
1	Lixiviate	-	0.127	
		0.600	0.504	62.8 %
2	Lixiviate	-	0.077	
		0.600	0.589	84.8 %
1	Tap water 1	-	0.084	
		0.600	0.649	94.2 %
2	Tap water 1	-	0.064	
		0.600	0.688	104 %
2	Tap water 2	-	0.031	
		0.250	0.285	102 %
2	Mineral Water	-	0.039	
		0.250	0.263	89.7%

P Procedure

Non-extractive methods can operate with higher repeatability and at measurement frequencies at 60 h^{-1} to 140 h^{-1} but to the cost of much lower sensitivity. [24,26,27] A similar performance in respect of time and sensitivity was achieved by Lindgren and Dasgupta (1992) while an interference study was missing in this work. [25] It should be pointed out that none of the given methods followed the standard procedure for the determination of DBAS, which could make a comparison of the results for complex matrices rather difficult.

In conclusion, the method proved to be applicable to water samples when extraction solvent washing is carried out. It could not overcome the typical AS interference and likewise require prior elimination of AS by anion exchange. However, due to the achieved miniaturization, the required amount of resin, operation time, and volume of solvent could be reduced and due to the high method sensitivity and possibility to perform in-system dilution of the sample with water, even solvent evaporation as part of the pretreatment step could be avoided.

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Conclusions

An automated method for the determination of CS from water samples was reported based on a novel configuration of in-syringe analysis, in which a denser solvent than water can be applied. In-system washing of the organic solvent was facilitated by the proposed analyzer configuration and a significant reduction of interferences was achieved. The method was applicable to the determination of CS in different water samples at sub-micromolar level. The interference of AS was not able to suppress but to diminish considerably by organic solvent washing with water, DSB and barium acetate solution. Repeatability, limit of detection, and analyte recovery were adequate for environmental studies of CS and the consumption of organic solvent and sample compared to the standard procedure was highly reduced.

Notes and references

[a] *Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, CZ-50005 Hradec Králové, Czech Republic. Fax: +420 495 067 504; Tel: +420 495 067 164; E-mail: Horstkob@faf.cuni.cz*

[b] *Laboratory of Environmental Analytical Chemistry – LQA2, University of the Balearic Islands, Cra. Valldemossa km 7.5, 07122 Palma de Mallorca, Spain. Fax: +34 971 173 462; Tel: +34 971 173 261; E-mail: Victor.Cerda@uib.es*

† Electronic Supplementary Information (ESI) available:
[ESI 1: Control circuit for the DC motor used for in-syringe stirring.
ESI 2: Procedure 1 for automated in-syringe stirring-assisted DLLME of cationic surfactants without organic phase washing.
ESI 3: Procedures 2 for automated in-syringe stirring-assisted DLLME of cationic surfactants with double organic phase washing.
ESI 4: Photo documentation of operation scheme of the simple extraction procedure 2.
ESI 5: Example of peak signals of calibrations wit both procedures.
ESI 6: Box-Behnken experimental design for the optimization of the volumes of buffer and DSB stock solutions and extraction time.
ESI 7: Optimization of parameters for simple extraction being the stirring time (a), volume of acetate buffer solution (b), and the volume of DSB solution (c).
ESI 8: Box-Behnken experimental design for the optimization of the volumes of barium acetate and DSB stock solutions for extraction solvent washing.
ESI 9: Optimisation of parameters for extract washing being the stirring time (a), volume of DSB solution (b), and the volume of barium acetate solution (c).]

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