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An *in-situ* extraction–preconcentration method using ionic liquid-based surfactants for the determination of organic contaminants contained in marine sediments

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ABSTRACT

The determination of a group of organic contaminants from marine sediments samples including three polycyclic aromatic hydrocarbons, five alkylphenols (*i.e.*, bisphenol-A or nonylphenol) and one paraben has been carried out using an extraction/preconcentration strategy with ionic liquid-based surfactants and high-performance liquid chromatography (HPLC) with diode array detection (DAD). Sediments are first extracted using two IL-based surfactants, specifically 1-hexadecyl-3-methyl imidazolium bromide (C_{16}MIm–Br) and 1-hexadecyl-3-butyl imidazolium bromide ($C_{16}C_4$Im–Br), as the extraction media in a microwave-assisted extraction (MAE) procedure, followed by a novel *in situ* preconcentration method. The optimized MAE method requires 0.1 g of sediment and 5 mL of 40 mM C_{16}MIm–Br IL solution. The extracts are then centrifuged and filtered. The subsequent preconcentration *in situ* approach consists of the insolubilization of an IL-based surfactant formed by a metathesis reaction using the anion-exchange reagent lithium bis[(trifluoromethane)sulfonyl]imide ($LiNTf_2$), which produces an analyte enriched IL microdroplet. The optimized *in situ* approach utilizes 4 mL of the filtered extract, which are mixed with acetonitrile and 92 μL of $LiNTf_2$ solution (0.5 g mL^{-1}), heated, vortexed and centrifuged. The formed IL microdroplet is then simply diluted with acetonitrile (~100 μL) and injected in the chromatograph without any further clean-up steps. The overall extraction/preconcentration method requires approximately 25 min in spite of dealing with complex solid samples, is nearly free of organic solvent (requires ~900 μL of acetonitrile per sample), and produces high preconcentration factors and quantification limits down to 0.04 mg kg^{-1} using HPLC–DAD.

1. Introduction

New trends in chemistry, and particularly in analytical chemistry, are shifted to the development of methods which minimize the amount of chemical waste generated in the laboratory. Green analytical chemistry intends to replace environmental-unfriendly methods [1]. Thus, the elimination or minimization of organic solvent consumption has been the core of microextraction techniques [2–4]. Other alternatives include the substitution of organic solvents for less harmful reagents [5]. In any case, the development of green extraction steps is particularly difficult when considering the extraction of complex solid samples.

Ionic liquids (ILs) are nonmolecular solvents which often can be used in place of organic solvents in extraction and microextraction procedures [5,6]. Their properties include negligible vapor pressure at ambient temperature, high thermal stability, tuned miscibility with water or a variety of organic solvents, and variable viscosity [7]. Their increasing applicability within analytical chemistry [8,9] arises from their structural tuneability and the ability to mix and match various cation/anion combinations. While certain classes of ILs have been pointed out to be toxic, current trends seek to couple suitable functional groups within their structures to produce greener ILs [10].

Some ILs have been described to undergo micellization, that is, to behave as surfactants when dissolved in water above a critical concentration [11,12]. Since 2004, approximately 48 IL-based

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surfactants have been reported [13]. These IL-based surfactants exhibit characteristics of cationic surfactants. However, the ability to modify the properties of the micellar solution by easily changing the identity of the cation/anion pair draws obvious comparisons with the tuneability of classic ILs. This same behavior is hardly observed with conventional cationic surfactants.

A number of applications have been described in sample preparation for IL-based surfactants [13]. Thus, they have been mainly used as extractant systems for organic compounds present in complex solid samples, including environmental [14,15], food [16,17] and medicinal plants [18–21], mainly in combination with microwave-assisted extraction (MAE). These procedures are carried out without the need of using organic solvents in the extraction step.

The main disadvantage of those MAE methods with IL-based surfactants lies with the difficulty in performing subsequent preconcentration steps. Therefore, the sensitivity of these methods is often limited.

Very recently, a novel preconcentration approach has been described for IL-based surfactants [22]. The method is based on transforming a water-soluble IL-based surfactant, specifically 1-hexadecyl-3-butylimidazolium bromide ($C_{16}C_4$Im–Br) into a water-insoluble surfactant, specifically 1-hexadecyl-3-butylimidazolium bis[(trifluoromethane)sulfonyl]imide ($C_{16}C_4$Im–NTf_2), via a metathesis reaction with a lithium salt ($LiNTf_2$) [22]. The application was developed for a group of hydrocarbons from toasted cereals. This preconcentration procedure followed previously described methods using several ILs (not IL-based surfactants) for the determination of metals [23–25] and organic compounds [26–29] in aqueous samples. The utility of the preconcentration step for IL-based surfactants rather than with regular ILs lies in the fact that IL-based surfactants can deal with complex solid samples; that is, using MAE (as an example) as a previous step of the preconcentration strategy.

The main purpose of this study is to expand the applicability of preconcentration procedures for IL-based surfactants towards complex solid samples. Thus, it is described for the first time the extraction/preconcentration procedure for the IL-based surfactant 1-hexadecyl-3-methylimidazolium bromide (C_{16}MIm–Br), and the modifications for the previously described method involving the $C_{16}C_4$Im–Br IL. The application is carried out with a group of three polycyclic aromatic hydrocarbons (PAHs), five alkylphenols including bisphenol-A (BPA) and nonylphenol (NP), and one paraben, from sediment samples; for being common pollutants environmentally monitored in the Canary Islands. The method is used in combination with high-performance liquid chromatography (HPLC) and diode array detection (DAD).

2. Experimental

2.1. Reagents and materials

The ionic liquid-based surfactants studied were 1-hexadecyl-3-methylimidazolium bromide (C_{16}MIm–Br) and 1-hexadecyl-3-buthylimidazolium bromide ($C_{16}C_4$Im–Br). These ionic liquids were synthesized and fully characterized according to a previous study [30]. The critical micelle concentration (CMC) values of these ILs-based surfactants in water have been previously determined to be 0.76 and 0.08 mM, respectively [31]. The preparation of $C_{16}C_4$Im–Br and C_{16}MIm–Br micellar solutions simply involves the dissolution of these ILs in water at a concentration above their respective CMC values.

The lithium bis[(trifluoromethane)sulfonyl]imide ($LiNTf_2$) salt was supplied by Sigma-Aldrich GmBH (Steinheim, Germany).

The polycyclic aromatic hydrocarbons studied were benzo(a)anthracene (BaA) and benzo(a)pyrene (BaP), both supplied by Aldrich Chem CO, and benzo(k)fluoranthene (BkF), supplied by Sigma-Aldrich Chemie GmBH (Steinheim, Germany). Individual standard solutions of these analytes were prepared in acetonitrile with concentrations ranging from 950 to 990 mg L^{-1}.

The alkylphenols used in this study were 4-*tert*-butylphenol (*t*-BP), 4-octylphenol (OP), 4-cumylphenol (4-CP), 4-*n*-nonylphenol (NP), and bisphenol-A (BPA). They were all supplied by Sigma-Aldrich Chemie GmBH, except NP, which was supplied by Alfa-Aesar (Karlsruhe, Germany). Individual standard solutions of these analytes were prepared in acetonitrile with concentrations ranging from 910 to 1170 mg L^{-1}.

The paraben used in this study was butylparaben (BuP) supplied by Sigma-Aldrich Chemie GmBH. Individual standard solution of this paraben was prepared in acetonitrile at 970 mg L^{-1}.

Main physicochemical characteristics of this heterogeneous group of contaminants are included in Table S1of the Supplementary material.

For the focused-microwave-assisted extraction procedure, standard solution mixtures containing paraben, alkylphenols and PAHs were prepared at concentration values ranging from 10 to 60 mg L^{-1} in acetonitrile. For the *in situ* preconcentration procedure, standard solution mixtures containing paraben, alkylphenols and PAHs were prepared at concentration values ranging from 0.2 to 1.2 mg L^{-1} in acetonitrile.

Acetonitrile of HPLC grade (Merck, Darmstadt, Germany) was used for such dilutions. Deionized water was obtained from a Milli-Q gradient A10 system (Millipore, Bedford, MA, USA). All the solvents were filtered through a 0.45 μm Durapore® membrane filter (Millipore) before being used in the chromatographic system.

KIMAX® centrifuge tubes (Sigma-Aldrich), with 35 mL of capacity, were used in the microwave. Stir bars of 10 × 3 mm were also obtained from Sigma-Aldrich. PIREX® centrifuge tubes with 20 mL of capacity were used for the *in situ* preconcentration procedure.

The method was validated using the certified reference material CRM-535, river harbor sediment, certified by BCR and revised under the responsibility of the Institute of Reference Materials and Measurements (IRMM, Geel, Belgium). This material was provided by LGC Promochem (Barcelona, Spain). This reference material is well-characterized, with an organic matter content of 12% (mass fraction), pore size < 2 mm, and certified for seven individual PAHs, three of them included in the present study. Other characteristics of this certified sediment are included in Table S2.

One real marine sediment sample was collected from the coasts of Tenerife (Canary Islands), in an area known for its low levels of contamination. This sediment, with an organic matter content of 27.3 g kg^{-1}, was used as a blank. Marine sediments were also sampled from a contaminated area, close to an oil-petroleum refinery. These contaminated sediments were characteristic for presenting an organic matter content of 59.2 g kg^{-1}. Table S2 also includes a summary of the main pollutants content in this contaminated sediment, obtained during routine analysis in an Environmental Lab using reference methods. After sieving, fractions of sediment with particle size < 250 μm were taken and used for subsequent analysis.

2.2. Instrumentation

Focused microwave-assisted extractions were performed at atmospheric pressure using a CEM Focused Microwave™ Synthesis System apparatus, model Discover (CEM corporation, Matthews, NC, USA) equipped with an infrared temperature control system, stirring and cooling options. Cooling is carried out by means of a flowing air.

A vortex model reax-control from Heidolph Instruments GMBH (Schwabach, Germany), and an Eppendorf Centrifuge model 5702

(Hamburg, Germany) were also used in the studies. The heating plate, model RCT Basic, was supplied by IKA® Werke (Staufen, Germany).

The HPLC consisted of a gradient system L-2130 Merck Hitachi Pump (supplied by Merck) and a Rheodyne valve (Supelco, Bellefonte, PA, USA) with a 20 μL loop. Detection was carried out using a ProStar 330 diode array detector (DAD) supplied by Varian (Palo Alto, CA, USA). Data acquisition was carried out with the Star 5.51 software by Varian.

The analytical column was a C18 Res Elut HPLC Column (5 μm, 150 × 4.6 mm) supplied by Varian, and protected by a Pelliguard LC-18 guard column (Supelco). A 100 μL Hamilton syringe (Reno, NV, USA) was used for all injections.

2.3. Procedures

2.3.1. Focused microwave-assisted extraction procedure

Three or five milliliters of an aqueous solution containing the IL-based surfactant was added to 0.1 g of the sediment and placed in a 35 mL tube. The IL concentration was dependent on the particular experiment. The optimum IL-based surfactant concentration for C_{16}MIm–Br and $C_{16}C_4$Im–Br was 40 mM. After adding an agitation bar to the extraction tube, it was introduced into the microwave cavity. The extraction was performed at a fixed maximum temperature of the vessels and at a fixed level of microwave oven power (both values dependant on the particular experiment). When the maximum temperature was reached, it was kept constant during the prefixed time. Afterwards, the tube was allowed to cool to room temperature. The supernatant was centrifuged for 5 min at 3600 rpm and then was quantitatively transferred and filtered through a CHROMAFIL® Xtra PET-45/25 disposable syringe filter (Machery-Nagel, Düren, Germany). The optimum microwave extraction conditions included a microwave power of 140 W to reach a maximum temperature of 90 °C. This temperature was then kept constant for 6 min.

2.3.2. In situ ionic liquid preconcentration procedure

During the optimization study of the *in situ* ionic liquid preconcentration procedure, non-spiked blank sediments were extracted with IL-based surfactants following the optimum conditions described in Section 2.3.1. Then, different volumes of these extract solutions (containing the IL-based surfactants C_{16}MIm–Br and $C_{16}C_4$Im–Br with a fixed concentration of 40 mM) were mixed with different aliquots of aqueous solution and a 0.5 g mL^{-1} LiNTf$_2$ solution. A 1:1 M ratio between the IL-based surfactant and the LiNTf$_2$ was kept constant. These mixtures were then mixed (or not) with acetonitrile, heated (or not), vortexed, centrifuged, and kept in the freezer (or not). The specific volume of the microdroplet formed was quantitatively measured and subjected to HPLC–DAD quantification if the analytes were present in the initial IL-based surfactant solution.

Under optimum conditions, 4 mL of the C_{16}MIm–Br microwave extract already filtered (from Section 2.3.1), were mixed with 800 μL of acetonitrile and 92 μL of the LiNTf$_2$ solution. Afterwards, the turbid solution was heated at 65 °C for 5 min, vortexed for 3 min, and then centrifuged for 4 min at 3400 rpm. A microdroplet of ~90 μL was formed at the bottom of the tube. The microdroplet was withdrawn with a 100 μL Hamilton syringe, diluted up to 200 μL with acetonitrile to decrease its viscosity as well as make it compatible for HPLC analysis, and vortexed for 1 min. The mixture was then injected in the HPLC without any additional clean-up step.

2.3.3. Spiking procedure for sediments

Spiking of marine sediment samples was performed according to the following procedure. A 0.1 g amount of the blank sediment was mixed with 45–200 μL of acetonitrile containing known concentrations of each analyte, dependent on the particular experiment, and then stirred for several seconds. The samples were then stored in the dark and allowed to dry. The concentration of spiked analytes oscillated between 0.89 and 5.3 mg kg^{-1} on dry-weight basis when using a low-spiked level, and between 19.8 and 118 mg kg^{-1} on dry-weight basis when using a higher spiked level.

Certified sediment, and non-spiked sediments originating from a contaminated marine area, were kept in the dark before the analysis and extracted without any spiking procedure.

2.3.4. HPLC method

The HPLC method used for the separation and determination of the analytes consisted of a gradient elution procedure. An acetonitrile and water mobile phase mixture was used at a flow-rate of 1 mL min^{-1}. A linear gradient was employed from 50 to 80% of acetonitrile over 8 min, then 80% of acetonitrile was maintained for 9 min; then from 80 to 100% of acetonitrile over 2 min and then 100% of acetonitrile was maintained for 3 min. Several wavelengths were selected for each analyte in the DAD and include: 228 nm for *t*-BP, OP, 4-CP, NP, and BPA; 255 nm for BuP; 275 nm for BaA; 238 nm for BkF; and 260 nm for BaP. 4-CP, BPA and *t*-BP were also measured at 238, 238 and 275 nm, respectively.

3. Results and discussion

3.1. HPLC separation of selected organic contaminants in the presence of IL-based surfactants

It is necessary to establish the influence of the IL-based surfactant solution on the chromatographic separation of the selected analytes. One of the problems often encountered when using micellar media with HPLC is that the chromatographic signal coming from the surfactant itself can prevent the quantification of the analytes of interest. This is especially significant when using nonionic surfactants containing alkylphenolic-polyoxyethylated chains, which produce a highly interfered fluorescence or absorbance signal [14,32]. Thus, analytes were dissolved in the maximum concentration (40 mM) tested for both IL-based surfactants. Fig. 1 shows the chromatograms of the analytes (at selected wavelengths), when dissolved in 40 mM C_{16}MIm–Br (Fig. 1(A)), in acetonitrile (Fig. 1(B)), and in 40 mM $C_{16}C_4$Im–Br (Fig. 1(C)). All chromatograms in the figure have been plotted with the same *y*-axis scale for comparative purposes.

It can be observed that all the studied compounds appeared to be completely separated from the IL in the chromatograms obtained if adequate wavelengths are selected. It can be observed that the IL presents a strong absorbance in the UV at 228 nm, which takes place at retention times lower than 5 min, and therefore, its elution interferes with the first eluting analytes. Therefore, it is necessary to make a proper selection of the λ in the DAD to avoid this interference. From the comparison of chromatograms included in Fig. 1, it can be observed that the IL-based surfactant C_{16}MIm–Br presents lower interfering signals in the chromatogram than $C_{16}C_4$Im–Br. In addition, it should be highlighted that there are no changes in retention times, peak areas or peak heights, when the analytes are dissolved in acetonitrile or in IL-based surfactants when proper wavelengths were selected.

Chromatographic calibration plots were constructed by dissolving the analytes in acetonitrile. The different analytes gave satisfactory retention times with relative standard deviation (RSD) values ranging from 0.6 to 5.2% (n=20). The quality parameters of the analytical calibration plots are included in Table 1. Calibration graphs were constructed by plotting the

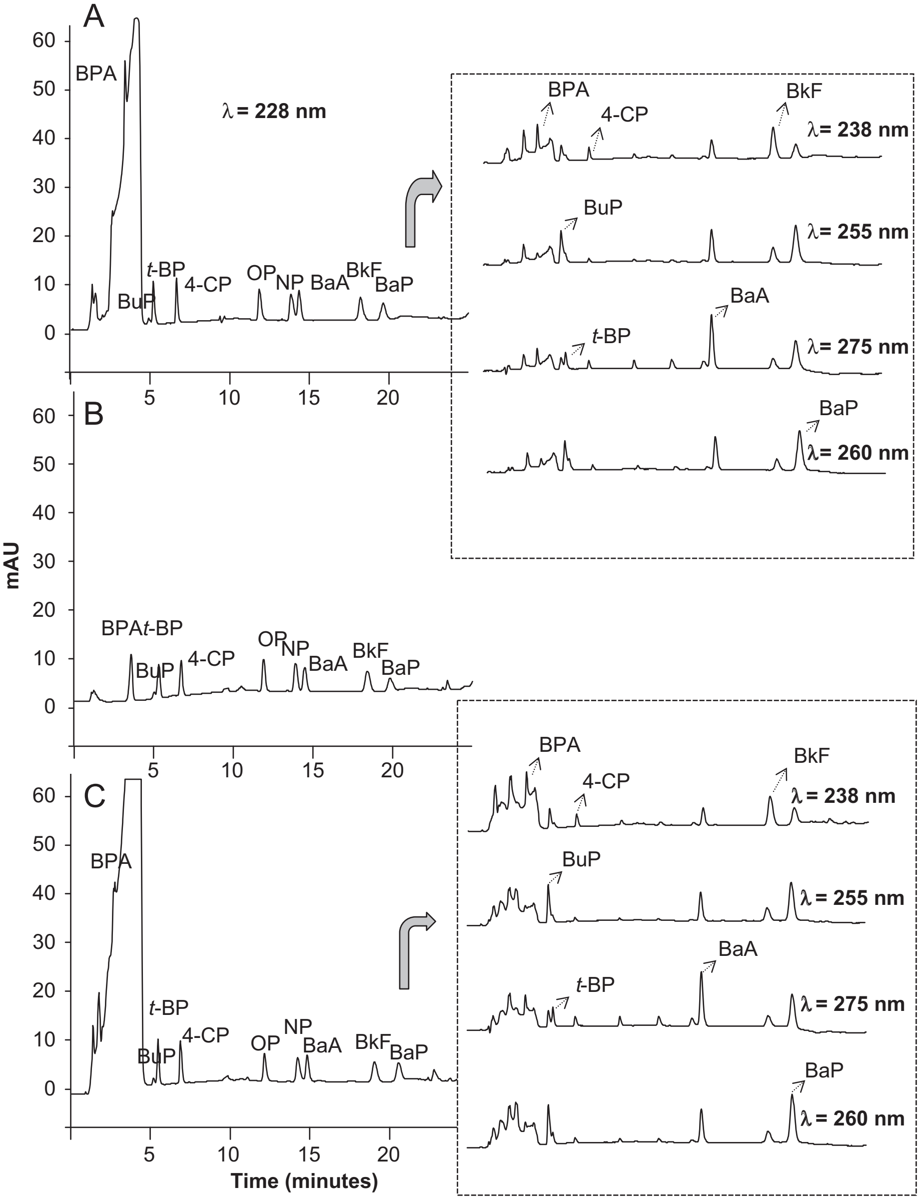


Fig. 1. Chromatograms of analytes dissolved in 40 mM C$_{16}$MIm–Br (A), in acetonitrile (B), or in 40 mM C$_{16}$C$_4$Im–Br (C). The analytes concentrations were 1.8 mg L^{-1} for BPA, 1.0 mg L^{-1} for BuP and BaP, 2.5 mg L^{-1} for *t*-BP, 1.6 mg L^{-1} for 4-CP, 2.6 mg L^{-1} for OP, 3.0 mg L^{-1} for NP, and 0.5 mg L^{-1} for BaA and BkF.

Table 1
Quality parameters of the chromatographic calibration plots for the studied analytes using HPLC–DAD.

Analyte	Wavelength (nm)	Calibration range[a] (mg L^{-1})	Slope ± SD[b]	Error of the estimate[c]	RSD[d] (%)	R^2	LOQ[e] (mg L^{-1})	Retention time ± SD[f] (min)
Bisphenol-A	228	0.10–2.6	75.3 ± 1.7	4.11	2.5 (1.4)	0.997	0.05	3.3 ± 0.2
	238		24.6 ± 0.5	1.23	3.4 (1.4)	0.997	0.05	
Butylparaben	255	0.06–1.4	95.5 ± 1.6	2.10	1.9 (0.8)	0.998	0.05	4.7 ± 0.2
4-*tert*-butylphenol	228	0.15–3.7	33.1 ± 0.6	2.03	1.7 (1.9)	0.998	0.08	5.0 ± 0.2
	275		11.9 ± 0.2	0.797	3.1 (1.9)	0.997	0.10	
4-cumylphenol	228	0.10–2.4	51.7 ± 0.9	2.00	1.6 (1.2)	0.998	0.05	6.5 ± 0.1
	238		14.4 ± 0.2	0.508	2.7 (1.2)	0.998	0.14	
Octylphenol	228	0.15–3.8	32.6 ± 0.7	2.37	1.5 (2.0)	0.997	0.08	11.7 ± 0.1
4-*n*-nonylphenol	228	0.18–4.4	28.3 ± 0.6	2.27	2.7 (2.3)	0.997	0.05	13.7 ± 0.1
Benzo(a)anthracene	275	0.03–0.70	285.4 ± 7.0	4.80	1.9 (0.4)	0.996	0.01	14.3 ± 0.1
Benzo(k)fluoranthene	238	0.03–0.70	255.5 ± 4.9	3.39	2.0 (0.4)	0.997	0.02	18.2 ± 0.3
Benzo(a)pyrene	260	0.06–1.4	158.7 ± 3.0	3.97	1.7 (0.7)	0.997	0.06	19.7 ± 0.4

[a] 8 calibration levels.

[b] Error of the slope for $n=8$ calibration levels.

[c] Standard deviation of the regression.

[d] Relative standard deviation for $n=7$. The concentration of the standard used is written in parenthesis (in mg L^{-1}).

[e] Limit of quantification, calculated as described in the text.

[f] Standard deviation for $n=20$.

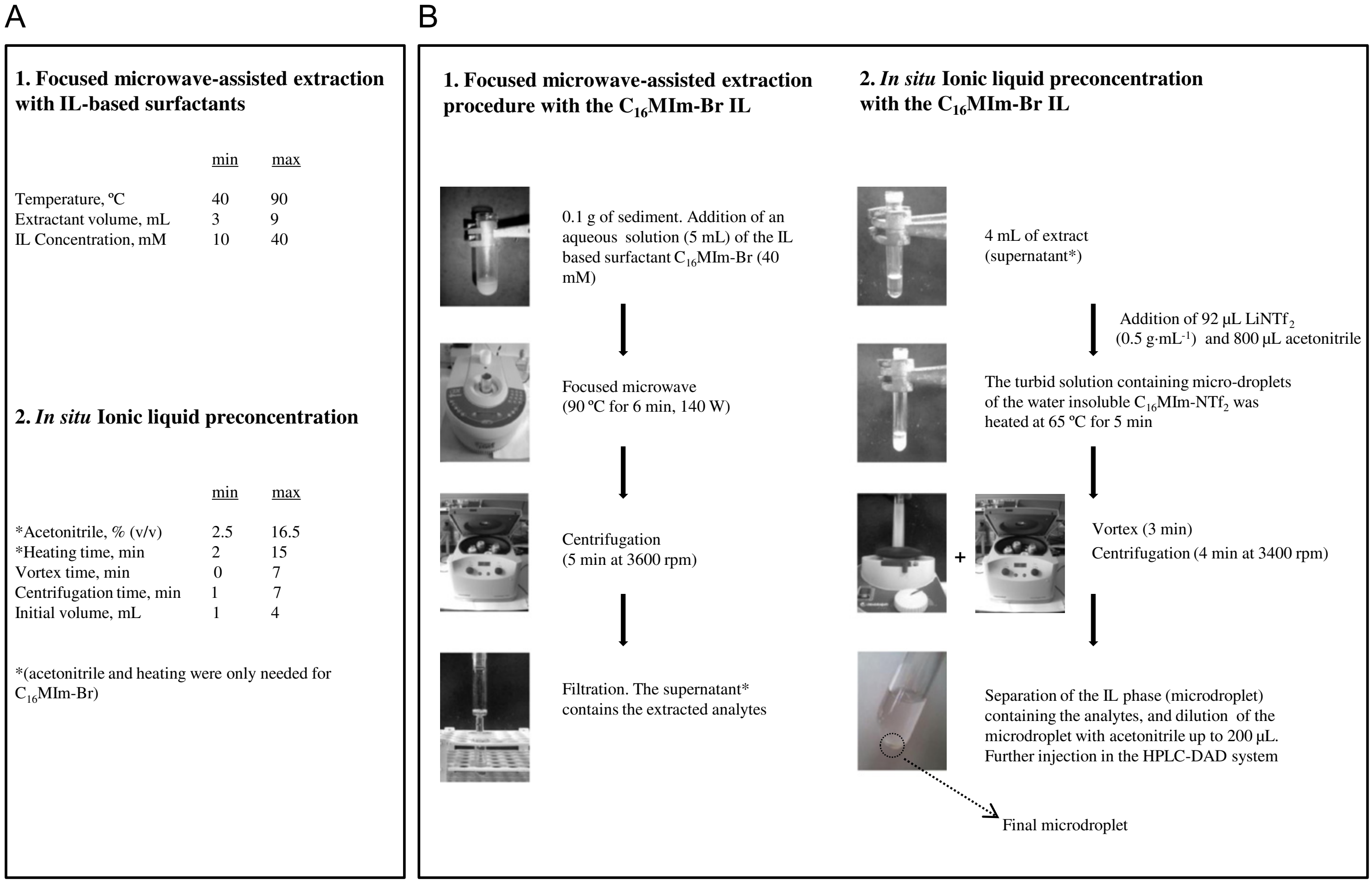
peak-area *versus* the analyte concentration. Linear relationships with R^2 values varying between 0.996 and 0.998 were obtained. Quantification limits for the calibration plots ranged from 0.01 mg L^{-1} for BaA to 0.1 mg L^{-1} for OP. They were calculated as 10 times the standard deviation of a standard prepared at very low concentration (half of the lower concentration tested in the calibration range). The precision (as RSD value) in the calculated concentration for seven consecutive injections of an intermediate standard varied between 1.5 and 3.4%.

The chromatographic calibrations of the studied analytes were also carried out using the maximum IL-based surfactant concentrations tested (40 mM). This study was carried out in order to compare the sensitivity of the calibrations obtained when analytes are dissolved in aqueous solutions of ionic-liquid based surfactants, with regards to the obtained sensitivity with calibrations in acetonitrile (Table 1). Table S3 shows such comparisons. The ratio of the slopes parameter (RSP, in %) has been used to easily compare the sensitivity values of the analytes dissolved in the IL-based surfactant solution *versus* the sensitivity obtained when dissolved in acetonitrile. It can be observed that RSP average values were of 103% and 101% for C_{16}MIm–Br and $C_{16}C_4$Im–Br, respectively, oscillating from 83.5% for BPA in $C_{16}C_4$Im–Br (at 238 nm), to 124% for BaP in $C_{16}C_4$Im–Br (at 260 nm). Furthermore, the similarity of slopes and intercepts of the calibration curves obtained were checked by means of an ANOVA analysis. The application of this test was not significant ($\alpha = 0.05$ as significance level), showing that the solvent media is not exerting a significant effect in the chromatographic calibrations. Therefore, analytes can be adequately quantified using calibrations obtained with standards dissolved in acetonitrile (Table 1).

3.2. Optimization of the microwave-assisted extraction procedure using IL-based surfactants

Focused microwave-assisted extraction of seven PAHs from sediments using the IL-based surfactant C_{16}MIm–Br has been previously described [14]. The purpose of the present work is not only to extract but to preconcentrate organic contaminants from sediments using IL-based surfactants. The analytes studied include three PAHs and also a group of endocrine disrupting chemicals including five alkylphenols and one paraben, which have been selected as representative contaminants of sediments in the Canary Islands. It was necessary to optimize the microwave extraction conditions for this group of chemicals using not only the C_{16}MIm–Br IL but also the $C_{16}C_4$Im–Br IL. A blank sediment, which was shown to be free of organic contaminants by an independent laboratory (Environmental Service of the University of La Laguna), was selected as a blank sample to carry out the spiking procedure and the optimization step.

Several variables can highly influence the microwave-assisted extraction procedure including the concentration of the IL-based surfactant solution and its volume, the microwave power, the maximum temperature to be reached in the focused-microwave system, and also the time at which this temperature must be kept fixed. Our previous experience in using the focused microwave system as well as several preliminary experiments indicated that a fixed time of 6 min and a microwave power at 140 W would be optimal in this study. Furthermore, the amount of sediment was fixed at 0.1 g. Given the short number of variables to optimize, we decide to follow a factor by factor optimization. Fig. 2(A) shows a



1. Focused microwave-assisted extraction with IL-based surfactants

	min	max
Temperature, °C	40	90
Extractant volume, mL	3	9
IL Concentration, mM	10	40

2. *In situ* Ionic liquid preconcentration

	min	max
*Acetonitrile, % (v/v)	2.5	16.5
*Heating time, min	2	15
Vortex time, min	0	7
Centrifugation time, min	1	7
Initial volume, mL	1	4

*(acetonitrile and heating were only needed for C_{16}MIm-Br)

Fig. 2. Scheme of the variables studied during the optimization step of both: the microwave-assisted extraction and the *in situ* approach with the IL-based surfactants C_{16}MIm–Br and $C_{16}C_4$Im–Br (A); and optimum variables for the microwave-assisted extraction and the *in situ* approach with the IL-based surfactant C_{16}MIm–Br (B).

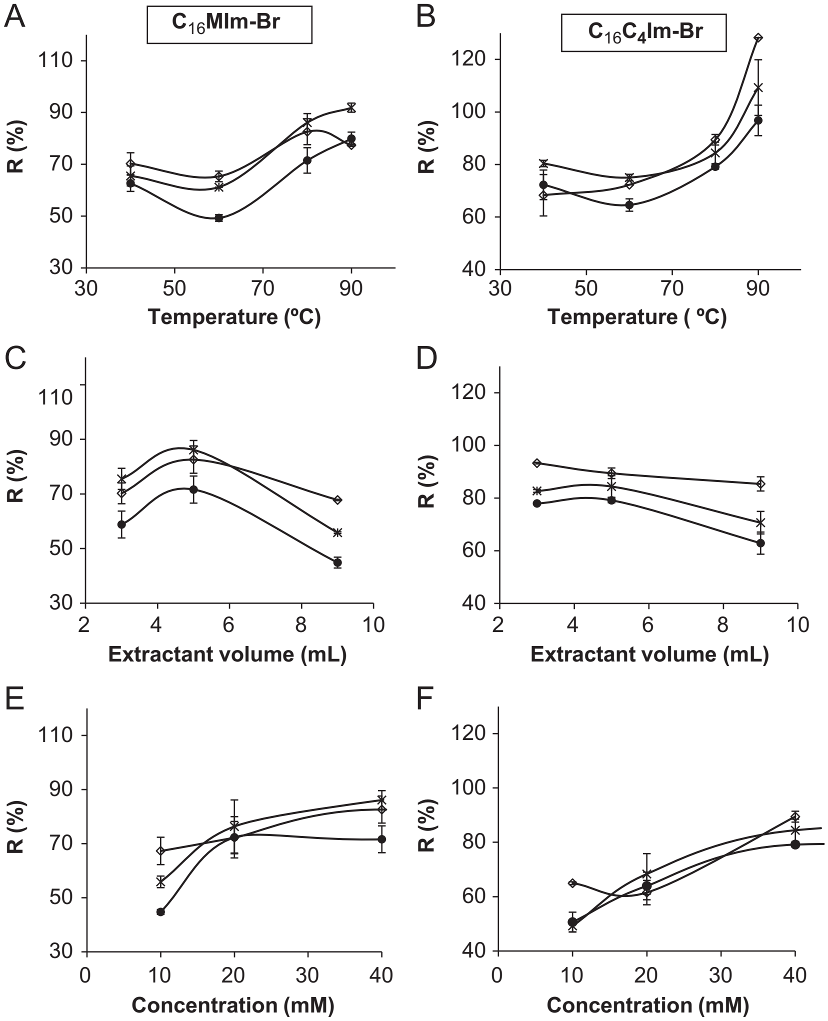


Fig. 3. Study of the variables influencing the microwave-assisted extraction step with IL-based surfactants: effect of the temperature (A) and (B), effect of the extractant volume (C) and (D), and effect of the IL-based surfactant concentration (E) and (F). Representative analytes included in the plots are: (◇) BuP, (●) NP, and (⁎) BaP. Plots on the left side (A), (C) and (E) correspond to C_{16}MIm–Br, and plots of the right side (B), (D) and (F) correspond to $C_{16}C_4$Im–Br.

scheme with the main variables optimized in this study, as well as their ranges.

The temperature was the first variable studied and was varied between 40 and 90 °C. Higher temperatures were not selected to avoid water evaporation and lower temperatures are not sufficient in a microwave-assisted step for complex solid samples such as sediments. The obtained recoveries for a spiked amount of 19.8–118 mg kg^{-1} (depending on the analyte) can be observed in Fig. 3(A) and (B) for a representative analyte of each family, namely, BaP for PAHs, NP for alkylphenols, and BuP as the paraben tested. The extraction recoveries were calculated for each analyte, taking into account the extractant volume used, the spiked amount and the chromatographic calibrations shown in Table 1. In general, it can be observed that high temperatures are preferred to reach high extraction efficiencies.

The second studied variable was the extractant volume with the extraction temperature in the microwave being held constant along with the concentration of the IL-based surfactant and the spiked amount in the sediment. The tested extractant volumes varied between 3 and 9 mL. Lower volumes were not tested in order to ensure adequate mixing between the sediment sample and the IL-based surfactant solution and higher volumes were not tested to avoid excessive dilution of the extracts. The results are shown in Fig. 3(C) and (D).

The last variable studied, the IL-based surfactant concentration, was optimized by keeping constant the extraction temperature in the microwave (80 °C during 6 min), The concentration was varied between 10 and 40 mM. A concentration of 40 mM is near the solubility limit of $C_{16}C_4$Im–Br in water at room temperature (~46 mM), and so higher amounts were not tested. The minimum concentration (10 mM) for C_{16}MIm–Br and $C_{16}C_4$Im–Br was chosen in order to have ranges of at least 12 and 100 times their respective CMC values. It is always desirable to work well above the CMC. The obtained recoveries are shown in Fig. 3(E) and (F). Clearly, the best results are obtained at higher surfactant concentrations.

Attending to the results obtained in these experiments, the selected temperature was 90 °C, the selected concentration for both IL-based surfactants was 40 mM, and the selected extraction volumes were 5 and 3 mL for C_{16}MIm–Br and $C_{16}C_4$Im–Br, respectively. Fig. 2(A) adequately summarizes the variables studied (and their studied ranges) during the optimization study of the microwave-assisted extraction step.

3.3. *Quality parameters of the microwave-assisted extraction method with IL-based surfactants*

The extraction efficiency (*R*%) and reproducibility (as RSD in %) of the optimized microwave-assisted extraction procedure using both IL-based surfactants in combination with HPLC–DAD was assessed by performing five extractions of the blank sediment, which was spiked at two different levels. One of the spiked levels corresponded to a low-intermediate level, with contents of organic contaminants varying between 7.4 and 44 mg kg^{-1}. The lowest spiked level corresponded to contents varying between 0.9 and 5.3 mg kg^{-1}, depending on the analyte. The obtained results can be observed in Table 2.

Average extraction efficiencies oscillated between 98.7% at the intermediate level to 104% at the lowest level for the $C_{16}C_4$Im–Br IL. For the C_{16}MIm–Br IL, average extraction efficiencies varied from 87.9% at lowest spiked level and 95.9% at intermediate spiked level. With regards to precision, RSD values varied from 4.4 to 21% for the lowest spiked level, and from 7.1 to 14% at the intermediate level. Poorer precision is normally obtained when working at low levels, close to the limit of quantification.

Limits of quantification (LOQ) were calculated taken into account the chromatographic LOQ values (calculated in Section 3.1, Table 1), and considering the extraction efficiencies obtained in the microwave-assisted extraction method when spiking at the lowest level. The calculated LOQs are also shown in Table 2. They oscillated between 0.42 and 3.4 mg kg^{-1} for the $C_{16}C_4$Im–Br IL, and between 0.78 and 4.2 for the C_{16}MIm–Br IL.

To validate the microwave-assisted extraction method with IL-based surfactants in combination with HPLC–DAD, three independent extractions of the certified material BCR-535 were carried out using the established experimental conditions. This sediment is certified for three of the analytes included in this study, namely, BaA, BkF and BaP, with certified values (in mg kg^{-1}, $\pm$ uncertainty with $K=2$) of 1.54 ± 0.10, 1.09 ± 0.15, and 1.16 ± 0.10, respectively.

If the $C_{16}C_4$Im–Br IL is used as extractant system in the microwaves, BaP cannot be determined because its certified content is below the quantification limit of the microwave-assisted extraction method. The obtained contents for the remaining PAHs were 1.4 ± 0.3 mg kg^{-1} for BaA (average extraction efficiency of 89%) and 0.5 ± 0.4 mg kg^{-1} for BkF (average extraction efficiency of 46%). If the C_{16}MIm–Br IL is used, only BaA could be quantified, and therefore, it was decided to do not extract the certified material with this IL-based surfactant.

The optimized microwave-assisted extraction method with IL-based surfactants was also intended for an application with several sediment samples, coming from a contaminated area in Tenerife. The usual levels of PAHs for sediments collected in this area normally oscillate between 0.03 and 0.25 mg kg^{-1}. Given these common levels and considering the limits of quantification achieved with the extraction procedure (Table 2), a preconcentration step is necessary in order to increase the overall sensitivity.

3.4. *In situ preconcentration method for IL-based surfactants*

On contrary to the common preconcentration approach for non-ionic surfactants (the cloud-point extraction) upon heating, cationic surfactants do not suffer cloud-point unless extreme pH values or high ionic strengths are used. Therefore, the development of preconcentration schemes for cationic surfactants is of interest as they have been widely used in analytical chemistry as extractant media [33–35].

Our group has recently proposed the utilization of a metathesis reaction to transform a water soluble IL-based surfactant (specifically $C_{16}C_4$Im–Br) into a water insoluble IL-based surfactant (specifically $C_{16}C_4$Im–NTf_2), in a specific application for toasted cereal samples [22]. Such metathesis reaction was successfully accomplished by adding a lithium bis[(trifluoromethane)sulfonyl]imide salt ($LiNTf_2$) while ensuring an appropriate mol:mol ratio between $C_{16}C_4$Im–Br and $LiNTf_2$. The preconcentration method was then followed by vortex, centrifugation, and a 1 h freezing time to ensure an adequate settling (and further manipulation) and formation of the $C_{16}C_4$Im–NTf_2 IL microdroplet.

The C_{16}MIm–Br IL-based surfactant has not been yet tested in any preconcentration scheme, and the $C_{16}C_4$Im–Br IL-based surfactant has not been tested with endocrine disrupting chemicals. The present work intends to find the optimum *in situ* preconcentration conditions not only for the $C_{16}C_4$Im–Br IL but also for the non-tested C_{16}MIm–Br IL, both in an application with sediment samples. It should be emphasized that the matrix has a crucial effect in the development of the metathesis reaction [22].

We chose a factor by factor optimization for the *in situ* approach involving both IL-based surfactants, given the (*a priori*) short number of variables to be controlled. The optimization study with IL-based surfactants was carried out by fixing the aqueous solution concentration of $LiNTf_2$ to 0.5 g mL^{-1}, and performing the *in situ* preconcentration approach with extracts originating from the optimized microwave-assisted method of non-spiked blank sediments using IL-based surfactants (Section 3.3). Thus, the extract contains 40 mM of the IL-based surfactants, and co-extracted components from the sediment sample by the employment of the microwaves. The conductivity of such extracts was 271 μS for the $C_{16}C_4$Im–Br IL and 168 μS for the C_{16}MIm–Br IL. All experiments for the *in situ* optimization were carried out in triplicate. The initial volume used for the IL-based solutions was 2 mL, unless otherwise stated.

3.4.1. *Optimization of the in situ approach with $C_{16}C_4$Im–Br and sediment samples*

Fig. 2(A) includes a summary of the optimization study carried out for the *in situ* approach. The optimization of the *in situ* preconcentration method for the $C_{16}C_4$Im–Br IL with sediment

Table 2
Limits of quantification (LOQs), average extraction efficiencies (*R*%), and precision (RSD in %), for the microwave-assisted extraction method in combination with HPLC–DAD, and using both IL-based surfactants under optimized conditions. Blank sediments were subjected to extraction after spiking them at different levels ($n=5$).

Analyte	Lowest spiked level (0.9–5.3 mg kg^{-1})				Intermediate spiked level (7.4–44 mg kg^{-1})				LOQ (mg kg^{-1})	
	$C_{16}C_4$Im–Br		C_{16}MIm–Br		$C_{16}C_4$Im–Br		C_{16}MIm–Br		$C_{16}C_4$Im–Br	C_{16}MIm–Br
	R (%)	RSD (%)	*R* (%)	RSD (%)	*R* (%)	RSD (%)	*R* (%)	RSD (%)		
Bisphenol-A	–	–	–	–	116	10	78.4	11	1.6	2.6
Butylparaben	112	17	91.0	9.2	109	14	96.4	13	1.4	2.9
4-*tert*-butylphenol	85.3	4.4	52.6	21	98.4	1.1	87.6	5.3	3.0	4.2
4-cumylphenol	106	11	105	10	100	8.4	108	8.8	1.6	2.6
Octylphenol	122	21	98.0	13	96.1	10	99.3	11	3.4	3.9
4-*n*-nonylphenol	93.8	17	87.1	20	85.7	9.6	90.3	11	1.6	2.9
Benzo(a)anthracene	102	14	91.8	17	89.5	10	100	13	0.42	0.78
Benzo(k)fluoranthene	88.3	16	75.7	13	77.8	11	86.5	14	0.71	1.4
Benzo(a)pyrene	124	12	102	18	116	10	117	11	1.5	3.0

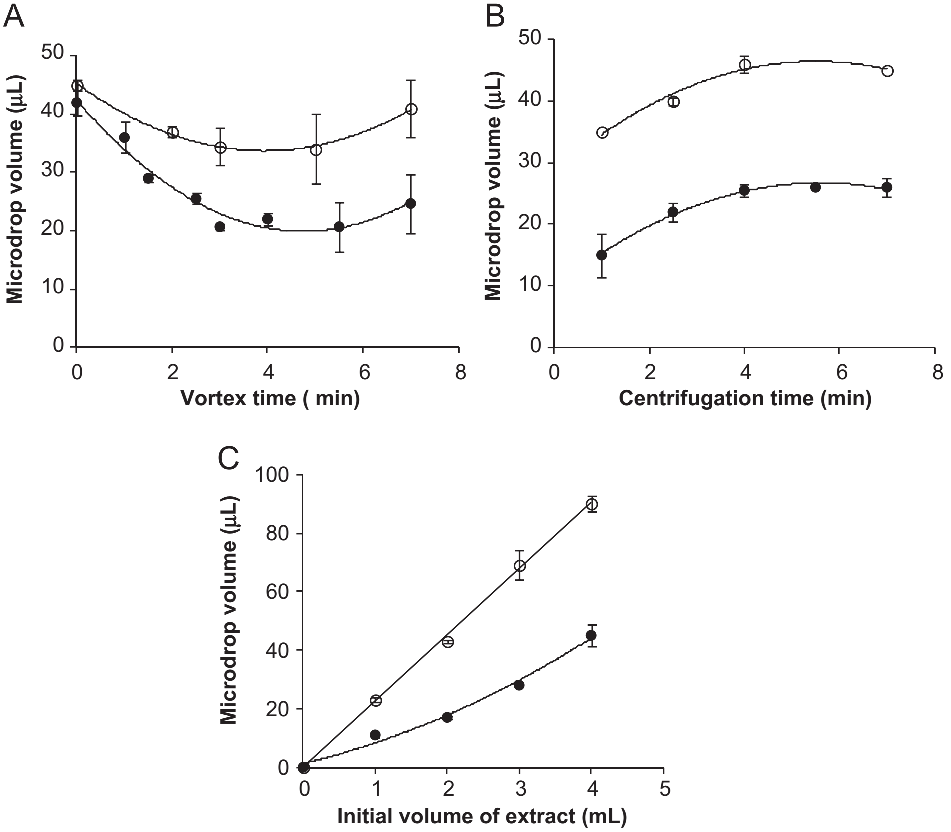


Fig. 4. Influence of several variables in the *in situ* preconcentration procedure for $C_{16}C_4$Im–Br (●) and C_{16}MIm–Br (○). (A) Influence of the vortex time. The variables fixed in this study were 1:1 M ratio between the IL-based surfactant and Li–NTf_2, and centrifugation time of 4 min (at 3400 rpm) after vortex. (B) Influence of the centrifugation time. The variables fixed in this study were 1:1 M ratio between the IL-based surfactant and Li–NTf_2, and 3 min of vortex time. (C) Influence of the initial volume of the IL-based surfactant solutions in the *in situ* performance. The fixed conditions were: 3 min of vortex time and 4 min of centrifugation. In all cases, 1 h of freezing time after centrifugation was used for $C_{16}C_4$Im–Br; whereas 16.5% (v/v) of acetonitrile and heating for 5 min at 65 °C before vortex were necessary for C_{16}MIm–Br.

extract samples was carried out modifying the procedure described for the same IL-based surfactant when used in the extraction of cereals [22]. Thus, variables which exerted an influence in cereal extracts were also examined in this study. The optimization was shifted towards obtaining a $C_{16}C_4$Im–NTf_2 IL microdroplet with a low volume (to ensure a high preconcentration factor), while also ensuring that it is easy to handle and is highly reproducible.

The effect of vortex time after mixing the $C_{16}C_4$Im–Br IL and LiNTf_2 in the sediment extract (1:1 M ratio) was studied between 0 and 7 min while the centrifugation time (4 min at 3400 rpm), and the freezing time (1 h) after centrifugation were kept constant. The volume of the microdroplet obtained at different vortex times is shown in Fig. 4(A). Every experiment was carried out in triplicate. It can be observed that 3 min is a sufficient time to obtain a stable microdroplet with minimum size (but still high enough to be easily manipulated), and with sufficient reproducibility. Under these conditions, the microdroplet size was 20.7 ± 1.2 µL.

The centrifugation time was varied between 1 and 7 min at 3400 rpm with all initial conditions from previous work being kept constant [22]: 2.5 min for the vortex time after mixing the $C_{16}C_4$Im–Br IL and LiNTf_2 in the sediment extract (1:1 M ratio), and 1 h in the freezer after centrifugation. It was observed (results included in Fig. 4(B)) that 4 min was sufficient to reach a reproducible and small microdroplet volume, of 25.5 ± 1.9 µL. Lower times were accompanied by low volumes and make the microdroplet more difficult to handle.

Finally, the freezing time necessary to obtain a clear microdroplet was evaluated, after mixing the $C_{16}C_4$Im–Br IL and LiNTf_2 in the sediment extract (1:1 M ratio), while the initial conditions of our previous work were kept constant [22]: vortex (2.5 min) and centrifuging (4 min at 3400 rpm). For sediment extracts, it was observed that the freezing step was not necessary and the microdroplet of the $C_{16}C_4$Im–NTf_2 IL was easy to settle to the bottom of the tube after centrifugation. Therefore, the tedious freezing step (needed when using $C_{16}C_4$Im–Br with cereal samples) was totally unnecessary with sediment samples.

In summary, for the extraction of endocrine disrupting chemicals and PAHs from sediments using the $C_{16}C_4$Im–Br IL, the *in situ* preconcentration step should be carried out after microwaves by mixing $C_{16}C_4$Im–Br and Li-NTf_2 with a 1:1 M ratio, followed by vortex during 3 min, centrifuging for 4 min, and removing the microdroplet with a syringe, which is then diluted up to 100 µL with acetonitrile when the initial volume of $C_{16}C_4$Im–Br is 2 mL. The diluted microdroplet is then ready for HPLC–DAD analysis.

The effect that the initial volume of the IL-based surfactant exerts on the preconcentration procedure must also be studied, especially since the main utility of this preconcentration step lies in its application towards extracts originating from MMAE of sediment samples. All previous experiments have been carried out using an initial IL-based surfactant solution volume of 2 mL. Fig. 4(C) shows the variation in the obtained microdroplet of the $C_{16}C_4$Im–NTf_2 IL when the initial volume of $C_{16}C_4$Im–Br coming from the microwave extract was varied between 1 and 4 mL. In

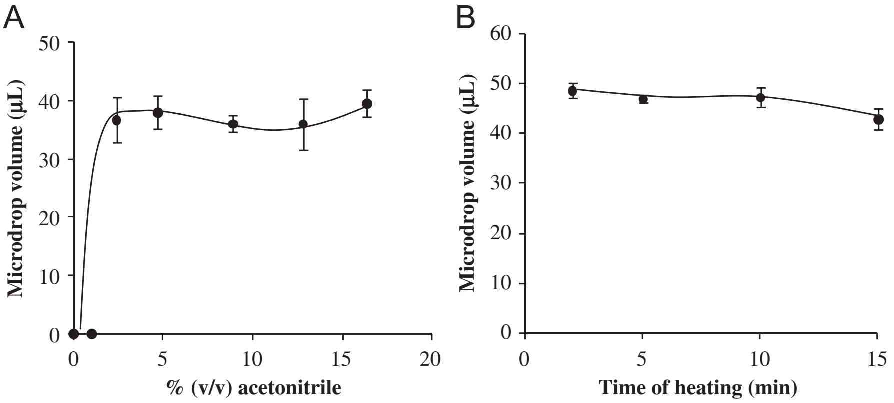


Fig. 5. Influence of the acetonitrile content (A) and heating time at 65 °C (B) in the *in situ* preconcentration performance (monitored as volume of C_{16}MIm–NTf_2 drop formed) for C_{16}MIm–Br. The fixed conditions were a 1:1 M ratio between the IL-based surfactant and $LiNTf_2$ and: (A) heating time of 5 min (at 65 °C), a vortex time of 3 min, and a centrifugation time of 4 min (no freezing step); and (B) 16.5% of acetonitrile (v/v), heating at 65 °C, 3 min of vortex time after heating, and 4 min of centrifugation time after vortex (no freezing step). The remaining conditions as described in the text.

the studied range, it can be observed that the final microdroplet volume varied from 11 ± 1 to 45 ± 7 μL.

3.4.2. *Optimization of the in situ approach with C_{16}MIm–Br and sediment samples*

Fig. 4(A) also includes a summary of the optimization study carried out for the *in situ* approach for C_{16}MIm–Br. The C_{16}MIm–Br IL has not been tested in any *in situ* preconcentration procedure. If the aforementioned *in situ* conditions for the $C_{16}C_4$Im–Br IL are used with the C_{16}MIm–Br IL, the solution does not become turbid and no microdroplet is formed. In other words, the addition of $LiNTf_2$ followed by vortex and centrifugation (or even freezing) is not sufficient to observe a final microdroplet. Indeed, the development of an *in situ* preconcentration step for the C_{16}MIm–Br IL required much more steps, such as the addition of acetonitrile followed by heating of the solution. Furthermore, the order of the addition of reagents was important to observe the formation of the C_{16}MIm–NTf_2 IL microdroplet, with the necessity of adding acetonitrile before $LiNTf_2$, followed by heating, vortexing, and centrifugation (in this order).

The addition of acetonitrile to the $C_{16}C_4$Im–Br IL has been studied in a previous work [31]. It was observed that the $C_{16}C_4$Im–Br IL kept its micellar integrity with acetonitrile content above 20% (v/v). The CMC value for the $C_{16}C_4$Im–Br IL is 0.463 mM with an acetonitrile content of 20% (v/v). A similar situation has been observed for C_{16}MIm–Br, but its CMC at high acetonitrile content has not been reported. The influence of acetonitrile in the *in situ* step for $C_{16}C_4$Im–Br was studied between 2.5 and 16.5% (v/v) (below 20%), while other variables such as a 1:1 M ratio with $LiNTf_2$, heating at 65 °C for 5 min, a vortex time of 3 min, and a centrifugation time of 4 min (no freezing) were kept constant. The obtained results can be observed in Fig. 5(A). The addition of ~5% (v/v) of acetonitrile is sufficient to observe a C_{16}MIm–NTf_2 IL microdroplet. However, it is necessary to work up to 16.5% (v/v) of acetonitrile in order to have enough reproducibility in the handling of the microdroplet.

The heating time after mixing the C_{16}MIm–Br IL and $LiNTf_2$ when using 16.5% of acetonitrile (v/v) was studied between 2 and 15 min. In all cases, temperatures of 65 °C were employed. The obtained results can be observed in Fig. 5(B). The conditions used for the subsequent experiments included 3 min of vortex after heating and 4 min of centrifugation time after vortex. It was observed that 5 min was sufficient to obtain an adequate microdroplet of the C_{16}MIm–NTf_2 IL.

The vortex time after heating was also optimized. It was studied up to 7 min, while keeping constant the remaining variables; 1:1 M ratio between C_{16}MIm–Br and $LiNTf_2$, 16.5% (v/v) of acetonitrile, heating for 5 min at 65 °C, and centrifugation after vortex for 4 min. The results can be observed in Fig. 4(A). It can be observed that a vortex time of 3 min was enough to obtain an adequate microdroplet of the C_{16}MIm–NTf_2 IL with a volume of 34.4 ± 3.6 μL.

The centrifugation time was also studied and the obtained results are included in Fig. 4(B). The following experimental conditions were kept fixed in the study: 16.5% (v/v) of acetonitrile, 5 min of heating, and 3 min of vortex. It was observed that 4 min was sufficient to obtain an adequate sized microdoplet of C_{16}MIm–NTf_2, and higher times did not influence the microdroplet size.

Under optimized conditions for C_{16}MIm–Br (1:1 M ratio between C_{16}MIm–Br and $LiNTf_2$, 16.5% of acetonitrile, 5 min of heating at 65 °C, 3 min of vortex and 4 min of centrifugation), the obtained microdroplet of C_{16}MIm–NTf_2 was 46.0 ± 0.2 μL.

Finally, the effect of the initial volume of the C_{16}MIm–Br IL was examined. It must be pointed out that in the optimization study of the C_{16}MIm–Br IL, an initial was of 2 mL was used. However, the initial volume is important considering that the *in situ* approach will be applied directly after microwave irradiation (in which the maximum volume that can be obtained after filtration of the microwave extract in this particular case is 4 mL). Thus, Fig. 4(C) shows the variation in the obtained microdroplet of C_{16}MIm–NTf_2 when the initial volume of C_{16}MIm–Br is varied between 1 and 4 mL, keeping constant the aforementioned optimized conditions. A microdroplet volume of 90 ± 6 μL was achieved using an initial volume of 4 mL.

3.5. *Quality parameters of the in situ preconcentration step with HPLC–DAD and its coupling the microwave-assisted extraction of sediments*

Calibrations for the *in situ* preconcentration method and HPLC–DAD were conducted in sediment extracts obtained in the microwave step (rather than in aqueous solutions of IL-based surfactants), utilizing the extracts as the solvent medium of analytes. Thus, different concentrations of analytes were spiked in sediment extracts, and such standards were subjected to the optimized *in situ* preconcentration procedure. It must be taken into account that such sediment extracts already contain $C_{16}C_4$Im–Br or C_{16}MIm–Br.

There were many interfering signals in the HPLC–DAD chromatograms when blank sediments samples extracted with $C_{16}C_4$Im–Br and microwaves were later subjected to the optimum *in situ* preconcentration method. Therefore, it was concluded that for sediment samples, the optimum IL-based surfactant to simultaneously carry out a microwave-assisted extraction followed by an *in situ* preconcentration was C_{16}MIm–Br. The calibrations used blank sediments, which were extracted by microwaves and the C_{16}MIm–Br IL using the optimum conditions (described in Section 2.3.1). Four milliliter of the filtered extract were then spiked with the analytes at different concentration levels and subjected to the optimum *in situ* preconcentration conditions: mixing with 800 μL of acetonitrile and 92 μL of $LiNTf_2$ (0.5 g mL^{-1}). The turbid solution was then heated at 65 °C during 5 min, vortexed for 3 min, and then centrifuged during 4 min at 3400 rpm. A C_{16}MIM–NTf_2 microdroplet of ~90 μL was later withdrawn with a 100 μL Hamilton syringe, diluted up to 200 μL with acetonitrile, and vortexed for 1 min. The mixture was then injected to the HPLC without any additional clean-up step. Fig. 2(B) summarizes the optimum conditions for C_{16}MIm–Br with regards both: the microwave-assisted extraction and the *in situ* approach. Calibrations plots for the *in situ* preconcentration method were constructed by plotting the peak-area of the analytes *versus* the initial concentration of the analytes spiked in the microwave extract. Table 3 includes several quality analytical parameters of such calibrations. Linear relationships with R^2 varying between 0.991 and 0.998 were obtained in all cases, except for 4-CP, in which the R^2 value was 0.948. The calibration for 4-CP and C_{16}MIm–Br was interfered by co-extracted components of the sediment, which are also preconcentrated with the *in situ* approach. Therefore, this analyte is only tentatively quantified with the overall approach. The quantification limits for the *in situ* preconcentration calibration plots ranged from 1 μg L^{-1} for BkF and BaA to 25 μg L^{-1} for 4-CP. It must be highlighted these low levels, which can be determined by HPLC–DAD given the high preconcentration achieved with the *in situ* approach. Quantification limits were calculated as ten times the standard deviation of standard prepared at very low concentration, and subjected to the overall *in situ* method ($n=6$). The obtained limits are 3 to 30 times (12 on average) lower than the chromatographic ones (Table 1), which tentatively shows the preconcentration achieved.

The extraction efficiency, precision and enrichment factor values obtained with the *in situ* preconcentration method are included in Table 4. This study was carried out at two different spiked levels in the sediment extract: from 4 to 26 μg L^{-1} (low level) and from 20 to 110 μg L^{-1} (intermediate level).

Relative recoveries (RR, in %) are obtained by comparing the performance of these sediments extracts spiked at both levels, with regards to the performance of extracts used in the calibrations included in Table 3. These RR values are very close to 100%. Average RR values were 107 and 105% at the low and intermediate spiked level, respectively.

The enrichment factor (E_F) was calculated using the following equation:

$$E_F = C_{drop}/C_{initial} \tag{1}$$

where C_{drop} is the concentration of the final IL microdroplet obtained by *in-situ* preconcentration and taking into account its dilution with acetonitrile before HPLC injection, and is obtained from the chromatographic calibration (Table 1). From Table 4, it can be observed that average E_F values oscillate between 13 at the low spiked level to 15 at the intermediate spiked level. This is in agreement with the aforementioned tentative preconcentration values obtained by comparing limits of quantification (12 on average). The precision of the method is also acceptable, with RSD values ranging from 3.8 to 19%.

The real extraction recovery (R or E_R) of the *in situ* preconcentration method, also known as relative enrichment factor, is calculated using Eq. 2:

$$R(\%) = E_R(\%) = RE_F(\%) = 100 \times E_F/E_{Fmax} \tag{2}$$

where E_{Fmax} is the maximum preconcentration achieved if all analytes are effectively concentrated in the final C_{16}MIm–NTf_2 microdroplet of the *in situ* preconcentration method. E_{Fmax} can be calculated from the ratio $V_{initial}/V_{drop}$, being $V_{initial}$ the sediment extract volume used (4 mL), and V_{drop} the microdroplet volume including its dilution factor with acetonitrile (200 μL). Therefore, E_{Fmax} is roughly 20. It can be observed average extraction efficiencies for all analytes studied of 67% at the low spiked level and of 76% at the intermediate spiked level. For the group of PAHs studied, the efficiency is quantitative with average extraction efficiencies of 99.8 and 94.6% at the low and intermediate level, respectively. Extraction efficiencies in microextraction procedures such as solid-phase microextraction (SPME) or dispersive liquid-liquid microextraction (DLLME) do not necessarily have to be 100%, as long as the reproducibility is acceptable. Indeed, E_R values of 29–91% [36] or 55–74% [37] have been reported in DLLME applications for various classes of analytes.

Finally, the overall limits of quantification for the proposed method were estimated. They are included in the last column of Table 3 and were calculated considering the LOQs of the *in situ* step altogether with the extraction efficiency obtained in the microwave-assisted extraction step. They oscillate between 0.04 mg kg^{-1} for BaA and BkF, and 1.0 mg kg^{-1} for 4-CP. These LOQs are quite acceptable considering that DAD is employed. Indeed, these LOQs are, on average, 17 times lower than those obtained by simply applying the microwaves step (without preconcentration), as it can be observed in Table 2.

Table 3
Quality analytical parameters of the *in situ* preconcentration calibrations using HPLC–DAD, and limits of quantification for the overall extraction/preconcentration method developed.

Analyte	Wavelength (nm)	Calibration range[a] (mg L^{-1})	Slope[a] ± SD	Error of the estimate[b]	R^2	LOQ[c] (mg L^{-1})	LOQ[d] (mg kg^{-1})
Bisphenol-A	228	0.010–0.132	764.9 ± 22.9	2.80	0.994	0.005	0.20
Butylparaben	255	0.009–0.073	942.6 ± 43.8	2.51	0.991	0.003	0.12
4-*tert*-butylphenol	275	0.023–0.188	113.1 ± 4.9	0.57	0.994	0.020	0.80
4-cumylphenol	238	0.046–0.120	123.5 ± 16.6	1.01	0.948	0.025	1.00
Octylphenol	228	0.023–0.190	496.3 ± 9.5	1.45	0.998	0.020	0.80
4-*n*-nonylphenol	228	0.016–0.221	498.6 ± 11.6	2.37	0.996	0.009	0.36
Benzo(a)anthracene	275	0.003–0.037	5390.7 ± 118.7	4.08	0.997	0.001	0.04
Benzo(k)fluoranthene	238	0.004–0.037	4214.3 ± 103.6	3.56	0.996	0.001	0.04
Benzo(a)pyrene	260	0.005–0.071	3316.3 ± 67.8	4.47	0.997	0.002	0.08

[a] 8 calibration levels.
[b] Standard deviation of the regression.
[c] Limit of quantification of the *in situ* preconcentration procedure, calculated as described in the text.
[d] Limit of quantification for the overall method: micellar microwave-assisted extraction + *in situ* preconcentration, calculated as described in the text.

Table 4
Analytical performance of the *in situ* preconcentration method with C_{16}MIm–Br and sediment extracts.

Analyte	Wavelength (nm)	Lowest spiked level (0.004–0.026 mg L^{-1})				Intermediate spiked level (0.020–0.110 mg L^{-1})			
		RR (%)	RSD (%)	E_F	E_R or *R* (%)	*RR* (%)	RSD (%)	E_F	E_R or *R* (%)
Bisphenol-A	228	109	7.5	5.4	27.1	109	11	9.7	48.6
Butylparaben	255	93.4	6.0	10	50.3	103	8.9	10	51.8
4-*tert*-butylphenol	275	107	13	7.7	38.3	100	10	9.0	44.8
4-cumylphenol	238	–	–	–	–	114	19	18	90.6
Octylphenol	228	109	10	8.9	44.7	101	3.8	14	68.4
4-*n*-nonylphenol	228	107	8.2	15	73.2	111	9.6	18	92.6
Benzo(a)anthracene	275	111	8.0	24	120	100	9.2	20	98.4
Benzo(k)fluoranthene	238	127	9.2	18	87.9	101	8.9	16	79.5
Benzo(a)pyrene	260	91.1	4.4	18	89.5	102	10	21	106

Table 5
Application of the overall extraction/preconcentration procedure with C_{16}MIm–Br to the certified sediment BCR-535.

Analyte	Certified value (mg kg^{-1}) $\pm$ uncertainty[a]	Found (mg kg^{-1}) $\pm$ SD[b]
Benzo(a)anthracene	1.54 ± 0.10	1.76 ± 0.09
Benzo(k)fluoranthene	1.09 ± 0.15	0.53 ± 0.06
Benzo(a)pyrene	1.16 ± 0.10	0.91 ± 0.11

[a] Uncertainty of certified values for an average of 13 accepted results (Uncertainty=$K \times$SD, being the coverage factor K=2).

[b] n=5.

3.6. *Applications of the overall extraction/preconcentration method with C_{16}MIm–Br*

To determine the accuracy of the overall extraction/preconcentration method, five independent extractions of the BCR-535 sediment were carried out keeping the established experimental conditions (Fig. 2(B)). Table 5 shows the average results obtained, and the comparison with the certified values. The inclusion of the *in situ* preconcentration step permits the use of the C_{16}MIm–Br IL as extractant agent for the certified sediment. As stated in Section 3.3, the sole use of microwaves with the C_{16}MIm–Br IL was not sufficient.

Finally, the overall method was applied to real sediments coming from a contaminated area, and also from a non-contaminated area, both sampled in Tenerife. Sediments originating from the non-contaminated area were analyzed by the overall extraction/preconcentration method, and they were shown to be free of the organic contaminants studied in this work (or at least, below the LOQ values reported in Table 3). With regards to the sediment sampled in the contaminated area, the following analytes were detected and quantified with the overall optimized method (amount $\pm$ standard deviation): 0.37 ± 0.06 mg kg^{-1} of BuP, 4.0 ± 1.0 mg kg^{-1} of OP, 1.1 ± 0.3 mg kg^{-1} of NP, 0.30 ± 0.09 mg kg^{-1} of BaA, and 0.19 ± 0.04 mg kg^{-1} of BaP. Samples were analyzed in triplicate. In any case, the levels founds for PAHs in this contaminated sediment are within the common range according to other local environmental studies.

Finally, a comparative of the performance of the proposed method using IL-based surfactants for the extraction of PAHs from sediments and HPLC–DAD has been carried out with regards to the performance of the conventional Soxhlet extraction and GC-FID. The main characteristics of such comparative can be observed in Table S4. The Soxhlet extraction is characterized for higher preconcentration factors, and lower limits of detection (however, it must be noticed that we are using in this work HPLC with DAD detection and not fluorescence detection). On the other hand, the proposed method with the IL-based surfactant C_{16}MIm–Br allows the using of lower amounts of sediments, the wasting of lower amounts of extraction solvent, and significantly decreasing the extraction time, keeping adequate quality analytical parameters.

4. Conclusions

It is reported for first time a combined extraction–preconcentration procedure for sediment samples utilizing two IL-based surfactants as extraction/preconcentration solvents. The procedure is based on utilizing a water-soluble IL-based surfactant ($C_{16}C_4$Im–Br or C_{16}MIm–Br) as extractant solvent in a microwave-assisted extraction (MAE) procedure. Then, the water-soluble IL containing the extracted analytes from the sediments is transformed into a water-insoluble IL ($C_{16}C_4$Im–NTf_2 or C_{16}MIm–NTf_2), via a simple metathesis reaction. Thus, extracted analytes experience an important preconcentration into the water-insoluble IL: a microdroplet of few μL, which is diluted with acetonitrile and subjected to HPLC–DAD.

The overall optimized method is practically organic solvent free, requiring only ~900 μL of acetonitrile per sample, and it takes ~25 min overall in spite of sample complexity.

The method has been applied to the determination of five alkylphenols (including bisphenol-A and nonylphenol), three polycyclic aromatic hydrocarbons (PAHs), and one paraben contained in sediments, exhibiting nice extraction efficiencies (67% in average when spiking at low levels, being quantitative for PAHs: 94.6–99.8%), and good precision values (lower than 19%). Certified sediment for PAHs was also used to evaluate this method with satisfactory results.

The developed *in situ* approach would enormously improve the sensitivity of extraction methods of other organic contaminants contained in complex sediment samples, by using IL-based surfactants.

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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.2012.07.073.

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