Simultaneous extraction and concentration of water pollution tracers using ionic-liquid-based systems

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ABSTRACT

Human activities are responsible for the release of innumerous substances into the aquatic environment. Some of these substances can be used as pollution tracers to identify contamination sources and to prioritize monitoring and remediation actions. Thus, their identification and quantification are of high priority. However, due to their presence in complex matrices and at significantly low concentrations, a pre-treatment/concentration step is always required. As an alternative to the currently used pre-treatment methods, mainly based on solid-phase extractions, aqueous biphasic systems (ABS) composed of ionic liquids (ILs) and $K_3C_6H_5O_7$ are here proposed for the simultaneous extraction and concentration of mixtures of two important pollution tracers, caffeine (CAF) and carbamazepine (CBZ). An initial screening of the IL chemical structure was carried out, with extraction efficiencies of both tracers to the IL-rich phase ranging between 95 and 100%, obtained in a single-step. These systems were then optimized in order to simultaneously concentrate CAF and CBZ from water samples followed by HPLC-UV analysis, for which no interferences of the ABS phase-forming components and other interferents present in a wastewater effluent sample have been found. Based on the saturation solubility data of both pollution tracers in the IL-rich phase, the maximum estimated concentration factors of CAF and CBZ are 28595- and 8259-fold. IL-based ABS can be thus envisioned as effective pre-treatment techniques of environmentally-related aqueous samples for a more accurate monitoring of mixtures of pollution tracers.

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1. Introduction

Recent investigations on persistent pharmaceutical compounds as pollution tracers have allowed to accurately track the source, fate and type of aquatic contamination [1–4]. Among these, the pharmacological markers caffeine (CAF) and carbamazepine (CBZ) have been amongst the most reported pollution tracers, found at the $\mu g\,L^{-1}$ level in diverse worldwide aqueous samples [5–10]. High performance liquid chromatography (HPLC) coupled with ultraviolet (UV) or fluorescence detectors have been recurrently used for the identification and quantification of pollution tracers in aqueous environments since these techniques offer a simple and more economical route, when compared, for instance, with mass spectrometry methods/detectors [11,12]. However, due to the high limits of detection of more conventional analytical equipment,

a pre-treatment and/or concentration of the target compounds from the original aqueous matrices is usually required [11,13]. Pre-treatment methods are usually applied to remove the organic matter aiming at decreasing interferences, also contributing to an increase in the quantification accuracy [11,12]. Several pre-treatment techniques have been proposed and optimized for CBZ present in water samples, such as solid-phase extraction (SPE) [14,15], solid-phase microextraction (SPME) [16], and liquid-phase microextraction (LPME) [17]. Due to its good recovery capacity, SPE has been widely reported and used as a *priori* concentration approach for CAF and CBZ [13,18–20]. Nevertheless, the pre-treatment methods commonly employed present several disadvantages: i) they are time-consuming, labor-intensive and costly [13]; ii) they require the use of large amounts of hazardous volatile organic compounds (VOCs)/solvents [13]; and iii) they present additional difficulties regarding the recovery of the adsorbed target analytes after the SPE process [13]. Therefore, a simpler and cost-effective pre-treatment technique which would allow a more

accurate monitoring of pollution tracers present in aquatic environments remains a challenge to the scientific community.

Aqueous biphasic systems (ABS) composed of ionic liquids (ILs) have been studied as pre-treatment techniques for a wide variety of samples [21]. ABS are more benign alternatives than conventional liquid-liquid extraction processes since the use of VOCs is avoided [21]. Conventional ABS are formed by the mixture of different pairs of solutes (polymer-polymer, polymer-salt or salt-salt) in aqueous media above specific concentrations. In addition to these more conventional ABS, the use of ILs as phase-forming components of ABS has led to remarkable extraction efficiencies and selectivity [21]. ILs are salts composed of a large organic cation and an organic or inorganic anion, by general definition liquid at temperatures below 373 K [22]. Besides their outstanding properties, such as a negligible vapor pressure at ambient conditions, and high thermal and chemical stabilities, one of the most relevant characteristics of ILs comprises their tunable ability, resulting from the large plethora of chemical structures available for their ions [22] - a feature that is transferable to IL-based ABS [21].

IL-based ABS have been investigated as pre-treatment techniques of several complex matrices targeting a more accurate identification/quantification of different compounds [21,23–26]. However, in most works, no concentration factors were explored [21,23,25–27]. Only few addressed the investigation of enrichment factors provided by IL-based ABS, with low values so far achieved [24,28]. Liu et al. [24] and Du et al. [28] reported concentration factors of 10 and 20 for steroid hormones and proteins, respectively, from human fluids. Up to now, and to the best of our knowledge, only three works reporting on the use of ABS composed of ILs and inorganic salts have taken the major steps on the development of IL-based ABS as viable concentration techniques. Passos et al. [29] reported the application of ABS composed of hydrophilic ILs and K_3PO_4 to completely extract and concentrate up to 100-fold the endocrine disruptor bisphenol A (BPA) from biological fluids. Additionally, Noorashikin et al. [30] demonstrated the performance of IL-based ABS in the presence of β-cyclodextrin to extract parabens from water samples, achieving concentration factors ranging from 95 to 103. When dealing with pharmaceutical contaminants in environmental aqueous samples, it is necessary to have even higher concentration factors due to their presence at vestigial concentrations. In this context, Dinis et al. [31] recently demonstrated that it is possible to completely extract and concentrate the synthetic hormone 17α-ethinylestradiol (EE2) from aqueous samples by manipulating the IL chemical structure and phases' volumes, where a concentration factor up to 1000-fold was reported, allowing therefore an adequate detection and quantification of EE2 in wastewater samples through HPLC equipped with a fluorescence detector. In all of these investigations no major interferences of the ILs as phase-forming components have been found. In addition to the use of ILs as extraction solvents, these have also been used as additives in mobile phases of liquid chromatography, showing to be able to improve the separation and resolution of this technique [32–34]. Although remarkable results have been reported with IL-based ABS [29–31], these works only address the extraction and concentration of individual compounds. Yet, when dealing with complex matrices, such as wastewater samples, the presence of a large number of pollution tracers has to be considered. In this context, the potential of IL-based ABS as pre-treatment strategies of a wider range of pollution tracers is still an unexplored issue.

In this work, we propose a more complete study on the potentiality of IL-based ABS to extract and concentrate mixtures of pollution tracers from wastewater matrices, in particular mixtures of CAF and CBZ. Their ubiquitous presence has been investigated throughout the past years, being these two compounds the most reported pollution tracers in wastewater samples [5,6,8–10,35], and most often present as mixtures [6,8,9]. The chemical structures

of the studied pollution tracers CAF and CBZ are depicted in Fig. 1. An initial screening of ABS composed of $K_3C_6H_5O_7$ and several hydrophilic ILs was carried out to identify the most promising systems to completely extract CAF and CBZ in a single-step, followed by studies on their mixtures, and application to real wastewater effluent samples. Finally, the maximum concentration factors achievable with these systems was estimated based on the saturation values of each pharmaceutical in the IL-rich phase.

2. Experimental section

2.1. Materials

The ILs studied in this work were: 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[C_4C_1im][CF_3SO_3]$, 1-butyl-3-methylimidazolium dicyanamide, $[C_4C_1im][N(CN)_2]$, 1-butyl-3-methylimidazolium thiocyanate, $[C_4C_1im][SCN]$, 1-butyl-3-methylimidazolium bromide, $[C_4C_1im]Br$, 1-butyl-3-methylimidazolium chloride, $[C_4C_1im]Cl$, 1-butyl-2,3-dimethylimidazolium chloride, $[C_4C_1C_1im]Cl$, tetrabutylammonium chloride, $[N_{4444}]Cl$, tetrabutylphosphonium chloride, $[P_{4444}]Cl$, 1-butyl-1-methylpyrrolidinium chloride, $[C_4C_1pyr]Cl$, and 1-butyl-1-methylpiperidinium chloride, $[C_4C_1pip]Cl$. The imidazolium-, pyrrolidinium-, and piperidinium-based ILs were purchased from Iolitec. Tetrabutylphosphonium chloride was kindly offered by Cytec Industries Inc. Tetrabutylammonium chloride was obtained from Sigma-Aldrich. All ILs used have a stated supplier purity of at least 98 wt%. Before use, aiming at reducing the water and volatile compounds contents to negligible values, all IL samples were dried under constant agitation at vacuum and at a temperature of 323 K for a minimum of 48 h. After this procedure, the purity of each IL was further checked by ^1H and ^{13}C NMR spectra. The chemical structures of the ILs studied in this work are depicted in Fig. 2. The potassium citrate tribasic mono-hydrated, $K_3C_6H_5O_7 \cdot H_2O$, >99.0 wt% pure, was acquired from Sigma-Aldrich. Caffeine (CAF) anhydrous, >99 wt% pure, was supplied by Fluka, and carbamazepine (CBZ) anhydrous, >99 wt%, was supplied by Sigma. Both compounds were used as received. The water employed was double distilled, passed across a reverse osmosis system, and further treated with a Milli-Q plus 185 water purification apparatus. HPLC grade acetonitrile, 99 wt% pure, was acquired from HiPerSolv Chromanorm.

2.2. Experimental procedure

2.2.1. Screening of IL-based ABS for the extraction of CAF and CBZ

For the selection of improved IL-based ABS for the one-step extraction and concentration of CAF and CBZ, several ternary systems (IL + $K_3C_6H_5O_7$ + H_2O) were prepared within the biphasic region at constant weight fraction percentages of each component: 40 wt% of IL + 20 wt% of $K_3C_6H_5O_7$ + 40 wt% of H_2O. To better characterize these mixtures, namely on the composition of the coexisting phases and achievable concentration factors, the ternary phase diagrams of the respective ABS were taken from the literature [36], with the exception of the phase diagram of the ABS formed by $[C_4C_1C_1im]Cl + K_3C_6H_5O_7 + H_2O$ that was experimentally determined in this work. Further details on the experimental determination of the phase diagrams and tie-lines (TLs), which describe the composition of each phase at a given mixture composition, are given in the Supporting Information. Aqueous solutions of CAF at 0.912 g L^{-1} were used as the water weight fraction that should be added to each ABS. Due to its low solubility in water, and thus to difficulties in preparing aqueous solutions of known composition, *ca.* 10^{-3} g of CBZ were added directly to each ternary mixture and individual experiment. The ternary mixtures were vig-
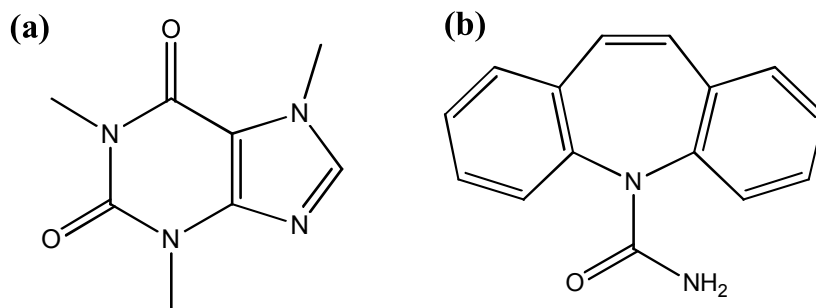
Fig. 1. Chemical structures of the investigated pharmaceuticals: (a) caffeine (CAF), and (b) carbamazepine (CBZ).
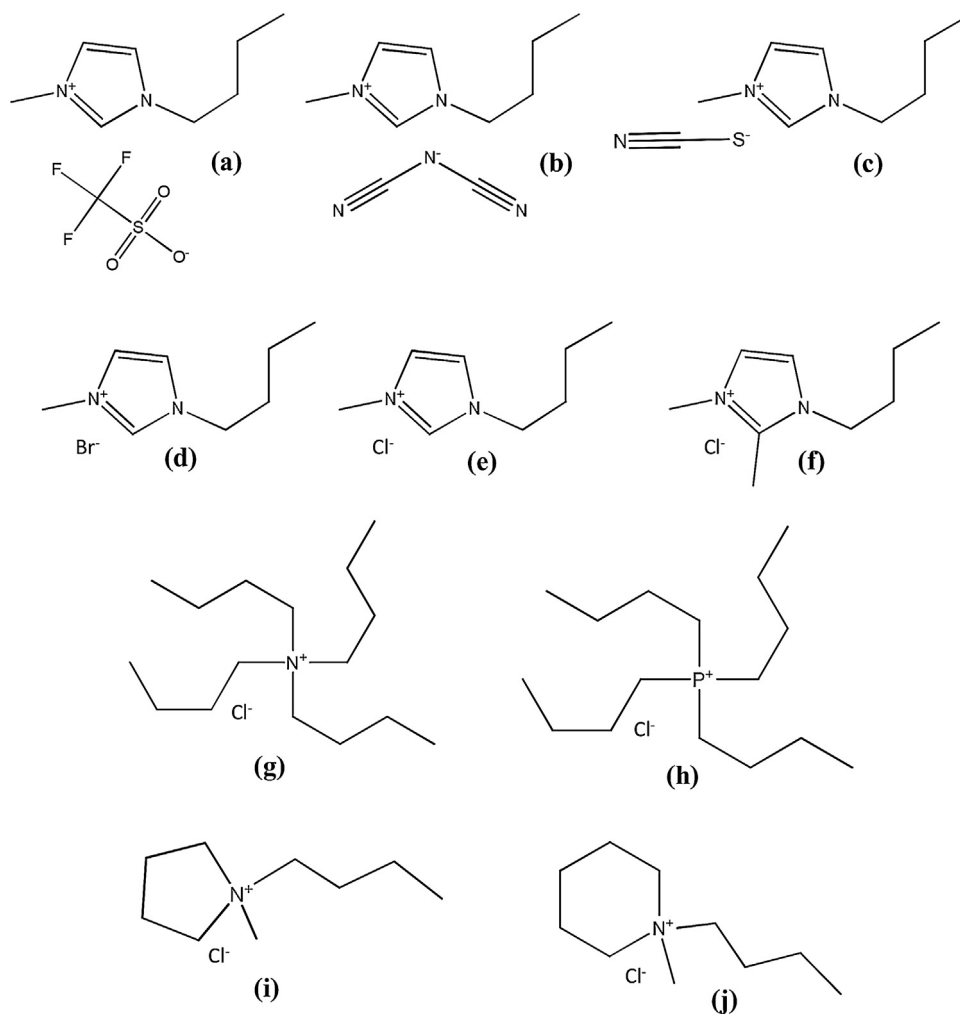
Fig. 2. Chemical structures of the investigated ILs: (a) $[C_4C_1im][CF_3SO_3]$, (b) $[C_4C_1im][N(CN)_2]$, (c) $[C_4C_1im][SCN]$, (d) $[C_4C_1im]Br$, (e) $[C_4C_1im]Cl$, (f) $[C_4C_1C_1im]Cl$, (g) $[N_{4444}]Cl$, (h) $[P_{4444}]Cl$, (i) $[C_4C_1pyr]Cl$, and (j) $[C_4C_1pip]Cl$.

orously stirred and left to equilibrate for at least 12 h at (298 ± 1) K. After the separation of the top and bottom phases, they were separated and weighted within $\pm 10^{-4}$ g to determine the TLs (*cf.* the Supporting Information), and the quantification of the pollution tracers in each phase was carried out through UV-spectroscopy, using a Synergy|HT Microplate Reader, from Biotek, at 272 nm for CAF and 285 nm for CBZ, using calibration curves previously established. Blank controls of each ternary system, without pollution tracers, were always prepared and used. Three samples of each phase were analyzed, in at least three individual systems, in order to determine the average extraction efficiencies of CAF and CBZ and the respective standard deviations.

The percentage extraction efficiencies of the pollution tracers, *EE%*, were determined by Eq. (1):

$$EE\% = \frac{w^{IL}}{w^{IL} + w^{salt}} \times 100 \qquad (1)$$

where w^{IL} and w^{salt} are the total weight of CAF and CBZ present in the IL-rich and in the $K_3C_6H_5O_7$-rich phases, respectively.

The pH values of both the IL-rich and salt-rich phases were measured at (298 ± 1) K using a Mettler Toledo SevenMulti pH meter within ± 0.02.

2.2.2. Validation of the IL-based ABS concentration technique by HPLC quantification of CAF and CBZ and use of real effluent samples

The ABS formed by $[N_{4444}]Cl + K_3C_6H_5O_7 + H_2O$ was used in the studies of the pollution tracers concentration, for which the tie-line length (TLL) of 79 was used. In order to confirm the TL and TLL data reported in the literature [36] obtained by a gravimetric method [37], they were additionally determined by an analytical approach (detailed information in the Supporting Information).

CAF and CBZ were quantified in the IL-rich phase using a Shimadzu High-Performance Liquid Chromatograph (HPLC) Prominence system equipped with a SPD-20A, UV detector, at a wavelength of 272 nm and 285 nm, respectively. The equipment comprises a degasser DGU-20A5, a bomb LC-20AD, and a column oven CTO-10ASVP. An ACE C18 column-PFP (5 μm, 150 mm × 4.6 mm) connected to an ACE 5 C18 4.6 mm *i.d.* guard column was used. Both the column and cell temperature were maintained at (298 ± 1) K. Standard aqueous solutions of $[N_{4444}]Cl$ at known concentrations, as well as IL aqueous solutions spiked with CAF and CBZ at known concentrations, were used in order to optimize the flow and composition of the mobile phase by gradient elution (detailed information in the Supporting Information). The mobile phase consisted of a water–acetonitrile gradient mixture, at a flow rate of 0.8 mL min^{-1}. Both solvents were filtered before use using 0.2 μm polyamide membrane filters from Whatman. The IL-rich phase containing CAF and CBZ was analyzed in order to determine the extraction efficiencies and concentration factors of both pollution tracers at the IL-rich phase. At least three replicates of each sample were carried out. Blank controls of the systems (no pollution tracers added) were always prepared and the corresponding IL-rich phases were also analyzed. A final concentration in the IL-rich phase was fixed at 1×10^{-3} g L^{-1} for both CAF and CBZ (5.15×10^{-6} mol L^{-1} and 4.23×10^{-6} mol L^{-1}, respectively). Individual standard stock aqueous solutions of CAF and CBZ were prepared at appropriate concentrations (1×10^{-3} g L^{-1}) in order to compare the peaks areas of the pharmaceuticals obtained in the aqueous standard solutions and in the IL-rich phase. Since this methodology is intended to be applied directly to environmental samples, an effluent sample from a wastewater treatment plant serving a population of about 20,000 inhabitants, located in central Portugal, was also used. As stated before, a final concentration in the IL-rich phase was fixed at 1×10^{-3} g L^{-1} for both CAF and CBZ. In order to compare the peak areas, individual standard stock solutions of CAF and CBZ at appropriate concentrations (1×10^{-3} g L^{-1}) using the effluent sample were additionally prepared. The effluent sample was filtered before use through a 0.45 μm regenerated cellulose membrane filters from Whatman.

2.2.3. Solubility of CAF and CBZ in the $[N_{4444}]Cl$-rich phase

In order to evaluate the maximum concentration factor of CAF and CBZ in the studied ABS, the solubility (saturation) of each compound was determined in the IL-rich phase of the respective ABS, at (298 ± 1) K and atmospheric pressure. To this end, each tracer was added in excess amounts to 1 g of individual samples of the $[N_{4444}]Cl$-rich phase (*ca.* 1.55×10^{-1} g for CAF and 1.00×10^{-2} g for CBZ) and left to equilibrate under constant agitation using an Eppendorf Thermomixer Comfort at (298 ± 1) K and atmospheric pressure. Previously optimized conditions were established: the agitation was settled at 850 rpm during 72 h. After saturation, all samples were centrifuged and left to equilibrate for 24 h at (298 ± 1) K. The liquid phases were carefully collected and the pollution tracers were quantified through UV-spectroscopy, using a Synergy|HT Microplate Reader, from Biotek, at a wavelength of 272 nm for CAF and 285 nm for CBZ, using calibration curves previously established. Blank controls were always prepared to eliminate interferences caused by the IL and the salt. Three samples
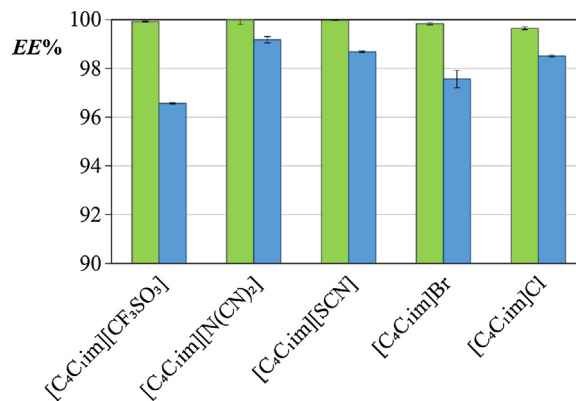

Fig. 3. IL anion effect on the ABS extraction efficiencies, *EE*%, of CAF (■) and CBZ (■).

of each aqueous phase were analyzed, in at least three individual systems, in order to determine the average solubility values of CAF and CBZ and the respective standard deviations.

3. Results and discussion

An initial optimization of IL-based ABS to act as extraction/concentration platforms is required in order to find the most adequate conditions, such as the nature of the phase-forming components and the composition of the initial mixture. This approach was taken into consideration by scanning the extraction efficiencies of IL-based ABS composed of different ILs for CAF and CBZ, as discussed below. For all ABS investigated, the top-rich phase corresponds to the IL-rich phase, whereas the bottom-phase represents the $K_3C_6H_5O_7$-rich phase.

3.1. Screening of IL-based ABS for the extraction of CAF and CBZ

All ternary phase diagrams studied for the extraction of pollution tracers were previously characterized and are reported in the literature [36], with the exception of the $[C_4C_1C_1im]Cl + K_3C_6H_5O_7 + H_2O$ ABS that was experimentally determined in this work. The corresponding liquid-liquid phase diagram, as well as the parameters from the data fitting, are provided in the Supporting Information. The pH values of the coexisting phases, the experimental TLs, *i.e.* the compositions of the coexisting phases, along with their respective lengths (TLL), are also reported in the Supporting Information.

The effects of the IL anion, the IL cation core, and number of aliphatic tails were evaluated regarding the partitioning of the two pollution tracers between the coexisting phases of all ABS at a fixed mixture composition (40 wt% of IL + 20 wt% of salt + 40 wt% of aqueous solutions containing CAF or CBZ). Figs. 3 and 4 depict the extraction efficiencies of the investigated IL-based ABS, formed with different ILs and $K_3C_6H_5O_7$, for CAF and CBZ. Detailed data are provided in the Supporting Information. In general, both CAF and CBZ preferentially partition to the IL-rich phase, with extraction efficiencies higher than 95% in all systems evaluated. Since the pH values of each phase range from neutral to slightly alkaline values (from 6.70 to 10.01; *cf.* the Supporting Information with detailed data), and CAF and CBZ are mostly present in their neutral form according to their pK_a values (pK_{a1}/pK_{a2} of 0.12/10.5 for CAF [38] and pK_{a1}/pK_{a2} of 2.3/13.9 for CBZ [35,39]), electrostatic interactions occurring between the salt or IL ions and charged solutes do not play a significant role in the partition trend observed with these systems/solutes.

The effect of the IL anion can be appraised in Fig. 3, where the results for ABS composed of ILs containing a common cation combined with different anions ($[C_4C_1im][CF_3SO_3]$, $[C_4C_1im][SCN]$,
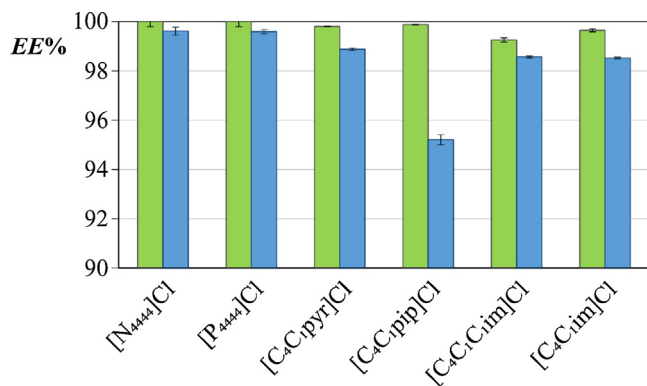
Fig. 4. IL cation effect on the ABS extraction efficiencies, *EE%*, of CAF (■) and CBZ (■).

[C_4C_1im]Br, [C_4C_1im]Cl) are depicted. The results for CBZ are similar in all studied ABS, with the complete extraction of CBZ to the IL-rich phase obtained in a single-step. This is in agreement with the high extraction efficiencies afforded by IL-based ABS when dealing with hydrophobic solutes [29,31]. Although the salting-out effect of the salt used cannot be discarded, this feature mirrors the affinity of CBZ for more hydrophobic phases, *i.e.* the IL-rich phase. In fact, CBZ is a highly hydrophobic substance with a logK_{ow} value of 2.32 [40]. On the other hand, the IL anion effect has a significant impact on the partition of CAF. CAF presents a more polar character than CBZ, as reflected by its lower logK_{ow} value (-0.07 [40]), and its partition is thus more dependent on the IL chemical structure. The extraction efficiencies of CAF (ranging from 96.57 to 99.18%) are lower than those observed with CBZ, decreasing in the following order: [C_4C_1im][$N(CN)_2$] > [C_4C_1im][SCN] > [C_4C_1im]Br ≈ [C_4C_1im]Cl > [C_4C_1im][CF_3SO_3]. According to this rank, CAF also has a higher affinity to more hydrophobic phases, closely following the ILs hydrogen-bond basicity [21]. Moreover, the trend obtained follows the decreased amount of IL at the IL-rich phase given by the TLs data for the fixed initial mixture composition studied (*cf.* the Supporting Information). The higher the amount of IL at the IL-rich phase, the higher is the partition of CAF to this phase. The main exception was observed with the system containing [C_4C_1im][CF_3SO_3]. This behavior was also previously observed with the extraction of alkaloids using ABS composed of ILs and K_3PO_4 [21], and may result from specific IL-CAF interactions. It should be stressed that higher differences in the effect of the IL chemical structure through the ABS extraction efficiencies for CAF are observed in this work when compared to the previous study in which the K_3PO_4 salt was used [21]. These lower differences are a consequence of the K_3PO_4 stronger salting-out effect [41] (when compared to the organic salt $K_3C_6H_5O_7$ used in this work).

In addition to the IL anion effect, the effects of the IL cation core and number of aliphatic tails on the ABS extraction efficiencies were investigated with ILs containing the common Cl⁻ anion, combined with the following cations: [N_{4444}]⁺, [P_{4444}]⁺, [C_4C_1pyr]⁺, [C_4C_1pip]⁺, [C_4C_1im]⁺ and [$C_4C_1C_1im$]⁺. As shown in Fig. 4, the extraction efficiencies of ABS composed of [N_{4444}]Cl, [P_{4444}]Cl, [C_4C_1pyr]Cl and [C_4C_1pip]Cl for CBZ are *ca.* 100%, obtained in a single-step. Slightly lower values were obtained with the ABS formed by [C_4C_1im]Cl and [$C_4C_1C_1im$]Cl, although always higher than 98.52%. On the other hand, and in accordance with the data obtained for the IL anion effect, the partitioning extent of CAF is more dependent on the IL chemical structure, with extraction efficiencies ranging from 95.21 to 99.61%, decreasing in the order: [N_{4444}]Cl > [P_{4444}]Cl > [C_4C_1pyr]Cl > [$C_4C_1C_1im$]Cl > [C_4C_1im]Cl > [C_4C_1pip]Cl. In general, it seems that the IL cation aromaticity has a low impact on the extraction of CAF, as previously observed in the extraction of CAF from biomass using

IL aqueous solutions [42]. The ability of the IL cation to establish hydrogen-bonds with CAF also has a negligible impact on the extraction efficiencies, as can be appraised from the data obtained for ABS composed of [C_4C_1im]Cl and [$C_4C_1C_1im$]Cl, in which the most acid hydrogen at the imidazolium ring was substituted by a methyl group in the former. Overall, the effect of the IL chemical structure seems to have a more significant impact on the partition of CAF than in CBZ, which seems to derive from the higher polarity of CAF and its lower affinity for organic fluids, as discussed before for the IL anion effect.

Although ammonium- and phosphonium-based ILs have been scarcely investigated as constituents of IL-based ABS when compared to imidazolium-based fluids [21], these cation-based ILs outstand as promising phase-forming constituents of IL-based ABS for the extraction of pollution tracers. In addition to their high ability to form ABS, ammonium- and phosphonium-based ILs offer additional advantages when compared with imidazolium-based ones, such as their lower cost, availability at a larger scale, and lower toxicity [43,44].

3.2. Concentration of CAF and CBZ with IL-based ABS

The detection and quantification of pollution tracers is of vital importance in pollution control, comprising an appropriate characterization of wastewater and surface water matrices. Nevertheless, pollution tracers are present in low amounts and will not be accurately identified or quantified if no concentration and pre-treatment steps of the samples are performed in advance. Fig. 5 represents a schematic illustration of the concept behind the concentration approach using IL-based ABS. When dealing with IL-based ABS as concentration techniques, the manipulation of the initial compositions along the same TL allows to obtain two-phase systems with the same compositions at the coexisting phases, while being able to decrease the IL-rich phase volume (and consequently increasing the volume of the salt-rich phase). Furthermore, higher TLL values lead to larger differences between the composition of the coexisting phases and to higher concentration factors that can be more easily experimentally achieved [31]. It should be noted that it is theoretically possible to reach a concentration factor up to infinite. However, the achievable concentration factors are restricted by the experimental feasibility of obtaining/recovering a low phase volume and by the maximum allowable concentration of the target analyte, which is further conditioned by its saturation solubility in the IL-rich phase.

According to the data previously shown and discussed, [N_{4444}]Cl- and [P_{4444}]Cl-based ABS lead to higher extraction efficiencies values for both CAF and CBZ. Despite the high extraction ability of the phosphonium-based IL, the ABS composed of [N_{4444}]Cl + $K_3C_6H_5O_7$ + H_2O (Fig. 5) was here selected to conduct the concentration studies due to its more benign character resulting from the lower toxicity of ammonium-based ILs [45]. Some quaternary ammonium-based ILs are also produced at a large scale and widely used in the extraction of metals, as phase transfer catalysts, surfactant agents, among others [43]. Moreover, as phosphonium-based, also ammonium-based ILs do not absorb in the UV region leading thus to lower interferences when dealing with analytical methods based on UV absorption. Accordingly, the ABS formed by [N_{4444}]Cl + $K_3C_6H_5O_7$ + H_2O was initially tested to individually extract and concentrate CAF and CBZ from aqueous samples. Then, the ability of this system to act as a single-step concentration platform of both pollution tracers was experimentally addressed, followed by the determination of the solubility of CAF and CBZ in the IL-rich phase to ascertain on the maximum concentration of tracer pollutants that can be extracted and respective concentration factors that could ideally be achieved.
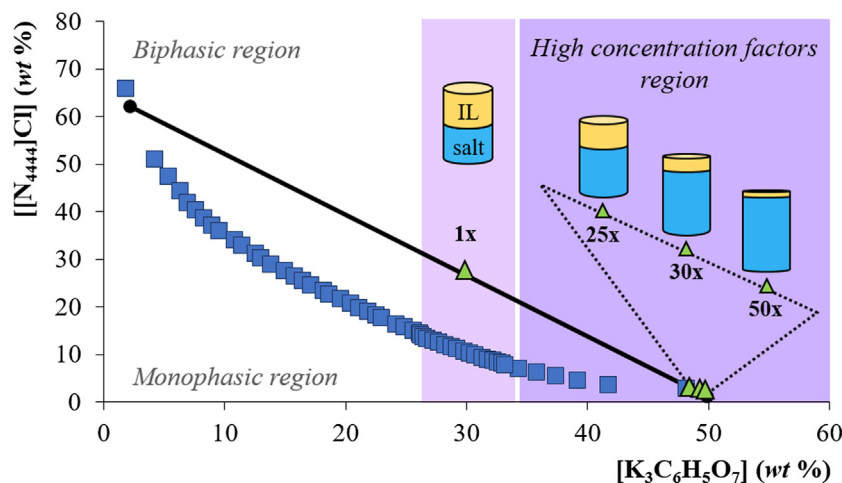
Fig. 5. Schematic illustration behind the use of IL-based ABS as concentration platforms. Phase diagram of the system composed of $[N_{4444}]Cl + K_3C_6H_5O_7 + H_2O$, at $(298 \pm 1)K$: binodal data (■); TL data (●); initial compositions for the corresponding concentration factor values (▲).

To demonstrate the ability of IL-based ABS to simultaneously extract and concentrate a mixture of CAF and CBZ, a concentration factor of 50 was here employed for an easier experimental manipulation. For the TLL of 79 previously described, the composition of the mixture which allows a concentration factor of 50 is: 1.18 wt% of $[N_{4444}]Cl + 49.85$ wt% of $K_3[C_6H_5O_7] + 48.98$ wt% of an aqueous solution containing the pollution tracers. The concentration factor (CF) value was determined by the ratio between the weight of the aqueous solutions containing the target pollution tracers and the weight of the IL-rich phase obtained after the separation of the coexisting phases.

The individual extraction/concentration of CAF and CBZ up to 50-fold was firstly addressed in order to validate the feasibility of using IL-based ABS for this purpose. The HPLC-UV chromatograms obtained are provided in the Supporting Information. In general, the complete extraction of CAF and CBZ and a concentration factor of 50-fold afforded by the IL-rich phase was confirmed by HPLC-UV identification/quantification. Based on these results, the simultaneous extraction and concentration of CAF and CBZ was further investigated, by applying the same IL-based ABS and the same concentration factor. Fig. 6A depicts the chromatogram resulting from the simultaneous extraction and 50-fold concentration of CAF and CBZ into the IL-rich phase of the $[N_{4444}]Cl$-based ABS. The recovery results obtained are $91 \pm 15\%$ for CAF and $104 \pm 5\%$ for CBZ. The detailed chromatographic data are presented in the Supporting Information. According to Fig. 6A, the peak areas of CAF (peak 1) and CBZ (peak 2) in the $[N_{4444}]Cl$-rich phases remain similar to those obtained with the standard spikes, revealing that both compounds can be simultaneously extracted and concentrated without saturating the IL-rich phase, and their quantification can be carried out by HPLC with UV detection without major interferences of the ABS phase-forming components. The chromatograms for the blank controls, which correspond to the chromatograms of the IL-rich phases (without pollution tracers added) are also shown in Fig. 6, confirming that there are no major interferences of the ABS phase-forming components in the analytical method used. Moreover, blank controls were analyzed for the experiments of individual extraction/concentration of CAF and CBZ (cf. the Supporting Information).

The simultaneous extraction and concentration of CAF and CBZ was also carried out with a real wastewater effluent sample to confirm the viability of the developed technique when applied to more complex matrices. With this more complex matrix, now comprising additional interferents, the recovery results for both pollution tracers were $90 \pm 16\%$ for CAF and $87 \pm 9\%$ for CBZ. These results

are satisfactory, showing that IL-based ABS can be applied to real effluent samples with no significant differences in the recovery results and concentration factors obtained − the respective chromatograms are shown in Fig. 6B and detailed information on the HPLC-UV conditions is given in the Supporting Information.

In order to determine the solubility and the maximum allowable concentration factor of the two pollution tracers in the IL-rich phase, i.e., the amount of CAF and CBZ that can be extracted without saturating the phase and/or without decreasing the ABS extraction efficiency, the solubility of both compounds was determined in the $[N_{4444}]Cl$-rich phase, at (298 ± 1) K and atmospheric pressure. The detailed experimental information is provided in the Supporting Information. The saturation solubility values of CAF and CBZ are $28.60 \pm 0.27\,g\,L^{-1}$ and $8.26 \pm 0.60\,g\,L^{-1}$, respectively. Accordingly, it is possible to significantly increase the concentration of CAF and CBZ in the IL-rich phase up to a 1.5- and 485-fold when compared to their solubility in pure water (ca. $21.6\,g\,L^{-1}$ for CAF and $16.8 \times 10^{-3}\,g\,L^{-1}$ for CBZ) [46]. This remarkable increase in the solubility of target solutes in aqueous solutions of ILs was recently reported and discussed as a result of an hydrotropic action afforded by ILs [47]. Based on this increase in the solubility, the detection of pollution tracers with poor-water solubility, and consequently difficult to be analyzed due to their presence in vestigial amounts in water samples, can be also improved by employing IL-based ABS.

Concentrations ranging from 0.47 to $11.16\,\mu g\,L^{-1}$ and from 0.27 to $1.02\,\mu g\,L^{-1}$ for CAF [6,8], and from 0.137 to 6.30 and from 0.022 to $2.5\,\mu g\,L^{-1}$ for CBZ [6,48,49], have been reported in wastewater effluents and in surface waters, respectively. The limit of detection (LOD) values associated to the HPLC equipment with UV detection (equipment employed in this work) ranges between $(0.1–1.0) \times 10^{-3}\,g\,L^{-1}$ for both pollution tracers, meaning that concentrations factors ranging from 100- to 1000-fold are required to accurately identify/quantify CAF and CBZ present in wastewater effluents and in surface waters. Taking into account the saturation solubility values of CAF and CBZ in the IL-rich phase discussed before and a final concentration of $1.0 \times 10^{-3}\,g\,L^{-1}$ for an accurate quantification of the two pollution tracers by HPLC-UV, it is reasonable to admit that IL-based ABS outstand as promising concentration strategies since "ideal" concentration factors up to 28595-fold for CAF and up to 8259-fold for CBZ could be attained up to the saturation of the IL-rich phase.

Based on the previous results, IL-based ABS are thus viable concentration techniques which allow a significant improvement on the detection and quantification of CAF and CBZ, typically found at very low concentrations in water matrices. Although some
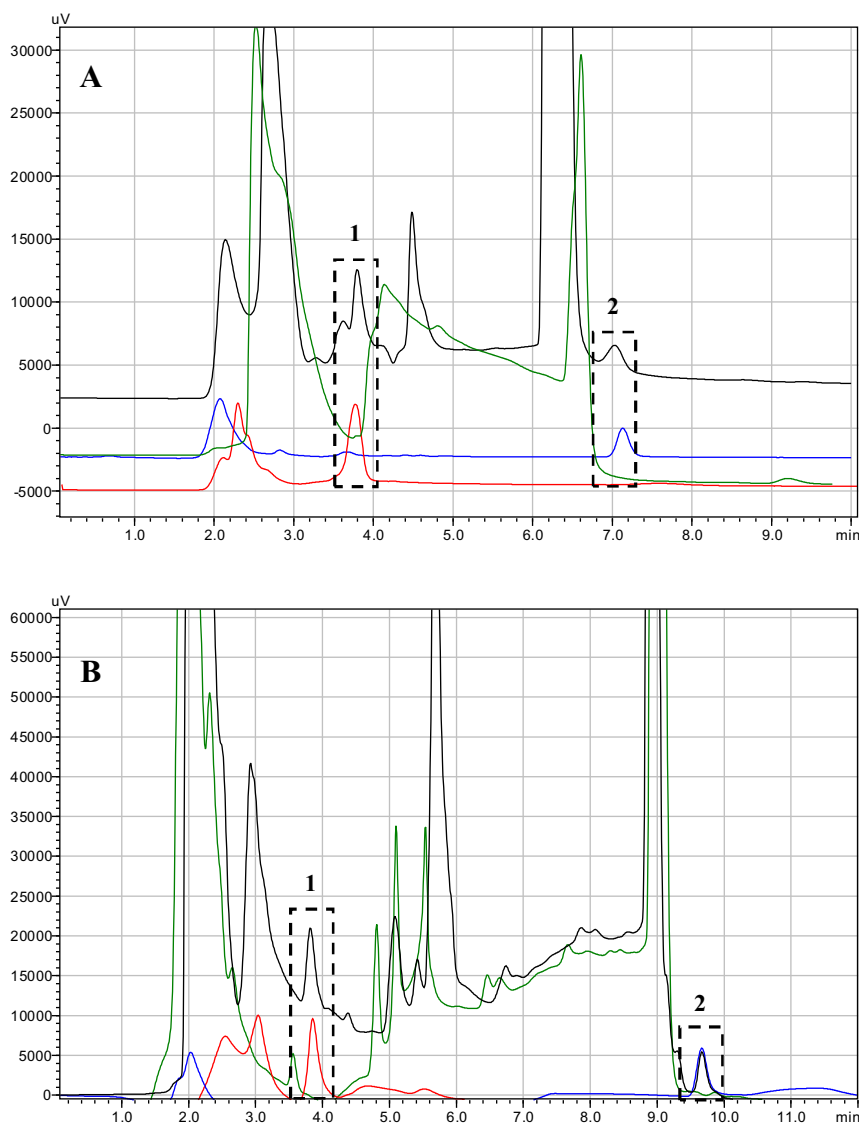
Fig. 6. HPLC-UV chromatograms corresponding to the identification/quantification of pollution tracers simultaneously extracted using **A**) model aqueous solutions and **B**) an effluent sample from a wastewater treatment plant. Chromatograms of standard solutions of CAF (red line); chromatograms of standard solutions of CBZ (blue line); Chromatograms of CAF and CBZ concomitantly extracted into the $[N_{4444}]$Cl-rich phase (black line); and Chromatograms of the $[N_{4444}]$Cl-rich phase (no pollution tracers added) (green line). Peaks: 1 – CAF and 2 – CBZ. The gradient elution used in these analyses is provided in detail in the Supporting Information. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

works have been found in the literature [14–20] regarding the pre-treatment of water samples for CAF and CBZ analysis, most of them reached low enrichment factors or do not highlight the enrichment factors experimentally achieved. For instance, Müller et al. [17] employed a solid-phase microextraction (SPME) approach based on a polypropylene hollow fiber for the pre-concentration of several compounds from water samples, including CBZ. Depending on the pH and presence of electrolytes, different concentration factors have been attained for CBZ: 39-, 60- and 30-fold in distilled water at pH = 2, pH = 7, and pH = 10, respectively, and 166-fold in a saturated sodium chloride aqueous solution [17]. González-Barreiro et al. [19] reported an enrichment factor of *ca.* two-orders of magnitude for CBZ from wastewater samples using SPE, followed by its analysis by HPLC with photochemically induced fluorescence (PIF). Santos et al. [9] achieved enrichment factors of 5 and 10 for CAF and CBZ, respectively, from wastewater samples using SPE as a pre-treatment method and HPLC coupled to a diode array (DAD) detector as the analytical technique. All the pre-treatment/concentration techniques referred above are based on SPE, further presenting the

disadvantage of requiring an additional desorption step and that is usually carried out with VOCs. Even so, the most important challenge in SPE techniques is the capability to simultaneously extract several classes of compounds with a wide range of polarities, solubilities and chemical stabilities that inherently compose the complex wastewater and surface water matrices [50]. As demonstrated here, IL-based ABS can overcome some of these drawbacks, namely by not requiring the use of an additional desorption step, by avoiding the use of VOCs, and by their tailoring ability that allow a simultaneous extraction/concentration step of mixtures of pollution tracers. Not only CAF and CBZ, but also an array of different classes of pollution tracers that compose the complex wastewater and surface water matrices can be simultaneously extracted and concentrated using IL-based ABS by an appropriate tailoring of the IL chemical structure and composition. In summary, IL-based ABS can be seen as efficient pre-treatment techniques within environmental analysis, aiming at better establishing the source, fate and type of aquatic contamination, as well as the risks posed to the

environment and human health by the long-term exposure to such pollutants.

4. Conclusions

Aiming at overcoming one of the major limitations in the environmental analysis and monitoring of aquatic samples, IL-based ABS are here proposed as pre-treatment/concentration techniques for mixtures of pollution tracers. The extraction ability of several IL-based ABS was initially addressed with two of the most commonly found anthropogenic tracers, namely caffeine (CAF) and carbamazepine (CBZ). Extraction efficiencies ranging between 95% and 100% for both compounds into the IL-rich phase were obtained in a single-step. After this initial screening, the [N$_{4444}$]Cl-based ABS was then used to simultaneously extract and concentrate CAF and CBZ, both from model aqueous solutions and from a real wastewater effluent sample. The maximum allowable concentration of the pollution tracers was assessed by determining the solubility (saturation values) of CAF and CBZ in the IL-rich phase, which can go up to 28595- and 8259-fold − values which are well above the required concentration factors to accurately monitoring CAF and CBZ in wastewater effluents and in surface waters. In summary, IL-based ABS are promising pre-treatment/concentration strategies for pollutant tracers, for which a proper detection and quantification still remain major challenges in the analytical field towards a better comprehension of their source, fate, type of aquatic contamination, and risks posed to the environment and human health.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.07.084.

References

[1] A. Daneshvar, K. Aboulfadl, L. Viglino, R. Broseus, S. Sauve, A.S. Madoux-Humery, G.A. Weyhenmeyer, M. Prevost, Evaluating pharmaceuticals and caffeine as indicators of fecal contamination in drinking water sources of the Greater Montreal region, Chemosphere 88 (2012) 131–139.

[2] G. Gasser, M. Rona, A. Voloshenko, R. Shelkov, N. Tal, I. Pankratov, S. Elhanany, O. Lev, Quantitative evaluation of tracers for quantification of wastewater contamination of potable water sources, Environ. Sci. Technol. 44 (2010) 3919–3925.

[3] M. Hajj-Mohamad, K. Aboulfadl, H. Darwano, A.S. Madoux-Humery, H. Guerineau, S. Sauve, M. Prevost, S. Dorner, Wastewater micropollutants as tracers of sewage contamination: analysis of combined sewer overflow and stream sediments, Environ. Sci.: Process. Impacts 16 (2014) 2442–2450.

[4] A.S. Madoux-Humery, S. Dorner, S. Sauve, K. Aboulfadl, M. Galarneau, P. Servais, M. Prevost, Temporal variability of combined sewer overflow contaminants: evaluation of wastewater micropollutants as tracers of fecal contamination, Water Res. 47 (2013) 4370–4382.

[5] M. Carballa, F. Omil, J.M. Lema, M. Llompart, C. Garcia-Jares, I. Rodriguez, M. Gomez, T. Ternes, Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant, Water Res. 38 (2004) 2918–2926.

[6] F. Gagne, C. Blaise, C. Andre, Occurrence of pharmaceutical products in a municipal effluent and toxicity to rainbow trout (Oncorhynchus mykiss) hepatocytes, Ecotoxicol. Environ. Saf. 64 (2006) 329–336.

[7] X.S. Miao, J.J. Yang, C.D. Metcalfe, Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant, Environ. Sci. Technol. 39 (2005) 7469–7475.

[8] M. Pedrouzo, S. Reverte, F. Borrull, E. Pocurull, R.M. Marce, Pharmaceutical determination in surface and wastewaters using high-performance liquid chromatography-(electrospray)-mass spectrometry, J. Sep. Sci. 30 (2007) 297–303.

[9] J.L. Santos, I. Aparicio, E. Alonso, M. Callejon, Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors, Anal. Chim. Acta 550 (2005) 116–122.

[10] S. Terzic, I. Senta, M. Ahel, M. Gros, M. Petrovic, D. Barcelo, J. Muller, T. Knepper, I. Marti, F. Ventura, P. Jovancic, D. Jabucar, Occurrence and fate of emerging wastewater contaminants in Western Balkan Region, Sci. Total Environ. 399 (2008) 66–77.

[11] D.P. Mohapatra, S.K. Brar, R.D. Tyagi, P. Picard, R.Y. Surampalli, Analysis and advanced oxidation treatment of a persistent pharmaceutical compound in wastewater and wastewater sludge-carbamazepine, Sci. Total Environ. 470 (2014) 58–75.

[12] V. Calisto, A. Bahlmann, R.J. Schneider, V.I. Esteves, Application of an ELISA to the quantification of carbamazepine in ground, surface and wastewaters and validation with LC-MS/MS, Chemosphere 84 (2011) 1708–1715.

[13] D. Fatta, A. Nikolaou, A. Achilleos, S. Meric, Analytical methods for tracing pharmaceutical residues in water and wastewater, TrAC Trends Anal. Chem. 26 (2007) 515–533.

[14] G. Gasser, M. Rona, A. Voloshenko, R. Shelkov, O. Lev, S. Elhanany, F.T. Lange, M. Scheurer, I. Pankratov, Evaluation of micropollutant tracers. II. Carbamazepine tracer for wastewater contamination from a nearby water recharge system and from non-specific sources, Desalination 273 (2011) 398–404.

[15] D.P. Mohapatra, S.K. Brar, R.D. Tyagi, P. Picard, R.Y. Surampalli, Carbamazepine in municipal wastewater and wastewater sludge: ultrafast quantification by laser diode thermal desorption-atmospheric pressure chemical ionization coupled with tandem mass spectrometry, Talanta 99 (2012) 247–255.

[16] M.D.G. García, F.C. Cañada, M.J. Culzoni, L. Vera-Candioti, G.G. Siano, H.C. Goicoechea, M.M. Galera, Chemometric tools improving the determination of anti-inflammatory and antiepileptic drugs in river and wastewater by solid-phase microextraction and liquid chromatography diode array detection, J. Chromatogr. A 1216 (2009) 5489–5496.

[17] S. Müller, M. Möder, S. Schrader, P. Popp, Semi-automated hollow-fibre membrane extraction, a novel enrichment technique for the determination of biologically active compounds in water samples, J. Chromatogr. A 985 (2003) 99–106.

[18] L.V. Cardoso, D. Tomasini, R.F. Sampaio, S.S. Caldas, N. Kleemann, E.G. Primel, F.F. Gonçalves, Optimization and validation of a method using SPE and LC-APCI-MS/MS for determination of pharmaceuticals in surface and public supply water, J. Braz. Chem. Soc. 22 (2011) 1944–1952.

[19] C. González-Barreiro, M. Lores, M.C. Casais, R. Cela, Simultaneous determination of neutral and acidic pharmaceuticals in wastewater by high-performance liquid chromatography–post-column photochemically induced fluorimetry, J. Chromatogr. A 993 (2003) 29–37.

[20] V. Camel, Solid phase extraction of trace elements, Spectrochim. Acta Part B 58 (2003) 1177–1233.

[21] M.G. Freire, A.F.M. Claudio, J.M.M. Araujo, J.A.P. Coutinho, I.M. Marrucho, J.N. Canongia Lopes, L.P.N. Rebelo, Aqueous biphasic systems: a boost brought about by using ionic liquids, Chem. Soc. Rev. 41 (2012) 4966–4995.

[22] K.R. Seddon, Ionic liquids for clean technology, J. Chem. Technol. Biotechnol. 68 (1997) 351–356.

[23] P. Berton, R.P. Monasterio, R.G. Wuilloud, Selective extraction and determination of vitamin B-12 in urine by ionic liquid-based aqueous two-phase system prior to high-performance liquid chromatography, Talanta 97 (2012) 521–526.

[24] Z. Du, Y.L. Yu, J.H. Wang, Extraction of proteins from biological fluids by use of an ionic liquid/aqueous two-phase system, Chem. Eur. J. 13 (2007) 2130–2137.

[25] J. Flieger, A. Czajkowska-Zelazko, Aqueous two phase system based on ionic liquid for isolation of quinine from human plasma sample, Food Chem. 166 (2015) 150–157.

[26] J.A. Han, Y. Wang, C.L. Yu, C.X. Li, Y.S. Yan, Y. Liu, L.A. Wang, Separation, concentration and determination of chloramphenicol in environment and food using an ionic liquid/salt aqueous two-phase flotation system coupled with high-performance liquid chromatography, Anal. Chim. Acta 685 (2011) 138–145.

[27] J. Han, Y. Wang, W.B. Kang, C.X. Li, Y.S. Yan, J.M. Pan, X.Q. Xie, Phase equilibrium and macrolide antibiotics partitioning in real water samples using a two-phase system composed of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate and an aqueous solution of an inorganic salt, Microchim. Acta 169 (2010) 15–22.

[28] C.Y. He, S.H. Li, H.W. Liu, K. Li, F. Liu, Extraction of testosterone and epitestosterone in human urine using aqueous two-phase systems of ionic liquid and salt, J. Chromatogr. A 1082 (2005) 143–149.

[29] H. Passos, A.C.A. Sousa, M. Ramiro Pastorinho, A.J.A. Nogueira, L.P.N. Rebelo, J.A.P. Coutinho, M.G. Freire, Ionic-liquid-based aqueous biphasic systems for improved detection of bisphenol A in human fluids, Anal. Methods 4 (2012) 2664–2667.

[30] M.S. Noorashikin, S. Mohamad, M.R. Abas, Extraction and determination of parabens in water samples using an aqueous two-phase system of ionic liquid and salts with beta-cyclodextrin as the modifier coupled with high performance liquid chromatography, Anal. Methods 6 (2014) 419–425.

[31] T.B.V. Dinis, H. Passos, D.L.D. Lima, V.I. Esteves, J.A.P. Coutinho, M.G. Freire, One-step extraction and concentration of estrogens for an adequate monitoring of wastewater using ionic-liquid-based aqueous biphasic systems, Green Chem. 17 (2015) 2570–2579.

[32] M.P. Collins, L. Zhou, S.E. Camp, N.D. Danielson, Isopropylammonium formate as a mobile phase modifier for liquid chromatography, J. Chromatogr. Sci. 50 (2012) 869–876.

[33] B. Soares, H. Passos, C.S.R. Freire, J.A.P. Coutinho, A.J.D. Silvestre, M.G. Freire, Ionic liquids in chromatographic and electrophoretic techniques: toward additional improvements in the separation of natural compounds, Green Chem. 18 (2016) 4582–4604.

[34] L. Zhou, N.D. Danielson, The ionic liquid isopropylammonium formate as a mobile phase modifier to improve protein stability during reversed phase liquid chromatography, J. Chromatogr. B 940 (2013) 112–120.

[35] O.A.H. Jones, N. Voulvoulis, J.N. Lester, Aquatic environmental assessment of the top 25 English prescription pharmaceuticals, Water Res. 36 (2002) 5013–5022.

[36] H. Passos, A.R. Ferreira, A.F.M. Claudio, J.A.P. Coutinho, M.G. Freire, Characterization of aqueous biphasic systems composed of ionic liquids and a citrate-based biodegradable salt, Biochem. Eng. J. 67 (2012) 68–76.

[37] J.C. Merchuk, B.A. Andrews, J.A. Asenjo, Aqueous two-phase systems for protein separation Studies on phase inversion, J. Chromatogr. B 711 (1998) 285–293.

[38] J.F.B. Pereira, A. Magri, M.V. Quental, M. Gonzalez-Miquel, M.G. Freire, J.A.P. Coutinho, Alkaloids as alternative probes to characterize the relative hydrophobicity of aqueous biphasic systems, ACS Sustain. Chem. Eng. 4 (2016) 1512–1520.

[39] L.D. Nghiem, A.I. Schafer, M. Elimelech, Pharmaceutical retention mechanisms by nanofiltration membranes, Environ. Sci. Technol. 39 (2005) 7698–7705.

[40] S.G. Machatha, S.H. Yalkowsky, Comparison of the octanol/water partition coefficients calculated by ClogP®, ACDlogP and KowWin® to experimentally determined values, Int. J. Pharm. 294 (2005) 185–192.

[41] F. Hofmeister, Zur Lehre von der Wirkung der Salze, Arch. Exp. Pathol. Pharmakol. 24 (1888) 247–260.

[42] A.F.M. Claudio, A.M. Ferreira, M.G. Freire, J.A.P. Coutinho, Enhanced extraction of caffeine from guarana seeds using aqueous solutions of ionic liquids, Green Chem. 15 (2013) 2002–2010.

[43] A. Stojanovic, B.K. Keppler, C. Morgenbesser, D. Kogelnig, R. Krachler, Quaternary Ammonium and Phosphonium Ionic Liquids in Chemical and Environmental Engineering, INTECH Open Access Publisher, 2011.

[44] P. Attri, P. Venkatesu, Ammonium ionic liquids as convenient co-solvents for the structure and stability of succinylated Con A, J. Chem. Thermodyn. 52 (2012) 78–88.

[45] D.J. Couling, R.J. Bernot, K.M. Docherty, J.K. Dixon, E.J. Maginn, Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure-property relationship modeling, Green Chem. 8 (2006) 82–90.

[46] PhysProp Database, 2016 (Accessed 22.12.16) http://srcinc.com/what-we-do/environment.aspx.

[47] A.F.M. Claudio, M.C. Neves, K. Shimizu, J.N. Canongia Lopes, M.G. Freire, J.A.P. Coutinho, The magic of aqueous solutions of ionic liquids: ionic liquids as a powerful class of catanionic hydrotropes, Green Chem. 17 (2015) 3948–3963.

[48] T.A. Ternes, Occurrence of drugs in German sewage treatment plants and rivers, Water Res. 32 (1998) 3245–3260.

[49] S. Wiegel, A. Aulinger, R. Brockmeyer, H. Harms, J. Loffler, H. Reincke, R. Schmidt, B. Stachel, W. von Tumpling, A. Wanke, Pharmaceuticals in the river Elbe and its tributaries, Chemosphere 57 (2004) 107–126.

[50] A.L. Batt, M.S. Kostich, J.M. Lazorchak, Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS, Anal. Chem. 80 (2008) 5021–5030.