Universidade de Aveiro Departamento de Química
2015

Teresa Beatriz Vide Dinis

Concentração de marcadores de poluição humana com líquidos iónicos

Concentration of human pollution tracers with ionic liquids

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, Ramo de Biotecnologia Industrial e Ambiental, realizada sob a orientação científica da Drª. Mara Guadalupe Freire Martins, Investigadora Coordenadora do Departamento de Química, CICECO, da Universidade de Aveiro e co-orientação do Professor Dr. Valdemar Inocêncio Esteves, Professor Auxiliar do Departamento de Química da Universidade de Aveiro.

Aos meus pais e irmão, que enchem cada página de amor, perseverança e vontade de ir mais além…

o júri

presidente

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palavras-chave

Carbamazepina, cafeína, marcadores de poluição antropogénica, análise de águas residuais, concentração, solubilidade, hidrótropos, sistemas aquosos bifásicos, líquidos iónicos.

resumo

A presente tese tem como objetivo o desenvolvimento de uma tecnologia de pré-concentração para uma avaliação correta da presença de marcadores de poluição antropogénica em águas residuais. Devido à capacidade excecional que os líquidos iónicos (LIs) apresentam no que respeita ao ajuste das suas propriedades físicas e químicas, os sistemas aquosos bifásicos (SAB) constituídos por LIs providenciam eficiências de extração mais elevadas e específicas para os mais variados compostos, e substituindo o uso de solventes orgânicos voláteis (SOV). Deste modo, SAB constituídos por LIs foram estudados e caracterizados neste trabalho como uma técnica de extração e concentração simultâneas.

Numa primeira etapa, os SAB constituídos por LIs foram estudados como potenciais plataformas para extrair e concentrar dois fármacos geralmente usados como marcadores de poluição humana, nomeadamente cafeína (CAF) e carbamazepina (CBZ). A presença destes dois poluentes persistentes em concentrações reduzidas (na ordem dos $\mu g \cdot dm^{-3}$ e $ng \cdot dm^{-3}$, respetivamente) nas águas residuais não permite uma correta deteção e quantificação pelos equipamentos normalmente utilizados para o efeito, sem que haja uma etapa prévia de concentração. Contudo, os métodos de pré-concentração normalmente aplicados apresentam várias desvantagens, tais como um custo elevado, um tempo laboral moroso, taxas de recuperação irregulares e o uso de SOV. Assim sendo, estudou-se um SAB constituído pelo LI cloreto de tetrabutilamónio ($[N_{4444}]Cl$) e pelo sal biodegradável citrato de potássio ($K_3[C_6H_5O_7]$) para extrair e concentrar CAF e CBZ num único passo, superando assim os baixos limites de deteção do equipamento analítico utilizado para a quantificação de marcadores de poluição humana.

Por último, foi estudado o efeito hidrotrópico providenciado pelos LIs que se refletem na capacidade destes sistemas para extrair e concentrar os mais variados compostos. Verificou-se que o LI é responsável pelo efeito hidrotrópico, permitindo um aumento da solubilidade da CAF em soluções aquosas, e até de 4 vezes. Além disso, uma escolha adequada do LI permite definir um sistema específico para melhorar a solubilidade de um composto na fase rica em LI, garantindo portanto o desenvolvimento de uma plataforma para extração e concentração com elevada eficiência.

Os SAB constituídos por LIs foram aqui demonstrados como uma técnica alternativa mais versátil e promissora para a extração e concentração simultâneas, permitindo uma monitorização adequada de compostos vestigiais em matrizes das águas residuais.

keywords

Carbamazepine, caffeine, anthropogenic pollution tracers, wastewater analysis, concentration, solubility, hydrotropes, aqueous biphasic systems, ionic liquids.

abstract

The main objective of the present thesis consists on the development of an analytical preconcentration technology for the concomitant extraction and concentration of human pollution tracers from wastewater streams. Due to the outstanding tunable properties of ionic liquids (ILs), aqueous biphasic systems (ABS) composed of ILs can provide higher and more selective extraction efficiencies for a wide range of compounds, being thus a promising alternative to the volatile and hazardous organic solvents (VOCs) typically used. For that purpose, IL-based ABS were employed and adequately characterized as an one-step extraction and concentration technique.

The applicability of IL-based ABS was verified by their potential to completely extract and concentrate two representative pharmaceutical pollution tracers, namely caffeine (CAF) and carbamazepine (CBZ), from wastewaters. The low concentration of these persistent pollutants (usually found in $\mu g \cdot dm^{-3}$ and $ng \cdot dm^{-3}$ levels, respectively) by conventional analytical equipment does not permit a proper detection and quantification without a previous concentration step. Preconcentration methods commonly applied are costly, time-consuming, with irregular recoveries and make use of VOCs. In this work, the ABS composed of the IL tetrabutylammonium chloride ($[N_{4444}]Cl$) and the salt potassium citrate ($K_3[C_6H_5O_7]$) was investigated while demonstrating to be able to completely extract and concentrate CAF and CBZ, in a single-step, overcoming thus the detection limit of the applied analytical equipment.

Finally, the hydrotropic effect responsible for the ability of IL-based ABS to extract and concentrate a wide variety of compounds was also investigated. It was shown that the IL rules the hydrotropic mechanism in the solubility of CAF in aqueous solutions, with an increase in solubility up to 4-fold. Moreover, the proper selection of the IL enables the design of the system that leads to a more enhanced solubility of a given solute in the IL-rich phase, while allowing a better extraction and concentration.

IL-based ABS are a promising and more versatile technique, and are straightforwardly envisaged as selective extraction and concentration routes of target micropollutants from wastewater matrices.

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wt % – weight percentage (%);

% wt_{IL}- weight percentage of the IL-rich phase (%);

% wt_{salt} - weight percentage of the salt-rich phase (%);

β – hydrogen-bond basicity;

λ – wavelength (nm);

σ – standard deviation;

Abs – absorbance (dimensionless);

Mw – molecular weight ($g \cdot mol^{-1}$);

K_a – acid dissociation constant ($mol \cdot dm^{-3}$);

K_{OW} – octanol-water partition coefficient (dimensionless);

R^2 – correlation coefficient (dimensionless);

α – ratio between the top weight and the total weight of the mixture (dimensionless);

$[IL]$ – concentration of ionic liquid (wt % or $mol \cdot dm^{-3}$);

$[IL]_{IL}$ – concentration of ionic liquid in the ionic-liquid-rich phase (wt %);

$[IL]_{Salt}$ – concentration of ionic liquid in the salt-rich phase (wt %);

$[IL]_{M}$ – concentration of ionic liquid in the mixture point (wt %);

$[Salt]$ – concentration of salt (wt % or $mol \cdot kg^{-1}$);

$[Salt]_{IL}$ – concentration of salt in the ionic-liquid-rich phase (wt %);

$[Salt]_{Salt}$ – concentration of salt in the salt-rich phase (wt %);

$[Salt]_{M}$ – concentration of salt in the mixture point (wt %);

$[Pha]_{IL}$ – concentration of an pharmaceutical in the ionic-liquid-rich phase ($g \cdot dm^{-3}$);

$[Pha]_{Salt}$ – concentration of an pharmaceutical in the salt-rich phase ($g \cdot dm^{-3}$);

[CAF] – concentration of caffeine ($g \cdot dm^{-3}$ or $mol \cdot dm^{-3}$);

$[CAF]_{IL}$ – concentration of caffeine in the ionic-liquid-rich phase ($mol \cdot dm^{-3}$);

[CBZ] – concentration of carbamazepine ($g \cdot dm^{-3}$);

$[CBZ]_{IL}$ – concentration of carbamazepine in the ionic-liquid-rich phase ($mol \cdot dm^{-3}$);

$[K_3[C_6H_5O_7]]$ – concentration of $K_3[C_6H_5O_7]$ (wt % or $mol \cdot dm^{-3}$);

w_{IL} – weight of the ionic-liquid-rich phase (g);

w_{salt} – weight of the salt-rich phase (g);

$EE_{Pha}\%$ – percentage extraction efficiencies of a given pharmaceutical (%);

$EE_{CAF}\%$ – percentage extraction efficiencies of caffeine (%);

K_{Hyd} – constant of hydrotropy ($g^{-1} \cdot mol$);

[Hyd] – concentration of the hydrotrope ($mol_{Hyd} \cdot dm_{water}^{-3}$);

S – solubility of a given compound ($mol \cdot dm^{-3}$);

S_0 – solubility of a given compound in pure water (mol·dm^{-3});

C_{Hyd} – concentration of the hydrotrope in aqueous solution (mol·dm^{-3}).

List of acronyms and abbreviations

ABS – aqueous biphasic system;

BPA – bisphenol A;

BR – biphasic region;

CAF – caffeine;

CBZ – carbamazepine;

CF – concentration factor;

CMC – critical micelle concentration;

EDC – endocrine disruptor compound;

EE2 – ethinylestradiol;

GC - gas chromatography;

HPLC – high performance liquid chromatography;

IL – ionic liquid;

LC – liquid chromatography;

LLE – liquid-liquid extraction;

LOD – limit of detection;

LPME – liquid-phase microextraction;

MR – monophasic region;

MS – mass spectrometry;

SPE – solid-phase extraction;

SPME – solid-phase microextraction;

TL – tie-line;

TLL – tie-line length;

UV-VIS – ultraviolet-Visible light;

VOC – volatile organic compound;

WWTP – wastewater treatment plant;

$[C_4C_1pyr]Cl$ – 1-butyl-3-methylpyrrolidinium chloride;

$[C_4C_1pip]Cl$ – 1-butyl-3-methylpiperidinium chloride;

$[C_4C_1im]Br$ – 1-butyl-3-methylimidazolium bromide;

$[C_4C_1im]Cl$ – 1-butyl-3-methylimidazolium chloride;

$[C_4C_1C_1im]Cl$ – 1-butyl-2,3-dimethylimidazolium chloride;

$[C_4C_1im][N(CN)_2]$ – 1-butyl-3-methylimidazolium dicyanamide;

$[C_4C_1im][BF_4]$ – 1-butyl-3-methylimidazolium tetrafluoroborate;

$[C_4C_1im][CH_3SO_4]$ – 1-butyl-3-methylimidazolium methylsulfate;

$[C_4C_1im][SCN]$ – 1-butyl-3-methylimidazolium thiocyanate;

$[C_4C_1im][TOS]$ – 1-butyl-3-methylimidazolium tosylate;

$[C_4C_1im][CF_3SO_3]$ – 1-butyl-3-methylimidazolium trifluoromethanesulfonate;

$[C_4C_1im][PF_6]$ – 1-butyl-3-methylimidazolium hexafluorophosphate;

$[C_6C_1im][PF_6]$ – 1-hexyl-3-methylimidazolium hexafluorophosphate;

$[C_4C_1py]Cl$ – 1-butyl-3-methylpyridinium chloride;

$[C_4C_1py][N(CN)_2]$ – 1-butyl-3-methylpyridinium dicyanamide;

$[N_{1112OH}]Cl$ – choline chloride;

$[N_{4444}]Cl$ – tetrabutylammonium chloride;

$[P_{4444}]Cl$ – tetrabutylphosphonium chloride.

“What is a scientist after all? It is a curious man looking through a keyhole, the keyhole of nature, trying to know what is going on.”

Jacques-Yves Cousteau

1

General introduction

1.1. Ionic-liquid–based aqueous biphasic systems (IL-based ABS) as one-step extraction and concentration technologies, for analysis of human pollution tracers in aquatic environments

1.1.1. IL-based ABS: a new platform for extraction and concentration approaches

In many biotechnological processes, downstream processing methods for the recovery of the final product are a key problem and represent the highest contribution in the overall process costs [2-3]. In 1958, Albertsson [4] revolutionized the technology of downstream processes: aqueous biphasic systems (ABS) came as to be more cost-effective in one-step separation, purification and concentration compared to other separation techniques, while preserving the biological activity of the final product - a main result of the high water content [3].

ABS fall within the liquid-liquid extraction (LLE) techniques and involve the partitioning of biomolecules from one aqueous phase to another [5-6]. Usually these systems are formed by different pairs of solutes miscible in water (polymer-polymer, polymer-salt or salt-salt), where above specific concentrations the system undergoes a two-phase separation [5, 7]. Because of the non-volatile nature of polymers and salts, these phase-forming components can be recovered and recycled, and are a more benign alternative to traditional liquid-liquid extraction routes which use hazardous volatile organic compounds (VOC) [5-6, 8]. In the last 50 years, conventional ABS mainly composed of polymers have been widely investigated [5, 7-19] and used to isolate and to purify several biological products [5, 12, 16, 19], drug compounds [10, 13-14, 17], and small organic molecules from complex matrices [7-9, 15, 18]. Besides, these ABS have already been investigated as extraction and concentration techniques [20-21]. However, these systems present many drawbacks: high Viscosities, high cost and require a long equilibration time to achieve the phases' separation [22-23]. In 2003, Gutowski *et al.* [24] demonstrated that hydrophilic ionic liquids (IL) can form ABS when combined with an inorganic salt in aqueous media being a possible alternative for extraction purposes. Since the past decade, ABS composed of ILs have been proposed in the design of novel separation and concentration processes [24].

ILs belongs to the molten salts category and are composed of a large organic cation and an organic or inorganic anion. Due to the large differences in size and shape between the ions, ILs cannot easily form an ordered crystalline structure and thus remain liquid at a general temperature below 373 K [25]. The most commonly studied ILs are nitrogen-based, such as those composed of pyrrolidinium, imidazolium, piperidinium, pyridinium, or quaternary ammonium cations. Their general cation molecular structures are depicted in Figure 1. On the other hand, their anions can

range from halogens to more complex organic and fluorinated structures. Some anion molecular structures, as well as the corresponding hydrogen-bond basicity (β) values are presented in Table 1. β indicates the hydrogen-bond accepting ability of the IL anion and it is the most used parameter to describe important IL properties, since solute-IL and solvent-IL interactions are mainly determined by the anion nature [26]. Due to their ionic character, most ILs present unique characteristics, such as a negligible vapor pressure [27], non-flammability, high thermal and chemical stabilities, and a high solvation capacity [25, 27-29], either for polar or nonpolar compounds [30-32]. Thereby, because of these crucial properties, ILs are usually described as "green solvents", being one of the main studied strategies to replace VOCs in many applications [25]. Further, the most important feature that has attracted both academy and industry is their aptitude as "designer solvents". This designation is given due to the numerous IL cations and anions combinations [33], and thus, ILs with the desired physicochemical properties (for example, hydrophobicity and their recovery capacity) for a specific extraction can always be foreseen [34-37]. Oppose to the limited range of polarities afforded by VOCs, it is theoretically possible to form at least 1 million of different ILs [29], which enables the possible creation of versatile ABS for enhanced separation processes [25].

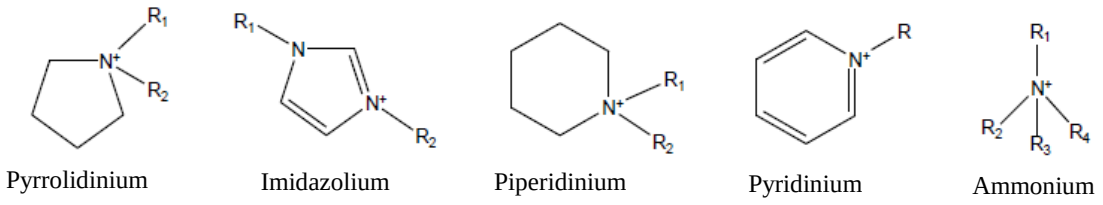
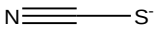
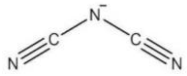
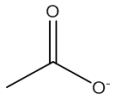
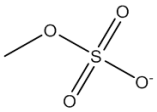
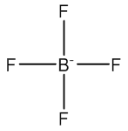
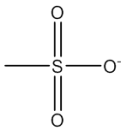
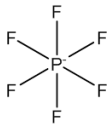
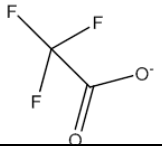
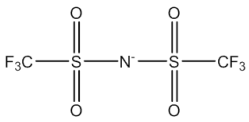


Figure 1. Cation molecular structures of nitrogen-based ILs.

IL-based ABS present several advantages over the typical polymer-based ABS, namely a low Viscosity, higher thermal stability, and enhanced and tailored extraction efficiencies for a large number of biomolecules [33, 38]. As polymer-based ABS present a restricted difference in polarities between the two immiscible phases, selective extractions of similar solutes are difficult to attain. Due to the inherent tailoring ability of ILs, IL-based ABS cover a wider hydrophilic-hydrophobic range and remarkable extractions and selectivities are possible to occur [39]. In this context, IL-based ABS meet all the requirements for an easy design and scale-up of a wide range of separation processes, becoming a target of high interest in concentration steps. In the last few years, there was an exponential growth in published articles, showing the interest by the scientific community on exploring these extraction processes [39], and as shown in Figure 2. IL-based ABS were extensively explored in the extraction of a wide range of solutes. The results gathered from literature are divided in main classes of partitioned solutes: alkaloids, amino acids and proteins, most of them with pharmacological relevance [39].

Table 1. Anion molecular structures and respective hydrogen-bond basicity (β) values (decreasing order) of imidazolium-based ILs with the solvatochromic probe $[Fe(phen)_2(CN)_2]ClO_4$ [40].

IL anion	β	Molecular structure	IL anion	β	Molecular structure
Chloride	0.95		Thiocyanate	0.71	
Bromide	0.87		Dicyanamide	0.64	
Acetate	0.85		Trifluoromethanesulfonate	0.57	
Methanesulfonate	0.85		Tetrafluoroborate	0.55	
Methylsulfate	0.75		Hexafluorophosphate	0.44	
Trifluoroacetate	0.74		Bis(trifluoromethanesulfonylimide)	0.42	

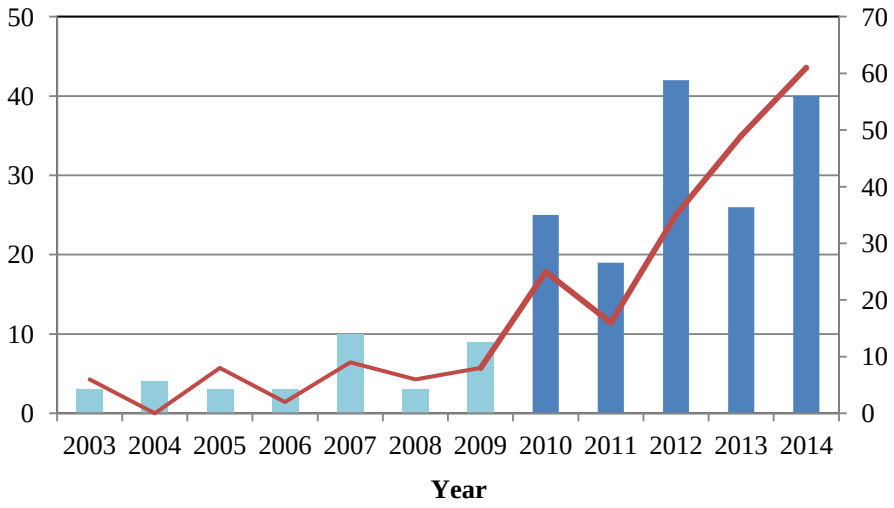


Figure 2. Published manuscripts on "IL-based ABS" from 2003 to 2014: number of articles *per* year relative to the characterization of IL-based ABS (blue bars); number of articles reported *per* year on the application of IL-based ABS for extraction processes (red line/scale). Values based on a search on *ISI Web of Knowledge* in 26th March, 2015. The following topics were used: "aqueous biphasic systems", "aqueous two-phase systems", "ionic liquids" and "partition coefficients" and "extraction efficiencies".

Li *et al.* [38] reported that IL-based ABS are able to combine with analytical techniques, such as High Performance Liquid Chromatography (HPLC), for the extraction and identification of

opium alkaloids, from real matrices, with more than 90% of recovery and an enrichment factor of 10. This combined method avoided the use of the pretreatment time-consuming step, as well as the use of VOCs.

Recently, IL-based ABS shown to be an efficient extraction route and concentration technique for target molecules present in biological matrices [41-42]. For instance, Passos et al. [42] reported that ABS composed of hydrophilic ILs and the inorganic salt potassium phosphate are capable to completely extract and concentrate the endocrine disruptor bisphenol A (BPA), up to 100-fold in a single-step, from biological fluids. In this way, it is possible to use IL-based ABS as a concentration technique for pretreatment steps in the analytical field.

In summary, IL-based ABS are a promising alternative, while offering the possibility to extract and highly concentrate (with tailored ability on the extraction efficiencies) a variety of solutes, in a single step procedure, allowing therefore their proper identification and quantification by analytical techniques and in more complex (real) aqueous matrices. These possibilities open new horizons to implement such processes in new areas, such as in the environmental field, where sewage epidemiology constitutes the major problem of aquatic sources, and where methods that correlate good detection with an efficient risk assessment of target micropollutants on human and wildlife health are also very difficult to achieve [43].

Most of the investigated IL-based ABS are composed of inorganic salts, with high concentrations of salt at the salt-rich phase [24], which results in serious environmental concerns [44]. Taking this drawback into consideration, recent works have successfully introduced biodegradable and non-toxic organic salts in the composition of IL-based ABS, such as citrate- [32, 45], tartrate- [45-46] or acetate-based [45, 47] salts. IL-based ABS with biodegradable salts have shown to be an optimal replacement of systems composed of inorganic salts in extraction steps [32], although no enrichment factors with these systems were investigated.

In a more recent work [48], hydrophilic IL-based ABS combined with the biodegradable salt potassium sodium tartrate were tested and optimized in order to extract and concentrate the synthetic hormone 17α-ethinylestradiol (EE2), which is one the most prominent endocrine disrupting compounds EDC found in wastewater treatment plant WWTP effluents and aquatic environments. After the complete extraction of EE2 obtained by these systems, the concentration factor of EE2 was further optimized, and it was found that it is possible to increase the EE2 concentration from wastewater samples at least up to 1000-fold in a single-step [48]. These IL-based ABS were coupled to HPLC as the analytical method. The combined methodology allowed to increase the EE2 concentration by three orders of magnitude (from $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$), while overcoming the detection limits of the conventional analytical equipment used for its quantification [48].

1.1.2. Development of a new technology

Given that IL-based ABS already showed to be of high value to concentrate EE2 from WWTPs [48], a more consistent and advanced technology can be enVisaged to be applied for human pollution tracers (present in low amounts in aqueous samples). To this end, the development of a preconcentration technology for sample preparation based on ABS composed of hydrophilic ILs and conventional salts as salting-out agents will be investigated.

Before the use of this technology, as both an extraction and concentration technique, the corresponding ABS ternary phase diagrams must be accurately determined aiming at establishing the biphasic region. Figure 3 represents a ternary system phase diagram composed of IL + Salt + H_2O. A ternary phase diagram (as can be seen in the left image of Figure 3) consists of a *binodal* or solubility curve, that separates the monophasic region (MR) from the biphasic region (BR), and depends on certain conditions (such as pH and temperature) [49]. The knowledge of the biphasic region, the initial mixture composition of the ABS, and the composition of the individual phases is crucial to any extraction process and to predict the mechanism allied to the partitioning of a solute between the two immiscible aqueous phases. For a given initial mixture composition, selected from the biphasic region, the composition of the two immiscible phases, the bottom and top phases in equilibrium, are represented by two extreme points, designated by *nodes* (A and C, respectively), which remain on the *binodal* curve [49]. The connection between these two *nodes* forms a well-defined line named *tie-line* (TL, ABC) [49]. Different TLs lay in a phase diagram and with different tie-line lengths (TLL). Higher TLL values lead to higher differences between the bottom and top phase compositions. The manipulation of the initial compositions along the same TL allows to obtain liquid-liquid systems with the same composition at the coexisting phases while differing in the volume ratios [49] (as can be seen in the right image of Figure 3). This is the *basis* for the development of the concentration technology carried out with ABS.

The procedure of a simultaneous extraction and concentration step, in an ABS, consists in the scanning of the concentration factor (CF) (Figure 3) through the compositions of an initial mixture along a given TL, in order to gradually decrease the volume ratio of the phase in which the solute (represented by gray spheres) is being extracted, in this work corresponding to the IL-rich phase. The decrease of the extractive phase volume leads to an increased concentration of the analyte. By applying the lever-arm rule, it is possible to determine the CF value corresponding to the mixture point composition (CF1 < CF2 < CF3). The concentration procedure is satisfied by two fundamental requisites: 1) to find an ABS with a TL capable of leading to the complete extraction and without the saturation of the extractive phase; and 2) to use a long TL (or with a proper length) in order to achieve CFs as high as possible. However, at least 50 wt% of water (containing the target analyte) in the overall system should be kept to avoid significant discrepancies in the desired

CF value. It should be clarified that the use of conventional salts as salting-out agents in IL-based ABS was already reported in the literature, and where concentration factors of 100- and 1000-fold have been obtained [42, 48]. For instance, no concentration investigations have been attempted with weaker salting-out species. This is due to the strong salting-out effect that conventional salts present over the other already employed salting-out species (amino acids [50-51], carbohydrates [52-59] and polymers [44, 60-62]), and thus, an easier separation and manipulation of the phases' properties can be made as well as the acquisition of phase diagrams with longer *tie-lines* (TL). In this work, ABS composed of ILs and strong salting-out species were investigated aiming at simultaneously extracting and concentrating a mixture of human micropollutants. The main goal is to overcome the detection limits of conventional analytical equipment commonly used in the analysis and monitoring of complex environmental aqueous samples.

Table 2 reviews the benefits associated to IL-based ABS when used as a concentration technology: sustainability, simplicity, and improvement of sensibility. The new technology will allow the finding of human contamination local origins, and to create relationships between local consumption patterns and mixture behaviors of these contaminants in aqueous matrices. It is also expected to create a new technology as a general pretreatment method able to be combined with several analytical equipments. An adequate manipulation of the IL-ABS will allow the selection of an adequate CF of the target analytes from real matrices so that the identification/quantification by several conventional analytical equipments can be easily attempted.

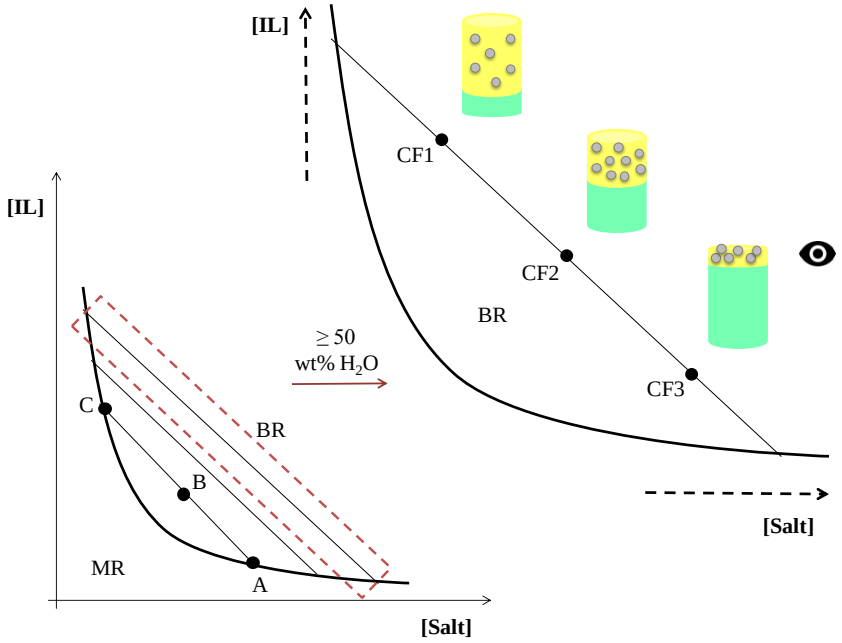


Figure 3. Ternary phase diagram (orthogonal representation) for a hypothetical system composed of IL + Salt + H_2O (left image); Schematic representation of the concentration factor achievable through the variation of the initial mixture composition along the TL (right image); MR – monophasic region; BR – biphasic region; [ABC] – TL formed by nodes A and C and by initial mixture B (composition B originates the top and bottom phases with the composition A and B, respectively; CF(1, 2, 3) - concentration factor 1, 2 and 3 (increasing order); IL-rich phase (◆), Salt-rich phase (■);extracted solute (●).

Table 2. Benefits associated with the preconcentration technology under development in this work.

Benefits	
Sustainability	"Greener" alternatives to systems using VOCs
	The concentration step implies a reduction of the phase-forming components with a significant reduction in the overall process costs
Simplicity	Extraction and concentration achievable in a single step
Improvement of sensibility	It is possible to manipulate the ABS compositions allowing the selection of the proper CF to be applied for the monitoring of micropollutants
	The tunable properties allied to ILs allow the design of proper ABS in order to obtain higher accuracy in the quantification of the tracer compounds

1.2. Scope and objectives

The main objective of this thesis consists on the development of a preconcentration technology for human pollution tracers from wastewater streams allowing therefore an adequate monitoring of their presence in different environmental matrices. For that purpose, it is essential an adequate characterization of the IL-based ABS as alternative preconcentration technologies, namely the characterization of their phase diagrams, TLs and TLLs, and on the evaluation of their applicability to extract and concentrate pharmaceutical tracers from real aqueous matrices.

Pharmaceuticals are ubiquitous micropollutants since their continuous consumption and consequent release *via* human excretions in aqueous systems are inevitable. Due to their presence at very low concentrations and in complex mixtures, a proper risk assessment on aquatic life and human health must be early established. Therefore, the development of a technology that will improve the sensibility while allowing the quantification and the monitoring of trace pollutants in complex wastewater matrices is of major relevance. In recent years, the quantification of persistent pharmaceuticals as human pollution tracers has been a target of various studies showing great potential on tracking the contamination. Conventional analytical equipment do not permit the detection and quantification of the low levels of these human pollution tracers if a previous concentration step is not carried out. However, common pretreatment processes commonly applied are costly and time-consuming, and make use of volatile and hazardous organic solvents. In the first part of this work, IL-based ABS were employed as the extraction and concentration technique. Two pharmaceuticals, as representative of human pollution markers, namely caffeine (CAF) and carbamazepine (CBZ), were investigated. The systems were formed by $[N_{4444}]Cl$, the strong *salting-out* salt $K_3[C_6H_5O_7]$ and water, with the goal of finding an improved TL capable of completely extracting and concentrating CAF and CBZ from wastewaters, while still guaranteeing the development of a cost-effective technique.

ILs have been reported in several studies as alternative solubilizing agents of pharmaceutical active ingredients [63-68]. It was recently reported that ILs are a novel class of catanionic hydrotropes responsible for the improved solvation capacity of a wide range of compounds [69]. In this context, the goal of the second part of this work consists on the investigation of the hydrotropic effect of ILs which could justify the enhanced extraction and concentration ability of IL-based ABS for CAF and CBZ.

2

Extraction and concentration of pharmaceuticals from wastewaters using IL-based ABS

2.1. Pharmacological contamination in WWTP effluents – a major concern as a result of human excretion

In the past few years, several studies have reported a class of emergent human source contaminants present in water [43, 70-72], the micropollutants. The term "micropollutant" is due to the presence of organic or mineral compounds in the aquatic environment at very low concentrations, in the order of $ng \cdot dm^{-3}$ to $\mu g \cdot dm^{-3}$ [70, 73]. Even at those levels, these compounds have been investigated to display toxic, persistent and bioaccumulative properties, particularly when present as components of complex mixtures [70, 73]. Within the micropollutants group, pharmaceutical compounds are largely consumed by humans around the world [74-78]. Only in industrialized countries, the consumption of pharmaceuticals *per* year is estimated to be from 50 to 150g *per capita* [79]. Despite the first report in 1960 referring the inability of WWTPs to remove some pharmaceuticals [80], only after 1990 they have gained a significant international concern on an environmental basis [81]. A large diversity of pharmaceuticals has been found in the environment, such as analgesics, antibiotics, antiepileptics, β-blockers, blood-lipid regulators, antidepressants, anxiolytics, sedatives, and contraceptives [82].

The human excretion of pharmaceutical compounds is considered the most relevant source of micropollutants in WWTPs, when compared with the other disposal forms, because these excreted compounds can be as high as 75% of a single dose [83]. Most pharmaceuticals have a high solubility in aqueous matrices and do not evaporate at ambient pressure and temperature [79]. Moreover, they are usually resistant to degradation (as a result of their design to display a high durability and to be easily absorbed by the organism) [79]. Consequently, these compounds are actually ubiquitous contaminants through worldwide wastewater effluents [70]. Recent advances in analytical methods confirmed the worldwide presence of pharmaceuticals in WWTP effluents, surface waters, groundwaters and drinking waters [84-89]. Nevertheless, their environmental impact is not easy to know since the concentration of pharmaceuticals found in water supplies is millions of times lower than a medical dose [83]. Conventional water treatment processes do not have specific infrastructures to efficiently remove these "micro" compounds, as they have largely different physical and chemical properties [71, 90-92]. Studies have demonstrated that additional advanced treatment technologies are effective in removing pharmaceuticals, but they depend on the chemical class of the contaminants [78, 93-94]. Several studies have reported that these compounds do not occur individually but as complex mixtures with potential toxicity to a large variety of organisms, mainly due to their combined and synergistic effects [94-101], and because these compounds are designed and produced to therapeutically interact with cellular receptors at low concentrations [102-103]. Furthermore, other studies over the world have revealed that the type and abundance of pharmaceuticals found in water is strictly related with the local consumption

behavior [83]. It thus essential to determine excretion patterns of mixtures of these compounds and other micropollutants, like EDCs, discharged into aquatic environments, and that could pose considerable health risks to humans and ecological systems [83, 85]. In this way, a more defined degradability and environmental persistence profiles can be established, to further understand the chronic and acute exposure of these compounds to living organisms.

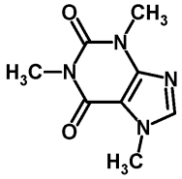
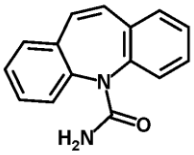
From the mentioned above, a proper analytical methodology for the monitoring of these mixtures of compounds should be implemented in WWTPs, because it will give the opportunity to select the proper treatment processes and to ascertain on the risk assessment through the aquatic environment and public health [94]. There are three important reasons why some legislation focuses on WWTPs as central points for pollution monitoring. The first is that WWTP effluents continuous release pharmaceuticals and are the major source of aquatic environmental contamination [94]. The second reason is that the concentrations of pharmaceuticals reported in surface waters sources are much lower than their levels in the associated wastewater effluents. This feature results in a series of factors that have to be taken into account during the analytical process regarding the evaluation of the environmental impact of these compounds: 1) mass flow of contaminants [87]; 2) level of dilution at the discharging point [104]; and 3) biotransformation, chemical transformation, phototransformation, and sorption ability (which control the fate and exposure of these compounds and resulting metabolites to human and wildlife health [105-107]). The last reason is due to the fact that pharmaceuticals are never completely metabolized by the human body, resulting in the excretion through feces and urine of the unchanged parent form and its metabolites [108]. Once in WWTPs, the inherent presence of the fecal bacteria *Escherichia coli* deconjugates the metabolites, increasing thus the amount of the parent compounds in wastewaters [109-110].

2.2. Caffeine (CAF) and carbamazepine (CBZ) as model human pollution tracers

In 1997, it was suggested that by tracing the contaminants present in water it could be possible to identify human activities [111]. The application of persistent pharmaceutical compounds as markers of anthropogenic contamination has permitted to quickly and accurately track the source, fate and type of aquatic contamination in natural waters [74, 77, 87, 112-113]. The selection of the best compounds as indicators of human pollution in aquatic systems must reflect their solubility in water [114], environmental significance, occurrence, resistance to treatment, and environmental persistence [87, 114]. The pharmacological micropollutants caffeine (CAF) and carbamazepine (CBZ) have been amongst the largest reported anthropogenic markers in the world [84, 115-120].

CAF, 1,3,7-trimethylpurine-2,6-dione, is an alkaloid with natural occurrence in a wide variety of plants [121]. It is one of the major ingredients in some food and beverages (soft and energetic drinks) and is also used as a pharmacological agent. CAF is the most consumed alkaloid in the world since it acts as a central nervous system stimulant, while increasing alertness [122]. The physicochemical properties of CAF and respective molecular structure are reported in Table 3. Its high water solubility (and small octanol-water partition coefficient, K_{OW}) confers to CAF a high solubility in aqueous streams. Besides its continuous consumption and consequent discharge by humans at considerable values in WWTPs, it presents relative low stability under environmental conditions, high mobility in water, and negligible volatility [123-124]. Consequently, the environmental occurrence of CAF appears amongst the higher levels of detected compounds in incorrect discharged WWTPs effluents around the world [87].

Table 3. Physicochemical properties of CAF [1, 122, 125-127] and CBZ [1, 122, 128].

Properties	**CAF**	**CBZ**
Molecular formula	$C_8H_{10}N_4O_2$	$C_{15}H_{12}N_2O$
Molecular weight (g·mol^{-1})	194.19	236.27
Vapor pressure at 298 K (mmHg)	7.3×10^{-09}	1.84×10^{-07}
Solubility in water at 298 K (g·dm^{-3})	21.60	0.017
logK_{OW}	-0.07	2.45
pK_a at 298 K	3.12 – 8.02	13.94
Relative chemical stability	Lower	Higher
Chemical structure		

CBZ, benzol[b][1]benzazepine-11-carboxamide, is a pharmacological drug designated to treat and prevent epilepsy and as a mood stabilizer [94]. CBZ is one of the most prescribed drugs in the treatment of epilepsy and other medical conditions [84]. The physicochemical properties of CBZ and respective molecular structure are reported in Table 3. Its relative high stability under environmental conditions, and negligible volatility [123], support why this drug has a negligible degradation in WWTP [129], even by photobleaching treatment, and therefore is one of the most persistent pharmaceuticals [130]. Consequently, CBZ appears as one of the micropollutants present in higher levels in WWTP effluents and their discharge into aquatic environments, namely in water sources [92, 131] and even in treated drinking water [132-133].

Table 4 presents some of the recently determined concentrations of CAF and CBZ, in surface waters, and in different stages of WWTPs, in different worldwide regions. From Table 4 it is clear the high accumulation of these two pharmaceuticals (reaching values in the order of $\mu g \cdot L^{-1}$). For these reasons, CAF has been studied as a potential anthropogenic tracer in wastewater contamination in surface waters [87, 123, 134], stormwater outfalls [135-136], and untreated wastewater [123, 137]. For example, a previous study reported that the co-presence of CAF with nitrate can be used as a tracer of leaching from household septic systems [138]. CBZ has also been proposed as a potential anthropogenic tracer of local cumulative wastewater discharges [86-87, 139]. Furthermore, Almeida *et al.* [84] reported that there is a growing bioaccumulation of CBZ in clams and in coastal systems, where their results demonstrated the high suspected toxicological risks to aquatic living organisms. Indeed, this drug has the potential to cause toxic effects on non-target organisms, and a complete trace of its toxicological risk profile is very important. CBZ has been reported in literature as "R52/53 harmful to aquatic organisms and may cause long term adverse effects in the aquatic environment" [140] (according to European legislation on the classification and labeling of chemicals (92/32/EEC)).

In summary, CAF and CBZ can be used as standard human pollutant markers of great interest, in particular when new advanced analytical procedures to detect and quantify micropollutants profiles in wastewater streams from WWTPs and in aquatic environment are under development.

Table 4. Quantification of CAF and CBZ in various stages of WWTPs of two world regions (input, after primary and secondary treatments, and final effluent) and in surface water.

Compound	Wastewater input ($\mu g \cdot dm^{-3}$)	After primary treatment ($\mu g \cdot dm^{-3}$)	After secondary treatment ($\mu g \cdot dm^{-3}$)	Wastewater final effluent ($\mu g \cdot dm^{-3}$)	Surface water ($\mu g \cdot dm^{-3}$)	Ref.
CAF	13.05	0.7	0.43	0.39	1.02	[113]
	27.7	13.6	(----)	0.038	(----)	[141]
CBZ	5.0	(----)	(----)	2.3	4.5	[132]
	0.44	0.49	(----)	0.495	0.146	[129]

2.3. Extraction and concentration of CAF and CBZ from wastewaters

The development of precise analytical measurement technologies for the monitoring of wastewater matrices remains a challenge due their complex matrices and very low concentrations of marker pollutants ($ng \cdot dm^{-3}$ to $\mu g \cdot dm^{-3}$) [114]. The application of liquid chromatographic methods has allowed the determination of a wide range of compounds, including pharmaceuticals,

and have therefore permitted a more comprehensive assessment of environmental contaminants [89, 142-143]. Liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography tandem mass spectrometry (GC-MS/MS) have been selected as the techniques of choice for the environmental analysis in wastewater matrices, either for CAF [113] or CBZ [94, 129]. However, these techniques present several drawbacks, turning them impracticable techniques for many analytical laboratories: they are based on expensive devices, require high maintenance costs and expertise people, and the matrix effect can interfere with the technique performance [48, 94]. HPLC coupled with ultraviolet (UV) or fluorescence detection is a simpler and cheaper technique [94]. In addition, it only requires a quick pretreatment method, since the presence of organic matter does not interfere with the quantification, minimizing thus the matrix effect [94, 129]. However, the sensitivity, or the lower limit of quantification of these methods depends on a proper pretreatment and/or concentration step [76, 94].

A pre-concentration step is fundamental for a precise and accurate quantification of all pharmaceutical compounds, since they are usually present in relative low concentrations and within a complex environmental sample [76, 144]. Several extraction and pre-concentration techniques have been developed and optimized before the quantification of CBZ in water samples, such as LLE [145], solid-phase extraction (SPE) [139, 146], solid-phase microextraction (SPME) [147], and liquid-phase microextraction (LPME) [148]. Due to the good recovery capacity, with both pretreatment and preconcentration purposes [76], the SPE pretreatment step has been widely reported as a *priori* concentration approach for the quantification of CAF and CBZ [76, 149-150]. However, all these pre-concentration methods present several disadvantages, such as: 1) they are time-consuming, labor-intensive and costly; 2) large use of hazardous VOCs are used ; and 3) the solid-phase extraction processes present some recovery problems [76]. All of these drawbacks indicate the need to find a more sustainable, simple and effective pre-concentration method, capable of pre-concentrating CAF and CBZ for their further proper detection and more sensitive quantification in wastewater and water samples. IL-based ABS have already been a target of scientific research on the extraction of pharmaceutical compounds within wastewater samples [39, 151]. These data prove that IL-based ABS are more effective alternatives over the traditional extraction techniques.

In this section, it was tested the ability of the IL $[N_{4444}]Cl$ combined with the organic salt $K_3[C_6H_5O_7]$ (a strong salting-out inducing agent) as constituents of ABS for the simultaneous extraction and concentration of CAF and CBZ, in order to improve their detection *via* conventional analytical techniques, namely by HPLC with UV-Vis detection.

2.4. Experimental section

2.4.1. Chemicals

The IL studied in this work was tetrabutylammonium chloride, $[N_{4444}]Cl$, obtained from Sigma-Aldrich. The IL used has a stated supplier purity of at least 97 wt%. The chemical structure of $[N_{4444}]Cl$ is depicted in Figure 4. Before use, aiming at reducing the water and volatile compounds contents to negligible values, the IL sample was 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 the IL was further checked by ^{1}H and ^{13}C NMR spectra and confirmed to be in accordance with the purity given by the supplier. The potassium citrate tribasic mono-hydrated, $K_3[C_6H_5O_7]\cdot H_2O$, > 99.0 wt% pure, was from Sigma-Aldrich and GPR. NMR spectra of $K_3[C_6H_5O_7]$ were made for both suppliers in order to verify the purity of the salt received - *cf.* NMR spectra in Appendix A (Figures A 1 and A 2). Potassium nitrate, KNO_3, > 98 wt%, was acquired from Panreac, acetic acid, $C_2H_4O_2$, > 99.5 wt%, was supplied by Labsolve JMGS, sodium acetate, $NaC_2H_3O_2$, 100 wt% pure, was acquired from Prolabo, and potassium chloride, KCl, > 99.5 wt%, was from Chem-Lab.

CAF anhydrous, > 99 wt % pure, was supplied by Fluka, and CBZ anhydrous, > 99 wt%, was supplied by Sigma. Both tracers were used as received.

The water employed was 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 from HiPerSolv Chromanorm.

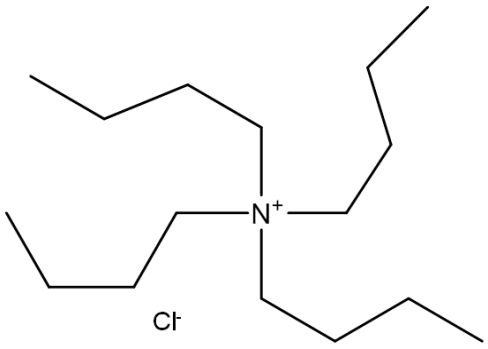


Figure 4. Chemical structure of the IL investigated: tetrabutylammonium chloride, $[N_{4444}]Cl$.

2.4.2. Experimental procedure

2.4.2.1. Analytical quantification of the TL for concentration analysis

A proper TL of the liquid–liquid aqueous biphasic systems composed of $[N_{4444}]Cl$ + $K_3[C_6H_5O_7]$ + H_2O was selected from literature [32]. Once the equation commonly applied to describe the binodal data is not able to correctly describe the regions of the solubility curve for high

IL and salt concentrations [152], the concentration of each compound in both phases of this TL was analytically determined. It was already reported that shorter TLs obtained by the quantification of all phase-forming components agree well with those obtained by the mass-balance method proposed by Merchuk et al. [153]. However, larger differences are observed at significantly long TLLs, or where the concentration of one of the phase-forming components in the initial mixture is very low [152]. $[N_{4444}]Cl$ was quantified in each phase through chloride quantification using a Metrohm 904 Titrando ion chloride electrode. For that, a stock aqueous solution of KCl of 1 $mol \cdot dm^{-3}$ was prepared and diluted at appropriate concentrations (between 0.1×10^{-3} $mol \cdot dm^{-3}$ and 100×10^{-3} $mol \cdot dm^{-3}$) in order to determine the calibration curve. A TISAB solution (mixture of aqueous solutions at 0.1 $mol \cdot dm^{-3}$ of KNO_3, $C_2H_4O_2$, and $C_2H_3NaO_2$) was prepared and added in all standard solutions and samples to maintain the ionic strength during the measurements. The water content in each phase was determined by evaporation, by means of an air oven at ~100 °C, until a constant weight of the non-volatile mixture $[N_{4444}]Cl + K_3[C_6H_5O_7]$ was achieved. The amount of $K_3[C_6H_5O_7]$ was determined by the weight difference. This process was carried out in triplicate to ascertain on the associated standard deviations.

2.4.2.2. Extraction and concentration of the pharmaceuticals in the $[N_{4444}]Cl$-based ABS

The concentration factor of CAF and CBZ along the characterized TL was evaluated by the preparation of ternary systems at a weight ratio (weight of water added to the system *per* weight of the IL-rich phase) of 1000-fold. Along the same TL, the composition of each phase is maintained while varying only the volume or weight ratio of the phases (Figure 5).

Aqueous solutions of CAF and CBZ were prepared with a concentration of 1×10^{-2} $g \cdot dm^{-3}$ (5.15×10^{-5} $mol \cdot dm^{-3}$ and 4.23×10^{-5} $mol \cdot dm^{-3}$, respectively). The ternary mixtures were vigorously stirred and left to achieve the equilibrium for at least 48 h, under controlled temperature using an air oven at (298 ± 1) K, as previously established [48]. This time allowed to achieve the complete partitioning of pharmaceuticals between the two phases. The top and bottom phases were carefully separated under controlled temperature, at (298 ± 1) K.

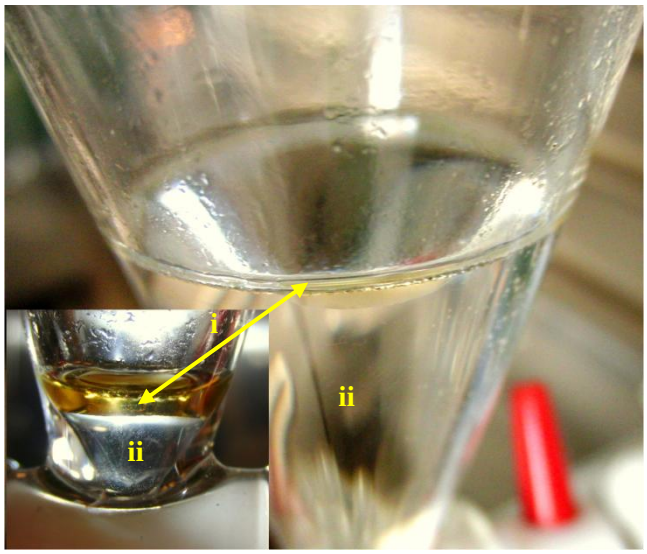


Figure 5. ABS formed by IL + Salt + H_2O: (i) IL-rich phase; (ii) salt-rich phase. The reduced IL-rich phase volume is due to the increased concentration along a given TL (estimated concentration factor of *ca.* 1000-fold).

2.4.2.3. HPLC quantification of the extracted and concentrated pharmaceuticals

CAF and CBZ were quantified using a Shimadzu High-Performance Liquid Chromatograph (HPLC) Prominence system equipped with a SPD-20A, UV-Vis detector. This device consists of 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 for the separation. The detection/quantification of CAF and CBZ was performed at a wavelength of 272 nm and 285 nm, respectively. Both the column and cell temperature were maintained at 298 K. Due to the distinct chemical properties of CAF and CBZ (Table 3), standard solutions of $[N_{4444}]Cl$ with appropriate concentration and spikes of CAF and CBZ of the standard solution were analyzed to optimize the flow and composition of the mobile phase for the quantification of CAF and CBZ. The mobile phase consisted of a water–acetonitrile gradient mixture, at a flow rate of 0.8 $mL \cdot min^{-1}$. Tables 5 and 6 report the time programming of the gradient elution developed for the optimization of the mobile phase for quantification of CAF and CBZ, respectively. Water and acetonitrile used in the mobile phase were filtered using 0.2 μm polyamide membrane filters from Whatman. Three samples of different concentrations of CAF and CBZ in the IL-rich phase were analyzed in order to determine the recovery of the pharmaceuticals as well as the respective standard deviations. Individual standard stock aqueous solutions of CAF and CBZ were prepared at appropriate concentrations (1×10^{-3} $g \cdot dm^{-3}$, 5.15×10^{-6} $mol \cdot dm^{-3}$ and

4.23×10^{-6} mol·dm^{-3}, respectively) in order to compare the peaks areas of the pharmaceuticals in an aqueous standard solution and in the IL-rich phase.

Table 5. Time programming of the gradient elution optimized to quantify CAF by HPLC. Solvent A: water; Solvent B: acetonitrile.

Time (min)	Solvent A (v/v%)	Solvent B (v/v%)	Wavelength (nm)
0	75	25	272
2	57.5	42.5	272
4	40	60	272
6	40	60	272
8	40	60	272
10		Stops	

Table 6. Time programming of the gradient elution optimized to quantify CBZ by HPLC. Solvent A: water; Solvent B: acetonitrile.

Time (min)	Solvent A (v/v%)	Solvent B (v/v%)	Wavelength (nm)
0	75	25	285
2	57.5	42.5	285
4	60	40	285
6	60	40	285
8	60	40	285
20		Stops	

2.4.2.4. pH measurement

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 an uncertainty of ± 0.02.

2.5. Results and discussion

The aqueous biphasic system composed of $[N_{4444}]Cl$ + $K_3[C_6H_5O_7]$ + H_2O was selected here since the IL $[N_{4444}]Cl$ is capable to undergo phase separation in the presence of aqueous solutions of several salts, including the salt $K_3[C_6H_5O_7]$ used in this work. Due to its high ability on undergoing liquid-liquid demixing it also allows achieving long TLs and thus high concentration factors [32, 42, 154]. Moreover, $[N_{4444}]Cl$ revealed to be one of the ILs with more ability to extract the most diverse biomolecules, and without saturation of the IL-rich phase [32, 42, 154]. Ammonium-based ILs also do not present absorption in the UV region not interfering with analytical methods based on UV absorption. In the studied ABS, CAF and CBZ were preferentially

partitioned for the IL-rich phase. The pH of the medium afforded by the salt (≈ 9) allows working with CAF and CBZ in their neutral form - *cf.* speciation curves in Appendix D (Figures D 1 and D 2) - thus avoiding their speciation and possible electrostatic contributions.

The main characteristic of ABS to be used as concentration platforms consists on the selection of a proper TL that is used to analyze and manipulate the CFs intended. The selection of the TL to be used in the concentration process was chosen based on data taken from the literature [32], and according with the criteria aforementioned in the general introduction. In this context, the TL with a length of *ca.*79 was used. As previously verified, the Merchuk equation [153] commonly applied to describe the binodal data is not able to correctly describe the regions of the solubility curve for high IL and salt concentrations [152]. Thus, with the aim to attain higher enrichment factors, in this work, the Merchuk equation [152] problem was firstly solved, by analytically determining the concentration of each compound in both phases of this TL and as described in the experimental section, in section 2.4.2.1. Secondly, the lever arm rule was applied, as indicated by Equations 1 and 2. These equations allow determining the weight percentage of each component, and thus it is possible to estimate the CFs intended – *cf.* lever arm rule application and estimated CF values in Appendix D – Table D 1.

$$\%\, wt_{IL} = \frac{[salt]_{salt} - [salt]_M}{([salt]_M - [salt]_{IL}) + ([salt]_{salt} - [salt]_M)} \times 100 \quad (1)$$

$$\%\, wt_{salt} = \frac{[salt]_M - [salt]_{IL}}{([salt]_M - [salt]_{IL}) + ([salt]_{salt} - [salt]_M)} \times 100 \quad (2)$$

where $\%\, wt_{IL}$ corresponds to the weight percentage of the IL-rich phase and $\%\, wt_{salt}$ corresponds to the weight percentage of the salt-rich phase, in the ternary system. The indexes *M*, *IL* and *salt* correspond to the mixture, IL-rich phase and salt-rich phase, respectively.

Since the aim of this section is to concentrate aqueous samples containing pharmaceuticals in wastewaters, it should be taken into account that the amount of the real sample added to the system is the same as the amount of water required to create the initial mixture point. Thus, in this situation, all the CF values in this work were determined by Equation 3 taking into account the total amount of water added to the mixture point, where after the phase equilibrium is reached it is expected the complete extraction of CAF and CBZ for the IL-rich phase.

$$CF = \frac{w_{H_2O}^M}{w_{IL}} \quad (3)$$

where, $w_{H_2O}^{M}$ corresponds to the weight of water added in the mixture point of the ABS and w_{IL} corresponds to the weight of the IL-rich phase obtained after phase separation.

In this work, the correct quantification of the TL selected was not successfully achieved, which led to real and experimental CF values distinct from the estimated values - *cf.* CF values obtained in Appendix D – Table D 2. Figure 6 depicts the solubility curve of the $[N_{4444}]Cl$ + $K_3[C_6H_5O_7]$ + H_2O ABS, as well as the TL selected, as determined initially by the Merchuk equation [152], as analytically quantified, and the expected quantified TL.

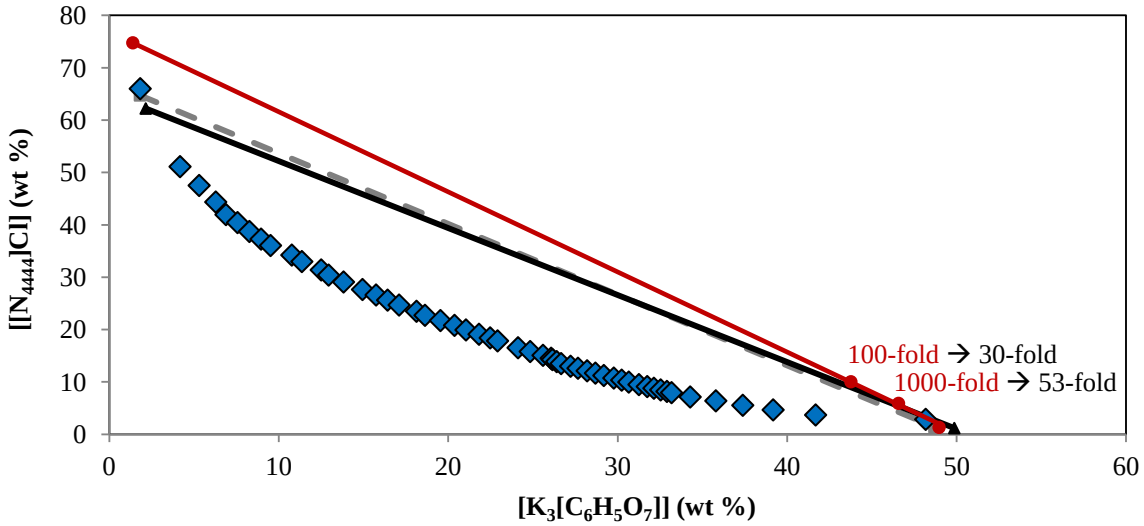


Figure 6. Evaluation of the CF along the selected TL for concentration approaches in ABS composed of $[N_{4444}]Cl$ + $K_3[C_6H_5O_7]$ + H_2O, at 298K: ◆, binodal curve data [32]; ■, TL quantified by the Merchuk equation [153]; ▲, TL analytically quantified; and ●, "ideally" quantified TL. According to the corresponding colors, 100-fold and 1000-fold correspond to the CF values ideally estimated which correspond to 30-fold and 53-fold, respectively, according to the experimental values obtained. ABS were composed of 1.44 wt% of $[N_{4444}]Cl$ + 49.65 wt% of $K_3[C_6H_5O_7]$ + 48.91 wt% of H_2O for estimated CF of 100-fold and 1.18 wt% of $[N_{4444}]Cl$ + 49.85 wt% of $K_3[C_6H_5O_7]$ + 48.98 wt% of H_2O for CF estimated of 1000-fold.

According to Figure 6, CFs of 100- and 1000-fold were intentioned to be evaluated in this work in order to prove the efficiency of IL-based ABS as a concentration platform for micropollutants in water samples. However, 30- and 53-fold CFs were respectively obtained. These values were significantly distinct from the estimated values. Moreover, these deviations increase along the TL, *i.e.*, for high concentrations of IL or salt, where differences in the order of two decimal oscillations is enough to generate great differences in the CF values. Therefore, the analytical quantification of the TL is a crucial requirement to obtain the CF required. CFs of 100- and 1000-fold were ideally planned for this work in order to allow the concentration of CAF and

CBZ, from those found in the wastewaters and reported in Table 4, to values that could be quantified by HPLC. Since the concentration levels found of CAF and CBZ in wastewaters are in the order of $\mu g \cdot dm^{-3}$ and $ng \cdot dm^{-3}$ (Table 4), respectively, a final CF value intended in this work of 1000-fold would increase these levels in three orders of magnitude, where a final concentration of 1.0×10^{-3} $g \cdot dm^{-3}$ was established to be reached after the concentration step for the IL-rich phase. In the HPLC quantification, if a final concentration of the pharmaceuticals of 1 $mg \cdot L^{-1}$ was verified, their complete extraction into the IL-rich phase (that means, in the $[N_{4444}]$Cl-rich phase) was reached as well as the CF intended. In this way, a concentration platform can be developed, envisaged as a more cost-effective technology, without the need to establish complex protocols for a proper quantification of the analytes.

Although a significant amount of experimental work is still necessary in order to correctly quantify the planned TL, in this section, the experimental TL was used in order to validate the ability of IL-based ABS to completely extract and concentrate both pharmaceuticals in a single-procedure and simultaneously. Thus, the CF of 53-fold obtained was used in order to improve the detection of CAF and CBZ in wastewaters. It should be referred that, in the following results regarding the CF values, volumes are considered instead of the weights of water and of the coexisting phases, in order to eliminate significant differences arising from the density of the IL-rich phase and water. The density of the $[N_{4444}]$Cl-rich phase density, at 25 °C and atmospheric pressure is 1.05 $g \cdot cm^{-3}$ (as experimentally verified in this work). Mixture compositions of the several ternary systems along the given TL were firstly prepared to extract and concentrate each pharmaceutical.

The chromatograms used for the identification and quantification of CAF and CBZ, individually extracted and concentrated, are presented in Figure 7 - *cf.* chromatographic data in Appendix D – Table D2. The quantification was made by HPLC with UV-Vis detection. Standard samples with initial concentrations of 1 $mg \cdot dm^{-3}$ of CAF and CBZ were used in order to evaluate the CAF and CBZ extraction efficiencies and the concentration factor of 53-fold afforded by the $[N_{4444}]$Cl-based ABS. As it can be seen, the peak areas of CAF (peak 1) and CBZ (peak 2) in the $[N_{4444}]$Cl-rich phases are similar with those obtained with the standard spikes. Thus, it can be assumed that the complete extraction of CAF and CBZ was always attained for all the mixture compositions and that a CF value of approximately 53-fold was obtained. The remaining peaks in the chromatograms are referent to possible interferences caused by the phase-forming components of the ABS. Further analyses are needed in order to understand such interferences by the phase-forming components. Based on these results, the simultaneous extraction of CAF and CBZ was further investigated, and using the same CF of 53. CAF and CBZ were initially introduced in the

same $[N_{4444}]Cl$ -based ABS with the same final concentration of 1.0 $\times 10^{-3}$ g·dm^{-3} (of each pharmaceutical).

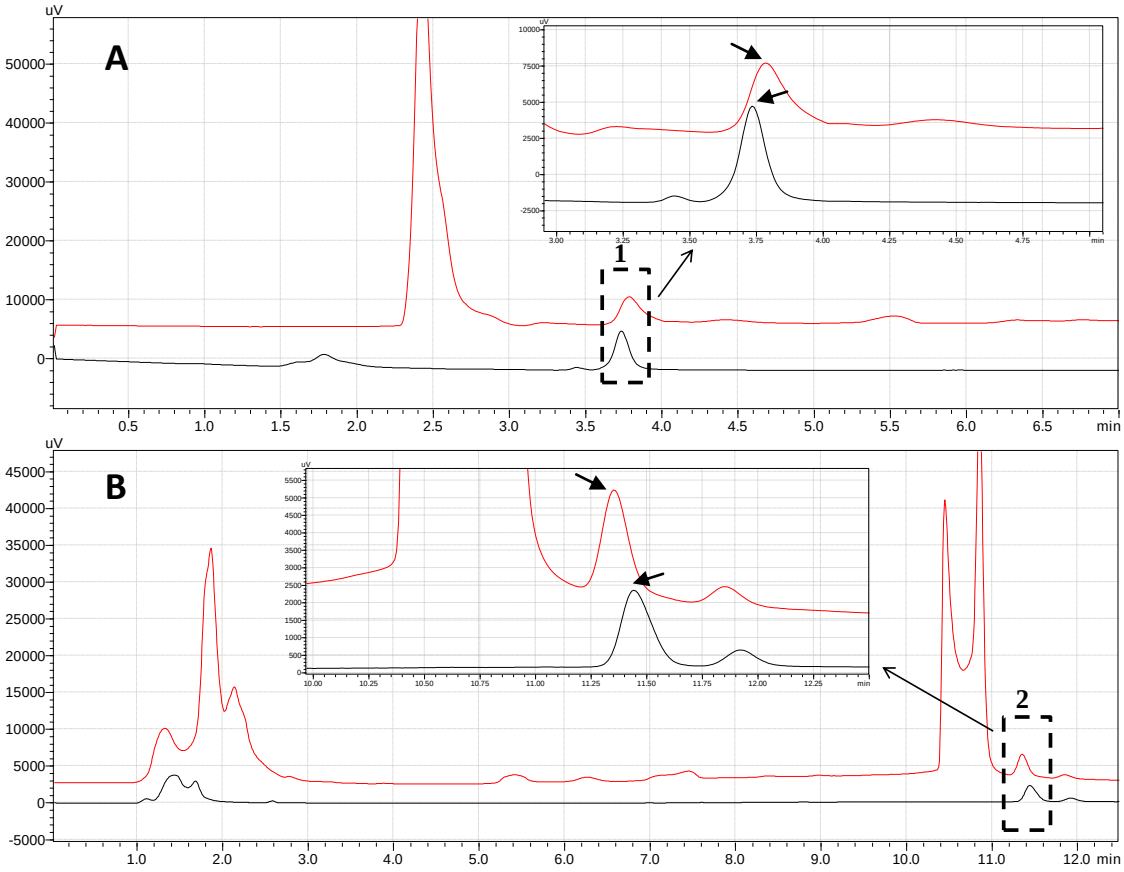


Figure 7. Chromatograms corresponding to the quantification of pharmaceuticals, individually extracted, in aqueous solutions. A) Chromatogram for the identification and quantification of CAF; B) Chromatogram for the identification and quantification of CBZ. Chromatograms of standard solutions of CAF and CBZ (black line) and chromatograms of aqueous solutions of CAF and CBZ extracted into the $[N_{4444}]Cl$-rich phase (red line). Peaks: 1 – CAF and 2 – CBZ. The remaining peaks correspond to possible interferences caused by the phase-forming components of the ABS. The quantification was made by HPLC with UV-Vis detection.

The chromatogram of CAF and CBZ, being simultaneously extracted, is depicted in Figure 8 - *cf.* chromatographic data in Appendix D – Table D 2. Standard samples with initial concentrations of 1 mg·dm^{-3} of CAF and CBZ were used in order to evaluate the CAF and CBZ extraction efficiencies and the concentration factor of 53 afforded by the $[N_{4444}]Cl$-based ABS. As it can be seen, the peak areas of CAF (peak 1) and CBZ (peak 2) in the $[N_{4444}]Cl$-rich phases remain similar with those obtained with the standard spikes, revealing that the IL-rich phase is not saturated and that both compounds can be extracted concomitantly. The recovery results obtained were 91 ± 15% for CAF and 104 ± 5% for CBZ. Therefore, the concentration of CAF and CBZ in wastewater can be increased at least up to 53-fold, in a single-step, simply by tuning the mixture

point composition which decreases the IL-rich phase volume. The mixture point required to create this condition is: 1.18 wt% of $[N_{4444}]Cl$ + 49.85 wt% of $K_3[C_6H_5O_7]$ + 48.98 wt% of H_2O.

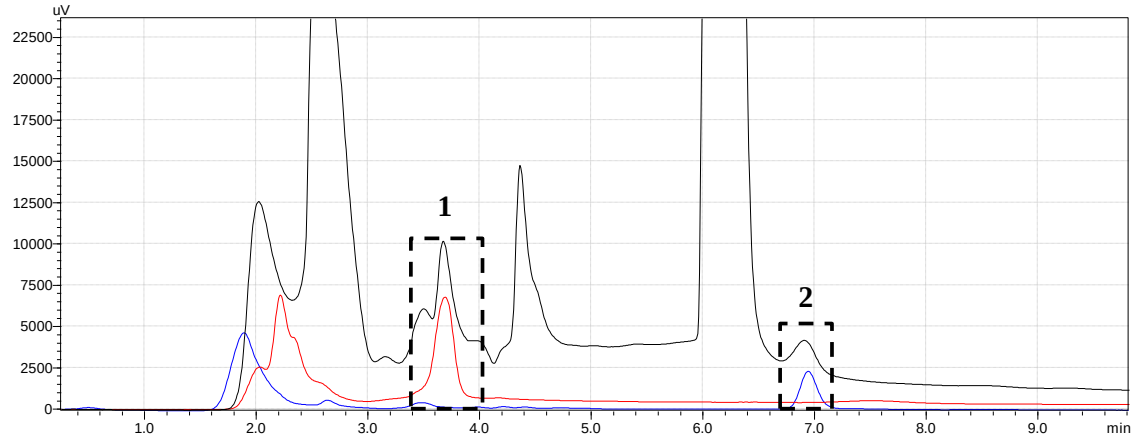


Figure 8. Chromatograms corresponding to the quantification of pharmaceuticals, simultaneously extracted, in aqueous solutions. Chromatogram of a standard solution of CAF (red line); Chromatogram of a standard solution of CAF (blue line); Chromatograms of aqueous solutions of CAF and CBZ extracted into the $[N_{4444}]Cl$-rich phase (black line). Peaks: 1 – CAF and 2 – CBZ. The remaining peaks are referent to possible interferences caused by the phase-forming components of the ABS. The quantification was made by HPLC with UV-Vis detection.

Although the concentration factor desired of 1000-fold was not reached in this work, the studied conditions guarantee the performance of the proposed technology to completely extract and concentrate the two human pollution tracers simultaneously. This achievement is of high importance to validate the efficient operation of this technique under the established requisites. The LOD values of the HPLC with UV-Vis detection for CAF and CBZ range between $(0.1 – 1.0) \times 10^{-3}$ g·dm^{-3}, for both compounds. The final concentration of CAF and CBZ achieved in this work is 1.0×10^{-3} g·dm^{-3}, starting from an initial concentration of 9.0×10^{-6} g·dm^{-3} in the system. As the initial concentration value is increased for a final concentration above the LOD values, the concentration for the quantitative analysis is improved having into account their detection by the analytical equipment. Despite the final concentration value is included in the LOD values range, the area values of the resulting peaks is four times higher than a minimum area value required to be well correlated with an accurate detection of the compounds. This proves that the IL-based ABS here studied as a preconcentration method ensure a good detection by conventional analytical methods, in this case by HPLC - UV-Vis, for a further and proper quantification of these pharmaceuticals in aqueous samples.

In a wastewater real sample, CAF and CBZ are present in concentrations in the order of μg·dm^{-3} and ng·dm^{-3}, respectively (Table 4). The major goal was to reach a final concentration of

CAF and CBZ at the IL-rich phase for which a real wastewater sample can be used. However, taking into account the concentrations of these two compounds in real aqueous matrices, the final concentration of 1.0×10^{-3} g·dm^{-3} established in this work was based in a target CF value of 1000-fold. Since a final CF value of only *ca.* 53-fold was reached, instead of the intended 1000-fold value, this concentration value is not still enough to be accurately applied to a real wastewater sample (to simultaneously detect and quantify these two anthropogenic pollutant markers). Previous works reported enrichment factors of *ca.* 100-fold for CBZ from wastewater samples, using SPE as preconcentration method [155] and HPLC - UV-Vis as the analytical method. This value was higher than the concentration attained in this work; however, it should be noted that the conditions are different and an adequate comparison between the two concentration methods is not possible. As referred in the general introduction, there are reports about the ability of the IL-based ABS with conventional salts that proved that these systems are capable to concentrate EDCs up to 100- [42] and 1000-fold [48]. In this work, it was possible to demonstrate the high ability of the IL-based ABS to completely extract and concentrate two pollutant tracers, independently of the CF value applied. Therefore, the real problem remains on the proper quantification of the TL that was initially established. However, further work is ongoing in order to understand how to correctly quantify this TL, which is the "key" for the $[N_{4444}]$Cl-based ABS to be capable of reaching the CF value of at least 1000-fold. Moreover, it should be noted that SPE is one of the most reported preconcentration methods for environmental analysis, but, as referred in Section 2.3, it presents several disadvantages such as the use of VOCs, it is time-consuming and leads to irregular recovery yields. IL-based ABS are thus a promising alternative to be applied as a preconcentration method in environmental analysis. With this technology it will be possible to establish the risks posed to human health from long-term, low level exposure to such human contaminants, as well as the behavior of mixtures of these compounds within the environment.

Finally, the economical and sustainable viability of the proposed method to detect micropollutants in WWTPs are ensured, due also to the use of more benign phase-forming components, and since the amount of IL used for aqueous biphasic system formation is inversely proportional to the concentration factor required; furthermore, the use of HPLC with an UV-Vis detector allows a simpler, cheaper and easy technique to use (compared for instance with the use of mass spectrometry [113, 129]).

2.6. Conclusion

Aiming at overcoming one of the major limitations in the analysis and monitoring of wastewaters, a novel methodology is here proposed to concentrate CAF and CBZ by the application of IL-based ABS. By tuning the mixture point composition, it was possible to

concentrate CAF and CBZ in the IL-rich phase up to 53-fold. IL-based ABS should allow to increase the concentration of CAF and CBZ, simultaneously, from their levels in wastewaters (μg·dm^{-3} and ng·dm^{-3}, respectively) up to the range (0.1 – 1.0) × 10^{-3} g·dm^{-3} (correspondent to the HPLC with UV-Vis LOD range for CAF and CBZ, respectively). However, a CF of 1000-fold is required to obtain a final concentration of 1.0 ×10^{-3} g·dm^{-3} for direct HPLC analysis. Recovery percentages of 91 ± 15% and 104 ± 5% for CAF and CBZ were obtained, respectively. Nevertheless, more experimental work is still required at this stage to improve the CF achievable. After this achievement, the proper evaluation of the environmental impact of pollution tracers by conventional and less expensive analytical equipment can be made. Since the detection and quantification of a wide array of micropollutants stills are a major challenge in the analytical field, these systems are also straightforwardly envisaged for the simultaneous extraction and concentration of a plethora of other components present in wastewaters. In the future, this preconcentration method is envisaged to be applied in the concentration and detection of other "microcompounds" present in more diluted concentrations, for instance in surface waters, where their proper analysis stills is a main challenge.

3

The hydrotropic effect responsible for the extraction and concentration ability of IL-based ABS

3.1. Hydrotropes: their role in aqueous solutions

Since 1916, when Carl Neuberg [156] reported for the first time the existence of substances that enhance the solubility in water of poor water soluble compounds, the hydrotropic phenomenon has gained a grown impact in industry (formulation of drugs, cleaning agents, personal care products, for extraction purposes, among others [69, 157-161]), being these substances defined as hydrotropes. Hydrotropes are a class of compounds, characterized by an amphiphilic structure with hydrophilic nature (K_{ow} lower than 1.0 [162]), and demonstrated to largely enhance the solubility of solutes in aqueous solutions, under ambient conditions [69]. Conventional hydrotropes applied in industry are composed of an aromatic ring (hydrophobic part) attached to an anionic group (hydrophilic part), with ammonium, calcium, potassium or sodium as counter ions. Examples of these typical compounds are sodium benzoate and sodium xylene sulphonate [161]. Figure 9 depicts a schematic representation of the solubility of octanoic acid in aqueous solutions of hydrotropes and of common surfactants [161]. The presence of the hydrotrope in aqueous media, at high concentrations, enabled the improvement of the solubility of octanoic acid (a very poor water soluble fatty acid) up to approximately four times higher than that afforded by a micellar solution. Contrarily to common micellar surfactants, hydrotropes present a smaller hydrophobic part. Therefore, they do not form micelles nor present a critical micellar concentration (CMC). However, the mechanism of action of these compounds is still not well understood [156]. In summary, hydrotropes have demonstrated a higher ability to solubilize hydrophobic compounds that are sparingly soluble in water over other solubilizing agents.

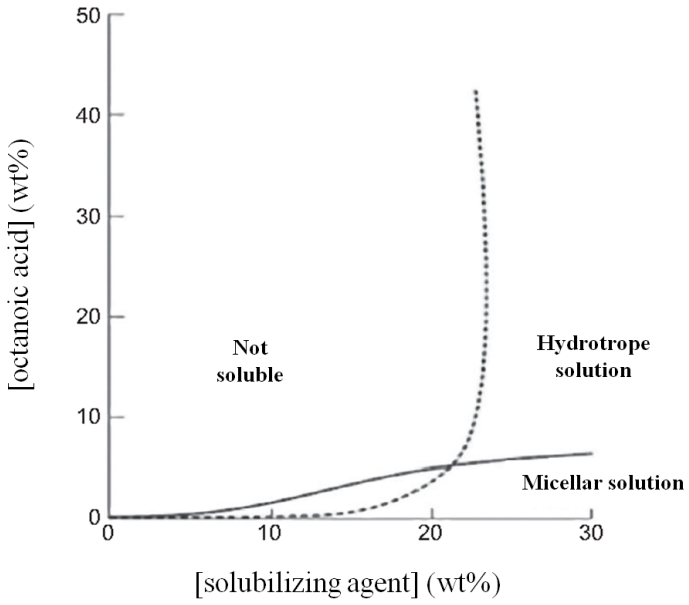


Figure 9. Schematic representation of the solubility of octanoic acid in water in presence of two solubilizing agents. Adapted from reference [161].

Innumerous works have been attempted addressing hydrotropes, mainly due to their outstanding characteristics: 1) stabilization and viscosity control of aqueous solutions [161]; 2) high ability to modify the liquid-liquid phase separation temperatures [161]; and 3) low toxicity, non-flammability and low bioaccumulation [161]. But the most attractable characteristic of these compounds is their ability to provide the greenest recovery of solutes, *i.e.*, since the solubility of the solutes depends on the hydrotrope concentration, and therefore the addition of water will simple lead to the solutes precipitation [161, 163]. This phenomenon opens new horizons to discover new compounds with potential hydrotropic effect where more cost-effective extraction and purification platforms can be developed.

3.2. ILs as a new class of hydrotropes: improving the solubility of pharmaceuticals in water

Taking into account their unique solvent abilities for a wide range of distinct solutes, in the recent years, ILs have been reported in several studies as alternative solubilizing agents [157, 159-161]. Many of these works are centered in the pharmaceutical field, where the potential of a range of ILs to successfully improve the solubility of poor water soluble drugs have demonstrated new opportunities to design the reformulation of drugs [66-67]. For example, it has been already investigated the use of ILs as drug delivery vehicles through the formation of stable micro-emulsions in order to dissolve and transdermally release poor water soluble pharmaceutical ingredients [164-165]. Further, imidazolium-based ILs have been target of alternative drug reservoirs for a controlled release or even as active pharmaceutical ingredients themselves [63-65, 166]. The solubility of paracetamol and ibuprofen, two important pharmaceuticals, were also investigated in the ILs 1-butyl-3-methylimidazolium hexafluorophosphate, $[C_4C_1im][PF_6]$, and 1-hexyl-3-methylimidazolium hexafluorophosphate, $[C_6C_1im][PF_6]$, in a wide range of temperatures [68]. In this study [68], both ILs demonstrated to be good solvents, where an increase in the temperature led to an increase in the concentration of the pharmaceutical. However, two opposite trends were observed for the pharmaceutical compounds, namely the solubility of ibuprofen follows the expected trend (water < $[C_4C_1im][PF_6]$ < $[C_6C_1im][PF_6]$) while the solubility of paracetamol follows a less expected order ($[C_4C_1im][PF_6]$ < $[C_6C_1im][PF_6]$ < water) [68]. These results reflect that ILs could have a specific-compound ability to dissolve these compounds, having into account the physico-chemical properties of the solutes. Nevertheless, further studies are necessary in order to better understand the mechanism of action ruling the interactions between the target solute and ILs.

ILs present an amphiphilic behavior similar to hydrotropes, which can be responsible for the solubilization/extraction of a wide number of nonpolar compounds. Figure 10 represents the

chemical structure of the IL 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[C_4C_1im][CF_3SO_3]$, as a potential hydrotrope.

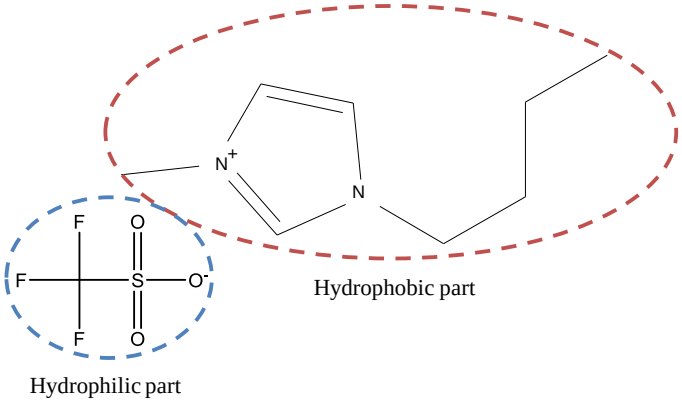


Figure 10. Chemical structure of 1-butyl-3-methylimidazolium trifluoromethanesulfonate ($[C_4C_1im][CF_3SO_3]$).

Cláudio *et al.* [69] recently investigated the hydrotropic ability of ILs, in comparison with conventional hydrotropes, to increase the solubility of phenolic compounds. Most ILs investigated revealed a high hydrotropic ability able to enhance the solubility in water of vanillin and gallic acid up to 40-fold. The IL concentration, the IL chemical structure and the temperature showed to have a significant impact on the solubility of these compounds [69]. An increase on the IL concentration, however passing through a maximum, as well as on the temperature led to a higher hydrotropic ability of the IL. Either the cation or the anion showed to influence the hydrotropic effect. In summary, Cláudio *et al.* [69] demonstrated that ILs constitute a novel class of catanionic hydrotropes and that the use of water as antisolvent conducts to the design of novel recovery strategies for both the solutes and IL solvents.

Taking into account the extraction and concentration approach proposed before using IL-based ABS, it is fundamental to understand, on a molecular-level scenario, their high ability to extract the two studied pharmaceuticals and without reaching the saturation of the phase. Therefore, in this section, it was investigated the hydrotropic mechanism responsible for the high solubility and extraction of CAF and CBZ using IL-based ABS. To this end, ABS composed of $K_3[C_6H_5O_7]$ and a wide range of ILs, and at different mixture compositions, were investigated for the extraction of CAF and CBZ. To support the results achieved, the solubility of caffeine was also determined in aqueous solutions of these ILs and compared with those in pure water, pure ILs, and aqueous solutions of $K_3[C_6H_5O_7]$ and some common hydrotropes and salting-in inducing agents, such as sodium benzoate, sodium citrate and sodium thiocyanate. Different initial concentrations of CAF were finally added into the IL-based ABS in order to infer on the saturation of the IL-rich phase.

3.3. Experimental section

3.3.1. Chemicals

The ILs studied in this work were: 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[C_4C_1im][CF_3SO_3]$, 1-butyl-3-methylimidazolium thiocyanate, $[C_4C_1im][SCN]$, 1-butyl-3-methylimidazolium methylsulfate, $[C_4C_1im][CH_3SO_4]$, 1-butyl-3-methylimidazolium tosylate, $[C_4C_1im][TOS]$, 1-butyl-3-methylimidazolium bromide, $[C_4C_1im]Br$, 1-butyl-3-methylimidazolium dicyanamide, $[C_4C_1im][N(CN)_2]$, 1-butyl-3-methylimidazolium chloride, $[C_4C_1im]Cl$, 1-butyl-2,3-dimethylimidazolium chloride, $[C_4C_1C_1im]Cl$, 1-butyl-1-methylpyridinium dicyanamide, $[C_4C_1py][N(CN)_2]$, 1-butyl-1-methylpyridinium chloride, $[C_4C_1py]Cl$, 1-butyl-1-methylpiperidinium chloride, $[C_4C_1pip]Cl$, 1-butyl-1-methylpyrrolidinium chloride, $[C_4C_1pyr]Cl$, tetrabutylammonium chloride, $[N_{4444}]Cl$, tetrabutylphosphonium chloride, $[P_{4444}]Cl$, and cholinium chloride, $[N_{1112(OH)}]Cl$. The imidazolium-, pyridinium-, and pyrrolidinium-based ILs were purchased from Iolitec. The tetrabutylphosphonium chloride was kindly offered by Cytec Industries Inc. The tetrabutylammonium chloride and cholinium chloride were 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 and confirmed to be > 98 wt%. The deuterium oxide used was acquired from Aldrich with > 99.96 % D atoms. 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) was from Aldrich with > 98 % D atoms. Sodium benzoate ($Na[C_7H_5O_2]$), > 99.0 wt% pure, was supplied by Panreac, sodium thiocyanate (Na[SCN]), > 98.0 wt % pure, was supplied by Fluka, sodium citrate ($Na_3[C_6H_5O_7]$), > 98.0 wt % pure, was from JMGS, and potassium citrate tribasic mono-hydrated ($K_3[C_6H_5O_7]\cdot H_2O$), > 99.0 wt % pure, was from Sigma-Aldrich. NMR spectrum was made for $K_3[C_6H_5O_7]$ in order to verify the purity of the salt received - *cf.* NMR spectrum in Appendix A (Figure A 1). All of these compounds were also dried before use. The chemical structures of anions and cations of all ILs and salts investigated are depicted in Figure 8.

CAF anhydrous, > 99 wt% pure, was supplied by Fluka and used as received. CBZ anhydrous, > 99 wt%, was supplied by Sigma and used as received.

The water employed was distilled, passed across a reverse osmosis system, and further treated with a Milli-Q plus 185 water purification apparatus.

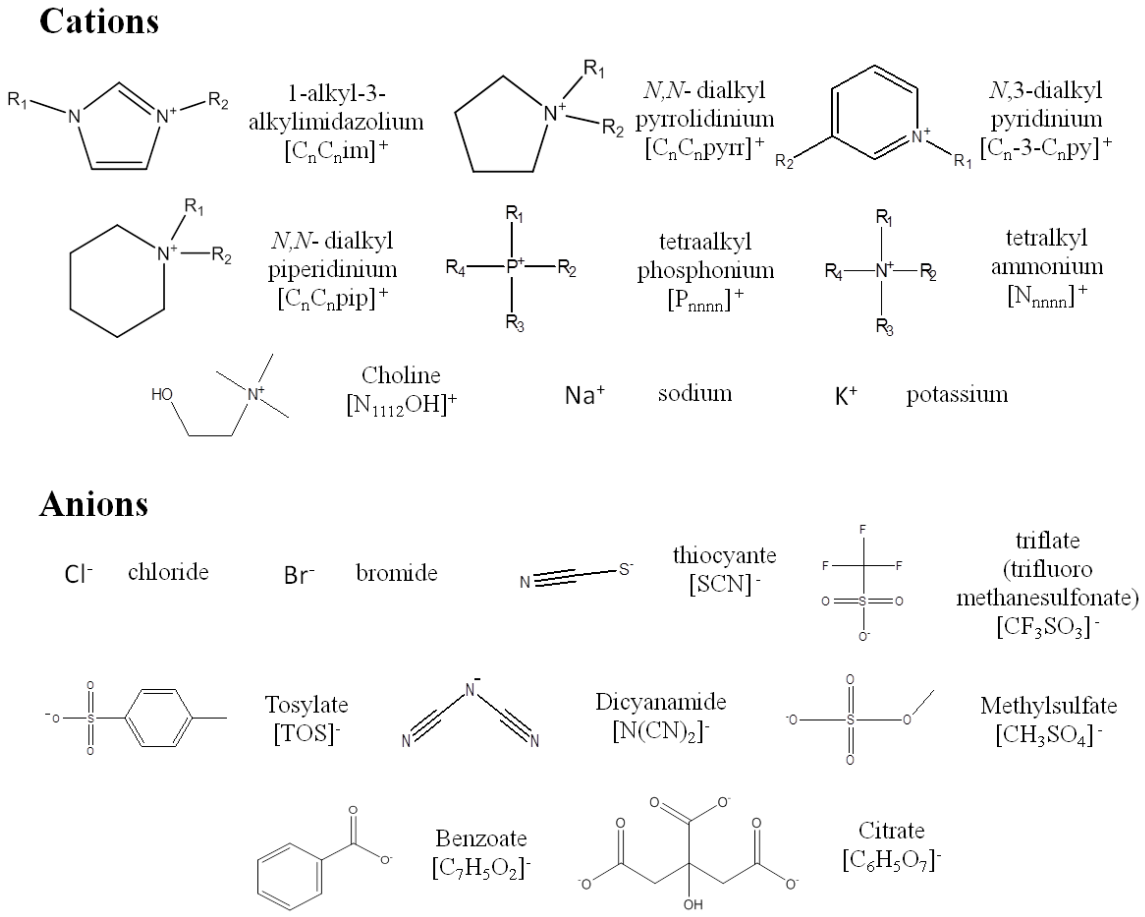


Figure 11. Chemical structures of the anions and cations of all ILs and salts investigated.

3.3.2. Experimental procedure

3.3.2.1. Extraction of pharmaceuticals with IL-based ABS

An aqueous solution of CAF was prepared with a concentration of 0.912 g·dm^{-3} (4.70 × 10^{-3} mol·dm^{-3}). Due to its low solubility in water, in each ternary mixture and individual experiment, *ca.* 1.0 × 10^{-3} g of CBZ was added, and in order to guarantee the same initial concentration of the two pharmaceuticals in these systems (mixtures with a total of 3 g were prepared). Several ternary systems were prepared within the biphasic region of the mixture of IL + $K_3[C_6H_5O_7]$ + aqueous solution of CAF with the following ILs: $[C_4C_1im][CF_3SO_3]$, $[C_4C_1im][SCN]$, $[C_4C_1im][CH_3SO_4]$, $[C_4C_1im]Br$, $[C_4C_1im][N(CN)_2]$, $[C_4C_1im]Cl$, $[C_4C_1C_1im]Cl$,

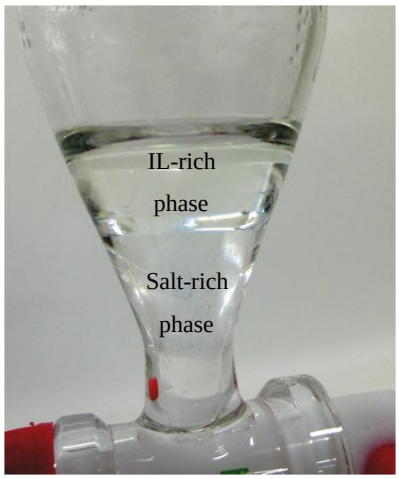


Figure 12. ABS formed by IL + $C_6H_5K_3O_7$ + H_2O.

$[C_4C_1py][N(CN)_2]$, $[C_4C_1py]Cl$, $[C_4C_1pip]Cl$, $[C_4C_1pyr]Cl$, $[N_{4444}]Cl$, and $[P_{4444}]Cl$. In order to avoid discrepancies in the results, the ternary systems were prepared at constant weight fraction percentage of each component: 40 wt% of IL + 20 wt% of $K_3[C_6H_5O_7]$ + 40 wt% H_2O. The mixture compositions for each ternary system are provided in Table 7. The ternary mixtures were vigorously stirred and left to achieve the equilibrium for at least 12 h, (298 ± 1) K (Figure 12). After the separation of the top and bottom phases, the detection and quantification of the pharmaceuticals were carried out 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 (*cf.* Appendix B - Figure B 1 to Figure B 2). For the measurement of the respective absorbance, blank controls of each ternary system were always prepared to eliminate interferences caused by the IL and $K_3[C_6H_5O_7]$. Three samples of each aqueous phase were analyzed, in at least three individual systems, in order to determine the extraction efficiencies of pharmaceuticals and the respective standard deviations. The percentage extraction efficiencies of the pharmaceuticals, EE_{Pha}%, were estimated by equation 4:

$$EE_{Pha}\% = \frac{w_{IL}[Pha]_{IL}}{w_{IL}[Pha]_{IL} + w_{salt}[Pha]_{salt}} \times 100 \quad (4)$$

where w_{IL} and w_{salt} are the weight of the IL-rich and $K_3[C_6H_5O_7]$-rich phases, and $[Pha]_{IL}$ and $[Pha]_{salt}$ are the concentration of each pharmaceutical in the IL-rich and $K_3[C_6H_5O_7]$-rich aqueous phases, respectively.

3.3.2.2. Optimization study for the extraction of CAF

Determination of tie-lines (TLs): The TLs were determined by a gravimetric method originally described by Merchuk et al. [153]. A mixture point at the biphasic region was gravimetrically prepared with IL + salt + water, vigorously stirred, and allowed to reach the equilibrium by the separation of both phases for at least 12 h at (298 ± 1) K. After the separation step, both top and bottom phases were weighted. Finally, each individual TL was determined by application of the lever-arm rule to the relationship between the top phase weight and the overall system composition.

For the determination of TLs, it was used the following system of four equations (equations 5 to 8) with four unknown variables ($[IL]_{\text{IL}}$, $[salt]_{\text{IL}}$, $[IL]_{\text{salt}}$, $[salt]_{\text{salt}}$):

$$[IL]_{\text{IL}} = A \exp\left(B[salt]_{\text{IL}}^{0.5} - C[salt]_{\text{IL}}^{3}\right) \quad (5)$$

$$[IL]_{\text{salt}} = A \exp\left(B[salt]_{\text{salt}}^{0.5} - C[salt]_{\text{salt}}^{3}\right) \quad (6)$$

$$[IL]_{\text{IL}} = \frac{[IL]_{\text{M}}}{\alpha} - \left(\frac{1-\alpha}{\alpha}\right)[IL]_{\text{salt}} \quad (7)$$

$$[salt]_{\text{IL}} = \frac{[salt]_{\text{M}}}{\alpha} - \left(\frac{1-\alpha}{\alpha}\right)[salt]_{\text{salt}} \quad (8)$$

where the indexes *M*, *IL* and *salt* correspond to the mixture, IL-rich phase and salt-rich phase, respectively. The parameter α is the ratio among the IL–rich phase and the total mixture weight. The solution of the referred system gives the concentration of IL and organic salt in the top and bottom phases, respectively.

The tie-line lengths (TLL) were calculated through equation 9:

$$TLL = \sqrt{([salt]_{\text{IL}} - [salt]_{\text{salt}})^2 + ([IL]_{\text{IL}} - [IL]_{\text{salt}})^2} \quad (9)$$

Extraction of CAF: The ternary system $[C_4C_1im]Br + K_3[C_6H_5O_7]$ + aqueous solution of CAF was selected for the optimization study. The influence of the tie-line length (TLL), *i.e.*, by varying the initial mixture composition, was analyzed aiming at reaching the complete extraction of the pharmaceutical in a single-step. The initial concentration of CAF added to each system was also studied to infer on its saturation at the IL-rich phase.

For the analysis of the TLL effect, the ABS were prepared at increased initial concentrations of the IL and salt as follows: 40 wt % of $[C_4C_1im]Br$ + 20 wt % of $K_3[C_6H_5O_7]$, 40 wt % of $[C_4C_1im]Br$ + 22.5 wt % of $K_3[C_6H_5O_7]$, 40 wt % of $[C_4C_1im]Br$ + 25 wt % of $K_3[C_6H_5O_7]$, 42.5 wt % of $[C_4C_1im]Br$ + 25 wt % of $K_3[C_6H_5O_7]$, and 45 wt % of $[C_4C_1im]Br$ + 25 wt % of $K_3[C_6H_5O_7]$. Then, for the analysis of the initial concentration of CAF in the system, three aqueous solutions with decreased concentrations of CAF were prepared: 0.912 $g \cdot dm^{-3}$ (4.70×10^{-3} $mol \cdot dm^{-3}$), 0.456 $g \cdot dm^{-3}$ (2.35×10^{-3} $mol \cdot dm^{-3}$), and 0.228 $g \cdot dm^{-3}$ (1.17×10^{-3} $mol \cdot dm^{-3}$).

The mixture compositions for each ternary system are provided in Table 7. All the partitioning experiments were performed accordingly to the conditions described in section 3.3.2.1.

3.3.2.3. Solubility of CAF in aqueous solutions

CAF (solid solute) was added in excess amounts to each IL aqueous solution, pure water or pure IL and $K_3[C_6H_5O_7]$ aqueous solution, and was then equilibrated in an air oven (at given temperatures (± 0.5 K)), under constant agitation, using an Eppendorf Thermomixer Comfort equipment. Before the solubilization study of CAF, equilibration

conditions were optimized and the following were established: stirring velocity of 750 rpm and equilibration time of at least 72 h. After the saturation was reached, all samples were centrifuged at the same temperature of equilibration in a Hettich Mikro 120 centrifuge during 20 min at 4500 rpm to separate the macroscopic solid and liquid phases. After centrifugation, all samples were placed in an air bath equipped with a Pt 100 probe and PID controller at the temperature used in equilibrium assays during 2 h. Then, the samples of the liquid phase were carefully collected and diluted in ultra-pure water, and the amount of CAF was quantified through UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at a wavelength of 274 nm, using a calibration curve previously established (*cf.* Appendix B - Figure B 3). In all experiments, control samples at the same compositions, but without solute, were used. At least three individual samples were quantified for each mixture and temperature. The hydrotropy constants, K_{Hyd}, were determined using the Setschenow equation [167], described by equation 10:

$$\log \left(S/S_0\right) = K_{Hyd} \times C_{Hyd} \quad (10)$$

where S and S_0 are, respectively, the solubility (mol·kg^{-1}) of CAF in the hydrotrope aqueous solution and in pure water, C_{Hyd} is the concentration of hydrotrope in aqueous solution (mol·kg^{-1}) and K_{Hyd} is the hydrotropy constant (kg^{-1}·mol).

3.3.2.4. Solubility of CAF in IL + $C_6H_5K_3O_7$ + H_2O ternary systems

The ternary systems selected, in the biphasic system, for the solubility studies of CAF were composed of the ILs [C_4C_1im]Cl and [C_4C_1im][$N(CN)_2$]. Various systems were prepared and pure CAF was added by an increased concentration in the ternary mixture, until the saturation of the pharmaceutical was reached: 10 g·dm^{-3} (5.2 × 10^{-2} mol·dm^{-3}) for [C_4C_1im]Cl and 10 g·dm^{-3} (5.2 × 10^{-2} mol·dm^{-3}), 25 g·dm^{-3} (13 × 10^{-2} mol·dm^{-3}), and 50 g·dm^{-3} (26 × 10^{-2} mol·dm^{-3}) for [C_4C_1im][$N(CN)_2$].The mixture compositions for each ternary system are provided in Table 7. All mixtures were treated according to the conditions described in section 3.3.2.1.

For the ternary systems where the saturation of CAF was reached, a previous and additional procedure was realized: all samples were centrifuged at (298 ± 1) K in an Eppendorf Centrifuge 5804, during 30 min at 3500 rpm to separate the solid (non-solubilized CAF) from the liquid phase. All samples were then separated according to the conditions described above and the CAF at both phases was quantified. The EE_{CAF} % values were also determined using equation 4.

3.3.2.5. pH measurements

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 an uncertainty of ± 0.02. The pH values obtained for each aqueous phase are provided in Table 7.

3.4. Results and discussion

All ABS applied in this work were previously characterized and are reported in the literature [32]. The effects of the IL anion, the IL cation core, and number of aliphatic tails were initially evaluated towards the partitioning of the pharmaceuticals between the two aqueous phases. The experimental data of the partitioning of CAF and CBZ are reported in Appendix (*cf.* Appendix D - Table D 3). Figures 13 and 14 depict the extraction efficiencies of the two pharmaceuticals, and respective standard deviations, in ABS formed with different ILs, $K_3[C_6H_5O_7]$ and H_2O, as well as the pH values of each phase. All the partitioning experiments were carried out at a fixed initial mixture composition of 20 wt % of salt and 40 wt % of IL.

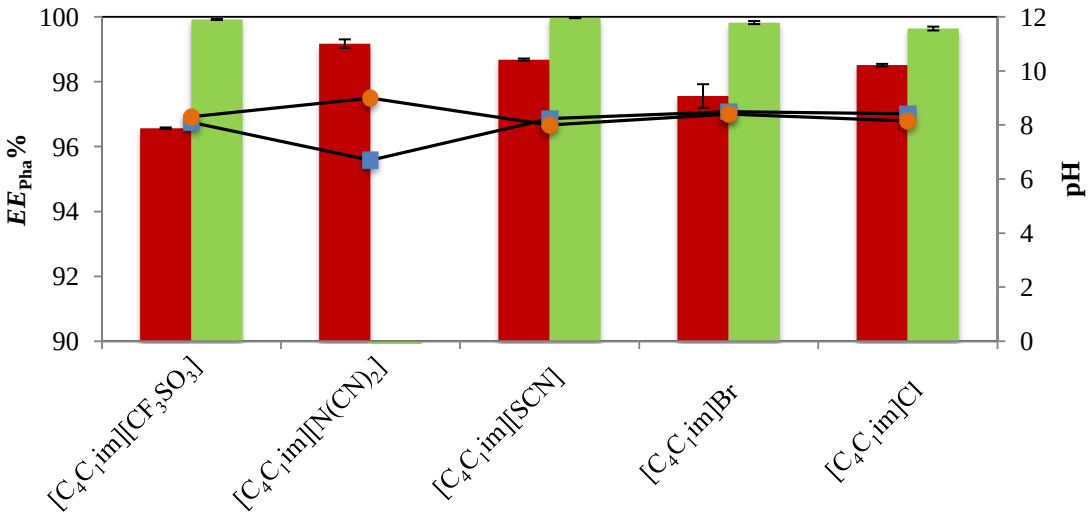


Figure 13. Percentage extraction efficiencies of CAF (▲) and CBZ (◆), EE_{Pha} %, in different ABS at 298 K, regarding the IL anion effect, and respective pH values of top (■) and bottom (●) phases. All ABS are composed of 20 wt% of $K_3[C_6H_5O_7]$ + 40 wt% of IL + 40 wt% of an aqueous phase.

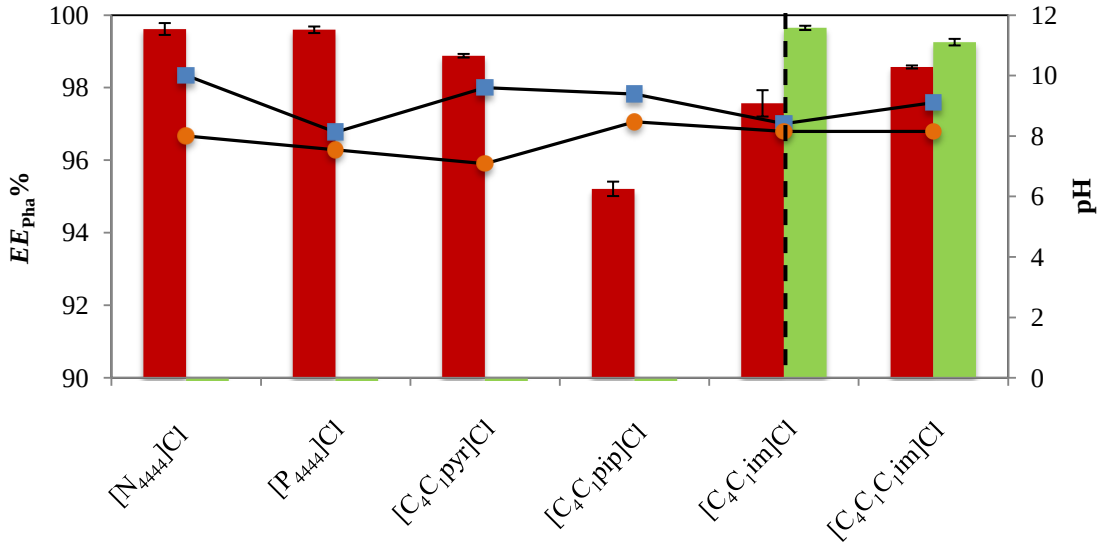


Figure 14. Percentage extraction efficiencies of CAF (▲) and CBZ (◆), EE_{Pha} %, in different ABS at 298 K, regarding the IL cation effect (on the left side of the dashed line) and the effect of the number of alkyl substitutions at the imidazolium cation (on the right side of the dashed line), and respective pH values of top (■) and bottom (●) phases. All ABS are composed of 20 wt% of $K_3[C_6H_5O_7]$ + 40 wt% of IL + 40 wt% of an aqueous phase.

CAF and CBZ were mainly extracted to the IL-rich phase in all ABS, with extraction efficiency values higher than 95%. The experimental TLs, that means the composition of the phases for a given mixture point, along with their respective lengths (TLL), are reported in Table 7. The pH values of both phases in each ABS and for the determined TLs were measured and are also shown. Since the pH values of each phase range between 6.70 and 10.01, and CAF and CBZ are mostly present in their neutral form – *cf.* speciation curves in Appendix D (Figures D 1 and D 2), electrostatic interactions occurring between the salt or IL ions and the charged solutes are not important and do not contribute to the partitioning behavior observed in these systems. The pH values of the initial $C_6H_5K_3O_7$ aqueous solutions used for the determination of the ABS presented a pH value around 9.3. As the $K_3[C_6H_5O_7]$ aqueous solution is not a buffer solution, the differences observed in the pH values of both phases are due to the concentration of the salt and the different ILs used as phase-forming components of ABS. However it should be noted that there is a more marked difference between IL- and salt-rich phases on the effect of the IL cation core due to the presence of lower differences between the concentration of IL in the IL-rich phase and concentration of salt in the salt-rich phase.

Table 7. Weight fraction compositions (wt %) for the TLs: IL-rich (IL) phase, the initial mixture (M) and salt-rich (salt) phase of the ternary systems composed of IL + $K_3[C_6H_5O_7]$ + H_2O at 298 K, and the respective concentrations of CAF, $[CAF]_{IL}$, and CBZ, $[CBZ]_{IL}$, in the IL-rich phase.

IL	Weight fraction composition (wt %)								TLL	$[CAF]_{IL}$ (mol·dm^{-3})	$[CBZ]_{IL}$ (mol·dm^{-3})
	$[IL]_{IL}$	$[Salt]_{IL}$	pH_{IL}	$[IL]_M$	$[Salt]_M$	$[IL]_{Salt}$	$[Salt]_{Salt}$	pH_{Salt}			
$[C_4C_1im][CF_3SO_3]$	84.50	1.19	8.10	40.54	18.96	1.02	34.93	8.31	90.04	0.0021	0.0052
$[C_4C_1im][N(CN)_2]$	85.81	0.62	6.70	39.81	20.12	0.31	36.87	9.00	92.87	0.0016	(---)[a]
$[C_4C_1im][SCN]$	72.91	1.16	8.24	39.57	19.94	0.00	42.23	8.00	83.68	0.0019	0.0073
$[C_4C_1im]Br$	62.53	2.93	8.49	39.22	20.15	2.18	48.65	8.40	74.73	0.0017	0.0068
	65.08	2.37	8.72	40.01	22.36	0.99	53.50	8.16	81.99	0.0016	(---)
	70.60	1.40	8.91	39.47	25.22	0.78	54.83	8.31	87.93	0.0010	(---)
	73.39	1.02	8.84	41.77	25.07	0.58	56.38	8.80	91.46	0.0010	(---)
	75.16	0.82	9.02	44.74	24.97	0.26	60.28	8.48	95.63	0.0011	(---)
$[C_4C_1im]Cl$	51.10	8.22	8.41	39.34	20.05	6.69	52.86	8.15	62.97	0.0014	0.0034
$[N_{4444}]Cl$	65.08	1.76	10.01	40.06	20.07	1.44	48.32	8.00	78.85	0.0014	(---)
$[P_{4444}]Cl$	66.65	3.74	8.13	39.54	20.02	2.06	42.53	7.54	75.34	0.0015	(---)
$[C_4C_1pyr]Cl$	54.77	4.83	9.60	40.01	19.97	4.71	56.18	7.08	71.71	0.0014	(---)
$[C_4C_1pip]Cl$	52.77	5.69	9.39	39.73	20.06	2.39	61.23	8.16	74.99	0.0018	(---)
$[C_4C_1C_1im]Cl$	77.89	0.23	9.11	40.20	25.27	18.76	39.13	7.25	71.58	(---)	(---)
	63.56	3.17	9.19	36.35	23.24	24.15	32.24	9.12	48.98	0.0011	0.0033

[a] (---) means that these values were not determined during experimental procedure

According to Figure 13, the effect of the IL anion was studied using several ILs containing the common $[C_4mim]^+$ cation, while combined with the following anions: $[CF_3SO_3]^-$, $[N(CN)_2]^-$, $[SCN]^-$, Br^-, and Cl^-. The partitioning of CBZ is similar for all ILs studied and practically a complete extraction was obtained for this compound with all systems investigated: $[C_4C_1im][CF_3SO_3] \approx [C_4C_1im][SCN] \approx [C_4C_1im]Br \approx [C_4C_1im]Cl$. This trend is in accordance with the higher extraction values obtained in a previous work with more hydrophobic molecules [48]. In fact, CBZ is a highly hydrophobic substance ($\log K_{ow}$ = 2.45 (Table 3)). On the other hand, the anions of imidazolium-based ILs present a more significant impact on the partitioning of CAF. The extraction efficiencies of CAF were lower than those observed for CBZ and decrease in the order: $[C_4C_1im][N(CN)_2] > [C_4C_1im][SCN] > [C_4C_1im]Cl > [C_4C_1im]Br > [C_4C_1im][CF_3SO_3]$. This trend is closely related with that reported in a previous work where ABS composed of ILs and K_3PO_4 were investigated [41], and where the partitioning of CAF follows the IL anion salting-in inducing capacity (Hofmeister-like series) [168]. In general, a more hydrated IL-rich phase, *i.e.*, IL with anions with higher β values leads to higher partition coefficients and extraction efficiencies. However, the ABS formed by the ILs $[C_4C_1im][SCN]$ and $[C_4C_1im][N(CN)_2]$ are outsiders to this trend, having achieved the highest values of EE_{CAF}%, namely 98.68 % and 99.18 %. According with Table 1, these two ILs present lower β values than $[C_4C_1im]Cl$ and $[C_4C_1im]Br$. The low

log(K_{ow}) value of CAF of -0.07 (Table 3) reflects its polarity characteristics and its low affinity for organic fluids. The polarity of CAF can be explained by its molecular structure, where two oxygen atoms are attached to a purine ring (Table 3). Indeed, water-CAF major interactions can be purposed as important since lower EE_{CAF}% were obtained with less hydrated IL-rich phases. The ABS formed by $[C_4C_1im][CF_3SO_3]$ led to the lowest EE_{CAF}% value of 96.6%. $[C_4C_1im][CF_3SO_3]$ is the most hydrophobic IL here investigated resulting in an IL-rich phase more concentrated in IL, and as can been seen by the TLs data shown in Table 7.

The effect of the IL cation core was investigated with ILs containing the common Cl^- anion, while combined with the following cations: $[N_{4444}]^+$, $[P_{4444}]^+$, $[C_4C_1pyr]^+$, $[C_4C_1pip]^+$ and $[C_4C_1im]^+$. As can be seen in Figure 14 (on the left side of the dashed line), EE_{CAF}% decreases in the order: $[N_{4444}]Cl$ > $[P_{4444}]Cl$ > $[C_4C_1pyr]Cl$ > $[C_4C_1im]Cl$ > $[C_4C_1pip]Cl$. For the differences observed in EE_{CAF}% values, the IL cation has a higher influence through the extraction of CAF, as already demonstrated in previous studies [41]. In general, $[N_{4444}]Cl$ and $[P_{4444}]Cl$ present similar and the highest EE_{CAF}% values. It seems that the imidazolium aromaticity has a low impact on the extraction of CAF. The low contribution of the aromatic ring of the imidazolium cation has already been suggested in the extraction of CAF using IL-based ABS [121].

The effect of the number of alkyl substitutions at the imidazolium cation core was also studied with the ILs $[C_4C_1im]Cl$ and $[C_4C_1C_1im]Cl$. According to Figure 14 (on the right side of dashed line), both ILs lead to similar extraction efficiencies values for CAF and CBZ. Therefore, the substitution of the most acidic hydrogen at C2 of $[C_4C_1im]Cl$ by an alkyl group, while reducing the ability of $[C_4C_1C_1im]Cl$ to form hydrogen-bonds with the solute, seems to have no major impact. This tendency clearly evidences that the hydrogen-bond interactions between the oxygen and nitrogen atoms of CAF and the oxygen atom of CBZ and the imidazolium cation do not play a significant role on the partitioning mechanism.

In general, the effect of the IL chemical structure seems to have a more significant impact on the partitioning of CAF than in CBZ. In fact, the extraction efficiencies values of CAF were lower and more divergent than those obtained for CBZ. This difference is due to the distinct properties that these two pharmaceuticals present, being CAF more polar than CBZ (Table 3), which gives to this last compound a higher affinity to the IL-rich phase. In order to achieve the complete extraction of CAF into the IL-rich phase, and taking into account the major goal of ABS as concentration techniques for the wastewaters monitoring, further studies were conducted, namely on the concentration of the phase-forming components of the ABS and concentration of CAF added (to infer if saturation is occurring in the IL-rich phase). For that purpose, the ABS composed of $[C_4C_1im]Br + K_3[C_6H_5O_7] + H_2O$ was chosen in order to optimize the EE_{CAF}% values. Since using an initial mixture point of 20 wt % of salt and 40 wt % of IL. this ABS presented an

EE_{CAF}% = 97.56%, by increasing the mixture compositions, through a TLL increase, it is expectable to enhance the extraction ability of the system. In the same way, by decreasing the initial concentration of CAF it is possible to verify the saturation (or not) of the IL-rich phase with the solute. The experimental data of the partitioning of CAF along the TLL are reported in Appendix (*cf.* Appendix D - Table D 3). The experimental TLLs studied to optimize the system $[C_4C_1im]Br + K_3[C_6H_5O_7] + H_2O$ are reported in Table 7 and illustrated in Figure 15.

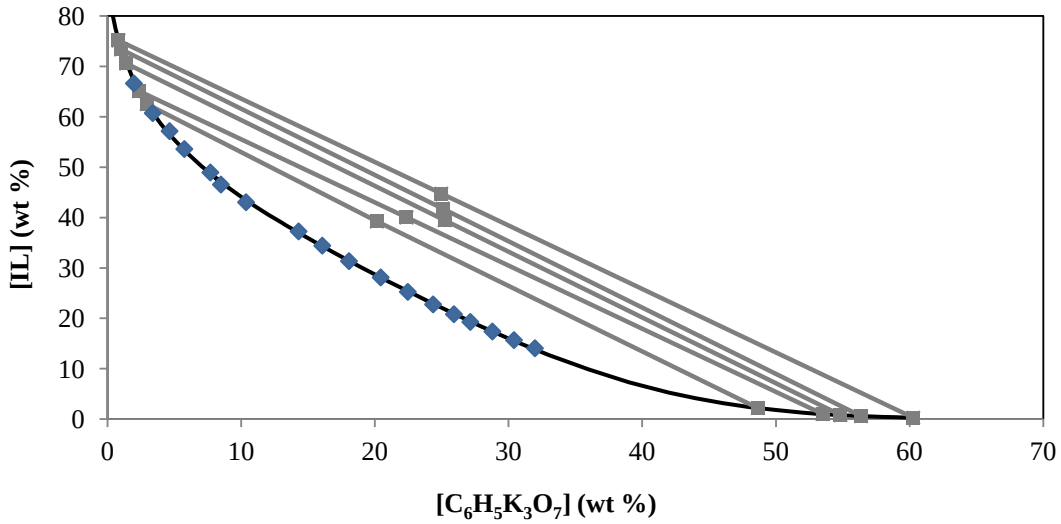


Figure 15. Phase diagram for the ternary system composed of $[C_4C_1im]Br + K_3[C_6H_5O_7] + H_2O$: binodal curve data (◆); TL data (▬); adjusted binodal data through Merchuk's equation [153] (–).

Figure 16 shows the results of the effect of the TLL and of the initial concentration of CAF through the optimization of the CAF extraction efficiency with $[C_4C_1im]Br$-based ABS, and respective standard deviations. It is possible to conclude that the TLL affects the extraction ability of IL-based ABS for CAF. In general, an increase in the IL concentration, *i.e.*, an increase in the TLL, leads to improved EE_{CAF}% values. A further increase in the TLL was not possible to implement for the extraction of CAF since the solid-phase equilibrium was verified thereinafter. On the other hand, to verify that the effect of the concentration of CAF in the system, initial concentrations of 0.912, 0.456, and 0.228 $g \cdot dm^{-3}$ were then studied in the same systems along with the TLL effect. In general, the increase of the concentration of CAF improves the extraction of CAF, meaning that the solubility characteristics of CAF in the IL-rich phase seem to be significant in the extraction efficiencies. Thus, at these concentrations, it seems that the saturation of the IL-rich phase is not reached. Taking into account the data shown in Figure 16, longer TLLs lead to better extraction profiles, regardless of the concentration of CAF added to the system. In summary, longer TLLs are preferable since the compositions of the two phases are significantly more

different. In this context, it is essential to better understand the hydrotropic mechanism afforded by ILs which improve the solubility of CAF.

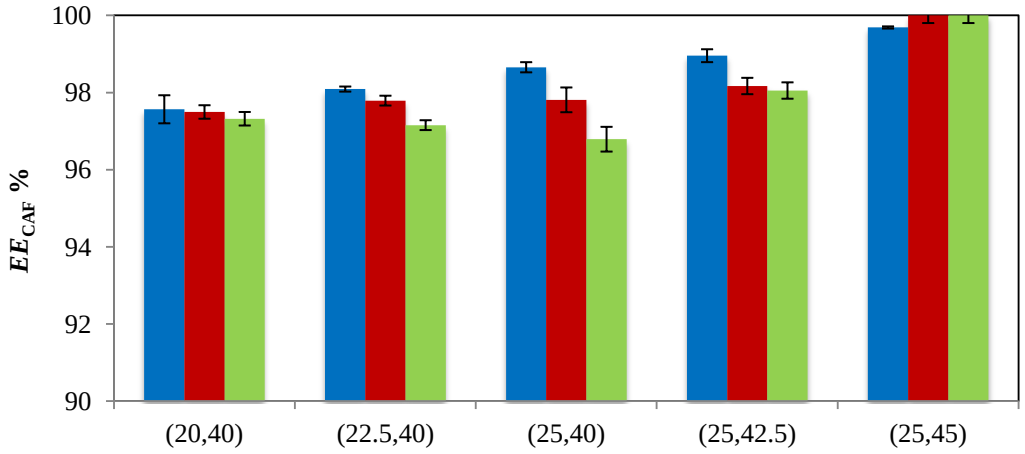


Figure 16. Percentage extraction efficiencies of CAF, EE_{CAF} %, at 298 K, at different initial concentrations (wt % salt, wt % IL) of the ABS $[C_4C_1im]Br + K_3[C_6H_5O_7] + H_2O$, and at different concentration of aqueous solution of CAF: 0.912 $g \cdot dm^{-3}$ (■), 0.456 $g \cdot dm^{-3}$ (●), and 0.228 $g \cdot dm^{-3}$ (◆).

In this section, the ability of ILs as hydrotropes was investigated by determining the solubility of CAF in several aqueous solutions. For that, different concentrations of ILs were compared with results obtained with common hydrotropes and salting-in inducing salts. Furthermore, the effects of the chemical structure and temperature on the hydrotropic capacity of the IL were also assessed. The obtained solubility of CAF in pure water was (23.09 ± 1.36) $g \cdot dm^{-3}$, which corresponds to (0.12 ± 0.01) $mol \cdot dm^{-3}$, at 303 K. This value is in accordance with the values reported in the literature at the same temperature [169-170].

Figure 17 depicts the solubility of CAF at 303 K in aqueous solutions of ILs. Aqueous solutions of ILs with concentrations up to 2.0 $mol_{Hyd} \cdot kg_{water}^{-1}$ were used. The experimental data of the solubility of CAF and CBZ are reported in Appendix (*cf.* Appendix C - Table C 1).

In general, ILs reveal a higher hydrotropic ability compared with the conventional hydrotropes and salting-in inducing salts, namely $Na_3[C_6H_5O_7]$, $K_3[C_6H_5O_7]$, Na[SCN], and $Na[C_7H_5O_2]$. The hydrotropy constants, K_{Hyd}, were also determined for each aqueous solution studied and are reported in Table 8 with the respective standard deviations. Higher K_{Hyd} values reflect a higher hydrotropic ability of the compound to enhance the solubility of CAF in aqueous medium. This capacity obeys the following order (where the ILs used in the formation of ABS for the extraction of CAF are highlighted in bold): $Na_3[C_6H_5O_7]$ < $K_3[C_6H_5O_7]$ < $[N_{1112}OH]Cl$ < **$[C_4C_1pip]Cl$** < **$[C_4C_1pyr]Cl$** < $[C_4C_1py]Cl$ < **$[C_4C_1C_1im]Cl$** < **$[C_4C_1im]Cl$** < **$[C_4C_1im]Br$** < **$[P_{4444}]Cl$** < $[C_4C_1im][CH_3SO_4]$ < **$[N_{4444}]Cl$** < Na[SCN] < **$[C_4C_1im][SCN]$** < **$[C_4C_1im][N(CN)_2]$** <

[C_4C_1im][CF_3SO_3] < Na[$C_7H_5O_2$] < [C_4C_1im][TOS] < [C_4C_1py][$N(CN)_2$]. It should be noted that the salts $Na_3[C_6H_5O_7]$ and $K_3[C_6H_5O_7]$, with the former used in the formation of the ABS investigated, display negative K_{Hyd} values, acting thus as salting-out agents (by decreasing the solubility of CAF in water). The $[CAF]_{IL}$ values shown in Table 7 closely correlate with the hydrotropic ability of the IL for CAF. Higher K_{Hyd} values correspond to higher $[CAF]_{IL}$ values. Higher TLLs in the corresponding systems also correspond to higher $[CAF]_{IL}$ values, since an increase in the IL concentration in the top phase leads to an increased concentration of CAF. This behavior is consistent with the solubility study depicted in Figure 17 where an increase in hydrotrope concentration leads to an increase in K_{Hyd}. Concluding, the TLL effect seems to be correlated with the hydrotropic ability of IL.

In the ABS studied above, the saturation of the phase was not verified. The K_{Hyd} values achieved for CAF are lower than those reported for vanillin and gallic acid [69], indicating that the hydrotropic effect is closely dependent on the solute properties.

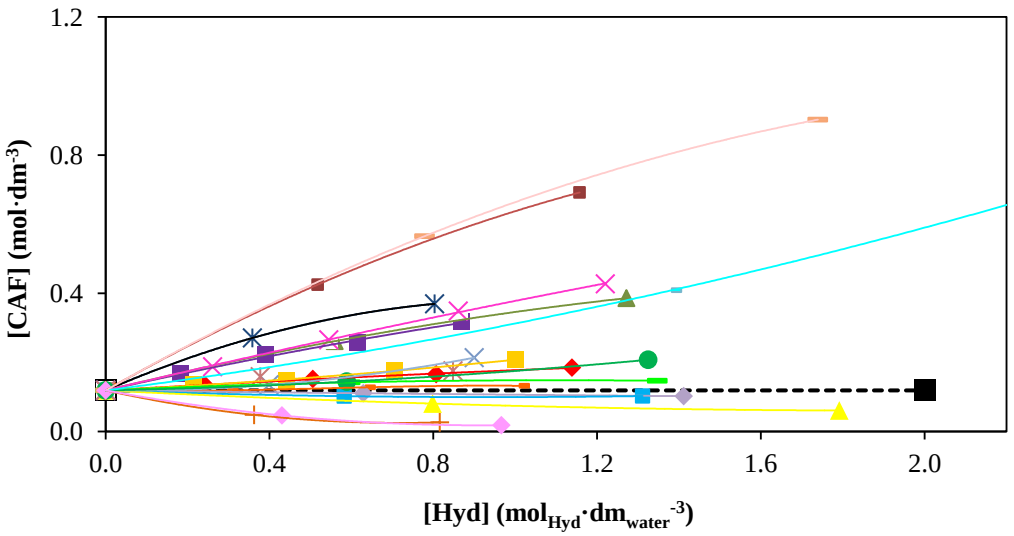


Figure 17. Influence of the hydrotropes (ionic liquids and conventional salts) concentration in the solubility of CAF in aqueous solutions at 303 K: pure water (---,■), **[C_4C_1im]Cl** (—), **[$C_4C_1C_1$im]Cl** (●), **[C_4C_1im][$N(CN)_2$]** (×), **[C_4C_1im][CF_3SO_3]** (■), [C_4C_1im][CH_3SO_4] (■), **[C_4C_1im]Br** (◆), [C_4C_1py][$N(CN)_2$] (■), [C_4C_1py]Cl (—), **[C_4C_1pyr]Cl** (◆), **[C_4C_1pip]Cl** (■), [C_4C_1im][TOS] (✳), **[C_4C_1im][SCN]** (▲), Na[$C_7H_5O_2$] (—), Na[SCN] (—), $Na_3[C_6H_5O_7]$ (◆), $K_3[C_6H_5O_7]$ (+), **[N_{4444}]Cl** (×), **[P_{4444}]Cl** (✳) and [N_{1112OH}]Cl (▲). Lines are guides for the eye.

Taking into account the results shown in Table 8, [C_4C_1im]Cl is one of the compounds with the lowest hydrotropic ability, while [C_4C_1im][$N(CN)_2$] is the IL with the highest hydrotropic

effect. These two ILs were further studied in order to understand their ability to solubilize CAF, *i.e.*, how much these two compounds can enhance the solubility of the biomolecule.

Figure 18 represents the influence of the IL concentration in the solubility of CAF, namely in aqueous solutions of $[C_4C_1im][N(CN)_2]$ and $[C_4C_1im]Cl$ – the respective data are reported in Appendix (*cf.* Appendix C - Table C 2). The solubility was studied in a complete concentration range, from pure water up to aqueous saturated solutions of these compounds at 303 K. S/S_0 represents how many times the solubility is enhanced, where S and S_0 represent the solubility ($mol \cdot kg^{-1}$) of each biomolecule in the aqueous solutions of ILs and in pure water, respectively.

As can be seen from Figure 18, the ability of aqueous solutions of ILs to solubilize the alkaloid is much higher than pure water. It is possible to enhance 4 times the solubility of CAF in aqueous solutions of ILs when compared with pure water. However, $[C_4C_1im]Cl$ has a less significant impact on solubilizing CAF when compared with $[C_4C_1im][N(CN)_2]$. In general, the solubility enhanced factors obtained for CAF are significantly lower than those reported for vanillin and gallic acid [69]. In this case, the values of maximum enhancement factors reported were 40-fold for vanillin, with $[C_4C_1im][N(CN)_2]$, and 20-fold for acid gallic with $[C_4C_1im]Cl$ [69]. Therefore, the hydrotropic effect is largely dependent on the nature of the target solute. CAF is a polar compound with higher affinity for water than for organic compounds, compared with the more water poor soluble vanillin and acid gallic, with $\log(K_{ow})$ values of 1.37 [122] and 0.70 [122], respectively. Thus, the increased addition of IL in aqueous solution is more significant for solubilizing these two biomolecules. In this line, it is expected a much higher hydrotropic phenomenon of ILs in respect to CBZ.

Table 8. K_{Hyd} values for the various hydrotropes studied in the solubility of CAF.

Hydrotrope	$K_{Hyd} \times 10^3$ ($g^{-1} \cdot mol$)
$Na_3[C_6H_5O_7]$	-0.8636
$K_3[C_6H_5O_7]$	-0.8485
$[N_{1112OH}]Cl$	-0.171
$[C_4C_1pip]Cl$	-0.0592
$[C_4C_1pyr]Cl$	-0.0469
$[C_4C_1py]Cl$	0.07
$[C_4C_1C_1im]Cl$	0.176
$[C_4C_1im]Cl$	0.1779
$[C_4C_1im]Br$	0.1779
$[P_{4444}]Cl$	0.2206
$[C_4C_1im][CH_3SO_4]$	0.2399
$[N_{4444}]Cl$	0.2586
Na[SCN]	0.3129
$[C_4C_1im][SCN]$	0.436
$[C_4C_1im][N(CN)_2]$	0.5091
$[C_4C_1im][CF_3SO_3]$	0.5371
$Na[C_7H_5O_2]$	0.5669
$[C_4C_1im][TOS]$	0.6766
$[C_4C_1py][N(CN)_2]$	0.7288

The influence of the temperature on the solubility of CAF in IL aqueous solutions was also evaluated and is depicted in Figure 19. Correspondent data are reported in Appendix (*cf.* Appendix C - Table C 3). A well correlated increase of solubility of the alkaloid is accompanied by an increase in the temperature. This trend is consistent with a previous work, where a similar study was realized for the solutes vanillin and acid gallic [69]. This suggests that the effects of the IL concentration and temperature can be considered in order to improve the target solute solubilization performance, in this case, up to 6 times with $[C_4C_1im]Cl$ in the temperature range studied.

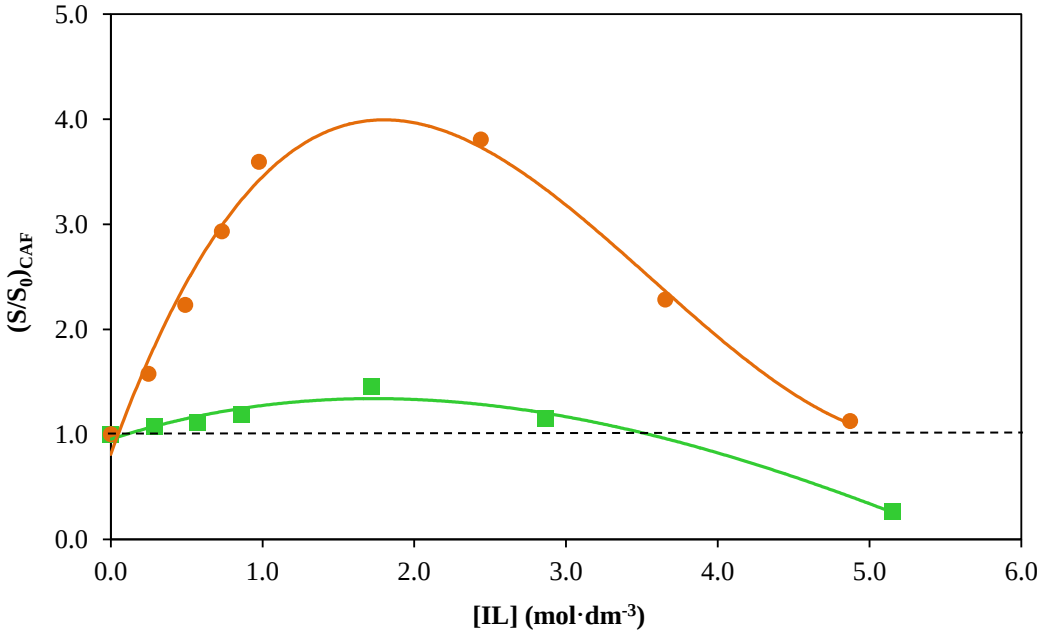


Figure 18. Influence of the ILs concentration in the solubility of CAF in aqueous solutions of (●) $[C_4C_1im][N(CN)_2]$ and (■) $[C_4C_1im]Cl$ at 303 K. Lines are guides for the eye.

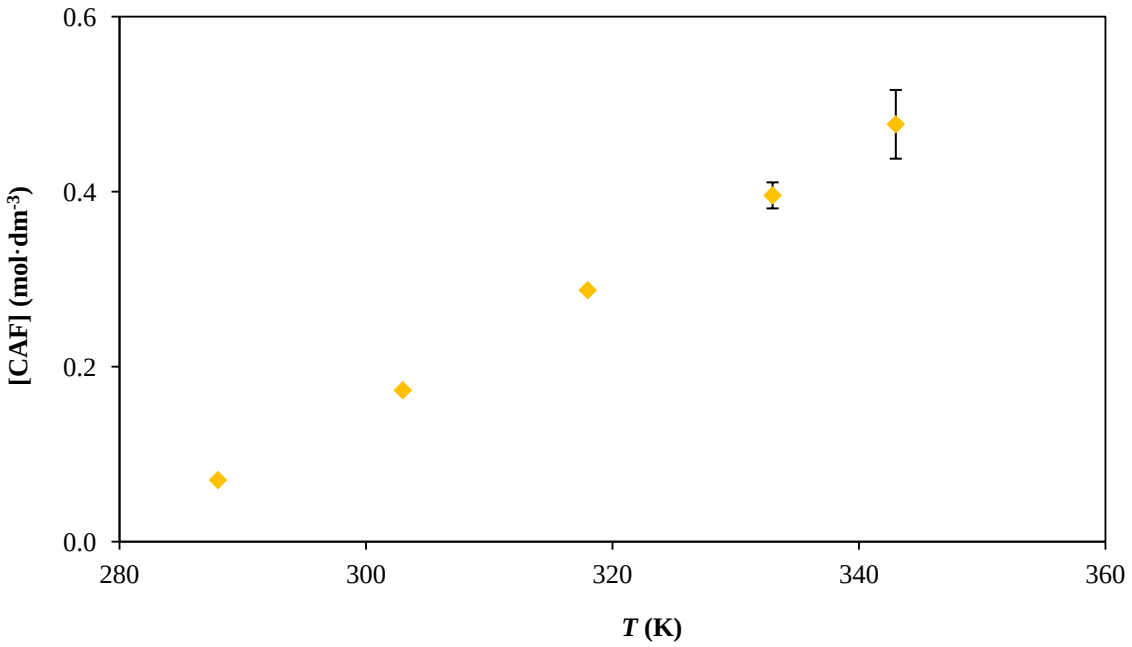


Figure 19. Temperature dependency for the solubility of CAF in aqueous solutions of $[C_4C_1im]Cl$ at 0.1731 mol·dm^{-3}.

Based on the previous results, the impact of the initial concentration of the solute (CAF) was evaluated. For that purpose, $[CAF]_{IL}$ and EE_{CAF} % were determined at different concentrations of CAF initially introduced into the system, and until the saturation of the solute has been reached,

in ABS composed of $[C_4C_1im]Cl$ + $K_3[C_6H_5O_7]$ + H_2O and $[C_4C_1im][N(CN)_2]$ + $K_3[C_6H_5O_7]$ + H_2O. The partitioning experiments were carried out at the same initial mixture composition of 20 wt % of salt and 40 wt % of IL. Figures 13 and 14 depict the EE_{CAF} % values obtained. Thus, for all the partitioning conditions, the pH values of the top and bottom phases, as well as the TL and TLL data, remain the same and are presented in Table 7.

As CAF is intended to be almost extracted to the IL-rich phase in all ABS, Figure 20 represents the influence of the initial concentration of CAF introduced in the system, in the solubility and extraction of CAF in the IL-rich phase. The experimental data of the partitioning of CAF are reported in Appendix (*cf.* Appendix D - Table D 4). It is possible to understand the hydrotropic ability of the IL to enhance the solubility of the solute in a ternary system. In general, for all the systems, an improved solubilization of CAF was observed, since $[CAF]_{IL}$ is higher than $[CAF]_{initial}$, while the high EE_{CAF} % values were maintained. As also verified above, the ability of $[C_4C_1im][N(CN)_2]$ to act as hydrotrope is significantly higher than $[C_4C_1im]Cl$, due to better capability on enhancing the solubilization of higher concentrations of CAF.

ILs show to have an enhanced hydrotropic ability by improving the solubilization of poorly water-soluble compounds. IL-based ABS are thus an alternative strategy, while it is possible to select the proper hydrotrope as well as to manipulate the TLL envisaging the optimization of the solubility of CAF in the IL-rich phase, and to better concentrate the pharmaceuticals that are found in trace amounts in wastewaters. Since the hydrotropic ability of ILs depend on the physico-chemical properties of the solute, this is outstandingly advantageous to design extraction and concentration routes, while adding substantial value to the technology under development in this work.

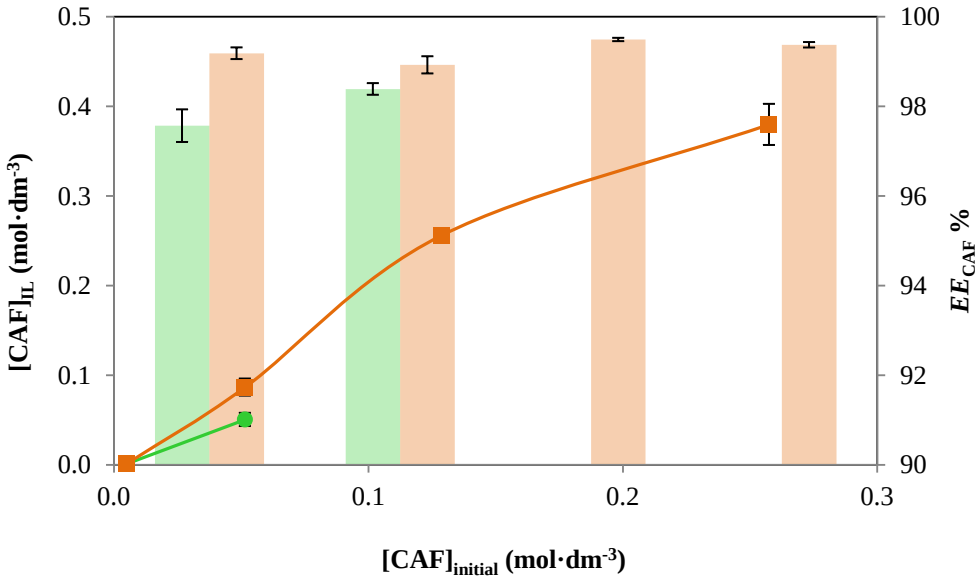


Figure 20. Influence of the initial concentration of CAF, introduced in the system, $[CAF]_{initial}$, in the solubility/extraction of CAF in the IL-rich phase of the ABS, composed of 20 wt% of 40 wt% of IL + $K_3[C_6H_5O_7]$ + 40 wt% of H_2O. $[CAF]_{IL}$ represents the concentration of CAF in the IL-rich phase for the ILs: (■) $[C_4C_1im][N(CN)_2]$ and (●) $[C_4C_1im]Cl$. EE_{CAF} % represents the percentage extraction efficiencies for the ILs: (◆) $[C_4C_1im][N(CN)_2]$ and (▲) $[C_4C_1im]Cl$. All the determinations were carried out at 298 K.

3.5. Conclusion

ILs are a novel class of catanionic hydrotropes. In this work, it was investigated the hydrotropic mechanism of ILs applied to the alkaloid caffeine. In general, the higher the IL concentration, the higher is the ability of the IL to act as hydrotrope (although moving through a maximum), which is improved by an increase in temperature On the other hand, the salt $K_3[C_6H_5O_7]$ used in the formulation of ABS acts a salting-out species. In summary, the TLL and K_{Hyd} of the IL allow a proper design of the system that leads to a better solubilization of the solute in the IL-rich phase, and thus to better extraction and concentration approaches.

4

Final remarks

4.1. Conclusions

Due to the innumerous advantageous properties that ILs offer, IL-based ABS have been studied in the last years as alternative one-step extraction and concentration technologies for a wide plethora of compounds. In this thesis, the development of an analytical preconcentration technology for human pollution tracers from wastewater streams was investigated. In this context, this work was divided into two main sections: (i) the potential applicability of IL-based ABS to extract and concentrate pharmaceutical tracers from wastewaters; and (ii) the hydrotropic ability of ILs which enhance the solubility of poor-water soluble compounds in aqueous media. In the first part, by tuning the mixture point composition, it was possible to concentrate CAF and CBZ in the IL-rich phase, overcoming one of the major limitations in the analysis and monitoring of wastewaters, while allowing an evaluation of their environmental impact. However, further studies are still in need and are ongoing aiming at reaching higher concentration factors. Even so, these systems are straightforwardly envisaged for the one-step extraction and concentration of a large plethora of components currently present in wastewaters while overcoming one of the major challenges in the analytical field. In the second part, ILs as a novel class of catanionic hydrotropes, were investigated regarding their hydrotropic effect which could be reflected on the extraction and concentration ability of IL-based ABS. In summary, the TLL and K_{Hyd} of the IL allow a proper design of the system that leads to an enhanced solubilization of the solute in the IL-rich phase, allowing thus better extraction and concentration approaches.

IL-based ABS are a promising and versatile alternative since they offer the opportunity to extract and concentrate (with a tailored ability) a variety of solutes present in trace amounts, in a single step, allowing therefore their proper identification and quantification in environmental matrices, such as surface waters, where a proper concentration of the compounds still is a main challenge.

4.2. Future work

In the future, the correct quantification of the TL applied in the concentration process is needed in order to achieve the high concentration factors initially established in this work. As demonstrated in previous studies, IL-based ABS are able to concentrate solutes from aqueous media up to 100- [42] and 1000-fold [48]. Therefore, only by attaining these values, this work would be comparable with the other processes already reported in the literature [76, 149-150, 155].

Due to the high versatility and analyte-specificity associated to IL-based ABS, it would be interesting to continue the study of finding novel ABS. composed of ILs and other organic salts. and to study their potential applicability in the concentration of other micropollutants currently difficult to monitor in wastewaters.

Finally, regarding the proposed technique, it would be of high value to test these systems with real water matrices, with different organic matter and ionic strength, aiming at providing their "real" applicability so that they could become a widespread applicable preconcentration technique in the near future.

5

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List of publications

Co-author in:

1. Teresa B. V. Dinis, Helena Passos, Diana L. D. Lima, Valdemar I. Esteves, João A. P. Coutinho and Mara G. Freire, *One-step extraction and concentration of estrogens for an adequate monitoring of wastewater using ionic-liquid-based aqueous biphasic systems*, Green Chemistry, 2015, 17, 2570-2579.
2. Andreia Luís, Teresa B. V. Dinis, Helena Passos, Mohamed Taha and Mara G. Freire, *Good's buffers as novel phase-forming components of ionic-liquid-based aqueous biphasic systems*, Biochemical Engineering Journal, 2015, 101, 142-149.

Appendix A

NMR spectra

A.1. NMR spectra

The ^{1}H NMR spectra of the pure potassium citrate used, from two different suppliers, were acquired in order to verify the presence of contaminants and are shown in Figures A 1 and A 2.

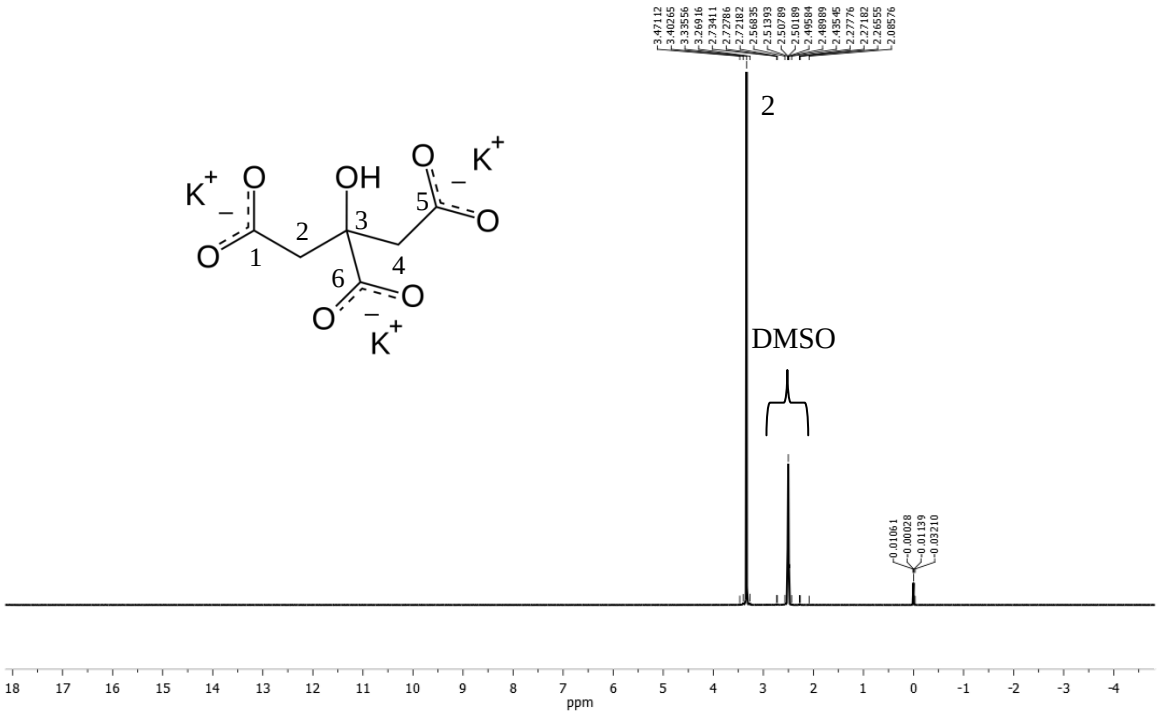

Figure A 1. ^{1}H NMR spectrum of the potassium citrate ($K_3[C_6H_5O_7]$), from Sigma-Aldrich, in DMSO.

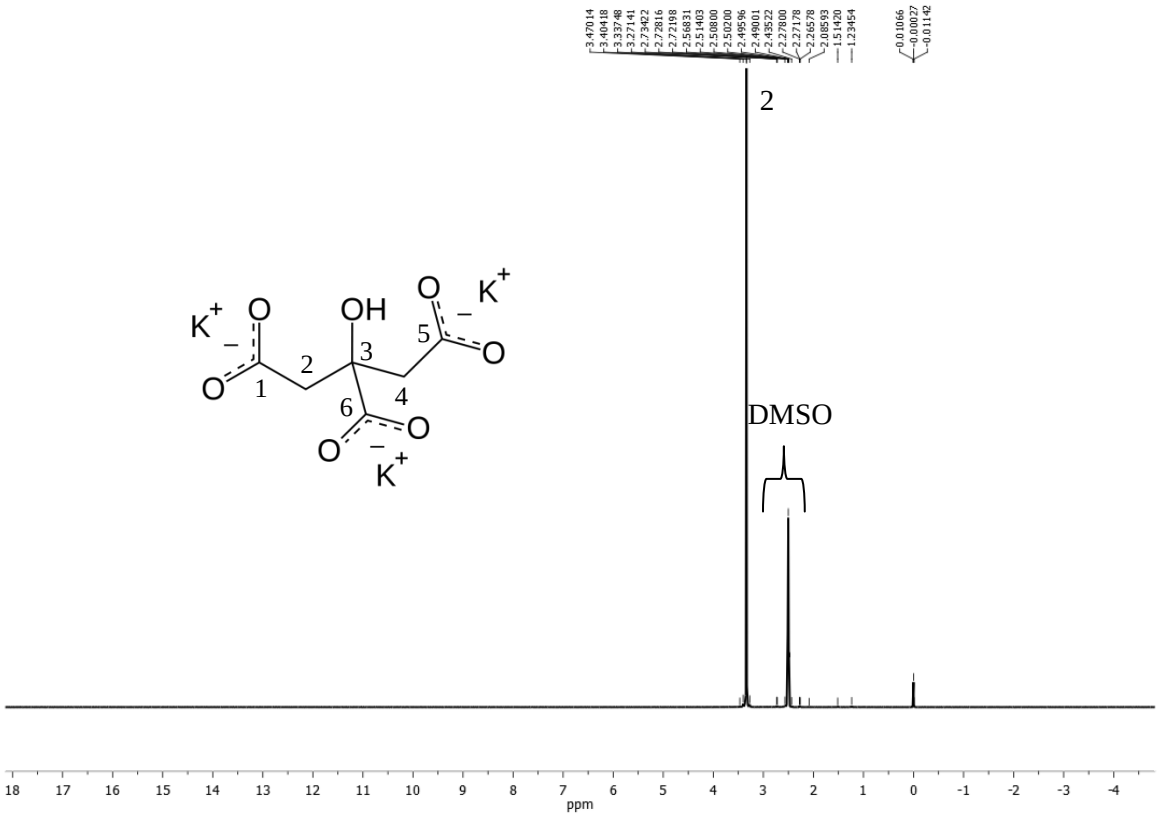

Figure A 2. ^{1}H NMR spectrum of the potassium citrate ($K_3[C_6H_5O_7]$), from GPR, in DMSO.

Appendix B

Calibration curves

B.1. UV-Vis calibration curves for the pharmaceuticals

Figures B 1 and B 2 depict the calibration curves (absorbance *vs.* concentration) of CAF and CBZ, using a Synergy|HT Microplate Reader, from Biotek, at 272 nm and 285 nm, respectively. Figures B 3 depicts the calibration curve (absorbance *vs.* concentration) using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at 274 nm.

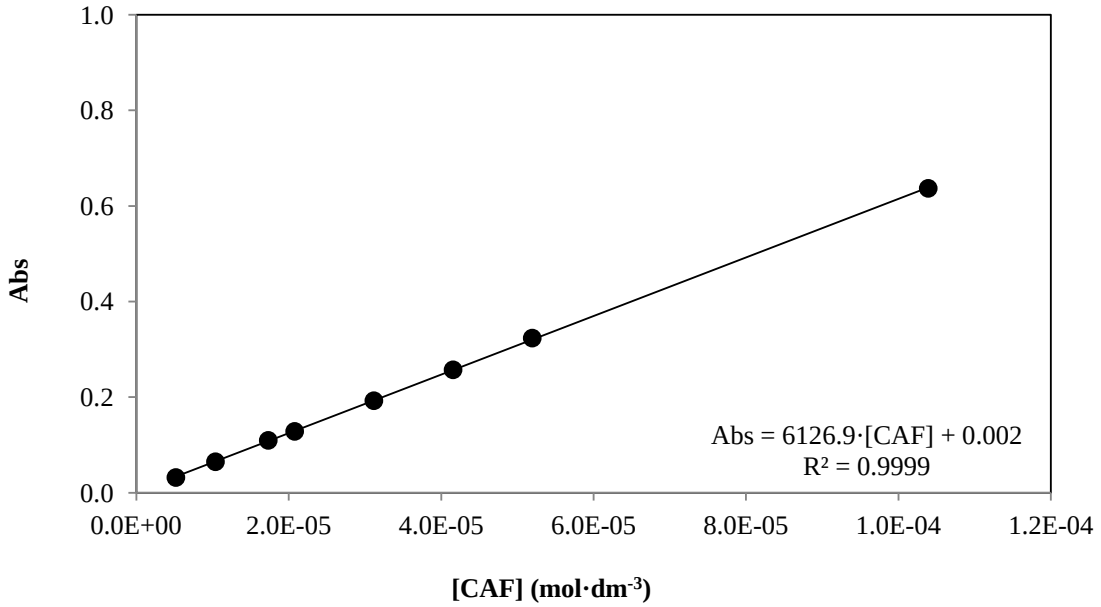

Figure B 1. Calibration curve for CAF, for Synergy|HT Microplate Reader, from Biotek, at λ = 272 nm.

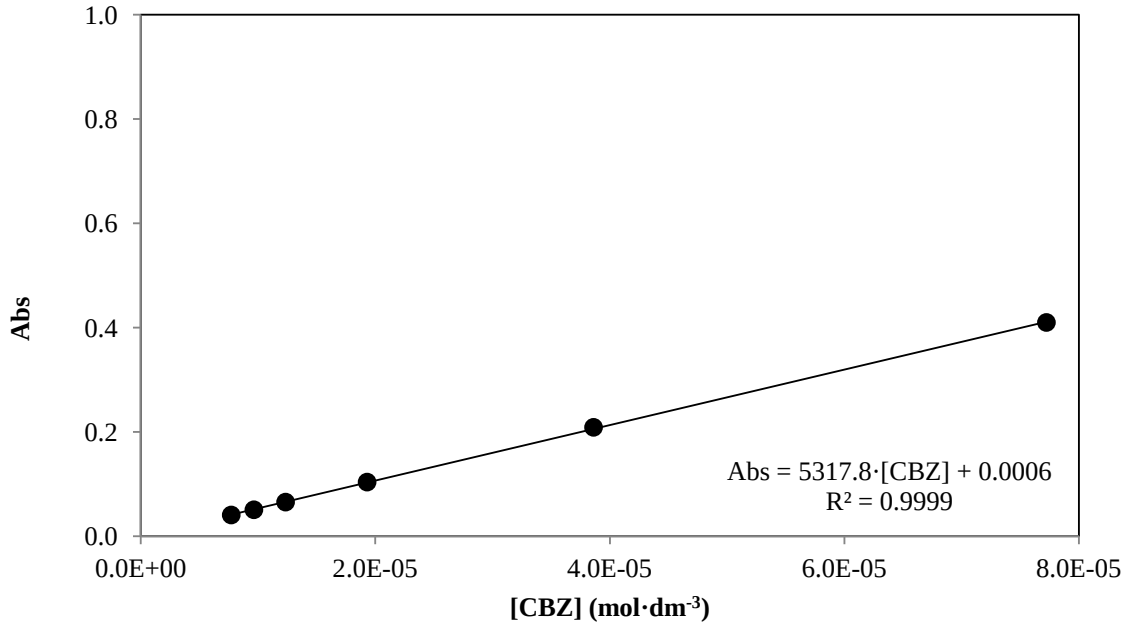

Figure B 2. Calibration curve for CBZ, for Synergy|HT Microplate Reader, from Biotek, at λ = 285 nm.

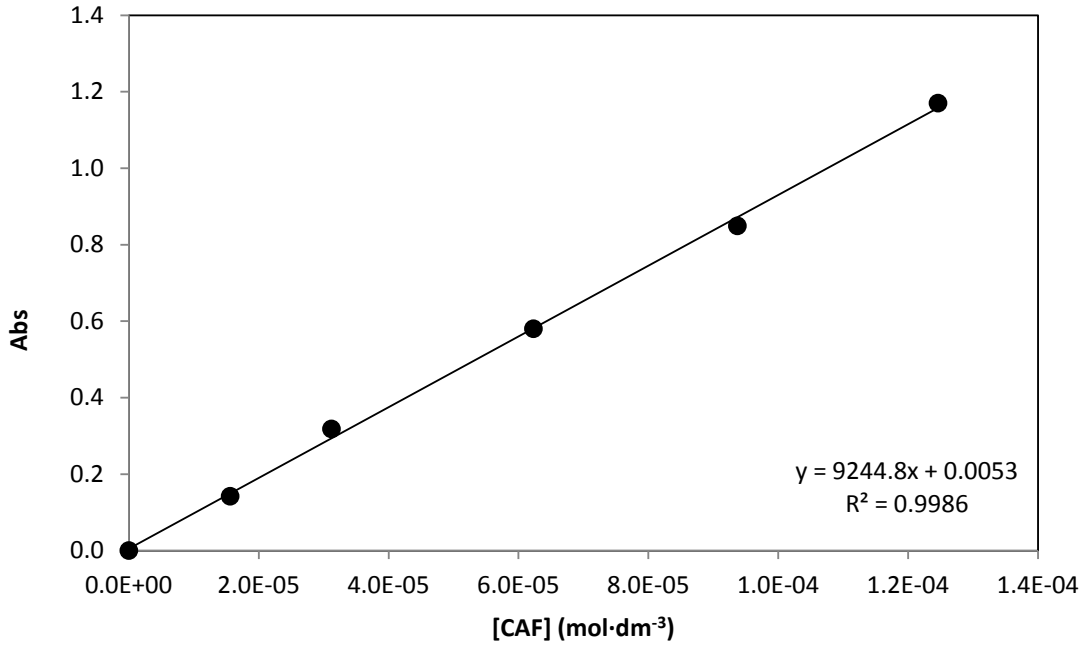

Figure B 3. Calibration curve for CAF, for UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at λ = 274 nm.

Appendix C

Solubility of caffeine

C.1. Experimental data for the solubility of caffeine

Table C 1 reports the concentration values of each hydrotrope studied, [Hyd], and the concentration values of CAF, [CAF], in aqueous solution of the corresponding hydrotrope. Table C 2 reports data relative to the influence of the ILs concentration on the solubility of CAF, $(S/S_0)_{CAF}$, in aqueous solutions of the corresponding ILs. Table C 3 reports the data corresponding to the temperature effect, T, on the solubility of CAF in aqueous solutions of the corresponding IL.

Table C 1. Experimental solubility of caffeine in aqueous solutions of hydrotropes, at 303 K.

Hydrotrope	[Hyd] (mol·dm^{-3})	[CAF] (mol·dm^{-3})	[CAF] (g·dm^{-3})	Hydrotrope	[Hyd] (mol·dm^{-3})	[CAF] (mol·dm^{-3})	[CAF] (g·dm^{-3})
H_2O	0.00	0.00	23.09± 1.36	$[C_4C_1im]Cl$	1.01	0.14	27.49 ± 0.91
$[C_4C_1im]Br$	0.24	0.14	26.36 ± 0.80		1.43	0.14	26.28 ± 1.20
	0.51	0.15	29.52 ± 2.22		2.45	0.17	33.62 ± 0.12
	0.81	0.17	32.20 ± 0.27		5.73	0.14	26.65 ± 0.53
	1.14	0.18	35.88 ± 0.14		51.53	0.03	6.03 ± 0.61
$[C_4C_1im][CF_3SO_3]$	0.18	0.17	32.40 ± 0.44	$[C_4C_1C_1]imCl$	0.59	0.14	28.01 ± 0.57
	0.39	0.22	43.06 ± 1.65		1.32	0.21	40.31 ± 1.13
	0.62	0.26	49.89 ± 1.17	$[C_4C_1py]Cl$	0.60	0.14	27.63 ± 2.09
	0.87	0.32	61.53 ± 1.99		1.35	0.15	28.51 ± 0.27
$[C_4C_1im][CH_3SO_4]$	0.22	0.14	26.40 ± 0.25	$[C_4C_1pyrr]Cl$	0.63	0.11	22.48 ± 2.53
	0.44	0.15	28.53 ± 1.89		1.41	0.10	19.86 ± 2.53
	0.70	0.18	34.48 ± 1.23	$[C_4C_1pip]Cl$	0.58	0.10	19.86 ± 4.27
	1.00	0.21	40.27 ± 2.57		1.31	0.10	19.93 ± 0.97
$[C_4C_1im][TOS]$	0.36	0.42	52.56 ± 1.36	$[N_{1112OH}]Cl$	0.80	0.08	15.68 ± 0.40

	0.80	0.69	71.74 ± 0.88		1.79	0.06	11.78 ± 0.96
$[C_4C_1py][N(CN)_2]$	0.52	0.42	82.53 ± 2.05	$[N_{4444}]Cl$	0.40	0.13	26.18 ± 0.62
	1.16	0.69	134.36 ± 1.65		0.90	0.21	41.47 ± 1.42
$[C_4C_1im][SCN]$	0.56	0.26	50.76 ± 3.38	$[P_{4444}]Cl$	0.38	0.16	30.74 ± 1.87
	1.27	0.39	74.94 ± 5.16		0.85	0.18	34.07 ± 3.97
$[C_4C_1im][N(CN)_2]$	0.26	0.19	36.37 ± 0.88	$Na_3[C_6H_5O_7]$	0.43	0.05	9.01 ± 2.07
	0.54	0.27	51.52 ± 2.02		0.97	0.02	3.51 ± 0.36
	0.86	0.35	67.69 ± 0.69	$Na[C_7H_5O_2]$	0.78	0.57	109.78 ± 1.27
	1.22	0.43	82.96 ± 1.17		1.74	0.90	175.28 ± 1.49
	4.88	0.45	87.87 ± 2.65	Na[SCN]	1.38	0.41	79.39 ± 2.60
	14.61	0.27	52.69 ± 0.73		3.08	0.99	191.37 ± 6.95
	48713.83	0.13	25.99 ± 2.67	$K_3[C_6H_5O_7]$	0.36	0.05	9.33 ± 0.31
$[C_4C_1im]Cl$	0.30	0.13	24.81 ± 1.36		0.82	0.03	5.12 ± 0.03
	0.63	0.13	25.67 ± 1.20				

Table C 2. Data relative to the influence of the ILs concentration in the solubility, S/S_0, of CAF in aqueous solutions of $[C_4C_1im][N(CN)_2]$ and $[C_4C_1im]Cl$.

Hydrotrope	**[Hyd] (mol·dm^{-3})**	**$(S/S_0)_{CAF}$**
$[C_4C_1im][N(CN)_2]$	0.00	1.00
	0.25	1.58
	0.49	2.23
	0.73	2.93
	0.98	3.59
	2.44	3.81
	3.65	2.28
	4.87	1.13
$[C_4C_1im]Cl$	0.00	1.00
	0.28	1.07
	0.57	1.11
	0.86	1.19
	1.72	1.46
	2.86	1.15
	5.15	0.26

Table C 3. Data relative to the temperature, T, dependency of the concentration of CAF (solubility) in aqueous solutions of $[C_4C_1im]Cl$ at 0.1731 mol·kg^{-1} and respective standard deviations.

T (K)	**[CAF] ± σ (mol·dm^{-3})**
288	0.070 ± 0.001
303	0.173 ± 0.001
318	0.287 ± 0.002
333	0.396 ± 0.015
343	0.477 ± 0.039

Appendix D

Partitioning of pharmaceuticals

D.1. Speciation data of the pharmaceuticals

In Figures D 1 and D 2 are presented the effect of the pH in the concentration of different species that can exist in aqueous solutions of CAF and CBZ, respectively.

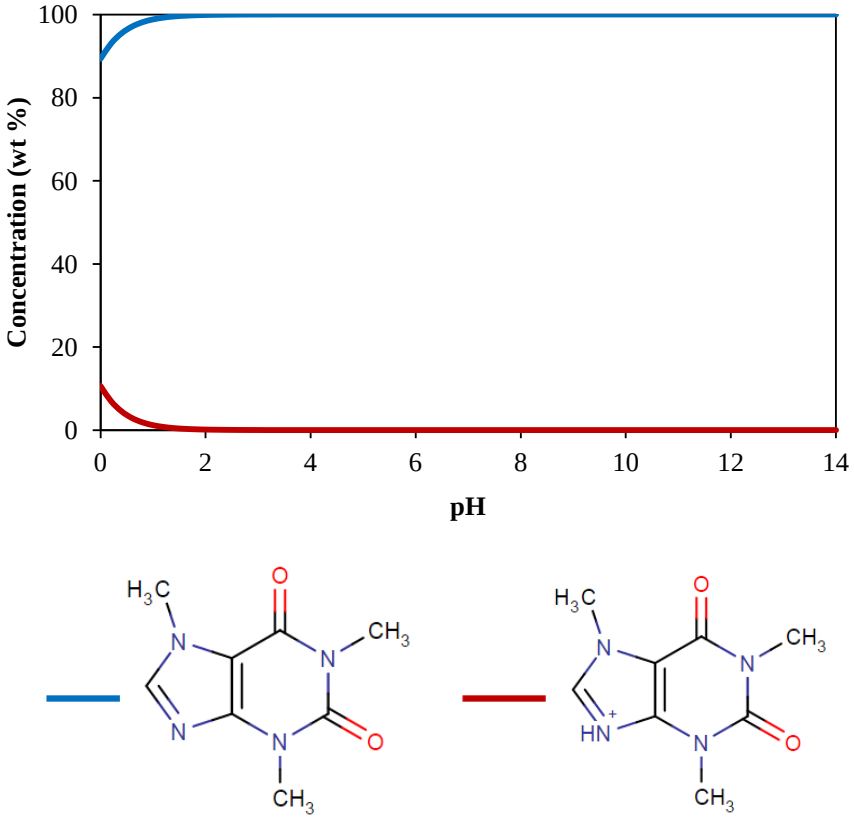

Figure D 1. Speciation diagram of CAF [1].

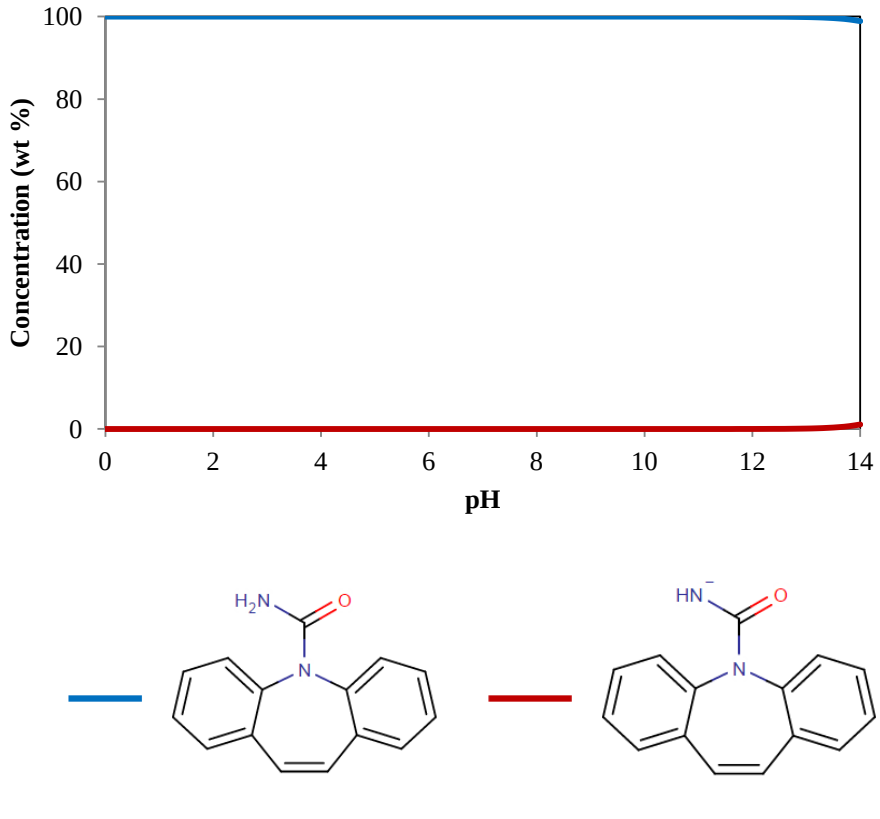

Figure D 2. Speciation diagram of CBZ [1].

D.2. Extraction data for the pharmaceuticals

Tables D 1 and D 2 present the concentration factors, composition of the phases and the recovery of caffeine determined by HPLC – UV-Vis.

Tables D 3 and D 4 present the initial mixture compositions, and respective TLLs, and the partition coefficients of the different pharmaceuticals.

Table D 1. Mixture compositions, weight percentages of the IL- and salt-rich phases obtained by the lever-arm rule and respective estimated concentration factor, CF, for the $[N_{4444}]Cl + K_3[C_6H_5O_7] + H_2O$ ABS. The TL used here was analytically quantified (TLL ≈ 79). The parameters of the TL equation are: slope = -1.28 and y-intercept = 64.96.

Weight fraction composition (wt %)					CF
$[IL]_M$	$[salt]_M$	$[water]_M$	[IL-rich phase]	[salt-rich phase]	
62.20	2.16	35.65	100.00	0.00	0.36
48.71	12.70	38.59	77.90	22.10	0.50
26.58	30.00	43.42	41.64	58.36	1.04
6.95	45.34	47.71	9.49	90.51	5.02
4.10	47.57	48.33	4.82	95.18	10.03
1.45	49.64	48.91	0.48	99.52	**101.46**
1.18	49.85	48.97	0.05	99.95	**1061.99**
1.16	49.87	48.97	0.00	100.00	N.D.[a]

[a] N.D. not determined

Table D 2. Chromatographic data for the quantification of CAF and CBZ, including the retention time, $t_{retention}$, peak area, A_{peak}, and percentage recoveries with the respective standard deviation values.

System	Pharmaceutical	$t_{retention}$ (min)	A_{peak}	% Recovery ± σ	CF
Single extraction	CAF	3.74	48973	98 ± (---)[a]	45.88
Single extraction	CBZ	11.33	29847	125 ± (---)	47.39
Simultaneous extraction	CAF	3.6915	88955	91 ± 15	48.97
	CBZ	8.7845	33037	104 ± 5	
Blank control	(---)	(---)	(---)	(---)	53.39

[a] (---) these values were not determined during the experimental procedure

Table D 3. Extraction efficiencies of CAF (EE_{CAF}%) in IL + $K_3[C_6H_5O_7]$ + H_2O ABS, and respective standard deviations, mixture compositions, and TLL values.

IL	Weight fraction composition (wt %)		TLL	EE_{CAF} % ± σ			EE_{CBZ} % ± σ
	$[IL]_M$	$[Salt]_M$		0.912 g·dm^{-3} CAF	0.456 g·dm^{-3} CAF	0.228 g·dm^{-3} CAF	
$[C_4C_1im][CF_3SO_3]$	40.54	18.96	90.04	96.57 ± 0.03	(---)[a]	(---)	99.92 ± 0.02
$[C_4C_1im][N(CN)_2]$	39.81	20.12	92.87	99.18 ± 0.13	(---)	(---)	(---)
$[C_4C_1im][SCN]$	39.57	19.94	83.68	98.68 ± 0.04	(---)	(---)	99.97 ± 0.01
$[C_4C_1im]Br$	39.22	20.15	74.73	97.56 ± 0.36	97.49 ± 0.04	97.32 ± 0.18	99.83 ± 0.05
	40.01	22.36	81.99	98.09 ± 0.07	97.79 ± 0.17	97.15 ± 0.13	(---)
	39.47	25.22	87.93	98.65 ± 0.13	97.81 ± 0.18	96.79 ± 0.32	(---)
	41.77	25.07	91.46	98.95 ± 0.17	98.17 ± 0.25	98.05 ± 0.21	(---)
	44.74	24.97	95.63	99.68 ± 0.03	100.00 ± 0.20	100.00 ± 0.20	(---)
$[C_4C_1im]Cl$	39.34	20.05	62.97	98.5 ± 0.04	(---)	(---)	99.64 ± 0.06
$[N_{4444}]Cl$	40.06	20.07	78.85	99.61 ± 0.16	(---)	(---)	(---)
$[P_{4444}]Cl$	39.54	20.02	75.34	99.59 ± 0.09	(---)	(---)	(---)
$[C_4C_1pyr]Cl$	40.01	19.97	71.71	98.88 ± 0.05	(---)	(---)	(---)
$[C_4C_1pip]Cl$	39.73	20.06	74.99	95.21 ± 0.20	(---)	(---)	(---)
$[C_4C_1C_1im]Cl$	36.35	23.24	48.98	98.57 ± 0.36	(---)	(---)	99.25 ± 0.09

[a] (---) values not determined during the experimental procedure

Table D 4. Extraction efficiencies of CAF (EE_{CAF}%) in IL + $K_3[C_6H_5O_7]$ + H_2O ABS, and respective standard deviations, mixture compositions, and concentration values of CAF initially added in the ABS ($[CAF]_{ABS}$) and in the IL-rich phase ($[CAF]_{IL}$).

IL	Weight fraction composition (wt %)		$[CAF]_{ABS}$ (mol·dm^{-3})	$[CAF]_{IL}$ (mol·dm^{-3})	EE_{CAF} % ± σ
	$[IL]_M$	$[Salt]_M$			
$[C_4C_1im][N(CN)_2]$	39.81	20.12	0.0051	0.0015	99.18 ± 0.13
			0.0515	0.0865	98.92 ± 0.19
			0.1287	0.2559	99.49 ± 0.04
			0.2575	0.3796	99.37 ± 0.06
$[C_4C_1im]Cl$	39.34	20.05	0.0051	0.0014	97.56 ± 0.36
			0.0515	0.0504	98.38 ± 0.13