Separation and Purification Technology 122 (2014) 315–322

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Recovery of paracetamol from pharmaceutical wastes

Francisca A. e Silva, Tânia Sintra, Sónia P.M. Ventura, João A.P. Coutinho *

Departamento de Química, CICECO, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form 11 September 2013
Accepted 14 November 2013
Available online 23 November 2013

Keywords:
Pharmaceutical wastes
Active compounds
Paracetamol
Aqueous biphasic systems
Ionic liquid
Extraction efficiency

ABSTRACT

The concern about the impact of active pharmaceutical compounds on the environment has been growing for decades. This work looks at pharmaceutical wastes as a source of valuable active compounds proposing the use of novel aqueous biphasic systems (ABS) composed of quaternary ammonium compounds and different salts to the extraction of active substances from pharmaceutical wastes. The phase diagrams of ABS of six quaternary ammonium halides ($[N_{2,2,2,2}]Br$, $[N_{2,2,2,2}]Cl$, $[N_{3,3,3,3}]Br$, $[N_{3,3,3,3}]Cl$, $[N_{4,4,4,4}]Br$ and $[N_{4,4,4,4}]Cl$) and three different salts (potassium citrate buffer, potassium carbonate and potassium phosphate buffer) were established at 298 (±1) K. These systems allow the investigation of the influence of the ammonium structure, the salting-out agent, and the pH of the aqueous medium. They were then applied to the extraction of paracetamol that displays an extensive partition towards the ammonium-rich phase. All the studied systems, employing both the model compound and real matrix reveal a great aptitude to recover paracetamol from pharmaceutical wastes, presenting extraction efficiencies ranging from around 80% up to 100%.

1. Introduction

The pharmaceutical industry in Europe has been growing and with it, the consumption of drugs and medicines. This increasing consumption is nowadays responsible for a big society problem due to the high level of waste produced, and its crescent environmental impact [1–4]. This demand can only be solved by a better control and processing of the pharmaceutical wastes. In Portugal, the pharmaceutical waste management company VALORMED collected, in 2011, 853.8 tons of pharmaceutical wastes (packaging and pharmaceutical drugs), of which 0.638 tons were composite packaging (blister) [5]. The pharmaceutical waste is sent to sorting centers, where it is directed to recycling centers or energy recovery [5]. This amount of pharmaceutical waste results from drugs past of expiration date that no longer can be used for human therapy or drugs that are not used due to the mismatch between the package content and the treatment needs [4,6]. The expiration date of drugs is assessed by the application of the knowledge acquired through stability studies [7]. By definition, during this period, the physical, chemical, microbiological, galenic, therapeutic and toxicological properties remain as unchanged within acceptable and well-defined limits [7]. However, even past the expiration date, drugs still retain their active substances, notwithstanding in quantity/quality that do not assure the therapeutic effect [5]. In fact, the intensive use and disposal of those substances results in their occurrence in natural environments such as water channels and soils [2]. Antibiotics, cancer and endocrine system drugs, anthelmintics and antidepressants are some examples of the pharmaceutical groups with more environmental persistence [8].

In this context, green and sustainable pharmacy appears as an emerging and essential topic [3,4]. The concepts brought by the fields of sustainability and green chemistry highlight the relevance of considering each stage comprising the life cycle of a compound to identify opportunities to manage and reduce risk potential [9]. Accordingly, the last chapter of drugs life represents an excellent opportunity to drug recovery, adding value to those active pharmaceutical substances. So, it is of great interest the development of novel strategies that combine the extraction and purification of drugs from pharmaceutical wastes. The high environmental and economic potential lies on the possibility to prevent the entrance of these compounds in the environment and to resell the recovered compounds for further purification and use.

Aqueous biphasic systems (ABS) are a well-known liquid–liquid extraction technique, formed by two aqueous phases of different compounds that are immiscible above given concentrations. These are highly versatile extraction systems, since a great variety of compounds can be used in their preparation [10–20]. IL-based ABS have been the subject of vast attention and widely used for the extraction of several biomolecules [21] such as alkaloids [22], antibiotics [23], amino acids [24] and enzymes [25,26], showing high effectiveness, yields and selectivities. They allow the concentration of biomolecules present in very low amounts in complex matrices in one aqueous phase [22,27] as also has been shown for the extraction of antibiotics from fermented broth [28].

* Corresponding author. Tel.: +351 234 401507; fax: +351 234 370084.
E-mail address: jcoutinho@ua.pt (J.A.P. Coutinho).

1383-5866/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.seppur.2013.11.018

In this work, ammonium-based ILs, reported as being cost-effective ILs [29,30] of low toxicity, and as promising biocompatible solvents to stabilize biomolecules [31], are investigated to extract active substances from pharmaceutical wastes. The paracetamol (Fig. 1) is here used as a model drug to evaluate the extraction capabilities of the IL-based ABS. Six short-chain quaternary ammonium compounds and three salts are used to prepare the ABS, aiming at investigating the influence of the alkyl chain of the cation and the anion effect. A systematic analysis of the aptitude of ammonium compounds to form ABS is performed by changing the salt, the ammonium type and the medium pH. These systems are further applied in the extraction of paracetamol from both model and real pharmaceutical matrices. The model matrix is adopted to develop an optimization study aiming at evaluating the operating conditions of the ABS (ammonium structure, salt agent, tie-line length – TLL – and pH of the aqueous medium), in order to achieve the highest extraction efficiencies (EE_{PC}, %). Subsequently, representative conditions are employed with the objective to demonstrate the possibility to apply these extraction systems to pharmaceutical matrices.

2. Materials and methods

2.1. Materials

The paracetamol, also known as N-(4-hydroxyphenyl)acetamide or acetaminophen, had a quoted purity ⩾99 wt% and was acquired at the Sigma–Aldrich (Fig. 1). The quaternary ammonium compounds were tetraethylammonium bromide $[N_{2,2,2,2}]Br$ (98 wt%); tetraethylammonium chloride $[N_{2,2,2,2}]Cl$ (98 wt%), tetrapropylammonium bromide, $[N_{3,3,3,3}]Br$ (98 wt%), tetrapropylammonium chloride $[N_{3,3,3,3}]Cl$ (98 wt%), tetrabutylammonium bromide $[N_{4,4,4,4}]Br$ (>98 wt%), tetrabutylammonium chloride $[N_{4,4,4,4}]Cl$ (97 wt%). All quaternary ammonium compounds were purchased from Sigma–Aldrich. The chemical structures of all compounds used in this work are depicted in Fig. 2. The purity of each ammonium was further checked by ^1H and ^{13}C NMR and found to be in accordance with the stated purity level provided by the suppliers. Potassium phosphate monobasic KH_2PO_4 (99.5 wt%), potassium carbonate K_2CO_3 (99 wt%) and potassium citrate tribasic monohydrate $C_6H_5K_3O_7{\cdot}H_2O$ (⩾99 wt%) were acquired from Sigma–Aldrich, potassium phosphate dibasic K_2HPO_4 (>98 wt%) was supplied by JMVP and the citric acid monohydrate $C_6H_8O_7{\cdot}H_2O$ (100 wt%) was purchased at Fisher Scientific. The water used was double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

The drug *Ben-u-ron® 500* was produced in Portugal by Neo-Farmacêutica.

2.2. Phase diagrams and tie-lines

The binodal data were determined through the cloud point titration method at 298 (±1) K and atmospheric pressure, as previously described in literature [17–20]. The ABS were measured by using ammonium aqueous solutions ($[N_{2,2,2,2}]Br$, $[N_{2,2,2,2}]Cl$, $[N_{3,3,3,3}]Br$, $[N_{3,3,3,3}]Cl$, $[N_{4,4,4,4}]Br$ and $[N_{4,4,4,4}]Cl$) at concentrations ranging from ≈40 wt% to ≈80 wt% and aqueous solutions of three

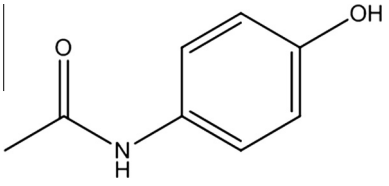

Fig. 1. Chemical structure of paracetamol.

different salt species. The potassium phosphate buffer solution (pH 7) was prepared by adding $K_2HPO_4 + KH_2PO_4$ (*at circa* 40 wt%) [19], the potassium citrate buffer solutions (pH 5–8) were composed of $C_6H_5K_3O_7 + C_6H_8O_7$ (*at circa* 50 wt%) [32] and the potassium carbonate aqueous solution was prepared *at circa* 50 wt% (pH ≈ 13).

Each tie-line (TL) was measured through a well-established gravimetric method firstly proposed by Merchuk and co-workers [33] and validated by us for this purpose [17–20]. A mixture at the biphasic region was prepared, vigorously stirred and allowed to reach the equilibrium by the phases separation for at least 18 h and at 298 (±1) K, using small ampoules (10 cm^3) specially designed for this purpose. After the separation of the co-existing phases, they were carefully and individually weighed. The tie-lines (TLs) were determined by the application of the lever-arm rule and the experimental solubility curves were correlated using the Merchuk Equation (Eq. (1)) [33],

$$[ammonium] = A\ exp[(B[salt]^{0.5}) - (C[salt]^3)] \quad (1)$$

where [ammonium] and [salt] represent, respectively, the ammonium and salt mass fraction percentages, and *A*, *B* and *C* are the constants obtained by the regression of the experimental binodal data. The compositions adopted at the biphasic region in order to determine the TLs were the same as those further applied in the paracetamol extraction studies (within ±10^{-4} g).

The tie-line length (TLL) was determined using the Eq. (2),

$$TLL = \sqrt{([salt]_T - [salt]_B)^2 + ([ammonium]_T - [ammonium]_B)^2} \quad (2)$$

and denotes the distance between the bottom (*B*) and the top (*T*) phase compositions.

2.3. Optimization study of the paracetamol partition

The systems for the optimization study of the paracetamol extraction were gravimetrically prepared in glass vials by adding the appropriate amounts of ammonium, of an aqueous solution of the salt component and 0.5 g of an aqueous solution containing the paracetamol (≈0.001 g mL^{-1}), for a total mass of the extraction systems prepared of 5 g. The influence of different parameters was analyzed, namely the ammonium structure, the salt component, the TLL and finally, the pH. The extraction points used were chosen in accordance with the conditions investigated and presented in Table 1.

Subsequently, the mixtures were vigorously stirred and the systems were placed at 298 (±1) K, for at least 18 h, to assure the complete equilibrium of the phases and the total paracetamol partition. The degree of agitation used in the ABS preparation was controlled and maintained at 2400 rpm, avoiding any limitations related with mass transfer problems and guarantying that equilibrium was reached while minimizing the time of each essay. At this point, the systems were clear and a well-defined interface between the coexisting phases was observed. Finally, the phases were carefully separated and collected for the measurement of their weight, volume and pH. At the conditions used in this work, the system resulted in the ammonium-rich phase as the top layer while the salt-rich is representing the bottom phase. In this work, the extraction efficiencies of paracetamol (EE_{PC}, %) were calculated for each system studied following the Eq. (3),

$$EE_{PC} = \frac{[PC]_T \times V_T}{[PC]_i \times V_i} \times 100 \quad (3)$$

where V_i and $[PC]_i$ are the initial volume and the initial paracetamol concentration added to prepare the extraction systems, while V_T and $[PC]_T$ represent the volume and paracetamol concentration of the top phase, respectively.

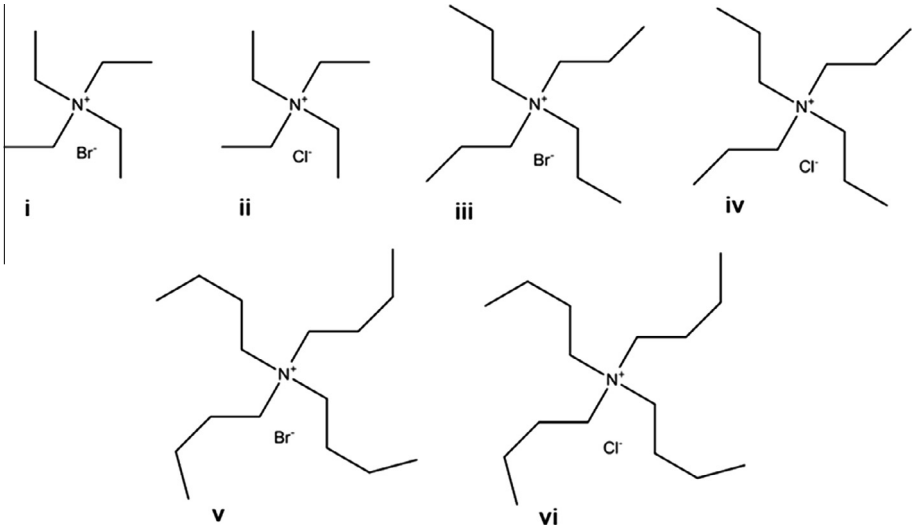

Fig. 2. Chemical structure of the ammonium compounds studied in this work: $[N_{2,2,2,2}]Br$ (i), $[N_{2,2,2,2}]Cl$ (ii), $[N_{3,3,3,3}]Br$ (iii), $[N_{3,3,3,3}]Cl$ (iv), $[N_{4,4,4,4}]Br$ (v) and $[N_{4,4,4,4}]Cl$ (vi).

Table 1
Conditions studied and approximate mass fraction compositions (in percentage) for all partition systems investigated.

	System		pH	100 × mass fraction composition (wt%)		
	Ammonium	Salt		Ammonium	Salt	Water
Ammonium structure	$[N_{4,4,4,4}]Cl$ $[N_{4,4,4,4}]Br$ $[N_{3,3,3,3}]Cl$ $[N_{3,3,3,3}]Br$ $[N_{2,2,2,2}]Br$	Potassium citrate buffer	7	40	25	35
	$[N_{2,2,2,2}]Cl$			33	26	41
Salt	$[N_{2,2,2,2}]Br$	Potassium phosphate buffer Potassium carbonate Potassium citrate buffer	7 ≈13 7	20 40	25 25	55 35
TLL	$[N_{2,2,2,2}]Br$	Potassium carbonate	≈13	20 30 40	25 25 25	55 45 35
pH	$[N_{2,2,2,2}]Br$	Potassium citrate buffer	5 6 7 8	33	26	41

The quantification of paracetamol was done in triplicate and, at least, three different assays for each system were performed (average values accompanied by the respective standard deviations are reported). Its concentration was assayed using a Shimadzu UV–VIS spectrophotometer UV mini-1240, at a wavelength of 242 nm, and using a calibration curve previously determined. The wavelength of the absorption peak of paracetamol was confirmed to remain unaffected, within the array of conditions tested. Possible interferences of the ammonium and the salt components with the paracetamol quantification method were eliminated by the regular application of blank controls, where the paracetamol aqueous solution is substituted by distilled water.

2.4. pH determination

The pH (±0.02) values of both phases for each system were measured at 298 (±1) K using an HI 9321 Microprocessor pH meter equipment (Hanna Instruments).

2.5. Recovery of paracetamol from Ben-u-ron®500

From the optimization study, representative conditions and systems were applied to the recovery of paracetamol from the *Ben-u-ron® 500* tablets (total weight *ca.* 617 mg), produced in Portugal by Neo-Farmacêutica. The recovery was carried out through the implementation of a two-stage extraction process comprising a first filtration step to remove some excipients, followed by the ABS application. For that purpose, the tablet was powdered and dissolved in distillated water in a concentration *at circa* 1 g dm^{-3} of paracetamol. The obtained solution was filtrated using syringe filters (0.45 μm) in order to remove the suspended solids (insoluble excipients used in the production of the tablets). Subsequently, the ABS were applied to recover the paracetamol from the filtered solution. The compositions were decided taking into account the EE_{PC} obtained in the optimization study. Three systems composed of 40 wt% of $[N_{4,4,4,4}]Br$ + 25 wt% of potassium citrate buffer pH 7, 40 wt% of $[N_{2,2,2,2}]Br$ + 25 wt% potassium citrate buffer pH 7 and 40 wt% of $[N_{2,2,2,2}]Br$ + 25 wt% of potassium carbonate were chosen aiming at maximizing the EE_{PC} from the pharmaceutical drug. The systems were prepared as described above. The filtered solution of paracetamol was added to the ternary mixtures, instead of the commercial sample of paracetamol from Sigma–Aldrich.

The exact concentration of paracetamol in the filtered solution was determined in order to calculate the EE_{PC} (Eq. (3)) of the process. The paracetamol quantification was performed by absorbance (λ = 242 nm). At least three independent extraction systems and

three assays for each, as well as blank controls were prepared, being reported the average values and corresponding standard deviations. The pH of both phases was also determined.

3. Results and discussion

3.1. Phase diagrams and tie-lines

In this work, the ABS formation capacity of six quaternary ammonium compounds with three different salts was evaluated. The phase diagrams were measured at 298 (±1) K and atmospheric pressure. The data in mass fraction units is provided in Supporting Information (Tables A.1–A.5). The binodal curves are plotted in molality units in Figs. 3–5 to remove any effects related to the differences in the molecular weight of the ammonium compounds and salts studied.

To assess the influence of the ammonium structure on the ABS formation (Fig. 3), two parameters were investigated, the anion type (Cl and Br) and the length of the alkyl chains of the cation (varying between 2 and 4). The $[N_{2,2,2,2}]Cl$, $[N_{2,2,2,2}]Br$, $[N_{3,3,3,3}]Cl$, $[N_{3,3,3,3}]Br$, $[N_{4,4,4,4}]Cl$ and $[N_{4,4,4,4}]Br$ were used and the potassium citrate buffer at pH 7 was fixed to obtain the binodal curves. The experimental curves of solubility, represented in Fig. 3, reveal that the ability of the ammonium compounds to form ABS increases in the order $[N_{2,2,2,2}]Cl < [N_{2,2,2,2}]Br \approx [N_{3,3,3,3}]Cl < [N_{3,3,3,3}]Br < [N_{4,4,4,4}]Cl < [N_{4,4,4,4}]Br$. Accordingly, the capacity of the salt to induce ABS formation is enhanced by the decreasing in the ammonium compounds affinity for water since the ammonium is salted-out by the salt ions [34–36]. Regarding separately the ammonium anion effect, it is clear that bromide-based ammonium compounds show higher aptitude to form ABS than their chloride-based equivalents. These results are in good agreement with data previously published [18,37] and results from the lower hydrogen-bond basicity (β) of the bromide when compared with the chloride anion. In what concerns the influence of the ammonium alkyl chain length, it follows the trend $[N_{2,2,2,2}] < [N_{3,3,3,3}] < [N_{4,4,4,4}]$ and results from the increase on the cation hydrophobicity [17,19]. The results presently available on the ability of ammonium and phosphonium compounds to form ABS are limited. They seem, however, to indicate that these compounds possess a greater ability to induce ABS formation when compared with the widely studied conventional aromatic-based cations [38–40].

The impact on the ABS formation of the salts studied, potassium citrate and phosphate buffers at pH 7 and potassium carbonate, in presence of $[N_{2,2,2,2}]Br$, is displayed in Fig. 4. Only a very tenuous difference, given by the slightly higher salting-out strength of the potassium phosphate buffer when compared with potassium citrate buffer and potassium carbonate salt is observed. Interestingly, this tendency is reversed at higher salt concentrations, being then the potassium carbonate responsible for the higher ability to induce the ABS formation.

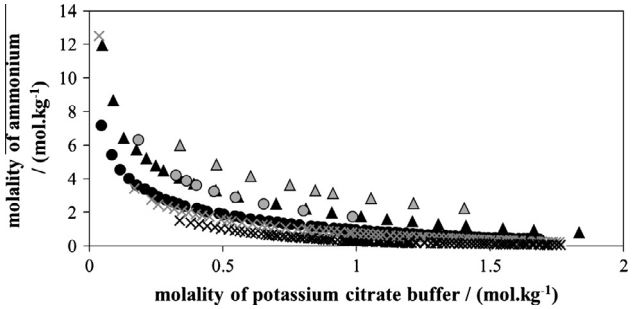

Fig. 3. Binodal curves for the systems composed of ammonium-based compounds + potassium citrate buffer at pH 7: $[N_{2,2,2,2}]Cl$ (▲), $[N_{2,2,2,2}]Br$ (▲), $[N_{3,3,3,3}]Cl$ (●), $[N_{3,3,3,3}]Br$ (●), $[N_{4,4,4,4}]Cl$ (×), $[N_{4,4,4,4}]Br$ (×).

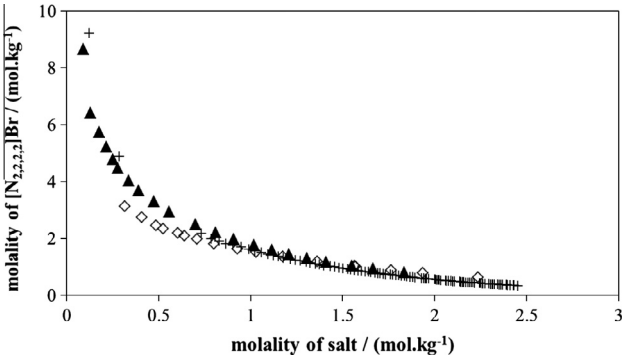

Fig. 4. Binodal curves for the systems composed of $[N_{2,2,2,2}]Br$ + water + salt: potassium citrate buffer pH 7 (▲), potassium phosphate buffer pH 7 (◇) and potassium carbonate salt (+).

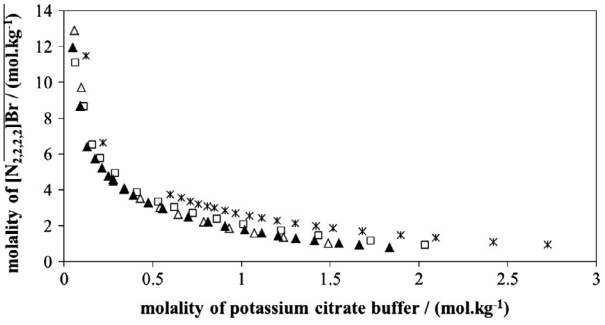

Fig. 5. Binodal curves for the system composed of $[N_{2,2,2,2}]Br$ + water + potassium citrate buffer at several pH values: pH 5 (∗), pH 6 (□), pH 7 (▲) and pH 8 (△).

The pH effect in the formation of $[N_{2,2,2,2}]Br$ + potassium citrate buffer + water systems was studied by varying the citric acid content to obtain pH values from 5 to 8. The results reported in Fig. 5 show that the immiscibility region is smaller at acidic pH. In good agreement with previous results [32] the ABS formation for the systems here studied is more favorable at neutral or alkaline pH values. The same tendency was observed by Zafarani-Moattar and Hamzehzadeh [32] for ABS composed of imidazolium-based ILs + potassium citrate buffer + water. The citrate anions ability to induce phase separation at different pH is likely related with its different degrees of protonation [32]. The triple charged citrate anion is a more effective salting-out agent than either double or single charged citrate anions, due to its stronger hydration. The results here reported indicate that the quaternary ammonium present good ability to form ABS in both acidic and neutral media, suggesting that they may present a high potential for the extraction of biomolecules with slightly low acid dissociation constants.

The binodal curves presented above were correlated using Eq. (1) firstly proposed by Merchuk et al. [33]. The regression parameters A, B and C and the corresponding standard deviations (σ), as well as the respective correlation coefficients (R^2) are reported in Table 2. The tie-lines (TLs) and respective tie-line lengths (TLLs) are provided in Supporting Information from Table A.6 to A.8, along with the composition of the ammonium and salt in both top and bottom phases. Also, the graphical representation of the phase diagrams and TLs determined for each system studied and further considered to the extraction systems is depicted in Supporting Information from Figs. A.1 to A.10. As an example, the ABS composed of $[N_{2,2,2,2}]Br$ + potassium citrate buffer + water at pH 7 and 298 (±1) K with its tie-lines is represented in Fig. 6.

Table 2
Parameters of Eq. (1) with the respective standard deviations (σ) and correlation coefficients (R^2) for the different ammonium + salt + water ternary systems studied.

Ammonium	Salt	$A \pm \sigma$	$B \pm \sigma$	$C \pm \sigma$	R^2
$[N_{4,4,4,4}]Cl$	Potassium citrate buffer pH 7	106.4 ± 1.3	−0.335 ± 0.004	$2.0 \times 10^{-5} \pm 6.4 \times 10^{-7}$	0.9968
$[N_{3,3,3,3}]Cl$		106.8 ± 0.7	−0.264 ± 0.002	$6.9 \times 10^{-6} \pm 5.8 \times 10^{-7}$	0.9999
$[N_{2,2,2,2}]Cl$		96.0 ± 0.9	−0.215 ± 0.003	$3.7 \times 10^{-6} \pm 3.0 \times 10^{-7}$	0.9999
$[N_{4,4,4,4}]Br$		163.3 ± 5.4	−0.505 ± 0.010	$3.4 \times 10^{-5} \pm 1.0 \times 10^{-6}$	0.9977
$[N_{3,3,3,3}]Br$		88.0 ± 0.2	−0.256 ± 0.001	$2.0 \times 10^{-5} \pm 1.2 \times 10^{-7}$	0.9999
$[N_{2,2,2,2}]Br$		92.3 ± 1.1	−0.227 ± 0.005	$1.1 \times 10^{-5} \pm 7.9 \times 10^{-7}$	0.9985
	Potassium phosphate buffer pH 7	73.5 ± 0.7	−0.284 ± 0.004	$2.2 \times 10^{-5} \pm 8.5 \times 10^{-7}$	0.9998
	Potassium carbonate	110.1 ± 0.5	−0.398 ± 0.002	$5.0 \times 10^{-5} \pm 4.5 \times 10^{-7}$	0.9996
	Potassium citrate buffer pH 5	102.3 ± 1.9	−0.223 ± 0.006	$5.0 \times 10^{-6} \pm 5.9 \times 10^{-7}$	0.9961
	Potassium citrate buffer pH 6	93.2 ± 1.1	−0.221 ± 0.005	$8.1 \times 10^{-6} \pm 7.6 \times 10^{-7}$	0.9988
	Potassium citrate buffer pH 8	101.8 ± 1.2	−0.251 ± 0.005	$1.0 \times 10^{-5} \pm 1.1 \times 10^{-6}$	0.9991

3.2. Optimization study of the paracetamol partition

The applicability of the studied ammonium-based ABS to the recovery of paracetamol is investigated using model systems for the optimization of the various operating conditions, namely the ammonium structure, the salt component, the TLL and the pH of the aqueous medium.

The paracetamol concentration was assayed through UV-spectroscopy and the maximum wavelength of absorbance at 242 nm, was assessed by the evaluation of its UV–VIS spectrum in water. It was verified that this wavelength did not change for all the conditions here studied. High deviations related with the quantification of paracetamol in the bottom phase (salt-rich phase) were obtained, due to the extensive partitioning of the molecule to the top-phase (ammonium-rich phase). Thus, the paracetamol concentration was only determined in the ammonium-rich phase and the biased spectroscopic method described by Rossmanith and co-workers [41] was applied. The corresponding blank controls (without paracetamol) were used to eliminate the negative interference of the ternary phase components, using a protocol recently validated and applied by us in ILs-based ABS [42]. After the validation of the quantification method used to measure the paracetamol concentration, the EE_{PC} for all ABS was gauged following Eq. (3). Table 3 presents the EE_{PC} achieved along with the average mass fraction compositions (in percentage) used to prepare each model system. In general, the investigated ABS reveal a good ability to extract the paracetamol, with EE_{PC} ranging from 81.6 ± 2.4 to 100%.

The preference of the paracetamol to the ammonium-rich phase is primarily related to its low polarity, due to the low number of hydrogen-bond donor/acceptor sites. The octanol–water partition coefficient available for this compound, K_{ow} = 3.02 [43], is a clear indication of the preference of paracetamol for the most hydrophobic phase. To better understand the influence of the other parameters tested in the ABS performance, several representations are provided and arranged according to the four parameters investigated: the ammonium structure (Fig. 7), the salt type (Table 4), the TLL (Fig. 8) and the pH of the aqueous extraction medium (Fig. 9).

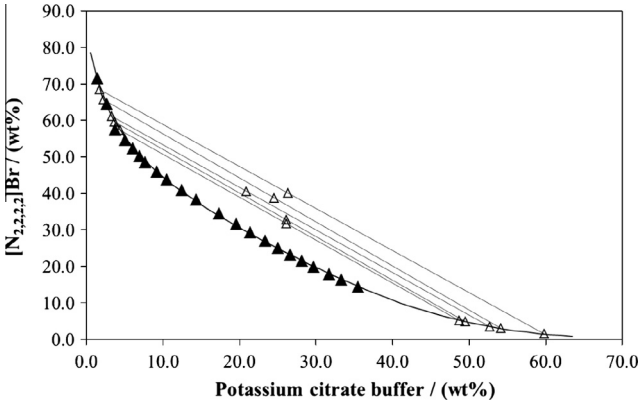

Fig. 6. Phase diagram of the system $[N_{2,2,2,2}]Br$ + potassium citrate buffer + water at pH 7 and 298 (±1) K: ▲, binodal curve data; △, TL data; —, fitting of the experimental data by Eq. (1).

To address the impact of the ammonium structure on the extraction of paracetamol, systems composed of *circa* 40 wt% of the quaternary ammonium compounds ($[N_{2,2,2,2}]Br$, $[N_{3,3,3,3}]Cl$, $[N_{3,3,3,3}]Br$, $[N_{4,4,4,4}]Cl$ and $[N_{4,4,4,4}]Br$) + 25 wt% of potassium citrate buffer pH 7 + 35 wt% of water and 33 wt% of $[N_{2,2,2,2}]Cl$ + 26 wt% of potassium citrate buffer pH 7 + 41 wt% of water were prepared. According to Fig. 7, it is possible to evaluate the effect of two ammonium features on the EE_{PC}: the anion, Br < Cl, and the alkyl chain length of the cation, from four to two carbons ($[N_{4,4,4,4}] < [N_{3,3,3,3}] < [N_{2,2,2,2}]$). In what concerns the anion effect, the chloride-based ammonium compounds are responsible for higher EE_{PC} when compared with their bromide counterparts. These results are in good agreement with Cláudio et al. [44], where the same trend (for these two anions) was obtained when the extraction of vanillin is considered (which is structurally similar to paracetamol). The apparent contradiction between the preferential partition towards the more hydrophobic phase and the larger EE_{PC} observed for the Cl anion and the ammonium with the smaller alkyl chains can be also explained based in the water content of the phases. Aiming at better understanding the partitioning of paracetamol, the water content of ammonium-rich phase is also plotted in Fig. 7. The EE_{PC} results are in close agreement with the increasing of the water concentration in the top phase (Fig. 7). A correlation of the EE_{PC} against the water content is shown in Fig. A.11 in the Supporting Information file. Here, it is clear the influence of the water content on the EE_{PC} showing that the apparent contradiction observed, results from a superposition of effects.

The influence of three different salts on the extraction of paracetamol was investigated through paired comparison (Table 4); the potassium carbonate (pH ≈ 13) was compared with the potassium phosphate buffer both at pH 7, by fixing the extraction point at 20 wt% of $[N_{2,2,2,2}]Br$ + 25 wt% of salt + 55 wt% of water, while the potassium citrate buffer pH 7 was compared with potassium carbonate by stipulating another extraction point (40 wt% of $[N_{2,2,2,2}]Br$ + 25 wt% of salt + 35 wt% of water). Two different points were selected because of the impossibility to prepare the extraction systems for the same point in the biphasic region, due to a salt precipitation phenomenon. The potassium phosphate buffered ABS reveals a greater aptitude to enhance the recovery of paracetamol (EE_{PC} = 92.3 ± 1.9%) when compared with the potassium carbonate salt (EE_{PC} = 81.6 ± 2.4%). The pH of the coexisting phases was measured for all systems and it was shown that the buffer condition for the ABS containing either potassium citrate or potassium phosphate-based salts was sustained (pH ≈ 7), whilst the potassium carbonate salt is inducing a strong alkaline environment (pH ≈ 13). The pH data are presented in Table A.9 in the Supporting

Table 3
Mass fraction compositions (in percentage) and extraction efficiencies (EE_{PC},%) plus the corresponding standard deviations (σ) for all the partition systems investigated.

Ammonium	Salt	pH	100 × mass fraction composition (wt%)			$EE_{PC} \pm \sigma$ (%)
			Ammonium	Salt	Water	
$[N_{4,4,4,4}]Cl$	Potassium citrate buffer	7	40.02	25.06	34.93	95.3 ± 3.1
$[N_{3,3,3,3}]Cl$			40.19	25.05	34.76	98.0 ± 3.4
$[N_{2,2,2,2}]Cl$			31.30	26.56	42.14	101.3 ± 3.4
$[N_{4,4,4,4}]Br$			39.69	25.02	35.29	91.1 ± 1.1
$[N_{3,3,3,3}]Br$			40.47	24.76	34.77	96.6 ± 1.5
$[N_{2,2,2,2}]Br$			38.80	24.39	36.81	99.7 ± 0.2
	Potassium phosphate buffer	7	20.67	25.17	54.16	92.3 ± 1.9
			40.12	24.85	35.03	98.2 ± 2.1
	Potassium carbonate	≈13	29.79	24.86	45.34	90.4 ± 1.2
			19.99	24.77	55.24	81.6 ± 2.4
	Potassium citrate buffer	5	32.84	25.83	41.32	95.8 ± 2.9
		6	31.20	24.86	43.94	92.3 ± 3.5
		7	32.59	26.09	41.32	98.3 ± 2.2
		8	32.54	25.86	41.60	89.7 ± 1.0

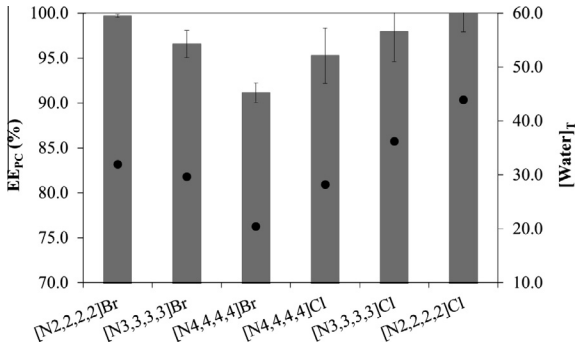

Fig. 7. Extraction efficiencies (EE_{PC}) (grey bars) obtained for the ABS composed of ammonium + potassium citrate buffer at pH 7 + water plus the corresponding water composition in the top phase (●).

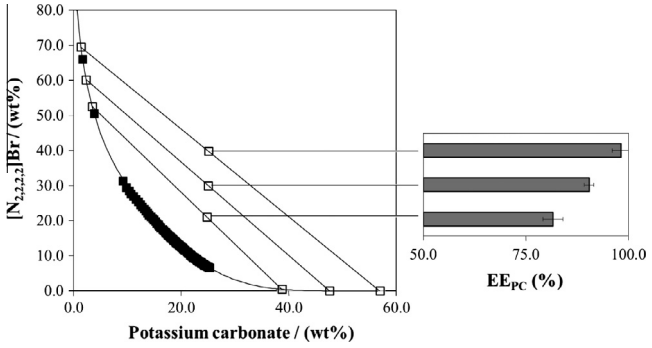

Fig. 8. Representation of the experimental binodal curve (■) and three different tie-lines (□), with distinct tie-line lengths (TLL) for the system composed of $[N_{2,2,2,2}]Br$ + potassium carbonate + water plus the extraction efficiency (EE_{PC}) of paracetamol (grey bars).

Information. Moreover, when the pH is investigated a careful analysis of the speciation curve of the target biomolecule must be used, aiming at the determination of the surface charges presented by the biomolecule with the pH (Fig. A.12 in Supporting Information). The paracetamol (pK_a = 9.5 [45]) changes its charge with the pH due to the deprotonation of its hydroxyl group (Fig. A.12 in Supporting Information). Thus, as the buffer condition was preserved for the systems comprising either potassium citrate or potassium phosphate-based salts, the ABS environment was favorable to the presence of the uncharged species. The alkaline medium of potassium carbonate systems favors the presence of the single charged paracetamol ions decreasing the hydrophobicity of the molecule, and thus its partition towards the ammonium-rich phase.

The picture that emerges from these results is that at neutral or acidic pH, the paracetamol is preferentially migrating to the ammonium-rich phase, due to its preference for the more hydrophobic phases. In strong alkaline media (i.e. potassium carbonate salt pH ≈ 13), other interactions appear between the charged structure of paracetamol and the components of the salt-rich phase, leading to a decrease in the EE_{PC}.

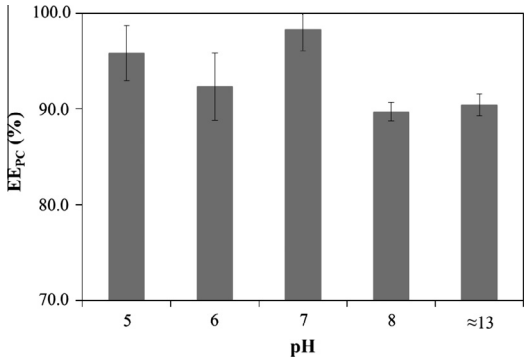

Fig. 9. Effect of pH on the extraction efficiency (EE_{PC}) of paracetamol (grey bars) using ABS composed of $[N_{2,2,2,2}]Br$ + potassium citrate buffer + water at pH ranging from 5 to 8 and $[N_{2,2,2,2}]Br$ + potassium carbonate + water system at pH ≈ 13.

The TLL impact on the extraction of paracetamol was also assessed and it is displayed in Fig. 8. Using the potassium

Table 4
Extraction efficiencies (EE_{PC}) obtained for the ABS composed of $[N_{2,2,2,2}]Br$ + salt + water along with the corresponding water composition in the top phase ($[water]_T$).

Salt	Ammonium	100 × mass fraction composition (wt%)		EE_{PC} (%)	$[water]_T$
		Ammonium	Salt		
Potassium phosphate buffer		20.67	25.17	92.3 ± 1.9	53.18
Potassium carbonate	$[N_{2,2,2,2}]Br$	19.99	24.77	81.6 ± 2.4	43.97
		40.12	24.85	98.2 ± 2.1	29.14
Potassium citrate buffer		38.80	24.39	99.7 ± 0.2	32.00

Table 5
Mass fraction composition (in percentage) and extraction efficiency (EE_{PC}) data for the extraction systems applied in the real pharmaceutical sample.

Salt	Ammonium	100 × mass fraction composition (wt%)			pH (±0.02)		EE_{PC} (%)
		Ammonium	Salt	Water	Top Phase	Bottom Phase	
Potassium citrate buffer pH 7	$[N_{2,2,2,2}]Br$	38.63	24.47	36.90	7.31	7.47	100
	$[N_{4,4,4,4}]Br$	39.84	25.70	34.46	7.16	7.11	100
Potassium carbonate	$[N_{2,2,2,2}]Br$	39.94	24.73	35.33	≈14	13.63	100

carbonate, three different extraction points (around 20 wt%, 30 wt% and 40 wt% of $[N_{2,2,2,2}]Br$ + 25 wt% of potassium carbonate + 55 wt%, 45 wt% and 35 wt% of water, respectively) were selected. Herein, decreasing the TLL also the EE_{PC} decreases (from EE_{PC} = 98.2 ± 2.1% to EE_{PC} = 81.6 ± 2.4%). Again, the paracetamol is more concentrated in the top phase, due to the favorable interactions between the biomolecule and the ammonium. However, in this specific case, the EE_{PC} is decreasing with the decrease in the TLL, i.e., with the decrease of the concentrations of both ABS components (ammonium and salt) in both (top and bottom) phases. Despite the significant capacity of paracetamol to interact with the water in the top phase, in this particular case, it is not possible to correlate both parameters. However, and because the change (decrease) in the TLL leads to a significant increase in the water present in both phases, the EE_{PC} of the biomolecule was correlated in this particular case, with the water content of the bottom phase (Fig. A.13 in the Supporting Information file). So, an opposite trend from that observed for the previous ABS was noticed, likely due to the fact that the molecule is singly charged (Fig. A.12 in Supporting Information) and starts to be more significantly partitioned between the two coexisting phases.

The influence of the pH medium on the EE_{PC} is reported in Fig. 9. The same extraction point (33 wt% of $[N_{2,2,2,2}]Br$ + 26 wt% of potassium citrate buffer + 41 wt% of water) was established for each ABS, varying only the $C_6H_5K_3O_7/C_6H_8O_7$ ratio of the buffer in order to achieve pH values within the range of 5 to 8. The EE_{PC} data obtained for the system composed of 30 wt% of $[N_{2,2,2,2}]Br$ + 25 wt% of potassium carbonate + 45 wt% of water is also plotted in Fig. 9, in order to infer the effect of a strong alkaline pH on the paracetamol partition. The EE_{PC} data obtained at different pH values is scattered. The results suggest the absence of a clear tendency when pH and EE_{PC} were correlated. However, it seems that similar values of EE_{PC} are obtained at mildly acidic or neutral conditions that decrease with increasing pH value due to the paracetamol becoming charged. Until now, the major key role controlling the paracetamol partition was attributed to the "biomolecule-ammonium" and "biomolecule–water" interactions, however when the pH is considered, these two major interactions are not capable to explain all the results. Looking for an explanation, the speciation curve of the citrate salt component was also investigated in the pH range tested (see Fig. A.14 in the Supporting Information file). It was verified that the citrate-based salts present several hydrogen-bond donor/acceptor sites and, consequently the speciation change with the pH will affect the interaction with the molecules, and thus the partition. In the mildly acidic and neutral pH region a good correlation between the partition coefficients and the water content is achieved (as shown in Fig. A.15 in the Supporting Information). Increasing in the alkalinity of the medium will change the charge of the paracetamol and thus the EE_{PC} decreases due to the interactions between the charged structure of paracetamol and the components present in the salt-rich phase.

3.3. Application of ABS to the recovery of paracetamol from Ben-u-ron® 500

The extraction of active substances from pharmaceutical wastes is the major goal of this work. After the optimization studies carried out using the model systems, the ABS with representative compositions were applied to the extraction of paracetamol from a real matrix, namely a solid state tablet of *Ben-u-ron® 500*. Therefore, systems composed of 40 wt% of ammonium ($[N_{2,2,2,2}]Br$ and $[N_{4,4,4,4}]Br$) + 25 wt% of potassium citrate buffer (pH 7) or potassium carbonate salt were applied. The influence of the potassium carbonate on the extraction of paracetamol from a more complex matrix was also assessed due to its strong alkaline nature and lower EE_{PC} achieved during the optimization stage. The *Ben-u-ron® 500* tablets containing 500 mg of paracetamol (total weight *at circa* 617 mg) were used. The drug tablet was grinded and the powder was dissolved in excess water, and left under continuous stirring overnight, to guarantee the complete dissolution of paracetamol, while the insoluble excipients remained in suspension. Hence, an extra step comprising one filtration was added to this approach, to remove the suspended solids and to obtain a clear paracetamol aqueous solution where the paracetamol concentration was measured. The solution thus obtained was used to prepare the ABS and the concentration of paracetamol was quantified in the ammonium-rich phase.

The application of the proposed ammonium-based ABS revealed a great performance in the extraction of paracetamol from the complex matrix as shown in Table 5. It is worth notice the complete extraction of paracetamol into the ammonium-rich phase. As observed in other studies [22,25,27,28], the real systems present higher EE_{PC} than the model systems. It seems that, despite the low concentrations of the six excipients, their presence affects positively the paracetamol migration to the ammonium-rich phase.

4. Conclusions

The ammonium-based ABS were successfully applied to the extraction of paracetamol from a real solid state pharmaceutical matrix. For that purpose, novel phase diagrams composed of quaternary ammonium and three different salts were determined. Ammonium compounds containing cation cores with longer alkyl chain lengths are more capable to induce phase splitting. Moreover, the pH of the aqueous media presents also an important role on the phase separation capacity which decreases for more acidic environments.

The aptitude of these ABS to extract paracetamol was assured and the operating conditions optimized. In the optimization study it was observed the preferential migration of paracetamol to the ammonium-rich phase that is independent of the ammonium structural features, pH and salt used, but at the same time, can be manipulated by these conditions to achieve the total extraction of the target compound. All systems studied show high EE_{PC}, ranging from 81.6 ± 2.4% to 100%. After the optimization step, some ABS were applied to the extraction of paracetamol from a real pharmaceutical matrix, in the solid state. Surprisingly, a complete recovery of the paracetamol present in the pharmaceutical sample to the top phase was accomplished, with EE_{PC} of 100% for all systems.

This work reveals the potential capacity of ammonium-based ABS to extract an active compound from a pharmaceutical waste, opening a new window to the management, reduction and valorization of pharmaceutical wastes.

Acknowledgements

The authors are grateful for financial support from FEDER funds through the program COMPETE and by national fund through the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects Pest-C/CTM/LA0011/2013 and PTDC/AAC-AMB/119172/2010. The authors also thank financial support through the post-doctoral and doctoral grants SFRH/BPD/79263/2011 and SFRH/BD/85871/2012of S.P.M. Ventura and T. Sintra, respectively.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.seppur.2013.11.018.

References

[1] N.F. Cruz, P. Simões, S.F. Ferreira, M.C. Pereira, R.C. Marques, EIMPack-Economic Impact of the Packaging and Packaging Waste Directive, Report from the Technical University of Lisbon, 2011, pp. 1–28.
[2] G.T. Ankley, B.W. Books, D.B. Hugget, J.P. Sumpter, Repeating history: pharmaceuticals in the environment, Environ. Sci. Technol. 41 (2007) 8211–8217.
[3] K. Kümmerer, The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges, J. Environ. Manag. 90 (2009) 2354–2366.
[4] European Environment Agency, Pharmaceuticals in the environment - Results of an EEA workshop, EEA Technical, Report No 1(2010), 2010, pp. 1–34.
[5] VALORMED, Relatório de actividades 2011. (accessed on 15th November 2012).
[6] G. Vollmer, Disposal of Pharmaceutical Waste in Households – A European Survey, in: K. Kümmerer, M. Hempel (Eds.), Green and Sustainable Pharmacy, Springer, Berlin Heidelberg, 2010, pp. 165–178.
[7] World Health Organization, Stability testing of active substances and pharmaceutical products, Working document QAS/06.179 (2006) 1–33.
[8] F. Carvalho, Impacto dos medicamentos no ambiente, Mundo Farmacêutico (2006) 12–13.
[9] P.T. Anastas, J.C. Warner, Green Chemistry. Theory and Practice, Oxford University Press, Oxford New York, 1998.
[10] J.F.B. Pereira, V.C. Santos, H.-O. Johansson, J.A.C. Teixeira, A. Pessoa Jr., A stable liquid–liquid extraction system for clavulanic acid using polymer-based aqueous two-phase systems, Sep. Pur. Technol. 98 (2012) 441–450.
[11] J.M.P. Barbosa, R.L. Souza, A.T. Fricks, G.M. Zanin, C.M.F. Soares, Á.S. Lima, Purification of lipase produced by a new source of Bacillus in submerged fermentation using an aqueous two-phase system, J. Chromatogr. 879 (2011) 3853–3858.
[12] D.A.V. Marques, A. Pessoa-Júnior, J.L. Lima-Filho, A. Converti, P. Perego, A.L.F. Porto, Extractive fermentation of clavulanic acid by Streptomyces DAUFPE 3060 using aqueous two-phase system, Biotechnol. Prog. 27 (2011) 95–103.
[13] I.A.O. Reis, S.B. Santos, L.A. Santos, N. Oliveira, M.G. Freire, J.F.B. Pereira, S.P.M. Ventura, J.A.P. Coutinho, C.M.F. Soares, Á.S. Lima, Increased significance of food wastes: selective recovery of added-value compounds, Food Chem. 135 (2012) 2453–2461.
[14] Y. Wang, Y. Liu, J. Han, S. Hu, Application of water-miscible alcohol-based aqueous two-phase systems for extraction of dyes, Sep. Sci. Technol. 46 (2011) 1283–1288.
[15] B. Wu, Y.M. Zhang, H.P. Wang, Aqueous biphasic systems of hydrophilic ionic liquids + sucrose for separation, J. Chem. Eng. Data 53 (2008) 983–985.
[16] G. de Brito Cardoso, T. Mourão, F.M. Pereira, M.G. Freire, A.T. Fricks, C.M.F. Soares, A.S. Lima, Aqueous two-phase systems based on acetonitrile and carbohydrates and their application to the extraction of vanillin, Sep. Pur. Technol. 104 (2013) 106–113.
[17] C.M.S.S. Neves, S.P.M. Ventura, M.G. Freire, I.M. Marrucho, J.A.P. Coutinho, Evaluation of cation influence on the formation and extraction capability of ionic-liquid-based aqueous biphasic systems, J. Phys. Chem. B 113 (2009) 5194–5199.
[18] S.P.M. Ventura, C.M.S.S. Neves, M.G. Freire, I.M. Marrucho, J. Oliveira, J.A.P. Coutinho, Evaluation of anion influence on the formation and extraction capacity of ionic-liquid-based aqueous biphasic systems, J. Phys. Chem. B 113 (2009) 9304–9310.
[19] S.P.M. Ventura, S.G. Sousa, L.S. Serafim, A.S. Lima, M.G. Freire, J.A.P. Coutinho, Ionic liquid based aqueous biphasic systems with controlled ph: the ionic liquid cation effect, J. Chem. Eng. Data 56 (2011) 4253–4260.
[20] S.P.M. Ventura, S.G. Sousa, L.S. Serafim, A.S. Lima, M.G. Freire, J.A.P. Coutinho, Ionic-liquid-based aqueous biphasic systems with controlled ph: the ionic liquid anion effect, J. Chem. Eng. Data 57 (2012) 507–512.
[21] M.G. Freire, A.F.M. Cláudio, J.M.M. Araújo, J.A.P. Coutinho, I.M. Marrucho, J.N.C. Lopes, L.P.N. Rebelo, Aqueous biphasic systems: a boost brought about by using ionic liquids, Chem. Soc. Rev. 41 (2012) 4966–4995.
[22] M.G. Freire, C.M.S.S. Neves, I.M. Marrucho, J.N. Canongia Lopes, L.P.N. Rebelo, J.A.P. Coutinho, High-performance extraction of alkaloids using aqueous two-phase systems with ionic liquids, Green Chem. 12 (2010) 1715–1718.
[23] Y. Jiang, H. Xia, J. Yu, C. Guo, H. Liu, Hydrophobic ionic liquids-assisted polymer recovery during penicillin extraction in aqueous two-phase system, Chem. Eng. J. 147 (2009) 22–26.
[24] M.T. Zafarani-Moattar, S. Hamzehzadeh, Partitioning of amino acids in the aqueous biphasic system containing the water-miscible ionic liquid 1-butyl-3-methylimidazolium bromide and the water-structuring salt potassium citrate, Biotechnol. Prog. 27 (2011) 986–997.
[25] S.P.M. Ventura, R.L.F. de Barros, J.M. de Pinho Barbosa, C.M.F. Soares, A.S. Lima, J.A.P. Coutinho, Production and purification of an extracellular lipolytic enzyme using ionic liquid-based aqueous two-phase systems, Green Chem. 14 (2012) 734–740.
[26] S.P.M. Ventura, S.G. Sousa, M.G. Freire, L.S. Serafim, Á.S. Lima, J.A.P. Coutinho, Design of ionic liquids for lipase purification, J. Chromatogr. B 879 (2011) 2679–2687.
[27] H. Passos, A.C.A. Sousa, M.R. Pastorinho, A.J.A. Nogueira, L.P.N. Rebelo, J.A.P. Coutinho, M.G. Freire, Ionic-liquid-based aqueous biphasic systems for improved detection of bisphenol A in human fluids, Anal. Methods 4 (2012) 2664–2667.
[28] J.F.B. Pereira, F. Vicente, V.C. Santos-Ebinuma, J.M. Araújo, A. Pessoa, M.G. Freire, J.A.P. Coutinho, Extraction of tetracycline from fermentation broth using aqueous two-phase systems composed of polyethylene glycol and cholinium-based salts, Proc. Biochem. 4 (2013) 716–722.
[29] S. Shahriari, L.C. Tome, J.M.M. Araujo, L.P.N. Rebelo, J.A.P. Coutinho, I.M. Marrucho, M.G. Freire, Aqueous biphasic systems: a benign route using cholinium-based ionic liquids, RSC Adv. 3 (2013) 1835–1843.
[30] S. Mallakpour, Z. Rafiee, Tetrabutylammonium bromide: an efficient, green and novel media for polycondensation of 4-(4-dimethylaminophenyl)-1,2,4-triazolidine-3,5-dione with diisocyanates, Eur. Polym. J. 43 (2007) 1510–1515.
[31] P. Attri, P. Venkatesu, Ammonium ionic liquids as convenient co-solvents for the structure and stability of succinylated Con A, J. Chem. Thermodyn. 52 (2012) 78–88.
[32] M.T. Zafarani-Moattar, S. Hamzehzadeh, Effect of pH on the phase separation in the ternary aqueous system containing the hydrophilic ionic liquid 1-butyl-3-methylimidazolium bromide and the kosmotropic salt potassium citrate at T = 298.15 K, Fluid Phase Equil. 304 (2011) 110–120.
[33] J.C. Merchuk, B.A. Andrews, J.A. Asenjo, Aqueous two-phase systems for protein separation: Studies on phase inversion, J. Chromatogr. B: Biomed. Sci. Appl. 711 (1998) 285–293.
[34] M.G. Freire, P.J. Carvalho, A.M.S. Silva, L.M.N.B.F. Santos, L.P.N. Rebelo, I.M. Marrucho, J.A.P. Coutinho, Ion specific effects on the mutual solubilities of water and hydrophobic ionic liquids, J. Phys. Chem. B 113 (2009) 202–211.
[35] M.G. Freire, C.M.S.S. Neves, A.M.S. Silva, L.M.N.B.F. Santos, I.M. Marrucho, L.P.N. Rebelo, J.K. Shah, E.J. Maginn, J.A.P. Coutinho, ^{1}H NMR and molecular dynamics evidence for an unexpected interaction on the origin of salting-in/salting-out phenomena, J. Phys. Chem. B 114 (2010) 2004–2014.
[36] L.I.N. Tomé, F.R. Varanda, M.G. Freire, I.M. Marrucho, J.A.P. Coutinho, Towards an understanding of the mutual solubilities of water and hydrophobic ionic liquids in the presence of salts: the anion effect, J. Phys. Chem. B 113 (2009) 2815–2825.
[37] Y. Pei, J. Wang, L. Liu, K. Wu, Y. Zhao, Liquid−liquid equilibria of aqueous biphasic systems containing selected imidazolium ionic liquids and salts, J. Chem. Eng. Data 52 (2007) 2026–2031.
[38] N.J. Bridges, K.E. Gutowski, R.D. Rogers, Investigation of aqueous biphasic systems formed from solutions of chaotropic salts with kosmotropic salts (salt–salt ABS), Green Chem. 9 (2007) 177–183.
[39] C.L.S. Louros, A.F.M. Cláudio, C.M.S.S. Neves, M.G. Freire, I.M. Marrucho, J. Pauly, J.A.P. Coutinho, Extraction of biomolecules using phosphonium-based ionic liquids + K_3PO_4 aqueous biphasic systems, Int. J. Mol. Sci. 11 (2010) 1777–1791.
[40] C.M.S.S. Neves, M.G. Freire, J.A.P. Coutinho, Improved recovery of ionic liquids from contaminated aqueous streams using aluminium-based salts, RSC Adv. 2 (2012) 10882–10890.
[41] P. Mester, M. Wagner, P. Rossmanith, Biased spectroscopic protein quantification in the presence of ionic liquids, Anal. Bioanal. Chem. 397 (2010) 1763–1766.
[42] S.P.M. Ventura, V.C. Santos-Ebinuma, J.F.B. Pereira, M.F.S. Teixeira, A. Pessoa, J.A.P. Coutinho, Isolation of natural red colorants from fermented broth using ionic liquid-based aqueous two-phase systems, J. Ind. Microbiol. Biotechnol. 40 (2013) 507–516.
[43] K. Carlsson, B. Karlberg, Determination of octanol–water partition coefficients using a micro-volume liquid–liquid flow extraction system, Anal. Chim. Acta 423 (2000) 137–144.
[44] A.F.M. Cláudio, M.G. Freire, C.S.R. Freire, A.J.D. Silvestre, J.A.P. Coutinho, Extraction of vanillin using ionic-liquid-based aqueous two-phase systems, Sep. Pur. Technol. 75 (2010) 39–47.
[45] ChemSpider – The free chemical database at www.chemspider.com. (Accessed on February 2013).