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Aqueous biphasic systems composed of ionic liquids and polymers: A platform for the purification of biomolecules

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ABSTRACT

The ability of alternative aqueous biphasic systems (ABS) composed of polyethylene glycol and imidazolium-based ionic liquids (ILs) to selectively separate similar biomolecules was here investigated. The preferential partitioning of three alkaloids (caffeine, xanthine and nicotine) was addressed by means of their partition coefficients and selectivity values. Aiming at optimizing the selectivity of the studied ABS, factors such as the chemical structure of the IL (cation side alkyl chain length, number of aliphatic moieties or their functionalization, and the anion nature) and the temperature of equilibrium were experimentally addressed. In almost all examples it was observed a preferential concentration of caffeine in the polymer-rich phase whereas nicotine and xanthine preferentially migrate to the (opposite) IL-rich phase. In spite of the alkaloids chemical similarities, the studied ABS presented selectivity values of xanthine vs. caffeine as large as 19. The gathered results show that polymer-IL-based ABS allow the selective separation of similar structures by an adequate manipulation of the IL chemical structure and temperature of equilibrium, and can be envisaged as potential platforms to be applied in countercurrent chromatography.

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1. Introduction

Aqueous biphasic systems (ABS) can be used as separation processes based on the partition of a target compound between two immiscible aqueous-rich phases formed by polymer–polymer, polymer–salt and salt–salt mixtures [1–3]. Since the publication of the first work showing the possibility of creating ABS by mixing ionic liquids (ILs) and inorganic salts in aqueous solution by Rogers and co-workers [4], the literature has witnessed an increasing number of works reporting either on the liquid–liquid phase diagrams of new IL-based ABS or on their application for the separation of a wide range of (bio)molecules [1].

ILs are organic salts generally found in the liquid state at temperatures below 100 °C. Since they are exclusively composed of ions, most of the ILs present a negligible vapour pressure, non-flammability at ambient conditions, high chemical and thermal stabilities [5], high ionic conductivity and a wide electrochemical potential window [6] – properties which are the basis for their use in a large range of applications. These include their use in coordination chemistry [7], analytical chemistry [8], polymer-based

materials [9], nanotechnology [10], (bio)catalysis [11,12] and finally, as high performance solvents, either as reaction media or as extractive solvents [13–15].

Due to the outstanding properties of ILs, IL-based ABS have led to enhanced performances on separation mainly because of the possibility of tailoring the polarity of both phases by a proper choice of the IL [16–18]. Polymer–polymer-based systems usually display two hydrophobic phases and their polarity differences essentially depend on the amount of water in each phase. On the other hand, polymer-salt ABS present a hydrophobic phase mainly constituted by the polymer and a highly hydrophilic (and ionic) phase, typically formed by high charge-density inorganic salts [19]. The limited range of polarity differences between the two phases limits thus the applicability of conventional polymer-based ABS for extraction and separation purposes. However, the substitution of a high charge density inorganic salt and/or a polymer by an IL may provide a precise control of the phases' polarities since they can be adequately chosen taking into account the vast array of their ionic structures (at least 10^6 simple ILs can be easily prepared in the laboratory) [20]. Further advantages can be associated to the use of ILs as constituents of ABS when compared with the more conventional high-melting inorganic salts, namely their lower corrosive character [21], and the possibility of controlling the salt precipitation since ILs display lower melting temperatures and

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hydrophilic ILs are usually miscible with water in the complete composition range. Despite the large amount of literature devoted to ABS composed of ILs and inorganic salts [1], recently some works have further reported the formation of aqueous systems involving polymers and ILs [21–24]. In those works the influence of the ILs' structure on the phase behaviour and ABS formation was addressed [21–24]. It was shown that the phase behaviour, as well as the phases polarities, of polymer-IL-based ABS can be properly tailored by a judicious selection of the polymer or IL employed [21–24]. In particular, a detailed understanding of the molecular-level interactions occurring in ABS composed of ILs and polyethylene glycol (PEG) was carried out by Freire et al. [21]. The liquid–liquid demixing ability in the IL-polymer-based ABS was explained and supported as dominated by the interactions taking place between the polymer and the ionic species and not by the hydration capability of the isolated IL ions [21].

Despite the reduced number of publications dealing with polymer-IL ABS [23–25], their singular nature and properties makes them promising liquid–liquid separation systems to apply into countercurrent chromatography (CCC) when envisaging large scale applications. The CCC is an emerging low pressure chromatographic technique which can leads to the separation of a large range of molecules on the basis of different partition coefficients obtained between the two immiscible liquid phases [26]. In fact, a significant number of works associating the CCC technique with ABS can be found in literature making use of polymer–polymer [27] or polymer–salt ABS [28–31] being, however, the use of IL-based ABS only limited to one literature study [32]. The use of IL-based ABS leads to a density difference that makes the liquid–liquid system easier to retain in a CCC column [32]. The use of a new chromatographic strategy for the quantitative determination of several alkaloids was recently reported by Wei et al. [33]. The authors applied an IL-based microwave-assisted extraction for a fast and safe quantitative analysis of the active components from pigeon pea leaves [33].

Taking into consideration the wide polarity range displayed by IL-based ABS, in the present work it was investigated the use of some novel PEG-IL-based ABS recently reported [21] for the selective separation of similar alkaloids, namely xanthine, caffeine and nicotine. As recently suggested [16], IL-based ABS are an enhanced strategy for concentrating alkaloids from human biological fluids in order to perform doping control analysis. Moreover, several biomass samples and pharmaceutical products present different types of alkaloids. Therefore, their isolated identification and analysis is of foremost importance.

The main objective of this paper is to show that, by the application of different ILs as main constituents of ABS, it is possible to manipulate the affinity of different alkaloids for different phases and, consequently, to selectively separate related chemical structures. The studied ABS are composed of polyethylene glycol (with an average molecular weight of 2000 g mol^{-1}) and different imidazolium-based ILs, so that the effects of the cation alkyl side chain length, the number of alkyl groups at the cation or their functionalization, as well as the anion nature, could be evaluated. The effect of temperature on the selective extraction was also investigated.

2. Experimental section

2.1. Materials

The ILs investigated as main constituents of IL-polymer-based ABS were 1-allyl-3-methylimidazolium chloride, [amim]Cl (98.0 wt.% pure), 1-(2-hydroxyethyl)-3-methylimidazolium chloride, [OHC$_2$mim]Cl (99.0 wt.% pure), 1-methylimidazolium chloride, [C$_1$im]Cl (98.0 wt.% pure), 1,3-dimethylimidazolium

chloride, [C$_1$mim]Cl (99.0 wt.% pure), 1-ethyl-3-methylimidazolium chloride, [C$_2$mim]Cl (98.0 wt.% pure), 1-ethyl-3-methylimidazolium acetate, [C$_2$mim][CH$_3$CO$_2$] (98.0wt% pure), 1-ethyl-3-methylimidazolium methanesulfonate, [C$_2$mim][CH$_3$SO$_3$] (99.0 wt.% pure), 1-ethyl-3-methylimidazolium hydrogenosulfate, [C$_2$mim][HSO$_4$] (99.0 wt.% pure) and 1-ethyl-3-methylimidazolium dimethylphosphate, [C$_2$mim][DMP] (98.0 wt.% pure). All ILs were supplied by Iolitec. Their ionic structures are presented in Fig. 1.

Before use all ILs were dried under constant stirring, moderate temperature (343 K) and high vacuum conditions (<0.1 mbar) for a minimum of 48 h. The purities of all ILs were additionally confirmed by ^{1}H and ^{13}C NMR and they are according to those given by the supplier. Polyethylene glycol, with an average molecular weight of 2000 g mol^{-1} (PEG 2000) was acquired at Sigma–Aldrich and used as received.

The water content of all ILs and polymer was measured by Karl-Fischer titration and was taken into account in the calculation of the global compositions of the PEG 2000 + IL + H$_2$O mixtures. In general, the water content of IL samples after the drying procedure was found to be <1000 ppm, whereas the water content in the polymer was <10,000 ppm. Ultrapure water that was doubly distilled, passed through a reverse-osmosis system, and further treated with a Milli-Q plus water purification apparatus was used.

The alkaloids used as partitioning solutes were xanthine (99.0 wt.% pure from Sigma–Aldrich), nicotine (⩾97.0 wt.% pure from Fluka) and caffeine (⩾99.5 wt.% pure from José Manuel Vaz Pereira). The molecular structures of the three alkaloids are depicted in Fig. 2.

2.2. Methods

2.2.1. Partitioning studies and selectivity of alkaloids

All the liquid–liquid partitioning systems or ABS were prepared in graduated centrifuge tubes by weighing the appropriate amounts of PEG 2000 (*ca.* 53 wt.%), IL (≈27 wt.%) and ≈20 wt.% of an aqueous solutions containing each one of the alkaloids, namely caffeine (15.1 g L^{-1}, 7.78 × 10^{-2} mol L^{-1}), xanthine (0.01 g L^{-1}, 6.57 × 10^{-5} mol L^{-1}) and nicotine (2.0 g L^{-1}, 1.23 × 10^{-2} mol L^{-1}). All the solutions were prepared in order to be guarantee that the alkaloids are at infinite dilution aiming at avoiding solute–solute interactions which could lead to different partitioning behaviours. The different alkaloid concentrations were selected to assure a maximum accuracy within the analytical technique employed. The selected mixtures form two immiscible aqueous phases as confirmed by the corresponding ternary phase diagrams previously published by us [21]. All mixtures were gravimetrically prepared within ±10^{-4} g. After a complete dissolution of all the components in the mixture by stirring, the mixture was left to equilibrate for 12 h in an air oven and at 323, 333, 343, 353 or 363 (±1) K, to achieve the complete partitioning of each alkaloid between the aqueous phases. The two phases were then carefully separated and the alkaloids were quantified in both the top and bottom phases by UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at the wavelengths of 261 nm (for nicotine), 267 nm (for xanthine) and 274 nm (for caffeine). Calibration curves for the determination of each alkaloid were previously established at the respective maximum absorption peaks. Possible interferences of the polymer or ILs investigated through the quantification of each alkaloid were taken into account and found to be of no concern at the dilutions carried out.

At least three individual ABS for each IL or alkaloid were prepared being the partition coefficient results presented as an average value and being the uncertainty associated to the alkaloids' partition coefficients within ±5%.

The alkaloids partition coefficients (K_{alk}) were estimated according to the following equation:
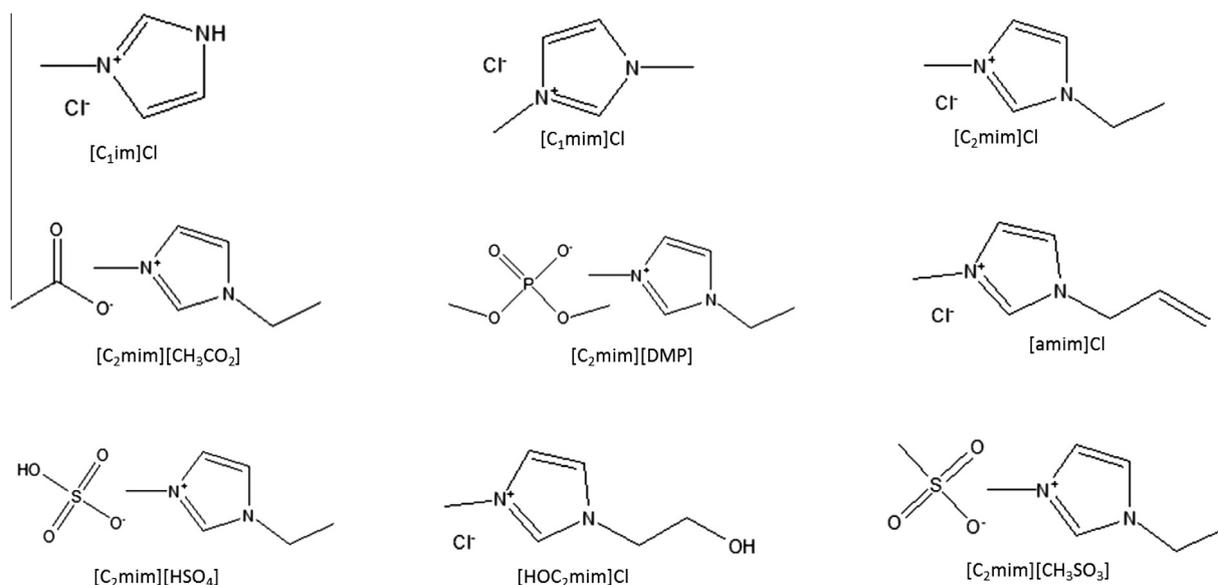
Fig. 1. Chemical structure and respective abbreviation name of all ILs investigated.
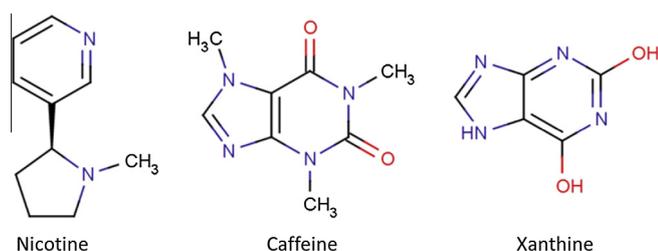

Fig. 2. Chemical structure of the three alkaloids investigated.

$$K_{alk} = \frac{[alk]_{IL}}{[alk]_{PEG}} \qquad (1)$$

where $[alk]_{IL}$ and $[alk]_{PEG}$ represent the concentration of each alkaloid in the IL- and PEG-2000-rich phases, respectively.

It should be remarked that the relative densities of the IL- or polymer-rich phases are not constant, i.e. the denser phase can be either the polymer- or the IL-phase, depending on the IL which constitutes the ABS (cf. Table A.1 in Supporting information).The temperature effect in the partitioning process was also evaluated for nicotine and caffeine. The respective thermodynamic parameters of phase transfer, such as the standard molar Gibbs energy ($\Delta_{tr}G_m^0$), the standard molar enthalpy ($\Delta_{tr}H_m^0$), and the standard molar entropy of transfer ($\Delta_{tr}S_m^0$) were determined according to Eqs. (2-4) [34,35],

$$\ln(K_{alk}) = -\frac{\Delta_{tr}H_m^0}{R} \times \frac{1}{T} + \frac{\Delta_{tr}S_m^0}{R} \qquad (2)$$

$$\Delta_{tr}G_m^0 = \Delta_{tr}H_m^0 - T\Delta_{tr}S_m^0 \qquad (3)$$

$$\Delta_{tr}G_m^0 = -RT\ln(K_{alk}) \qquad (4)$$

where K_{alk} is the partition coefficient of the alkaloid, R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is the temperature (K).

The selectivity on the partition of nicotine ($S_{nic/caf}$) or xanthine ($S_{xant/caf}$) in respect to caffeine was calculated according to Eqs. (5) and (6), respectively:

$$S_{nic/caf} = \frac{K_{nic}}{K_{caf}} \qquad (5)$$

$$S_{xant/caf} = \frac{K_{xant}}{K_{caf}} \qquad (6)$$

2.2.2. pH determination

The pH (±0.02) of the top and bottom phases was measured at 323 (±1) K using an HI 9321 Microprocessor pH meter (HANNA instruments). The calibration of the pH meter was carried out with two buffers (pH values of 4.00 and 7.00). The compositions adopted at the biphasic region mixture correspond to those used in the partitioning experiments. All mixtures were gravimetrically prepared within ±10^{-4} g.

3. Results and discussion

The migration of a target molecule in an ABS depends on the physico-chemical properties of the two phases so that specific extractions or its selectivity can be manipulated. Several conditions can be used to control the molecules partitioning, such as the chemical composition of the system, temperature, pH, and the inclusion of adjuvants, affinity ligands or amphiphilic structures [22,36].

In this work, it was first evaluated the effect of the IL which composes the IL-PEG-2000-based ABS on the partitioning of three alkaloids, namely caffeine, nicotine and xanthine at a similar composition (27 wt.% of IL + 53 wt.% of PEG 2000 + 20 wt.% of an aqueous solution containing the alkaloid) and temperature (323 ± 1 K). This first set of results allows the analysis of the impact of the IL on the partition coefficients of the alkaloids, as well as the nature of the solute and its partitioning pattern. Regarding the structural variations at the IL cation, the selected ILs allow the study of the effects of the alkyl side chain length, the aliphatic moiety functionalization and of the number of alkyl groups present at the imidazolium ring. For that study the chloride anion was fixed and was combined with the following cations: $[C_1im]^+$, $[C_1mim]^+$, $[C_2mim]^+$, $[amim]^+$ and $[OHC_2mim]^+$. The logarithm of the partition coefficients ($\log K_{alk}$) for the three alkaloids at 323 (±1) K, is depicted in Fig. 3 (the detailed partition coefficients, and respective standard deviations, and extraction efficiencies are reported in Table A.2 in Supporting information). The gathered results indicate that all the alkaloids preferentially migrate for the IL-rich phase with the exception of caffeine that favourably partitions for the
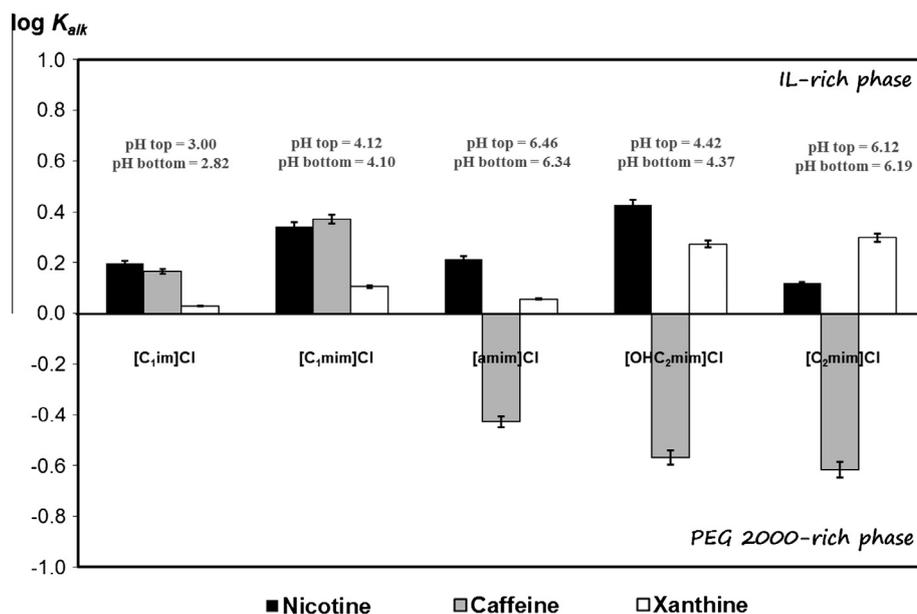
Fig. 3. Logarithm of the alkaloids partition coefficients ($\log K_{alk}$) in the ABS composed of PEG 2000 ($\approx$53 wt.%) + chloride-based ILs ($\approx$27 wt.%) and at 323 ($\pm$1) K. The pH values of the top and bottom phases are also displayed.

PEG-2000-rich phase in the ABS composed of $[C_2mim]Cl$, $[amim]Cl$ and $[OHC_2mim]Cl$.

The pH values of the coexisting phases of the systems shown in Fig. 3 range between 2.8 and 6.5. Taking into account the alkaloids speciation as a function of the pH in the systems investigated (the individual speciation curves are presented in Figs. A.1–A.3 in Supporting information), both xanthine and caffeine are present mainly as neutral molecules, whereas nicotine is present mostly as a positively charged species.

From the three alkaloids studied, nicotine is the one with the lowest affinity for water, as expressed by the higher octanol–water partition coefficient, K_{ow} = 3.71 [37]. However, despite this higher affinity for more hydrophobic phases and an expected preferential migration for the polymeric phase, nicotine, being present mostly as a positively charged species in all the investigated systems, preferentially partitions for the most hydrophilic and charged IL-rich phase. In addition, when comparing the trends collected with the two neutral alkaloids in the systems displayed in Fig. 3, xanthine always migrates preferentially for the IL-rich phase while caffeine shows an inversion to this pattern with more hydrophobic ILs. The K_{ow} values of caffeine and xanthine are 0.23 [37] and 0.74 [37], respectively, indicating that contrarily to nicotine, caffeine and xanthine display a higher affinity for more hydrophilic and water-rich phases. This preferential affinity of caffeine for hydrophilic phases is reflected in the inversion on its partition coefficients for the polymer-rich phase that occurs when ILs with longer aliphatic moieties (i.e. more hydrophobic ILs) are present.

The effect of the IL anion on the partitioning of the three alkaloids was also investigated with the results depicted in Fig. 4. As observed before with caffeine and the ABS composed of $[C_2mim]Cl$, for all the systems composed of $[C_2mim]$-based ILs, caffeine preferentially migrates for the polymer-rich phase, contrarily to what is observed with nicotine and xanthine. At the pH values of the coexisting phases of the systems considered in Fig. 4 (1.5 < pH < 9.2), xanthine and caffeine are present mainly as neutral species while nicotine is mostly as a positively mono-charged species – cf. speciation curves in Supporting information (Figs. A.1–A.3). Therefore, as observed before, nicotine always favourably migrates for the charged IL-rich phase despite the higher affinity of its neutral form for more organic phases. Xanthine, albeit present as a neutral

species, partitions in most examples for the most hydrophilic and IL-rich phase due to its inherent hydrophilic nature.

In summary, both xanthine and nicotine preferentially migrate for the IL-rich phase in practically all the studied ABS whereas the migration of caffeine can be tailored by the choice of the IL which constitutes a given ABS. Therefore, these results suggest that there is an inherent selectivity of the studied ABS for similar chemical structures such as alkaloids. To investigate the selective character of the IL-polymer-ABS studied, the selectivity of nicotine and xanthine in respect to caffeine for the IL-rich phase, $S_{nic/caf}$ and $S_{xant/caf}$, respectively, were further calculated. The corresponding results are presented in Fig. 5. The data shown in Fig. 5A allow the evaluation of the IL structural influence towards the selectivity at a fixed temperature and mixture composition. The selectivity data indicate that the modification of the IL chemical structure has a huge influence on the selective separation of caffeine from the remaining alkaloids.

Despite the molecular structure similarities between caffeine and xanthine, selectivity values up to 19 can be achieved with the ABS composed of $[C_2mim][CH_3CO_2]$. Indeed, the selectivity values cover a wider range for the caffeine–xanthine binary mixture than for the caffeine–nicotine pair. In addition to these calculations based on the isolated partition coefficients, the experimental investigation on the partitioning behaviour of a mixture of alkaloids, namely caffeine and nicotine, was also carried out. For that purpose, a system composed of $[OHC_2mim]Cl$ + PEG 2000 + an aqueous solution containing both nicotine (2.0 g L^{-1}) and caffeine (2.0 g L^{-1}) was used – one of the systems leading to higher selectivity values.

The UV–Vis spectra of the aqueous solutions of the pure compounds (caffeine and nicotine) and in each IL- or polymer-rich phase are presented in Fig. 6. The results show that nicotine is preferentially present in the IL-rich phase whereas caffeine is mainly concentrated in the polymer phase. Using a deconvolution technique to separate the peaks of the spectra of the two compounds, the respective K_{alk} values were calculated and the experimental selectivity parameter was further determined. The respective value is presented in Table 1. The results obtained with the real mixture of alkaloids ($S_{nic/caf}$ = 6.01) confirm the high selectivity values obtained with the isolated partition coefficients and validate all the
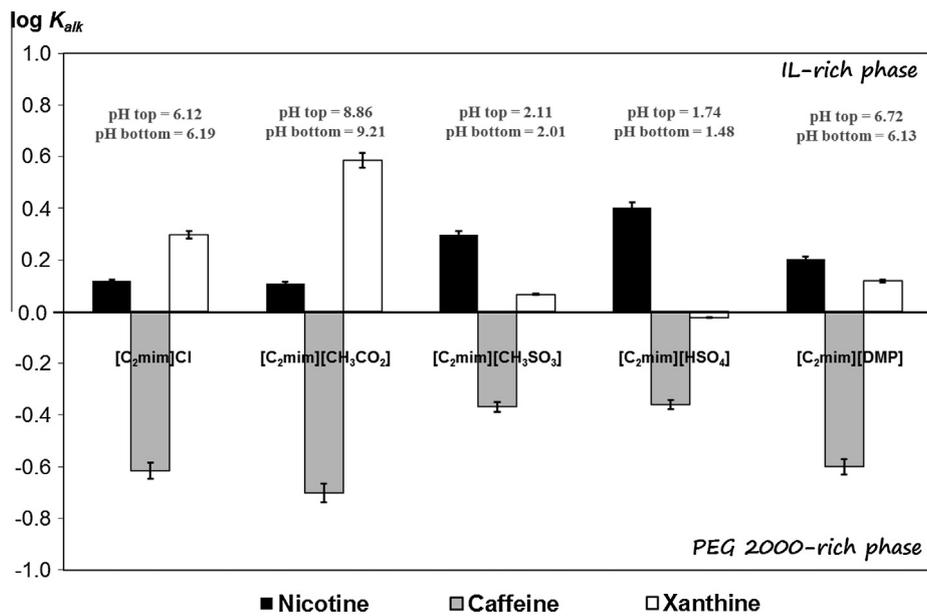
Fig. 4. Logarithm of the alkaloids partition coefficients ($\log K_{alk}$) in the ABS composed of PEG 2000 ($\approx$53 wt.%) + [C$_2$mim]-based ILs ($\approx$27 wt.%) and at 323 ($\pm$1) K. The pH values of the top and bottom phases are also displayed.
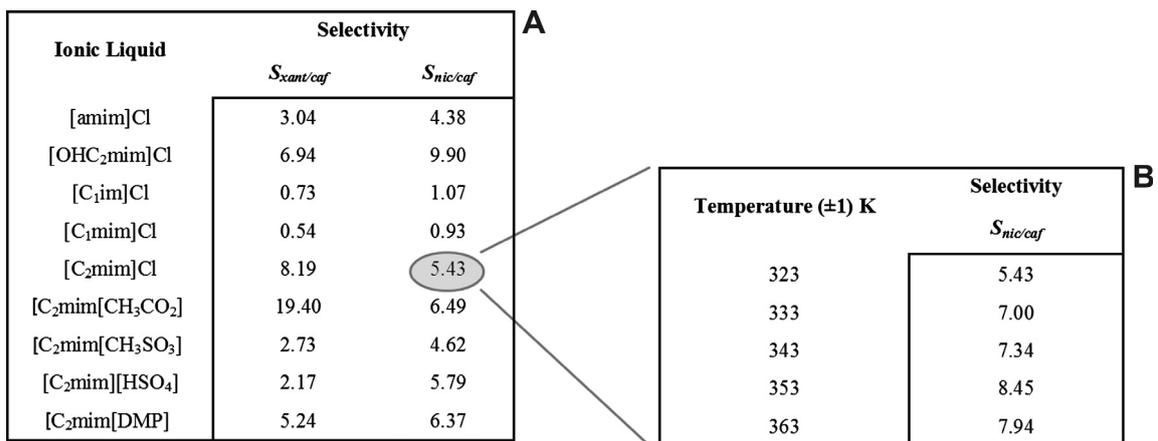

Fig. 5. Selectivity parameters for: A) xanthine/caffeine ($S_{xant/caf}$) and nicotine/caffeine ($S_{nic/caf}$) pairs considering all PEG-2000-ILs-based ABS tested and B) nicotine/caffeine ($S_{nic/caf}$) pair considering the PEG-2000-[C$_2$mim]Cl-based ABS and different temperatures of extraction.
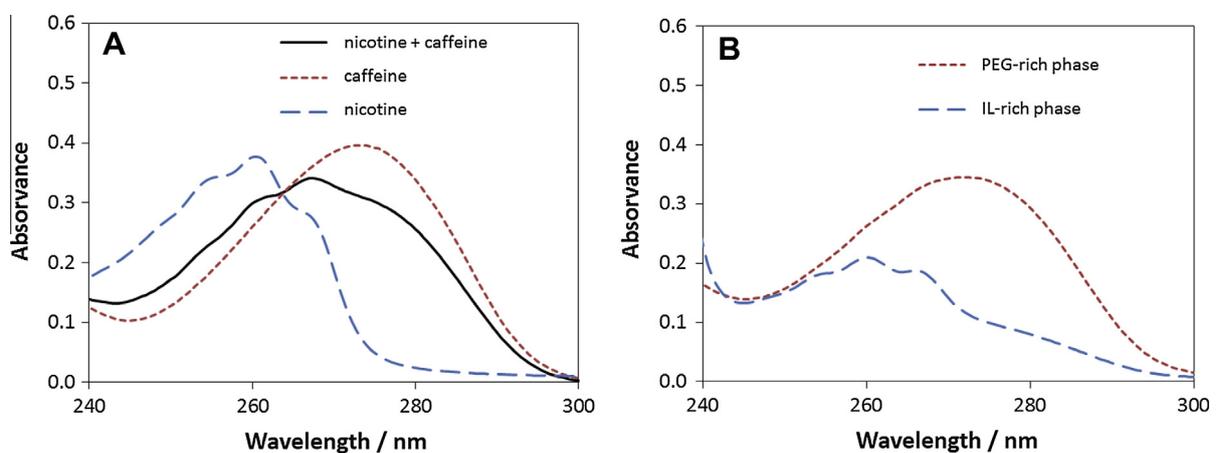

Fig. 6. (A) Spectra of the diluted aqueous solutions of nicotine, caffeine and the mixture nicotine + caffeine. (B) Spectra of the diluted IL- and PEG-rich phases considering the ABS composed of PEG 2000 ($\approx$53 wt.%) + [OHC$_2$mim]Cl ($\approx$27 wt.%), at 323 ($\pm$1) K.

Table 1

Partition coefficients of nicotine (K_{nic}) and caffeine (K_{caf}), and selectivity parameters for the nicotine + caffeine ($S_{nic/caf}$) pair in the ABS composed of PEG 2000 ($\approx$53 wt.%) + [OHC$_2$mim]Cl ($\approx$27 wt.%) at 323 ($\pm$1) K.

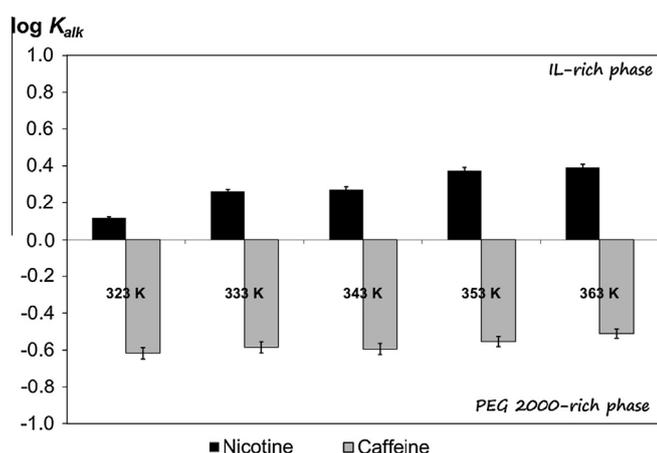| Alkaloid aqueous solution	K_{nic}	K_{caf}	$S_{nic/caf}$
Nicotine	2.67	–	9.90
Caffeine	–	0.27	
Nicotine + caffeine	1.86	0.31	6.01

Fig. 7. Logarithmic function of the alkaloids partition coefficients ($\log K_{alk}$) in the ABS composed of PEG 2000 ($\approx$53 wt.%) + [C$_2$mim]Cl ($\approx$27 wt.%) and different temperatures ($\pm$1) K.

Table 2

Standard molar thermodynamic functions of transfer for nicotine and caffeine, at 323 ($\pm$1) K.

Alkaloid	$\Delta_{tr}H_m^0$ (kJ mol^{-1})	$\Delta_{tr}S_m^0$ (J mol^{-1} K^{-1})	$\Delta_{tr}G_m^0$ (kJ mol^{-1})	$T\,\Delta_{tr}S_m^0$ (kJ mol^{-1})	$\ln K_{alk}$
Caffeine	−5.44	−28.74	3.84	−9.29	−1.43
Nicotine	−14.85	−43.13	−0.91	−13.94	0.34

optimization studies previously performed. These new IL-PEG-2000-based ABS can thus be used as viable separation systems of high selectivity for compounds of the same family or with similar structures if a proper selection of the IL is carried out. Since this is the first time that these polymer-IL-based ABS are applied to the partitioning of alkaloids, the comparison with literature data is difficult. However, several [C$_2$mim]- and [C$_4$mim]-based IL-ABS were previously studied in combination with K$_3$PO$_4$ as partitioning systems for caffeine and nicotine [16]. For these systems much higher partition coefficients were observed, and even complete extractions were verified in the presence of the strong salting-out species, K$_3$PO$_4$ [16]. Nevertheless, if we consider their capability to selectively separate the alkaloids they are by far less efficient than the systems studied in this work (0.93 < $S_{nic/caf}$ (PEG 2000 + IL) < 9.90 and 1.01 < $S_{nic/caf}$ (K$_3$PO$_4$ + IL) < 2.02). The systems here proposed provide a milder extraction but with a far more selective character allowing the separation of very similar biomolecules such the caffeine and xanthine.

The temperature of equilibrium for the alkaloids' separation was also investigated making use of the ABS composed of PEG 2000 + [C$_2$mim]Cl. With this system the partition coefficients of both nicotine and caffeine were determined. The detailed partition coefficients are presented in Supporting information. The gathered results are depicted in Fig. 7.

The data obtained show that an increase in temperature leads to a higher extraction of nicotine for the IL-rich phase and to a decrease on the partition coefficient of caffeine for the polymer-rich phase. Nevertheless, the effect of temperature is more evident in the extraction of nicotine for the IL-rich phase in the 323–363 ($\pm$1) K temperature range.

The preferential partitioning of both alkaloids and their different dependence with temperature has a significant impact in the selectivity values. The selectivity values of nicotine to caffeine as a function of temperature are presented in Fig. 5B. The increase in temperature improves the selectivity of the system with a maximum that seems to be achieved at around 353 ($\pm$1) K, meaning that there is an optimal temperature with the highest selectivity.

For a better understanding of the molecular mechanisms responsible for the migration of the alkaloids between the coexisting phases, the thermodynamic parameters of transfer, namely the standard molar Gibbs energy ($\Delta_{tr}G_m^0$), the standard molar enthalpy ($\Delta_{tr}H_m^0$) and the standard molar entropy of transfer ($\Delta_{tr}S_m^0$) were calculated using Eqs. (2-4) [34,35]. The plots of ln K_{alk} vs. $1/T$ are shown in Supporting information (Fig. A.4). The representation of ln $Kalk$ as a function of $1/T$ is well described by a linear function indicating that the molar enthalpy of transfer for caffeine and nicotine is temperature independent (at least in the temperature range studied in this work).

The thermodynamic parameters of transfer are presented in Table 2. The $\Delta_{tr}G_m^0$ of nicotine is negative and reflects the spontaneous and preferential partitioning of this alkaloid for the IL-rich phase with K_{nic} > 1. On the other hand, for caffeine, the $\Delta_{tr}G_m^0$ is positive – a result of the preferential migration of caffeine for the polymer-rich phase (K_{caf} < 1).

The $\Delta_{tr}H_m^0$ is negative for both alkaloids, suggesting that the transference of nicotine and caffeine from the polymer- to the IL-rich phase is an exothermic process, e.g., an increase in temperature favours the migration for the IL-rich phase. Although the partitioning of nicotine is an exothermic process it was verified that an increase in temperature seems to contradict this behaviour. Albeit this trend was unexpected, this lower affinity for the PEG-2000-rich phase with an increase in temperature can be related with the lower water content and more "hydrophobic" character of the PEG-rich phase at higher temperatures [38]. Finally, when comparing the absolute values of $T\,\Delta_{tr}S_m^0$ and $\Delta_{tr}H_m^0$ for the systems with the two alkaloids it can be seen that the enthalpy of transfer is lower for the caffeine migration and slightly higher when considering the nicotine partitioning. Therefore, the enthalpic effects are of more importance in the systems comprising nicotine.

Taking into account all the gathered results it can be concluded that the IL-polymer-based ABS here studied can be used to selectively separate similar alkaloids. These ABS can be envisaged as potential separation platforms to be applied in CCC techniques.

4. Conclusions

In this work, alternative IL-PEG-2000-based ABS were investigated as novel extractive systems aiming at gathering a selective separation of similar solutes. To such an aim it was studied the effect of the IL chemical structure and temperature of equilibrium. The preferential partitioning of three alkaloids (caffeine, xanthine and nicotine) was addressed by means of the measured partition coefficients and calculated selectivity values. In almost all examples it was observed a preferential concentration of caffeine into the PEG-2000-rich phase whereas nicotine and xanthine preferentially migrate to the (opposite) IL-rich phase. Therefore, despite their chemical similarities, the three alkaloids suffer different partitioning trends between the coexisting phases of the studied ABS. Selectivity values of xanthine in respect to caffeine up to 19 were attained with the system composed of [C$_2$mim][CH$_3$CO$_2$]. The promising results here obtained show that polymer-IL-based ABS

allow the selective separation of similar structures by a proper manipulation of the ILs chemical structure and temperature of equilibrium, and can be envisaged as potential platforms to be applied in CCC.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.seppur.2013.04.004.

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