

# High-performance extraction of alkaloids using aqueous two-phase systems with ionic liquids†

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**Ionic-liquid-based aqueous two-phase systems are great candidates for the replacement of volatile organic compounds in typical liquid–liquid extractions. This work shows clear evidence for the complete extraction of alkaloids such as caffeine and nicotine using a single-step procedure.**

The use of doping agents by athletes to improve athletic performance is a major concern in endurance sports. The International Olympic Committee listed caffeine and nicotine, two easily obtainable alkaloids (see Fig. 1), as stimulant, ergogenic and restricted drugs.<sup>1–3</sup> Although not prohibited by the World Anti-Doping Agency, their use is limited to specific levels. For instance, in the case of caffeine, threshold urinary levels of 12  $\mu\text{g mL}^{-1}$  have been established by several sports federations.<sup>1–3</sup> Levels above such a threshold are viewed as being achieved through a deliberate attempt at doping by the athlete. Drugs and/or metabolites in human fluid samples are usually identified by mass spectrometry.<sup>4–5</sup> Although most analyses are qualitative, in some cases quantitative determinations are required (e.g. caffeine, ephedrine and opium alkaloids). Quantitative determinations are regularly accomplished by chromatographic and spectroscopic methods, which inherently require the pre-treatment of samples by the application of extraction methods (mainly to increase the metabolites and drug concentration from the original sample); later, if appropriate, hydrolysis and/or derivatization steps are performed.<sup>4–5</sup> There is already

evidence that, for instance, the direct quantification of opium alkaloids, testosterone and epitestosterone in ionic-liquid-rich phases using high performance liquid chromatography (HPLC) is possible, and no interferences with the ionic liquid were found.<sup>6–7</sup>

Aqueous two-phase systems or aqueous biphasic systems (ABS) are composed of two immiscible phases, both of which are water-rich phases. ABS constitute a “greener” and potentially more efficient pre-treatment solution in liquid–liquid extractions. ABS were first introduced<sup>8</sup> in the eighties and have since been applied for separation, fractionation, and molecular characterization of biological macromolecules. In the past few years, ABS have been intensely explored for the recovery of metal ions, radiochemicals, dyes, drug molecules, small organic species and inorganic particles.<sup>9–11</sup>

Recently, Rogers and co-workers<sup>12</sup> have demonstrated the ability of ionic liquids (ILs) to induce ABS in the presence of inorganic salts. Attempts to extract (bio)molecules and/or drugs using such IL-based ABS are rare: merely short chain alcohols,<sup>12</sup> phenol,<sup>13</sup> bovine serum albumin,<sup>14–15</sup> testosterone,<sup>6</sup> epitestosterone,<sup>6</sup> penicillin G,<sup>16</sup> opium alkaloids,<sup>7</sup> L-tryptophan,<sup>17–19</sup> and antibiotics<sup>20</sup> have been investigated as partitioning solutes. Efforts to extract alkaloids such as caffeine, nicotine and opium alkaloids using IL-based ABS may be highly relevant as a new approach in liquid–liquid extraction trials. To the best of our knowledge, we report here for the first time, a remarkably strong ability of IL-based ABS to extract caffeine and nicotine and show that such drugs can, in a single-step procedure, be completely extracted from one aqueous phase to another. Therefore, there is potential both for achieving highly concentrated samples of alkaloids for further quantitative analysis and for engineering strategies to obtain alkaloid-free matrixes. We show that due to the very high concentration effect attained by using IL-based ABS on alkaloid-containing solutions, human fluid samples can, for example, be effortlessly checked for their alkaloid content. In addition, the required quantities of ionic liquid can be reduced to very low levels.

Most previous studies<sup>6–7,13–16</sup> have focused on just one ionic liquid (usually  $[\text{C}_4\text{mim}]\text{Cl}$  or  $[\text{C}_n\text{mim}]\text{Br}$  with  $n = 4, 6$  and  $8$ ) and on the influence of several inorganic salts in phase diagrams (where the ion's influence follows the well-known Hofmeister series<sup>21–23</sup>). However, the efficiency of IL-based ABS extractions should also be planned taking into account the correct choice of the anion and/or cation that make up the ionic liquid. Therefore, in this communication, we focus on testing the marked ability of several distinct ionic liquids as constituents of the extraction media—both in aqueous phases and more

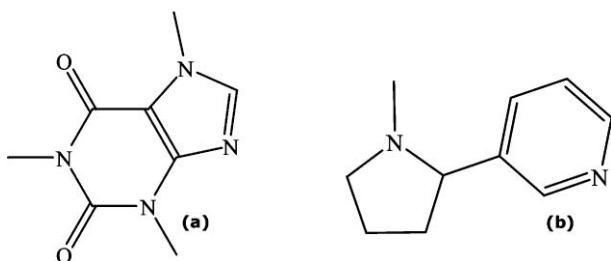


Fig. 1 Molecular structures of caffeine (a) and nicotine (b).

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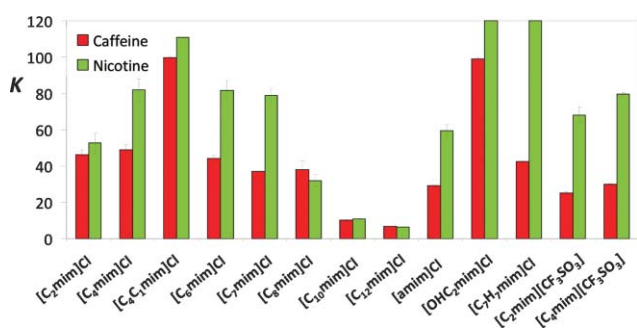
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† Electronic supplementary information (ESI) available: Experimental procedure, extraction efficiencies and tie-lines and tie-lines' length for the investigated ternary systems. See DOI: 10.1039/c0gc00179a

complex matrixes, such as human urine-type samples—and on the influence of ionic liquid/inorganic salt concentrations. To this end, the partition coefficients,  $K$ , of two main alkaloids—caffeine and nicotine—between an aqueous IL-rich phase and an aqueous  $K_3PO_4$ -rich phase were measured. The partition coefficients of caffeine ( $K_{caf}$ ) or nicotine ( $K_{nic}$ ) are defined as the ratio between the concentration of the alkaloids in the IL- and  $K_3PO_4$ -rich phases. Details regarding equilibration time and experimental procedures, as well as the mapping of ternary phase behaviour, can be found elsewhere.<sup>17–18,24–25</sup> Rather than attempting to provide the partition coefficients of both alkaloids, this work is focused on providing a comprehensive emphasis on the improved ability of ionic liquids as extraction media.

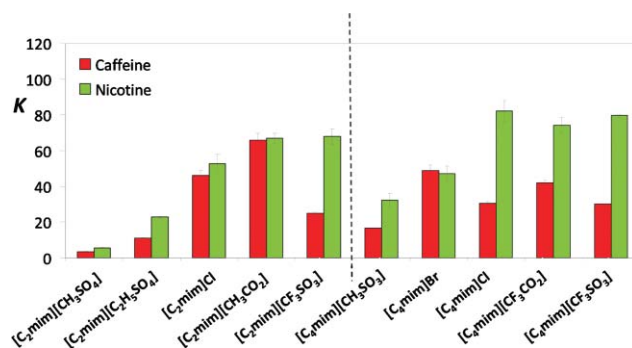
The influence of both ionic liquid cation and anion in extracting caffeine and nicotine while maintaining the same inorganic salt ( $K_3PO_4$ ) for ABS formation, as well the composition in the ternary phase diagrams, have been evaluated. We have used several ionic liquids [a list, including a definition of their acronyms, is provided as a footnote<sup>‡</sup>; details on their purification and purity control are found in the Electronic Supporting Information (ESI)<sup>†</sup>] to assess the impact of their chemical nature on the study. The quantification of the alkaloids in both phases was carried out by UV spectroscopy. Initial concentrations of caffeine and nicotine for phase distribution at the water ternary composition were, respectively,  $2.6 \times 10^{-2} \text{ mol}\cdot\text{dm}^{-3}$  and  $2.5 \times 10^{-2} \text{ mol}\cdot\text{dm}^{-3}$ . Possible interferences of the inorganic salt, ionic liquid or urea with the analytical method were taken into account and blank control samples were used whenever necessary. The partition coefficients and respective standard deviations are the result of at least three independent determinations. The partition coefficients of caffeine and nicotine in the ABS containing  $[C_n\text{mim}]\text{Cl}$ ,  $[C_4C_1\text{mim}]\text{Cl}$ ,  $[\text{amim}]\text{Cl}$ ,  $[\text{OHC}_2\text{mim}]\text{Cl}$ ,  $[\text{C}_7\text{H}_7\text{mim}]\text{Cl}$  and  $[C_n\text{mim}][\text{CF}_3\text{SO}_3]$ , at 298 K and at atmospheric pressure, are depicted in Fig. 2. The partition coefficients at the same temperature for  $[C_2\text{mim}]$ - and  $[C_4\text{mim}]$ -based ILs containing distinct anions<sup>‡</sup> are presented in Fig. 3.



**Fig. 2** Partition coefficients,  $K$ , of caffeine and nicotine in different ILs/ $K_3PO_4$  ABS at 298 K. All ABS are based on chloride or triflate ionic liquids and contain 25 wt% of ionic liquid and 15 wt% of  $K_3PO_4$ , except in the case of  $[\text{OHC}_2\text{mim}]\text{Cl}$  in which its concentration is 40 wt%.

The efficiency of the extractions, reported in detail in the ESI<sup>†</sup>, show that complete extraction (no detection of alkaloid in the  $K_3PO_4$ -rich phase) of each alkaloid is attained at a partition coefficient greater than *circa* 120.

In previous works,<sup>17–19</sup> we have demonstrated that the addition of solutes at infinite dilution to the ternary ABS does not have



**Fig. 3** Partition coefficients,  $K$ , of caffeine and nicotine in different ILs/ $K_3PO_4$  ABS at 298 K. All ABS are based on 1-ethyl-3-methylimidazolium (left) or 1-butyl-3-methylimidazolium (right) ionic liquids and contain 25 wt% of ionic liquid and 15 wt% of  $K_3PO_4$ . The different anions were ordered according to a Hofmeister-like series.<sup>18</sup>

a significant impact on the tie-lines and tie-line lengths (tie-lines and tie-line lengths are presented in the ESI<sup>†</sup>).

From the results depicted in Fig. 2 and 3, it is clear that nicotine presents higher partition coefficients compared to caffeine in basically all the studied ionic liquids-based systems. This feature mirrors that of the affinity for organic-rich phases, *i.e.*, similar behaviour is displayed in octanol-water partition coefficients ( $K_{ow}$ ), where reported  $K_{ow}$  values<sup>26</sup> for caffeine and nicotine are, respectively, 0.85 and 14.79. Although both compounds are water soluble, nicotine's methyl-pyrrolidine ring accounts for its less-polar character and its concomitant affinity for organic phases. On the contrary, the caffeine purine ring, with two attached oxygen atoms, contributes to the compound's polarity, and thus, to a lower affinity for organic fluids. However, one should note that these ( $K_{ow}$ ) values are many times smaller than the  $K$  values reported here. Part of the difference may be attributed to the fact that ABS include a  $K_3PO_4$ -rich aqueous phase where the salting-out effect of the inorganic salt promotes the extraction of the alkaloids to the other (IL-rich) phase. Nevertheless, the presence of the ionic liquid also plays an important role in the process, as can be seen in Fig. 2 and 3, with  $K$  values ranging from 3 to complete extraction.

In general terms, the influence of the ionic liquid on the complete extraction of the alkaloids depends much more on the nature of the cations than that of the anions. The results seem to indicate that extractions are driven by different factors including: a) hydrogen-bond interactions between the non-bonding electron pairs present in the oxygen and nitrogen atoms of the alkaloids; b) acidic hydrogen atoms present in the cations of the ionic liquid; c)  $\pi \cdots \pi$  interactions between the aromatic part of the solutes and the imidazolium cation; and d) dispersive-type interactions between alkyl groups of the alkaloids and the alkyl side chains of the imidazolium-based ions. Examples of such factors at work can be noticed in systems like  $[\text{C}_7\text{H}_7\text{mim}]\text{Cl}$  (enhanced aromatic interactions) or  $[\text{OHC}_2\text{mim}]\text{Cl}$  (enhanced hydrogen-bonding capabilities) that have performed specially well. The effect of the length of the alkyl side chain can be seen in the imidazolium-based IL series (left of Fig. 2), with the  $K$  values achieving a maximum at  $[C_4C_1\text{mim}]\text{Cl}$ , followed by a decrease to  $[C_{12}\text{mim}]\text{Cl}$ . This can be rationalized in terms of the competition between the need to accommodate the non-polar

parts of the alkaloid solutes and the progressive dilution of the ionic part of the ionic liquid as one progresses along the family.<sup>27</sup>

Concerning the influence of the ionic liquid anion, there seems to be a close relation to the ionic liquid salting-in/-out behaviour. From a molecular point of view, salting-in/-out effects can be understood as a delicate balance between the interactions of the two salts (ionic liquid and  $K_3PO_4$ ) and the solvent (water). Previously<sup>18</sup> we have demonstrated the relationship between the ionic liquid anion hydrogen-bonding accepting ability and the ionic liquid ABS formation capability, and we have ranked the ionic liquid anions according to their salting-in/-out inducing capacity (Hofmeister-like series). Generally, and as shown in Fig. 3, salting-in inducing anions such as  $[CF_3SO_3]^-$  are more efficient at extracting solutes from a second liquid phase than salting-out inducing anions, such as  $[CH_3SO_3]^-$ ,  $[CH_3SO_4]^-$  and  $[C_2H_5SO_4]^-$ .

Fig. 2 and 3 present the results for a fixed ternary system composition (ionic liquid at 25 wt% and  $K_3PO_4$  at 15 wt%). Yet we have extended the study of the ILs' extraction ability through a range of ionic liquid concentrations. Fig. 4 and 5, illustrate the effect of increasing the ionic liquid mass fraction in the partition coefficients of caffeine and nicotine, respectively.

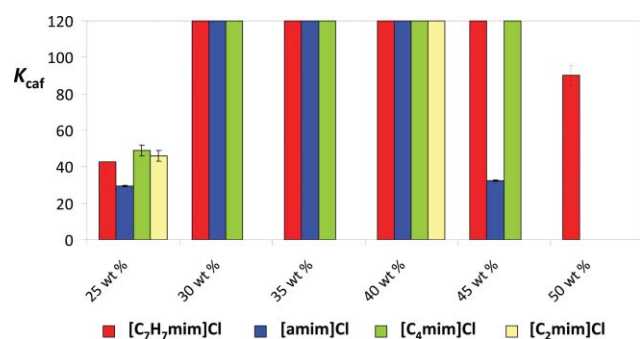


Fig. 4 Partition coefficients of caffeine in different ILs/ $K_3PO_4$  ABS at 298 K, with  $K_3PO_4$  at 15 wt% and different wt% of ionic liquid.

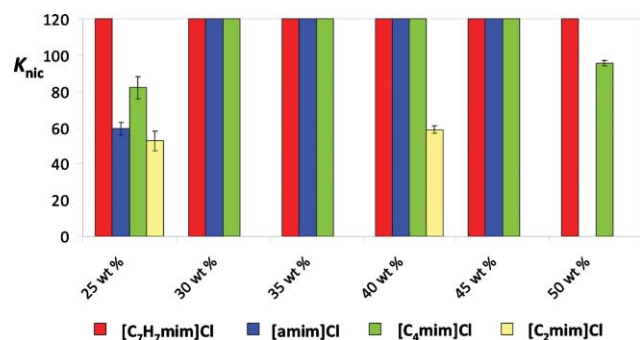


Fig. 5 Partition coefficients of nicotine in different ILs/ $K_3PO_4$  ABS at 298 K, with  $K_3PO_4$  at 15 wt% and different wt% of ionic liquid.

The results show that an increase of 5 wt% of ionic liquid in the overall system leads to the complete extraction of both alkaloids. In this sequence, the ionic liquid  $[OHC_2mim]Cl$  was also studied at 40 wt% with 15 wt% of  $K_3PO_4$ , and also showed complete extraction of nicotine and a high extraction efficiency for caffeine. The results indicate that there seems to be an optimum value for the ionic liquid concentration above which

the partition coefficients start to decrease. From a molecular perspective, this maximum of partition coefficients is probably related to salting-out effects of the ionic liquid over the solute. At high concentrations of ionic liquids, competition begins between the inorganic salt ions and the ionic liquid ions to salt-out such solutes. At specific concentrations, ionic liquid ions start to salt-out caffeine and nicotine to the aqueous  $K_3PO_4$ -rich phase. Curiously, the trend observed (the concentration at which each ionic liquid starts to promote salting-out) intrinsically follows the cation sequence previously observed<sup>17</sup> in respect to their salting-in/-out ability.

In the case of the system containing  $[C_7H_7mim]Cl$ , the mass fraction of  $K_3PO_4$  was further adjusted from 15 wt% to 30 wt% in order to evaluate the inorganic salt's impact on the partitioning coefficients of nicotine and caffeine. All the results (not shown graphically but given in the ESI†) led to the complete extraction of the alkaloids. This behaviour corroborates the previous results, and no maximum in the partition coefficients was found—the inorganic salt is a much stronger salting-out agent than the ionic liquid is. For economical purposes the ionic liquid can be maintained at 25 wt% while increasing the inorganic salt concentration aiming at completely extracting both alkaloids from aqueous phases.

After the fine-tuning of ionic liquids and respective mass fraction compositions, the direct extraction of alkaloids from a synthetic biological sample—artificial human urine (with a composition described in ESI†)—was further evaluated. Quantitative extraction of caffeine and nicotine into the IL-rich phase from complex matrixes of artificial human urine is displayed in Fig. 6 and 7. Partition coefficients obtained when

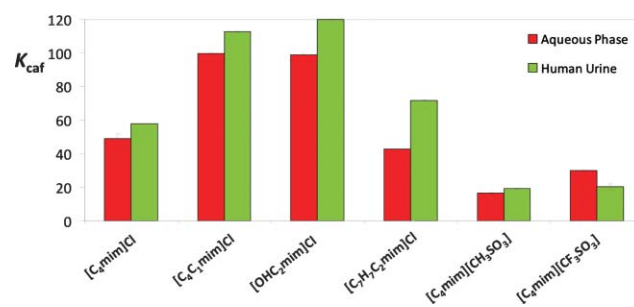


Fig. 6 Partition coefficients of caffeine in different ABS at 298 K. All ABS contain 25 wt% of ionic liquid and 15 wt% of  $K_3PO_4$ , except the case of  $[OHC_2mim]Cl$  in which its concentration is 40 wt%.

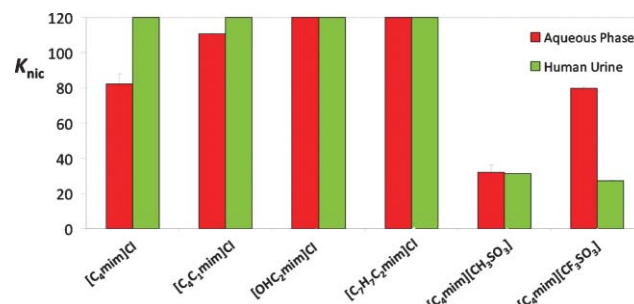


Fig. 7 Partition coefficients of nicotine in different ABS at 298 K. All ABS contain 25 wt% of ionic liquid and 15 wt% of  $K_3PO_4$ , except the case of  $[OHC_2mim]Cl$  in which its concentration is 40 wt%.

employing simple aqueous phases, as previously shown, are also included for comparison.

Aiming at isolating the IL cation and anion contributions, ionic liquids based on several combinations were used to perform the extraction experiments with human urine samples. The results show that with human urine samples the extraction performances of both alkaloids are significantly improved. The presence of a more complex matrix, which now includes NaCl and urea, favours the alkaloids partitioning for the IL-rich phase. Indeed, there are particular examples showing complete extraction of both alkaloids which were not previously observed with simpler aqueous phases. The main exception is the system containing the  $[C_4mim][CF_3SO_3]$  ionic liquid, where a decrease in  $K$  was observed for the two partitioning solutes (nicotine and caffeine). It must be stressed that in this case the IL-rich phase is much more concentrated in ionic liquid, as compared with the other studied ABS (see tie-lines in the ESI†). This fact—the relative water depletion in the IL-rich phase—may explain the anomalous trend between the aqueous solutions with and without urea. The poorer hydration of solutes and ions at the IL-rich phase with  $[C_4mim][CF_3SO_3]$  involves a higher competition between water-ion, water-urea and water-alkaloid interactions.

We have shown that complete extractions of archetypal alkaloids using IL-based aqueous biphasic systems (ABS) have been successfully accomplished, and thus, new applications for analytical purposes are immediately envisaged. The method also opens new views for the separation and concentration of other bioactive drugs. Compared to conventional liquid–liquid or solid–liquid extractions, this new approach avoids the use of volatile organic solvents, replacing them with relatively small amounts of (recyclable) ionic liquid solvents in a second aqueous phase.

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## Notes and references

‡ IL cations: 1-alkyl-3-methylimidazolium ( $[C_nmim]^+$  with  $n = 2, 4, 6, 7, 8, 10$  and  $12$ ), 1-butyl-2,3-dimethylimidazolium ( $[C_4C_1mim]^+$ ), 1-allyl-3-methylimidazolium ( $[amim]^+$ ), 1-hydroxyethyl-3-methylimidazolium ( $[OHC_2mim]^+$ ) and 1-benzyl-3-methylimidazolium ( $[C_7H_2mim]^+$ ); IL anions: chloride ( $Cl^-$ ), bromide ( $Br^-$ ), methylsulfate ( $[CH_3SO_4]^-$ ), ethylsulfate ( $[C_2H_5SO_4]^-$ ), acetate ( $[CH_3CO_2]^-$ ), trifluoroacetate ( $[CF_3CO_2]^-$ ), methanesulfonate ( $[CH_3SO_3]^-$ ) and triflate ( $[CF_3SO_3]^-$ ). All the ILs were commercially acquired at Iolitec, with the exception of  $[C_4mim][CF_3CO_2]$  which was purchased from Solchemar.

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