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Selective partition of caffeine from coffee bean and guaraná seed extracts using alcohol–salt aqueous two-phase systems

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ABSTRACT

This work proposes the application of flexible alcoholic aqueous two-phase systems to manipulate the partition of caffeine. An optimization study was performed the caffeine partitioning towards top phase (60.0 wt% 2-propanol + 20.0 wt% K_3PO_4 at 303 K – K_{caf} = 3.4 and R_T = 89.1%) and bottom phase (60.0 wt% methanol + 17.5 wt% K_2HPO_4/KH_2PO_4 at 278 K – K_{caf} = 0.06 and R_B = 81.1%). These processes were evaluated by applying them to real biomass as guaraná seeds (purification factor (PF) = 6.59-fold) and coffee beans (PF = 3.24-fold) for the 2-propanol and K_2HPO_4/KH_2PO_4 system.

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Introduction

Caffeine is chemically known as 1,3,7-trimethylxanthine or 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione ($C_8H_{10}N_4O_2$). This alkaloid belongs to the xanthine group, being found in several edible plants, such as green tea leaves (*Camellia sinensis*),[1] guaraná seeds (*Paullinia cupana*)[2] and coffee beans (*Coffea canephora, Coffea arabica*).[3]

Extensive research has been carried over the years on the extraction of caffeine, which may serve the purposes of obtaining decaffeinated foods and to obtain pure caffeine for other applications, namely in the food and pharmaceutical industries. Many processes have been proposed for the recovery of caffeine from coffee beans and other raw materials. Most extraction processes studied use solid–liquid techniques with the application of organic solvents, such as chloroform, acetone, methanol, ethanol or acetonitrile,[1,4] or alternatively use of supercritical fluids.[5] Other unconventional approaches propose the use of high temperature and ultrasounds,[6] column chromatography,[7] microwave-enhanced vacuum ice water extraction[8] and aqueous solution of ionic liquids (ILs).[9] However, to enhance the purity level of caffeine, a purification step must be applied in most cases to the crude extract. Among the various examples of purification processes applicable are the aqueous two-phase systems (ATPS), extensively studied in the last decades.

ATPS are formed by two immiscible aqueous phases and the main approach used to prepare ATPS is the addition of two water-miscible polymers,[10] or a polymer and an inorganic salt.[11,12] Recently, systems based on ILs and salts,[13–15] organic solvents and salts,[16–19] organic solvents and ILs,[20] besides organic solvents and carbohydrates[21] have been proposed showing the flexibility of these systems that can be easily used to complement the extraction processes by including a fractionation step also designated by purification step. ATPS are considered as an important tool for an efficient fractionation/purification of simple biomolecules, such as alkaloids,[22] food flavours,[23–25] drugs[26–30] and natural dyes,[31] as well as more complex structures, namely enzymes[14,19,32,33] or antibodies.[34,35] However, polymer-based ATPS present high viscosities, which may be a significant concern in the separation process due to the mass transfer problems associated with the biomolecule partition. Moreover, while polymer–polymer ATPS present a limited polarity difference between the phases, thus limiting the partition coefficients of the smaller biomolecules, polymer–salt systems on their side present a too large polarity difference limiting their selectivity. In this context, the present work is carried out considering the use of alcohol–salt ATPS,[36] thus reducing the high viscosities obtained when polymer-based ATPS[33] are applied. In addition, the alcohol-based ATPS are low-cost separation techniques because the recovery of the phase-forming

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alcohols is easily achieved through evaporation and they may present a significant polarity window, for example, when compared with the polymer–polymer ATPS. Although the alcohol–salt ATPS can have limited biocompatibility with some complex biomolecules (such as enzymes, causing their inactivation or even denaturation[37]), for simpler biomolecules, such as phenolic compounds, antioxidants or xanthenes, their negative impact in the molecules structures is not so pronounced.

The present work addresses the use of alcohol–salt ATPS for the recovery of caffeine from guaraná seeds and coffee beans. The optimization of the caffeine partition between the two phases, the alcoholic (methanol, ethanol, 1-propanol and 2-propanol) top phase and the saline (potassium phosphate salts, namely K_3PO_4, K_2HPO_4 and potassium phosphate buffer composed of K_2HPO_4/KH_2PO_4) bottom phase, was first studied. The concentration of each one of the ATPS components (tie-lines composition) and the temperature of the partition systems, between 278 and 308 (±1 K), were the parameters investigated. The potential of these alcoholic-based liquid–liquid extraction systems promoting the migration of caffeine (commercial standard) for the alcohol-rich phase or its re-concentration into the salt-rich phase, simply by the manipulation of the conditions described above, was successfully achieved. After the optimization process, the best operational conditions were applied in the extraction and purification of caffeine from two different sources, namely coffee beans and guaraná seeds, one of the most abundant natural sources of caffeine found in Brazil, aiming to demonstrate the potential of these systems to manipulate the caffeine partition.

Materials and methods

Materials

Caffeine with purity higher than 99.5 wt% was purchased at José M. Vaz Pereira, SA (Lisboa, Portugal). The alcohols (methanol, ethanol, 1-propanol, 2-propanol) and inorganic salts (potassium phosphate, K_3PO_4, potassium hydrogen phosphate, K_2HPO_4, and potassium dihydrogen phosphate, KH_2PO_4) were obtained from Vetec (Rio de Janeiro, Brazil). The alcohols and salts present a purity level higher than 98 wt%. Guaraná seeds and coffee beans were purchased at a local market in Aracaju-Sergipe, Brazil. Ultrapure water that was double-distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus was used.

Caffeine partition in model systems

The model systems studied are comprised of alcohols (methanol, ethanol, 1-propanol and 2-propanol) and aqueous solutions of potassium phosphate salts (K_3PO_4, K_2HPO_4 and the phosphate buffer composed of two inorganic salts K_2HPO_4 and KH_2PO_4) (pH 7.0; Henderson-Hasselbalch equation equivalents = 1.087).

The partition systems were prepared in graduated centrifuge tubes (15 mL) by weighting the appropriate amounts of alcohol (40–60 wt%) and potassium phosphate salts (10–20 wt%), as described by Lima and coworkers,[24] and a caffeine aqueous solution (10 mg·L^{-1}) in a total mass of 14.0 g. The mixtures were then gently stirred and centrifuged at 2000 rpm for 10 min at 278–308 K. The graduated tubes were placed at the respective temperature, between 278 and 308 (±1 K) and atmospheric pressure, using a thermostatic bath MARCONI MA-127, for at circa 12 h, ensuring that the thermodynamic equilibrium is reached and guaranteeing the complete caffeine partition. The vials were closed during this period to prevent alcohol evaporation. The two phases were then carefully collected for the determination of their volume and weight, and the caffeine was quantified in the top phase (in triplicate) using a Varian Cary-50 Bio UV-visible Spectrophotometer, at the wavelength of 279 nm. The respective calibration curve was properly established for the wavelength considered and the mass balance of caffeine was used to determine the caffeine concentration in the bottom phase due to the phosphate salt interference in the caffeine determination in the lower layer. The caffeine concentration described is thus the average of the caffeine concentration determined without any dilution being applied.

It should be remarked that for all ATPS studied the top phase was the alcohol-rich phase while the bottom phase corresponds to the salt-rich phase.

The caffeine partition coefficient (K_{caf}) was defined as the ratio between the concentration of caffeine (C_{caf}) in the top (T) and bottom phases (B) (Eq. (1)). In order to evaluate the partition process, the volume ratio (R_v) and the respective caffeine recovery for the top and bottom phases (R_T and R_B %) were estimated according to literature[19,38] and following Eqs. (2)–(4).

$$K_{caf} = \frac{C_{cafT}}{C_{cafB}} \quad (1)$$

$$R_v = \frac{V_T}{V_B} \quad (2)$$

$$R_T = \left(\frac{100}{1 + \frac{1}{K_{caf} \times R_v}} \right) \quad (3)$$

$$R_B = \frac{100}{1 + K_{caf} \times R_v} \quad (4)$$

where C is the concentration of caffeine, V is the phase volume, and T and B correspond to the top and bottom phases, respectively.

At least three individual ATPS for each condition studied were prepared, the partition coefficient and recovery results being presented as an average value and the uncertainty associated to both parameters being less than 5.0%.

The temperature effect in the caffeine partition process was also evaluated. The respective thermodynamic parameters of phase transfer, such as the standard molar Gibbs energy ($\Delta_{tr}G°_m$ – kJ·mol^{-1}), the standard molar enthalpy ($\Delta_{tr}H°_m$ – kJ·mol^{-1}) and the standard molar entropy of transfer ($\Delta_{tr}S°_m$ – J·mol^{-1}·K^{-1}), were calculated according to Eqs. (5)–(7):

$$\ln(K_{caf}) = -\frac{\Delta_{tr}H^0_m}{R} \times \frac{1}{T_{ref}} + \frac{\Delta_{tr}S^0_m}{R} \quad (5)$$

$$\Delta_{tr}G^0_m = \Delta_{tr}H^0_m - T_{ref}\Delta_{tr}S^0_m \quad (6)$$

$$\Delta_{tr}G^0_m = -RT_{ref}\ln(K_{caf}) \quad (7)$$

where R is the universal gas constant (8.314 J·mol^{-1}·K^{-1}) and T_{ref} is the temperature of reference (±1 K).

Recovery and purification of caffeine from guaraná seeds and coffee beans

The selective recovery of caffeine was carried out using two natural sources widely available in Brazil: guaraná seeds and coffee beans. The compositions of the preferred ATPS to selectively fractionate the caffeine from the remaining contaminants were chosen considering the best results of partition coefficients and recovery identified in the optimization study aiming at the recovery of caffeine. In this context, the system composed of 60.0 wt% of 2-propanol + 20.0 wt% of K_3PO_4 + 20.0 wt% of H_2O was used aiming to promote the migration of caffeine to the top phase, while the system composed of 60.0 wt% of methanol + 17.5 wt% of potassium phosphate buffer + 22.5 wt% of H_2O was able to manipulate the migration of caffeine to the salt-rich phase.

Around 1.07 ± 0.04 g of coffee beans and 0.309 ± 0.002 g of guaraná seeds were dispersed in 30 mL of aqueous solutions of alcohol (2-propanol or methanol at 60.0 wt%) and then incubated at 278 and 303 (±1 K) for 12 h, depending on the system applied. The alcoholic-based extracts prepared rich in caffeine were then filtered (0.42 μm) to eliminate the bean/seed particles. The inorganic salts (K_2HPO_4/KH_2PO_4 at 17.5 wt% or K_3PO_4 at 20.0 wt%) and water were then added to prepare the respective ATPS in the required concentrations (total mass of 14 g). The mixtures were gently stirred for 5 min and centrifuged at 2000 rpm at 298 or 303 K for 10 min. The extraction systems were allowed to reach the equilibrium during 12 h at least. The vials were closed during this period to prevent alcohol evaporation. Finally, both phases were carefully separated and weighed, the volume of each phase was measured and the caffeine was quantified in the top phase. The caffeine quantification was carried out as previously described.

Before calculation of the purification factor (PF), the analysis of the total phenol content (PC) in the extract of coffee beans and guaraná seeds was assessed and quantified in each phase by spectrophotometry using the colorimetric Folin–Ciocalteu test, using gallic acid as standard.[39] Then, the PF_{caf} was determined by the ratio between the specific concentration of the caffeine (SC_{caf}) extract and in each phase (subscribe T for top and B for bottom) according to Eqs. (8) and (9), respectively.

$$SC_{caf} = \frac{C_{caf}}{C_{PC}} \quad (8)$$

$$PF_{caf} = \frac{(SC_{caf})_{TorB}}{(SC_{caf})_E} \quad (9)$$

Results and discussion

This work studies the potential of alcohol–salt ATPS in the purification of alcoholic caffeine extracts. Since one of its objectives is to evaluate the possibility of manipulating the migration of caffeine from an alcoholic to an aqueous phase, the results will be analysed through the recovery of the biomolecule for the top and bottom phase, taking into account its concentration in the alcohol- and salt-rich phases, respectively. The use of these systems as potential platforms of caffeine recovery from biomass or food wastes sources is thus envisaged. The concentration of caffeine used was the same for all experiments (10 mg·L^{-1}), independently of the conditions under investigation. Thus, different parameters were optimized, namely the alcohol, the phosphate salt type and concentration and, finally, the temperature of the partition processes.

Influence of the ATPS components on the caffeine partition

The effect of the alcohol (methanol, ethanol, 1-propanol and 2-propanol) and potassium phosphate salt (K_3PO_4, K_2HPO_4 and K_2HPO_4/KH_2PO_4) in the caffeine

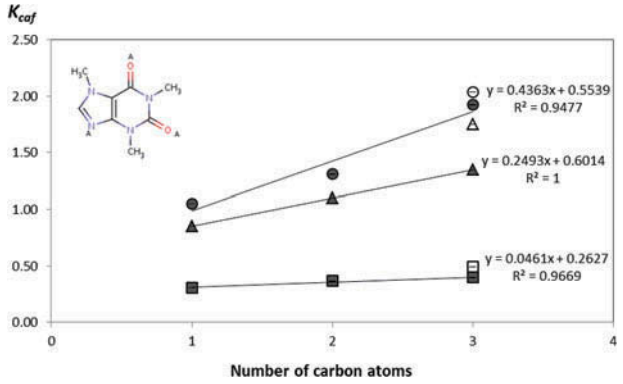

Figure 1. Influence of the alcohol (number of carbon atoms) on the K_{caf}: (1) methanol, (2) ethanol and (3) 1-propanol or 2-propanol (open symbol) conjugated with the salts. (●) K_3PO_4, (▲) K_2HPO_4 and (■) K_2HPO_4/KH_2PO_4 for the ATPS composed of 50.0 wt% of alcohol + 15.0 wt% of salt + 35.0 wt% of H_2O (at 298 ± 1 K).

partition was first evaluated (Table 1). To assess the partition coefficients experimentally obtained for caffeine, the quantification method was carefully evaluated, considering the possible effect of the ATPS components, the influence of the type of salt and the pH values on the caffeine quantification (see supplementary Table S1) in each system.[24] Because the caffeine partition is pH independent since its charge does not change with the pH (see supplementary Figure S1), a blank system (without caffeine) was prepared for each system studied to eliminate the possible interferences in caffeine quantification. The effect of the alcohol on the partition is reported in Fig. 1 (see supplementary Tables S2 and S3). The ATPS were prepared using 50.0 wt% of alcohol + 15.0 wt% of the inorganic salt + 35.0 wt% of a caffeine aqueous solution (≈10 mg·L^{-1}). The partition coefficient of a biomolecule is the result of several competing interactions between the biomolecule and the ATPS phase formers. Moreover, distinct phenomena besides the interaction between the target biomolecule and the system components must also be considered,[26] namely the two-phase polarity and the salting-out effect of the inorganic salt.[22]

The results reported in Fig. 1 show that there is an effect of the size of the alcohol and the inorganic salt on the caffeine partition. In what concerns the salt effect, the K_{caf} seems to closely follow the Hofmeister series,[40] K_3PO_4 > K_2HPO_4 > K_2HPO_4/KH_2PO_4, as observed in the partition of other biomolecules.[41] The alcohol also seems to have an influence on the K_{caf}, which increases with the alcohol size, this effect being more pronounced when conjugated with stronger salting-out species (K_2HPO_4 and K_3PO_4). Besides the increase in the hydrophobicity resulting from the increase in the alkyl chain of the alcohol, and its impact on the partition coefficients of the biomolecules,[14, 33] in our case the influence of the caffeine solubility in the alcohol-rich phase by the increase of the "alcohol–water" interactions is also an important issue.[17] Wang and coworkers[17] described that the "ion–dipole" interactions between the salt ions and the water molecules are responsible for a better ionic hydration, and that the phase-forming salts can be dissolved in the bottom phase. Meanwhile, the above interactions decrease the amount of free water molecules in the aqueous phase, leading to the exclusion of the alcohol and caffeine from the lower phase. In the same way, the authors explain that a large amount of a hydrophilic alcohol (*e.g.* methanol) in the alcohol-rich phase will interact with the water molecules, influencing the solubility of the target biomolecule in this system. This behaviour is described by the dielectric constants of the alcohols investigated: methanol (33.30) > ethanol (25.02) > 1-propanol (20.33) > 2-propanol (18.22).[42,43] This parameter represents the capacity of a material to be "polarized" by an environment imposition, with this "induced-polarization" being responsible for the increased alcohol solubility in water (*e.g.* methanol). Consequently, the caffeine solubility in the alcohol-rich phase is decreased,[26] therefore justifying the lower K_{caf} for the alcohol-rich phase investigated. The dielectric constant can also justify the partition coefficient behaviour of both propanol isomers. The 2-propanol shows a slightly higher K_{caf} when compared with the 1-propanol results (which is independent of the inorganic salt used) since the higher solubility of 1-propanol in water described by its higher dielectric constant is also responsible for its lower affinity with caffeine. Despite the lack of studies using alcohol–salt ATPS to extract caffeine,

Table 1. Experimental results of K_{caf} (uncertainty < 5.0%) and recovery data for both phases (uncertainty < 8.0%), top (R_T) and bottom phases (R_B) for all the systems investigated composed of 50.0 wt% of alcohol + 15.0 wt% of salt + 35.0 wt% of a caffeine aqueous solution, at 298 ± 1 K.

	K_3PO_4			K_2HPO_4			K_2HPO_4/KH_2PO_4		
Alcohol	K_{caf}	R_T (%)	R_B (%)	K_{caf}	R_T (%)	R_B (%)	K_{caf}	R_T (%)	R_B (%)
Methanol	1.05	84.4	15.6	0.85	81.7	18.8	0.30	60.4	40.1
Ethanol	1.31	83.4	16.7	1.10	82.8	17.3	0.36	55.1	45.2
1-Propanol	1.92	81.8	18.3	1.35	75.2	24.5	0.40	56.2	44.2
2-Propanol	2.04	83.7	16.5	1.75	84.6	14.7	0.49	59.5	40.7

Coutinho and coworkers[22] have reported the use of IL-based ATPS to separate the same biomolecule, where caffeine appears concentrated in the (more organic) IL-rich phase (K_{caf} = 120).

Despite the lowest K_{caf} reported in Fig. 1, one of the advantages of these ATPS is their versatility and capacity to manipulate the caffeine partition, which is not verified using IL-based ATPS,[22] where all the K_{caf} obtained were higher than 1, indicating the migration of caffeine for the (IL)-rich phase. Using the alcohol-based ATPS, it is possible to induce the migration of caffeine for either the top or bottom phases only by adjusting the components of the system. This tailored ability is a peculiar characteristic of these alcohol-based methodologies with a large potential for the selective extraction or purification processes of caffeine, or other biomolecules, from different natural sources (*e.g.* processed food and/or fruit raw materials). In this context, in the following two sections, the manipulation of the alcohol–salt ATPS to optimize the caffeine partition for each of the two phases, by changing temperature, type and concentration of the inorganic salt and the alcohol, is presented and further discussed.

Caffeine partition into the alcohol-rich phase

In this section, the best conditions to promote the partition of caffeine towards the alcohol-rich phase were checked by changing (increasing) the tie-line length, which consequently changed the mass composition of each one of the phases, thus maximizing the favourable interactions between the alcohol and caffeine (addition of higher amounts of alcohol) or increasing the salting-out nature of the bottom phase (addition of higher amounts of salt). In terms of experimental procedure, both the amount of the inorganic salt and alcohol used in the ATPS formation were increased. This optimization is proposed since the recovery of pure caffeine from the alcohol rich-phase is easily carried out by the alcohol evaporation. Thus, in this specific case, the partition system was studied using three different approaches, namely the effect of K_3PO_4 and 2-propanol concentration and the temperature of the process.

The effects of different mass fraction percentages of K_3PO_4 in the K_{caf} were studied and the respective results are depicted in Fig. 2A (detailed in supplementary Table S4). The results suggest that the increase in the salt concentration enhances the exclusion of caffeine from the salt- to the alcohol-rich phase. This behaviour is explained by the higher affinity of the salt to be hydrated by the water molecules (high ionic strength, or "salting-out" nature), forcing the caffeine migration towards the top phase.[33] Through the increase of the salt concentration, it is possible to increase the K_{caf} from 1.89 to 2.20. However, the recovery parameter for the top phase is not affected being approximately constant for all systems (83.7% < R_T < 85.7%).

The second approach used to manipulate the caffeine migration into the top phase was based on the use of different mass fractions of 2-propanol (from 40.0 to 60.0 wt%). As already discussed, also in this case the tie-line (and tie-line length) was changed and with it both phases were enriched in the principal components. In this case, it is expected to maximize the favourable interactions between caffeine and alcohol. The associated results are reported in Fig. 2B (detailed in supplementary Table S5), using K_3PO_4 at 20.0 wt% (the best condition optimized above). The increase in the 2-propanol concentration is responsible for the increase of K_{caf} from 1.43 to 3.17. The migration of caffeine to the top-rich phase is explained by the theory reported by Ling and coworkers [37]. The authors explained that the increase in the 2-propanol solubility in water and the consequent hydration of the top phase causes the increase in the "caffeine–water" interactions, promoting the biomolecule migration for the top phase. Also, changing the 2-propanol concentration, the R_T was increased from 74.3% (40 wt% of 2-propanol) to 89.9% (60 wt% of 2-propanol).

In order to conclude regards the performance of our partition systems, the values of R_T achieved in this work needs to be analysed and compared with those found in literature.[3,5,7]. Despite the fact that the values of R_T increase due to the increase of the volume of the bottom phase, it is possible to compare our results with some of the recovery results found in the literature. In fact, and in our case using just step of purification described by the application of an ATPS, it was proved that the best R_T found by us is similar to the highly efficient separation process constituted by a column-chromatographic extraction, followed by a sequential adsorption to separate the caffeine from green tea. In this case, not only the chromatographic technique used is much more sensitive but also the process is much more complex. In this case, the authors reported a recovery of 90%, but using a fourfold excess of water circulating five times among different columns (R = 90%).[7] Other authors investigating the extraction of caffeine from *C. sinensis* leaves and *C. arabica* beans and its purification employed a liquid–liquid extraction followed by a step of chromatography.[44] In this work, and despite the absence of numerical results, the authors attest the complete purification of caffeine. Another work reporting the partitioning of caffeine was reported by Coutinho and collaborators,[45] using ATPS

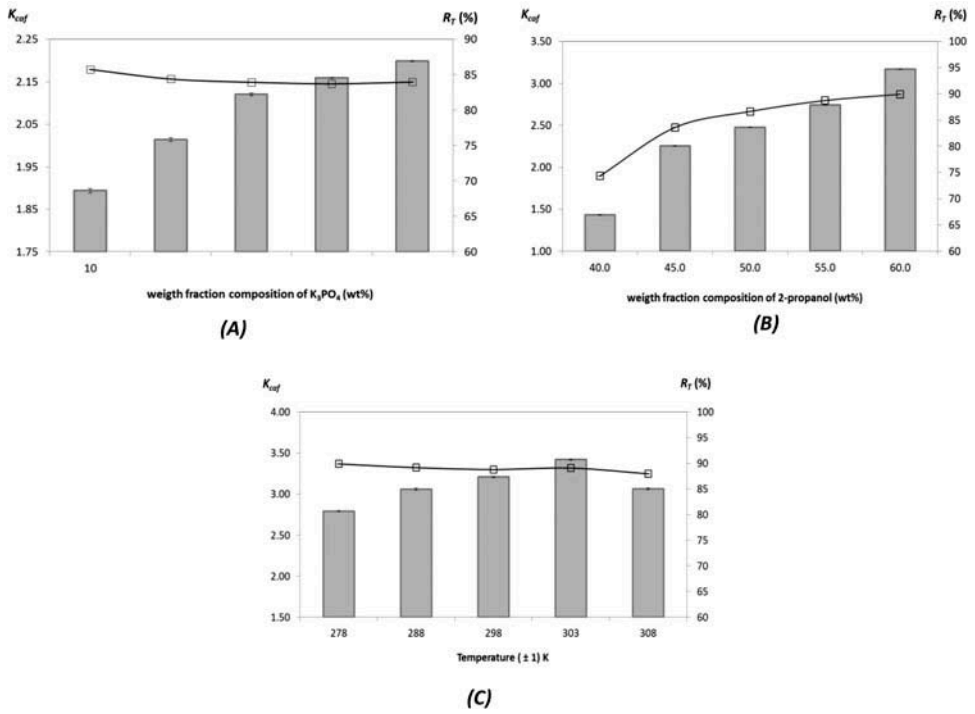

Figure 2. Experimental results for the dependency of K_{caf} (grey bars) and R_T % (□) with the change of the weight fraction compositions of the ATPS components: (A) 60.0 wt% of 2-propanol + 10.0–20.0 wt% of K_3PO_4; (B) 40.0–60.0 wt% of 2-propanol + 20.0 wt% of K_3PO_4 at 298 ± 1 K, and the temperature (± 1 K); (C) 60.0 wt% of 2-propanol + 20.0 wt% of K_3PO_4.

based in ILs and polymers, the partition coefficient results reported being similar to those obtained in this work. In this sense, and considering the similar results found in both works, this study seems to develop a more advantageous process because it is not only equally efficient but it is of lower economic impact (alcohols are cheaper than polymers and ILs, principally in Brazil) and more environmental friendly (ILs are not easily distillable[46] and polymers are not easily recycled).

Finally, in order to maximize the partition of caffeine for the top phase, the effect of temperature from 278 to 303 (±1) K was evaluated and the experimental results depicted in Fig. 2C (detailed in supplementary Table S6). The selected system was based on the best conditions found to maximize the caffeine migration to the top phase, namely 2-propanol at 60 wt% and K_3PO_4 at 20 wt%. From the results presented in Fig. 2C, it can be seen that the K_{caf} of caffeine increases with temperature, with a maximum of 3.42 obtained at 303 ± 1 K. This behaviour is justified by the increase in the solubility of caffeine into the top phase, promoted by entropic factors. Meanwhile, the R_T is not significantly affected by the temperature effect changing only from 87.8% to 88.9%. Beyond the study of the temperature effect in the caffeine migration, in this work, the thermodynamic parameters of the partition process were also evaluated.

For a better understanding of the molecular mechanisms responsible for the caffeine partition between the coexisting phases, the thermodynamic parameters of transfer, namely the standard molar Gibbs energy ($\Delta_{tr}G°_m$), enthalpy ($\Delta_{tr}H°_m$) and entropy ($\Delta_{tr}S°_m$), were calculated through Eqs. (4)–(6). The plot of ln K_{caf} versus $1/T$ is shown in the supplementary Figure S2. It is possible to conclude that the caffeine migration for the alcohol-rich phase using these specific ATPS is a spontaneous process ($\Delta_{tr}G°_m$ = −2.55 kJ·mol^{-1}). Moreover, the $\Delta_{tr}H°_m$ is negative, suggesting that the transference of caffeine from the salt to the alcohol-rich phase is an exothermic process ($\Delta_{tr}H°_m$ = −5.35 kJ·mol^{-1}). Furthermore, comparing the absolute values of $T\Delta_{tr}S°_m$ ($\Delta_{tr}S°_m$ = −9.40 J·mol^{-1}·K^{-1}) and $\Delta_{tr}H°_m$, it can be seen that the enthalpy of transfer is the highest, meaning that the enthalpic effects have a crucial role in the caffeine migration process for the specific ATPS studied in this section. Finally, through the analysis of the thermodynamic parameters, particularly the $\Delta_{tr}G°_m$, the spontaneity of the caffeine migration to the alcohol-rich phase is verified simply by manipulation of the system conditions, therefore making unnecessary to consider any additional components to assist the controlled extraction of caffeine, such as the commonly used electrolytes.

Caffeine partitioning to the salt-rich phase

Since one of the main objectives of this work is to assess the possibility of manipulating the partition of caffeine between the alcohol- and salt-rich phases, the preferential partition of caffeine for the salt-rich phase was also investigated. In this sense, the concentration of caffeine in the salt-rich phase was studied using ATPS comprised of a phosphate buffer solution (K_2HPO_4/KH_2PO_4) and methanol. This ternary system (50.0 wt% of methanol + 15.0 wt% of K_2HPO_4/KH_2PO_4) was considered due to its low partition coefficient, K_{caf} = 0.30, which represents the higher concentration of caffeine in the bottom phase.

To promote and optimize the migration of caffeine into the bottom phase, different concentrations of K_2HPO_4/KH_2PO_4 (10.0–17.5 wt%) were studied at 298 ± 1 K, the results being shown in Fig. 3A (see supplementary Table S7). The results imply that the use of different phosphate buffer mass fraction percentages have a proven effect on the caffeine partition. However, the influence of these particular concentrations was not sufficient to significantly decrease the partition coefficient obtained in the original partition system. It seems that the partition of caffeine for the bottom phase is favoured by higher concentrations of the inorganic salt (15.0 or 17.5 wt%). These systems are also represented by lower R_T, indicating that the amount of caffeine is higher in the salt-rich phase. These results can be explained by the "salting-out" capacity of the salt used. The potassium phosphate buffer is formed by a mixture of two inorganic salts, K_2HPO_4 and KH_2PO_4. Because KH_2PO_4 exhibits a lower "salting-out" capacity and, consequently, a weaker interaction with the water molecules when compared with K_3PO_4 and K_2HPO_4, it is not capable of self-promoting the formation of alcohol-salt ATPS. The variable "salting-out" capacity[33] of these phosphate salts is normally described by the Hofmeister series.[40] The lower "salting-out" capacity and affinity with water of the potassium phosphate buffer, which is associated with the higher affinity of the alcohol-rich phase and the water, helps to maintain caffeine in the salt-rich phase.

The effect of diverse methanol concentrations was also investigated aiming to force the migration of caffeine into the bottom phase. Here, the partition process of caffeine was assessed using systems composed of 17.5 wt% of the K_2HPO_4/KH_2PO_4 and distinct methanol concentrations, from 40.0 to 60.0 wt%, the experimental results being depicted in Fig. 3B (see supplementary Table S8), at 298 ± 1 K. The increase in the methanol concentration provided, as expected, a significant

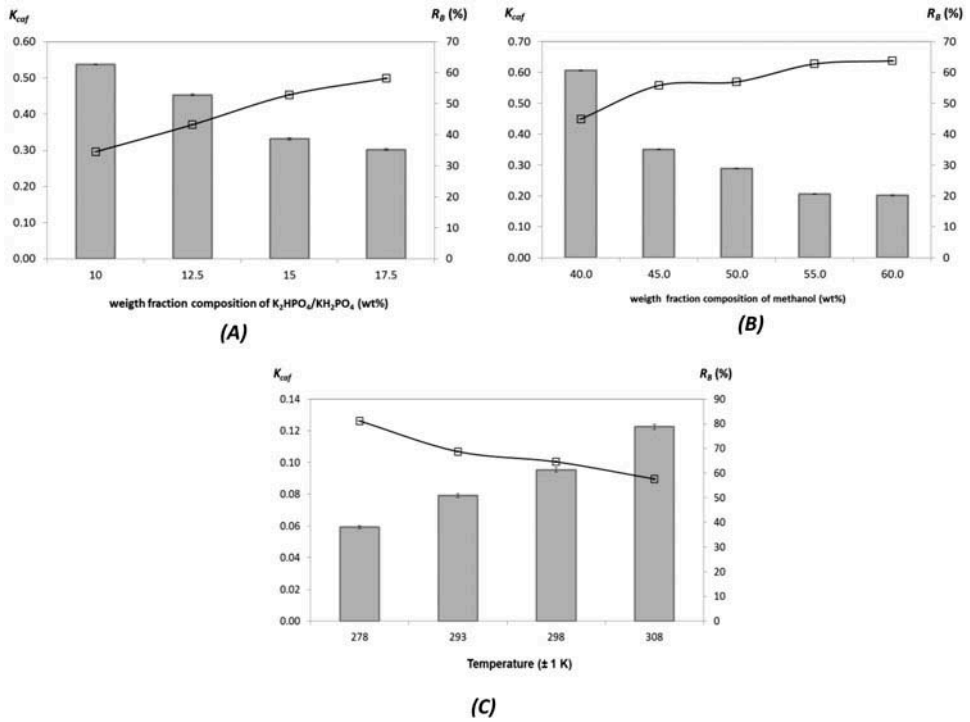

Figure 3. Experimental results for the dependency of K_{caf} (grey bars) and R_T % (□) with the change of the weight fraction compositions of the ATPS components: (A) 50.0 wt% of methanol + 10.0–17.5 wt% of K_2HPO_4/KH_2PO_4; (B) 40.0–60.0 wt% of methanol + 17.5 wt% of K_2HPO_4/KH_2PO_4 at 298 ± 1 K, and the temperature (± 1 K); (C) 60.0 wt% of methanol + 17.5 wt% of K_2HPO_4/KH_2PO_4.

decrease of K_{caf} and the increase of R_B, which means the preferential migration of caffeine for the bottom phase, with the best results found for 60.0 wt% of methanol (K_{caf} = 0.20 and R_B = 63.7%). As described above, methanol presents the highest dielectric constant, as a result of its higher affinity with water, and the smallest capacity to solubilize the caffeine (see section "Influence of the ATPS components on the caffeine partition"). It is evident that, with the increase in the methanol concentration, more methanol–water hydrogen–bond interactions can be established, reducing considerably the solubility of caffeine into the top layer. This methanol–water affinity is thus the main driving force controlling the migration of caffeine into the bottom (salt-rich) phase, justifying both parameters, K_{caf} and R_T. This tendency of decreasing the caffeine recovery in the top phase by increasing the amount of alcohol was also found in the literature,[6] where the extraction yield was decreased with the increase in the ethanol concentration (50 and 100 wt% of alcohol), from 4.0 to 1.5 wt%, respectively.

The temperature effect in the caffeine partition was evaluated, once again using the same ternary system composed of 60.0 wt% of methanol + 17.5 wt% of K_2HPO_4/KH_2PO_4 + 22.5 wt% of H_2O. The results shown in Fig. 3C (see supplementary Table S9) pointed out that the variation of K_{caf} slightly increases with temperature. This conclusion is also confirmed by the R_B since this parameter decreases with the increase in temperature of equilibrium, thus indicating the forced migration of caffeine into the alcohol-rich phase at higher temperatures. Thus, the best results for the preferential migration of caffeine to the bottom phase are achieved at 278 ± 1 K (K_{caf} = 0.06 and R_T = 81.1%). Finally, the thermodynamic parameters were also calculated for the migration of caffeine for the bottom phase (see supplementary Figure S3), the results being depicted in Fig. 3. One more time, and for these specific ATPS, the migration of caffeine for the salt-rich phase is a spontaneous process ($\Delta_{tr}G°_m$ = −0.64 kJ□mol^{-1}), although to a less extent, when compared with the results previously discussed (section "Caffeine partition into the alcohol-rich phase"). This result is in fact in a close agreement with the partition coefficient results since the migration of caffeine for the bottom phase is less extensive when compared with the migration of caffeine for the top phase. The $\Delta_{tr}H°_m$ is also negative in this case (−11.29 kJ·mol^{-1}), suggesting that the transference of caffeine from the alcohol- to the salt-rich phase is an exothermic process, for these specific ATPS. When comparing the absolute values of $T\Delta_{tr}S°_m$ ($\Delta_{tr}S°_m$ = −35.71 J·mol^{-1}K^{-1}) and $\Delta_{tr}H°_m$, it seems that the enthalpy of transfer value is the highest, which means that the enthalpic effects are in this case of utmost importance.

From the main results achieved and analysed in this work, it is concluded that the caffeine partition can be manipulated for the bottom and top phases, and that this behaviour is particularly dependent on the alcohol–salt ATPS used.

Extraction and purification of caffeine from guaraná seeds and coffee beans

To complement and prove the applicability of the systems developed in this work in terms of recovery of caffeine and show that they can be used in the purification of natural extracts, solid–liquid extractions were carried in guaraná seeds and coffee beans. These systems used aqueous solutions of alcohols as solvents to extract caffeine from the natural biomass (solid–liquid extraction) and then those extracts were concentrated by adding K_3PO_4 in appropriate amounts capable to form the ATPS. First, solutions of 2-propanol and methanol at 60.0 wt% were used to extract caffeine from the raw matrices. The caffeine content of the extracted aqueous solutions of 2-propanol and methanol were statistically similar either in guaraná seeds (189.5 ± 2.5 mg caffeine/g sample and 180.7 ± 1.8 mg caffeine/g sample, respectively) or in coffee beans (135.7 ± 9.1 mg caffeine/g sample and 128.6 ± 3.3 mg caffeine/g sample, respectively). These results agree with literature,[47,48] in which extractions of caffeine from guaraná seeds using an aqueous ethanol solution at 60% (155 mg caffeine/g sample) and from robusta coffee with pure ethanol (84.1 mg caffeine/g sample) were tested.

After the solid–liquid extraction, ATPS were prepared by adding the potassium phosphate salts into the alcohol extracts (20.0 wt% of K_3PO_4 or 17.5 wt% of K_2HPO_4/KH_2PO_4). Again, the same capacity to manipulate the caffeine migration is described here, in the same line previously discussed in the optimization step of this work. In this case, systems consisting of 2-propanol and K_3PO_4 present partition coefficients higher than the unit (1.77 < K_{caf} < 2.31), denoting the caffeine migration for the alcohol (top)-rich phase (72.5% < R_T < 77.6%) with the highest PF in top phase (3.24 < PF_T < 6.59). For systems composed of methanol plus the K_2HPO_4/KH_2PO_4, the partition coefficients are denoting the migration of the biomolecule for the bottom phase (0.28 < K_{caf} < 0.45; 79.1% < R_B < 85.8% and 1.53 < PF_B < 2.68) as expected (Table 2; see supplementary Tables S10 and S11). Despite the same trend of extraction, the values found for the partition coefficient were below those for the model

Table 2. Experimental results for K_{caf} (uncertainty associated < 5.0%) and R % (uncertainty associated < 5.0 %) for the systems optimized and respective data obtained for the recovery of caffeine from the coffee beans and guaraná seeds.

	Optimized system		Guaraná seeds			Coffee beans		
ATPS	K_{caf}	R (%)	K_{caf}	R (%)	PF (fold)	K_{caf}	R (%)	PF (fold)
60.0 wt% of 2-propanol+ 20.0 wt% of K_3PO_4 at 303 ± 1 K (top phase)	3.42	89.1	2.31	77.6	6.59	1.76	72.5	3.24
60.0 wt% of methanol+ 17.5 wt% of K_2HPO_4/KH_2PO_4 at 278 ± 1 K (bottom phase)	0.06	81.1	0.45	79.1	1.53	0.28	85.8	2.68

systems, which is attributed to the complexity of the hydroalcoholic extract obtained from the comparison of the two matrices with the model systems.

Regarding the industrial application of the processes here proposed, the concentration of caffeine in both alcohol- and salt-rich phases and the recycling of the phase components must be addressed. In this work, a simple integrated process focusing on the partition of caffeine is proposed and presented. The flow chart reported in Fig. 4 shows how the manipulation of caffeine migration can be achieved and identifies how to promote the recycling of both phases. In this sense, if caffeine is concentrated in the alcohol-rich phase, the phase components will be recycled by evaporation; if caffeine is concentrated in the salt-rich phase, the components can be recovered and reused after the removal of the phenolic compounds by precipitation at very low pH values.[49]

Conclusions

The possibility of manipulating the caffeine partition by applying ATPS based in alcohol–potassium phosphate inorganic salts for both alcohol- and salt-rich phases was successfully presented and discussed. In this context, an optimization study considering the migration of caffeine was performed not only by the change in the alcohol and salt type, but also by the application of different concentrations of each one of these phase formers and the temperature considered. It was shown that the increase on the affinity of both alcohol and salt for the water molecules is one of the most important driven forces for the success of the caffeine partition manipulation. The caffeine migration for the alcohol (top)-rich phase is thus favoured by the application of the system based in 60.0 wt% of 2-propanol + 20.0 wt% of K_3PO_4, at 303 ± 1 K (K_{caf} = 3.42 and

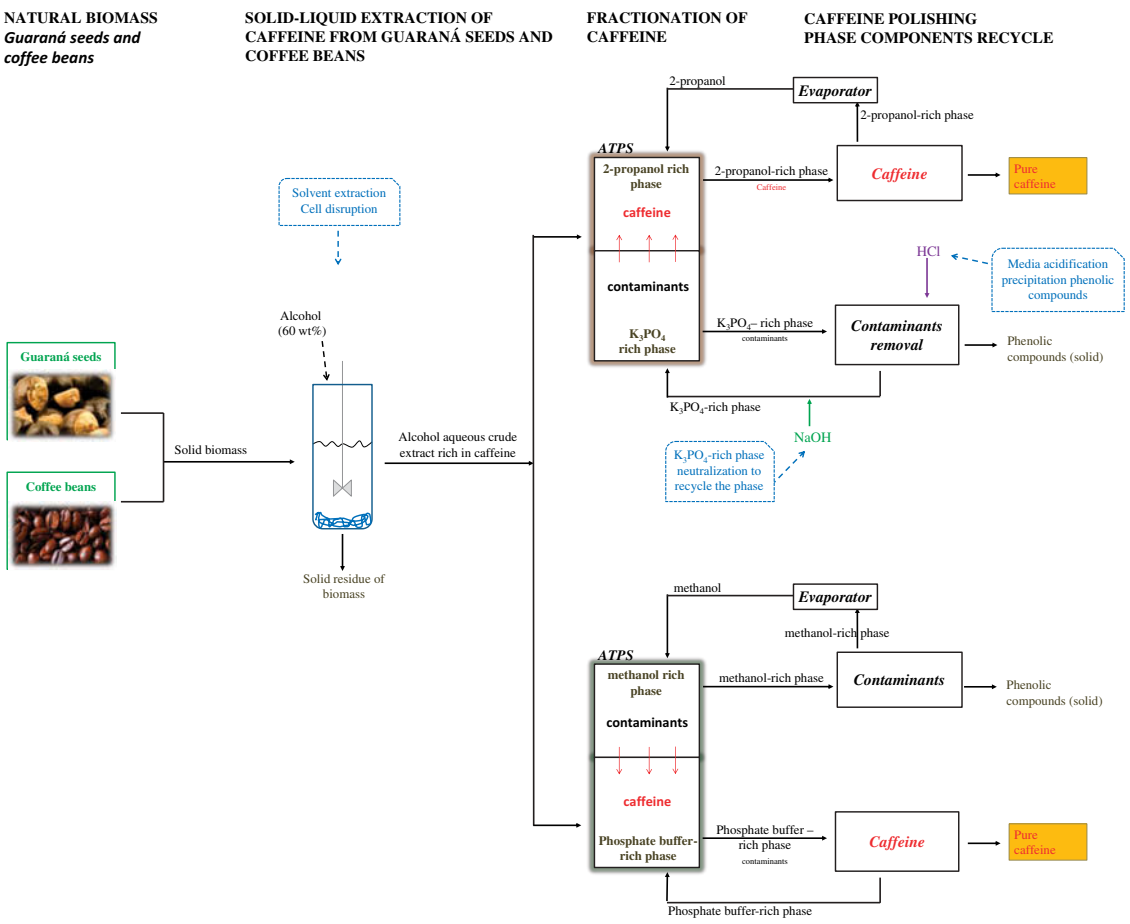

Figure 4. Flow chart of the integrated process in the two processes under study, the purification of caffeine and the decaffeination of alcoholic extracts. The caffeine here represented has its origin in two distinct food residues, namely Guaraná seeds and coffee beans.

R_T = 89.1%), while the caffeine partition for the salt (bottom)-rich phase is favoured by the application of the system 60.0 wt% of methanol + 17.5 wt% of K_2HPO_4/KH_2PO_4, at 278 ± 1 K (K_{caf} = 0.06 and R_B = 81.1%). After optimization, the recovery of caffeine was performed considering two distinct samples, guaraná seeds and coffee beans, which confirmed the main trends discussed in the optimization study. Using the system based in methanol and K_2HPO_4/KH_2PO_4, the PF_B were 1.53 (guaraná seeds) and 2.68 (coffee beans); whereas using the 2-propanol and K_2HPO_4/KH_2PO_4-based ATPS, the PF_T were 6.59-fold (guaraná seeds) and 3.24-fold (coffee beans). Taking into account the results obtained, it is possible to envisage the potential application of these alcoholic ATPS to perform the purification and recovery of caffeine from natural sources or even raw materials.

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