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Optimization of the gallic acid extraction using ionic-liquid-based aqueous two-phase systems

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ABSTRACT

Gallic acid is an interesting natural compound because of its antioxidant, anti-inflammatory, antifungal and antitumor properties. It is present in relatively high concentrations in a number of biomass sources and in industrial wastes from where it could be extracted. Aiming at developing benign and efficient extraction/purification processes for gallic acid, aqueous two-phase systems (ATPS) composed of ionic liquids (ILs) and inorganic salts were investigated. Several combinations of ionic liquids and inorganic salts were studied to understand the influence of the ionic liquid structure and of the pH of the aqueous medium on the gallic acid partitioning. It is here shown that at low pH values the non-charged form of gallic acid (or other phenolic compounds) preferentially migrates for the ionic-liquid-rich phase whereas its conjugate base preferentially partitions for the salt-rich phase. The results indicate that IL-based ATPS can be the basis of new extraction/purification processes of gallic acid from natural matrices, and improved extractions are obtained using acidic aqueous solutions.

1. Introduction

Aqueous two-phase systems (ATPS) consist in two macroscopic aqueous-rich phases formed by mixing in water two incompatible polymers, one polymer and one inorganic salt, or by the combination of two different salts [1]. Above a critical concentration of the two solutes the phase separation takes place. The purification of complex biomolecules using ATPS has been shown to have a number of advantages since the aqueous nature of the coexisting phases prevents their denaturation or loss of activity. Moreover, ATPS processes are simple to scale-up, and can be used in continuous operation. Currently, a number of proteins are purified at an industrial level using ATPS [2].

In recent years, ionic-liquid-based ATPS have been object of a growing interest due to their potential in the design of novel separation processes. Gutowski et al. [3] were the first to show that aqueous solutions of imidazolium-based ionic liquids (ILs) can form ATPS in the presence of inorganic salts. Since then, the extractive potential of IL-based ATPS has been studied with distinct compounds, such as testosterone and epitestosterone, alkaloids, antibiotics, amino acids, phenolic compounds, terpenoids, among other biological molecules of interest [4–11]. Although the partitioning of biomolecules in ATPS depends on inherent parameters such as the biomolecule size, surface properties, molecular weight, temperature, pH, net charge, among others [12], the tunability of the extraction efficiencies using IL-based ATPS has been previously demonstrated [4–11]. The physicochemical properties of ILs can be tailored by the proper choice of the cation and/or anion that compose a particular fluid, allowing, therefore, the optimization of their characteristics for a specific extraction [11,13]. A further advantage of IL-based ATPS relays on the low viscosity of the coexisting phases, contrarily to that typically observed in polymer-based ATPS [4,8]. This is particularly relevant for the biomolecules processing at both analytical and industrial levels. The low viscosities facilitate the analysis of the metabolites, guarantee a faster segregation of the two phases after the extraction procedures, enhance the mass transfer of the solute between the two aqueous phases, and reduce the energy required for pumping the fluids [14,15].

Phenolic compounds display relevant properties in the health and nutrition fields [16,17]. These features are mainly related to the antioxidative and radical scavenging properties of some phenolic structures [18], but also to their anticholesterolemic, hypertension depression and protection against cardiovascular diseases [19]. The use of natural phenolic compounds for nutraceutical and cosmetic applications is highly advantageous compared to synthetic substitutes that usually present adverse effects [20]. Besides, some phenolic compounds are also phytotoxic and toxic to bacteria, and can be used in biological wastewater treatments [21–23].

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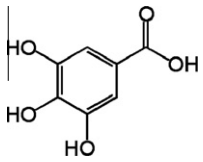

Fig. 1. Chemical structure of gallic acid.

Examples of simple phenolic structures are vanillic, gallic, protocatechuic, ellagic, and syringic acids and quercetin, vanillin and resveratrol. These compounds are usually present in natural sources such as wood, barks, fruits and vegetables [16,17,21,22]. They may, in many cases, be isolated from residues of industrial or agricultural activities, such as wastewaters from olive mills, cork powder and black condensates [21,24]. For instance, olive mills residues may contain 4–16% of organic matter, and from which phenolic compounds represent 2–15% [21]. To decrease the load of phenolic compounds on aqueous effluents, and at the same time to add economical value to them, there is a growing interest in their extraction from both industrial or agricultural wastes.

Hasmann et al. [25] have used aqueous two-phase systems composed of two thermoseparating copolymers (ethylene oxide and propylene oxide) to remove phenolic compounds from a hydrolysate of rice straw. The extraction efficiencies of phenolic compounds (ferulic acid, syringic acid, furfural, vanillin and syringaldehyde) varied between 6% and 80% depending on the copolymer used [25]. Moreover, the authors [25] found that in a system containing 50 wt.% of the copolymer with the higher molecular weight, no extraction of phenolic compounds was observed due to the high viscosity of the system. The application of IL-based systems to the extraction of phenolic compounds from natural sources has also been reported in literature [16]. Du et al. [16] applied a microwave-assisted technique to extract gallic acid, ellagic acid, quercentin and trans-resveratrol from medicinal plants using aqueous solutions of hydrophilic ILs. The extraction yield of polyphenolic compounds was greatly improved by the addition of ILs when compared with pure water, and reached extraction yields equivalent to those obtained with methanol [16]. Furthermore, by using different ILs it was also observed that the ions that compose the ionic fluid, and especially the anions, control the extraction efficiencies [16]. In a previous work, we have studied the partitioning of vanillin with IL-based ATPS composed of a large range of ionic liquids and a common inorganic salt – K_3PO_4 [4]. Additional parameters that could affect the partitioning of the molecule, namely the temperature and the available concentration of vanillin in the global system, were also evaluated [4]. In all systems and conditions tested, vanillin preferentially migrates for the IL-rich phase [4].

In this work, ATPS formed by various ILs and different inorganic salts were explored for the extraction of gallic acid (3,4,5-trihydroxybenzoic acid, $C_6H_2(OH)_3COOH$, pK_a = 4.41 at 298 K) [26] aiming at investigating the effect of the structural features of the ionic liquid and the medium pH on the extraction efficiency. The molecular structure of gallic acid is depicted in Fig. 1.

2. Experimental section

2.1. Materials

The ILs used in this work to study the partitioning of gallic acid were 1-ethyl-3-methylimidazolium trifluoromethanesulfonate, $[C_2mim][CF_3SO_3]$; 1-butyl-3-methylimidazolium bromide, $[C_4mim]Br$; 1-butyl-3-methylimidazolium methylsulfate $[C_4mim][CH_3SO_4]$; 1-butyl-3-methylimidazolium ethylsulfate, $[C_4mim][C_2H_5SO_4]$; 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[C_4mim][CF_3SO_3]$; 1-butyl-3-methylimidazolium dicyanamide, $[C_4mim][N(CN)_2]$, 1-heptyl-3-methylimidazolium chloride, $[C_7mim]Cl$, 1-methyl-3-octylimidazolium chloride, $[C_8mim]Cl$, and 1-butyl-3-methylimidazolium octylsulfate, $[C_4mim][OctylSO_4]$. All ILs were supplied by Iolitec, with the exception of $[C_4mim][OctylSO_4]$ which was acquired from Merck. To reduce the volatile impurities content to negligible values, ILs individual samples were kept at constant agitation under vacuum and at a moderate temperature (343 K), for a minimum of 24 h. After this purification step, the purity of each IL was further checked by ^1H, ^{13}C and ^{19}F NMR (when applicable) spectra and found to be >99 wt.% for all samples. Na_2SO_4 was acquired at LabSolve

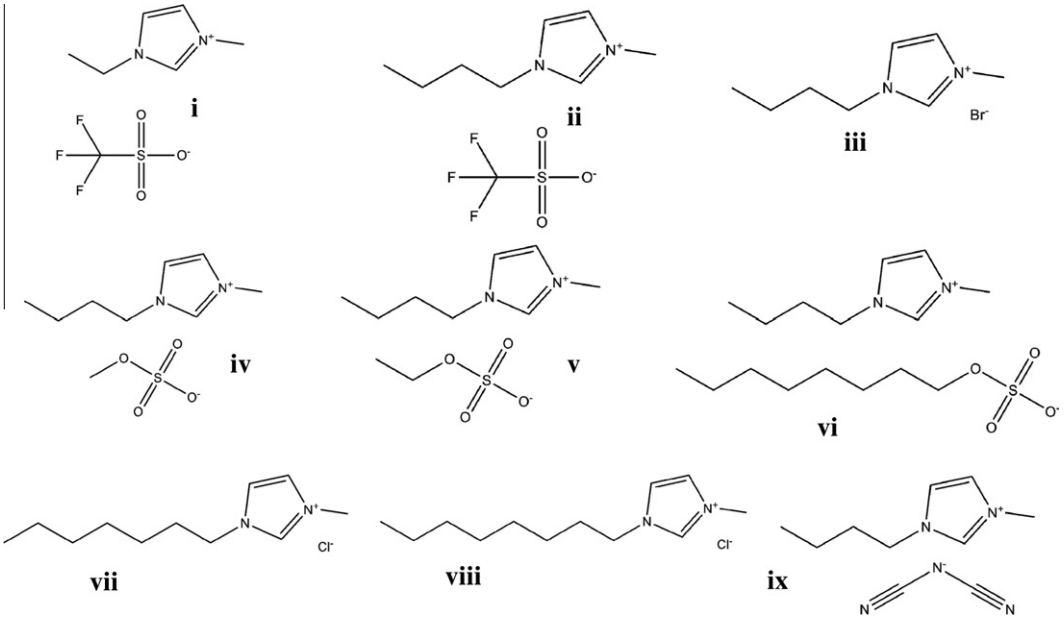

Fig. 2. Chemical structures of the studied ILs: (i) $[C_2mim][CF_3SO_3]$; (ii) $[C_4mim][CF_3SO_3]$; (iii) $[C_4mim]Br$; (iv) $[C_4mim][CH_3SO_4]$; (v) $[C_4mim][C_2H_5SO_4]$; (vi)$[C_4mim][OctylSO_4]$; (vii) $[C_7mim]Cl$;(viii) $[C_8mim]Cl$; and (ix) $[C_4mim][N(CN)_2]$.

Table 1
pH values of the aqueous coexisting phases in IL-based ATPS at 298 K.

IL + inorganic salt + water system		Na_2SO_4 pH	K_2HPO_4/KH_2PO_4	K_3PO_4
$[C_2mim][CF_3SO_3]$	Salt-rich phase	3.32	7.28	13.09
	IL-rich phase	2.71	7.57	13.15
$[C_4mim][CF_3SO_3]$	Salt-rich phase	3.04	7.10	12.85
	IL-rich phase	3.12	7.37	13.10
$[C_4mim][CH_3SO_4]$	Salt-rich phase	1.57	7.33	13.12
	IL-rich phase	1.88	7.6	13.33
$[C_4mim][C_2H_5SO_4]$	Salt-rich phase	1.29	7.15	12.88
	IL-rich phase	2.29	7.40	13.28
$[C_4mim][OctylSO_4]$	Salt-rich phase	3.52	7.01	12.89
	IL-rich phase	3.64	7.23	13.01
$[C_4mim][N(CN)_2]$	Salt-rich phase	8.07	7.53	13.48
	IL-rich phase	8.54	7.82	13.98
$[C_4mim]Br$	Salt-rich phase	5.22	7.08	12.97
	IL-rich phase	5.43	7.31	13.27
$[C_7mim]Cl$	Salt-rich phase	6.63	7.22	12.85
	IL-rich phase	6.05	7.45	12.99
$[C_8mim]Cl$	Salt-rich phase	6.72	7.55	13.41
	IL-rich phase	6.69	7.69	13.29

(purity > 99.8 wt.%), K_3PO_4 was from Sigma (purity > 98 wt.%), K_2HPO_4 was from Riedel-de Haën (purity > 99 wt.%), and KH_2PO_4 was from Panreac (purity > 99 wt.%). NaOH was from EKA (purity 98 wt.%). Gallic acid was acquired at Merck (>99.5 wt.% pure). The water used was double distilled, passed across a reverse osmosis system and further treated with a Milli-Q plus 185 water purification equipment. The buffers used in the calibration of the pH meter equipment were the citric acid/sodium hydroxide/sodium chloride solution with a pH value of 4.00 (± 0.02), and the potassium dihydrogen phosphate/disodium hydrogen phosphate solution with a pH value of 7.00 (± 0.02), acquired from Fluka.

The ionic structures of the ILs used for the partitioning of gallic acid are shown in Fig. 2.

2.2. Partitioning of gallic acid

The compositions of the ternary mixtures used for the gallic acid partitioning were chosen based on the phase diagrams determined in previous works [9,11,27–29]. The composition adopted at the biphasic region was 15 wt.% of inorganic salt (Na_2SO_4, K_3PO_4, or KH_2PO_4/K_2HPO_4 (phosphate buffer)), 60 wt.% of an aqueous solution containing gallic acid (at 3.06×10^{-3} mol dm^{-3}), and 25 wt.% of each IL. Since it was not possible to perform extractions for all the biphasic systems with the phosphate buffer containing 25 wt.% of IL, additional mixtures of 15 wt.% of KH_2PO_4/K_2HPO_4, 55 wt.% of a gallic acid aqueous solution and 30 wt.% of different ILs were also prepared. To better understand the influence of the pH on the extraction of gallic acid further experimental tests were conducted. For the system composed of 15 wt.% of Na_2SO_4, 60 wt.% of an aqueous solution containing gallic acid (at 3.06×10^{-3} mol dm^{-3}), and 25 wt.% of $[C_4mim][CF_3SO_3]$, small amounts of NaOH were progressively added to increase the pH of the aqueous media. All mixtures were prepared by weight with an uncertainty of $\pm 10^{-4}$ g. The detailed compositions used for the determination of the partition coefficients are reported in Supporting information. The mixtures were prepared and vigorously stirred in small ampoules of approximately 10 cm^3 especially built for extraction procedures [4,8,9,11]. After stirring, the biphasic systems were allowed to equilibrate and phase separate for at least 12 h at (298.15 ± 0.01) K. The temperature was controlled by keeping the glass ampoules in an air bath equipped with a Pt 100 probe and a temperature controller. Preliminary optimization studies showed that the partitioning of gallic acid was completely attained after 12 h of equilibrium.

After the separation of the phases, the amount of gallic acid was quantified through UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at a wavelength of 262 nm, and using a calibration curve previously established. Moreover, the effects of the salt and medium pH towards the quantification of gallic acid by UV spectroscopy were taken into account. At the dilutions carried out, and salt compositions employed, no significant deviations due to these factors were observed in the values of the partition coefficients. Only slight interferences of the imidazolium-based ILs with the analytical method were observed. Therefore, to minimize these interferences, ternary mixtures at the same mass fraction composition were prepared for individual systems, using pure water instead of the gallic acid aqueous solution, and to be used as blank samples. Further experimental tests were conducted in order to guarantee that the amount of gallic acid added does not modify the concentration of each ionic liquid in the coexisting phases. At least three individual samples were quantified to determine the gallic acid partition coefficients and the respective standard deviations – numerical values are presented in Supporting information. The partition coefficients of gallic acid, K_{GA}, were determined as the ratio of its concentration in the IL and in the inorganic salt (Na_2SO_4, K_3PO_4 or K_2HPO_4/KH_2PO_4) aqueous-rich phases, according to the following equation,

$$K_{GA} = \frac{[GA]_{IL}}{[GA]_x} \quad (1)$$

where $[GA]_{IL}$ and $[GA]_x$ are the concentration of gallic acid in the IL- and in each of the inorganic salt-rich aqueous phases, respectively.

The extraction efficiencies of gallic acid were determined accordingly to Eq. (2),

$$\%EE_{GA} = \frac{[GA]_{IL} \times W_{IL}}{[GA]_{IL} \times W_{IL} + [GA]_x \times W_x} \times 100 \quad (2)$$

where W_{IL} and W_x are the weight of the IL-rich phase and of the inorganic-salt-rich phase, respectively.

At the conditions used in this work, in general, the top layer is the IL-rich phase while the bottom phase is the inorganic-salt-rich

phase. Few exceptions were verified with the systems [C_2mim][CF_3SO_3] + K_3PO_4 + aqueous solution, [C_4mim][CF_3SO_3] + K_3PO_4 + aqueous solution, and [C_4mim][CF_3SO_3] + Na_2SO_4 + aqueous solution, where it was observed an inversion on the phase densities. This is a direct result of the high density of the fluorinated ILs [C_2mim][CF_3SO_3] and [C_4mim][CF_3SO_3] [30]. Each phase was qualitatively identified (IL-rich or inorganic-salt rich) by UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at wavelength of 211 nm (absorption peak for the imidazolium core).

2.3. pH measurements

The pH of the IL- and inorganic-salt-rich aqueous phases was measured at (298 ± 1) K using an HI 9321 Microprocessor pH meter (HANNA instruments) with an uncertainty of ±0.02. The calibration of the pH meter was carried out with two buffers (pH values of 4.00 and 7.00). The ternary mixtures were prepared by weight, vigorously stirred, and further kept still in small ampoules for phase separation, and for at least 12 h at (298.15 ± 0.01) K using the air bath previously described. After the careful separation of the phases, the pH of each aqueous phase was measured.

3. Results and discussion

3.1. Effect of the inorganic salt in the gallic acid partitioning

The effect of the inorganic salt in the partitioning of gallic acid in IL-based ATPS was evaluated using Na_2SO_4, K_3PO_4, and a K_2HPO_4/KH_2PO_4 buffer solution. The three salt solutions employed were chosen based on the different pH values that they confer to the aqueous phases. In general, imidazolium-based ionic liquids lead to acidic aqueous solutions. The pH of their solutions may be, or not, changed by the presence of the salt. The sodium sulfate, being a neutral salt, will maintain the acidity of the aqueous phase (except for the systems composed of dicyanamide, or other strong alkaline anions); the mixture of salts K_2HPO_4/KH_2PO_4 (phosphate buffer) leads to a pH close to a neutral value; the K_3PO_4 produces alkaline aqueous solutions.

All the pH values of both top and bottom phases of the systems used for the partitioning studies of gallic acid are presented in Table 1. These co-existing phases are the result of an overall mixture composition of 15 wt.% of inorganic salt, 60 wt.% of water and 25 wt.% of each IL for the salts K_3PO_4 and Na_2SO_4, and 15 wt.% of inorganic salt, 55 wt.% of water and 30 wt.% of each IL for the system employing the phosphate buffer. The detailed compositions are listed in Supporting information and correspond to the mass fraction percentages used in the partitioning experiments. For all the systems, no significant deviations between the pH of the salt-rich phase and the IL-rich aqueous phase were observed. The differences observed in the pH values between the various Na_2SO_4-based ATPS are more pronounced when compared with the remaining salts. Na_2SO_4 is a neutral salt and therefore the pH of the medium is more dependent on the ionic liquid employed.

The partition coefficients obtained for gallic acid are depicted in Fig. 3. The salts studied lead to very different behaviors in the partitioning of the biomolecule, suggesting that the choice of the inorganic salt is a dominant parameter in the optimization of the extraction of gallic acid.

From Fig. 3 it is possible to observe that, for most of the ionic liquids investigated, the partition coefficients of gallic acid decrease in the following order of inorganic salts: Na_2SO_4 >> K_2HPO_4/KH_2PO_4 > K_3PO_4. However, this tendency is not obeyed for the systems with ILs with longer alkyl side chains at the cation, namely [C_7mim]Cl and [C_8mim]Cl. The extraction of gallic acid with [C_7mim]Cl is better with K_3PO_4 (if compared with the phosphate buffer), while with [C_8mim]Cl the phosphate buffer seems to yield enhanced extractions. This different pattern must be related with the ability of these ionic liquids to form micelles, and thus with the influence of each salt in reducing or increasing the critical micelle concentration [31].

Although K_3PO_4 is the strongest salting-out salt studied in this work, it seems that the pH of the aqueous media plays a major role in the partition behavior observed. For instance, the partition coefficients obtained with the systems containing [C_4mim][CF_3SO_3] range between 0.18 and 21.98 when different salts are employed. Considering the pH values reported in Table 1, the partition coefficients obtained suggest that the neutral and less hydrophilic form of gallic acid, present in the acidic media of Na_2SO_4-based systems, is more easily extracted into the IL-rich phase than gallate. On the other hand, gallate, the charged conjugate base of gallic acid present in neutral or alkaline pH solutions formed by the phosphate-based salts, preferentially migrates for the salt-rich phase. In the systems considered here there are two aqueous phases of different nature: a predominant hydrophobic phase composed mainly of ionic liquid, and a more hydrophilic phase constituted majorly by the high charge density salts. These differences in the phases' polarities coupled to the charged or non-charged nature of gallic acid control the partition coefficients observed. Non-charged species tend to migrate to the most hydrophobic phase (IL-rich phase) while the charged species preferentially partition for the salt-rich-phase.

3.2. Effect of the IL ions in the gallic acid partitioning

The extraction of biomolecules using ATPS (among other factors) is the result of their interactions with the compounds dissolved in the aqueous medium. Therefore, the chemical

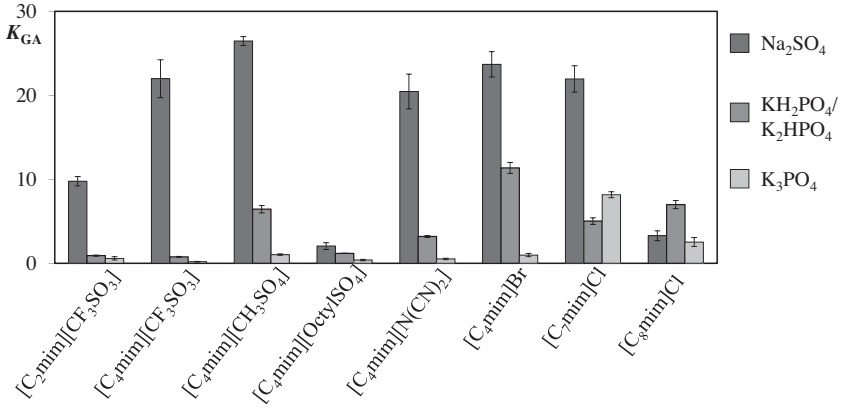

Fig. 3. Partition coefficients of gallic acid in IL-based ATPS formed by different inorganic salts at 298.15 K.

structures of the ionic liquid ions may have a significant impact on the gallic acid partitioning.

Fig. 4 depicts the partition data obtained for gallic acid in ATPS formed by alkaline aqueous solutions of K_3PO_4. The extraction efficiencies of gallic acid and the pH of the coexisting phases are also shown to allow comprehensive comparisons among different systems. The K_{GA} values in systems containing K_3PO_4 range between 0.18 and 8.16. The partitioning of the biomolecule towards the ionic-liquid-rich phase is favorable for the [C_4mim][CH_3SO_4], [C_4mim]Br, [C_7mim]Cl and [C_8mim]Cl containing systems (extraction efficiencies higher than 50%). Moreover, among the remaining systems, gallate preferentially migrates for the K_3PO_4-rich phase, and strongly depends on the IL structural features. In summary, the partition coefficients of gallic acid in K_3PO_4-based ATPS decrease in the following order: [C_7mim]Cl >> [C_8mim]Cl > [C_4mim][CH_3SO_4] ≈ [C_4mim]Br > [C_2mim][CF_3SO_3] ≈ [C_4mim][N(CN)$_2$] > [C_4mim][OctylSO$_4$] > [C_4mim][CF_3SO_3].

It should be stressed that an increase in the K_{GA} values does not necessarily involves the increase in the $\%EE_{GA}$ values. The $\%EE_{GA}$ values are a result of the concentration of gallic acid in each phase combined with their total weight.

The systems containing [C_4mim]Br and [C_4mim][CH_3SO_4] show extraction efficiencies *ca.* 50%. For the systems containing [C_2mim][CF_3SO_3], [C_4mim][N(CN)$_2$], [C_4mim][OctylSO$_4$] and [C_4mim][CF_3SO_3], the partition coefficient values are lower than 0.60, revealing that gallic acid migrates preferentially for the K_3PO_4-rich phase (extraction efficiencies below 35%). The higher hydrophobicity of these ionic liquids makes them less able to solvate the negative charged form of gallic acid. The hydrophobicity of these fluids can be confirmed by the ability of their anions to accept a proton (or donate an electron pair) in a solute–solvent hydrogen-bond [34]. The β values (hydrogen bond basicity values determined using solvatochromic probes for the distinct [C_4mim]-based ionic liquids investigated are according to: [C_4mim][CF_3SO_3] (β = 0.49) [35] > [C_4mim][N(CN)$_2$] (β = 0.60) [35] > [C_4mim][CH_3SO_4] (β = 0.75) [36] > [C_4mim]Br (β = 0.87) [36], and thus support the idea of their higher hydrophobicity.

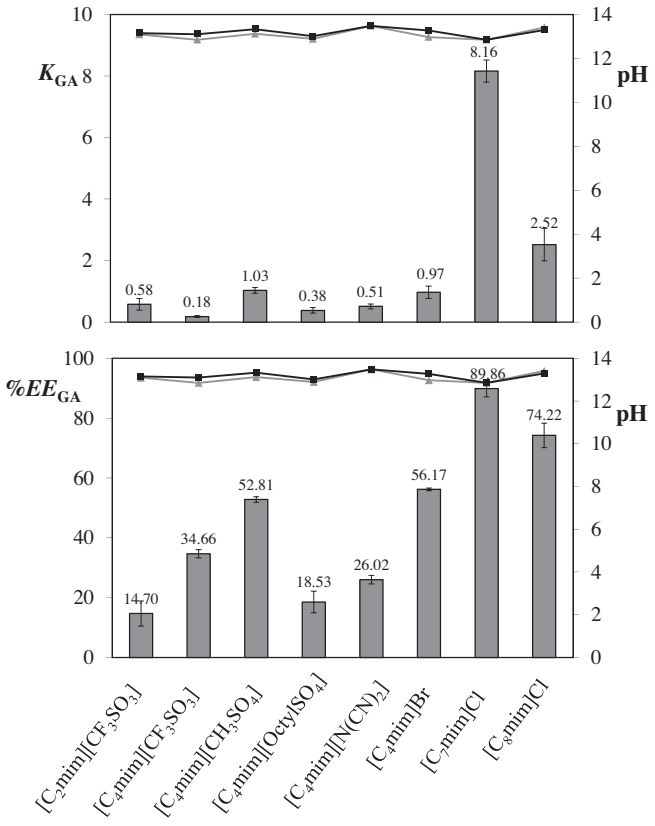

Fig. 4. Partition coefficients (K_{GA}) and extraction efficiencies percentages ($\%EE_{GA}$) of gallic acid, and pH of both IL- (squares) and salt-rich phases (triangles), for different IL-K_3PO_4-based ATPS at 298.15 K.

Albeit an increase in the cation side alkyl chain length also enhances the hydrophobicity of the ionic liquids [37], the extraction efficiencies observed for [C_7mim]Cl and [C_8mim]Cl, of 90% and 74%, respectively, suggest that the mechanism driving the partitioning of gallic acid in these systems is indeed more complex than expected, and must be certainly related with the ability of these long alkyl chain ionic liquids to form micelles in aqueous media. With the gathered data we can postulate that the mechanism of extraction is not related with the solvation of the conjugate base of the gallic acid inside the micelle; instead, gallate reduces the repulsion between the imidazolium head groups and stabilizes the micelle, enhancing thus the formation of self-aggregated structures. This would also explain why the octylsulfate-based system, also able to form micelles in aqueous solutions, has a poorer performance in extracting gallate since both species are negatively charged.

In a previous work [4], the extraction of vanillin was evaluated using systems composed of K_3PO_4 and several ILs. The partition coefficients of gallic acid and vanillin for a similar composition at the biphasic region (25 wt.% of IL + 15 wt.% of K_3PO_4), and at the same temperature in common IL-K_3PO_4-based ATPS, are compared in Supporting information. For all the studied systems, and at all the conditions analyzed, vanillin preferentially partitioned for the IL-rich phase [4]. Nonetheless, this pattern was not observed with gallic acid. Moreover, the partition coefficients obtained for vanillin [4] were substantially higher than those observed with gallic acid. This results from the fact that vanillin is less polar than gallic acid and is more easily extracted into the IL-rich phase.

Since the biphasic region for some IL-based ABS containing the phosphate buffer, K_2HPO_4/KH_2PO_4 [28,29], is smaller than for the remaining salts, the concentration of the IL used in the phosphate-buffer-containing systems was of 30 wt.%. Aiming at avoiding influences in the partition coefficient values that could be a merely result of the IL concentration, the systems containing the ILs [C_4mim][CF_3SO_3], [C_2mim][CF_3SO_3], and [C_4mim][N(CN)$_2$] were also evaluated at 25 wt.% with the phosphate buffer aqueous solution.

The partition coefficients and extraction efficiencies of gallic acid for the IL-rich phase and the pH of the coexisting phases of each system are sketched in Fig. 5. The change in the partition coefficients when increasing the ionic liquid concentration from 25 wt.% to 30 wt.% is almost insignificant. The difference of 5 wt.% in the ionic liquid concentration has thus a minor effect in the gallic acid partitioning that does not overlap with the larger differences observed among diverse ILs.

For the systems with 30 wt.% of IL + 15 wt.% of K_2HPO_4/KH_2PO_4 + 55 wt.% of an aqueous solution of gallic acid (Fig. 5), the K_{GA} values range between 0.84 and 11.35, and hence strongly depend on the IL employed. At the conditions investigated the partition coefficients of gallic acid in K_2HPO_4/KH_2PO_4-based ATPS decrease in the following order: [C_4mim]Br > [C_8mim] Cl > [C_4mim][CH_3SO_4] > [C_7mim]Cl > [C_4mim][N(CN)$_2$] > [C_4mim] [CF_3SO_3] > [C_2mim][CF_3SO_3].

In the systems composed of 25 wt.% of [C_2mim][CF_3SO_3] or [C_4mim][CF_3SO_3], and 30 wt.% of [C_2mim][CF_3SO_3], the partition coefficients observed are lower than 1, meaning that the gallic acid preferentially partitions towards the salt-rich phase. For the remaining systems, gallic acid partitions for the IL-rich phase. Although the partition of the gallic acid towards the ionic-liquid rich phase is favored by the phosphate buffer when compared with the K_3PO_4 salt, the general behavior observed is identical to that

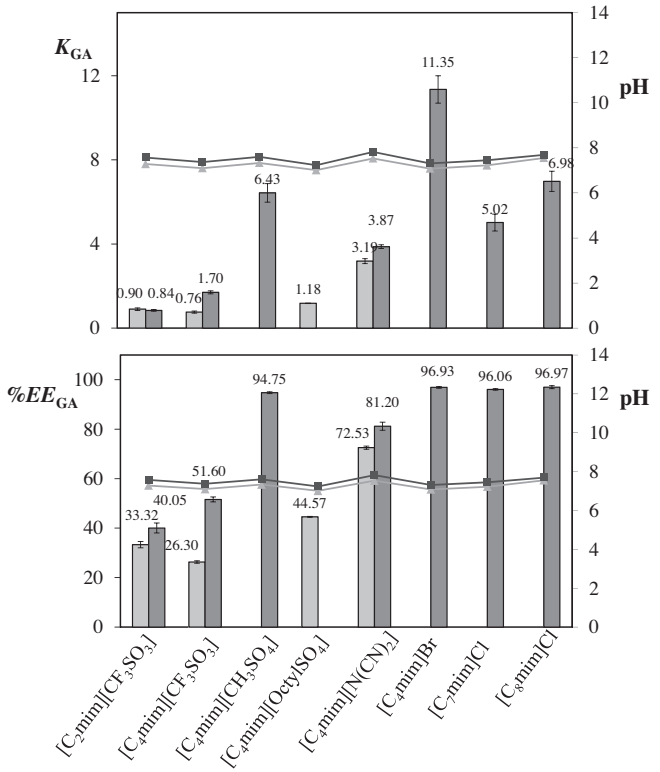

Fig. 5. Partition coefficients (K_{GA}) and extraction efficiencies percentages ($\%EE_{GA}$) of gallic acid, and pH of both IL- (squares) and salt-rich phases (triangles), for different IL-K_2HPO_4/KH_2PO_4-based ATPS at 298.15 K. The bars depicted in light gray correspond to systems containing 25 wt.% of IL, while the bars in dark grey refer to systems with 30 wt.% of IL.

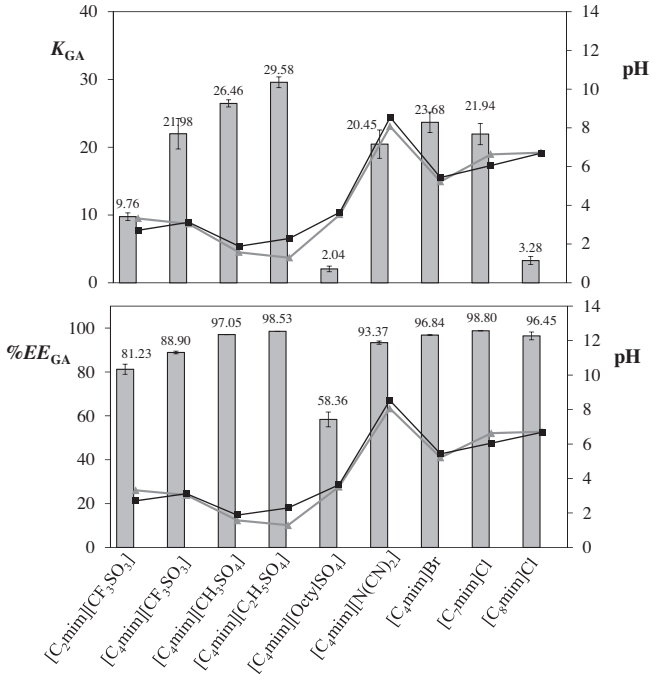

Fig. 6. Partition coefficients (K_{GA}) and extraction efficiencies percentages ($\%EE_{GA}$) of gallic acid, and pH of both IL- (squares) and salt-rich phases (triangles), for different IL-Na_2SO_4-based ATPS at 298.15 K.

previously discussed: there is a preferential partitioning of the biomolecule towards the salt-rich phase when the most hydrophobic ionic liquids are employed. Systems containing the most hydrophilic ionic liquids are now favored with extraction efficiencies increasing from 50% to 80–96%. However, the partitioning in the systems with [C_7mim]Cl and [C_8mim]Cl, with extraction efficiencies >96%, seems still to be driven by a different extraction mechanism.

The partition coefficients and extraction efficiencies of gallic acid in aqueous biphasic systems composed of Na_2SO_4 and distinct ILs, at 298.15 K, are reported in Fig. 6. The pH values of each aqueous phase are also displayed in Fig. 6. At pH values below 4.41 the dominant species is the non-charged form of gallic acid and the partitions are now all towards the ionic-liquid-rich phase with K_{GA} values ranging from 2.04 to 29.58 (extraction efficiencies from 58% to 99%).

At the conditions evaluated, the partition coefficients of gallic acid in Na_2SO_4-based ATPS decrease in the following order: [C_4mim][$C_2H_5SO_4$] > [C_4mim][CH_3SO_4] > [C_4mim]Br ≈ [C_4mim][CF_3SO_3] ≈ [C_7mim]Cl ≈ [C_4mim][$N(CN)_2$] >> [C_2mim][CF_3SO_3] >> [C_8mim]Cl > [C_4mim][OctylSO_4]. The sulfate-based ILs are, in this case, the improved ILs to extract gallic acid from aqueous media. The highly hydrophobic anions, such as $[CF_3SO_3]^-$ and $[OctylSO_4]^-$ present, on the other hand, the lowest extraction efficiencies. The good performance of the systems containing the [C_7mim]Cl, [C_8mim]Cl and [C_4mim][$N(CN)_2$] is related with their ability to extract gallate, present at the higher pH values observed for these systems, through the formation of micelles [31,32].

Having established that acidic media favors the partition of gallic acid towards the IL-rich phase, this approach was also applied to the vanillin partitioning which was previously studied in alkaline medium [4]. It was observed that the partition coefficients of vanillin (pKa = 8.2) [33] (for 25 wt.% of IL + 15 wt.% of inorganic salt, and at 298.15 K) ranged between 9.75 and 31.87 in K_3PO_4-IL-based ATPS, and between 59.32 and 70.03 in Na_2SO_4-IL-based ATPS for the ILs [C_4mim][$N(CN)_2$] and [C_4mim][CF_3SO_3], respectively. Further details on these values are provided at Supporting information. Therefore, the use of Na_2SO_4 (although a weaker salting-out agent than K_3PO_4) enhances the partitioning of added-value phenolic compounds for the IL-rich phase, since in the Na_2SO_4-based systems the pH is lower than in the K_3PO_4-based systems. Moreover, and as shown before, the extraction of vanillin is always superior to that observed with gallic acid using common IL-based systems due to its lower polarity.

To better understand the influence of the pH on the extraction of gallic acid and aiming at getting a more comprehensive analysis on the mechanism driving its partitioning, further experimental studies were conducted. For the system composed of Na_2SO_4,

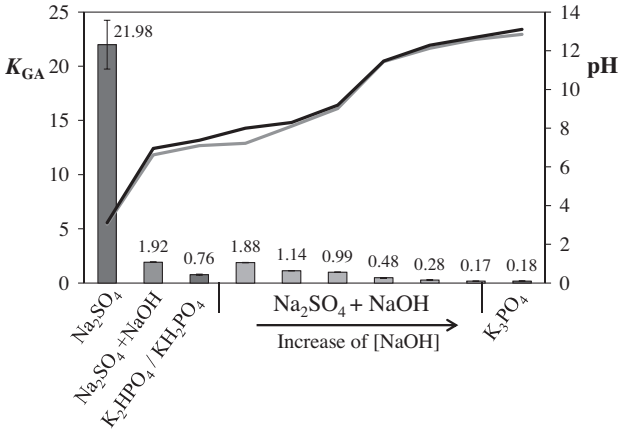

Fig. 7. Partition coefficients of gallic acid (K_{GA}), and pH of both IL- (black) and salt-rich phases (gray), for [C_4mim][CF_3SO_3]-based systems composed of different inorganic salts at 298.15 K.

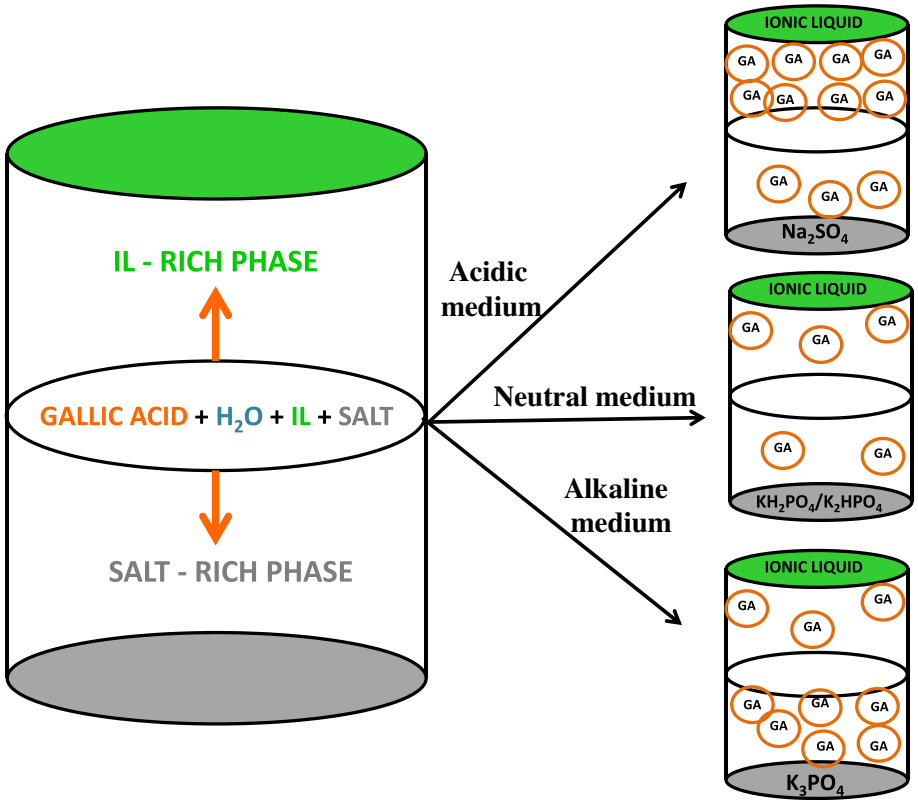

Fig. 8. Partitioning of gallic acid in different IL-based ATPS.

$[C_4mim][CF_3SO_3]$, and an aqueous solution of gallic acid, small amounts of NaOH were progressively added to increase the pH of the coexisting phases. The results obtained are depicted in Fig. 7. For pH values below the pKa of gallic acid (pKa = 4.4 [26]), the uncharged molecule preferentially migrates for the IL-rich phase. On the opposite, when NaOH is added and the pH of the medium is superior to 4.4, gallate migrates for the salt-rich phase. Indeed, the higher the pH value the lower is the partition coefficient of gallic acid. A graphical representation of the extraction mechanism of gallic acid.

A graphical representation of the extraction mechanism of gallic acid in the presence of different inorganic salts is summarized in Fig. 8. In general, the results gathered in this work support the idea that the neutral form of a biomolecule is more easily extracted into an IL-rich phase than its charged conjugate base and thus the pH can be used to control the direction of the partitioning.

4. Conclusions

Diverse IL-based ATPS were investigated regarding their extraction abilities for gallic acid – a phenolic compound of well-known interest. Several combinations of ILs and inorganic salts capable of creating ATPS were explored. Comparing the extractions performed with ATPS containing different inorganic salts (Na_2SO_4, K_2HPO_4/KH_2PO_4 and K_3PO_4) it was observed that Na_2SO_4-based systems provide an enhanced recovery of gallic acid at the IL-rich phase. From the results gathered at different pH values it was possible to recognize that there is a preferential partitioning of the non-charged molecule for the IL-rich phase, whereas the conjugate base tends to migrate for the salt-rich phase (more hydrophilic phase). Therefore, the choice of the inorganic salt, which in turns leads to different pH values, is a crucial factor in extraction approaches using IL-based ATPS – especially with compounds that exhibit acid dissociation constants. This concept was also tested with success in the extraction of vanillin with systems composed of Na_2SO_4 instead of K_3PO_4.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.seppur.2012.02.036.

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