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Ionic liquids as additives to enhance the extraction of antioxidants in aqueous two-phase systems

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ABSTRACT

Aqueous two-phase systems (ATPS) have been proposed as an alternative technique for the extraction, separation and/or purification of diverse biomolecules. Besides the typical polymer–salt ATPS, recently, ionic-liquid-(IL)–salt combinations have been reported to present higher extraction performances than the former systems are able to provide. Therefore, aiming at using the tailoring ability and high extraction efficiencies offered by ILs, yet with lower IL amounts, in this work novel ATPS composed of polyethylene glycol (PEG) and Na_2SO_4, using ILs as additives (at 5 or 10 wt%), were studied. Both the determination of the phase diagrams and their extraction efficiencies for gallic, vanillic and syringic acids were determined at 298 K. Furthermore, the effects of the molecular weight of PEG (200, 300, 400 and 600 g mol^{-1}) and of the IL chemical structure were investigated. The two-phase formation ability increases with the increase of the PEG molecular weight. Moreover, the addition of low amounts of ILs is favorable for the liquid–liquid demixing. The results obtained indicate that all the antioxidants investigated preferentially partition for the PEG-rich phase although depending on the PEG molecular weight and IL employed. The addition of 5 wt% of IL leads to extraction efficiencies ranging between 80% and 99%. These results clearly demonstrate the ability of the IL to tune the polarity of the PEG-rich phase and where the IL chemical structure plays a dominant role in the extraction of phenolic acids. PEG–salt–IL ATPS represent thus an interesting advance in separation processes and open the door for a new range of IL-based extraction processes.

1. Introduction

Aqueous two-phase systems (ATPS) consist on two macroscopic liquid phases formed by the dissolution in water, above certain concentrations, of two incompatible hydrophilic solutes [1]. These solutes can be two polymers, a polymer and a salt, or two salts [2–5]. Due to the existence of their liquid coexisting phases, ATPS can be regarded as a powerful and non-chromatographic process for the separation and/or purification of the most diverse biomolecules. In fact, conventional ATPS have been successfully applied in the purification of different biological materials, such as cells, nucleic acids, lipids, amino acids, proteins, antibodies and enzymes without significant denaturing effects [1,2,6–8]. Both phases in ATPS mainly consist of water (*ca.* 80–90 wt%) and most of the polymers have a stabilizing effect on the proteins tertiary structure [7,9,10]. This technique is relatively simple and inexpensive, of easy operation allowing its scale-up, and further ensures the purification and concentration stages to be integrated in a single step procedure [7,9].

Conventional ATPS are typically formed by polymer–polymer or by polymer–salt mixtures [1]. Polyethylene glycol (PEG) is commonly used as one of the phase-forming polymers in ATPS because it presents high biodegradability, low toxicity, low volatility, low melting temperature, large water miscibility and low cost [6,11]. PEG is a polyether diol that is commercially available in a wide variety of molecular weights. Salt-polymer-type ATPS provide advantages over systems formed by polymer–polymer combinations, such as a low interfacial tension, fast and high phase separation rates and low cost, which makes them practical for downstream processing [6]. Despite all these advantages, the narrow tailoring nature of PEG, which can be achieved only by changes in the molecular weight or by the polymer structural modification, limits its applicability through the complete extraction of several biomolecules to the polymer-rich phase [6]. To overcome this limitation, recent works have introduced ionic

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liquids (ILs) to tune the physicochemical properties of the PEG-rich phase, either by using them as adjuvants or by the synthesis of PEG-functionalized ILs foreseeing high extraction yields [6,12,13]. The reported results [6,12,13] suggest that the use of ILs in conventional ATPS provide tailored and optimized extractions by a proper choice of the chemical structure of the IL.

ILs are salts that are liquid below a conventional temperature of 100 °C and they are usually constituted by a large asymmetric organic cation and either an organic or inorganic anion. Due to their inherent ionic character, most of these fluids present remarkable properties, such as a negligible vapor pressure, null flammability, high ionic conductivity, as well as high thermal and electrochemical stabilities [14–17]. In addition to the ILs negligible volatility and non-flammability – the main features which have contributed to their recurrent classification as "green solvents" – one of the main advantages of ILs as phase-forming components in ATPS is the possibility of tailoring their phases' polarities and affinities by an adequate manipulation of the cation/anion combinations ("designer solvents") [18]. Indeed, ATPS constituted by ILs cover a much wider hydrophilic–lipophilic range allowing for more extensive and selective separations [19]. Due to these outstanding features, ATPS composed of ILs have been intensively investigated in the last decade for the extraction of the most diverse (bio)molecules [2,6,20–23], where results up to complete extraction and concentration factors up to 100 times were achieved.

Antioxidants are phenolic compounds that exhibit relevant properties in the health and nutrition fields. These compounds are widely used in dietary supplements and they have been investigated for the prevention of cancer, coronary heart disease and even altitude sickness due to their antioxidant and radical scavenging properties [24]. Antioxidants are also commonly used and/or added in nutraceutical and cosmetic-related products [25]. Examples of simple antioxidants structures are vanillic, gallic, protocatechuic, ellagic and syringic acids that are typically present in natural sources such as wood, barks, fruits and vegetables [26,27]. In the past few years, there has been a great demand for antioxidants extracted from natural sources to substitute synthetic counterparts that can lead to adverse effects in human health [25,28].

In order to develop new systems for the extraction and concentration of antioxidants, in this work, the ternary phase diagrams of ATPS composed of PEG + $NaSO_4$ were firstly determined at 298 K. The effect of the molecular weight of PEG (200, 300, 400 and 600 g mol^{-1}) was also addressed through the phase diagrams behavior. These ATPS were then evaluated in what concerns their extractive performance for three antioxidants, namely gallic acid (3,4,5-trihydroxybenzoic acid, $C_6H_2(OH)_3COOH$), vanillic acid (4-hydroxy-3-methoxybenzoic acid, $C_6H_3(OH)(OCH_3)COOH$) and syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid, $C_6H_2(OH)(OCH_3)_2COOH$). The molecular structures of the antioxidants investigated are depicted in Fig. 1. Aiming at tailoring the properties of the coexisting phases in the studied polymer–salt ATPS, ILs were additionally evaluated as potential adjuvants to tune the partitioning of the biomolecules for the PEG-rich phase. The effect of eight ILs and their concentration (5 and 10 wt%) in the phase diagrams of the systems constituted by water + PEG + Na_2SO_4 was addressed. Moreover, the influence of the IL chemical structure and pH of the medium on the partition coefficients and extraction efficiencies of gallic, vanillic and syringic acids were evaluated and compared with the results where no IL was added. The chemical structures of the ILs and polymer investigated are shown in Fig. 2.

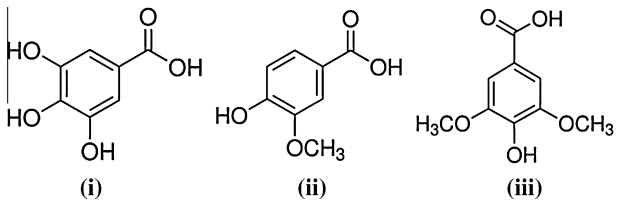

Fig. 1. Chemical structure of the antioxidants studied: (i) gallic acid; (ii) vanillic acid; (iii) syringic acid.

2. Experimental section

2.1. Materials

The ATPS studied in this work were established by using an aqueous solution of sodium sulfate, Na_2SO_4 (anhydrous, 100 wt% pure from Prolabo), and several solutions of PEGs. The PEGs studied were of molecular weight 200 g mol^{-1}, 300 g mol^{-1}, 400 g mol^{-1} and 600 g mol^{-1} and are abbreviated as PEG 200, PEG 300, PEG 400 and PEG 600, respectively. All the polymers were acquired from Fluka with the exception of PEG 300 that was from Sigma–Aldrich. Besides the determination of the PEG–salt systems, the effect of ILs through the phase diagrams and partitioning behavior was also investigated. The ILs studied were: 1-butyl-3-methylimidazolium thiocyanate, [C_4mim][SCN] (>98 wt% pure); 1-butyl-3-methylimidazolium tosylate, [C_4mim][TOS] (98 wt% pure); 1-butyl-3-methylimidazolium dicyanamide, [C_4mim][$N(CN)_2$] (>98 wt% pure); 1-butyl-3-methylimidazolium acetate, [C_4mim][CH_3CO_2] (98 wt% pure); 1-butyl-3-methylimidazolium chloride, [C_4mim]Cl (>99 wt% pure); 1-butyl-1-methylpiperidinium chloride, [C_4mpip]Cl (99 wt% pure); and 1-butyl-1-methylpyrrolidinium chloride, [C_4mpyr]Cl (>99 wt% pure). All ILs were purchased from Iolitec and their chemical structures are shown in Fig. 2. To reduce the content of water and other volatile compounds to negligible values, ILs individual samples were dried under constant agitation, at vacuum and moderate temperature (≈323 K), for a minimum of 24 h. After this process, the purity of each IL was further checked by ^1H and ^{13}C NMR spectra and found to be in accordance with the purity levels given by the supplier.

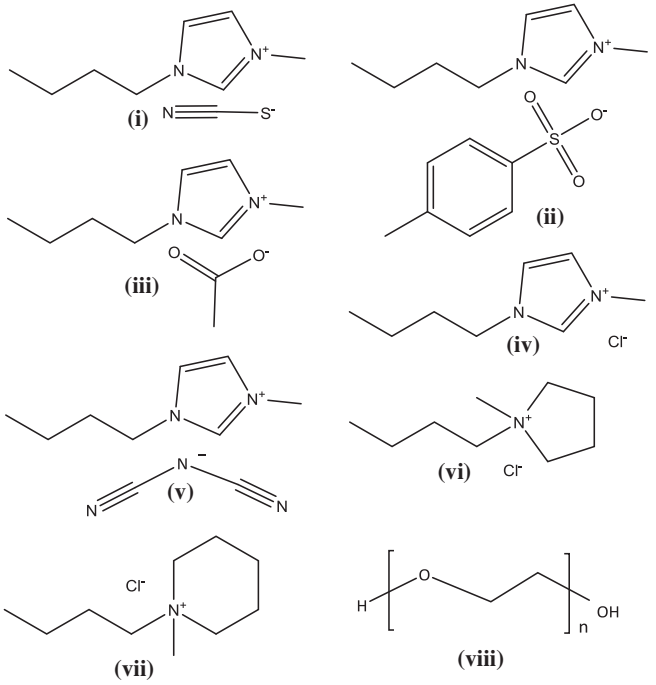

Fig. 2. Chemical structure of the studied ILs and PEG: (i) [C_4mim][SCN]; (ii) [C_4mim][TOS]; (iii) [C_4mim][CH_3CO_2]; (iv) [C_4mim]Cl; (v) [C_4mim][$N(CN)_2$]; (vi) [C_4mpyr]Cl; (vii) [C_4mpip]Cl; (viii) PEG.