Design of novel aqueous micellar two-phase systems using ionic liquids as co-surfactants for the selective extraction of (bio)molecules

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ABSTRACT

Aqueous micellar two-phase systems (AMTPS) using surfactants have been widely applied as an advantageous extraction technology for a wide range of (bio)molecules. When composed by two surfactants (the second one used as the co-surfactant), the extractive performance of such systems is considerably boosted. In this context, this work looks at ionic liquids (ILs) as a new class of tunable co-surfactants to be applied in AMTPS. Three distinct families of ILs, namely imidazolium, phosphonium and quaternary ammonium were applied in the design of the binodal curves of novel AMTPS based on the nonionic surfactant Triton X-114. On these systems it was investigated the impact of the IL absence/presence, concentration and structural features on the binodal curves behavior. Aiming at evaluating their applicability as extraction systems, partitioning studies of two targeted (bio)molecules, namely the protein Cytochrome c (Cyt c) and the dye/model drug Rhodamine 6G (R6G), were carried out. It is shown that the presence of ILs as co-surfactants is able not only to enhance the partition coefficients of Cyt c (indicated as $\log K_{\text{Cyt c}}$) from -0.59 ± 0.12 up to -1.51 ± 0.14, but also to improve the selectivity parameter ($S_{\text{R6G/Cyt c}}$) from 925.25 up to 3418.89. The results here obtained open new perspectives in the design of liquid–liquid separation processes, transversal to various fields and a wide range of applications.

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1. Introduction

During the last few years, with the appearance of new biopharmaceuticals and other complex molecules of biotechnological origin, there is a stringent need for improvements at the level of the downstream processing. While several advances in large-scale production methodologies were attained, the downstream still remains the main drawback for the scale-up of various processes towards industrial implementation. This is mainly due to technological limitations and the need for multiple unit operations to obtain a final product fulfilling the strict purity and safety requirements [1]. Chromatographic techniques are the most usual choice in these fields, owing to their simplicity, selectivity and accurate resolution; however, such methodologies present some limitations related with their scalability and economic viability [2]. Thus, there is an urgent demand for new separation/purification techniques, that present good extractive performances, as well as guarantee

the chemical structure and activity of the biomolecule isolated, while maintaining the economic viability of the entire process.

Liquid–liquid extraction (LLE) has been identified as a suitable technique for downstream processes due to its simple and fast operation. Traditionally, LLE was mostly accomplished by applying environmentally nefarious and expensive organic solvents [3]. In this context, aqueous two-phase systems (ATPS) emerged as appellative types of LLE, since they are mainly composed of water and do not require the use of organic solvents in the whole process, providing mild operation conditions [4,5]. These systems consist of two aqueous-rich phases of two structurally different compounds that are immiscible above stated values of concentration, undergoing phase separation. At the end, ATPS give rise to highly flexible approaches, since a considerable array of compounds, e.g. polymers, salts or surfactants, can be combined in order to strategically design them to achieve high extraction and purification effectiveness, selectivities and yields [4].

Aqueous micellar two-phase systems (AMTPS) are specific types of ATPS that use surfactants and appear as promising techniques for bioseparation purposes, due to their remarkable ability of keeping the native conformations and activities of biomolecules [6],

while they are migrating between the aqueous-rich coexisting phases. Surfactants are amphiphilic molecules, *i.e.* present a polar, hydrophilic and sometimes charged 'head' and a non-polar, hydrophobic 'tail'. When its concentration is above a certain value, *i.e.* the critical micelle concentration (CMC), the surfactant molecules form self-assembling aggregates [6,7]. Within micelles, each surfactant molecule displays its 'tail' in the interior, while its 'head' plays an important role in the interface between the aqueous solution and the aggregate's core [6–8]. AMTPS appear when the surfactants present in aqueous solution are able to form two macroscopic phases under specific conditions. These systems are dependent on temperature and surfactant concentration, displaying a single phase below a temperature [7], known as cloud point [9] or T_{cloud} [8], and undergoing phase separation above T_{cloud}. AMTPS are composed of two distinct phases, one micelle-rich and one micelle-poor, that can be either the top or bottom layers, depending on the surfactant adopted [6–9]. The phase separation behavior for different surfactants can be described by establishing the binodal curves, *i.e.* plotting T_{cloud} *versus* surfactant concentration, which represent the boundary between the conditions at which the system presents a single phase (below/outside the curve) or two macroscopic phases (above/inside the curve) [7]. Meanwhile, some additives, depending on their physico-chemical properties, were shown to affect the phase separation behavior of surfactant-based mixtures [10,11].

The pioneering work of Bordier [12], in which the differential partitioning of a plethora of proteins within AMTPS phases was successfully carried out, triggered the publication of many other works applying this type of systems in bioseparation. Since then, not only the separation, concentration and purification of several proteins using AMTPS were addressed [6–8,13,14], but also of a wider array of biocompounds such as viruses [6], DNA [15], bacteriocins [16], antibiotics [17] and porphyrins [18]. Moreover, Kamei and co-workers [19] focused on the balance of interactions affecting the effectiveness of such systems and proved that the electrostatic forces between biomolecules and micelles play a crucial role in the migration phenomenon in AMTPS composed of a pair of distinct surfactants; the authors brought new evidences that this technique could be an enhanced route for the selective extraction, if properly designed. Based on this concept, this work envisages the exploitation of this type of interactions in the partitioning phenomenon in AMTPS by introducing a novel class of ionic solvents – the ionic liquids (ILs). During the last decade, ILs have attracted the attention from both academia and industry in several areas of application; one of those includes the use of ILs as suitable and effective separation and purification agents in ATPS, boosting the extractive performance as well as the selectivity parameters [20]. This crescent interest relays on their unique properties, such as negligible vapor pressure, high chemical and thermal stability, non-flammability, low melting point among others [20–22]. Moreover, ILs are liquid in a wide range of temperatures and are able to solvate solutes from a broad spectrum of polarities, and owing to the countless cation/anion possible combinations their properties can be tuned to fit the specific purposes of a given application [20,21]. The application of such solvents in AMTPS can be supported by the evidence produced by Bowers et al. [23], describing the possibility of ILs to self-aggregate because when they have a long enough alkyl chain they are amphiphilic molecules. Since then, a number of authors have studied the aggregation and micelle formation of ILs in aqueous solutions [24–28], their incorporation in mixed micelles [26–34] and their contribution to the modification of the physico-chemical properties of surfactant micelles [24,26–34]. ILs with long alkyl chains were also found to be able to self-aggregate, promoting significant increments in enzymatic activity [35]. The addition of ILs to a surfactant can either decrease [26,29,31] or increase [28,32,33] the CMC and also

affect the aggregation number [23,24,29], depending on the ILs structural features (alkyl side chain, cation and anion) and the surfactant head group [26,29,34]. The ILs ability to act as surfactants has been evaluated by the determination of their CMCs using several methods, such as, electric conductivity [23–25,27,29,31], surface tension [23,28,32,34], fluorescence [24,26] and isothermal titration calorimetry [25,31] measurements.

Herein, a novel class of AMTPS based on the nonionic surfactant Triton X-114 as the main surfactant, the McIlvaine buffer and ILs belonging to three distinct families, namely the imidazolium, the phosphonium and the quaternary ammonium as co-surfactants (the use of ILs as co-surfactants to form mixed micelles was already briefly described [36]) is assessed. The solubility curves of such systems were established to evaluate the impact of the presence of the IL as co-surfactant in conventional surfactant/salt-based AMTPS, as well as its concentration and structural features. Moreover the potential of these AMTPS to be used in (bio)separation processes was evaluated. They were investigated in terms of their capacity to selectively separate two model (bio)molecules: the protein Cytochrome c (Cyt c), which possesses an essential biological role and here used as a model protein [6], and Rhodamine 6G (R6G) used as a model drug [37].

2. Experimental section

2.1. Material

The imidazolium-based ILs 1-decyl-3-methylimidazolium chloride [C_{10}mim]Cl (purity >98 wt%), 1-dodecyl-3-methylimidazolium chloride [C_{12}mim]Cl (purity >98 wt%) and 1-methyl-3-tetradecylimidazolium chloride [C_{14}mim]Cl (purity >98 wt%) were acquired at Iolitec (Ionic Liquid Technologies, Heilbronn, Germany). All the phosphonium-based ILs, namely trihexyltetradecylphosphonium chloride [$P_{6,6,6,14}$]Cl (purity = 99.0 wt%), trihexyltetradecylphosphonium bromide [$P_{6,6,6,14}$]Br (purity = 99.0 wt%), trihexyltetradecylphosphonium decanoate [$P_{6,6,6,14}$][Dec] (purity = 99 wt%), trihexyltetradecylphosphonium dicyanamide [$P_{6,6,6,14}$][N(CN)$_2$] (purity = 99.0 wt%), trihexyltetradecylphosphonium bis (2,4,4-trimethylpentyl)phosphinate [$P_{6,6,6,14}$][TMPP] (purity = 93.0 wt%) and tetraoctylphosphonium bromide [$P_{8,8,8,8}$]Br (purity = 95.0 wt%) were kindly supplied by Cytec. The ammonium-based IL tetraoctylammonium bromide [$N_{8,8,8,8}$]Br (purity = 98 wt%) was purchased from Sigma–Aldrich®. The chemical structures of the cations and anions composing the list of ILs herein investigated are depicted in Fig. 1a. Triton X-114 (laboratory grade) was supplied by Sigma–Aldrich®and the McIlvaine buffer components, namely sodium phosphate dibasic anhydrous Na$_2$HPO$_4$ (purity ⩾99%) and citric acid anhydrous C$_6$H$_8$O$_7$ (purity = 99.5%) were acquired at Fisher Chemical and Synth, respectively. The Cytochrome c (Cyt c) from horse heart (purity ⩾95 wt%) and Rhodamine 6G (R6G) (purity ≈95 wt%), depicted in Fig. 1b, were both acquired at Sigma–Aldrich®.

2.2. Methods

2.2.1. Binodal curves

The binodal curves of the AMTPS composed of Triton X-114 and the McIlvaine buffer at pH 7 (82.35 mL of 0.2 M Na$_2$HPO$_4$ + 17.65 mL of 0.1 M C$_6$H$_8$O$_7$), using different ILs as co-surfactants were determined using the cloud point method, whose experimental protocol is well described in literature [38]. This methodology consists of a visual identification, while raising the temperature, of the point at which a mixture with known compositions becomes turbid and cloudy, *i.e.* T_{cloud}. Then, the experimental binodal curves are obtained by plotting the T_{cloud} *versus* the surfactant mass