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Ionic liquids as a novel class of electrolytes in polymeric aqueous biphasic systems

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ABSTRACT

The use of ionic liquids (ILs) as electrolytes in aqueous biphasic systems (ABS) composed exclusively of polymers and their application to the extraction of biomolecules is here studied. The binodal curves of the quaternary systems formed by polyethylene glycol (PEG 8000), sodium poly(acrylate) (NaPA 8000), water and ILs or inorganic salts as electrolytes are established. These systems enable the assessment of the influence of the salt nature, chemical structure and concentration on the two-phase formation. These systems were characterized regarding the pH and the ILs' partition between both phases. Moreover the extractive potential of these ABS is evaluated using the protein cytochrome *c* (Cyt *c*) and the dye chloranilic acid (CA). The main results on the extraction efficiencies show that Cyt *c* is recovered in the NaPA 8000-rich phase (EE_{Cyt *c*} > 96.13 \pm 3.22%), while CA preferentially migrates towards the PEG 8000-rich phase. These results indicate that this class of polymeric ABS may be an advantageous tool in the development of novel extractive platforms.

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1. Introduction

Polyethylene glycol (PEG) is a non-ionic polymer commonly used in the preparation of aqueous biphasic systems (ABS), since it presents advantageous characteristics in terms of biodegradability, toxicity and cost-effectiveness. In order to create polymeric ABS, blends of PEG with other polymers, such as maltodextrin [1,2] and specially dextran [3,4], are the most common options. The number of water soluble polymers and of their mixtures is very large; yet, some of the potential pairs of polymers are not able to form two-phase systems at operationally convenient amounts. This limitation triggered the creation of a novel type of ABS, capable of minimizing the polymers concentrations required, while increasing those of water [5–7]. This can be attained through the addition of small amounts of a salt (1-5 wt%) [6] to an aqueous solution of polymers, where they act as electrolytes and induce phase separation. When no salt is added the two-phase formation does not occur or is only observed at very high polymer concentrations, and

http://dx.doi.org/10.1016/j.procbio.2015.02.001 1359-5113/© 2015 Elsevier Ltd. All rights reserved. therefore, the presence of one electrolyte is essential for this type of ABS formation [5–7]. Molecular mechanisms ruling the phase separation phenomenon were proposed by Johansson et al. [6]. They showed that the phase separation was promoted by the presence of the electrolyte ions which decrease the entropy of compartmentalization of the counter ions in the co-existing phases. The adequate selection of the electrolyte allows the control of the width of the biphasic regions [6], in which it is possible to work at very low polymers' concentrations (i.e. lower than 10 wt% of each polymer), and the partition of the solutes between the phases. These systems have been applied to the extraction and purification of an extensive range of molecules of biotechnological importance from several sources namely, distinct proteins [8,9], enzymes [8,10–12], DNA [13,14] and antibiotics [15]. Since such systems were shown to maintain the native conformation and biological activity of various biomolecules including proteins, and allow high yields in the purification processes [8,9], they are believed to be biocompatible and effective extraction processes.

Ionic liquids (ILs) are salts, liquid at temperatures below 100 °C, composed of large organic cations with dispersed charge, and anions of either inorganic or organic nature [16]. They present unique characteristics [16] and an outstanding ability to solvate

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Fig. 1. Chemical structures of the polymers and the molecules under study.

compounds from a wide spectrum of polarities [17,18]. The tunability of the ILs' properties by anion–cation combinations [19] makes it possible to control the formation and design of ABS by the manipulation of the phases' polarities allowing the creation of tailored systems for a given purpose. Their applications are well beyond the ABS domain since these ionic compounds are widely used in several areas of expertise such as organic chemistry [20], electrochemistry [21] and nanotechnology [22].

This work aims at studying the use of ILs as electrolytes to prepare polymeric ABS. Firstly, binodal curves of quaternary systems composed of PEG+sodium polyacrylate (NaPA) (both with an average molecular weight of 8000 g mol⁻¹)+ILs or inorganic salts (as electrolytes)+water were established at (298 ± 1) K and atmospheric pressure. Several ILs formed by distinct cations and anions and inorganic salts were applied as electrolytes, allowing the comparative assessment of their performance. The second part of this work evaluates the use of these systems in extraction processes, in which cytochrome c (Cyt c) and chloranilic acid (CA) were tested as model molecules. For this purpose only ABS containing imidazolium-based ILs and inorganic salts at either fixed or variable concentrations were considered. The ILs partition among the two phases, the pH and extraction parameters (extraction efficiencies for the two model compounds and partition coefficients for CA) were determined. Insights into the partitioning phenomenon are provided, allowing the development of heuristic rules for the design of these systems as powerful extraction platforms.

2. Materials and methods

2.1. Materials

PEG 8000 and the aqueous solution of NaPA 8000 (45 wt%) were used as received from Sigma-Aldrich[®]. Na₂SO₄ and NaCl were acquired from Synth[®] with 98% of purity. Chloranilic acid (purity >99 wt%) and cytochrome *c* from equine heart (purity =95 wt%) were purchased from Merck[®] and Sigma-Aldrich[®], respectively. The chemical structures of the polymers and the model molecules used are provided in Fig. 1. Eleven ILs sharing distinct cations and anions were investigated and their names, acronyms, chemical structures, purities and suppliers are listed in Table 1. The purity of all ILs was confirmed by ¹H and ¹³C NMR and found to match the

suppliers' information. The water used was double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

2.2. Binodal curves determination

The experimental binodal curves were determined gravimetrically, within an uncertainty of $\pm 10^{-4}$ g, using the cloud point titration method [23] at (298 ± 1) K and atmospheric pressure. Briefly, three stock aqueous solutions were prepared: 50.0 wt% of PEG 8000+5.0 wt% of IL or inorganic salt, 18.0 wt% of NaPA 8000+5.0 wt% of IL or inorganic salt and water+5.0 wt% of IL or inorganic salt. Additionally, four distinct IL and inorganic salt concentrations (used as electrolytes) were tested, namely 5.0, 2.5, 1.0 and 0.1 wt%. Drop-wise addition of the PEG 8000-containing solution was carried out to the solution of NaPA 8000 (and/or vice versa when necessary) until the visual detection of two phases. Subsequently, drop-wise addition of the IL/inorganic salt-containing solution was conducted until the two phases disappear (monophasic region). This procedure was repeated several times in order to obtain the binodal curve, being performed under constant stirring. The experimental phase diagrams data were correlated using the Merchuk equation [24] (Eq. (1)):

$$[PEG \ 8000] = A \ \exp[(B \times [NaPA \ 8000]^{0.5}) - (C \times [NaPa \ 8000]^3)$$
(1)

where [PEG 8000] and [NaPA 8000] represent the weight percentages of PEG 8000 and NaPA 8000, respectively. *A*, *B* and *C* are constants obtained by the regression of the experimental data. The Merchuk equation was chosen since it has a low number of adjustable parameters to correlate these data and it is the most commonly applied [25].

2.3. Partition of Cyt c, CA and imidazolium-based ILs

For the partition studies, only the imidazolium-based ILs and the inorganic salts were used. A mixture point within the biphasic region was selected and prepared by weighing the appropriate amounts of each compound: 15.0 wt% of PEG 8000+4.5 wt% of NaPA 8000 + 5.0 wt% of electrolyte + 0.1 g of a Cyt c stock aqueous solution (at circa 5.0 gL^{-1}) or 1 g of a CA stock aqueous solution (at circa 0.72 gL^{-1})+water (fulfilling a total mass of 5 g). For further optimization, the effect of electrolyte concentration on the partition of CA was addressed by using systems containing either [C₂mim][N(CN)₂] or NaCl. In these cases, the same mixture compositions aforementioned were prepared by varying only the amount of electrolyte: 5.0; 2.5; 1.0 and 0.1 wt%. All the components were mixed together using a Vortex agitator (IKA, C-MAG HS7), and then equilibrated at (298 ± 1) K and atmospheric pressure during 12 h, allowing the equilibrium to be reached. At the end, the final systems generated two clear phases, a PEG 8000rich as the top and a NaPA 8000-rich as the bottom layer, with a well-defined interface in between. The coexisting phases were carefully separated and collected for the measurement of their volumes and pH values, as well as for the molecules quantification assavs.

The target compounds and the imidazolium-based ILs content was determined using a Molecular Devices Spectramax 384 Plus | UV–Vis Microplate Reader, at the respective maximum wavelength of absorbance, namely 409 nm for Cyt *c*, 330 nm for CA, and 211 nm for the [C_2 mim]X. At least three independent samples of each system were prepared, being the average values reported along with the respective standard deviations.

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