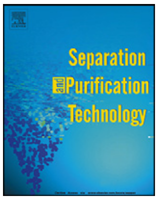
Separation and Purification Technology 72 (2010) 85–91

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur



(Extraction of biomolecules using) aqueous biphasic systems formed by ionic liquids and aminoacids

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ARTICLE INFO

Article history:
Received 29 September 2009
Received in revised form 11 January 2010
Accepted 15 January 2010

Keywords:
Aqueous biphasic systems
Extraction
Aminoacids
Ionic liquids

ABSTRACT

The increasing emphasis on cleaner and environmentally benign extraction procedures has led to the systematic investigation of systems containing ionic liquids (ILs)—a new class of non-volatile alternative solvents. In this work, aqueous biphasic systems (ABS) composed by hydrophilic ILs and aminoacids were studied aiming at obtaining new evidences regarding their ABS formation ability and their capacity for the extraction of specific biomolecules. On the basis of the IL cation 1-butyl-3-methylimidazolium, the IL anion influence on ABS formation was assessed through its combination with tetrafluoroborate, triflate, and dicyanamide anions, with three different aminoacids: L-lysine, D,L-lysine HCl and L-proline. Ternary phase diagrams (and respective tie-lines) formed by these aqueous solutions of the ILs and the selected aminoacids, were measured at 298 K and atmospheric pressure. The results indicate that the ability of an IL to produce ABS closely follows the decrease in the hydrogen-bond accepting strength of the IL anion. In addition, the ability of aminoacids to form ABS follows the order: L-lysine ≈ D,L-lysine HCl > L-proline. Finally, the extraction capability of the studied ABS was evaluated through their application to the extraction of three biomolecules (caffeine, ciprofloxacin and ciprofloxacin HCl).

1. Introduction

Separation and purification stages of biotechnological processes usually require numerous steps with high energy and chemical consumption and have often a large impact on the cost of the final product [1,2]. One of the major industrial challenges in biotechnology is the development of fast, efficient and cost-effective downstream processes for the recovery of biomolecules from fermentation media, while minimizing the environmental impact of the processes adopted. Among others [3–6], liquid–liquid extraction techniques seem to have the greatest potential, offering advantages such as higher capacity, better selectivity and integration between recovery and purification, and providing higher yields, purities and lower costs [2,7]. In spite of its effectiveness, liquid–liquid extraction processes may have some negative environmental impact and often rise concerns related with the purity and quality of the extracted biomolecules due to the toxic and denaturating characters of most volatile organic solvents commonly employed [2]. For this reason, in the past few years, there has been an active search for alternative approaches [8–10] to overcome those environmental and operational problems. In this context, the replacement of ordinary solvents by ionic liquids (ILs) as extraction media has emerged as one of the most promising techniques. In fact, besides their ambient-friendly thermophysical properties such as negligible vapour pressure, non-flammability and high chemical stability [11], ILs present excellent solvation qualities [12] and usually provide a non-denaturing environment for biomolecules, maintaining protein structure and enzymatic activity [13,14]. Since these properties are strongly dependent on the solvent nature, the possibility of customizing and manipulating the properties of the IL through the selection of the anions and cations that compose them represents an additional advantage. While the low vapour pressures of these compounds and consequent reduced air pollution risks is one of their most claimed advantages, the release of these liquids into aquatic environments may lead to water contamination because they have at least some miscibility with water. Recent reports [15–17] show that the ecotoxic character of ILs seems to increase with their hydrophobicity; hydrophilic ILs such as those most convenient for biochemical separations present very low toxicities.

Most biotechnological applications of liquid–liquid extraction using ILs are based on aqueous biphasic systems (ABS). Since

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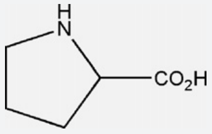
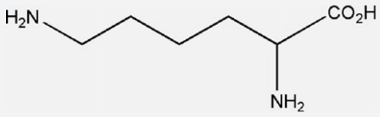
doi:10.1016/j.seppur.2010.01.008

Table 1
Structure of the aminoacids studied and their dissociation constants [43,44] and solubilities in water (*s*) [44].

Aminoacid	Molecular structure	pK_a (–COOH)	pK_a (–NH_3)	pK_a (–R)	*s*/g (100 g^{-1})
Pro		2.00	10.60	–	162.3
Lys		2.18	8.95	10.53	Very soluble

Gutowski et al. [18] reported that the addition of potassium phosphate to an aqueous solution of a hydrophilic IL produces ABS, the advantages of ABS technology – clarification, concentration and purification integrated in one step and high selectivity and scaling-up facility – have been investigated the tailoring properties of ILs to develop specific extraction and isolation procedures. A large amount of studies on the phase behaviour of ternary systems composed of ILs + water + salting-out inducing inorganic ions and on the ability of the last to form IL-based ABS can now actually be found in literature [19–26], along with some reports on the extraction potential of those systems for some biomolecules, such as testosterone and epitestosterone [23], opium alkaloids [24], antibiotics [25,26] and aminoacids [20,21]. Although often used, these kinds of IL/inorganic salt separation systems are highly ionic media and therefore might not be compatible with the extracted product, since many biomolecules present narrow tolerance limits of ionic strength. Moreover, the ion exchange between salt and IL can complicate the separation procedure and the recyclability of the IL from aqueous solutions while the salt disposal might cause a significant impact on wastewater treatment [27]. The use of sugars [28,29] and, very recently, of aminoacids [30] to form more environmentally benign and less aggressive IL-based ABS has been suggested to overcome these issues. Nevertheless, both liquid–liquid equilibrium results and extraction ability data are still very scarce and systematic work is still required to develop the potentialities of those new ABS. Actually, to the best of our knowledge there is a single investigation study [30] concerning aminoacid-ILs ABS-promoting capability and no reports on the extraction ability of those systems for biomolecules were found.

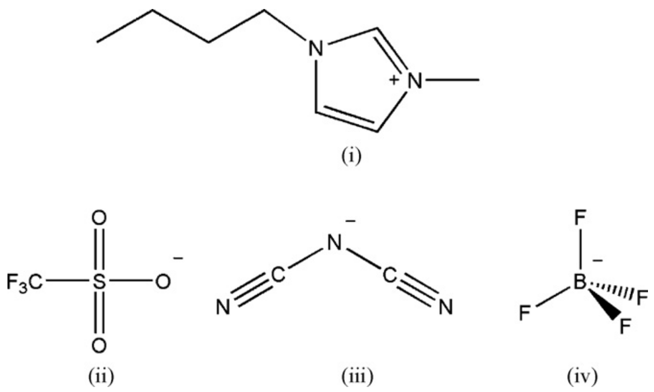
In this work, we evaluate the capability of ILs and aminoacids to promote ABS and study the influence of both the IL anion nature and the aminoacid structure on ABS formation. For that purpose, three imidazolium-based ILs—1-butyl-3-methylimidazolium triflate ([C_4mim][CF_3SO_3]), 1-butyl-3-methylimidazolium dicyanamide ([C_4mim][$N(CN)_2$]) and 1-butyl-3-methylimidazolium tetrafluoroborate ([C_4mim][BF_4])—and two structurally different aminoacids—proline (Pro) and lysine (Lys)—were selected. Additionally, the monohydrochloride form of lysine (Lys·HCl) was also considered in order to assess the effect of the addition of the HCl group on the promotion of the ABS. The ILs were chosen on the basis of a previous systematic study [20] which indicates that imidazolium-based ILs comprising triflate and dicyanamide anions present higher ABS formation capability in the presence of K_3PO_4. This fact is very important in the present work since aminoacids have a less pronounced salting-out inducing behaviour than the inorganic salts commonly used (e.g. K_3PO_4). In addition, imidazolium-based ILs with short cation alkyl chains are known to have low toxicities [15–17,31,32].

Ternary phase diagrams for different systems composed by hydrophilic IL + aminoacid + water were determined, at 298 K and atmospheric pressure, maintaining either the aminoacid or the IL to establish, respectively, the effect of the IL anion nature and of aminoacid structure on promoting ABS. The binodal curves were fitted to a three-parameter equation, and the tie-lines were estimated using the Merchuck et al. [33] approach. The ABS studies were further analysed according to their potential for the extraction of biomolecules, for which one alkaloid (caffeine) and two forms of an antibiotic with different aqueous solubilities (ciprofloxacin and ciprofloxacin·HCl) were selected as model compounds of biotechnological interest. The development of methods for the recovery and determination of alkaloids [24] and for the production of antibiotics at lower manufacturing costs [25,34,35] is still a significant challenge. Besides this specific practical interest, this work opens up new possibilities in the separation of other drugs from biological samples and gives another contribution towards the understanding of the molecular interactions which control the separation and purification processes by ILs [36–39].

2. Experimental

2.1. Materials

The ABS studied in this work were established using an aqueous solution of each aminoacid and different aqueous solutions of hydrophilic ILs. The aminoacids—L-proline (Sigma, >99%), L-lysine (Fluka, >98%) and D,L-lysine monohydrochloride (BHD Chemicals, >98.5%) were used as received without further purification. Their molecular structures are represented in Table 1. The ILs studied – [C_4mim][CF_3SO_3], [C_4mim][$N(CN)_2$] and [C_4mim][BF_4] – were supplied by Iolitec and are depicted in Fig. 1. To reduce the water and volatile compound contents to negligible values, IL individual samples were dried under constant agitation at vacuum and moderate temperature (353 K) for a minimum of 48 h. After this procedure, the purity of each IL was further checked by ^{1}H, ^{13}C



(i) (ii) (iii) (iv)

Fig. 1. Chemical structure of the cation and anions composing the ILs studied: (i) [C_4mim]$^+$; (ii) [CF_3SO_3]$^-$; (iii) [$N(CN)_2$]$^-$; (iv) [BF_4]$^-$.