Biochemical Engineering Journal 101 (2015) 142–149

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Good's buffers as novel phase-forming components of ionic-liquid-based aqueous biphasic systems

Andreia Luís, Teresa B.V. Dinis, Helena Passos, Mohamed Taha, Mara G. Freire*

CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 23 February 2015
Received in revised form 11 April 2015
Accepted 10 May 2015
Available online 14 May 2015

Keywords:
Liquid–liquid extraction
Bioseparation
Aqueous biphasic system
Ionic liquid
Good's buffer
Amino acid

ABSTRACT

Aiming at the development of self-buffering and benign extraction/separation processes, this work reports a novel class of aqueous biphasic systems (ABS) composed of ionic liquids (ILs) and organic biological buffers (Good's buffers, GBs). A large array of ILs and GBs was investigated, revealing than only the more hydrophobic and fluorinated ILs are able to form ABS. For these systems, the phase diagrams, tie-lines, tie-line lengths, and critical points were determined at 25 °C. The ABS were then evaluated as alternative liquid–liquid extraction strategies for two amino acids (L-phenylalanine and L-tryptophan). The single-step extraction efficiencies for the GB-rich phase range between 22.4 and 100.0% (complete extraction). Contrarily to the most conventional IL-salt ABS, in most of the systems investigated, the amino acids preferentially migrate for the more biocompatible and hydrophilic GB-rich phase. Remarkably, in two of the studied ABS, L-phenylalanine completely partitions to the GB-rich phase while L-tryptophan shows a preferential affinity for the opposite phase. These results show that the extraction efficiencies of similar amino acids can be tailored by the design of the chemical structures of the phase-forming components, creating thus new possibilities for the use of IL-based ABS in biotechnological separations.
© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The development of benign separation techniques has been a hot topic of research envisaging the extraction and purification of added-value compounds from biological media [1,2]. Biocompatibility is a crucial feature in the design of these platforms, particularly when dealing with biologically active products [3]. Typical organic solvents employed in liquid–liquid extractions from aqueous media are usually highly volatile and toxic. In this context, aqueous biphasic systems (ABS) – liquid–liquid systems mostly composed of water – are potential alternatives for extraction and purification processes. These systems consist in two aqueous-rich phases and can be created by the mixture of two polymers, a polymer with a salt or two salts. Liquid–liquid extractions by ABS have been intensively explored and used to separate and purify several biological products [4–6] and also to recover metal ions, radiochemicals, drugs molecules, dyes, among others, from complex mixtures [7,8].

In the last decade, it was demonstrated that ionic liquids (ILs) can be also employed as phase-forming components of ABS [9]. In general, ILs are organic salts with a melting temperature below 100 °C. Due to their ionic nature, ILs display exceptional properties, such as a negligible vapor pressure, a wide liquid temperature range, a high solvating ability for a wide variety of compounds or materials, and high thermal and chemical stabilities [10]. ILs are also good extraction solvents, both neat and in aqueous solutions, and are able to increase the stability of added-value biomolecules, namely proteins, enzymes and DNA [11–15]. Taking into account these features, IL-based ABS have been extensively investigated as alternative liquid–liquid extraction processes, allowing enhanced and selective extractions [16]. These ternary systems consist of two immiscible aqueous-rich phases, and could be designed by IL + salt, IL + carbohydrate, IL + amino acid or IL + polymer pairs dissolved in aqueous solution [12]. However, most of the investigations carried out with IL-based ABS are focused on combinations of ILs and high-charge density salts [16]. The major reason behind such a selection is related to the large ability of high-charge density salts, and thus to their salting-out nature, to create IL-based ABS. The salting-out effect is mostly a result of the formation of water–ion complexes that results on the "dehydration" of the IL [17–19]. Nevertheless, these systems suffer from major drawbacks resulting from the use of inorganic salts, namely their high charge–density and the creation of solutions of high ionic strength and extreme pH values that may damage the biological products, such as proteins,

* Corresponding author. Tel.: +351 234401422; fax: +351 234370084.
E-mail address: maragfreire@ua.pt (M.G. Freire).

http://dx.doi.org/10.1016/j.bej.2015.05.008
1369-703X/© 2015 Elsevier B.V. All rights reserved.

if buffers combinations or mixtures of salts are not employed. Moreover, the high concentration of these salts may be deleterious when discharged into aqueous effluents [20]. Even so, most studies regarding IL-based ABS comprised inorganic salts based on phosphate, carbonate and sulphate anions [14,21–23]. Most of the systems investigated lead to the formation of alkaline aqueous salt solutions (*e.g.*, with the use of K_2HPO_4, K_3PO_4, K_2CO_3, KOH, Na_2HPO_4 and NaOH) [9,21,23,24]. On the other hand, the use of salt mixtures and buffered solutions with pH close to biological values has been less explored [25,26]. In this context, the phase-forming ability of IL-based ABS by the addition of non-electrolytes, self-buffering and biocompatible salting-out agents (Good's buffers, GBs) was here investigated for the first time.

GBs, recognized as inert biological buffers for biochemical and biological studies, were developed by Good and co-workers [27–29]. GBs are zwitterionic compounds consisting of *N*-substituted taurine and glycine derivatives. Most of these buffers display pK_a values between 6 and 8, have a good solubility in water, do not readily permeate through cell membranes, do not chelate with metal-ions, are chemically stable and resist to enzymatic degradation, and are easily prepared and purified from low-cost materials [27,29,30]. Taking into account the collective benefits of GBs and ILs, we propose here their combined use as phase-forming components of ABS. Among the available GBs, *N*-tris(hydroxymethyl) methylglycine (Tricine), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), and 2-(*N*-morpholino) ethanesulfonic acid (MES) were selected because they are suitable buffers for pH control in the physiological pH region (although an alkaline species needs to be added for this purpose) [29,30]. Novel ternary phase diagrams for ABS composed of GBs and ILs were determined at 25 °C, and their ability as novel liquid–liquid systems for extraction/purification purposes was evaluated by partitioning studies carried out with standard aqueous solutions of two amino acids that can be produced in fermentative medium (L-tryptophan and L-phenylalanine) [31,32].

2. Material and Methods

2.1. Materials

The GBs investigated were Tricine (purity > 99 wt%), HEPES (purity > 99.5 wt%), TES (purity > 99 wt%), CHES (purity > 99 wt%), and MES (purity > 99 wt%), all purchased from Sigma–Aldrich. The ILs studied for ABS formation were 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([C_4mim][CF_3SO_3], purity ≥ 99 wt%), 1-butyl-3-methylimidazolium tetrafluoroborate ([C_4mim][BF_4], purity ≥ 99 wt%), 1-butyl-3-methylimidazolium dicyanamide ([C_4mim][$N(CN)_2$], purity > 98 wt%), 1-butyl-3-methylimidazolium thiocyanate ([C_4mim][SCN], purity > 98 wt%), 1-butyl-3-methylimidazolium tosylate ([C_4mim][TOS], purity 99 wt%), 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([C_2mim][CF_3SO_3], purity 99 wt%), 1-ethyl-1-methylpyrrolidinium trifluoromethanesulfonate ([C_2mpyr][CF_3SO_3], purity 99 wt%), all purchased from Iolitec. Tetrabutylphosphonium bromide ([P_{4444}]Br, purity > 95 wt%), kindly supplied by Cytec Industries Inc. was also investigated. To reduce the volatile impurities to negligible values, IL samples were purified under constant agitation, under vacuum, and at moderate temperature (60 °C), for a minimum of 24 h. After this procedure, the purity of each IL was further checked by ^{1}H, ^{13}C and ^{19}F NMR spectra (whenever applicable) and found to be in accordance with the stated purity level provided by the suppliers. L-Tryptophan (purity > 99.0 wt%) and L-phenylalanine (purity 99.0 wt%) were acquired from Sigma–Aldrich and Merck, respectively. Although several combinations of ILs and GBs were tested to create novel ABS, only aqueous mixtures composed of the more hydrophobic ILs ([C_4mim][CF_3SO_3] and [C_4mim][BF_4]) and the GBs Tricine, HEPES and TES were able to form ABS. The chemical structures of the GBs and ILs able to undergo liquid–liquid demixing toward the formation of ABS, and of the amino acids used in the extraction experiments, are shown in Fig. 1. The water used was double distilled, passed through a reverse osmosis system, and additionally treated with a Milli-Q plus 185 purification apparatus.

2.2. Methods

2.2.1. Phase diagrams, tie-lines, tie-line lengths and critical points

The solubility (saturation) curves were determined through the cloud point titration method [33] at (25 ± 1) °C and atmospheric pressure. Aqueous solutions of ILs at 80–90 wt% and aqueous solutions of the different GBs at concentrations below, yet close, to their saturation values in water (Tricine at 20 wt%, HEPES at 50 wt% and TES at 55 wt%) were prepared gravimetrically and used for the determination of the binodal curves. Repetitive drop wise addition of the aqueous IL solution to the aqueous solution of each GB was carried out until the detection of a cloudy (biphasic) mixture, followed by the drop wise addition of ultrapure water until the observation of the monophasic region (clear and limpid solution). The ternary system compositions were determined by weight quantification within $\pm 10^{-4}$ g. The experimental binodal curves were fitted to Eq. (1) [34]:

$$[\mathrm{IL}] = A\exp[(B[\mathrm{GB}]^{0.5}) - (C[\mathrm{GB}]^3)] \quad (1)$$

where [IL] and [GB] are the IL and GB weight fraction percentages, respectively, and the coefficients *A*, *B*, and *C* are the fitting parameters.

The tie-lines (TLs) were determined by a gravimetric method originally proposed by Merchuk et al. [34] for polymer-based ABS, applied later by Gutowski et al. [7] and by us to IL-based ABS [33]. A ternary mixture composed of IL + GB + water at the biphasic region was gravimetrically prepared within $\pm 10^{-4}$ g, vigorously agitated, and left to equilibrate for at least 12 h and at (25 ± 1) °C, aiming a complete separation of the coexisting phases. After this period, both phases were carefully separated and individually weighed. Each TL was determined by the lever-arm rule through the relationship between the IL-rich phase composition and the overall system composition, for which the following system of four equations (Eqs. (2)–(5)) and four unknown values ($[\mathrm{IL}]_{\mathrm{IL}}$, $[\mathrm{IL}]_{\mathrm{GB}}$, $[\mathrm{GB}]_{\mathrm{IL}}$, and $[\mathrm{GB}]_{\mathrm{GB}}$) was solved [34]:

$$[\mathrm{IL}]_{\mathrm{IL}} = A\exp[(B \times [\mathrm{GB}]_{\mathrm{IL}}^{0.5}) - (C \times [\mathrm{GB}]_{\mathrm{IL}}^3)] \quad (2)$$

$$[\mathrm{IL}]_{\mathrm{IL}} = A\exp[(B \times [\mathrm{GB}]_{\mathrm{GB}}^{0.5}) - (C \times [\mathrm{GB}]_{\mathrm{GB}}^3)] \quad (3)$$

$$[\mathrm{IL}]_{\mathrm{IL}} = \frac{[\mathrm{IL}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha}[\mathrm{IL}]_{\mathrm{GB}} \quad (4)$$

$$[\mathrm{GB}]_{\mathrm{IL}} = \frac{[\mathrm{GB}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha}[\mathrm{GB}]_{\mathrm{GB}} \quad (5)$$

where subscripts "IL", "GB" and "M" designate the IL-rich phase, the GB-rich phase and the mixture composition, respectively; α is the ratio between the mass of the IL-rich phase and the total mass of the mixture. The system solution results in the composition (wt%) of the GB and IL in the top and bottom phases. The identification of the IL- and GB-rich aqueous phases was carried out through the IL quantification in each phase at 212 nm by UV-spectroscopy using a BioTeck Synergy HT microplate reader.

For the calculation of each tie-line length (TLL) the following equation was used:

$$\mathrm{TLL} = \sqrt{([\mathrm{GB}]_{\mathrm{IL}} - [\mathrm{GB}]_{\mathrm{GB}})^2 + ([\mathrm{IL}]_{\mathrm{IL}} - [\mathrm{IL}]_{\mathrm{GB}})^2} \quad (6)$$

Download English Version:

https://daneshyari.com/en/article/2850

Download Persian Version:

https://daneshyari.com/article/2850

Daneshyari.com