Separation and Purification Technology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Effect of salt-based adjuvant on partition behaviour of protein in aqueous two-phase systems composed of polypropylene glycol and cholinium glycinate

Cher Pin Song[a], Ramakrishnan Nagasundara Ramanan[a], R. Vijayaraghavan[b], Douglas R. MacFarlane[b], Eng-Seng Chan[a], Pau Loke Show[c], Siek Ting Yong[a], Chien-Wei Ooi[a,*]

[a] Chemical Engineering Discipline, School of Engineering, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor, Malaysia
[b] School of Chemistry, Faculty of Science, Monash University, Clayton, VIC 3800, Australia
[c] Bioseparation Research Group, Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia

ARTICLE INFO

Keywords:
Aqueous two-phase system
Cholinium aminoate ionic liquid
Adjuvant
Partition behaviour
Protein

ABSTRACT

Aqueous two-phase systems (ATPSs) composed of ionic liquids (ILs) have been widely explored over the past decade for application in protein purification. The interaction between the phase components of ATPSs and the target protein is one of the factors governing the partition behaviour of the protein. The addition of adjuvants like inorganic salts at a low concentration in an ATPS could manipulate the partitioning of the protein between phases. In this study, the partitioning behaviour of model proteins in ATPSs composed of cholinium glycinate, poly(propylene) glycol 400 and a salt-based adjuvant (i.e., sodium chloride, magnesium chloride and sodium sulfate) was investigated systematically. The dissociated ions with a greater hydration capacity preferentially partitioned to the phase having a higher water content. Also, the partition coefficient of the dissociated ions was independent of the concentration of salt adjuvant. The affinity of adjuvant ions for the IL-rich bottom phase was in the decreasing order of: sulfate ion > chloride ion > magnesium ion > sodium ion. This non-uniform distribution of cations and anions of ionic adjuvants in the ATPS had an influence of the partitioning of proteins in ATPS. Bovine serum albumin and lysozyme, which differ in isoelectric point and surface charge density, were used as the model proteins. The partitioning behaviour of the proteins was affected by the distribution of the dissociated ions of the ionic adjuvant in both phases of the ATPS. The electrostatic interaction between proteins and the dissociated ions of the ionic adjuvant was governed by factors such as types and charge density of the dissociated ions of the salt-based adjuvants, isoelectric point of the protein, and pH of the ATPS. The results proved the feasibility of manipulating the partitioning of target proteins in ATPSs by using inorganic salts as the adjuvant, which provides a means to improve the protein separation in IL-based ATPSs.

1. Introduction

An aqueous two-phase system (ATPS) has been regarded as an alternative to the traditional methods of protein purification [1]. It offers advantages such as cost-effectiveness, high productivity, simplicity, short processing time and good scalability. In addition, an ATPS is mainly composed of aqueous phases that are biocompatible to proteins [2]. As a result, ATPSs have been explored intensively for the separation of various molecules, for instances, small organic species [3,4], metallic ions [5,6], nanoparticles [7] and drug molecules [8]. The traditional ATPSs are typically prepared by mixing either two different hydrophilic polymers, or polymer with salt. Other ATPS-forming components, including alcohols [9,10], carbohydrates [11,12] and surfactants [13,14], have also been well-documented. Nonetheless, the limited range of the polarities of the coexisting phases in most of the conventional ATPSs has restricted widespread use of these systems for purification purposes.

An ionic liquid (IL) is a type of salt which exists in liquid state at ambient temperature due to its low melting pointing [15]. ILs possess distinctive properties such as low vapour pressure [16] as well as the tunable properties (polarity and affinity) of its cation and anion groups [17]. ILs have been gaining tremendous interest in the scientific community due to their potential as green solvents that can possibly replace the traditional volatile and toxic organic solvents in many applications

* Corresponding author.
E-mail address: ooi.chien.wei@monash.edu (C.-W. Ooi).

http://dx.doi.org/10.1016/j.seppur.2017.09.017
Received 31 May 2017; Received in revised form 19 August 2017; Accepted 6 September 2017
1383-5866/ © 2017 Elsevier B.V. All rights reserved.

[18–20]. Furthermore, it has been reported that ILs are effective extraction solvents for a wide variety of compounds [21]. They also possess the ability to improve the stability of various biomolecules such as proteins, DNA and enzymes [22,23].

The pioneering work of Rogers and co-workers [24] on forming ATPSs with ILs and water-structuring salts has stimulated the development of IL-based ATPS variants, and the widespread use of IL-based ATPSs in downstream processing [25]. The wide range of hydrophobicity offered by ILs (as achieved by a proper manipulation of the cation/anion combination) provides the possibility to tailor the polarity and affinity of both phases in the ATPS to interact specifically with the target protein [26]. This advantageous property makes ILs an attractive ATPS phase-forming component. Despite the fact that ILs have been widely recognized as 'green' solvents, past studies have indicated the toxicity and the poor biodegradability of the conventional ILs (i.e., imidazolium-, pyridinium- and halide-based ILs), which are commonly used as the phase-forming components of ATPSs [27]. To overcome these drawbacks, considerable efforts have been devoted to synthesize ILs that have a reasonable environmental footprint [28,29].

Previously, our research group successfully designed a series of ATPSs composed of a thermo-separable polymer, i.e., polypropylene glycol 400 (PPG 400) and an environmentally benign IL, i.e., cholinium aminoate-based ionic liquid ([Ch][AA]) [30,31]. We also discovered that the charge of the [AA] anion species is tunable as a function of system pH. The interaction between the phase component and the model proteins can be controlled, thereby promoting the selective distribution of a target protein in the ATPS. Furthermore, the PPG 400 could be easily recovered by inducing the formation of a secondary ATPS through thermo-separation at a relatively low temperature (35 °C).

Salts are often used as an additive or adjuvant in ATPSs to direct the partitioning of target proteins between phases [32–34]. With the presence of salt-based adjuvants, the partition behaviour of proteins can be controlled in order to enhance their separation efficiency. Cascone et al. [35] reported the influence of sodium chloride (NaCl) as an additive on the partition behaviour of thaumatin in a poly(ethylene) glycol (PEG) + phosphate ATPS; the partition coefficient (K) of thaumatin was increased by 39-fold, upon the addition of 1.5 M NaCl in the ATPS. Lee and Sandler [36] reported that an increasing concentration of either NaCl or sodium sulfate (Na_2SO_4), led to an exponential increase in the K of vancomycin in a PEG + dextran ATPS. On the other hand, Hodgson [37] also discovered that the purification of a plasminogen activator from the contaminant cell culture could be achieved efficiently using PEG + phosphate ATPS supplemented with 0.5 M NaCl.

Here we aimed to study the effect of salt-based adjuvants on the partition behaviour of proteins in PPG 400 + cholinium glycinate ([Ch][Gly]) ATPSs. The salt-based adjuvants with different charge densities, namely NaCl, magnesium chloride ($MgCl_2$) and Na_2SO_4, were used in this study. The distribution of salt ions in the PPG 400 + [Ch][Gly] system was evaluated. Bovine serum albumin (BSA) and lysozyme were used as the model proteins in this study, and their partition behaviour under the influence of adjuvant ions in the ATPS were investigated systematically. By correlating the partition behaviour of proteins with the properties of salt-based adjuvants used in the ATPS, the mechanism of protein separation could be understood and the salt-based adjuvants could be adopted for controlling the partition behaviour of target protein in ATPS.

2. Materials and methods

2.1. Materials

Choline hydroxide (20 wt% in water) was obtained from Sigma-Aldrich (Australia), while glycine (99% purity) was purchased from Merck (Australia). PPG 400 (M_n = 446 g mol^{-1}, 99.5% purity), BSA, lysozyme, NaCl, Na_2SO_4 and $MgCl_2$ were purchased from Sigma-Aldrich (St. Louis, USA). Bradford assay dye reagent was purchased from Bio-Rad (USA). All chemicals were used without further purification. The synthesis process and the characterization of [Ch][Gly] have been reported in our previous study [30].

2.2. Partitioning of adjuvants ions and model proteins in ATPSs

The 2-g ATPSs composed of 50 wt% PPG 400 solution, 4 wt% [Ch][Gly] solution, 0–20 wt% protein solution, 0–2 wt% inorganic salt, and deionized water were prepared in 2-ml micro-centrifuge tubes. All the systems were mixed vigorously by using a vortex mixer, before being subjected to settling at 25 °C for 3 h to attain the equilibrium of both phases. To ensure the complete phase separation, the ATPSs were centrifuged at 2000 rpm for 5 min. The volumes of the top and bottom phases were measured.

For ATPSs involving the partitioning of model proteins, Bradford assay was used to determine the protein concentration in each phase. As per the instructions of the manufacturer (Bio-Rad, USA), 1 part of the Bio-Rad protein assay dye reagent was first diluted with 4 parts of deionized water. Then, 10 μl sample was mixed with 200 μl of the diluted dye reagent in a 96-well microplate. Subsequently, the mixture was incubated at room temperature for 5 min. The absorbance of the mixture was measured at 595 nm using a microplate absorbance reader (Sunrise, Tecan). The phase solution of blank ATPS was used as a control.

To assess the degree of separation achieved in the ATPS, the K of compound (i.e., protein and ions) was calculated as the ratio of equilibrium concentration of compound in the bottom phase (C_B) to equilibrium concentration of compound in the top phase (C_T) [Eq. (1)]

$$K = \frac{C_B}{C_T} \tag{1}$$

The percent yield (Y_B) of the protein in bottom phase of ATPS was calculated using Eq. (2) as shown below:

$$Y_B(\%) = \frac{C_B V_B}{C_T V_T + C_B V_B} \times 100 \tag{2}$$

where V_T and V_B are the top-phase volume and bottom-phase volume, respectively.

2.3. Quantification of adjuvants ions in ATPSs

The concentrations of Na^+ and Mg^{2+} in both phases were quantified using an Atomic Absorption Spectrometer (200 Series AA, Agilent Technologies). The calibration graph was generated using the standard salt solutions (0–1.2 mg/l).

The Mohr method [38] was used to determine the concentration of Cl^- in both phases by titration with silver nitrate. In brief, 0.5 ml of the sample was first added into a conical flask. Then, 0.02 ml of potassium chromate indicator solution was mixed with the sample. Next, the sample was titrated with silver nitrate solution (0.0141 N) until the colour of sample solution turned pinkish yellow. The volume of the titrant used was recorded and the Cl^- concentration was calculated using Eq. (3) as shown below:

$$[Cl^-]\ (mg/l) = \frac{(\ A \times N \times 35.45) \times 1000}{V_{sample}} \tag{3}$$

where A is the volume (ml) of titrant used; N is the normality of the titrant and V_{sample} is the volume (ml) of the sample (in this case, the N and V_{sample} values were 0.0141 and 0.5, respectively).

The concentration of SO_4^{2-} was measured using a turbidimetric method based on the formation of barium sulphate precipitates. In brief, a buffer solution was first prepared by dissolving 30 g $MgCl_2$, 5 g sodium acetate, 1 g potassium nitrate and 20 ml acetic acid in 1000 ml deionized water. This buffer solution was used to enhance the formation of barium sulfate (precipitate in colloidal form). 2 ml of buffer

Download English Version:

https://daneshyari.com/en/article/7043990

Download Persian Version:

https://daneshyari.com/article/7043990

Daneshyari.com