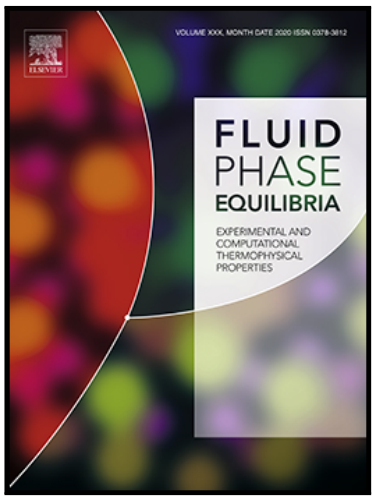

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Mafalda R. Almeida , Ana F.C.S. Rufino , Diana C.V. Belchior , Pedro J. Carvalho , Mara G. Freire

PII: S0378-3812(22)00083-8
DOI: https://doi.org/10.1016/j.fluid.2022.113458
Reference: FLUID 113458

To appear in: *Fluid Phase Equilibria*

Received date: 31 October 2021
Revised date: 18 March 2022
Accepted date: 21 March 2022

Please cite this article as: Mafalda R. Almeida , Ana F.C.S. Rufino , Diana C.V. Belchior , Pedro J. Carvalho , Mara G. Freire , Characterization of cholinium-carboxylate-based aqueous biphasic systems, *Fluid Phase Equilibria* (2022), doi: https://doi.org/10.1016/j.fluid.2022.113458

Characterization of cholinium-carboxylate-based aqueous biphasic systems

Mafalda R. Almeida[†], *Ana F. C. S. Rufino*[†], *Diana C. V. Belchior*, *Pedro J. Carvalho and Mara G. Freire*[*]

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

[†]These authors contributed equally to this work

* Corresponding author:

E-mail address: maragfreire@ua.pt

Abstract

In the past years large efforts have been placed in the development of novel separation techniques with improved resolution, simplicity, speed and easy to scale-up. Among these, ionic-liquid-based (IL-based) aqueous biphasic systems (ABS) have been broadly proposed for the separation of high-value compounds, allowing improved extraction performance and purification. More recently, significant efforts have been placed on the synthesis and use of novel ILs with both an acceptable environmental footprint and enhanced biocompatibility. In this sense, this work aims to characterize ABS composed of cholinium carboxylate ILs ([Ch][C_nO_2], with n = 2 to 7),

K_3PO_4 and water. The respective ternary phase diagrams, including binodal curves, tie-lines and tie-line lengths, were determined at (298 ± 1) K and at atmospheric pressure. The ability to form ABS (or of the IL to be salted-out) increases with the increase of the alkyl chain length of the IL anion, up to [Ch][C_5O_2]; nevertheless, for longer anion alkyl chain lengths ([Ch][C_6O_2] and [Ch][C_7O_2]) the ILs self-aggregation leads to a decrease of the ILs ability to form ABS. The liquid−liquid equilibrium data experimentally determined were modeled using the local composition activity coefficient model NRTL (Non-Random Two Liquid). Finally, the partition behavior of three alkaloids (nicotine, caffeine and theobromine), used here as hydrophobicity probes, was evaluated. In all studied systems, alkaloids preferentially migrate to the IL-rich phase, with partition coefficients (*K*) ranging between 2.23 and complete extraction in a single-step. Furthermore, the set of ILs investigated allowed identifying an odd-even effect in the alkaloids partitioning derived from the IL anion alkyl chain length. These results support the salting-out effect exerted by K_3PO_4 and favorable dispersive interactions established between the IL-rich phase-forming components and the alkaloids.

Keywords: Aqueous biphasic systems; phase diagrams; NRTL; cholinium carboxylate; ionic liquids; alkaloids

1. Introduction

The need to develop novel separation techniques with improved resolution, simplicity, speed, easy to scale-up and able to operate in continuous mode is an important topic of modern bioseparation [1,2]. This is particularly relevant in fields involving biomolecules usually present in complex media, whose current separation and purification techniques require several steps, with high energy and chemical demands

[1,3]. Techniques like chromatography, commonly applied in the purification of biomolecules, lead to high purity, but as well as to high purification costs [2,3]. Aiming at minimizing operation costs in this field, different techniques have been proposed, namely cross-flow electrofiltration, reversed micelles, centrifugal partitioning, membrane chromatography and liquid-liquid extraction with aqueous biphasic systems [1].

Aqueous biphasic systems (ABS) fall within the liquid–liquid extraction techniques, and may lead to high yields, high selectivity and improved purification, and a good combination between the recovery and purification steps, while keeping technological simplicity and a low associated cost [3,4]. These systems are formed when at least two water-soluble components, such as polymers, ionic liquids (ILs), alcohols, salts, sugars, among others, are mixed in water at given concentrations [5]. Polymer-based ABS usually generate systems with high viscosity, which can be a drawback in extraction processes, and may have their selectivity compromised due to their low tunable nature. Therefore, IL-based ABS are preferable choices since they allow to decrease the phases' viscosity and have a tunable character [6]. In the past years, significant efforts have been placed to synthesize and apply novel ILs with both an acceptable environmental footprint and enhanced biocompatibility [7]. Among these, ILs comprising the 2-hydroxyethyl-N,N,N-trimethylammonium (cholinium) cation stand out due to their high biodegradability and marginal toxicity if correctly designed [8]. However, cholinium-based ILs have a tendency to be highly hydrophilic and only form ABS with polymers (e.g., polypropylene glycol (PPG) 400 and 1000 [9–12], polyethylene glycol (PEG) 400, 600 and 1000 [13]), in which the IL may act as the salting-out species. On the other hand, more hydrophobic (still water miscible) cholinium-based ILs can be

designed to create ABS with strong salting-out salts (e.g., K_3PO_4 [6,14,15] and K_2CO_3 [7,14]).

Although Berton et al. [6] previously characterized ABS composed of cholinium carboxylate ILs and K_3PO_4 at controlled pH values, most of the investigated ILs comprise an even alkyl side chain and other species were added to control the pH. With this work we aim to enlarge the characterization of ABS formed by cholinium-based ILs, combining cholinium carboxylate ILs (with a relevant number of both odd and even alkyl side chain lengths) with K_3PO4 in aqueous media, without adding other species or pH control. To this end, ternary phase diagrams, including binodal curves and tie-lines, were determined at (298 ± 1) K and at atmospheric pressure. The liquid-liquid equilibrium data experimentally determined were modeled using the local composition activity coefficient model NRTL (Non-Random Two Liquid) [16]. Finally, a study on the anion alkyl side chain length effect on the partition of a series of alkaloids (caffeine, nicotine and theobromine) of variable hydrophobicity was performed. This group of alkaloids was selected since it allows to study a family of molecules with different hydrophobicity, and to identify differences in partitioning that could arise from the dispersive interactions established with the IL anion aliphatic moieties (and identify odd-even effects). To the best of our knowledge, several works reporting alkaloids partitioning in IL-based ABS can be found in the literature [3,17–19], but only applying imidazolium-based ILs.

2. Materials and methods

2.1. Materials

The cholinium carboxylate ILs evaluated in this study, namely cholinium propanoate ([Ch][C_3O_2]), cholinium butanoate ([Ch][C_4O_2]), cholinium pentanoate ([Ch][C_5O_2]), cholinium hexanoate ([Ch][C_6O_2]) and cholinium heptanoate ([Ch][C_7O_2]), were synthetized via neutralization, following reported methods [20]. Cholinium acetate ([Ch][C_2O_2]) was supplied by Iolitec (>99 wt%). For the ILs synthesized by us, the synthesis started with a solution of cholinium bicarbonate, to which the selected carboxylic acid was added in a molar ratio of 1.1:1. The mixture was agitated overnight at room temperature. Then, the water was removed in a rotary evaporator, and the IL washed with ethyl acetate to remove unreacted compounds. Finally, ethyl acetate and residual water were removed under high vacuum (1 Pa), moderate temperature (343 K) and continuous stirring for at least 72 h. All ILs showed high purity (>97 wt%), confirmed by ^{1}H and ^{13}C NMR (spectra given in the Supporting Information, with a maximum uncertainty of 10%). The required precursors, namely cholinium bicarbonate (80 wt% in water, Sigma-Aldrich) and propanoic (99 wt%, Acros Organics), butanoic (99 wt%, Acros Organics), pentanoic (99 wt%, Riedel-de Haen), hexanoic (99.5 wt%, Sigma-Aldrich) and heptanoic acids (99 wt%, Acros Organics) were used as received. The IL-based ABS studied in this work were prepared with potassium phosphate, K_3PO_4 (>97 wt%), purchased from Alfa Aesar. For the extraction studies, caffeine anhydrous (99 wt%, Sigma-Aldrich), nicotine (99 wt%, Alfa Aesar) and theobromine (99 wt%, Sigma-Aldrich) were commercially acquired and used as received. The compound name, CAS number, supplier and mass fraction purity of all compounds used are reported in Table

1. The chemical structures of the studied ILs and alkaloids are depicted in Figure 1. The water used was double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

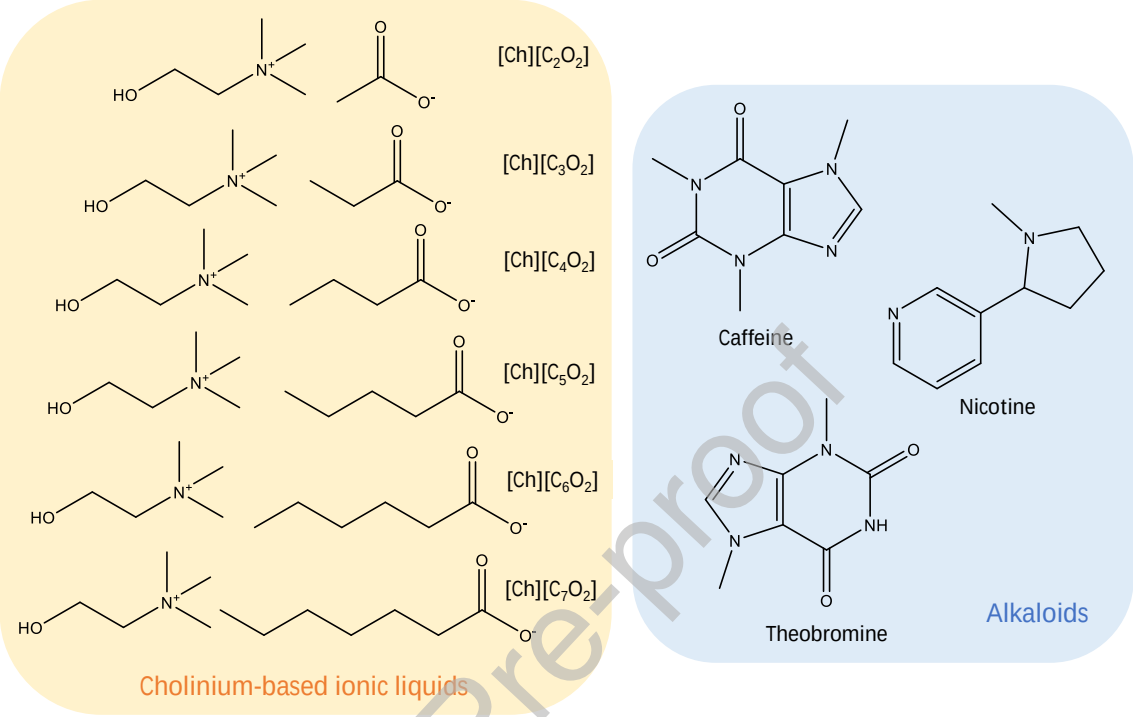

Figure 1. Chemical structures of the studied cholinium-based ILs and alkaloids.

Table 1. Compound name, CAS number, supplier and mass fraction purity of all compounds used.

Compound	CAS Reg. No.	Supplier	Mass fraction purity (wt%)
Cholinium bicarbonate	78-73-9	Sigma-Aldrich	80[a]
Propanoic acid	79-09-4	Acros Organics	99[a]
Butanoic acid	107-92-6	Acros Organics	99[a]
Pentanoic acid	109-52-4	Riedel-de Haen	99[a]
Hexanoic acid	142-62-1	Sigma-Aldrich	99.5[a]
Heptanoic acid	111-14-8	Acros Organics	99[a]
Potassium phosphate	7778-53-2	Alfa Aesar	>97[a]
[Ch][C_2O_2]	14586-35-7	Iolitec	>99[a]
[Ch][C_3O_2]	n.a.	Synthetized in this work	>97[b]
[Ch][C_4O_2]	n.a.	Synthetized in this work	>97[b]
[Ch][C_5O_2]	n.a.	Synthetized in this work	>97[b]
[Ch][C_6O_2]	n.a.	Synthetized in this work	>97[b]
[Ch][C_7O_2]	n.a.	Synthetized in this work	>97[b]
Caffeine	58-08-2	Sigma-Aldrich	99[a]
Nicotine	54-11-5	Alfa Aesar	99[a]
Theobromine	83-67-0	Sigma-Aldrich	99[a]

[a]reported by the supplier; [b]determined by ^{1}H and ^{13}C Nuclear Magnetic Ressonance with a maximum uncertainty of 10%; n.a. not available

2.2. Phase diagrams and NRTL modeling

The binodal data were determined through the cloud point titration method at T = (298 ± 1) K and atmospheric pressure [21,22]. An aqueous solution of K_3PO_4 at 50 wt% and aqueous solutions of the different ILs at 60 wt% were prepared and used for the determination of the binodal curves. The repetitive dropwise addition of the salt aqueous solution into the aqueous solutions of each IL was carried out until the detection of a cloudy (biphasic) solution, followed by the dropwise addition of ultrapure water until the observation of a limpid solution (monophasic region). All additions were made under continuous stirring. The binodal curves were determined by the weight quantification of all components added ($u(m)$= 10^{-4} g), in which both the weight mass fractions corresponding to the cloud and limpid points were used to describe each binodal curve. The experimental solubility curves were correlated using Eq. (1) [23]:

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where IL and Salt represent the IL and salt weight fraction percentages, respectively, and A, B, and C are constants obtained by regression.

Tie-lines (TLs) associated with each binodal curve, i.e., the composition of each phase at equilibrium for a specific mixture composition, were additionally determined. To this end, ternary mixtures composed of IL + K_3PO_4 + water were gravimetrically prepared ($u(m)$= 10^{-4} g) in sealed glass vials at compositions within the biphasic region. Upon preparation of the selected mixture point, vigorous stirring was applied. After stirring, the system was centrifuged for 30 min at (298 ± 1) K and both phases were

In addition to the characterization of the studied ABS, their performance to extract a series of alkaloids of different hydrophobicity was evaluated, namely with nicotine, caffeine and theobromine, at a fixed TLL (54 ± 2). The systems investigated allow partition coefficients ranging from 2.23 to Ꮒ complete extraction to the IL-rich phase. In general, ABS constituted by ILs with even alkyl chains at the anion ([Ch][C_2O_2], [Ch][C_4O_2] and [Ch][C_6O_2]), which correspond to those with a higher water content in the IL-rich phase, lead to higher partition coefficients. These results suggest the occurrence of an odd-even effect in the alkaloids extraction along the size of the anion aliphatic moiety. Overall, factors ruling the alkaloids partitioning in the studied ABS correspond to the salting-out effect exerted by the salt and dispersive forces.

The of results here provided contribute to a better characterization of IL-based ABS composed of cholinium carboxylate ILs and salts, while foreseeing their application in the separation of added-value compounds.

Mafalda R. Almeida: Investigation; Writing - original draft

Ana F. C. S. Rufino: Investigation; Writing - review & editing

Diana C. V. Belchior: Investigation; Writing - review & editing

Pedro J. Carvalho: Project administration; Resources; Writing - review & editing

Mara G. Freire: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing - review & editing

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. This research was funded by FEDER through COMPETE 2020—Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES from the project "IL2BioPro"–PTDC/BII-BBF/30840/2017. A.F.C.S. Rufino acknowledges FCT for the PhD grant SFRH/BD/138997/2018.

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