



Hydrodynamics of cholinium chloride-based aqueous biphasic systems (ABS): A key study for their industrial implementation

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ABSTRACT

Aqueous biphasic systems (ABS) have been widely studied for extraction and purification processes. Although they are considered biocompatible, amenable, and sustainable separation platforms, their application on an industrial scale remains impractical without proper scaling and integration into existing processes. To better understand the hydrodynamics of ABS formation, three cholinium chloride ([Ch]Cl)-based ABS composed of polypropylene glycol with a molecular weight of 400 g.mol⁻¹ (PPG-400), tripotassium phosphate (K₃PO₄), and dipotassium hydrogen-phosphate (K₂HPO₄) were studied. The hydrodynamics of phase separation of ABS composed of PPG-400/[Ch]Cl, [Ch]Cl/K₃PO₄ and [Ch]Cl/K₂HPO₄ was studied by analysing the relationship between the mixing time (T_m) and the phase settling time (T_s), at 25 °C and 50 °C. The results showed that T_s is independent of T_m, which is very long for the polymer/salt systems (T_s > 6 h), while for salt/salt ABS, a very fast phase settling was observed (T_s < 150 s). The hydrodynamics of each salt/salt system was then correlated with the physicochemical properties of the coexisting phases and the nature of the phase-forming compounds. The increase in the salting-out effect of the inorganic salts, and the consequent larger differences between the compositions of the coexisting phases, improved the hydrodynamics of the [Ch]Cl-based ABS. With the increase of the tie-line lengths, the composition of the phases stabilized, resulting also in more stable physicochemical properties in each phase and constant T_s. The correlations obtained in this work allow the definition of the best operating region within the biphasic (liquid-liquid) region of [Ch]Cl/salt-based ABS as being the largest TLLs within the LLE region. The definition of these criteria and region of operation is crucial for the design and industrial implementation of these types of LLE processes using conventional mixer-settler units.

1. Introduction

Aqueous biphasic systems (ABS), also known as Aqueous Two-Phase Systems (ATPS), can be used in liquid-liquid extraction (LLE) processes. They are prepared by mixing at least two different water-soluble compounds in an aqueous medium, such as polymers [1,2], salts [3,4], sugars [5,6], alcohols [5,7], or surfactants [8,9,10], that form two liquid phases coexisting in equilibrium above certain concentrations [11,12]. These systems have been extensively studied for over five decades as an efficient platform for extracting and separating target compounds in various processes, as they are simple, biocompatible, environmentally safe, and recyclable [11,12]. ABS have been widely applied in the separation, purification, and recovery of various types of (bio)molecules, such as proteins [13], enzymes [14], antibiotics [15], carotenoids [16] or dyes [11]. Despite all their advantages, the industrial use of ABS is

still limited due to the poor understanding of the partition/separation mechanisms, a limited number of predictive models, incompatibility with current extraction equipment/facilities, and a lack of large-scale studies [17].

The first ABS widely studied were composed of two hydrophilic polymers [1]. However, these systems have limitations such as reduced polarity differences between coexisting phases at equilibrium and narrow ability to manipulate phase separation, slow phase separation, along with high viscosity and relatively high cost [18,19]. Additionally, these polymer/polymer ABS have limited extraction and purification capabilities [18,19]. To overcome these limitations, other phase-forming compounds such as ionic liquids (ILs) [3,20] and salts [2,21,22] have been combined with polymers to form ABS, which have more distinct phases' characteristics and enable faster phase separation [18,22]. In addition, due to the tunability of ILs, the entire

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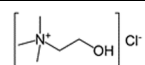
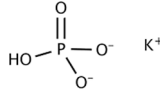
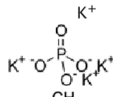
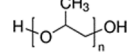
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Table 1

Chemical formula and structure of compounds used in this work, as well as information regarding the producer, purity (%), molecular weight ($\text{g}\cdot\text{mol}^{-1}$), and density ($\text{g}\cdot\text{cm}^{-3}$).

Compound	Chemical structure	Producer	Purity (%wt.)	Molecular weight ($\text{g}\cdot\text{mol}^{-1}$)	Density ($\text{g}\cdot\text{cm}^{-3}$)
Cholinium chloride ([Ch]Cl)		Tokyo Chemical Industry (China)	≥ 98	139.62	1.02
Dipotassium phosphate (K_2HPO_4)		PanReac (France)	≥ 98	174.18	2.44
Tripotassium phosphate (K_3PO_4)		Chem-Lab (Belgium)	≥ 98	212.28	2.56
Polypropylene glycol 400 (PPG-400)		Thermo Fischer Scientific (USA)	100	134.18	1.01

hydrophilicity and hydrophobicity range can be covered through judicious ions' selection. As a result, the use of salt/salt ABS [19,23,24,25,26] as separation platforms for a wide range of compounds has exponentially grown in recent years [12].

The thermodynamic fundamentals and separation mechanisms of ABS are complex and unpredictable. They depend on the thermodynamic equilibrium of the system, which is determined by the type, chemical nature and concentration of ABS constituents (e.g., polymer's molecular weight, anionic and cation of salt/IL, relative hydrophobicity, pH), the characteristics of the target solute, as well as the physicochemical properties of the coexisting phases (temperature, density, viscosity and interfacial tension) [12]. As a result, the selection of the best ABS must be based first on defining its constituents and subsequently on the composition of each system's mixture, which guarantees not only the formation of two immiscible aqueous phases but also fulfils the proposed application requirements, such as recovery efficiencies, selectivity, purification factor, among others [11,12].

To use ABS at large scale, studies on the fundamental aspects of the systems' hydrodynamics are necessary to learn about their stability and to achieve a proper equipment design for the desired industrial process [17,21]. However, studies focused on the operational conditions that govern ABS hydrodynamics are still scarce, and most of the existing studies are limited to conventional polymer/polymer [21,27] or polymer/salt [4,21,27,28,29,30] ABS. Although several studies have addressed the ABS formation of distinct mixtures of salts and ILs through the determination of binodal curves, tie-lines, and physicochemical characterization of the coexisting phases [19,23,24], there are no studies and it persists a lack of understanding regarding the hydrodynamics of salt/salt ABS.

A wide range of ionic compounds have been used to form IL-based polymer/salt [3,20] and salt/salt [15,19,24,31] ABS, including cholinium-based ILs, which have emerged as one of the most interesting and studied families. Cholinium-based ILs are particularly attractive due to their high biodegradability and solubility in water, "eco-friendliness", low toxicity, negligible vapour pressure at ambient conditions and enabling milder work conditions [23,32,33]. Several studies have investigated the use of cholinium-based compounds (ILs and salts) in ABS formation, applying them as efficient platforms for the recovery and extraction of wide range of (bio)molecules [15,34,35].

Cholinium chloride ([Ch]Cl), also known 2-hydroxyethyltrimethylammonium chloride, is an essential nutrient for various biological functions [23,33]. It is hygroscopic, having a strong affinity for water molecules (with an octanol/water partition coefficient ($\log K_{ow}$) of -3.70), and therefore requires the use of salts with high salting-out ability, such as dipotassium hydrogen phosphate (K_2HPO_4) [15] and tripotassium phosphate (K_3PO_4) [23,31] or polymers, such as propylene

glycol (PPG) or polyethylene glycol (PEG) [3,20,36], to achieve phase separation in water. Studies involving [Ch]Cl-based ABS typically present the phase equilibria, including phase diagrams and tie-lines [3,23,31,36], physicochemical properties [3,23] and their application for the extraction/separation of several biomolecules [15,23,31]. Despite the promising properties and exciting results demonstrated for [Ch]Cl-based ABS, no studies have been reported on the hydrodynamics of these systems, not even the simple assessment of the separation kinetics.

As this study aims to discuss the hydrodynamics of [Ch]Cl-based ABS and their integration at an industrial scale, the implementation of the studied systems using traditional mixer-settler units is the most interesting approach. Mixer-settlers are the simpler and more commonly used industrial units in conventional liquid-liquid extraction processes, as they incorporate mixing, coalescence, and separation stages in a single unit [17]. While some successful examples of ABS-based mixer-settler units have been reported for the purification of biomolecules at laboratory and pilot-scales, the large number of ABS variations and their distinct properties makes the hydrodynamics of mixing and separation phenomena, as well as the partition mechanisms of contaminants and target-molecules, highly empirical, unpredictable, and dependent on the specific systems being studied [17].

Therefore, the aim of this work is to evaluate and compare the hydrodynamics of [Ch]Cl-based ABS composed of PPG-400 (polymer/salt system), K_3PO_4 and K_2HPO_4 (two salt/salt systems) and to provide critical insights on phase separation for future scale-up of these LLE processes. In this study we determined phase diagrams, tie-lines (TLs), tie-lines length (TLL), physicochemical properties (density, ρ , viscosity, μ , pH, and surface tension, SFT) of the coexisting phases, and the time required for complete phase separation (T_s) after mixing (T_m) for each ABS. The results were used to obtain correlations for ABS hydrodynamics, by relating T_s with the physicochemical properties of the systems' phases and the characteristics of the phase-forming compounds and compositions.

2. Experimental

2.1. Material

The chemical compounds used in the ABS formation include cholinium chloride ([Ch]Cl); polypropylene glycol with a molecular weight of $400\text{ g}\cdot\text{mol}^{-1}$ (PPG-400); tripotassium phosphate (K_3PO_4); and dipotassium phosphate (K_2HPO_4). Please refer to Table 1 for the structures and specifications of each compound. The water used was double distilled, passed across a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

2.2. Determination of phase diagrams

Aqueous solutions of [Ch]Cl (aqueous solution at $\approx 70\%$ wt.), PPG-400 (pure), K_3PO_4 (aqueous solution at $\approx 40\%$ wt) and K_2HPO_4 (aqueous solution at $\approx 50\%$ wt.) were prepared and used for the determination of the binodal curves. The phase diagrams of [Ch]Cl/PPG-400, [Ch]Cl/ K_3PO_4 , and [Ch]Cl/ K_2HPO_4 ABS were determined by the cloud point titration method [13] at atmospheric pressure and $25^\circ C$, and for the salt/salt ABS also at $50^\circ C$. The experimental procedure employed was previously described in detail elsewhere [13]. The composition of the system was determined by weighing all phase-forming compounds added within an uncertainty of $\pm 10^{-4}$ g, and the respective binodal curves constructed.

The binodal curves of each [Ch]Cl-based ABS were correlated using the empiric equation proposed by Merchuk *et al.* [13] (Eq. (1):

$$Y = A.\exp[(B.X^{0.5}) - (C.X^3)] \quad (1)$$

where X is the mass fraction of PPG-400, K_2HPO_4 or K_3PO_4 and Y is the mass fraction of [Ch]Cl, both expressed as weight percentages. A , B and C are fitting parameters.

2.3. Determination of tie-lines

The determination of the TLs was accomplished by using the gravimetric method described by Merchuk *et al.* [13]. For the TLs determination, mixtures at the biphasic region were prepared (in 15 mL tubes), vigorously stirred and left to equilibrate for 12 h at $25^\circ C$, in order to obtain two clear and well separated coexisting (top and bottom) phases. After the equilibration time, both phases were carefully separated, collected, and weighed. Each individual TL was determined by applying the lever rule to the relationship between the bottom mass composition and the overall system composition and, therefore, by solving the following systems of four equations (Equations 2 to 5)⁴⁶. From the simultaneous resolution of these equations, the composition of respective phase-forming compound (*i.e.*, X_b , X_t , Y_b e Y_t) in each of the coexisting phases were determined.

$$Y_t = A.\exp[(B.X_t^{0.5}) - (C.X_t^3)] \quad (2)$$

$$Y_b = A.\exp[(B.X_b^{0.5}) - (C.X_b^3)] \quad (3)$$

$$Y_t = \left(\frac{Y_m}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right)Y_b \quad (4)$$

$$X_t = \left(\frac{X_m}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right)X_b \quad (5)$$

where X_t and X_b represent the PPG-400, K_2HPO_4 or K_3PO_4 , Y_t and Y_b correspond to [Ch]Cl, at the top and bottom phases, respectively. X_m is the initial concentration of PPG-400, K_2HPO_4 or K_3PO_4 , in the mixture, while Y_m is the initial concentration of [Ch]Cl in the same solution. The parameter α is the ratio between the bottom phase and the total weight of the mixture.

Then, the tie-line length (TLL) was determined according to Eq. (6).

$$TLL = \sqrt{(X_t - X_b)^2 + (Y_t - Y_b)^2} \quad (6)$$

2.4. Physicochemical characterization of ABS coexisting phases

After the TLs determination, the coexisting phases of the [Ch]Cl/PPG-400 ABS were characterized for viscosity and density, while the phases of the [Ch]Cl/ K_3PO_4 and [Ch]Cl/ K_2HPO_4 were characterized for pH, viscosity, density, and conductivity.

Density and viscosity measurements were performed using a viscosimeter-densimeter rotational Stabinger SVM3000 - EXW Anton

Paar® (Graz, Austria) at 25.00 and $50 (\pm 0.02)^\circ C$, and atmospheric pressure. The dynamic viscosities have a relative uncertainty of $\pm 0.35\%$ while the absolute uncertainty for the density is $5 \times 10^{-4} g.cm^{-3}$.

The pH values (± 0.02) of the top and bottom phases were measured using the pH-meter Metrohm - Model 914 from Metrohm® (Switzerland). The calibration of the pH-meter was performed using two standard buffers (pH values of 7.00 and 4.00 ± 0.02).

The SFT values of each phase were recorded applying the pendant drop method using the equipment Dataphysics Instruments GmbH, OCA20 (Germany) at room temperature, following the methodology described in Berry *et al.* [37]. A Hamilton DS 500/GT syringe was connected to a stainless steel needle with a diameter of 1.65 mm, filled with the tested phases and the liquids dispensed in air. A very slow continuous dispense ($0.10 \mu L s^{-1}$) of $5 \mu L$ of the test liquid was selected, with the image of the drop being taken when the complete profile of the drop was observed. With this image, the shape of the pendant drop was analysed by fitting to the Young-Laplace equation with the software modules SCA20, using the gravitational acceleration ($g = 9.8018 m.s^{-2}$) and latitude ($lat = 40$) values in accordance with the assay location. The phases' densities required for the calculation of the SFT from the drop image data were obtained as previously described in this section. In order to compare the results between the studied systems, the SFT values were normalized by the drops' volume since this parameter significantly alters the obtained results and was not constant for all assays.

The water content in each phase was determined using the Karl Fischer titration method using Karl-Fischer 890 Titrand from Metrohm® (Switzerland) ($\pm 0.5\%$) at room temperature, with HYDRANAL-methanol rapid (reagent for accelerated volumetric one-component Karl-Fischer titration) and HYDRANAL-Composite 5 (reagent for volumetric one-component Karl-Fischer titration methanol free), both supplied by Sigma-Aldrich®. At least three measurements were made for each sample.

2.5. Hydrodynamics ABS determination

To understand the kinetics of phase separation hydrodynamics of each [Ch]Cl-based ABS, different mixture points were prepared within the biphasic region, which were selected according to data from the binodal curves. All the mixture points were prepared in 15 mL conical centrifuge plastic tubes, with the systems presenting a total mass of 10 g. They were kept for 15 min at 25 or $50 (\pm 1)^\circ C$ using a thermostatic bath to reach the required temperature. Subsequently, they were poured into a 25 mL glass flask and subjected to vigorous agitation using a magnetic stirrer (22×5 mm) at 1200 rpm. The mixing time (T_m) varied from 15 to 600 s. Afterward, the mixed systems were poured back into the initial conical centrifuge tube and left at the defined temperature until the phases are completely settled, which took the time for settling of the phases (T_s). The complete settling was defined when two clear and completely immiscible phases were visually observed, with no emulsion layer between the phases (*cf.* Figure S1 in the Supplementary Information (SI)).

All T_s were registered in the different mixture compositions for each [Ch]Cl-based ABS and both temperatures (25 and $50^\circ C$) as a function of the T_m . The hydrodynamic behaviour of each ABS was determined as the relation of T_m versus T_s , being the last parameter correlated with the composition and physicochemical properties of the coexisting phases ABS, namely: salt or polymer composition (X_m), composition of [Ch]Cl in the mixture (Y_m); salt or polymer composition in the top phase (X_t); [Ch]Cl composition in the top phase (Y_t), salt or polymer composition in the bottom phase (X_b); [Ch]Cl composition in the bottom phase (Y_b); viscosity of the top and bottom phases (μ_t and μ_b , respectively); density of the top and bottom phases (ρ_t and ρ_b , respectively). Note that the subscripts "t" and "b" correspond to the "top" and "bottom" phases, respectively.

For the hydrodynamic studies of the salt-salt ABS, T_s was recorded using 10 g of the biphasic mixtures. These systems were agitated at a

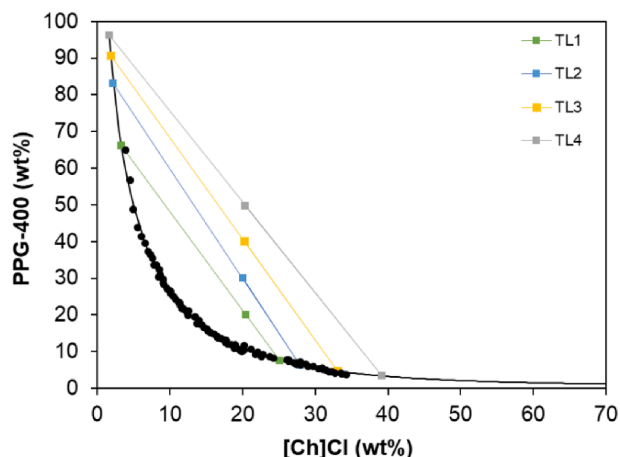


Fig. 1. Ternary phase diagram (at 25 °C and atmospheric pressure) in weight fraction (wt%) for the system composed of PPG-400, [Ch]Cl and H₂O. The black circles (●) and lines (–) correspond to the experimental and adjusted binodal curves, while coloured squares and lines correspond to the respective tie-lines (i.e., TL1, TL2, TL3 and TL4).

constant 1200 rpm for 300 s, using the same 25 mL glass flask and 22 × 5 mm magnetic stirrer that were used to establish the relation between T_s and T_m . To determine if the mixing duration was sufficient for the droplet size of the samples to reach a steady state, the mean surface diameter (D32) of the agitated biphasic mixtures from TL1 to TL5 of both salt/salt ABS was evaluated. This approach was done using a Laser Diffraction (LD) technique with a Mastersizer 2000 from Malvern Instruments (USA). For this analysis, 300 mL of a solution comprising the composition of all the mixture points was prepared in a 400 mL beaker (13.5 × 7.5 cm, approximately). The beaker was agitated at 1200 rpm at 25 °C using the equipment's mixing 3-blade folding propellers. The agitation of the systems only started when a complete phase separation was observed, and the D32 of the systems' was recorded every 30 s for a total of 600 s. The results of these analyses are presented in Figure S2 in SI. Finally, the obtained T_s were correlated with the composition and respective physicochemical properties of the tested systems.

2.6. Statistical analysis

All experiments were performed in triplicate and the results are expressed as the average of three independent assays with the corresponding errors at a 95% confidence level. Statistical analyses were performed using the JMP® Pro 17 software (USA). Values of $p \leq 0.05$ were considered statistically.

3. Results and discussion

To define the biphasic systems for the hydrodynamic studies, the phase diagrams of the distinct types of [Ch]Cl-based ABS were determined, namely: one polymer/salt system using PPG-400 as the polymer (at 25 °C and atmospheric pressure); and two salt/salt systems using K₃PO₄ and K₂HPO₄ as the second ionic compound of the mixture (at 25 °C and 50 °C, both at atmospheric pressure). The experimental data was adjusted using the empirical Merchuk's equation (Eq. (1)) and the experimental TLs determined (cf. Eq. 2 – 5), along with the respective TLL. The experimental data and respective correlations are reported in Table S1, and the phases' physicochemical properties in Table S2, provided as SI. The phase diagrams allow to identify the limit in which the composition of the phases in equilibrium are completely miscible (monophasic region, under the binodal curve) or completely immiscible (biphasic region, above the binodal curve) [12], as well as to define biphasic mixtures with different TLLs for the hydrodynamic studies.

Considering the potential application of ABS for extraction of added-

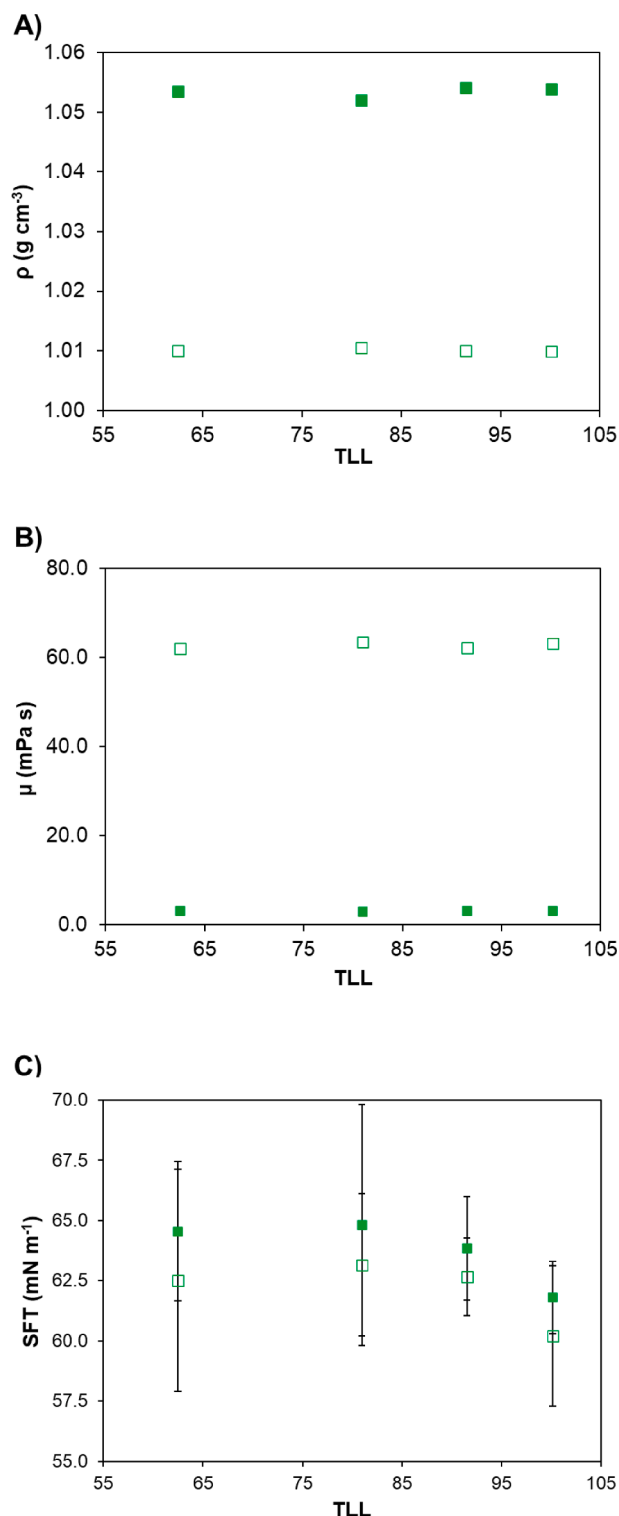


Fig. 2. Physicochemical properties of the phases in equilibrium for PPG-400/[Ch]Cl ABS (■ – salt-rich bottom phase; □ – polymer-rich top phase) determined at 25 °C, namely: A) Densities; B) viscosities and C) surface tensions (SFT). The TLL correspond to the following mixture points: TL1 = 62.48; TL2 = 80.95; TL3 = 91.52; TL4 = 100.24.

value molecules and the required biocompatibility and biodegradability, the hydrodynamics studies focused on [Ch]Cl-based ABS. As previously demonstrated, the formation of biphasic regimes using [Ch]Cl at milder temperatures and low concentration of phase-forming components requires the use of moderately hydrophobic polymers (such as PPG-400

[35,36]) or a highly charged density salt, such as K_3PO_4 [23,31] and K_2HPO_4 [15]. Herein, the hydrodynamics of these ABS was evaluated, but due to their distinct nature (*i.e.*, main mechanisms behind phase separation), the results will be separately discussed in two different subsections, *i.e.*, 3.1. for polymer/salt and 3.2. for salt/salt ABS.

3.1. ABS composed of [Ch]Cl and PPG-400

3.1.1. Phase diagram and physicochemical characterization

This hydrodynamics study started with the [Ch]Cl/PPG-400 ABS. Since the phase equilibria can suffer some small deviation according to the purity of commercial solutions of the phase-forming components, the ternary phase diagram composed of PPG-400, [Ch]Cl and H_2O at 25 °C and atmospheric pressure was firstly characterized, through the determination of the respective binodal curve and tie-lines for four mixture points at the biphasic region (*cf.* Fig. 1). The detailed weight fraction experimental data is presented in Table S3 (SI).

As shown in Fig. 1, there is no intersection between the four TLs and with the $R^2 \approx 1.0$ (Table S1) for the adjusted binodal curve, it is possible to affirm that the Merchuk's method is adequate for the determination of TLs and fitting of the binodal curve [38]. As detailed in Table S1, the top phase of all ABS is mostly composed of PPG-400 (polymer-rich phase), while the bottom phase is mostly constituted of the [Ch]Cl (salt-rich phase). An increase in the amount of PPG-400 added to a $\approx 20\%$ wt. solution of [Ch]Cl led to an increase in the concentration of polymer in the polymer-rich phase and of the salt in the salt-rich phase.

After determining the PPG-400/[Ch]Cl phase diagram, the physicochemical characterization of the coexisting phases in equilibrium was carried out. This characterization is of utmost importance since the mechanisms behind phase separation are governed by the thermodynamic equilibrium of the studied ABS and the phases' equilibrium kinetics and hydrodynamics depend on the properties of phase-forming constituents [12]. Three main forces, gravitational, flotational and frictional, have been reported as significant in the drop movement during coalescence, which forms the ABS. These forces depend on the physicochemical properties of each phase in equilibrium, such as density, viscosity, and interfacial tension. Therefore, to fully understand the hydrodynamics behind ABS formation, it is important to relate the phase separation ability to the physicochemical properties of each phase in equilibrium [12]. The coexisting phases of the four mixtures at the biphasic region were then characterized relatively to their density, viscosity and SFT values, at 25 °C and atmospheric pressure. Note that due to detection limit of the Tensiometer, it was not possible to measure the interfacial tension, being in that case measured the superficial tension of a drop of the phase relatively to the air. The detailed results are presented in Table S2, while, for a better visualization, the properties of each phase were plotted as a function of the TLL in Fig. 2.

The results presented in Fig. 2.A confirm that, regardless of the mixture point, the density values of both phases are similar, *i.e.*, 1.01 g.cm^{-3} and 1.06 g.cm^{-3} for the polymer- and salt-rich phases, respectively. In contrast, the viscosity values between the phases showed significant differences (*cf.* Fig. 2.B), with the viscosity of the polymer-rich phase being 20x higher than that of the salt-rich phase. Interestingly, the increase in PPG-400 concentration (from mixture points 1 to 4) and, consequently, the TLL increase did not affect the absolute values of these two properties, which remained approximately constant. Similarly to density values, as shown in Fig. 2.C the surface tensions are also almost constant (*i.e.*, varying between 60 and $65 \text{ mN.m}^{-1}.\mu\text{L}^{-1}$) for the various TLL and similar for both phases. As the SFT indicates the cohesion forces in each phase, the small differences between the phases' values suggest that both possess similar intermolecular forces, reducing their immiscibility with each other and increasing the time for the ABS formation, namely, to achieve a complete biphasic demixing [19,39]. The characterization of the phases' properties is very important, as it can allow to

Table 2

Settling times (T_s) as a function of mixing times (T_m) for different PPG-400/[Ch]Cl ABS at 25 °C and atmospheric pressure.

PPG-400/[Ch]Cl		T_m (s)						
		15	100	200	300	400	500	600
T_s (h)	TL1	>6	>6	>6	>6	>6	>6	>6
	TL2	>6	>6	>6	>6	>6	>6	>6
	TL3	>6	>6	>6	>6	>6	>6	>6
	TL4	>6	>6	>6	>6	>6	>6	>6

understand the separation hydrodynamics and, as a consequence, help designing the operations for their implementation at industrial scale [12,40].

3.1.2. Hydrodynamic study

After completing the physicochemical characterization of the PPG-400/[Ch]Cl systems, the hydrodynamic studies were carried out. For that purpose, the time required for each mixture point to achieve the complete separation of the two-phases, T_s , was registered as a function of different mixing times T_m (*i.e.*, with T_m varying from 15 to 600 s). These results help explaining how T_m influences the kinetics of phase separation in the studied systems, since this variable has paramount relevance for the industrial application of ABS [24]. Table 2 presents the obtained T_s for the studied polymer/salt system, considering different T_m .

The results presented in Table 2 for all PPG-400/[Ch]Cl systems showed very long T_s , higher than 6 h, regardless of the very distinct T_m . It should be noted that this corresponds to the maximum time continuously recorded, which means that the separation was obtained after that time but not necessarily at 6 h after stabilization. The need for longer settling times lead to longer operation times and limited processual flexibility, which results in more difficulties for the implementation of these processes at industrial scale, requiring the use of gravitational acceleration in centrifugal-type operations [25,41]. From the analysis of physicochemical properties of the phases, the long T_s recorded for the PPG-400/[Ch]Cl systems are expected, resulting from the low differences of densities between coexisting phases (of circa 0.03 g.cm^{-3})^{31,51}. In addition, the phase separation is dependent on the prior mixing step and rate of coalescence, which are associated with the interfacial tension and, consequently, the surface tension of each phase [25,40]. Since the values of SFT in each phase are very close to each other, the intermolecular forces in the coexisting phases are similar as well, resulting in a more difficult phase separation and an increase in T_s [25,39,40]. Moreover, the high viscosity of the PPG-400-rich phase can also affect the diffusion of little droplet, created during the mixing, and their coalescence, resulting in long T_s [24].

Considering the significant amount of time required for phases' settling, it is evident that this type of polymer/salt ABS may not be the suitable choice for implementation in industrial mixed-settler units, which are commonly used in LLE processes. This poses additional challenges for process design and raises concerns regarding the commercial viability of PPG-400/[Ch]Cl ABS [25]. Despite of the high extraction efficiencies and selectivity [35], or even their intrinsic biocompatibility [2,35], the industrial use of polymer/salt ABS, since all will have similar restrictions in terms of physicochemical properties of the phases in equilibrium, remains challenging, requiring adequate hydrodynamics and separation kinetics before their scaling-up, as this type of systems will, probably, require more complex and energy-consuming equipment [25,41] such as centrifuges, or counter current or centrifugal partition chromatography (CPC) apparatus to enhance phase separation [18,41].

Since the results for the polymer/salt system were unfavourable for the idealized hydrodynamic mixing-settling study, taking several hours

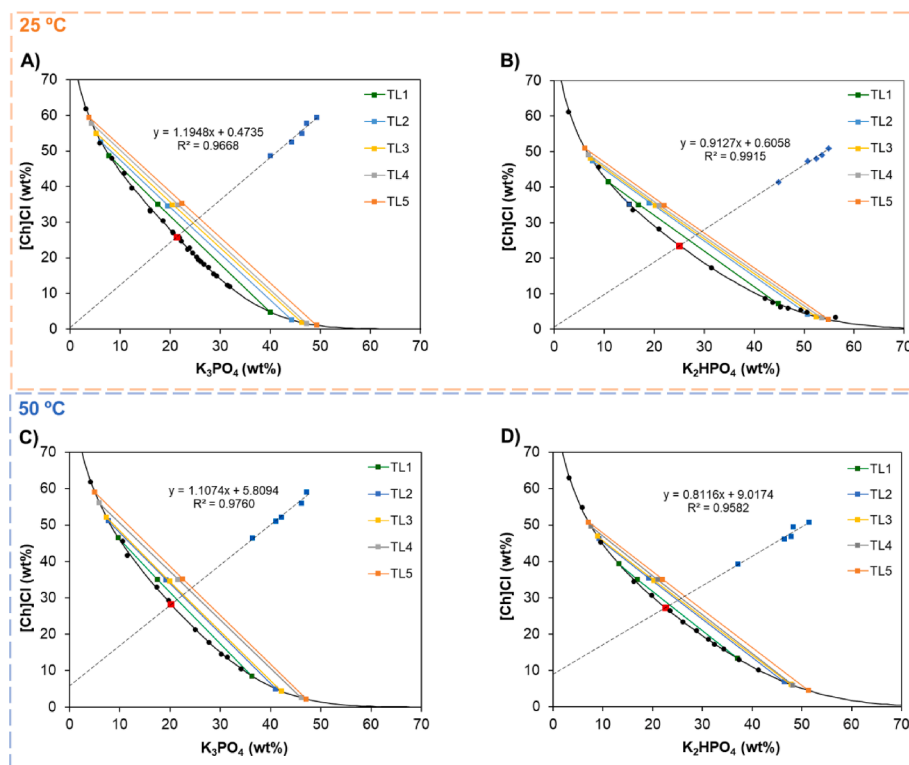


Fig. 3. Phase diagrams (at 25 °C and 50 °C, both at atmospheric pressure) in weight fraction (wt%) for the systems: **A** and **C**) [Ch]Cl/ K_3PO_4 ; and **B** and **D**) [Ch]Cl/ K_2HPO_4 . The black circles (●) and lines (–) correspond to the experimental and adjusted binodal curves, the blue squares are the TLs' relation and the remaining coloured squares and lines correspond to the respective tie-lines (i.e., TL1, TL2, TL3, TL4 and TL5). The critical point for both systems at both temperatures (red square, ■) were determined through the extrapolation the TLs' slopes of distinct systems.

for phase splitting under gravitational force, the system PPG-400/[Ch]Cl was no longer considered for the remaining work.

3.2. ABS composed of [Ch]Cl/ K_3PO_4 and [Ch]Cl/ K_2HPO_4

3.2.1. Phase diagrams and physicochemical characterization

Since the PPG-400/[Ch]Cl ABS's hydrodynamics were found to be not suitable for mixer-settler units, we shifted our focus to the study of [Ch]Cl-based salt/salt ABS composed of K_3PO_4 or K_2HPO_4 as the salting-out inorganic salt. We began by determining the phase diagrams of the ABS [Ch]Cl/ K_3PO_4 and [Ch]Cl/ K_2HPO_4 at 25 and 50 °C, and atmospheric pressure. The binodal curves, TLs and critical point (CP) are presented in Fig. 3, while the phases' compositions are provided in Table S1. Detailed experimental data of binodal curves can be found in Tables S4 to S7 in the SI. Since the T_s of these two salt/salt ABS were considerably lower than those obtained for the PPG-400/[Ch]Cl ABS (cf. hydrodynamics results presented in the next sub-section), we also evaluated the effect of temperature increase.

Since there is no intersection between the TLs and $R^2 \approx 1.0$ for all the ABS, a good adjustment of the binodal curve and the experimental data are obtained with the Merchuk's method [38]. As previously stated, [Ch]Cl has a considerable hydrophilic character and requires salts with high salting-out ability [23], such as the inorganic phosphate salts used in this study, to induce the phase separation [23]. Upon examination of the biphasic region sizes of both salt/salt systems (Fig. 3.A versus 3.B or 3.C versus 3.D), it is evident that the ABS containing K_3PO_4 display a larger region of immiscibility compared to those with K_2HPO_4 . This is consistent with the salting-out aptitude of each salt and follows the Hofmeister series rank ($K_3PO_4 > K_2HPO_4$) [18,23]. The ability to induce salting-out is determined by the anions' Gibbs' hydration energy, $-\Delta G_{hyd}$ (kJ/mol). Anions with higher hydration capacities have more negative ΔG_{hyd} values due to stronger interactions between these anions and water molecules than between water molecules themselves. The decrease of the hydration capacities will lead to a decrease on the ability to induce the formation of [Ch]Cl-based ABS [18,25], as can be confirmed comparing the ΔG_{hyd} values [18,25] of both phosphate salts,

namely, the anion PO_4^{3-} shows a hydration energy of $-2765 \text{ kJ}\cdot\text{mol}^{-1}$ and the anion HPO_4^{2-} presents a hydration energy of $-1125 \text{ kJ}\cdot\text{mol}^{-1}$. It should be noted that salts with lower hydration energies are not able to form biphasic systems when mixed with [Ch]Cl in water. Fig. 3.C and 3.D demonstrate that a temperature increase of 25 °C had only a minor effect on the biphasic region of both salt/salt systems. There was only a slight decrease in this region for both systems.

In summary, as detailed in Table 1, for both [Ch]Cl/ K_3PO_4 and [Ch]Cl/ K_2HPO_4 systems at 25 °C, the bottom phase is the inorganic salt-rich phase, with the concentration of phosphate salts (varying from 30 to 50 wt%) being higher than that of [Ch]Cl (from 3 to 16 wt%). On the other hand, the top phase is the organic salt-rich phase, with higher concentration of [Ch]Cl than the phosphate salt.

To understand the phase separation mechanisms of these ABS, the physicochemical properties of the coexisting phases for the different TLs were evaluated. The density, viscosity, pH and surface tension for each phase were determined and presented as a function of TLL in Fig. 3.

Contrarily to what was observed in the PPG-400/[Ch]Cl, the coexisting phases of both salt/salt ABS exhibit clear differences for the three physicochemical properties under study. In Fig. 4.A, a linear relation between density values and the increase in the TLL is observed, resulting from an increase in the densities of the inorganic salt-rich phases with a slight decrease in the densities of the [Ch]Cl-rich phases as the TLL increases. This suggests that the salt/salt systems have an easier phase separation compared to the polymer/salt ABS due to the larger density difference ($\Delta\rho$) between the coexisting phases, mainly governed by the salt-rich phase densities. The concentration of the phosphate in the salt-rich phase modifies the water ratio in the ABS, resulting in a larger difference in density between the phases with the increase of salt content. Therefore, the considerably higher $\Delta\rho$ promotes a quicker phase separation of ABS due to a higher rate of phase sedimentation (as shown below) [12,25].

On the other hand, regardless of the coexisting phase, an increase of the values of the other two properties (viscosity and SFT) was observed with the increase of TLL. As shown in Fig. 4.B, the viscosity values increase linearly in both phases, although more pronounced in the salt-

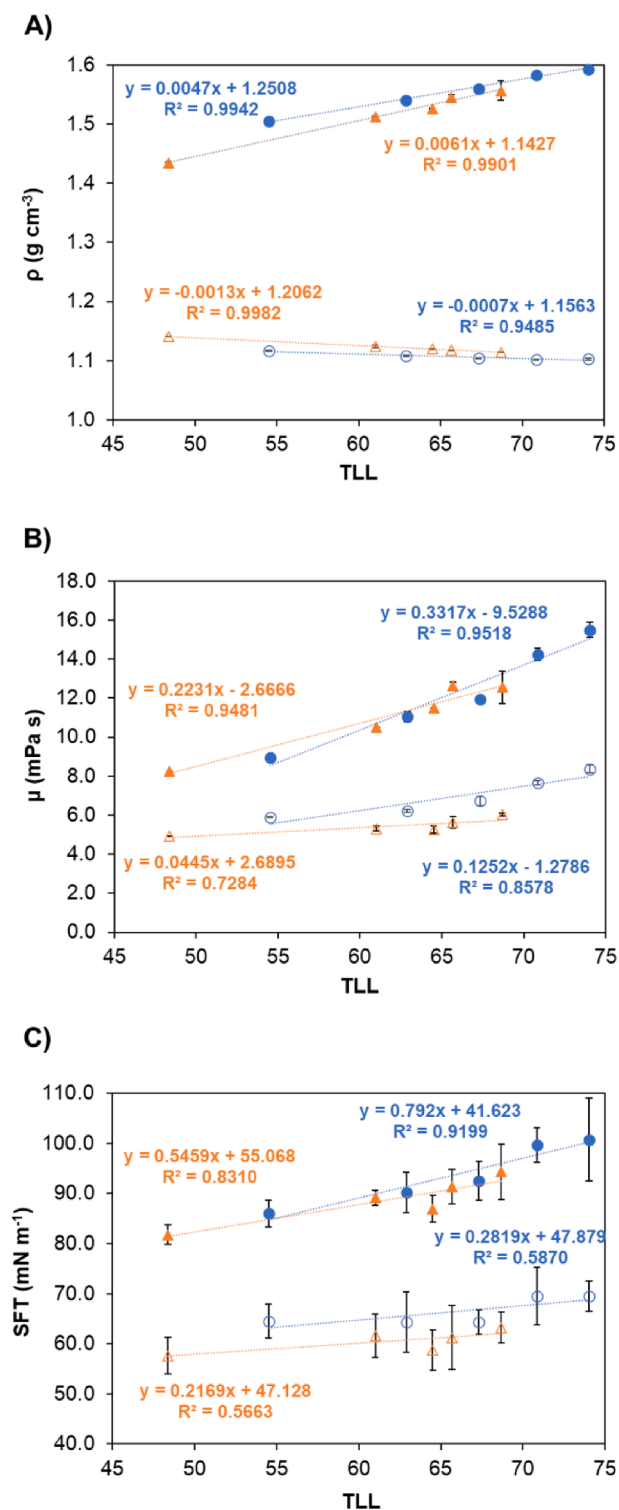


Fig. 4. Physicochemical properties of the phases in equilibrium for of the [Ch]Cl/salt systems at 25 °C (■ – [Ch]Cl/ K_3PO_4 ; ▲ – [Ch]Cl/ K_2HPO_4 , in which the phosphate salt-rich phase are the full symbols and the [Ch]Cl-rich top phase the empty symbols), namely: Density (A), viscosity (B) and surface tension (C) of The TLL of [Ch]Cl/ K_3PO_4 correspond to the following mixture points: TL1 = 54.50; TL2 = 62.89; TL3 = 67.32; TL4 = 70.84 and TL5 = 74.03. The TLL of [Ch]Cl/ K_2HPO_4 correspond to the following mixture points: TL1 = 48.37; TL2 = 61.01; TL3 = 63.50; TL4 = 65.67 and TL5 = 68.68.

rich phase. Comparing these two salt/salt ABS with the PPG-400/[Ch]Cl systems (Fig. 2.B), a very large difference in the viscosity of the coexisting phases ($\Delta\mu$) is observed, which is around 60 $\text{mPa}\cdot\text{s}$ for the polymer/salt system and between 3 and 8 $\text{mPa}\cdot\text{s}$ for the salt/salt systems. Consequently, longer phase separation times are expected for the polymer/salt ABS in comparison to the salt/salt systems, as the rate of drop coalescence and consequent phase separation is slower [12,18,25]. It should be noted, as shown in Table S2, that the values of pH for both coexisting phases are similar (i.e., [Ch]Cl/ K_3PO_4 with pH values of ≈ 13 and [Ch]Cl/ K_2HPO_4 with pH values of ≈ 10), confirming identical chemical potential and that equilibrium is reached [42]. Comparing the SFT results for the salt/salt ABS with the PPG-400/[Ch]Cl ABS (Fig. 2.C and 4.C, respectively), an increasing trend of SFT values of both phases with the increase of TLL, with the SFT difference between the phases considerably higher than the difference attained for the polymer/salt system. The increase in SFT indicates superior cohesion forces within the phases, which facilitates the respective phase demixing and ABS stabilization [12,19,39].

3.2.2. Hydrodynamics study

After the physicochemical characterization of the salt/salt ABS properties, the hydrodynamics studies for these ABS were carried out. Similarly to polymer/salt system, the T_s of different mixtures of both salt/salt systems were recorded for different T_m , at 25 °C and 50 °C. In Fig. 5, the hydrodynamics of phase separation of each system was presented as the variation of T_s with T_m for each TL, at both temperatures under study.

The results from Fig. 5 show that T_s is independent of T_m since there are no significant changes of T_s for the various T_m used. Therefore, the latter does not act as critical variable for the formation of two phases in equilibrium in these salt/salt ABS, with T_s ranging between 54.78 ± 1.03 s and 57.40 ± 0.93 s for the ABS composed of [Ch]Cl and K_3PO_4 (Fig. 5.A) and between 67.16 ± 0.64 s and 69.13 ± 1.68 s for the ABS composed of [Ch]Cl and K_2HPO_4 (Fig. 5.B) at 25 °C. The lower T_s for the system with K_3PO_4 is associated with its higher hydration ability in comparison to K_2HPO_4 , according to the Hoffmeister series [18]. The “stronger” salt being more strongly hydrated, retains more water molecules to the salt-rich phase, enhancing the phase separation and settling. In both salt/salt systems, this behaviour results in more distinct phases’ physicochemical properties (detailed below) and a faster phase separation of the ABS [25].

For both salt/salt systems, it is observed that an increase in phosphate salt concentration, and consequently in TLL, induced a reduction in the T_s values. These results imply that, as the mixture point moves away from the binodal curve and the TLL increases, the required settling time decreases. Faster phase settling seems to be related to the larger density differences between the phases in salt/salt systems, when compared to the studied polymer/salt ABS, ranging between 0.29 and 0.50 g cm^{-3} (Fig. 3.A). These differences in density increase the sedimentation rate and promote separation kinetics [12,25,40]. This shorter separation times obtained for salt/salt ABS are expected, since the phases $\Delta\rho$ values fall outside the critical range (from 0.05 to 0.15 g cm^{-3}) for ABS that require long T_s for the formation of two phases in equilibrium, as reported by Hustedt et al. [40]. In addition, comparing with polymer/salt ABS, due to the lower viscosity differences between the phases (Fig. 3.B), the phase separation phenomenon is facilitated, as it improves the systems’ fluid dynamics and decreases the settling time of the phases after mixing [12,31,40]. The larger differences between the SFT values of both phases (Fig. 3.C) also indicate larger differences in the cohesion forces between the molecules of the two phases and their greater immiscibility [12,19,39]. All these observations explain the significant differences observed in T_s for the polymer/salt and the salt/salt systems, as the major drawbacks of the first are the small differences in density and low SFT values, as well as the high viscosity differences between phases, which result in slow phase separations [12,25].

Concerning the effect of the temperature, the phase diagrams of both

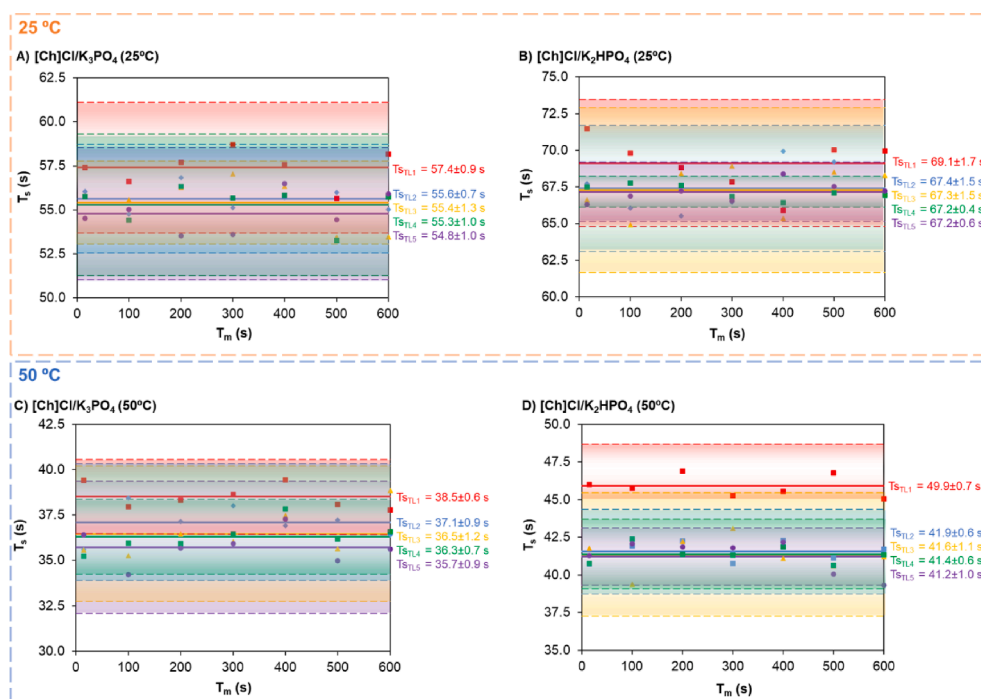


Fig. 5. T_s values of different T_m for [Ch]Cl/ K_3PO_4 and [Ch]Cl/ K_2HPO_4 at 25 °C (A and B, respectively) and 50 °C (C and D, respectively). The coloured squares indicate the registered T_s values obtained for each TL (TL1 – red square, ■; TL2 – blue square, ■; TL3 – yellow square, ■; TL4 – green square, ■; TL5 – purple square, ▲). The full lines and dash lines correspond to the mean T_s , and upper and lower limits according to IMR control charts, respectively. The mean T_s value for each TL is near the respective full line. The shaded area was used for a clear understanding of the variation of T_s described by the upper and lower limits.

systems at 50 °C presented lower biphasic regions when compared to those at 25 °C, which is in accordance with the upper critical solution temperature (UCST) behaviour usually reported for salt/salt systems [12]. The analysis of the T_s results showed a decrease in the registered T_s at 50 °C in comparison to the values obtained at 25 °C for both ABS, with values between 35.73 ± 0.91 and 38.52 ± 0.62 s for the system with K_3PO_4 (Fig. 5.C) and between 41.23 ± 1.03 and 49.91 ± 0.66 s for the system with K_2HPO_4 (Fig. 5.D). As reported by other authors, the density and viscosity of both phases tend to decrease with the increase of the temperature [24]. Although, since the decrease in the values of these properties is similar for both phases, the recorded differences between the phases physicochemical properties of each phase will remain almost constant and cannot entirely justify the faster phase separation of the ABS (confirmed by the density values obtained at 50 °C for the same mixture points, as detailed in Table S2). Therefore, the reduction in T_s with the increase in the temperature may be a result of a decrease on enthalpic contributions and increase of entropic forces. Nevertheless, the salting-out ability of the systems still remains important to the hydrodynamics of each ABS, as the system with the salt with lower Gibbs hydration energy and a consequently higher hydration capacity, K_3PO_4 , still presented T_s values lower than the system with K_2HPO_4 [19].

The favourable results obtained for these [Ch]Cl-based salt/salt ABS suggest that their large scale implementation is promising and straightforward, since it can be based in conventional mixed-settler units. The time required for the phase separation is very short and independent of T_m . Therefore, it means that this type of ABS can be agitated for the required time to guarantee the complete partition of the compound of interest, without affecting T_s . This allows quick phase separation and overcomes one of the major drawbacks of the conventional ABS polymer/polymer and polymer/salt systems [12,25]. In salt/salt systems, it should also be considered that ion-exchange may occur between the phases. This leads to more species being in equilibrium and results in more complex systems. It can potentially cause significant changes in the hydrodynamics of ABS and require the quantification of all ions in the systems' phases [17]. However, as previously

demonstrated [17], the ion-exchange effects in this type of ABS are insignificant, due to the strong salting-out aptitude of the inorganic phosphate salts. Anyway, this trend cannot be generalized, and it may not apply to other salt/salt systems, where the ion-exchange phenomenon can affect the ABS' hydrodynamics and partition mechanisms [17].

In the next section we will analyse the hydrodynamics of the various system studied aiming at drawing correlations between the hydrodynamic behaviour and the physicochemical properties of the ABS. This should provide a clear picture of what phase properties are governing the differences between the two salt/salt ABS, as well as how they change with the mixture compositions.

3.2.3. Hydrodynamic and physicochemical properties correlations

To gain a deeper understanding of the influence of physicochemical property differences on the hydrodynamics of ABS, the properties of the systems were correlated with T_s . To ensure that the droplet sizes in both salt/salt systems reached a steady state before recording T_s and to establish a comparable starting point for all biphasic mixtures, the mean surface diameter (D_{32}) from TL₁ to TL₅ of each salt/salt ABS were registered and are presented in Figure S2 in the SI. The results indicate that the D_{32} of all system particles remained constant after 60 s.

Although T_s was recorded for biphasic mixtures agitated with a magnetic stirrer instead of the LD equipment's propellers, it is worth noting that agitating a 10 g mixture using a magnetic stirrer in a small 25 mL glass flask would still ensure a constant D_{32} , as observed when using the equipment's propellers in a 300 mL solution within a 400 mL beaker (approximately 350 g). While the mixing efficiency of the magnetic stirrer may not be the same as the propellers, both types of agitation reached 1200 rpm. Additionally, the magnetic stirrer agitates a smaller amount of biphasic mixture compared to the propellers, resulting in a more turbulent flow regime (as easily observed with naked eye). Based on the data presented in Figure S2, it was defined the use of 1200 rpm and a mixture time higher than 60 s to guarantee that all tested biphasic mixtures are in steady state when T_s was registered. Therefore, in Fig. 6, the T_s values recorded after 300 s of mixing at 25 °C were

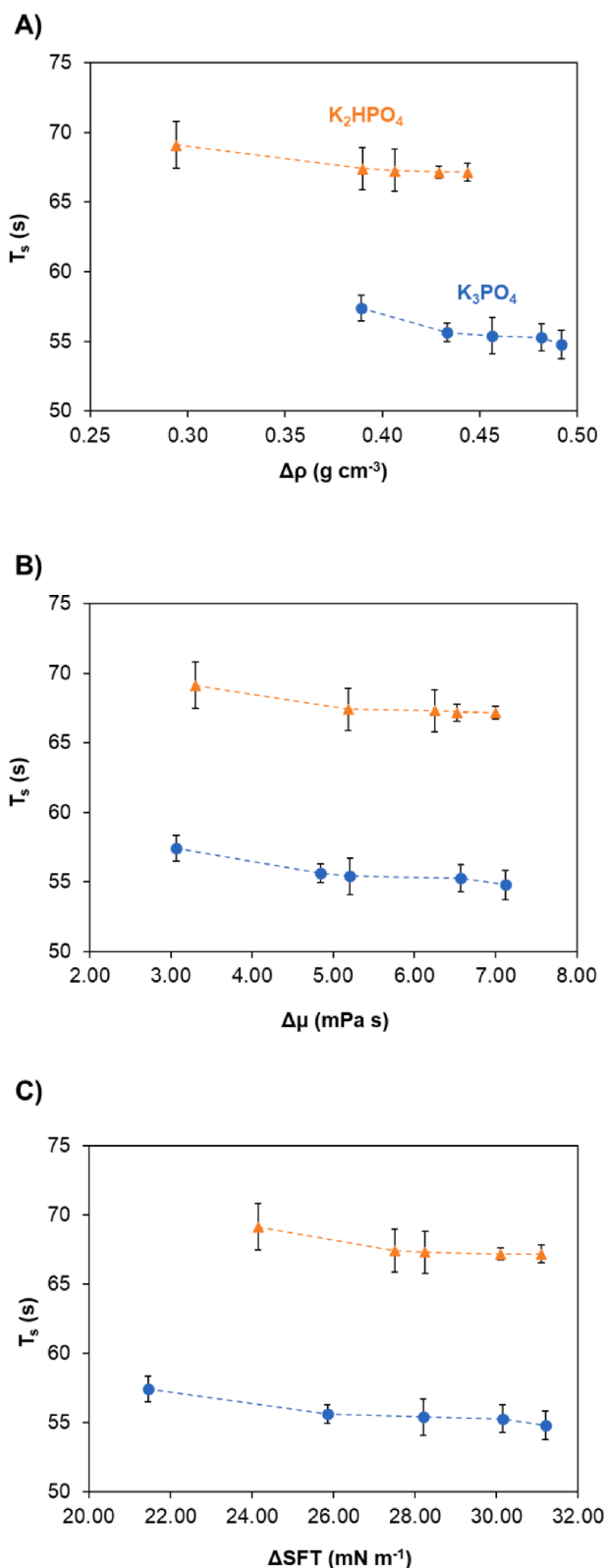


Fig. 6. Relation between T_s and $\Delta\rho$ (A), $\Delta\mu$ (B) and ΔSFT (C) for each salt/salt ABS at 25 °C (■ – [Ch]Cl/ K_3PO_4 ; ■ – [Ch]Cl/ K_2HPO_4). The dashed lines are only guides to the eye.

plotted as a function of density difference ($\Delta\rho$), viscosity difference ($\Delta\mu$) and surface tension difference (ΔSFT) between the [Ch]Cl-rich and the salt-rich phases for each system.

The results reported in Fig. 6 show that T_s decreases with the increase

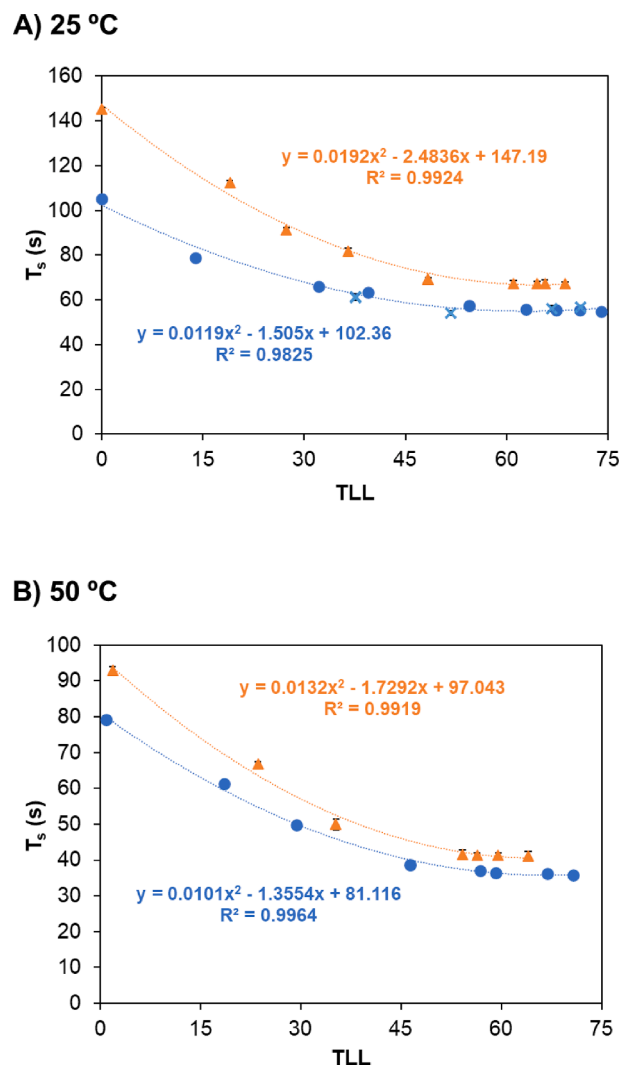


Fig. 7. Determined T_s values as a function of TLL at 25 °C (A) and 50 °C (B) (■ – mixture points of [Ch]Cl/ K_3PO_4 ; ■ – mixture points of [Ch]Cl/ K_2HPO_4 ; × – mixture points from Shahrihari et al. [23]).

in the differences between properties of the ABS phases, being very distinct for [Ch]Cl/ K_3PO_4 and [Ch]Cl/ K_2HPO_4 . In Fig. 6.A, the $\Delta\rho$ values for [Ch]Cl/ K_3PO_4 ranged from 0.38 to 0.50 g cm^{-3} , while for [Ch]Cl/ K_2HPO_4 ranged from 0.29 to 0.45 g cm^{-3} . Regarding $\Delta\mu$ and ΔSFT (Fig. 6.B and 6.C, respectively), the results for both systems are similar, being within the same range of values. Since the recorded T_s are very different between the two ABS, the similar results obtained for $\Delta\mu$ and ΔSFT cannot explain the significantly different T_s observed, otherwise the results of T_s for both systems should be similar for both systems as well. Therefore, only the different values of $\Delta\rho$ can be associated with the distinct T_s determined for each system, being identified as the most important property to assess the salt/salt ABS hydrodynamics [24].

Considering the observations discussed above showing that the increase in TLL increases the salt-rich phase density and, consequently, the $\Delta\rho$ values between the coexisting phases (Fig. 4.A), the distinct T_s registered for each of the systems were also related with the different salting-out (i.e., hydration) ability of each phosphate salt used [15,31].

Since it was observed that the decrease in TLL lead to more identical compositions between the coexisting phases of the ABS and longer T_s , it was hypothesised that the maximum T_s would be registered at the critical point (CP) of the systems (where the composition of the phases is identical). To prove this premise and understand how the T_s varies as a function of the composition of the coexisting phases, the CP and three

new mixture points (with lower TLL than the five initial mixture points were produced) were produced and their T_s registered at 25 and 50 °C (detailed information of the new points are in **Table S.8** in SI). Moreover, the mixture points reported by Shahriari *et al.* [23] for the system [Ch]Cl/K₃PO₄ were reproduced and their T_s obtained at 25 °C only (since it was the temperature at which the phase diagram was obtained and corresponding T_s were displayed as a function of the TLL registered in that work), in order to perceive if the obtained T_s for those mixture points would be within the trends observed in this study. All the obtained T_s are displayed as a function of the respective TLL in **Fig. 7**.

The results in **Fig. 7.A** confirmed that the phase separation for both systems is more difficult as the TLL becomes smaller, reaching their maximum T_s at TLL = 0 (CP), and decaying exponentially from the CP until TLL ≤ 60. For TLL ≥ 60 T_s becomes constant. This indicates that, above a certain TLL, the time necessary for the systems to reach the equilibrium will not present significant variations. Since this equilibrium is governed by the differences between the phase's compositions and respective properties, it is implied that these differences will also tend to stabilize above that TLL, with the T_s reaching their minimum and the ABS hydrodynamics their maximum. These results also show that mixture points with the same TLL possess similar T_s , since the two mixture points with equal TLL reported by Shahriari *et al.* [23] presented identical results. These data confirmed the influence of the phases' composition and physicochemical properties on the ABS hydrodynamics, with different mixture points possessing the same phases' composition and obtaining identical T_s . Besides, the T_s observed using mixture points from other authors regarding the systems [Ch]Cl/K₃PO₄ [23] showed the same tendency as those observed in this work, validating our hypothesis.

At 50 °C, some of the new mixture points do not form an ABS, since the biphasic regions of the studied salt/salt systems were slightly smaller at 50 °C (see **Table S8** in SI). Therefore, only the mixture points that produced a ABS were evaluated and the results are displayed in **Fig. 7.B**. The mixture points that were able to form an ABS at this temperature presented the same behavior observed at 25 °C, exponentially decreasing in both systems for 0 ≤ TLL ≤ 50, and becoming practically constant for the mixture points with TLL ≥ 50. The increase in temperature tends to decrease the T_s values, regarding of the composition differences between the ABS phases, since the intensity of H-bonding interactions within the system are reduced, consequently decreasing the phases' viscosity and improving phase separation [24]. It is also observed that the values of T_s at the same TLL for the two systems at 50 °C are closer to each other than at 25 °C, which indicates that the increase in temperature enhances the transport properties reducing the influence of the other phase properties on the ABS hydrodynamics.

Ventura *et al.* [19] showed that the salting-out effect of ILs is ruled by entropic contributions from the formation of water-ion complexes, and that the molar volume of the organic salt cation controls their abilities to form ABS.²⁴ Here the partial molar volume of [Ch]Cl ($V_{m[Ch]Cl}$) in the salt-rich phase of each mixture point was used to evaluate the salting-out effect of the used phosphate salts in the studied ABS, then being associated with their respective T_s . The values of $V_{m[Ch]Cl}$ in the salt-rich phase, at 25 and 50 °C, were determined using **equation (7)**:

$$V_{m[Ch]Cl} = \frac{x_{[Ch]Cl} \cdot M_{w[Ch]Cl}}{\rho_{[Ch]Cl}} \quad (7)$$

where $V_{m[Ch]Cl}$ and $x_{[Ch]Cl}$ are the partial molar volume and molar fraction of [Ch]Cl in the salt-rich phase, respectively, $M_{w[Ch]Cl}$ is the molecular weight of [Ch]Cl and $\rho_{[Ch]Cl}$ the density of [Ch]Cl as a liquid, reported in **Table 1**. The obtained results are represented in **Figure S3** (SI).

Similar tendencies to the ones previously reported in **Fig. 7** are observed in **Figure S3**, with T_s decreasing with the decrease of $V_{m[Ch]Cl}$. This information proves that the phase separation hydrodynamics of [Ch]Cl/salt ABS is mainly dominated by the salting-out capacity of the

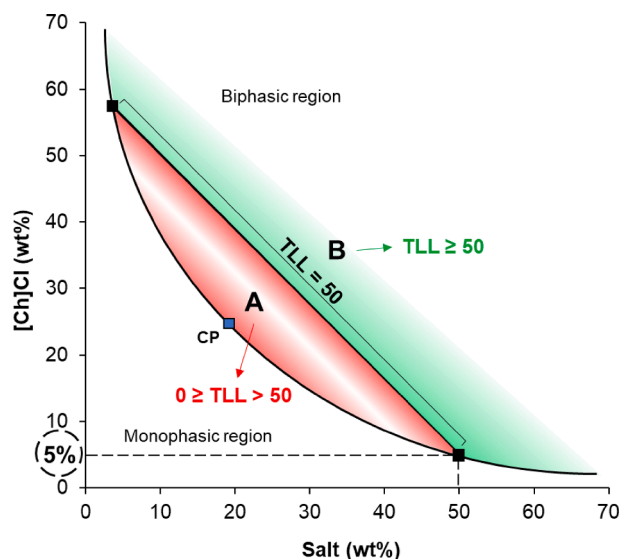


Fig. 8. Schematics of the best region of operation for a general [Ch]Cl-based salt/salt system. The blue square, ■ represents the CP (TLL = 0). **Region A** (red area) represents the section of the biphasic region where the mixture points present 0 ≥ TLL > 50, and where the ABS' point of operation is not recommended. **Region B** (green area) is defined as the best region of operation where all the mixture points present TLL ≥ 50 and the best ABS' hydrodynamics.

used salt, with this effect being possibly quantified using $V_{m[Ch]Cl}$ in the salt-rich phase. Higher concentration of phosphate salt in the systems will increase the salting-out effect, forcing the [Ch]Cl to migrate towards a [Ch]Cl-rich phase, decreasing its concentration in the salt-rich phase and, consequently, reducing the values of $V_{m[Ch]Cl}$. From the maximum $V_{m[Ch]Cl}$ obtained at the CP, where the differences between the phases' composition are as minimal as they can be, this parameter will decrease with the increase in TLL of the mixture points, exponentially decreasing the T_s due to the consequently higher differences between the phases' composition and respective properties. The increase in temperature also affects pronouncedly the phase separation hydrodynamics, reducing the intermolecular forces between the constituents and favouring the phase separation, but the results are still dependent on the salting-out ability of the salts used and the differences between the phases' composition and physicochemical properties.

It can be hypothesized that the values of T_s for the generality of salt/salt ABS will follow the reported profiles. This hypothesis is based on the evidence reported by other authors studying other types of salt/salt ABS formation, where the salting-out effect of one of the salts with higher hydration capacity rules the phase separation hydrodynamics [19,24]. From a process optimization perspective, these insights are key for industrial implementation of ABS, as they allow to establish the best regions of operation inside the biphasic area of this type of extraction systems, where T_s is minimum and the ABS hydrodynamics reached its maximum. **Fig. 8** illustrates a general example of an [Ch]Cl-based salt/salt ABS. If it is considered that, for an hypothetical [Ch]Cl-based salt/salt ABS, T_s decreases exponentially for all the mixture points between the CP (TLL = 0) until the concentration of [Ch]Cl in the salt-rich bottom phase in those points is below 5 wt% (at a TLL = 50) (**Region A**, 0 ≥ TLL > 50), and T_s becomes almost constant for TLL ≥ 50 (**Region B**), this region will be the best region of operation for this process. Within that region, the ABS hydrodynamics has reached its maximum, with all the produced ABS satisfying that restriction presenting the minimum T_s possible. Inside this region, the optimization of the mass transfer kinetics for the target compounds can take place and the best operation point selected, considering all the process requirements and restrictions.

The deeper understanding of the hydrodynamics for this type of systems makes possible to reduce the degrees of liberty in the selection

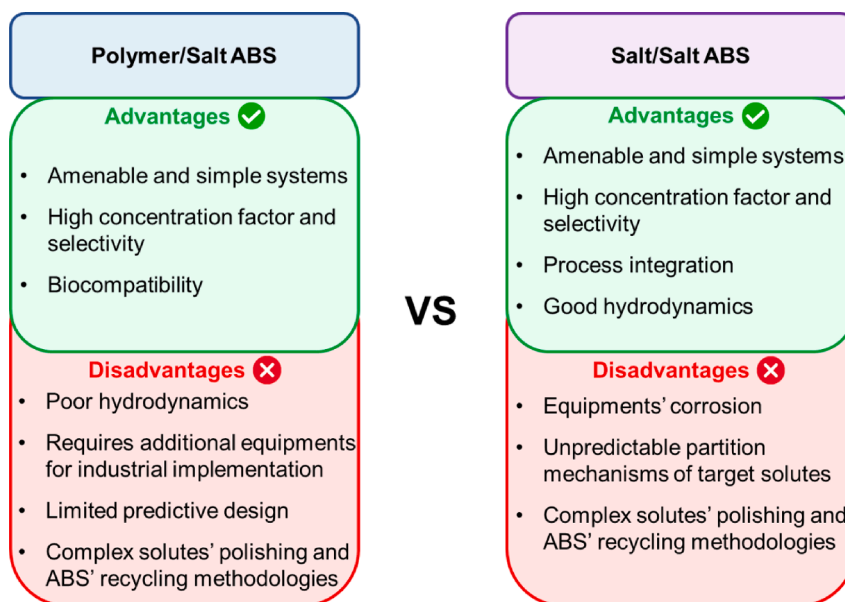


Fig. 9. Main advantages and disadvantages for the industrial implementation of polymer/salt and IL/salt ABS.

of the best operation point in [Ch]Cl-based salt systems, helping in the development of efficient and economically viable extraction projects using these ABS. From the phase diagrams, TLs and respective composition of the coexisting phases, a predictive model based on these findings can be created and used to define the best region of operation within the biphasic region of cholinium-based ABS, that probably applies for other types of salt/salt systems, as well.

To facilitate the comparison of the potential industrial implementation of polymer/salt and IL/salt ABS, the main advantages and disadvantages for both systems are summarized in Fig. 9.

From a general perspective, it is possible to state that both the polymer/salt and the salt/salt systems are amenable and simple systems, and if properly designed can allow to obtain high concentration factors and selectivities for the separation and purification of (bio)molecules, respectively. However, they require complex methodologies for guaranteeing a proper polishing of the target-solutes and the recycling of the ABS-forming components.

On the other hand, as clearly demonstrated in this work, salt/salt ABS demonstrates quicker phase separation when compared to the polymer/salt systems, highlighting the superior hydrodynamics of salt/salt systems. This facilitates their industrial implementation, albeit still complex, by reducing the degrees of freedom in the idealized industrial process. Additionally, the high viscosity of the polymer-rich phases in polymer/salt systems poses challenges for scaling-up these types in industrial extraction processes since they will require additional equipment to achieve an effective and viable separation process. The lack of predictive design regarding hydrodynamics and partition mechanisms is also an issue that needs to be addressed prior to scaling-up of polymer/salt ABS, ensuring their effective industrial application.

Even with disadvantages of salt/salt ABS such as unpredictable partition mechanisms of solutes and some equipment' corrosion issues due to the use of high concentration of phosphate salts, the quick phase separation and easier process integration make these systems more attractive for the industrial implementation. Therefore, this work not only enabled the establishment of an optimal operating region for salt/salt [Ch]Cl-based ABS, which will aid in improving the predictive design necessary future industrial implementation of these systems, but also highlights the advantages of using these systems in comparison to

polymer/salt ABS. Future studies should focus on addressing the drawbacks to effectively implement salt/salt systems at an industrial scale.

4. Conclusions

In this work, the hydrodynamics of two distinct types [Ch]Cl-based ABS (polymer/salt and salt/salt systems) were compared and related with their phases' physicochemical properties and characteristics of the phase-forming compounds. The system PPG-400/[Ch]Cl presented very long T_s (>6 h), being very disadvantageous from a hydrodynamic point-of-view as it requires the use of additional equipment and higher initial cost to obtain a viable project. The systems [Ch]Cl/ K_3PO_4 and [Ch]Cl/ K_2HPO_4 presented considerably shorter T_s (<150 s), with enhanced hydrodynamics when compared to the studied polymer/salt system and overcoming one major drawback of conventional ABS. Density was the physicochemical property that most influenced the phase splitting ability of the salt/salt systems, with the salting-out effect of K_3PO_4 and K_2HPO_4 being the main drive-force of the phase separation hydrodynamics of these ABS, as it alters the phases' composition, their physicochemical properties and the phases' settling time. For the first time, it is shown that an optimal region of operation can be established inside the biphasic region of [Ch]Cl-based salt/salt ABS, reducing the degrees of liberty in the selection of the best operation point and enabling a quicker and more efficient scale-up of these systems at industrial level. These understandings are key for the industrial implementation of ABS in different extraction and purification processes, being crucial to model and predict the ABS formation in any type of complex system and facilitate their industrial scale-up and further commercialization.

CRediT authorship contribution statement

Alexandre M.S. Jorge: Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft. **João A.P. Coutinho:** Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Jorge F.B. Pereira:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seppur.2023.124183>.

References

- [1] P.-Å. Albertsson, Partition of Proteins in Liquid Polymer-Polymer Two-Phase Systems, *Nature* 182 (4637) (Sep. 1958) 709–711, <https://doi.org/10.1038/182709a0>.
- [2] M. Mastiani, N. Firoozi, N. Petrozzi, S. Seo, M. Kim, Polymer-Salt Aqueous Two-Phase System (ATPS) Micro-Droplets for Cell Encapsulation, *Sci. Rep.* 9 (1) (Oct. 2019) 15561, <https://doi.org/10.1038/s41598-019-51958-4>.
- [3] J.F.B. Pereira, K.A. Kurnia, O.A. Cojocaru, G. Gurau, L.P.N. Rebelo, R.D. Rogers, M. G. Freire, J.A.P. Coutinho, Molecular interactions in aqueous biphasic systems composed of polyethylene glycol and crystalline vs. liquid cholinium-based salts, *PCCP* 16 (12) (2014) 5723, <https://doi.org/10.1039/C3CP54907K>.
- [4] H. Yuan, Y. Liu, W. Wei, Y. Zhao, Phase Separation Behavior and System Properties of Aqueous Two-Phase Systems with Polyethylene Glycol and Different Salts: Experiment and Correlation, *J. Fluids* 2015 (Feb. 2015) 1–10, <https://doi.org/10.1155/2015/682476>.
- [5] Y.H. Chow, A. Sahlini, H.-S. Ng, J.-C.-W. Lan, Efficacy of alcohol/sugar aqueous biphasic system on partition of bovine serum albumin, *Bioresour. Bioprocess* 8 (1) (Dec. 2021) 7, <https://doi.org/10.1186/s40643-021-00360-y>.
- [6] A.M. Ferreira, P.D.O. Esteves, I. Boal-Palheiros, A.B. Pereira, L.P.N. Rebelo, M. G. Freire, Enhanced tunability afforded by aqueous biphasic systems formed by fluorinated ionic liquids and carbohydrates, *Green Chem.* 18 (4) (2016) 1070–1079, <https://doi.org/10.1039/C5GC01610J>.
- [7] C.W. Ooi, B.T. Tey, S.L. Hii, S.M.M. Kamal, J.C.W. Lan, A. Ariff, T.C. Ling, Purification of lipase derived from Burkholderia pseudomallei with alcohol/salt-based aqueous two-phase systems, *Process Biochem.* 44 (10) (Oct. 2009) 1083–1087, <https://doi.org/10.1016/j.procbio.2009.05.008>.
- [8] N. Schaeffer, G. Pérez-Sánchez, H. Passos, J.R.B. Gomes, N. Papaiconomou, J.A. P. Coutinho, Mechanisms of phase separation in temperature-responsive acidic aqueous biphasic systems, *PCCP* 21 (14) (2019) 7462–7473, <https://doi.org/10.1039/C8CP07750A>.
- [9] B. Madadi, G. Pazuki, B. Nasernejad, Partitioning of Cefazolin in Biocompatible Aqueous Biphasic Systems Based on Surfactant, *J. Chem. Eng. Data* 58 (10) (Oct. 2013) 2785–2792, <https://doi.org/10.1021/je4004756>.
- [10] M.S. Álvarez, F. Moscoso, A. Rodríguez, M.A. Sanromán, F.J. Deive, Triton X surfactants to form aqueous biphasic systems: Experiment and correlation, *J. Chem. Thermodyn.* 54 (Nov. 2012) 385–392, <https://doi.org/10.1016/j.jct.2012.05.022>.
- [11] L. V. T. D. Alencar, L. M. S. Passos, R. N. Soriano, R. N. Bharagava, L. F. R. Ferreira, and R. L. de Souza, Aqueous Two-Phase Systems: An Alternative Process for Industrial Dye Recovery, 2021, pp. 35–55. https://doi.org/10.1007/978-981-15-6564-9_3.
- [12] J.F.B. Pereira, J.A.P. Coutinho, Aqueous Two-Phase Systems, in: *Liquid-Phase Extraction*, Elsevier, 2020, pp. 157–182, <https://doi.org/10.1016/B978-0-12-816911-7.00005-0>.
- [13] J.C. Merchuk, B.A. Andrews, J.A. Asenjo, Aqueous two-phase systems for protein separation, *J. Chromatogr. B Biomed. Sci. Appl.* 711 (1–2) (Jun. 1998) 285–293, [https://doi.org/10.1016/S0378-4347\(97\)00594-X](https://doi.org/10.1016/S0378-4347(97)00594-X).
- [14] V. Ramakrishnan, L.C. Goveas, N. Suralikerimath, C. Jampani, P.M. Halami, B. Narayan, Extraction and purification of lipase from *Enterococcus faecium* MTCC5695 by PEG/phosphate aqueous two-phase system (ATPS) and its biochemical characterization, *Biocatal. Agric. Biotechnol.* 6 (Apr. 2016) 19–27, <https://doi.org/10.1016/j.bcab.2016.02.005>.
- [15] M. Osloob, A. Roosta, Experimental study of choline chloride and K_2HPO_4 aqueous two-phase system, and its application in the partitioning of penicillin G, *J. Mol. Liq.* 279 (Apr. 2019) 171–176, <https://doi.org/10.1016/j.molliq.2019.01.134>.
- [16] C.U. Mussagy, D. Remonato, A.V. Paula, R.D. Herculanio, V.C. Santos-Ebinuma, J. A.P. Coutinho, J.F.B. Pereira, Selective recovery and purification of carotenoids and fatty acids from *Rhodotorula glutinis* using mixtures of biosolvents, *Sep. Purif. Technol.* 266 (Jul. 2021), 118548, <https://doi.org/10.1016/j.seppur.2021.118548>.
- [17] J.F.B. Pereira, M.G. Freire, J.A.P. Coutinho, Aqueous two-phase systems: Towards novel and more disruptive applications, *Fluid Phase Equilib.* 505 (Feb. 2020), 112341, <https://doi.org/10.1016/j.fluid.2019.112341>.
- [18] M. G. Freire, *Ionic-Liquid-Based Aqueous Biphasic Systems*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2016, <https://doi.org/10.1007/978-3-662-52875-4>.
- [19] S.P.M. Ventura, S.G. Sousa, L.S. Serafim, A.S. Lima, M.G. Freire, J.A.P. Coutinho, Ionic-Liquid-Based Aqueous Biphasic Systems with Controlled pH: The Ionic Liquid Anion Effect, *J. Chem. Eng. Data* 57 (2) (Feb. 2012) 507–512, <https://doi.org/10.1021/je2010787>.
- [20] C.M.S.S. Neves, S. Shahriari, J. Lemus, J.F.B. Pereira, M.G. Freire, J.A.P. Coutinho, Aqueous biphasic systems composed of ionic liquids and polypropylene glycol: insights into their liquid-liquid demixing mechanisms, *PCCP* 18 (30) (2016) 20571–20582, <https://doi.org/10.1039/C6CP04023C>.
- [21] J.A. Asenjo, B.A. Andrews, Aqueous two-phase systems for protein separation: Phase separation and applications, *J. Chromatogr. A* 1238 (May 2012) 1–10, <https://doi.org/10.1016/j.chroma.2012.03.049>.
- [22] L. McQueen, D. Lai, Ionic Liquid Aqueous Two-Phase Systems From a Pharmaceutical Perspective, *Front. Chem.* 7 (Mar. 2019), <https://doi.org/10.3389/fchem.2019.00135>.
- [23] S. Shahriari, L.C. Tomé, J.M.M. Araújo, L.P.N. Rebelo, J.A.P. Coutinho, I. M. Marrucho, M.G. Freire, Aqueous biphasic systems: a benign route using cholinium-based ionic liquids, *RSC Adv.* 3 (6) (2013) 1835–1843, <https://doi.org/10.1039/C2RA22972B>.
- [24] M.V. Quental, H. Passos, K.A. Kurnia, J.A.P. Coutinho, M.G. Freire, Aqueous Biphasic Systems Composed of Ionic Liquids and Acetate-Based Salts: Phase Diagrams, Densities, and Viscosities, *J. Chem. Eng. Data* 60 (6) (Jun. 2015) 1674–1682, <https://doi.org/10.1021/je501044u>.
- [25] Y.-V. Phakoukaki, P. O'Shaughnessy, P. Angeli, Flow patterns of ionic liquid based aqueous biphasic systems in small channels, *Chem. Eng. Sci.* 265 (Jan. 2023), 118197, <https://doi.org/10.1016/j.ces.2022.118197>.
- [26] P. Berton, H. Tian, R.D. Rogers, Phase Behavior of Aqueous Biphasic Systems with Choline Alkanoate Ionic Liquids and Phosphate Solutions: The Influence of pH, *Molecules* 26 (6) (Mar. 2021) 1702, <https://doi.org/10.3390/molecules26061702>.
- [27] A.R. Titus, P.P. Madeira, L.A. Ferreira, V.Y. Chernyak, V.N. Uversky, B. Y. Zaslavsky, Mechanism of Phase Separation in Aqueous Two-Phase Systems, *Int. J. Mol. Sci.* 23 (22) (Nov. 2022) 14366, <https://doi.org/10.3390/ijms232214366>.
- [28] J.A. Asenjo, B.A. Andrews, Aqueous two-phase systems for protein separation: A perspective, *J. Chromatogr. A* 1218 (49) (Dec. 2011) 8826–8835, <https://doi.org/10.1016/j.chroma.2011.06.051>.
- [29] J.A. Asenjo, S.L. Mistry, B.A. Andrews, J.C. Merchuk, Phase separation rates of aqueous two-phase systems: Correlation with system properties, *Biotechnol. Bioeng.* 79 (2) (Jul. 2002) 217–223, <https://doi.org/10.1002/bit.10273>.
- [30] M.H. Salamanca, J.C. Merchuk, B.A. Andrews, J.A. Asenjo, On the kinetics of phase separation in aqueous two-phase systems, *J. Chromatogr. B Biomed. Sci. Appl.* 711 (1–2) (Jun. 1998) 319–329, [https://doi.org/10.1016/S0378-4347\(98\)00173-X](https://doi.org/10.1016/S0378-4347(98)00173-X).
- [31] T. Abolghasembeyk, S.h. Shahriari, M. Salehifar, Extraction of stevioside using aqueous two-phase systems formed by choline chloride and K_2PO_4 , *Food Bioprod. Process.* 102 (Mar. 2017) 107–115, <https://doi.org/10.1016/j.fbp.2016.12.011>.
- [32] K. Radošević, M. Cvjetko Bubalo, V. Gaurina Srček, D. Grgas, T. Landeka Dragičević, I. Radojčić Redovniković, Evaluation of toxicity and biodegradability of choline chloride based deep eutectic solvents, *Ecotoxicol. Environ. Saf.* 112 (Feb. 2015) 46–53, <https://doi.org/10.1016/j.ecoenv.2014.09.034>.
- [33] S.H. Zeisel, K.-A. da Costa, Choline: an essential nutrient for public health, *Nutr. Rev.* 67 (11) (Nov. 2009) 615–623, <https://doi.org/10.1111/j.1753-4887.2009.00246.x>.
- [34] P. Panas, C. Lopes, M.O. Cerri, S.P.M. Ventura, V.C. Santos-Ebinuma, J.F.B. Pereira, Purification of clavulanic acid produced by *Streptomyces clavuligerus* via submerged fermentation using polyethylene glycol/cholinium chloride aqueous two-phase systems, *Fluid Phase Equilib.* 450 (Oct. 2017) 42–50, <https://doi.org/10.1016/j.fluid.2017.07.005>.
- [35] N.v. dos Santos, M. Martins, V.C. Santos-Ebinuma, S.P.M. Ventura, J.A.P. Coutinho, S.R. Valentini, J.F.B. Pereira, Aqueous Biphasic Systems Composed of Cholinium Chloride and Polymers as Effective Platforms for the Purification of Recombinant Green Fluorescent Protein, *ACS Sustain. Chem. Eng.* Jul. 2018 6(7) 9383–9393, <https://doi.org/10.1021/acssuschemeng.8b01730>.
- [36] X. Liu, Z. Li, Y. Pei, H. Wang, J. Wang, (Liquid+liquid) equilibria for (cholinium-based ionic liquids+polymers) aqueous two-phase systems, *J. Chem. Thermodyn.* 60 (May 2013) 1–8, <https://doi.org/10.1016/j.jct.2013.01.002>.

- [37] J.D. Berry, M.J. Neeson, R.R. Dagastine, D.Y.C. Chan, R.F. Tabor, Measurement of surface and interfacial tension using pendant drop tensiometry, *J. Colloid Interface Sci.* 454 (Sep. 2015) 226–237, <https://doi.org/10.1016/j.jcis.2015.05.012>.
- [38] A.K. Smith, J.H. Freed, Determination of Tie-Line Fields for Coexisting Lipid Phases: An ESR Study, *J. Phys. Chem. B* 113 (12) (Mar. 2009) 3957–3971, <https://doi.org/10.1021/jp808412x>.
- [39] B. Kronberg, K. Holmberg, B. Lindman, Surface and Interfacial Tension, in: *Surface Chemistry of Surfactants and Polymers*, Chichester, UK: John Wiley & Sons, Ltd, 2014, pp. 231–249, <https://doi.org/10.1002/9781118695968.ch12>.
- [40] H. Hustedt, K.H. Kroner, M.-R. Kula, Applications of Phase Partitioning in Biotechnology, in *Partitioning in Aqueous Two-Phase System*, Elsevier, 1985, pp. 529–587, <https://doi.org/10.1016/B978-0-12-733860-6.50022-0>.
- [41] F. Bezold, J. Goll, M. Minceva, Study of the applicability of non-conventional aqueous two-phase systems in counter-current and centrifugal partition chromatography, *J. Chromatogr. A* 1388 (Apr. 2015) 126–132, <https://doi.org/10.1016/j.chroma.2015.02.021>.
- [42] G. Job, F. Herrmann, Chemical potential—a quantity in search of recognition, *Eur. J. Phys.* 27 (2) (Mar. 2006) 353–371, <https://doi.org/10.1088/0143-0807/27/2/018>.