

Ionic liquid-based three phase partitioning (ILTPP) systems: Ionic liquid recovery and recycling

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ABSTRACT

Ionic liquid-based three phase partitioning (ILTPP) is a promising technique to recover lactoferrin, a high-added value whey protein, because it combines the advantages associated with the use of ionic liquids and the feasibility of the product recovery characteristic of three phase partitioning. The recyclability of the ionic liquid is essential for the feasibility and development of ILTPP technique, due to the high cost and environmental impact associated with the discharge of this type of compounds. For this purpose, the thermodynamic characterization of the systems of interest for ILTPP, which are based on the use of 1-butyl-3-methylimidazolium trifluoromethanesulfonate (BmimTfO) and sodium dihydrogenophosphate (NaH_2PO_4), is here performed to determine the composition of the liquid phases in equilibrium and the distribution coefficient between them. In addition, the fraction of ionic liquid that cannot be reused in the ILTPP process has been assessed, concluding that the recyclability of this compound is highly dependent on the protein concentration in the feed stream. At high protein concentrations (2 g L^{-1}) and moderate ILTPP efficiencies (around 80%) it is possible to recycle more than 99% of the ionic liquid, which improves both the economic and environmental performance of the ILTPP process.

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1. Introduction

The separation and purification of proteins is a bottleneck in the biotechnology field because traditional protein purification techniques (e.g. electrophoresis, ion-exchange chromatography, affinity chromatography or ammonium sulfate precipitation) are time and cost consuming [1,2]. Other techniques such as liquid–liquid extraction with hydrophobic ionic liquids show very promising results, but they may produce conformational changes in proteins that prevent their back-extraction [3]. In this way, a novel technique called ionic liquid-based three phase partitioning (ILTPP) has been proposed to recover proteins from waste streams. This technique leads to the interfacial partitioning of the target protein to recover it by means of ionic liquid/salt systems; it combines the results characteristic of three phase partitioning (TPP) with the advantages associated with ionic liquid-based aqueous two phase systems (ILATPS).

TPP is an emerging technique for protein separation that involves the accumulation of the target protein at the liquid–liquid interface between an organic phase, usually *t*-butanol, and an

aqueous solution of ammonium sulfate [4,5]. It can be applied to purify and concentrate proteins, commonly as a one-step purification protocol [6], and it stands out for being simple, inexpensive, scalable and a rapid procedure that may lead to purifications greater than 100-fold, 70–85% of final purity and at least a 50-fold decrease in volume [7,8]. Nevertheless, the main drawback of TPP is the use of a volatile organic solvent such as *t*-butanol which may limit the large-scale use of this technique [4].

Regarding ILATPS, they are usually formed by a hydrophilic ionic liquid and a salt, which are mutually incompatible though both miscible in water so that two aqueous phases are obtained (an ionic liquid-rich phase and a salt-rich phase) [9,10]. They have been considered an alternative to biomolecule extraction processes due to their relevant advantages: gentle biocompatible environment, high extraction efficiency, relatively low viscosity, quick phase separation, no need of using volatile organic solvents and the high control of the phases' polarities due to the vast number of ionic liquids available [11–13]. Nevertheless, most studies concerning ILATPS do not address the biomolecule recovery [14], which represents the major obstacle to assure the process feasibility [3]. Moreover, the difficulty in recycling ionic liquids and the environmental problems associated with effluent streams with high concentration of inorganic salts are also important concerns about this technique [15,16].

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Nomenclature

A	adjusted parameter
B	adjusted parameter
C	adjusted parameter
D	distribution coefficient of the ionic liquid between the two liquid phases
k	conductivity (S m^{-1})
k_1	adjusted parameter
k_2	adjusted parameter
LF_i	fraction of lactoferrin that can be recovered at the liquid–liquid interface
LF_S	fraction of lactoferrin that remains in the salt-rich phase
m_F	mass fraction of the feed stream
m_S^A	mass fraction of the overall system constituted by the salt added to the system so that the salt concentration remains constant
m_S^R	mass fraction of the overall system constituted by the salt-rich phase which is recycled
m_1	adjusted parameter
n_1	adjusted parameter
p	pressure (MPa)
R	fraction of ionic liquid recycled
T	temperature (K)
$[X]_i$	mass fraction, expressed as percentage (%), of the component X in the phase/stream i , where:

X can be:

IL	ionic liquid
LF	lactoferrin
S	salt

i can be:

F	feed stream
IL	ionic liquid-rich phase
M	mixture ($IL + S$)
S	salt-rich phase

Greek letters

α	mass fraction of the ionic liquid-rich phase
μ	dynamic viscosity (Pa s)
ρ	density (kg m^{-3})
$1-\alpha$	mass fraction of the salt-rich phase

ILTPP combines the advantages of both TPP and ILATPS, overcoming the main disadvantages of these techniques. It does not use volatile organic solvents and the target protein is accumulated at the interface, so back extraction steps are not required. ILTPP has been applied to the recovery of lactoferrin (LF) [17,18], a bovine whey protein that stands out because of its important biological functions [19]. The system based on BmimTfO and NaH_2PO_4 shows very promising results, since it combines high LF recoveries (between 83% and more than 98%), the use of stable ionic liquids such as BmimTfO and, under certain experimental conditions, the re-dissolution of the protein [18]. However, despite these promising results, the study of the fraction of the ionic liquid that can be reused after each cycle of the process has not been carried out, which is essential to minimize both economic and environmental impacts of this technique and is one of the concerns related to the use of ionic liquid-based systems. Furthermore, it should be taken into account that the toxicity of many ionic liquids such as BmimTfO is somewhat higher than some conventional volatile organic solvents (e.g. *t*-butanol) [20–22], which reinforces the importance of

their recyclability, since they must not be present in waste streams that are discharged into the environment.

The aim of this work is the study of the recyclability of the ionic liquid used in the ILTPP technique. For this purpose, the characterization of the thermodynamic equilibrium of the principal components of ILTPP (ionic liquid, salt and water) has been carried out. In this way, the objective of the present work is completely different from the aim of previous references about ILTPP. Following a process engineering approach, the first step in the development of ILTPP is the identification of suitable ionic liquid/salt systems and conditions to achieve the recovery of the target protein (LF), which has been reported by previous works of Alvarez-Guerra and Irabien [17,18]. Therefore, whereas previous studies about ILTPP are focused on the protein recovery, the present work is centered in the ionic liquid recyclability of the ionic liquid/salt systems of interest for the performance of ILTPP. Furthermore, the conclusions of this work are also valid for ILATPS if efficient solute recovery strategies are to be developed, since ILTPP and ILATPS are based on the same thermodynamic systems. Moreover, in contrast to the few previous studies about the recovery of the ionic liquid of ILATPS that are only based on experimental data of the thermodynamic equilibrium [23–26], the ILTPP technique applied to the LF recovery is modeled so that operational variables and constraints characteristic of this process can be taken into account in the analysis. This will make it possible to identify the operating conditions which minimize the ionic liquid losses in the process.

2. Materials and methods

2.1. Materials

The ionic liquid used in the present work, 1-butyl-3-methylimidazolium trifluoromethanesulfonate (BmimTfO) (99%), was purchased from IoLiTec (Ionic Liquid Technologies, Germany). Panreac Química (Spain) supplied the reagents to prepare the salt fraction of the system: sodium dihydrogenophosphate, NaH_2PO_4 (122018); and sodium hydroxide, NaOH (131687). The water used in the ILTPP systems was obtained by means of double distillation, reverse osmosis and, eventually, its treatment with Milli-Q plus 185 water purification equipment. Detailed information about the compounds used in this work can be found in Table S1 as Supplementary data.

2.2. Methods

2.2.1. Characterization of the thermodynamic equilibrium

The characterization of the thermodynamic equilibrium of the ILTPP systems implies the determination of their binodal curves and the tie-lines in the region of compositions of interest. The binodal curves were determined at 298 ± 1 K and atmospheric pressure by means of the cloud-point titration method, widely described in literature [27–29]. For this purpose, aqueous solutions containing 45 wt% of NaH_2PO_4 and 42 wt% of $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (prepared with a $\text{NaH}_2\text{PO}_4:\text{NaOH}$ mass ratio equal to 18) were used. Aqueous solutions containing 30 wt% of BmimTfO were added to the salt solution to obtain the region of the binodal curve at high salt mass fractions. However, the salt solutions were added to concentrated BmimTfO solutions (80 wt%) for the determination of the binodal curves at high ionic liquid mass fractions. The concentration of the experimental points of the binodal curve was determined by the weight quantification of the system components within $\pm 10^{-4}$ g with a Mettler Toledo Excellence XS205 Dual Range analytical balance.

The tie-lines were determined by the gravimetric method previously reported by Merchuk et al. [30]. The experimental details

for the measurement of the tie-lines are described in the previous work of Neves et al. [23], with the unique exception that in the present work, centrifugation at 3000 rpm during 5 min with a Centrifuge 5804 from Eppendorf was performed to promote the phase separation before the system equilibration. In addition, it should be specified that in all the experiments the top phase corresponds to the ionic liquid-rich phase whereas the bottom phase is constituted essentially by the inorganic salt. In the ternary diagram, the critical point was also determined with the same procedure reported in cited Ref. [23].

The ionic liquid content of the experimental points of the binodal curves with the highest salt concentrations is extremely low, so even small absolute errors may imply very notable relative errors. It should be noticed that this region of the ternary diagram is essential for the analysis of the ionic liquid recyclability. For this reason, a detailed study with a higher precision was carried out, in which the ionic liquid mass fraction of the salt-rich phase of the tie-lines was measured by means of UV-spectroscopy at 211 nm with a Shimadzu UV-1700 Pharma-Spec Spectrophotometer. To avoid any possible interference, blank solutions containing the same salt concentration of the bottom phases were used.

2.2.2. Properties of the ILTPP systems

The pH, conductivity, density and dynamic viscosity of both salt- and ionic liquid-rich phases of the ILTPP systems were measured. The BmimTfO/NaH₂PO₄ system is composed of 23.3 wt% of ionic liquid and 26.3 wt% of salt, whereas the BmimTfO/(NaH₂PO₄/Na₂HPO₄) system is formed by 24.6 wt% of ionic liquid and 22.0 wt% of salt. The pH and the conductivity were measured at 298.0 ± 0.1 K with a Mettler Toledo S47 SevenMulti™ dual meter pH/conductivity equipment. Buffer solutions with pH values equal to 4.00 and 7.00 were required to calibrate the pH meter, whereas the conductivity scale was calibrated with KCl aqueous solutions. As the conductivity of the samples was out of the range for which the conductivity meter was calibrated, 500-fold dilutions of all the samples were carried out. Regarding the density and viscosity, they were determined at 298.00 ± 0.02 K by means of an automated SVM 3000 Anton Paar rotational Stabinger viscometer–densimeter [23].

3. Results and discussion

3.1. Characterization of the thermodynamic equilibrium of ILTPP systems

The ionic liquid-based three phase partitioning (ILTPP) technique has demonstrated that can recover most of the lactoferrin (LF) contained in aqueous solutions. Specifically, two systems have shown very promising results for the recovery of this protein [18]. Both systems are based on the use of BmimTfO as ionic liquid and NaH₂PO₄ as salt. The first system, denoted as BmimTfO/NaH₂PO₄, is composed of 23.3 wt% of ionic liquid and 26.3 wt% of salt (the rest of the system is composed of water). The second system, denoted as BmimTfO/(NaH₂PO₄/Na₂HPO₄), is very similar to the previous, with the exception that a small amount of NaOH is added: the NaH₂PO₄:NaOH mass ratio is equal to 18. This NaH₂PO₄:NaOH mass ratio is kept constant during all the experiments. Even though two species of salt coexist in this second system (NaH₂PO₄ and Na₂HPO₄), the composition of the system will be described by means of the total ionic liquid and salt mass concentrations because the system composition is perfectly described if this constant NaH₂PO₄:NaOH ratio is taken into account. This BmimTfO/(NaH₂PO₄/Na₂HPO₄) system is composed of 24.6 wt% of ionic liquid and 22.0 wt% of salt so that the ionic

strength due to the inorganic salt is kept constant with respect to the BmimTfO/NaH₂PO₄ system.

Table 1 reports the pH, conductivity, density and dynamic viscosity of both salt- and ionic liquid-rich phases of the previously described systems to characterize the most relevant properties for the ILTPP process. As can be seen, the pH of both phases of the BmimTfO/(NaH₂PO₄/Na₂HPO₄) system is higher than the pH of the phases derived from the BmimTfO/NaH₂PO₄ system, as expected. However, their conductivities are very similar, which implies that the ionic strength of both systems is also similar. Therefore, ionic strength, which is a variable that has influence on the protein structure [3], should not be used to explain the differences in the ILTPP performance obtained with the two systems. Regarding the density and viscosity, the values reported in Table 1 are in the range of the densities and viscosities of other BmimTfO-based ILATPS reported in literature [23]. Furthermore, all these viscosities are considerably lower than those exhibited by typical polymer-rich phases of conventional polymer-based ATPS (around 0.04 Pa s), which reinforces the advantages associated with the use of ionic liquids in aqueous biphasic systems [9].

The assessment of the fraction of the ionic liquid which can be recycled, *R*, in the previous systems that lead to promising results in the ILTPP performance, involves the determination of the ionic liquid and salt concentrations in both phases as well as the mass fraction that each liquid phase represents, as can be seen in Section 3.2. For this purpose, the ternary phase diagrams of these systems should be determined, which implies that the binodal curve and the tie-lines in the region of compositions that are relevant for the ILTPP process ought to be known.

Fig. 1 shows the solubility phase diagrams, in which the binodal curve and several tie-lines are plotted for the two ILTPP systems that lead to successful LF recoveries. The concentrations of the components are reported in mass fraction expressed as percentage. The mixture points that are used to build the tie-lines correspond to the compositions that are obtained by addition of pure salt (NaH₂PO₄ or NaH₂PO₄/Na₂HPO₄) to the systems under study, whose tie-lines are also determined. The addition of salt to increase its mass fraction is one of the alternatives that are usually considered to reduce the ionic liquid that is contained in the salt-rich phase, which may reduce the amount of ionic liquid that cannot be recycled in the process [24]. Furthermore, an additional point for a lower salt concentration is also measured for each system to broaden the range of studied compositions and, in this way, assure the validity of the relationships described below. This point at lower salt concentration is obtained by reducing the salt added to the system, when compared with the other systems of interest studied.

The conventional way of describing the equilibria of ionic liquid/salt systems implies the simultaneous solution of a non-linear system of four equations based on the binodal curve (Eqs. (1) and (2)), the lever rule (3) and the line used to determine the critical point of the system (4) [9,23,30]:

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

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

$$\frac{[IL]_M - [IL]_{IL}}{[S]_M - [S]_{IL}} = \frac{[IL]_S - [IL]_M}{[S]_S - [S]_M} \quad (3)$$

$$[IL]_{IL} = m_1[S]_S + n_1 \quad (4)$$

where $[IL]$ and $[S]$ are the ionic liquid and salt mass fractions expressed as percentages; the subscripts S, IL and M refer the compositions to the salt-rich phase, ionic liquid-rich phase and the mixture point, respectively; and *A*, *B*, *C*, *m*₁ and *n*₁ are adjusted parameters. It should be noted that the equation of the binodal curve is the same in Eqs. (1) and (2), but it has to be applied twice

Table 1
Properties at the temperature $T=298$ K and at the pressure $p=0.1$ MPa of the salt and ionic liquid-rich phases formed from the mixture points of the BmimTfO/ NaH_2PO_4 and BmimTfO/ $(\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4)$ systems that are interesting for the lactoferrin recovery by means of ILTPP.

Property	BmimTfO/ NaH_2PO_4 ($[\text{IL}]_{\text{M}}, [\text{S}]_{\text{M}} = (23.3, 26.3)$)		BmimTfO/ $(\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4)$ ($[\text{IL}]_{\text{M}}, [\text{S}]_{\text{M}} = (24.6, 22.0)^{\text{a}}$)	
	IL-rich phase	Salt-rich phase	IL-rich phase	Salt-rich phase
pH ^b	4.051	4.106	5.227	4.961
k (S m^{-1}) ^c	22.048	25.023	21.318	25.574
ρ (kg m^{-3}) ^d	1241.9	1307.3	1231.5	1275.3
μ (Pa s) ^e	9.4693×10^{-3}	6.2730×10^{-3}	8.4159×10^{-3}	5.0677×10^{-3}

^a $[\text{NaH}_2\text{PO}_4]_{\text{M}} = 17.8$ (wt%); $[\text{Na}_2\text{HPO}_4]_{\text{M}} = 4.2$ (wt%).

^b Standard uncertainties u in the determination of pH are $u(\text{pH}) = 0.001$, $u(T) = 0.1$ K, and $u(p) = 0.01$ MPa.

^c Standard uncertainties u in the determination of k are $u(k) = 0.001$ S m^{-1} , $u(T) = 0.1$ K, and $u(p) = 0.01$ MPa.

^d Standard uncertainties u in the determination of ρ are $u(\rho) = 0.1$ kg m^{-3} , $u(T) = 0.02$ K, and $u(p) = 0.01$ MPa.

^e Standard uncertainties u in the determination of μ are $u(\mu) = 10^{-7}$ Pa s, $u(T) = 0.02$ K, and $u(p) = 0.01$ MPa.

to establish the relationship between the ionic liquid and salt in both phases (ionic liquid- and salt-rich phases).

The values of the adjusted parameters (A , B and C) used to model the binodal curve in the two systems are reported in Table 2. The quality of the adjustment that provides the equation of the binodal curve is notably high, since the value of R^2 is higher than 0.99 in both cases. The values of A and B are very similar for the two systems, whereas C is significantly different

(almost 50% higher in the BmimTfO/ NaH_2PO_4 system). This difference in the values of adjusted parameters implies that the BmimTfO/ $(\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4)$ system leads to a larger biphasic region since the binodal curve is closer to the axis of the diagram (regardless of the compositions being expressed in mass fraction or molality), which means that the system of higher pH has a stronger ability to induce the formation of two separated phases. It should be noted that the same trend is reported by Shahriari et al. [31], since the binodal curves of both BmimTfO/ NaH_2PO_4 and BmimTfO/ Na_2HPO_4 were also assessed and the first system also shows a smaller biphasic region. This behavior is also in agreement with the general trend observed for the effect of phosphate salts on the formation of ILATPS [32]. Despite the fact that the binodal curve of BmimTfO/ NaH_2PO_4 has already been studied in the mentioned reference, the experimental points used to determine it only contain a maximum of 13% of salt content, which is considerably lower than the region of salt concentrations that is of interest for the ILTPP process. In this way, even though the binodal curves of the system BmimTfO/ NaH_2PO_4 are similar in both works in terms of adjusted parameter values (Shahriari et al. [31] and the present work), the small differences detected should be attributed to the considerably wider range of compositions of the experimental points with which the binodal curve is built in the present work.

As can be seen in Fig. 1, the concentration of ionic liquid in the salt-rich phase is very low in both systems, because most of BmimTfO is present in the ionic liquid-rich phase at very high mass fractions in all cases. Due to this very low concentration of BmimTfO in the bottom phase, even low absolute errors in the description of this region of the binodal curve may lead to very significant relative errors in the results. For this reason a detailed study of this part of the binodal curve is carried out. For that purpose, the ionic liquid content of the bottom phases derived from the tie-lines is determined by the measurement of the absorbance at 211 nm of these bottom phases, since the absorbance of BmimTfO exhibits a maximum at this wavelength. According to the experimental results, the following relationship is proposed to describe the mass fraction of BmimTfO in the salt-rich phase:

$$[\text{IL}]_{\text{S}} = k_1 \times \exp(k_2[\text{S}]_{\text{S}}) \quad (5)$$

Table 2

Adjusted parameters of the binodal curve at 298 ± 1 K and at 0.10 ± 0.01 MPa for the BmimTfO/ NaH_2PO_4 and BmimTfO/ $(\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4)$ systems when the mass fractions are expressed as percentage (confidence level at 95%).

Parameter/coefficient	BmimTfO/ NaH_2PO_4	BmimTfO/ $(\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4)$
A	148.1 ± 4.8	150.9 ± 4.2
B	-0.7180 ± 0.0177	-0.7703 ± 0.0149
C	$(7.598 \pm 2.716) \times 10^{-5}$	$(5.284 \pm 2.015) \times 10^{-5}$
R^2	0.9909	0.9936

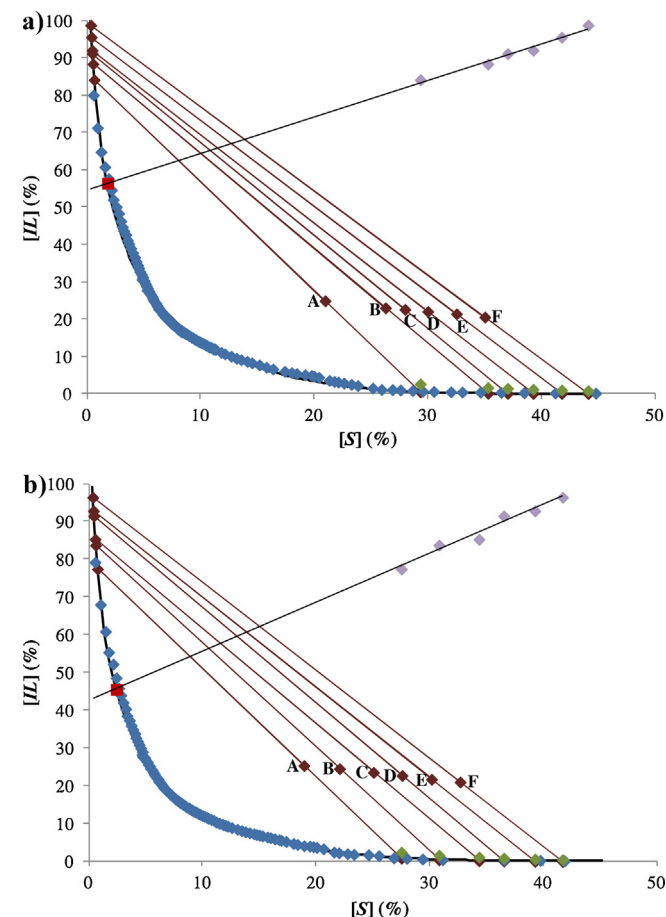


Fig. 1. Ternary phase diagrams at 298 ± 1 K and at 0.10 ± 0.01 MPa for the following systems: (a) BmimTfO/ NaH_2PO_4 ; (b) BmimTfO/ $(\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4)$. Notation: \blacklozenge Experimental binodal curve; \blacklozenge Tie lines; \blacklozenge Experimental points of the binodal curve at high salt mass fractions measured by means of UV absorbance; \blacksquare Critical point; \blacklozenge ($[\text{S}]_{\text{S}}, [\text{IL}]_{\text{S}}$) points used to determine the critical point; — Fitted binodal curve according to Eqs. (1) and (2) (values of adjusted parameters can be found in Table 2); — Fitted curve of ($[\text{S}]_{\text{S}}, [\text{IL}]_{\text{S}}$) points according to Eq. (4) (values of adjusted parameters can be found in Table 3). Detailed experimental data of the binodal curve (Tables S2 and S3) and the tie lines, (A)–(F) (Tables S4 and S5), can be found as Supplementary data.

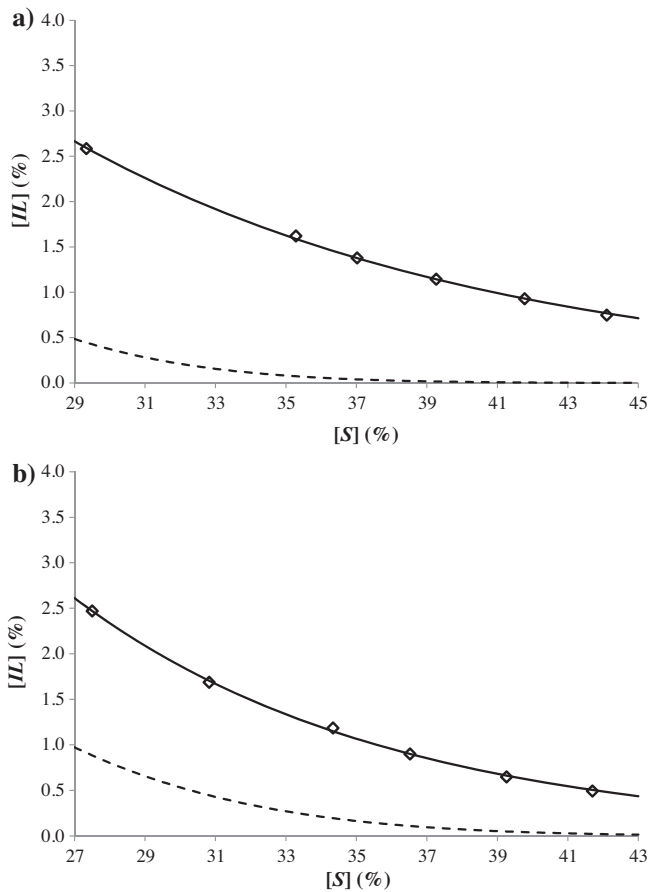


Fig. 2. Region of the binodal curve at very high salt mass fractions for the (a) BmimTfO/NaH₂PO₄ and (b) BmimTfO/(NaH₂PO₄/Na₂HPO₄) systems. (◆) Experimental points measured by means of absorbance; (—) binodal curve predicted by Eq. (5); (---) binodal curve predicted by Eq. (2).

where $[IL]_S$ and $[S]_S$ are the ionic liquid and salt mass fractions in the salt-rich phase, and k_1 and k_2 are adjusted parameters. Table 3 contains the values of k_1 and k_2 for both systems when the mass fractions are expressed as percentage and it reflects the perfect agreement between the experimental data and Eq. (5) ($R^2 > 0.999$). This equation can be considered as a simplification of the equation that models the complete binodal curve (Eq. (1)), and it is only valid

Table 3

Values of the adjusted parameters when the mass fractions are expressed as percentage and R^2 of Eqs. (4) and (5) for both BmimTfO/NaH₂PO₄ and BmimTfO/(NaH₂PO₄/Na₂HPO₄) systems (confidence level at 95%).

Parameter/coefficient	BmimTfO/NaH ₂ PO ₄	BmimTfO/ (NaH ₂ PO ₄ /Na ₂ HPO ₄)
	Eq. (4): $[IL]_{IL} = m_1[S]_S + n_1$	
m_1	0.9789 ± 0.2153	1.299 ± 0.336
n_1	54.65 ± 8.21	42.44 ± 11.87
R^2	0.9755	0.9665
	Eq. (5): $[IL]_S = k_1 \times \exp(k_2[S]_S)$	
k_1	29.08 ± 2.98	53.62 ± 7.72
k_2	-0.0824 ± 0.0030	-0.1119 ± 0.0047
R^2	0.9993	0.9993

for the description of the salt-rich phase at very high salt mass fractions (the region of compositions that are interesting for the ILTPP process). Fig. 2 shows the experimental points at high salt concentrations of the binodal curve measured by means of absorbance and the binodal curves predicted by Eqs. (1) and (5). It can be concluded that Eq. (1) clearly underestimates the ionic liquid content in the salt-rich phase, in contrast to the good agreement of Eq. (5). Even though the absolute errors in $[IL]_S$ introduced by Eq. (1) are relatively low (between 0.5 and 2%), the associated relative errors are considerably higher than 100% and cannot be accepted in calculations in which the mentioned variable is involved. It should be noted that $[IL]_S$ is the key variable to assess the losses of ionic liquid in the process, because the salt-rich phase may not be recycled and the ionic liquid contained in this phase may not be reused (Section 3.2). For this reason, Eq. (1) is replaced by Eq. (5) in the determination of the composition of both phases.

The critical point of each system, which is defined by the intersection of Eq. (4) and the binodal curve, is also drawn in the phase diagram of Fig. 1, because it is required to determine the composition of new tie-lines, so the relationship between $[IL]_{IL}$ and $[S]_S$ stated by Eq. (4) must be known. The values of m_1 and n_1 , which are the adjusted parameters of the mentioned equation, are contained in Table 3. Despite the sensitivity to the experimental errors associated with the determination of the tie lines, the values of R^2 are relatively high so these adjustments can successfully describe the critical point.

As a result, the salt- and ionic liquid-rich phase compositions obtained from any mixture point that belongs to the region of the ternary diagram interesting for the ILTPP performance can be calculated with Eqs. (2)–(5), as can be seen in Fig. 3. This thermodynamic equilibrium characterization is required to assess the fraction of

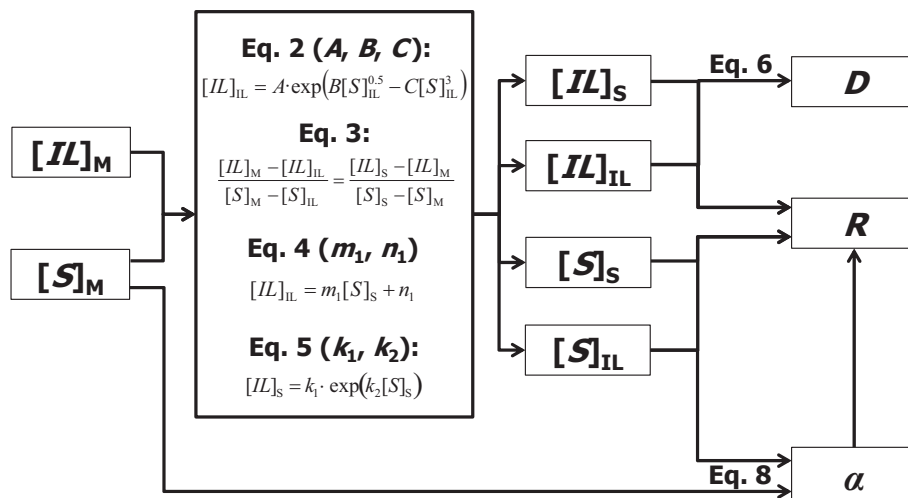


Fig. 3. Characterization of the thermodynamic equilibrium of the ionic liquid/salt systems used in the ILTPP systems.

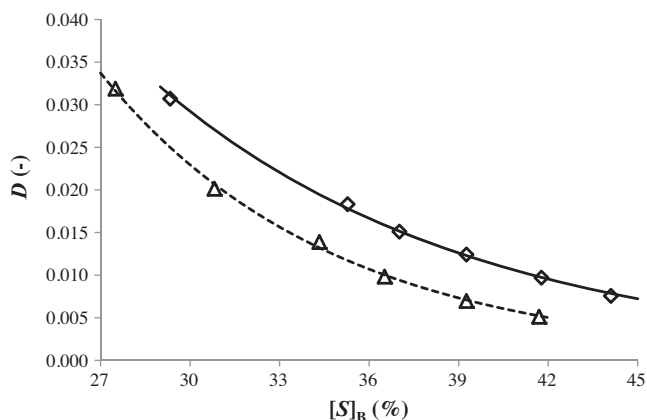


Fig. 4. Distribution coefficient, D , vs. salt mass fraction in the salt-rich phase, $[S]_B$ (%). (\diamond) Experimental and (—) simulated data for the BmimTfO/NaH₂PO₄ system; (\triangle) experimental and (---) simulated data for the BmimTfO/(NaH₂PO₄/Na₂HPO₄) system.

ionic liquid used in the ILTPP technique that cannot be recovered. In a first approach, the distribution coefficient, D , of the ionic liquid between the two liquid phases can be used to measure the relative importance of the ionic liquid that may not be reused:

$$D = \frac{[IL]_S}{[IL]_{IL}} \quad (6)$$

The ionic liquid present in the salt-rich phase can be considered as the losses of this component from the ionic liquid-rich phase that are determined by the thermodynamic equilibrium. As a result, the lower the D , the lower the concentration of ionic liquid in the salt-rich phase, which implies that the equilibrium favors the ionic liquid recyclability since the relative importance of $[IL]_{IL}$ with respect to $[IL]_S$ increases.

Fig. 4 shows the experimental and simulated values of D with respect to the salt mass fraction in the salt-rich phase, $[S]_S$. For a given value of $[S]_S$, the simulated D is obtained by means of Eqs. (4) and (5) with which $[IL]_{IL}$ and $[IL]_S$ can be calculated, as Fig. 3 states. The relatively high quality of the adjustment of these equations can be appreciated in Fig. 4, where experimental and simulated data are almost identical. As can be seen, the distribution coefficient decreases notably with $[S]_S$ because reductions in D of around 80% are obtained in the range of values of $[S]_S$ that are interesting for the performance of ILTPP. This conclusion is in agreement with previous studies that suggest an increase of the salt concentration for the improvement of the ionic liquid recyclability [24,25]. Therefore, D is useful to describe the thermodynamic equilibrium when the reuse of the ionic liquid is the aim of the study, since it provides a clear idea about how favorable is this equilibrium to that purpose.

Nevertheless, thermodynamics must be coupled with the mass balances of the process and its operating constraints to provide a precise assessment of the fraction of ionic liquid that can be recovered. This study is carried out hereafter in Section 3.2.

3.2. Assessment of ionic liquid recyclability in ILTPP systems

The analysis of the ionic liquid recyclability should be carried out in terms of the amount of ionic liquid that cannot be reused after each cycle of the ILTPP technique, which is represented in Fig. 5. According to this figure, all the ionic liquid-rich phase is recycled, because most of it is present in this phase, so this action guarantees a high recovery of this component. However, only a fraction of the salt-rich phase can be recycled so that the protein feed stream and the salt lost in the cycle can be added to the system without suffering from an increase in its total mass. The fraction that the

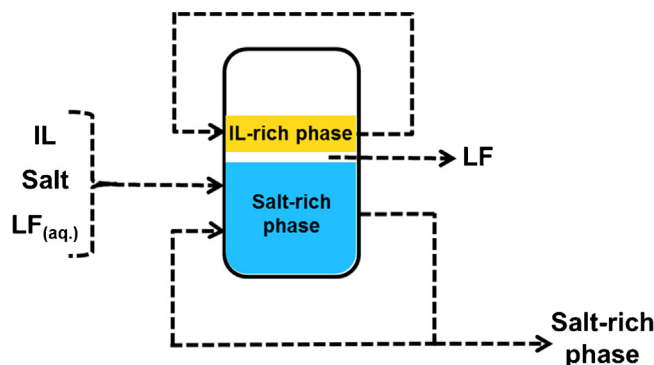


Fig. 5. Schematic diagram of the ILTPP process.

protein feed stream represents in the whole system depends on two variables: the protein concentration in the feed stream and the efficiency of the ILTPP, which is measured by the amount of protein (LF) that is recovered at the liquid–liquid interface, LF_1 . The mean concentration of LF in the bovine whey is equal to 100 mg L⁻¹, but ultrafiltration membranes can be used to reach more than 20-fold concentration increases of whey proteins [33]. As a result, the maximum LF concentration that is considered in the feed solution for the analysis is 2000 mg L⁻¹. The LF content in the overall system is kept constant at a mass fraction equal to 1.6×10^{-4} , since this is the concentration assumed as reference in previous works [17,18].

For the modeling of the ionic liquid recycled, three main assumptions are considered:

- LF present at the ionic liquid-rich phase can be considered negligible, according to previous experimental results [18].
- Considering the low protein mass fraction (1.6×10^{-4}) and that the ionic liquid recyclability takes place in the ILTPP process once most of the protein has been recovered at the interface, LF does not have any impact on the reuse of the reagents.
- The amount of ionic liquid that has to be added to the system can be considered negligible, since the complete recirculation of the ionic liquid-rich phase assures that most of this component can be reused.

The fraction of the ionic liquid recovered after each cycle, R , is calculated by means of Eq. (7):

$$R = \frac{\alpha[IL]_{IL} + m_S^R[IL]_S}{\alpha[IL]_{IL} + (1 - \alpha)[IL]_S} \quad (7)$$

where m_S^R is the mass fraction of the overall system constituted by the salt-rich phase that is recycled and α is the mass fraction of the ionic liquid-rich phase with respect to the overall system, which can be calculated by means of the lever rule once the compositions of both phases have been determined (Fig. 3):

$$\alpha = \frac{[S]_S - [S]_M}{[S]_S - [S]_{IL}} \quad (8)$$

Regarding m_S^R , it is determined from the overall mass balance of the system:

$$m_S^R = 1 - \alpha - m_F - m_S^A \quad (9)$$

where m_F is the mass fraction of the feed stream and m_S^A is the mass fraction of the overall system constituted by the salt added to the system after each cycle. These variables make it possible to remain constant the concentrations of both protein and salt, and they are derived from the partial mass balances to these components:

$$[LF]_M = m_F[LF]_F + \left(\frac{m_S^R}{1 - \alpha} \right) LF_S \times [LF]_M \quad (10)$$

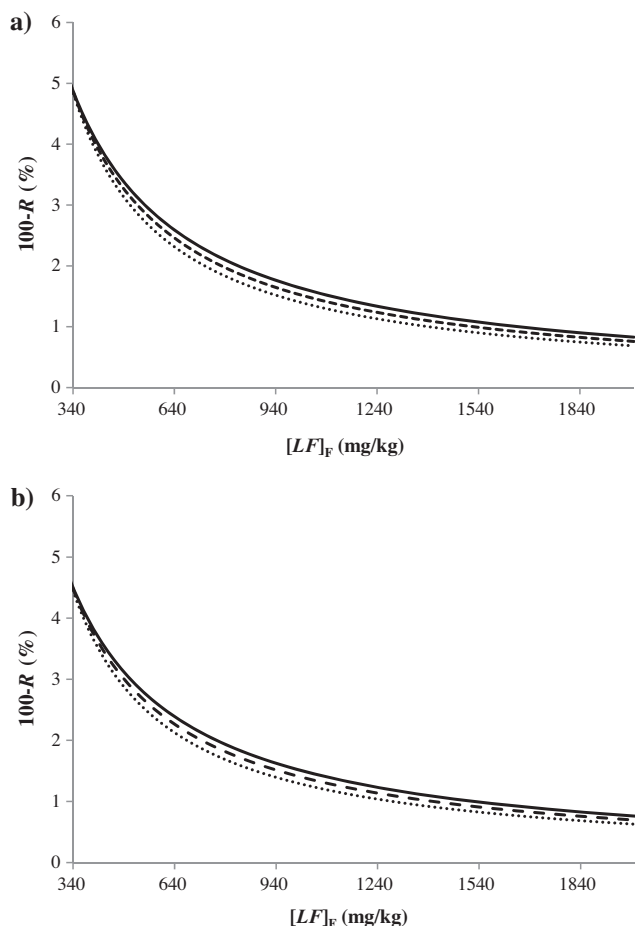


Fig. 6. Percentage of ionic liquid lost in each cycle, $100-R(\%)$, when no additional recovery steps are considered, at different LF_1 (— $LF_1=100\%$; --- $LF_1=90\%$; ... $LF_1=80\%$), for: (a) the BmimTfO/NaH₂PO₄ system; (b) BmimTfO/(NaH₂PO₄/Na₂HPO₄) system.

$$[S]_M = 100m_S^A + \alpha[S]_{IL} + m_S^R[S]_S \quad (11)$$

where $[LF]_M$ and $[LF]_F$ are the mass fractions of LF in the mixture point (overall system) and in the feed stream, respectively; LF_S is the fraction of LF that remains in the salt-rich phase; and $[S]_M$ is the mass fraction of salt in the mixture point. From Eqs. (9)–(11), m_F and m_S^A can be calculated as follows:

$$m_F = \frac{[LF]_M - LF_S \times [LF]_M + \frac{LF_S \times [LF]_M}{1-\alpha} \left[\frac{[S]_M - \alpha[S]_{IL} - (1-\alpha)[S]_S}{100 - [S]_S} \right]}{[LF]_F - \frac{LF_S \times [LF]_M}{1-\alpha} \left(1 + \frac{[S]_S}{100 - [S]_S} \right)} \quad (12)$$

$$m_S^A = \frac{[S]_M - \alpha[S]_{IL} - (1-\alpha - m_F)[S]_S}{100 - [S]_S} \quad (13)$$

The sum of the mass fractions related to the ionic liquid-rich phase, the feed stream and the salt added to the system must be at maximum equal to 1 (if the sum is equal to 1, it implies that no fraction of the salt-rich phase can be recycled), because otherwise the total mass of the system will increase in each cycle. This constraint is mathematically expressed as:

$$\alpha + m_F + m_S^A \leq 1 \quad (14)$$

Fig. 6 shows the fraction of ionic liquid that cannot be reused after each cycle, $100 - R$, expressed as percentage, for different values of LF_1 and for the both systems that lead to successful results with respect to LF recovery and that were described at the beginning of Section 3.1. It can be deduced from Fig. 6 that, the higher the

protein concentration in the feed stream, the lower the $100 - R(\%)$, because the feed stream required to reach the established LF concentration in the system decreases and, as a consequence, a higher fraction of the salt-rich phase can be recycled. If the salt-rich phase cannot be reused, the 4.97% and 4.71% of the ionic liquid present in the BmimTfO/NaH₂PO₄ and BmimTfO/(NaH₂PO₄/Na₂HPO₄) systems, respectively, are lost in each cycle. However, if a concentrated protein feed stream is used (e.g. 2000 mg L⁻¹), only 0.83% and 0.76% of the ionic liquid of these systems, respectively, cannot be recycled.

Regarding the influence of the ILTPP efficiency, the lower the LF_1 , the lower the $100 - R(\%)$, because lower values of LF_1 imply that the amount of LF contained in the salt-rich phase increases, so the feed stream mass required to keep constant the protein concentration is reduced. Therefore, the fraction of the salt-rich phase that can be recycled increases with the decrease of LF_1 .

Nevertheless, the effect of LF_1 is limited, especially at low protein concentrations in the feed stream, because at low values of $[LF]_F$ the fraction of the salt-rich phase recycled is very low, so the amount of protein recirculated with this phase is small. In the extreme case, $100 - R(\%)$ is independent on LF_1 at the protein concentration at which any fraction of the salt-rich phase is not recycled, so $\alpha + m_F + m_S^A = 1$. This LF concentration is equal to 333.8 mg L⁻¹ for the BmimTfO/NaH₂PO₄ system and 325.2 mg L⁻¹ for the BmimTfO/(NaH₂PO₄/Na₂HPO₄) system. Lower protein concentrations introduce such high water content to the system that the ionic liquid and salt mass fractions of the mixture point cannot be reached.

Comparing the behavior of both systems, the amount of the ionic liquid that cannot be reused is around 8% lower with BmimTfO/(NaH₂PO₄/Na₂HPO₄). This can be explained considering that the binodal curve of this system is closer to the axis of the ternary phase diagram so a bigger biphasic region is obtained, as discussed above in Section 3.1.

This study indicates that the reuse of the ionic liquid depends mostly on the protein concentration in the feed stream, whereas the effects of the efficiency of the ILTPP process or the differences in composition for similar ionic liquid/salt systems are much more limited. In this way, recoveries higher than 99% of the ionic liquid can be reached at high protein concentrations. Nevertheless, even though higher protein concentrations enhance the ionic liquid recovery, they also increase the energy consumption associated with the previous concentration processes (e.g. ultrafiltration membranes) which are not considered in this study, so a compromise between energy consumption and ionic liquid losses must be achieved. In future developments additional recovery steps can be included in the ILTPP process to increase the ionic liquid that is recycled. For this purpose, alternatives such as the increase of the salt mass fraction once the protein has been recovered [24,25] or the concentration of the salt-rich phase by means of evaporation [23,34] can be taken into account. In addition, the continuous version of the process should also be investigated and its feasibility by means of the $IL_{lost}/LF_{recovered}$ ratio should be assessed, because in this way the disadvantages associated with discontinuous processes are avoided.

4. Conclusions

The description of the thermodynamic equilibrium of the ILTPP systems that lead to successful lactoferrin recoveries is carried out in this work, as required to study the ionic liquid recyclability in this process. The distribution coefficient of the ionic liquid between the liquid phases decreases notably with the salt content, which favors the ionic liquid recyclability. For the ionic liquid/salt systems at which the ILTPP process is successfully applied, the fraction of ionic liquid that cannot be reused in each cycle varies

from almost 5% at low protein concentrations in the feed stream to values around 0.8% at high protein mass fractions, so this is the key operating variable on which the recovery of the ionic liquid depends. In contrast, the influence of the ILTPP efficiency in terms of protein recovery and the composition of similar ionic liquid/salt systems are notably lower, around 10% of the total ionic liquid that is lost in each cycle. Relatively low ILTPP efficiencies and the BmimTfO/(NaH₂PO₄/Na₂HPO₄) system slightly increases the fraction of ionic liquid recycled. Consequently, the present work demonstrates that ILTPP can potentially reuse more than 99% of the ionic liquid involved in the process according to the thermodynamic data and mass balances developed, which reduces the economic and environmental impact of the process due to the consumption of this type of compounds. In the future, additional studies should be carried aiming at introducing additional steps to enhance the fraction of the ionic liquid recovered.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.fluid.2014.03.009.

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