

Effect of ionic liquids as adjuvants on PEG-based ABS formation and the extraction of two probe dyes



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ABSTRACT

Aqueous biphasic systems (ABS) are relevant for the development of environmentally friendly and “bio-compatible” separation processes. However, the common polyethylene glycol (PEG) polymers present a limited range of applicability, due to the low polarity of the PEG-rich phase. To overcome this limitation, a new approach was recently proposed based on the use of ionic liquids (ILs) as adjuvants in ABS, enlarging the polarity range of these systems. This work addresses the use of imidazolium-based ILs as ionic adjuvant compounds in the formation of ABS, namely potassium salts + water + PEG (1500, 4000, 6000 and 8000). To explore the differences induced by the presence of the IL as adjuvant the partition behavior of two dyes, Chloranilic Acid and Rhodamine 6G, is analyzed and correlated with the phase behavior and the IL distribution on the ABS under study.

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1. Introduction

The application, of aqueous biphasic systems (ABS) for liquid–liquid extraction processes was originally proposed by Albertsson in 1958 [1]. These systems form two aqueous phases that coexist in equilibrium due to the dissolution, at appropriate concentrations, of pairs of solutes in water [2]. ABS composed of polymers (namely polymer–polymer, or polymer–salt), were recognized as “biocompatible” systems to cells, organelles and biologically active substances, properties that make them well known as good systems to be applied in the recovery and purification of biomolecules [3]. Despite the well-known advantages offered by these systems, such as low interfacial tension, good biocompatibility, fast and high phase separation rates and low cost [4,5], their performance is however significantly affected by the limited range of polarities of the coexisting phases, that can be an important issue to take into account when the goal is to apply these systems in the extraction of biomolecules.

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Since 2003 [6], alternative ABS constituted by ionic liquids (ILs) have been proposed to replace ABS based on polymeric matrices. Contrarily to the common polymer-based ABS, they do not suffer from high viscosity [7,8], formation of opaque aqueous solutions, and display a much broader range of polarities [2,9] since ILs cover the whole hydrophilicity/hydrophobicity range [10,11]. One of the main advantages of the application of ILs in ABS is the possibility of manipulating their physicochemical properties [12] by a proper combination/manipulation of the cation, anion and alkyl chains of the ILs [13]. Due to their advantages, these systems have been extensively studied [14–16] and applied in the extraction of wide variety of compounds such as amino-acids [17–21], drugs [22], phenolic compounds [21], alkaloids [23–27], antibiotics [24,28] and anti-inflammatory compounds [29], proteins [30–33], enzymes [30,34,35] and natural colorants [36]. When dealing with ILs very miscible in water at room temperature, large concentrations of salts are necessary to promote the ABS formation, making the extraction process more expensive and less sustainable. The use of small quantities of ILs as adjuvants [20] appears as an alternative to overcome this difficulty. Pereira et al. [20] have demonstrated that the incorporation of 5 wt% of an IL in a polymer + salt ABS is capable of modifying the polarities of both phases leading to more advantageous separation processes, with improved extraction parameters (*i.e.* higher partition coefficients and extraction efficiencies).

This work focuses in the design of several quaternary systems, based in four polymers (PEG 1500, PEG 4000, PEG 6000 and PEG 8000), three potassium salts (K_3PO_4 , K_2HPO_4 and the potassium phosphate buffer K_2HPO_4/KH_2PO_4) and four imidazolium-based ILs as adjuvants ($[C_2mim]Cl$, $[C_4mim]Cl$, $[C_6mim]Cl$ and $[C_8mim]Cl$) at 5 wt%. Thus, various parameters were investigated in terms of their effect on the characteristics of the ABS, namely the ILs cation alkyl chain length, the polymers molecular weights, different potassium-based salts and finally, different pH values associated with the salt type applied. To evaluate this effect upon the separation potential, the partition of two dyes, Rhodamine 6G (R6G) and Chloranilic Acid (CA), was investigated, and the results discussed based on the partition of each IL in the various ABS systems.

2. Experimental

2.1. Materials

The present study was carried out using different polyethylene glycol polymers (average molecular weight of 1500, 4000, 6000 and 8000 $g\ mol^{-1}$), abbreviated as PEG 1500, PEG 4000, PEG 6000 and PEG 8000, respectively. These polymers were supplied by Sigma–Aldrich and were used as received. The inorganic salts used in the formation of the phase diagrams were the potassium phosphate tribasic (K_3PO_4), the potassium phosphate dibasic (K_2HPO_4) and the potassium phosphate buffer composed of K_2HPO_4/KH_2PO_4 at pH 7 [37]. These salts were purchased from Sigma–Aldrich®, with purities higher than 98 wt%. The ILs studied were 1-ethyl-3-methylimidazolium chloride ($[C_2mim]Cl$), 1-butyl-3-methylimidazolium chloride ($[C_4mim]Cl$), 1-hexyl-3-methylimidazolium chloride ($[C_6mim]Cl$) and 1-methyl-3-octylimidazolium chloride ($[C_8mim]Cl$). All ILs were purchased from Iolitec (Ionic Liquid Technologies, Germany). The Chloranilic Acid [CA, purity >99 wt%] was purchased from Merck and the Rhodamine 6G (R6G, content \approx 95 wt%) was supplied by Sigma–Aldrich®. The chemical structures of the ILs and dyes are presented in Fig. 1A and B along with the abbreviations used.

2.2. Phase diagrams

The quaternary phase diagrams were determined at 298 (\pm 1) K and at atmospheric pressure, by the cloud point titration method [20]. The quaternary systems were prepared considering stock solutions of each salt at 25 wt% plus 5 wt% of each IL, PEG at 40 wt% plus 5 wt% of each IL and finally, water solutions with 5 wt% of each IL studied. The systems composition was determined by the weight quantification of all components added within an uncertainty of $\pm 10^{-4}$ g. The binodal curves data were correlated using the Merchuk equation [38], described as follows:

$$Y = A \times \exp[(BX^{0.5}) - (CX^3)] \quad (1)$$

where Y and X are respectively, the PEG and inorganic salt weight percentages.

2.3. Dyes partition

A mixture point into the biphasic region was selected, composed of PEG at 15 wt%, salt at 15 wt% and IL at 5 wt%, being used to evaluate the partition of both dyes, the Chloranilic Acid (CA) and the Rhodamine 6G (R6G), by the combination of four imidazolium-based ILs ($[C_2mim]Cl$, $[C_4mim]Cl$, $[C_6mim]Cl$ and $[C_8mim]Cl$), two polymers PEG 1500 and PEG 8000, and by the application of the potassium phosphate buffer.

In the preparation of each extraction system, *circa* 0.30 mg of each dye were introduced into the glass tubes already containing a total mass of the quaternary system of 5 g. The ABS was

then allowed to equilibrate at 298 (\pm 1) K and atmospheric pressure conditions during 12 h to reach equilibrium. The top and bottom phases were then carefully separated, and the partition coefficients of each dye and the IL were evaluated. Thus, the ILs (211 nm) and the dyes (332 nm for CA and 527 nm for R6G) were quantified in both phases, through UV spectroscopy using a SHIMADZU UV-1700 Pharma-Spec spectrometer. The possible interferences from the phase promoters (salt, IL and polymer) were taken into account and found to be of no significance at the dilution levels used. Moreover, at least three samples of each extraction system were prepared, being the IL and dyes precisely quantified in both aqueous phases. Thus, the partition coefficients of both ILs (K_{IL}) and dyes (K_{dye}) were determined, in accordance with Eqs. (2) and (3):

$$K_{IL} = \frac{Abs_{IL_T}}{Abs_{IL_B}} \times df \quad (2)$$

$$K_{dye} = \frac{Abs_{dye_T}}{Abs_{dye_B}} \times df \quad (3)$$

where Abs_{IL_T} and Abs_{IL_B} are the absorbance data of IL in the top (PEG-rich) and bottom (salt-rich) phases, Abs_{dye_T} and Abs_{dye_B} represent the dye absorbance data in the top and bottom phases, respectively and df represents the dilution factor.

3. Results and discussion

One of the major drawbacks in the application of conventional polymer–salt based ABS is their limited polarity window between both aqueous phases. In this context, the number of studies describing new ABS and their application as extraction techniques is increasing. Initially, this work studies the phase behavior of different quaternary ABS formed by different combinations of PEG + potassium salts + water + imidazolium-based ILs, being ILs here applied as adjuvants. In this work several parameters were investigated, namely the salt, their pH, the PEG molecular weight and the IL alkyl chain length.

The mass fraction solubility data of all systems are presented in Supporting Information (Tables A.1–A.6). The set of solubility curves obtained is discussed in this work, having into account two criteria: (a) the effect of the various salts in the ABS formation of PEG 1500-based systems with and without ILs, and (b) the effect of the PEG molecular weight in the formation of ABS, using the potassium buffer solution (K_2HPO_4/KH_2PO_4) at pH 7 and ILs as adjuvants. All phase diagrams are presented in molality units to avoid discrepancies in the phase diagrams behavior, which could be a result of the differences between the PEG, salt and IL molecular weights. All binodal curves were determined at 298 (\pm 1) K and atmospheric pressure. The data was correlated using Eq. (1) with the regression parameters reported in Tables A.7 and A.8. Furthermore, the partition of two different dyes (here utilized as probe molecules) is discussed based on the information collected about the phase diagrams, since our main objective is to evaluate the differences in the extraction capacity of these ABS induced by the addition of ILs as adjuvants.

3.1. Analysis of the phase diagrams

3.1.1. Effect of salts

The effect of using ILs as additives upon the formation of ABS composed by PEG 1500, potassium phosphate salts [phosphate buffer K_2HPO_4/KH_2PO_4 (pH 7), K_2HPO_4 or K_3PO_4] was analyzed in this study (Fig. 2 and Figs. A.1 and A.2, Supporting Information). The results depicted in Fig. 2 show that the presence of 5 wt% of $[C_nmim]Cl$ -based ILs produce a small effect in the ABS formation. In general, it seems that ILs with smaller chains, namely the $[C_2mim]Cl$, tend to increase the two phase

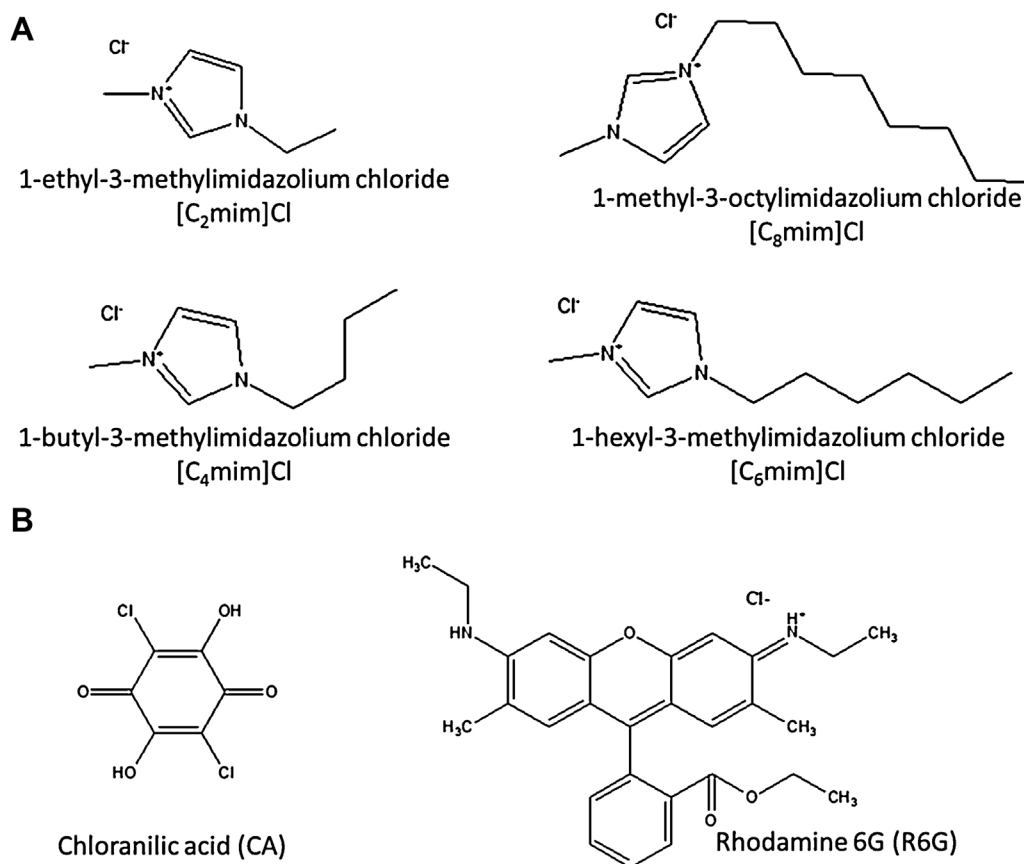


Fig. 1. Chemical structure, full name and abbreviation of the ILs (A) and dyes (B) investigated.

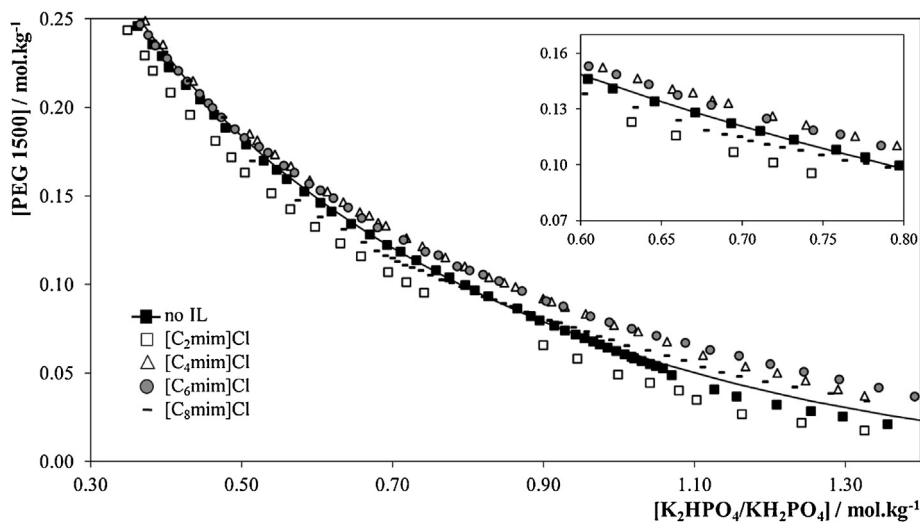


Fig. 2. Binodal curves for the quaternary systems composed of PEG 1500 + K₂HPO₄/KH₂PO₄ (pH 7) + 5 wt% of ILs (when present) + water, at 298 (±1) K.

region when compared with the remaining ILs, thus following the tendency: [C₂mim]Cl > [C₄mim]Cl > [C₆mim]Cl. This behavior is independent of the salt used (K₂HPO₄/KH₂PO₄, K₂HPO₄ and K₃PO₄ are depicted in Figs. 2, A.1 and A.2) and it is in close agreement with the increase in the ILs hydrophobic nature, from [C₂mim]Cl to [C₆mim]Cl. The analysis of the phase diagrams should start by the less complex ABS, namely the PEG + salt + water (Fig. A.3, Supporting Information). In this figure, the PEG 1500 + salt + water systems were depicted and the results suggest that the ability of the various potassium phosphate salts to form ABS follows

the trend: K₃PO₄ > K₂HPO₄ > K₂HPO₄/KH₂PO₄, describing the well-known Hofmeister series [39] and the “salting-out” ability of these salts. When IL is added, the changes in the binodal curves follow the same trend observed for the PEG + IL + water systems [2], indicating that the ILs are preferentially interacting with the polymer-rich phase, when the quaternary systems are considered. Because the interaction of the IL as adjuvant is important to understand the characteristics of these new systems, the partition coefficient of each IL (K_{IL}) tested was determined considering the PEG 1500 and PEG 8000 systems, both with the buffer solution (Fig. 3). This salt

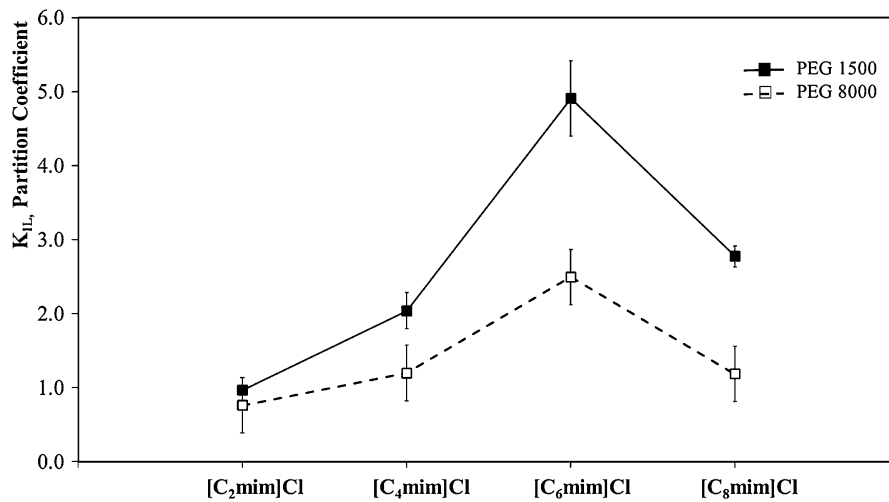


Fig. 3. ILs distribution between the two phases in terms of their partition coefficient (K_{IL}) and their respective standard deviations, for systems based in PEG (1500 and 8000) + 5 wt% of $[C_n\text{mim}]\text{Cl}$ + water + $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (pH 7) at $298 (\pm 1) \text{K}$.

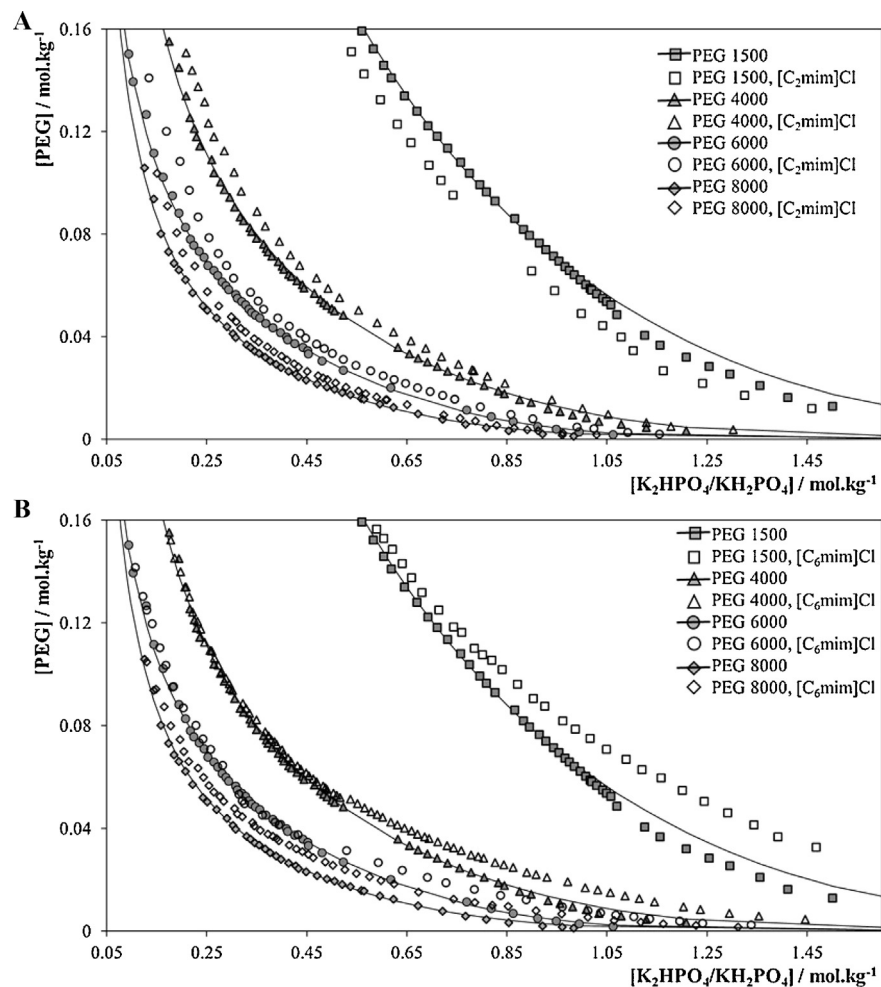


Fig. 4. Binodal curves for the quaternary systems, at $298 (\pm 1) \text{K}$, composed of PEG (1500, 4000, 6000, and 8000) + $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (pH 7) + water + 5 wt% of $[\text{C}_2\text{mim}]\text{Cl}$ (A), or 5 wt% of $[\text{C}_6\text{mim}]\text{Cl}$ (B).

was used, since it allows the pH to be kept constant during the partition experiments avoiding changes in the charge of the molecules [29,40]. The K_{IL} data were calculated according to Eq. (2), taking into account the PEG-rich phase as reference. Here, it seems that the various ILs have different affinities for the PEG-rich phase, which

can be explained by their interactions with each one of the phases. Moreover, when the results obtained for the K_{IL} were compared considering both PEG 1500 and PEG 8000, in general, it becomes clear that the IL migration for the top phase is favored for polymers with lower molecular weight. Meanwhile, it is observed that

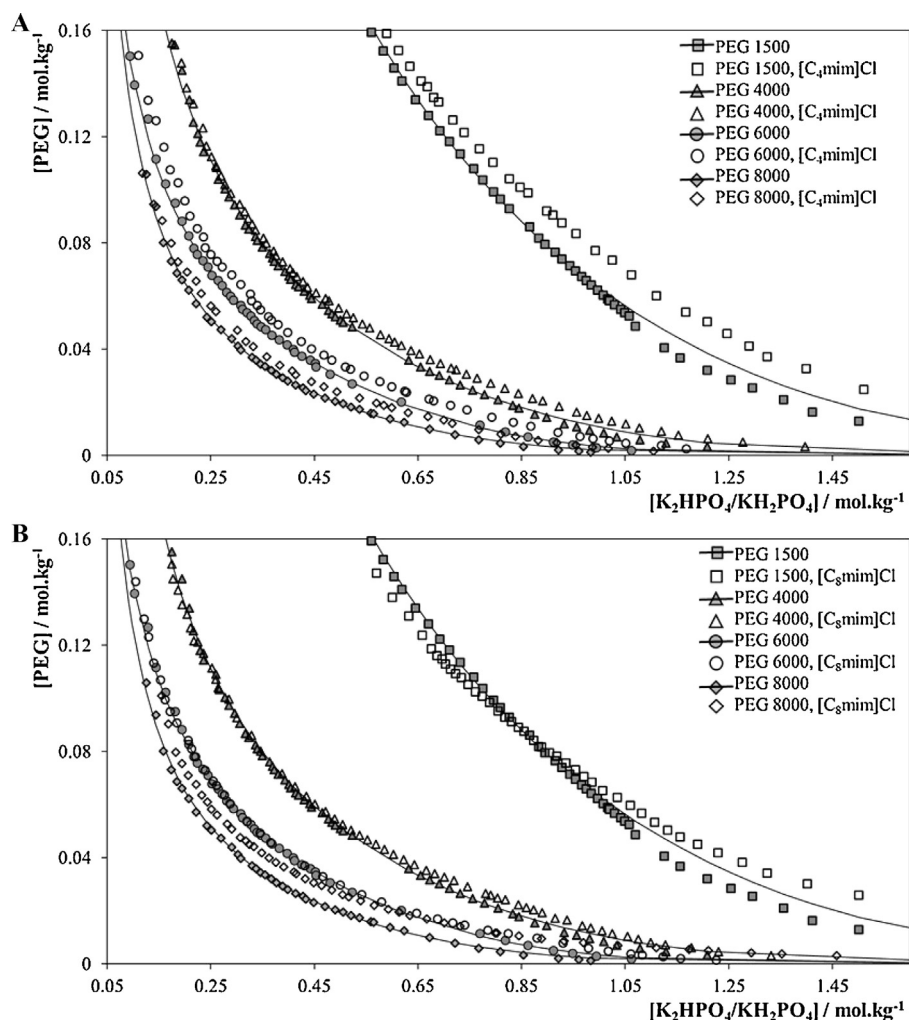


Fig. 5. Binodal curves for the quaternary systems, at 298 (± 1) K, composed of PEG (1500, 4000, 6000, and 8000) + K_2HPO_4/KH_2PO_4 (pH 7) + water + 5 wt% of $[C_4mim]Cl$ (A), or 5 wt% of $[C_8mim]Cl$ (B).

the migration of the ILs species increases with the IL hydrophobicity. The exception observed for the $[C_8mim]Cl$, seems to be related with the possible micelle formation by the IL self-aggregation, which is promoted by the longer alkyl chains of this IL, helped by the presence of the salt, which is acting as a “salting-out” agent. As recently discussed, the phase formation is always the result of a delicate balance between entropic effects (described by the decrease in the solubility and the consequent “salting-out” effects promoted by the presence of ILs) and the tendency of ILs to auto-aggregate when in aqueous media [41]. Their self-aggregation only happens for ILs containing longer alkyl chains higher than 6 carbons (not included) and above their corresponding critical micelle concentration (CMC) [42]. Furthermore, it is well-known that the presence of “salting-out” inducing salts is also responsible for the decrease of the CMC, facilitating more the aggregation of ILs, due to the decreased reduction of the repulsion between the cation core groups [41].

3.1.2. Effect of polymer

In this work, four PEGs (PEG 1500, PEG 4000, PEG 6000 and PEG 8000) were used to test the formation of quaternary ABS using the same ILs as adjuvants. In this case, the polymer also offers a certain level of tunability by the variation of the polymeric chain length and the average molecular weight. This effect was analyzed by studying ABS formed by the co-dissolution of the potassium phosphate buffer (K_2HPO_4/KH_2PO_4 at pH 7)

and 5 wt% of imidazolium-based ILs. Fig. 4 presents the binodal curves for PEG (1500, 4000, 6000 and 8000) + K_2HPO_4/KH_2PO_4 (pH 7) + water + IL. The influence of the PEG molecular weight (*i.e.* alkyl chain length) on the phase diagrams is clear with the two phase region increasing in the order: PEG 1500 < PEG 4000 < PEG 6000 < PEG 8000. This effect is well described in literature [43–45]. PEGs with a higher molecular weight are more hydrophobic, facilitating the ABS formation, since these polymers present a lower affinity for the water molecules, being more easily “salted-out”.

Fig. 4 also shows the impact of the $[C_nmim]Cl$ series on the phase separation. The binodal curves are depicted in Fig. 4A and B for $[C_2mim]Cl$ and $[C_6mim]Cl$ (respectively) for a better analysis of their effects in the ABS formation. The remaining systems are depicted in Fig. 5A and B for $[C_4mim]Cl$ and $[C_8mim]Cl$, respectively (Supporting Information), their behaviors being similar to those reported in Fig. 4. The binodal curves of Fig. 4A and B indicate that the IL effect upon the phase separation is stronger for the IL with shorter alkyl chains, *i.e.* $[C_2mim]Cl$. In this case, the binodal data of the systems with and without IL are more deviated from each other, and the biphasic region of the system with IL is, in general, larger for the ternary system, being the only exception observed for PEG 1500, the more hydrophilic polymer, with $[C_2mim]Cl$. This synergistic effect upon ABS formation of the more hydrophilic ILs was previously observed for other low molecular weight PEG with various ILs [20].

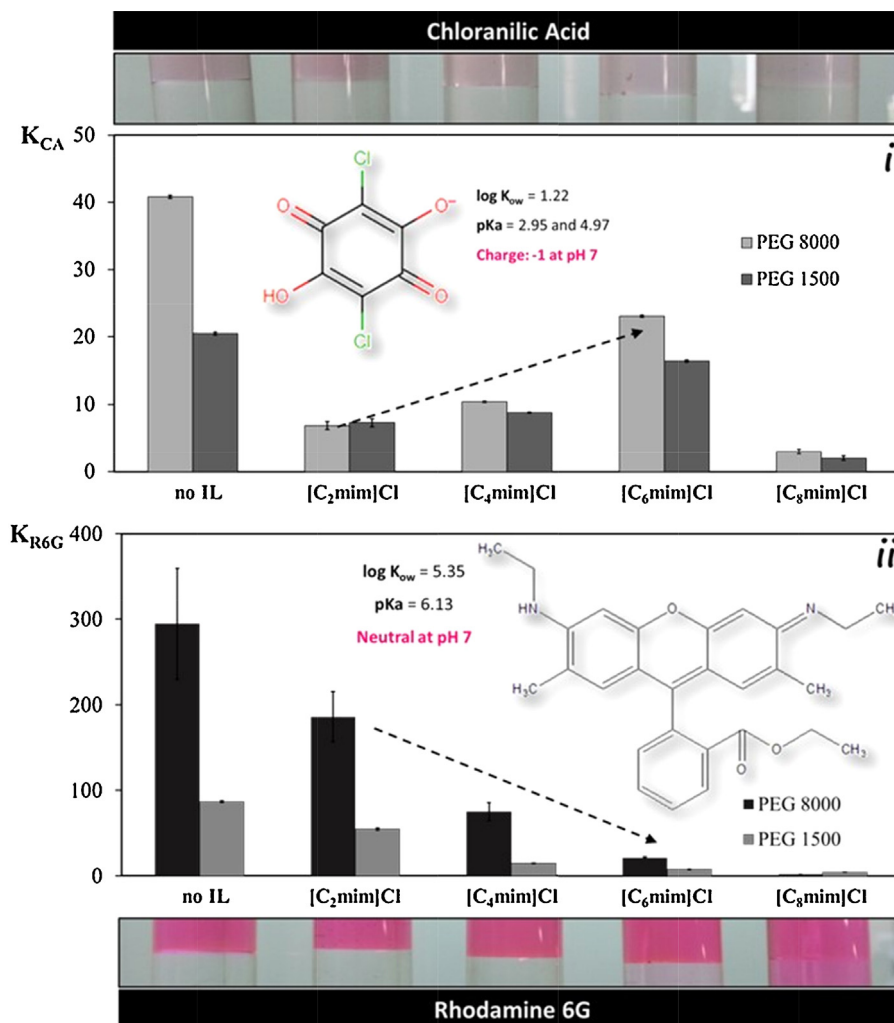


Fig. 6. Partition coefficient results for the (i) CA (K_{CA}) and (ii) R6G (K_{R6G}), by applying the systems based in PEG (1500 and 8000) + water + K_2HPO_4/KH_2PO_4 (pH 7) without and with 5 wt% of $[C_n\text{mim}]Cl$, at $298 (\pm 1) K$. The visual aspect of the extraction systems for CA and R6G is depicted for systems based in PEG 8000.

3.2. Dyes partition

It is previously shown that the presence of the IL does not have a significant impact upon the phase separation. To further understand the nature of these systems, and the impact of the use of the IL as adjuvant, partition studies of two probe dyes in ABS systems with and without ILs were carried out. Rhodamine 6G (R6G) and the Chloranilic Acid (CA) (their structures being presented in Fig. 1B) were adopted in the partition tests due to their different natures since at pH 7, the CA is negatively charged and R6G is mainly in its neutral form (the speciation curves for these molecules are reported in Supporting Information, Figs. A.4 and A.5). The results of the partition experiments are depicted in Fig. 6(i) and (ii). These were done for the following ABS: PEG 1500 and PEG 8000 + KH_2PO_4/K_2HPO_4 + $[C_n\text{mim}]Cl$. In addition, the mass fraction of the systems, and the partition coefficients of ILs, CA and R6G, are shown respectively in Tables A.9, A.10 and A.11 (in Supporting Information). The results of Fig. 6 show that both dyes have more affinity for the more hydrophobic phase ($K_{dyes} > 1$), the PEG-rich phase, which is in agreement with their octanol–water partition coefficients ($\log K_{ow} > 1$), suggesting the hydrophobic interactions as the controlling forces of the dyes partition. However, based on the K_{dyes} data, it seems that more than just the hydrophobic interactions control the partition, which is modulated by the ILs presence. The impact of the alkyl chain of the IL cation upon the partition

coefficients is opposite when both dyes are analyzed. While the anionic CA becomes more concentrated, the neutral R6G follows the opposite trend and becomes less concentrated in the PEG-rich phase, as the additives change $[C_2\text{mim}]Cl$ to $[C_6\text{mim}]Cl$. These results may be explained by additional interactions between the anionic CA and ILs, in particular the electrostatic interactions. The more concentrated the IL in the PEG-rich phase (according to Fig. 3 this increases with the alkyl chain length), the larger the partition of CA toward this phase. The decrease observed for $[C_8\text{mim}]Cl$ further supports this behavior ($K_{[C_8\text{mim}]Cl}$ is the lowest). Associated with the lowest amount of $[C_8\text{mim}]Cl$ system in the PEG-rich phase (Fig. 3), the CA partition coefficient is also influenced by the IL self-aggregation [46], since this phenomenon is promoting the alteration of the interactions acting in the partition of the dye when different ILs with shorter and longer alkyl chains are compared. A decrease in the IL concentration in the PEG-rich phase has a direct and proportional impact in the CA migration. The neutral R6G presents a different behavior. Since it is not charged, the presence of the IL in the PEG-rich phase becomes deleterious to the partition of the compound toward this phase, due to the enhanced polar and Coulombic interactions that would be present on this phase due to the IL. As the concentration of the IL in the PEG-rich phase increases with the alkyl chain length then the partition becomes less favorable toward this phase. Meanwhile, when the whole picture is observed, it seems that, despite the clear affinity of both dyes

for the PEG-rich phase, when the ILs are not present, the partition coefficients of both dyes are higher. This can be easily explained by the increased hydrophobic nature of the PEG-rich phase [47]. It is noticeable the huge impact that a small quantity of IL has on the dyes partition.

4. Conclusions

The effect of ILs used as adjuvants in small concentrations (5 wt%), in the ABS composed of PEG with some potassium salts is here investigated. It is shown that, while in most cases the effect is small, it seems that the presence of ILs with smaller chains tend to increase the two phase region, being this behavior independent of the salt associated. In this specific work, all ILs have higher interaction with the most hydrophobic phase, the polymer-rich phase, which is shown by the partition coefficients of the ILs investigated. This migration of the adjuvants seems to be favored when polymers with lower molecular weights are presented. The results obtained from a more general analysis suggest that synergistic effects are playing a key role upon ABS formation.

The performance of the ILs as additives was further investigated through the study of the partition coefficients of Rhodamine 6G and the Chloranilic Acid, being established that while the presence of a small concentration of additive may have a minor effect upon the ABS formation it has a major impact in the PEG-rich phase characteristics, and thus on the partition coefficients of the dyes studied. Although both dyes have a higher affinity for the PEG-rich phase, their partition coefficients react differently to the presence of the IL depending on the charge of the dye, and thus on the additional interactions that the IL induces in the PEG-rich phase.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.fluid.2014.04.011](https://doi.org/10.1016/j.fluid.2014.04.011).

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