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Article

Imidazolium-based Ionic Liquids as Adjuvants to Form Polyethylene Glycol with Salt Buffer Aqueous Biphasic Systems

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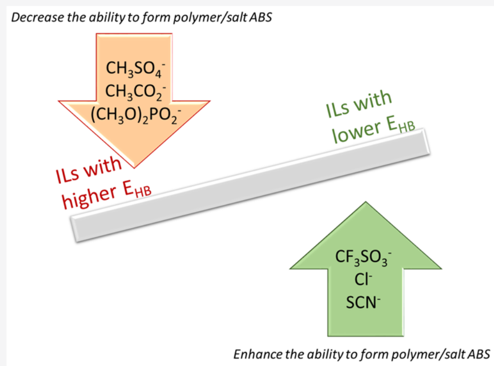
ABSTRACT: Aqueous biphasic systems (ABS) are biocompatible systems applied in the extraction of biomolecules. Despite the biocompatibility of polymers and, particularly polyethylene glycol (PEG), to form ABS, their limitation in terms of phase separation is recognized. A new approach was recently proposed based on the use of ionic liquids (ILs) as adjuvants in ABS, enlarging the polarity range of these systems. Up to now, the effects of ILs in PEG-salt ABS have been poorly described. To overcome this limitation, the phase diagrams of imidazolium-based ILs acting as adjuvants in ABS based in PEG with potassium salt buffers (pH = 7), that is potassium citrate ($C_6H_5K_3O_7/C_6H_8O_7$) and potassium phosphate (K_2HPO_4/KH_2PO_4) buffers, are herein addressed. Imidazolium-based ILs were focused in this work, since they have been applied on the purification of several biomolecules with success, even as adjuvants or electrolytes. The phase diagrams were mapped out for PEG/salt ABS without adjuvants. In this work, systems composed of PEG (1000, 1500, 2000, 3350, 4000, 6000, and 8000) with potassium phosphate buffer and PEG (2000, 6000, 10 000, and 20 000) with potassium citrate buffer were tested. Moreover, the presence of 5 wt % of imidazolium-based ILs (varying the anion moiety) for the system PEG 1500 with potassium phosphate buffer was also investigated. Imidazolium-based ILs with different anions were tested to investigate a large range of polarities attributed to the adjuvant. Moreover, the effect of the adjuvant content (5, 10, and 20 wt %) in the PEG 2000 with potassium citrate buffer system was studied for two distinct ILs, namely $[C_4mim][CF_3SO_3]$ and $[C_4mim][(CH_3O)_2PO_2]$, with lower and higher energy of intramolecular hydrogen bond, E_{HB}, respectively, a parameter representing the ions' hydration. A correlation between the anion moiety of imidazolium-based IL and the ability to form two phases was observed, being this related to the ILs' anion E_{HB} value. The concentration of the adjuvant confirmed the effects of enhancing or decreasing the ability to form two phases for ILs with lower and higher E_{HB} value, respectively.



1. INTRODUCTION

Aqueous biphasic systems (ABS) are formed by the dissolution of two polymers, a polymer and a salt, or two salts in water. These systems allow liquid−liquid extraction processes originally proposed by Albertsson in 1958.[1] ABS composed of polymers (namely polymer−polymer or polymer−salt) were recognized as biocompatible systems to cells, organelles, and biologically active substances, turning them as suitable systems to be applied on the recovery and purification of biomolecules.[2,3] Conventionally, from the polymers applied to form the two-phase systems polyethylene glycol (PEG) takes the lead as the most used as phase forming in combination with other polymers or salts.[4,5] The PEG/salt ABS offer plenty of advantages, such as their low interfacial tension, biocompatibility, fast and high phase separation rates, and low cost.[6,7] However, their performance is significantly affected by the limited range of polarity of the coexisting phases, which affect the extraction and purification performance of the biomolecules. To overcome this drawback, the use of small amounts of ionic liquids (ILs) has been proposed to extend the hydrophilicity/hydrophobicity range between the two aqueous phases.[8,9] Contrary to the common polymer/salt-based ABS, in general ILs do not suffer from high viscosity[10] or the formation of opaque aqueous solutions and display a much broader range of polarity.[11] One of the main advantages of ILs for ABS formulation is related to the designer solvents inner characteristics tuning the physicochemical properties[12] by the proper combination/manipulation of the ILs' cation, anion, and alkyl chains.[13] Due to their advantages, ILs have been extensively studied as adjuvants in PEG/salt ABS[14−16] and applied in the extraction of a wide variety of compounds such

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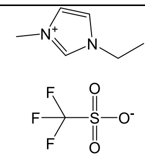
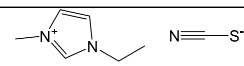
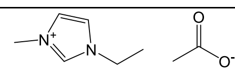
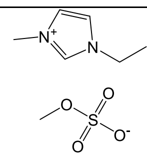
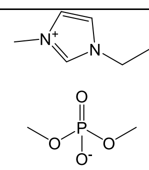
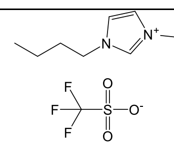
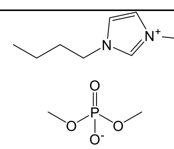
Table 1. CAS number, Mass Fraction Purity, Water Content, and Suppliers of the Chemicals

chemicals	CAS no.	suppliers	purity (wt %)	water content (wt %)
polyethylene glycol	25322-68-3	Sigma-Aldrich	≥99	<0.1
potassium phosphate dibasic	7758-11-4	Sigma-Aldrich	≥98	<0.1
potassium phosphate monobasic	7778-77-0	Sigma-Aldrich	99	<0.1
1-ethyl-3-methylimidazolium acetate	143314-17-4	Iolitec	98	4.9
1-ethyl-3-methylimidazolium methylsulfate	516474-01-4	Iolitec	98	1.5
1-ethyl-3-methylimidazolium thiocyanate	331717-63-6	Iolitec	98	1.6
1-ethyl-3-methylimidazolium triflate	145022-44-2	Iolitec	99	<0.1
1-ethyl-3-methylimidazolium dimethylphosphate	945611-27-8	Iolitec	98	2.5
1-butyl-3-methylimidazolium dimethylphosphate	891772-94-4	Iolitec	98	2.0
1-butyl-3-methylimidazolium triflate	174899-66-2	Iolitec	99	<0.1

Table 2. List of the ILs Used in This Work as Adjuvants

Acronym	IUPAC nomenclature	Chemical structure
$[C_2mim][CF_3SO_3]$	1-ethyl-3-methylimidazolium triflate	
$[C_2mim][SCN]$	1-ethyl-3-methylimidazolium thiocyanate	
$[C_2mim][CH_3CO_2]$	1-ethyl-3-methylimidazolium acetate	
$[C_2mim][CH_3SO_4]$	1-ethyl-3-methylimidazolium methylsulfate	
$[C_2mim][(CH_3O)_2PO_2]$	1-ethyl-3-methylimidazolium dimethylphosphate	
$[C_4mim][CF_3SO_3]$	1-butyl-3-methylimidazolium triflate	
$[C_4mim][(CH_3O)_2PO_2]$	1-butyl-3-methylimidazolium dimethylphosphate	

as proteins,[14,17−19] probe dyes,[20] phenolic compounds,[21] and amino acids.[15,21] Moreover, the use of small quantities of ILs in comparison with the use of ILs as main phase forming compounds, in which greater concentrations are required, make these systems less expensive and more sustainable. However, few studies focused on understanding the effect of IL characteristics on ABS formation. With this study, it is our intention to report the phase diagrams for several PEGs with potassium buffer ABS and PEG with potassium buffer systems with different ILs acting as adjuvants. The effects of the anion moiety of the ILs and ILs' concentration were evaluated to predict a correlation between the IL structure and the ABS formation.

2. MATERIALS AND METHODS

2.1. Materials. In Table 1, all the reagents are depicted along with CAS registry number, mass fraction purity, and supplier. Polyethylene glycol polymers of different molecular weights were used with an average molecular weight of 1000, 1500, 2000, 3350, 4000, 6000, 8000, 10 000 and 20 000 g·mol^{-1} respectively abbreviated as PEG 1000, PEG 1500, PEG 2000, PEG 3350, PEG 4000, PEG 6000, PEG 8000, PEG 10 000, and PEG 20 000. These polymers were supplied by

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Sigma-Aldrich and were used as received. The inorganic salts used in the formation of the phase diagrams were the potassium phosphate buffer composed of potassium phosphate monobasic/potassium phosphate dibasic K_2HPO_4/KH_2PO_4 (weight ratio 52.1 and 47.9 wt %, respectively) at pH 7 and potassium citrate buffer composed of potassium citrate/citric acid $C_6H_5K_3O_7/C_6H_8O_7$ (weight ratio 96.2 and 3.8 wt %, respectively) at pH 7. These salts were purchased from Sigma-Aldrich with purities higher than 98 wt %. The ILs studied were the 1-ethyl-3-methylimidazolium acetate ($[C_2mim][CH_3CO_2]$), 1-ethyl-3-methylimidazolium methylsulfate ($[C_2mim][CH_3SO_4]$), 1-ethyl-3-methylimidazolium thiocyanate ($[C_2mim][SCN]$), 1-ethyl-3-methylimidazolium triflate ($[C_2mim][CF_3SO_3]$), 1-ethyl-3-methylimidazolium dimethylphosphate ($[C_2mim][(CH_3O)_2PO_2]$), 1-butyl-3-methylimidazolium triflate ($[C_4mim][CF_3SO_3]$), and 1-butyl-3-methylimidazolium dimethylphosphate ($[C_4mim][(CH_3O)_2PO_2]$). All ILs (Table 2) were purchased from Iolitec (Ionic Liquid Technologies, Germany) with purity >98 wt %.

2.2. Characterization of ILs, PEGs, and Salts. The water content of ILs, PEGs, and salts was determined by coulometric Karl Fischer titration (Metrohm, model 831). The chemical structure of the imidazolium-based ILs was confirmed by ^{13}C NMR spectroscopy (Bruker, model AMX 300) operating at 75 MHz as reported in Supporting Information (^{13}C NMR of ILs) and Figures S.3−S.9.

2.3. Determination of the Ternary Phase Diagrams. The ternary phase diagrams were determined at 298 (±1) K and at atmospheric pressure by the cloud point titration method.[22] The ternary systems were prepared considering stock solutions of each potassium salt buffer (phosphate or citrate) at 25 wt % and PEG at 40 wt %. The systems' composition was determined by the weight quantification of all components added within an uncertainty of $\pm 10^{-4}$ g. The molecular weight of PEG was evaluated for the construction of the phase diagrams of PEG with potassium salt buffer and water.

The tie-lines (TLs) for the polymer-salt ABS were determined. The ternary phase diagrams were correlated using the Merchuk equation[23] (eq 1)

$$w_{PEG} = A\ \exp[(B[w_{Salt}]^{0.5}) - (C[w_{Salt}]^{3})] \quad (1)$$

where w_{PEG} and w_{Salt} are, respectively, the polymer and inorganic salt weight percentages (wt %), and A, B, and C are the Merchuk parameters.

The TLs were determined by a gravimetric method originally proposed by Merchuk et al.[23] to calculate the composition of the two-phases in equilibrium. The TLs were calculated for the system based in PEG/salt buffer at pH 7, considering two mixture points for each system. The weight compositions of both phase formers (PEG and salt) in the top and bottom phases (w_{PEG}^{Top}, w_{Salt}^{Top}, w_{PEG}^{Bottom}, w_{Salt}^{Bottom}) were obtained by solving the following system of four equations and four unknown variables (eqs 2−5)

$$w_{PEG}^{Top} = A\ \exp[(B\cdot w_{Salt}^{Top^{0.5}}) - (C\cdot w_{Salt}^{Top^{3}})] \quad (2)$$

$$w_{PEG}^{Bottom} = A\exp[(B\cdot w_{Salt}^{Bottom^{0.5}}) - (C\cdot w_{Salt}^{Top^{3}})] \quad (3)$$

$$w_{PEG}^{Top} = \frac{100 w_{PEG}^{M}}{\alpha} - \frac{1-\alpha}{\alpha} w_{PEG}^{Bottom} \quad (4)$$

$$w_{Salt}^{Top} = \frac{100 w_{Salt}^{M}}{\alpha} - \frac{1-\alpha}{\alpha} w_{Salt}^{Bottom} \quad (5)$$

The superscripts Top and Bottom designate the PEG-rich and salt-rich phases, respectively, and M represents the initial mixture point composition. The parameter α is the ratio between the top weight and total weight of the mixture. The solution of the referred system gives the concentration of the polymer and salt in the top and bottom phases. The tie-line length (TLL) is defined in eq 6

$$\mathrm{TLL} = \sqrt{(w_{Salt}^{Top} - w_{Salt}^{Bottom})^2 + (w_{PEG}^{Top} - w_{PEG}^{Bottom})^2} \quad (6)$$

2.4. Design of Quaternary Phase Diagrams. The quaternary phase diagrams were determined at 298 (±1) K and at atmospheric pressure by the cloud point titration method.[24] The quaternary systems were prepared considering stock solutions of potassium phosphate buffer at 25 wt % + 5 wt % of each IL, PEG 1500 at 40 wt % + 5 wt % of each IL, and finally water solutions with 5 wt % of each IL studied. For the quaternary systems with PEG 2000 with potassium citrate buffer, the IL was studied as adjuvant at 5, 10, and 20 wt %. The systems' compositions were determined by the weight quantification of all components added within an uncertainty of $\pm 10^{-4}$ g. The partitioning of ILs adjuvants was measured for the mixture point with 30 wt % PEG + 15 wt % salt buffer +5 wt % of IL through the partition coefficient (K_{IL}), which corresponds to the ratio of IL concentration in top and bottom phases. The concentration of IL in each phase is measured by the absorbance determination at 280 nm and using calibration curves previously determined in the same wavelength (eq 7)

$$K_{IL} = \frac{[IL]_{Top}}{[IL]_{Bottom}} \quad (7)$$

3. RESULTS AND DISCUSSION

3.1. Phase Diagrams of PEG with Potassium Salt Buffer-based ABS. The binodal curves for the ternary system composed of PEG with potassium salt buffers and water and the quaternary systems based on PEG with potassium salt buffers and water and IL were determined at 298 (±1) K and atmospheric pressure. The respective phase diagrams were depicted in an orthogonal representation, where the amount of water is omitted. The mass fraction solubility data of all systems are presented in Supporting Information (Tables S1−S10).

The ternary phase diagrams were mapped out for PEGs with different molar weights (1000, 1500, 2000, 3350, 4000, 6000, 8000, 10 000, and 20 000 g·mol^{-1}) and the potassium phosphate and citrate buffers at pH ≈ 7 in aqueous media. Phosphate- and citrate-buffered solutions are widely employed in ABS due to their biocompatible nature when in the presence of several biomolecules, including enzymes, which can have their activity and structure maintained.[25,26] Furthermore, these buffers are classically employed to guarantee the pH of the coexisting phases of ABS. It should be stressed that phosphate-based salts exhibit a high-charge density and are currently replaced by more biocompatible and biodegradable organic salts, such as the citrate-based salts, which combined with citric acid, also afford a wide-buffered pH region. Moreover, citrate-based salts also have a strong salting-out character being therefore able to provide reasonable biphasic regions to work.[8]

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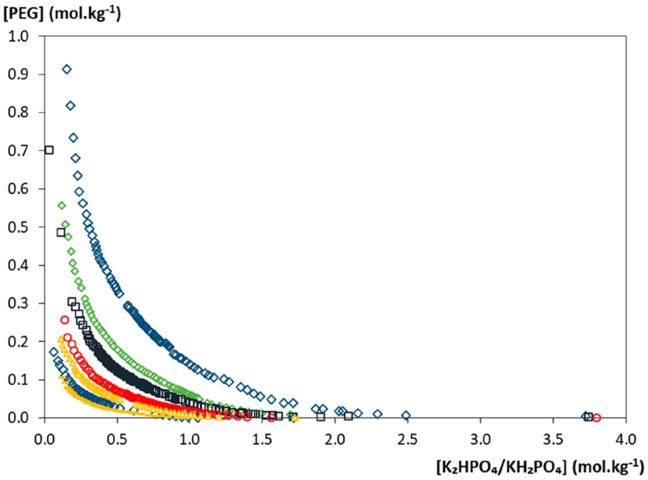

Figure 1. Phase diagrams for the systems composed of PEG ((blue ◇) PEG 1000, (green ◆) PEG 1500, (black □) PEG 2000, (red ○) PEG 3350, (yellow △) PEG 4000, (blue ◇) PEG 6000, and (yellow —) PEG 8000) + K_2HPO_4/KH_2PO_4 (pH 7) + water, at 298 (±1) K. Data regarding the systems of PEG 1500, PEG 4000, PEG 6000, and PEG 8000 were obtained from literature.[20] The phase diagrams for the systems of PEG 1000, 2000, and 3350 were experimentally obtained through cloud point titration method.

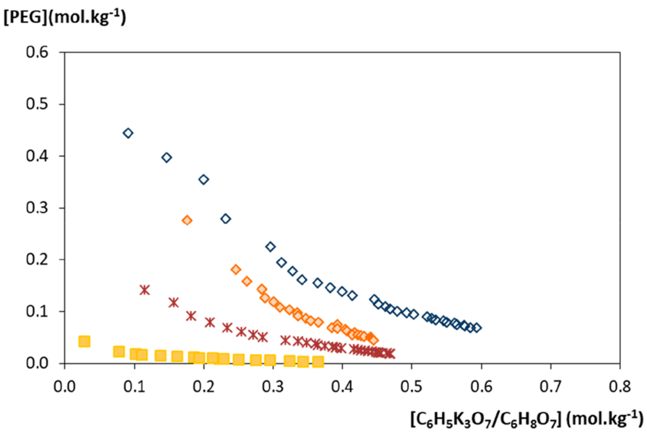

Figure 2. Phase diagrams for the ternary systems composed of PEG ((black ◇) PEG 2000, (orange ◆) PEG 6000, (red X) PEG 10 000, and (yellow ■) PEG 20 000) + $C_6H_5K_3O_7/C_6H_8O_7$ (pH 7) + water, at 298 (±1) K.

The ternary phase diagrams obtained at 298 (±1) K are illustrated in Figure 1 for K_2HPO_4/KH_2PO_4 (pH ≈ 7) and Figure 2 for $C_6H_5K_3O_7/C_6H_8O_7$ (pH ≈ 7). The biphasic region is located above the solubility curve, and the larger this region is the higher the ability of PEG to undergo liquid−liquid demixing in aqueous media is. As it can be seen from Figures 1 and 2, the ability to form ABS increases with the PEG molecular weight. In literature, phase diagrams for ABS composed of PEG with K_2HPO_4/KH_2PO_4[20,27] and PEG with $C_6H_5K_3O_7/C_6H_8O_7$[19] with a small range of PEG molecular weights were already described as having the same tendency. Likewise, similar trends have been observed in other ABS composed of polymer−salt[27] or PEG−IL pairs.[7] Comparing the two salting-out agents, the ability to form ABS is quite similar, which is somehow different from some of the conclusions[8] and tendencies[28] previously taken. Despite the accuracy of the Merchuk equation not being as great as the analytical determination of the concentration of each compound in both phases,[29] this is only verified for the regions of the solubility curve for very high PEG and salt concentrations.[30] The Merchuk equation was adopted to correlate the phase diagrams for each PEG/salt buffer system with the correlation parameters A, B, and C and the respective standard deviation (σ) depicted in Table 3. Moreover, for each ternary system, two TLs were measured for PEG with salt buffer ABS at 298 K, as presented in Table 4.

3.2. Quaternary Phase Diagrams of PEG with Potassium Salt Buffer and IL (Adjuvant)-based ABS.

One of the major drawbacks in the application of conventional polymer−salt-based ABS is their limited polarity window between the aqueous phases. In this context, the number of studies describing new ABS with the presence of small amounts of adjuvants and their application for biomolecules extraction is increasing. From the adjuvants applied in the polymer−salt-based ABS, ILs appear as one of the best possibilities since they are designer solvents and the proper hydrophilicity balance can be tailored. In this work, quaternary ABS formed by different combinations of PEG with potassium salt buffers, water, and ILs as adjuvants were designed. Several parameters were investigated, namely the salt, the PEG molecular weight, the IL anion moiety, and IL concentration. The set of phase diagrams was discussed taking into account two criteria: (i) the effect of the IL anion moiety in the binodal curve of PEG 1500 with K_2HPO_4/KH_2PO_4 (pH ≈ 7) based systems and (ii) the effect of the IL concentration for two types of adjuvants (with high and low values of E_{HB}) toward

Table 3. Correlation Parameters Used to Describe the Experimental Phase Diagram Data by Equation 1 and Respective Standard Deviation (σ)

PEG	buffer	$A \pm \sigma$	$B \pm \sigma$	$10^4 \times (C \pm \sigma)$	R^2
1000	phosphate	100 ± 4	−0.50 ± 0.02	1.6 ± 0.2	0.9851
1500	phosphate	92 ± 1	−0.510 ± 0.006	2.0 ± 0.06	0.9996
2000	phosphate	90.0 ± 0.9	−0.510 ± 0.005	2.60 ± 0.08	0.9969
3350	phosphate	106 ± 1	−0.590 ± 0.005	3.30 ± 0.05	0.9992
4000	phosphate	103 ± 1	−0.620 ± 0.007	4.0 ± 0.1	0.9991
6000	phosphate	97 ± 1	−0.600 ± 0.008	7.4 ± 0.3	0.9988
8000	phosphate	124 ± 3	−0.73 ± 0.01	7.2 ± 0.4	0.9987
2000	citrate	83 ± 6	−0.31 ± 0.03	2.00 ± 0.09	0.9855
6000	citrate	118 ± 6	−0.50 ± 0.02	3.0 ± 0.2	0.9967
10,000	citrate	171 ± 22	−0.67 ± 0.05	4.0 ± 0.5	0.9937
20,000	citrate	74 ± 3	−0.53 ± 0.02	6.0 ± 0.8	0.9942

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Table 4. Experimental Data for TLs and TLLs of PEG + Salt Buffer ABS at 298 (±1) K[a]

phase forming compounds	mixture point [PEG]/[salt] (wt %)	top-phase (wt %) [PEG]	top-phase (wt %) [salt]	bottom-phase (wt %) [PEG]	bottom-phase (wt %) [salt]	TLL
PEG 1000 + K_2HPO_4/KH_2PO_4	20:10	42.05	3.00	0.73	24.92	46.78
PEG 1000 + K_2HPO_4/KH_2PO_4	20:15	58.4	1.17	0.44	26.32	63.19
PEG 1500 + K_2HPO_4/KH_2PO_4	20:10	46.06	1.83	0.33	24.78	51.21
PEG 1500 + K_2HPO_4/KH_2PO_4	20:15	51.55	1.29	0.091	27.63	57.81
PEG 2000 + K_2HPO_4/KH_2PO_4	20:10	52.70	1.09	0.16	24.36	57.46
PEG 2000 + K_2HPO_4/KH_2PO_4	20:15	57.29	0.78	0.040	26.80	62.89
PEG 3350 + K_2HPO_4/KH_2PO_4	20:15	65.41	0.69	0.020	25.74	70.02
PEG 3350 + K_2HPO_4/KH_2PO_4	20:20	77.17	0.30	0.0085	26.89	81.61
PEG 4000 + K_2HPO_4/KH_2PO_4	20:10	56.19	0.98	0.0185	24.11	60.75
PEG 4000 + K_2HPO_4/KH_2PO_4	20:15	61.07	0.73	0.0031	26.27	66.19
PEG 6000 + K_2HPO_4/KH_2PO_4	20:10	82.23	0.073	9.7×10^{-4}	22.76	85.30
PEG 6000 + K_2HPO_4/KH_2PO_4	20:15	67.32	0.37	2.0×10^{-5}	25.63	71.91
PEG 8000 + K_2HPO_4/KH_2PO_4	20:10	47.28	1.74	4.6×10^{-5}	24.90	52.65
PEG 8000 + K_2HPO_4/KH_2PO_4	20:15	57.49	1.12	4.9×10^{-6}	26.67	62.91
PEG 2000 + $C_6H_5K_3O_7/C_6H_8O_7$	8:22	34.78	4.61	12.27	13.31	24.13
PEG 2000 + $C_6H_5K_3O_7/C_6H_8O_7$	8:26	34.22	4.73	11.68	13.70	24.26
PEG 2000 + $C_6H_5K_3O_7/C_6H_8O_7$	12:22	41.12	3.45	5.05	19.58	39.51
PEG 6000 + $C_6H_5K_3O_7/C_6H_8O_7$	10:24	39.14	5.91	7.60	14.43	32.67
PEG 10,000 + $C_6H_5K_3O_7/C_6H_8O_7$	15:15	34.79	5.31	0.089	22.30	38.64
PEG 10,000 + $C_6H_5K_3O_7/C_6H_8O_7$	15:10	27.84	6.56	0.47	19.51	30.29
PEG 20,000 + $C_6H_5K_3O_7/C_6H_8O_7$	15:15	36.81	1.76	0.0016	24.11	43.06
PEG 20,000 + $C_6H_5K_3O_7/C_6H_8O_7$	15:10	31.37	2.61	0.036	20.78	36.22

[a]Uncertainties lower than 10%.

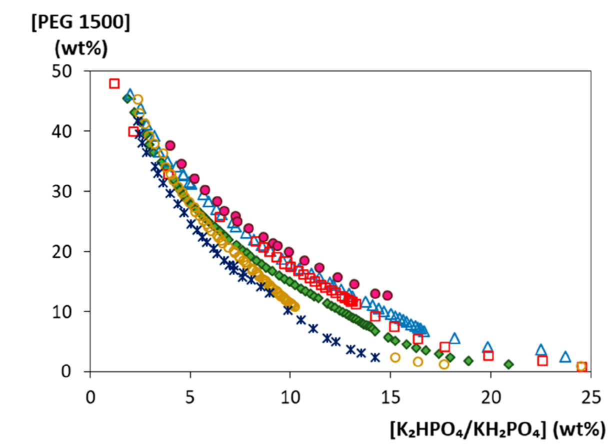

Figure 3. Phase diagrams for the systems composed of PEG 1500 + K_2HPO_4/KH_2PO_4 (pH 7) + ILs as adjuvants ((green ◆) without IL, (blue △) [C_2mim][CH_3CO_2], (red □) [C_2mim][CH_3SO_4], (yellow ○) [C_2mim][SCN], (dark blue X) [C_2mim][CF_3SO_3], and (pink ●) [C_2mim][$(CH_3O)_2PO_2$]) + water at 298 (±1) K.

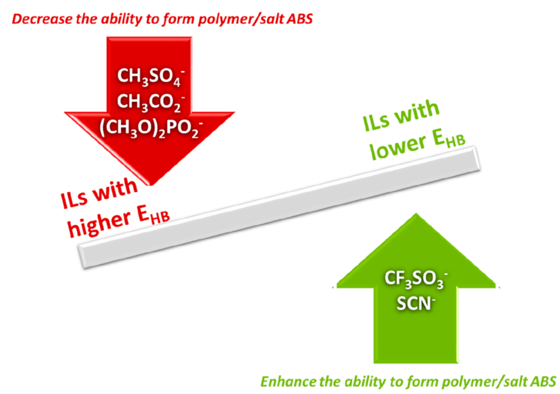

Figure 4. Effect of IL anion moiety regarding the ability to form PEG + salt buffer ABS with IL acting as adjuvants.

Table 5. Hydrogen-Bonding Interaction Energy of the Equimolar Cation-Anion Mixture (E_{HB}/(kJ·mol^{-1})) Taken from COSMO-RS Calculations for [C_4mim]-based ILs[a]

[C_4mim]/imidazolium-based ILs		
anion	abbreviation	E_{HB}/(kJ mol^{-1})
acetate	$[CH_3CO_2]^-$	−40.17
dimethylphosphate	$[(CH_3O)_2PO_2]^-$	−32.85
chloride	Cl^-	−30.72
triflate	$[CF_3SO_3]^-$	−17.11
thiocyanate	$[SCN]^-$	−17.01

[a]The list of anions is presented in a decreasing order of their hydrogen bond basicity. The data was adopted from literature.[31]

the binodal curve of systems composed of PEG 2000 with $C_6H_5K_3O_7/C_6H_8O_7$ (pH ≈ 7) and water.

3.2.1. Effect of Anion Moiety of IL as Adjuvant. The study of IL cation nature[24] and alkyl chain length[20] was already described for ILs as adjuvants in PEG/salt quaternary phase diagrams. Yet, the effect of IL anion moiety was never addressed for adjuvated PEG/salt-based ABS and is worth investigating due to its important role in PEG-based ABS formation. The ability of ILs as adjuvants to induce phase-separation is depicted in Figure 3 and follows the trend [C_2mim][CF_3SO_3] > [C_2mim][SCN] > [C_2mim][CH_3SO_4] ≈ [C_2mim][CH_3CO_2] > [C_2mim][$(CH_3O)_2PO_2$]. The anion moiety has a pronounced influence in the capacity to form ABS causing two distinct behaviors: (i) improving the ability to form two phases or (ii) decreasing it. The first class of ILs exhibit anions with a stronger hydrogen-bonding basicity and the second-class exhibit anions with a weak hydrogen-bonding basicity. The hydrogen-bond basicity is one of the most

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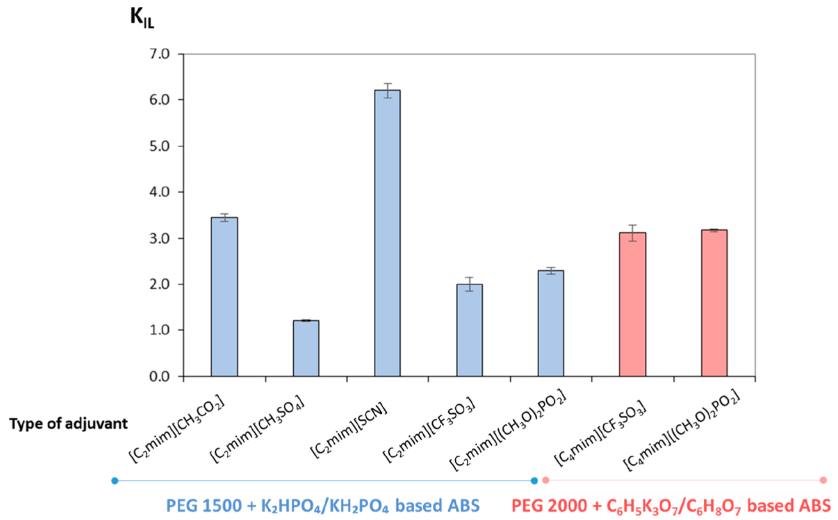

Figure 5. Graphical representation of K_{IL} values determined for the imidazolium-based ILs of different PEG + salt buffer ABS for a biphasic mixture point: 30 wt % PEG + 15 wt % salt buffer +5 wt % of IL.

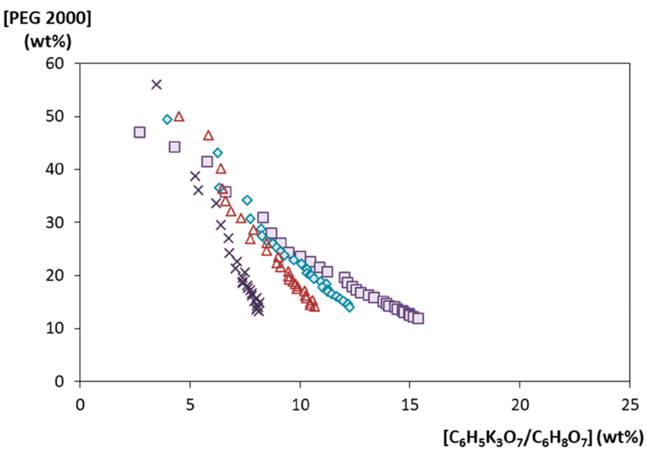

Figure 6. Phase diagrams for the systems composed of PEG 2000 + $C_6H_5K_3O_7/C_6H_8O_7$ (pH 7) + water at (purple ■) 0 wt %, (blue ◆) 5 wt %, (red △) 10 wt %, and (black X) 20 wt % of $[C_4mim][CF_3SO_3]$ as adjuvant at 298 (±1) K.

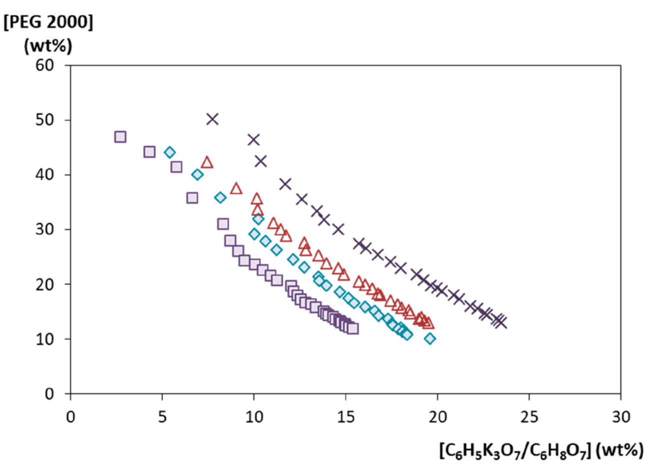

Figure 7. Phase diagrams for the systems composed of PEG 2000 + $C_6H_5K_3O_7/C_6H_8O_7$ (pH 7) + $[C_4mim][(CH_3O)_2PO_2]$ adjuvant at (blue ◆) 5 wt %, (red △) 10 wt %, and (black X) 20 wt % and (purple ■) without IL + water at 298 (±1) K.

important parameters reflecting the hydrogen-bond accepting ability of the IL anion and correlates with the hydrogen-bonding interaction energy in the equimolar cation−anion mixture $[E_{HB}/(kJ \cdot mol^{-1})]$ obtained from COSMO-RS. The E_{HB} values of the studied ILs are depicted in the Table 5, these data were previously detailed in literature.[31] Anions such as acetate, dimethylphosphate, and methylsulfate present high hydrogen-bond basicities (E_{HB}> 20 kJ·mol^{-1}) and thus an expected strong coordinating ability in aqueous solutions or other media able to donate protons.[31] On the other hand, the cyano (i.e. SCN^-) and fluorinated-based (i.e., $CF_3SO_3^-$) ILs are weak hydrogen-bond acceptors (E_{HB} < 20 kJ·mol^{-1}) and when combined with the imidazolium cation, result in noncoordinating ILs.[29] Moreover, for PEG with IL-based ABS the same phenomena was verified. Again, the ability of a specific anion to be preferentially hydrated largely depends on the anions' hydrogen bonding accepting ability, which consequently dictates their ability to form ABS.[11] Furthermore, for ILs with phosphate salt-based ABS, the effect of the anion moiety was also preponderant in the ability to form ABS.[32] This effect is schematically represented in Figure 4 in which the phase diagram region of PEG with salt-based ABS can be tuned by the use of appropriate IL anions, considering the E_{HB} value. To prove the effect of the IL hydrogen-bond basicity values, tests were performed varying the concentrations of ILs (5, 10, and 20 wt %) with low and high E_{HB} values, respectively, $[C_4mim][CF_3SO_3]$ and $[C_4mim][(CH_3O)_2PO_2]$. To understand the partitioning of the adjuvants, the K_{IL} was measured and is presented in Figure 5 and Table S11. For all systems, the IL partitioned preferentially to the top-phase with the highest value corresponding to the $[C_2mim][SCN]$ and the lowest to $[C_2mim][CH_3SO_4]$ (i.e., $K_{IL} \approx 1$).

3.2.2. Effect of IL Concentration as Adjuvant. The phase diagrams showing the effect of the IL concentration in the ABS formation of PEG 2000 with $C_6H_5K_3O_7/C_6H_8O_7$ (pH ≈ 7) systems are depicted in Figures 6 and 7 for $[C_4mim][CF_3SO_3]$ and $[C_4mim][(CH_3O)_2PO_2]$, respectively, with low and high E_{HB}. Although the IL concentration increased, the effects of enhancing or decreasing the ability to form ABS were even more pronounced for both cases. Therefore, an effect of the IL anion moiety in the binodal curves is clearly present. For instance, the biphasic point of 30 wt % of PEG 2000 and 10 wt

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% of $C_6H_5K_3O_7/C_6H_8O_7$ in the system with 5 wt % of $[C_4mim][CF_3SO_3]$ can be adjusted to 10 wt % of PEG 2000 and 10 wt % of $C_6H_5K_3O_7/C_6H_8O_7$ with the use of 20 wt % of $[C_4mim][CF_3SO_3]$, forming a biphasic mixture but using less amounts of polymer (about 20 wt %) to promote phase separation. For biphasic points with less than 10 wt % of PEG, the higher amount of $[C_4mim][CF_3SO_3]$ promoted the phase separation at lower buffer salt concentrations. The opposite effect is verified for $[C_4mim][(CH_3O)_2PO_2]$ as adjuvant. In this case, the biphasic point of 30 wt % of PEG 2000 and 10 wt % of $C_6H_5K_3O_7/C_6H_8O_7$ is observed in the system with 5 wt % of $[C_4mim][(CH_3O)_2PO_2]$ but for 20 wt % of $[C_4mim][(CH_3O)_2PO_2]$, 60 wt % of PEG 2000 is required to promote the ABS formation with 10 wt % $C_6H_5K_3O_7/C_6H_8O_7$. Since the polymer amount is reasonably higher, polymer precipitation could take place (solid−liquid equilibria).

4. CONCLUSIONS

ILs have been applied as adjuvants to induce phase separation of PEG with potassium salt buffer solutions. Therefore, new experimental equilibrium data for the compositions of the coexisting phases of ABS involving PEG with potassium (phosphate or citrate) buffer, hydrophilic imidazolium-based ILs and H_2O, at the same conditions of temperature and pressure (298 ± 1 K and atmospheric pressure), were studied and reported. The ability of imidazolium-based ILs as adjuvants to promote phase was shown to closely follow the increase of the hydrogen bond basicity value of the anions composing the IL. The results indicate that the PEG with potassium salt buffer systems can be finely tuned by the adjustment of the IL anion and concentration.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jced.9b01199.

> Compilation of weight fraction data (w) for the studied ABS; figures of phase diagrams in weight fraction units; K_{IL} values determined for the imidazolium-based ILs of different PEG + salt buffer-based ABS; and the ^{13}C NMR spectra of the studied ILs confirming their structure (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Albertsson, P. Partition of Proteins in Liquid Polymer-Polymer Two-Phase Systems. *Nature* **1958**, *182*, 709−711.

(2) Hatti-Kaul, R. Aqueous two-phase systems. A general overview. *Mol. Biotechnol.* **2001**, *19*, 269−277.

(3) Peters, T. J. Partition of cell particles and macromolecules: Separation and purification of biomolecules, cell organelles, membranes and cells in aqueous polymer two phase systems and their use in biochemical analysis and biotechnology. *Cell Biochem. Funct.* **1987**, *5*, 233−234.

(4) Silvério, S. C.; Wegrzyn, A.; Lladosa, E.; Rodríguez, O.; Macedo, E. A. Effect of aqueous two-phase system constituents in different poly(ethylene glycol)-salt phase diagrams. *J. Chem. Eng. Data* **2012**, *57*, 1203−1208.

(5) Delgado, C.; Malik, F.; Selisko, B.; Fischer, D.; Francis, G. E. Quantitative analysis of polyethylene glycol (PEG) in PEG-modified proteins/cytokines by aqueous two-phase systems. *J. Biochem. Biophys. Methods* **1994**, *29*, 237−250.

(6) Rito-Palomares, M.; Nunez, L.; Amador, D. Practical application of aqueous two-phase systems for the development of a prototype process for c-phycocyanin recovery from *Spirulina maxima*. *J. Chem. Technol. Biotechnol.* **2001**, *76*, 1273−1280.

(7) Pereira, J. F. B.; Kurnia, K. A.; Cojocaru, O. A.; Gurau, G.; Rebelo, L. P. N.; Rogers, R. D.; Freire, M. G.; Coutinho, J. A. P. Molecular interactions in aqueous biphasic systems composed of polyethylene glycol and crystalline *vs.* liquid cholinium-based salts. *Phys. Chem. Chem. Phys.* **2014**, *16*, 5723−31.

(8) Passos, H.; Ferreira, A. R.; Cláudio, A. F. M.; Coutinho, J. A. P.; Freire, M. G. Characterization of aqueous biphasic systems composed of ionic liquids and a citrate-based biodegradable salt. *Biochem. Eng. J.* **2012**, *67*, 68−76.

(9) Pereira, J. F. B.; Rebelo, L. P. N.; Rogers, R. D.; Coutinho, J. A. P.; Freire, M. G. Combining ionic liquids and polyethylene glycols to boost the hydrophobic-hydrophilic range of aqueous biphasic systems. *Phys. Chem. Chem. Phys.* **2013**, *15*, 19580−3.

(10) Galiński, M.; Lewandowski, A.; Stepniak, I. Ionic liquids as electrolytes. *Electrochim. Acta* **2006**, *51*, 5567−5580.

(11) Ventura, S. P. M.; Neves, C. M. S. S.; Freire, M. G.; Marrucho, I. M.; Oliveira, J.; Coutinho, J. A. P. Evaluation of Anion Influence on the Formation and Extraction Capacity of Ionic-Liquid-Based Aqueous Biphasic Systems. *J. Phys. Chem. B* **2009**, *113*, 9304−9310.

(12) Naushad, M.; Alothman, Z. A.; Khan, A. B.; Ali, M. Effect of ionic liquid on activity, stability, and structure of enzymes: a review. *Int. J. Biol. Macromol.* **2012**, *51*, 555−560.

(13) Freemantle, M. Designer solvents. *Chem. Eng. News* **1998**, *76*, 32−37.

(14) Lucena, I. V.; Brandão, I. V.; Mattedi, S.; Souza, R. L.; Soares, C. M. F.; Fricks, A. T.; Lima, Á. S. Use of protic ionic liquids as adjuvants in PEG-based ATPS for the purification of radish peroxidase. *Fluid Phase Equilib.* **2017**, *452*, 1−8.

(15) Yang, H.; Chen, L.; Zhou, C.; Yu, X.; Yagoub, A. E. G. A.; Ma, H. Improving the extraction of L-phenylalanine by the use of ionic liquids as adjuvants in aqueous biphasic systems. *Food Chem.* **2018**, *245*, 346−352.

(16) Santos, J. H.; e Silva, F. A.; Ventura, S. P. M.; Coutinho, J. A. P.; de Souza, R. L.; Soares, C. M. F.; Lima, Á. S. Ionic liquid-based aqueous biphasic systems as a versatile tool for the recovery of antioxidant compounds. *Biotechnol. Prog.* **2015**, *31*, 70−77.

(17) Santos, J. H. P. M.; Flores-Santos, J. C.; Meneguetti, G. P.; Rangel-Yagui, C. O.; Coutinho, J. A. P.; Vitolo, M.; Ventura, S. P. M.; Pessoa, A. *In situ* purification of periplasmatic L-asparaginase by aqueous two phase systems with ionic liquids (ILs) as adjuvants. *J. Chem. Technol. Biotechnol.* **2018**, *93*, 1871−1880.

(18) Souza, R. L.; Ventura, S. P. M.; Soares, C. M. F.; Coutinho, J. A. P.; Lima, Á. S. Lipase purification using ionic liquids as adjuvants in aqueous two-phase systems. *Green Chem.* **2015**, *17*, 3026−3034.

(19) Ferreira, A. M.; Faustino, V. F. M.; Mondal, D.; Coutinho, J. A. P.; Freire, M. G. Improving the extraction and purification of immunoglobulin G by the use of ionic liquids as adjuvants in aqueous biphasic systems. *J. Biotechnol.* **2016**, *236*, 166−175.

(20) de Souza, R. L.; Campos, V. C.; Ventura, S. P. M.; Soares, C. M. F.; Coutinho, J. A. P; Lima, Á. S. Effect of ionic liquids as adjuvants on PEG-based ABS formation and the extraction of two probe dyes. *Fluid Phase Equilib.* **2014**, *375*, 30−36.

(21) Sousa, R. d. C. S.; Pereira, M. M.; Freire, M. G.; Coutinho, J. A.P. Evaluation of the effect of ionic liquids as adjuvants in polymer-based aqueous biphasic systems using biomolecules as molecular probes. *Sep. Purif. Technol.* **2018**, *196*, 244−253.

(22) Neves, C. M. S. S.; Ventura, S. P. M.; Freire, M. G.; Marrucho, I. M.; Coutinho, J. A. P. Evaluation of cation influence on the formation and extraction capability of ionic-liquid-based aqueous biphasic systems. *J. Phys. Chem. B* **2009**, *113*, 5194−5199.

(23) Merchuk, J. C.; Andrews, B. A.; Asenjo, J. A. Aqueous two-phase systems for protein separation. Studies on phase inversion. *J. Chromatogr., Biomed. Appl.* **1998**, *711*, 285−293.

(24) Neves, C. M.S.S.; Sousa, R. d. C. S.; Pereira, M. M.; Freire, M. G.; Coutinho, J. A.P. Understanding the Effect of Ionic Liquids as Adjuvants in the Partition of Biomolecules in Aqueous Two-Phase Systems Formed by Polymers and Weak Salting-Out Agents. *Biochem. Eng. J.* **2019**, *141*, 239−246.

(25) Azevedo, A. M.; Gomes, A. G.; Rosa, P. A. J.; Ferreira, I. F.; Pisco, A. M. M. O.; Aires-Barros, M. R. Partitioning of human antibodies in polyethylene glycol - sodium citrate aqueous two-phase systems. *Sep. Purif. Technol.* **2009**, *65*, 14−21.

(26) Zafarani-Moattar, M. T.; Hamzehzadeh, S. Partitioning of amino acids in the aqueous biphasic system containing the water-miscible ionic liquid 1-butyl-3-methylimidazolium bromide and the water-structuring salt potassium citrate. *Biotechnol. Prog.* **2011**, *27*, 986−997.

(27) da Silva, L. H. M.; Coimbra, J. S. R.; Meirelles, A. J. de A. Equilibrium Phase Behavior of Poly(ethylene glycol) + Potassium Phosphate + Water Two-Phase Systems at Various pH and Temperatures. *J. Chem. Eng. Data* **1997**, *42*, 398−401.

(28) Shahriari, S.; Neves, C. M. S. S.; Freire, M. G.; Coutinho, J. A. P. Role of the Hofmeister Series in the Formation of Ionic-Liquid-Based Aqueous Biphasic Systems. *J. Phys. Chem. B* **2012**, *116*, 7252−7258.

(29) Alvarez-Guerra, E.; Ventura, S. P. M.; Coutinho, J. A. P.; Irabien, A. Ionic liquid-based three phase partitioning (ILTPP) systems: Ionic liquid recovery and recycling. *Fluid Phase Equilib.* **2014**, *371*, 67−74.

(30) Dinis, T. B.V; Passos, H.; Lima, D. L. D.; Esteves, V. I.; Coutinho, J. A. P.; Freire, M. G. One-step extraction and concentration of estrogens for an adequate monitoring of wastewaters using ionic-liquid based aqueous biphasic systems. *Green Chem.* **2015**, *17*, 2570−2579.

(31) Cláudio, A. F. M.; Swift, L.; Hallett, J. P.; Welton, T.; Coutinho, J. A. P.; Freire, M. G. Extended scale for the hydrogen-bond basicity of ionic liquids. *Phys. Chem. Chem. Phys.* **2014**, *16*, 6593−6601.

(32) Ventura, S. P. M.; Sousa, S. G.; Serafim, L. S.; Lima, Á. S.; Freire, M. G.; Coutinho, J. A. P. Ionic-Liquid-Based Aqueous Biphasic Systems with Controlled pH: The Ionic Liquid Anion Effect. *J. Chem. Eng. Data* **2012**, *57*, 507−512.

https://dx.doi.org/10.1021/acs.jced.9b01199
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