

Free Energy of Transfer of a Methylene Group in Biphasic Systems of Water and Ionic Liquids [C₃mpip][NTf₂], [C₃mpyrr][NTf₂], and [C₄mpyrr][NTf₂]

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ABSTRACT: Three hydrophobic ionic liquids (ILs) based on the bis(trifluoromethylsulfonyl)imide anion were studied in terms of the relative hydrophobicity of the equilibrium phases. The ILs were 1-methyl-1-propylpiperidinium bis(trifluoromethylsulfonyl)imide, [C₃mpip][NTf₂]; 1-methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide, [C₃mpyrr][NTf₂]; and 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide, [C₄mpyrr][NTf₂]. In addition, the liquid–liquid phase diagrams were determined for two of the ILs, namely, [C₃mpip][NTf₂] and [C₃mpyrr][NTf₂]. The compositions of the equilibrium phases were obtained using a gravimetric technique in the temperature range from 278.15 to 343.15 K. The immiscibility gaps for these systems were found to be very large, with that for the system [C₃mpip][NTf₂] + water being slightly wider than that for the system [C₃mpyrr][NTf₂] + water. Partition coefficients for a series of dinitrophenylated (DNP) amino acids in the three systems at 296.15 K were experimentally determined. The DNP amino acids were found to distribute preferentially to the water-rich phase, and the partition was more extreme for the system with [C₃mpip][NTf₂]. The experimental partition coefficients decreased as the size of the alkyl side chain in the solutes increased. The relative hydrophobicity of the equilibrium phases was assessed by means of the free energy of transfer of a methylene group between the phases, $\Delta G(\text{CH}_2)$, calculated from the partition coefficients of the series of solutes. It was found that the relative hydrophobicity between the phases follows the order [C₄mpyrr][NTf₂] > [C₃mpip][NTf₂] > [C₃mpyrr][NTf₂].

INTRODUCTION

Since the beginning of the 21st century, a new class of compounds called ILs has been emerging and gaining a great deal of attention from the scientific community. The attractive properties of these compounds, such as very low vapor pressure, high selectivity and solvation capability, low flammability, chemical and thermal stability, and ease of recovery,^{1–4} have led to their application in a series of different areas. Some of them are reaction media,^{1,5,6} catalysis,^{1,2,7} analytical chemistry,⁸ electrochemistry,⁹ liquid–liquid extraction,^{10,11} and biotechnology.^{12–18}

Considering the area of biotechnology, ILs have been widely used for several purposes. Lu et al.¹⁹ developed a new composite material based on chitosan and 1-butyl-3-methylimidazolium tetrafluoroborate ([C₄mim][BF₄]), which they used as an immobilization matrix for proteins and enzymes. They expect that this unique composite material can be applied in electrochemistry, biosensors, and biocatalysis. Li et al.²⁰ also developed a new composite system based on biomolecule-functionalized multiwalled carbon nanotubes and the IL 1-butyl-3-methylimidazolium hexafluorophosphate ([C₄mim][PF₆]). They concluded that the biocatalytic activity of the composite toward tryptophan was enhanced by the addition of the IL. Cai et al.²¹ reported the development of a new biosensor based on SBA-15. They added the IL [C₄mim][BF₄] to the biosensor and claimed that this improved its performance. They expect that the high sensitivity obtained in this way will allow for the application of the biosensor to the clinical diagnosis of breast cancer.

ILs have been widely used in biocatalysis.^{2,22} For example, Stahlberg et al.²³ reported the enzymatic conversion of glucose to fructose and xylose to xylulose in several ILs. They managed to recover the total amount of sugars after the reaction by extraction with aqueous HCl, which makes this procedure interesting for continuous operation. Li et al.²⁴ tested seven ILs for use in the regioselective acylation of 1- β -D-arabinofuranosylcytosine catalyzed by lipase. They found that the reaction rate was improved in the presence of small concentrations of most of the ILs studied. In addition, several studies have shown that biocatalytic reactions in ILs present higher enantioselectivities, yields, operational stabilities, and rates.^{25–28}

ILs have also been applied to the extraction and recovery of several biomolecules. The separation and purification of biomolecules is of the utmost importance in the field of biotechnology, so liquid–liquid extraction is commonly used in this area, for example, for the extraction of a specific biomolecule from a fermentation broth. Because ILs are good solvents for liquid–liquid extraction, their application to the recovery of several kinds of biomolecules has been frequent in the recent past, for example, in studies regarding the partitioning of proteins,^{29,30} amino acids,^{31–33} antibiotics,^{34–39} hormones,⁴⁰ DNA,⁴¹ and alkaloids,⁴² among others.

The application of ILs in these extraction processes is justified not only by their already-mentioned advantageous

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properties, but also by several studies that demonstrated that ILs can be biocompatible solvents. Numerous studies have reported that the stability of proteins and enzymes is maintained or even improved in the presence of ILs.^{43–49} The stabilization effect of different ILs on the enzyme α -chymotrypsin has been verified. For example, 1-butyl-3-methylimidazolium hexafluorophosphate ([C₄mim][PF₆]) increases the half-life of the enzyme by 200 times with respect to 1-propanol.⁵⁰ Also, Diego et al.⁴⁶ showed that the IL 1-ethyl-3-methyl-imidazolium bis(trifluoromethylsulfonyl)imide ([C₂mim][NTf₂]) has a stabilizing effect on the structure of α -chymotrypsin. Attri et al.⁵¹ also studied the stability and activity of α -chymotrypsin in the presence of five different ILs and concluded that the ILs triethyl ammonium acetate (TEAA) and triethyl ammonium phosphate (TEAP) are strong stabilizers for α -chymotrypsin. Weaver et al.⁵² found that the thermal stabilities of lysozyme and interleukin-2 increase with the concentration of the IL choline dihydrogen phosphate (CDHP). They also observed that the overall tertiary conformation of lysozyme remained unaltered in the presence of the same IL at 298.15 K, even though small changes were observed after incubation at 310.15 K. Even the recovery of protein activity after denaturation using the IL ethylammonium nitrate has been reported.⁵³ All of these examples demonstrate that ILs are suitable solvents for biomolecules.

The availability of experimental data about the liquid–liquid equilibria (LLE) of hydrophobic ILs and water is very important for certain applications, and such equilibria have been studied for several ILs. Our group has measured the liquid–liquid phase diagrams for the family of ionic liquids [C_{*n*}mim][BF₄] (with *n* = 6, 8, and 10) with water.^{54,55} Also, Freire et al.^{56,57} measured the same kind of phase diagrams for several [PF₆][−] and [NTf₂][−] ILs. Wang et al.⁵⁸ also measured the LLE of several ILs with the anions [PF₆][−] and [NTf₂][−]. Domanska et al.⁵⁹ determined the phase diagram for the LLE of the system [C₂mpip][NTf₂] + water.




In this work, the liquid–liquid phase diagrams of two hydrophobic ILs with water have been determined for a wide range of temperatures. The ILs were 1-methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide ([C₃mpyr][NTf₂]) and 1-methyl-1-propylpiperidinium bis(trifluoromethylsulfonyl)imide ([C₃mpip][NTf₂]). Additionally, the partition coefficients of a series of four dinitrophenylated (DNP) amino acids in the two systems were experimentally determined, as well as in the system with the IL 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide ([C₄mpyr][NTf₂]). These three ILs were chosen for several reasons: They are liquids at 296.15 K; their viscosities are low compared to those of other ILs; and they are hydrophobic, so when they are in contact with water, a two-phase system is formed. The four DNP-amino acids were *N*-(2,4-dinitrophenyl)glycine (DNP-Gly), *N*-(2,4-dinitrophenyl)-L-alanine (DNP-Ala), *N*-(2,4-dinitrophenyl)-DL-*n*-valine (DNP-Val), and *N*-(2,4-dinitrophenyl)-DL-*n*-leucine (DNP-Leu), which differ only in the size of the alkyl side chain of the molecule, namely, in the number of methylene groups. Consequently, the experimental partition coefficients allow for the determination of the free energy of transfer of a methylene group between the equilibrium phases.^{55,60} This property gives a measure of the relative hydrophobicity of the phases, which can provide some insight regarding the ability of ILs to participate in hydrophobic interactions. This information can be useful in understanding protein partitioning behavior in

water + IL biphasic systems. In fact, these types of experiments have been used previously to characterize the hydrophobic character of other types of systems^{61–63} (such as oil–water, 1-octanol–water, and aqueous biphasic systems) and to model the partitioning of solutes (namely, proteins and other biomolecules) in the mentioned systems.

MATERIALS AND METHODS

Materials. The ILs 1-methyl-1-propylpiperidinium bis(trifluoromethylsulfonyl)imide ([C₃mpip][NTf₂], 99%), 1-methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide ([C₃mpyr][NTf₂], 99%), and 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide ([C₄mpyr][NTf₂], 99%) were purchased from Iolitec. The chemical structures of the three ILs are presented in Table 1. The water contents of the ILs were measured by Karl Fischer titration using a Metrohm 870 KF Titrino plus titrator. The results are presented in Table 1.

Table 1. Chemical Structures, Short Names, and Water Content of the Investigated ILs

IL	Chemical structure	Short name	Water content
1-methyl-1-propylpiperidinium bis(trifluoromethylsulfonyl)imide		[C ₃ mpip][NTf ₂]	200 ppm
1-methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide		[C ₃ mpyr][NTf ₂]	250 ppm
1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide		[C ₄ mpyr][NTf ₂]	370 ppm

Dinitrophenylated (DNP) amino acids were obtained from Sigma [*N*-(2,4-dinitrophenyl)glycine, ≥99%; *N*-(2,4-dinitrophenyl)-DL-*n*-valine, ≥97%] and Research Organics [*N*-(2,4-dinitrophenyl)-L-alanine, ≥97%; *N*-(2,4-dinitrophenyl)-DL-*n*-leucine, ≥97%]. 1-Propanol was obtained from Merck (99.8% for liquid chromatography).

All products were used as received without further purification. Deionized water was used for all diluting purposes. All weighing was carried out on an Adam Equipment balance model AAA250L, precise to within ±0.2 mg.

Methods. Phase Diagrams. Phase diagrams of the systems [C₃mpyr][NTf₂] + water and [C₃mpip][NTf₂] + water were experimentally determined, for temperatures ranging from 278.15 to 343.15 K. Mixtures of each IL and water were added to jacketed glass cells (approximate volume of 50 mL) and were vigorously stirred for 2 h with a magnetic stirrer (IKA RO 15 Power). The cells were connected to a temperature-controlled (±0.10 K) water circulating bath (Grant LTC1). The temperatures inside the cells were monitored with a four-wire platinum resistance probe (Pt100) placed inside the cell with water, connected in parallel to the water bath and connected to an Agilent 34970A data acquisition unit (±0.01 K). This temperature measuring system was certified, presenting a maximum deviation of 0.06 K at 303.67 K. After being stirred, the mixtures were left to settle for at least 20 h, allowing phase separation to occur. Preliminary experiments showed that the stirring time of 2 h was adequate for reaching equilibrium. After phase separation, samples were collected from each phase in triplicate. Samples from the upper phase were collected using a volumetric pipet, and samples from the bottom phase were

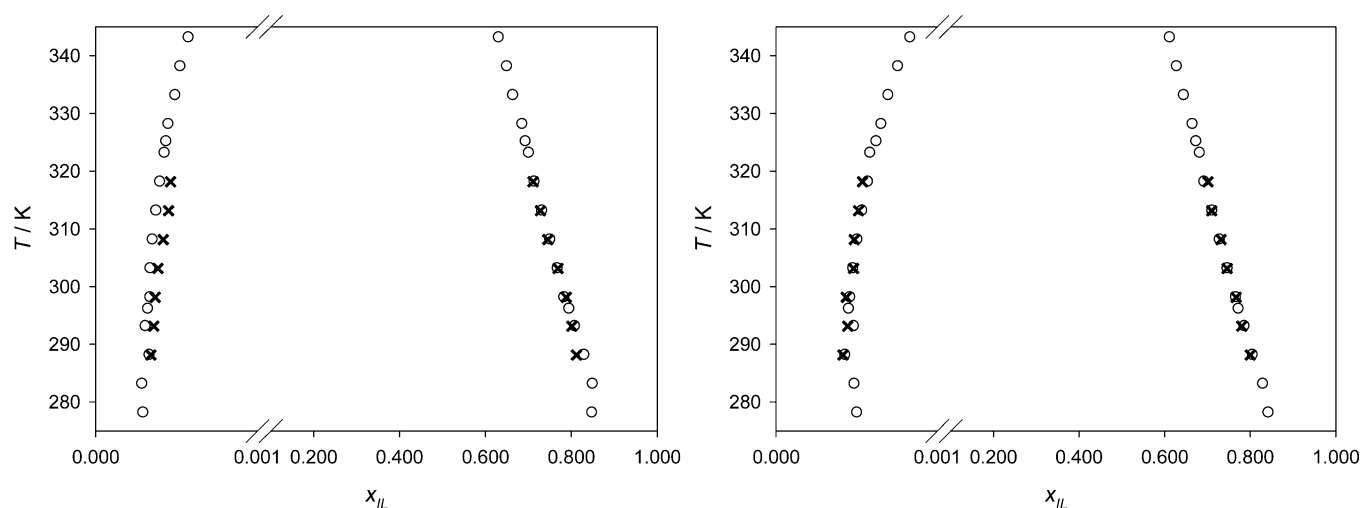


Figure 1. Phase diagrams of the systems (left) $[C_3mpip][NTf_2]$ + water and (right) $[C_3mpyr][NTf_2]$ + water: (O) this work, (x) literature.^{56,57}

Table 2. Compositions of the Equilibrium Phases for the Systems $[C_3mpyr][NTf_2]$ + Water and $[C_3mpip][NTf_2]$ + Water

T (K)	$[C_3mpip][NTf_2]$				$[C_3mpyr][NTf_2]$			
	x_{IL}^I	SD^a	$x_{IL}^{II} \times 10^4$	$SD \times 10^4^a$	w_{IL}^I	SD^a	$x_{IL}^{II} \times 10^4$	$SD \times 10^4^a$
278.15	0.8485	0.0019	2.95	0.04	0.8429	0.0033	5.03	1.24
283.15	0.8500	0.0043	2.88	0.11	0.8302	0.0027	4.87	0.82
288.15	0.8304	0.0014	3.34	0.23	0.8064	0.0005	4.30	0.03
293.15	0.8086	0.0007	3.10	0.01	0.7869	0.0029	4.84	0.52
296.15	0.7954	0.0007	3.25	0.08	0.7732	0.0014	4.53	0.03
298.15	0.7836	0.0016	3.38	0.34	0.7669	0.0025	4.60	0.02
303.15	0.7684	0.0028	3.39	0.03	0.7473	0.0012	4.79	0.01
308.15	0.7509	0.0008	3.53	0.01	0.7290	0.0005	5.06	0.01
313.15	0.7320	0.0018	3.76	0.09	0.7115	0.0018	5.36	0.01
318.15	0.7143	0.0019	3.99	0.03	0.6929	0.0003	5.71	0.02
323.15	0.7013	0.0012	4.27	0.06	0.6828	0.0028	5.85	0.41
325.15	0.6938	0.0011	4.37	0.08	0.6739	0.0003	6.23	0.01
328.15	0.6862	0.0047	4.51	0.01	0.6658	0.0006	6.54	0.06
333.15	0.6646	0.0031	4.94	0.22	0.6454	0.0016	6.98	0.08
338.15	0.6503	0.0002	5.25	0.00	0.6288	0.0008	7.58	0.08
343.15	0.6316	0.0004	5.75	0.02	0.6127	0.0009	8.34	0.40

$$^aSD = \{[\sum_{i=1}^N (x_i - \bar{x})^2] / (N - 1)\}^{1/2}.$$

collected using a syringe. The samples were then placed on previously weighed glass vials, which were weighed again and placed to dry on a heating plate, at an approximate temperature of 338.15 K to achieve the slow evaporation of water. The masses of the glass vials were monitored through time until the values of consecutive weighings remained unchanged, which meant that all of the water had evaporated. Using this gravimetric method, it was possible to determine the masses of both IL and water in all samples, thus allowing for the calculation of the equilibrium compositions in both phases. Different tie lines were obtained by changing the temperature of the circulating water bath. The accuracy of the gravimetric method was tested in preliminary experiments. Samples of known composition were prepared by adding known quantities of water and IL. Samples with different compositions were prepared in the range of compositions of the water-rich phases, as well as in the range of compositions of the IL-rich phases. Then, the gravimetric method was used to experimentally determine the compositions of the samples. The experimentally determined compositions were compared with the real

compositions of the samples, giving an average relative deviation of 0.11% and a maximum relative deviation of 0.22%.

DNP Amino Acid Partition Coefficients. Partition coefficients of four dinitrophenylated (DNP) amino acids in both systems were determined experimentally at 296.15 K. Aqueous stock solutions of DNP amino acids with a concentration of 0.2 wt % were prepared. IL + water biphasic systems were obtained by mixing precisely known volumes of the two components. Volumes were measured with an automatic pipet (Eppendorf Multipette Xstream). The volumes used to prepare the biphasic systems depended on how extreme the solute partition was for the different DNP amino acids. Six replicates of the systems were prepared in 2 mL Eppendorf tubes. Volumes of 0 μ L (blank) to 100 μ L of a given DNP amino acid stock solution were added to each tube. The corresponding volume of water (100 to 0 μ L) was also added to keep the total IL + water volume constant in all tubes. The tubes were thoroughly vortex mixed for 5 min and then centrifuged at 10⁴ rpm for 15 min to accelerate phase separation. Samples from the top and bottom phases were collected and conveniently diluted for analysis by UV/vis spectroscopy (Thermo Electron Corp., model UV1) at

a wavelength of 362 nm. Absorbance was lower than 2.0 in all cases. Water was used for dilution of samples from the top phases (water-rich), and 1-propanol was used for dilution of samples from the bottom phases (IL-rich). Calibration lines for the concentration range studied were obtained for each solute in each solvent (water and 1-propanol) with each IL present at the same composition in which it was present in the diluted samples. The calibration lines were linear in all cases. The partition coefficient for each DNP amino acid was calculated as the slope of the straight line obtained when the concentration in the top phase was plotted against the concentration in the bottom phase. The increasing concentration of solute in different replicates allowed the thermodynamic partition coefficients to be obtained independent of solute concentration, confirming that there were no aggregation effects.

RESULTS AND DISCUSSION

Phase Diagrams. Experimental liquid–liquid phase diagrams for the systems $[\text{C}_3\text{mpip}][\text{NTf}_2]$ + water and $[\text{C}_3\text{mpyr}][\text{NTf}_2]$ + water are presented in Figure 1. The compositions of the equilibrium phases together with their standard deviations are given in Table 2. The average standard deviations were 9.0×10^{-4} for the system $[\text{C}_3\text{mpip}][\text{NTf}_2]$ + water and 7.6×10^{-4} for the system $[\text{C}_3\text{mpyr}][\text{NTf}_2]$ + water. The maximum standard deviations were 4.7×10^{-3} and 3.3×10^{-3} , respectively. The analytical method proved to be very accurate, even for samples with very low mass fractions of one of the compounds. In Figure 1, results from the literature for the same phase diagrams are also represented. Freire et al.^{56,57} measured the mutual solubilities of $[\text{C}_3\text{mpip}][\text{NTf}_2]$ and $[\text{C}_3\text{mpyr}][\text{NTf}_2]$ and water for some temperatures ranging from 288.15 to 318.15 K. When comparing the results from this work with the previous results from Freire et al.,^{56,57} it is possible to observe an excellent agreement between the two sets of experimental data, even though the analytical techniques for the determination of phase compositions were not the same. Freire et al. determined the solubilities of water in the ILs using a Karl Fischer coulometer, and they determined the solubilities of the ILs in water by mass spectroscopy. This confirms the accuracy of the analytical technique (gravimetric method) chosen in this work.

The immiscibility gap for these two systems is very large. The solubility of water in the ILs is shown in Figure 2. The water uptake by the IL $[\text{C}_3\text{mpip}][\text{NTf}_2]$ was found to vary between 15.0 and 36.8 mol %, and that for $[\text{C}_3\text{mpyr}][\text{NTf}_2]$ varied between 15.7 and 38.7 mol %. On the other hand, the molar percentage of IL in the water-rich phases varied between 0.028 and 0.058 mol % for $[\text{C}_3\text{mpip}][\text{NTf}_2]$ and between 0.043 and 0.083 mol % for $[\text{C}_3\text{mpyr}][\text{NTf}_2]$. The solubility of the ILs in water is represented in Figure 3. It is possible to observe, from Table 2 and Figures 2 and 3, that the mutual solubilities between both ILs and water are very low, although slightly higher for $[\text{C}_3\text{mpyr}][\text{NTf}_2]$; that is, the immiscibility gap is slightly wider for the IL with the piperidinium-based cation than for that with the pyrrolidinium-based cation. As expected, both the fraction of IL in the water-rich phases and the fraction of water in the IL-rich phases increased slightly with temperature. Therefore, it is safe to say that the systems would present an upper critical solution temperature (UCST) at higher temperatures, if $\text{UCST} < 373.15 \text{ K}$ (boiling temperature for water).

In Figures 2 and 3, previously published results for the solubilities of water in IL and IL in water are also presented for

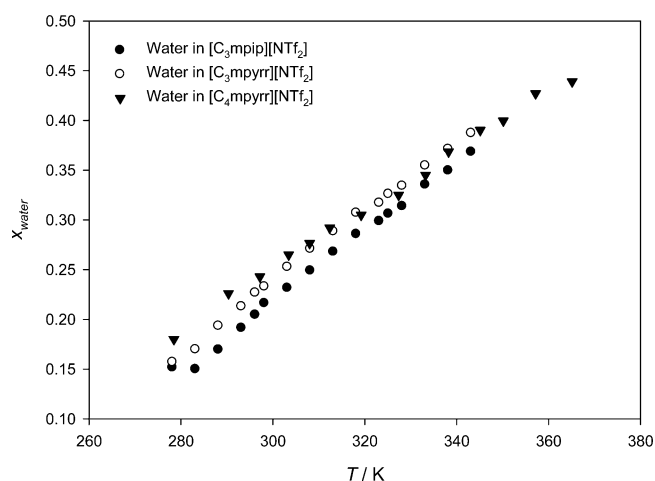


Figure 2. Mole fraction solubilities of water in ILs. Results for IL $[\text{C}_4\text{mpyr}][\text{NTf}_2]$ are from ref 58.

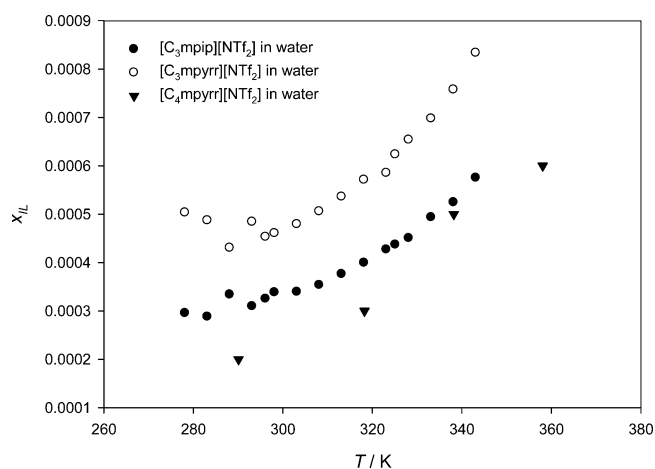


Figure 3. Mole fraction solubilities of ILs in water. Results for IL $[\text{C}_4\text{mpyr}][\text{NTf}_2]$ are from ref 58.

$[\text{C}_4\text{mpyr}][\text{NTf}_2]$.⁵⁸ Regarding the solubility of water in the IL, it can be observed that, for low temperatures (lower than approximately 300 K), the uptake of water from $[\text{C}_4\text{mpyr}][\text{NTf}_2]$ is higher than that for the rest of the ILs. For temperatures higher than 300 K, the solubility of water in $[\text{C}_4\text{mpyr}][\text{NTf}_2]$ is still higher than the solubility in $[\text{C}_3\text{mpip}][\text{NTf}_2]$, but smaller than that in $[\text{C}_3\text{mpyr}][\text{NTf}_2]$. However, the three solubilities are very similar. Concerning the IL solubilities in water, they vary in the order $[\text{C}_4\text{mpyr}][\text{NTf}_2] < [\text{C}_3\text{mpip}][\text{NTf}_2] < [\text{C}_3\text{mpyr}][\text{NTf}_2]$. It is important to note that the cloud-point method was used to determine the phase diagram for $[\text{C}_4\text{mpyr}][\text{NTf}_2]$,⁵⁸ which is different from the technique used in this work. Domanska et al.⁵⁹ determined the LLE of the system $[\text{C}_2\text{mpip}][\text{NTf}_2]$ + water. When comparing with results from this work for the system $[\text{C}_3\text{mpip}][\text{NTf}_2]$ + water, it can be observed that both mutual solubilities (IL in water and water in IL) were higher for the system $[\text{C}_2\text{mpip}][\text{NTf}_2]$ + water, which is in accordance with lower hydrophobicities associated with smaller alkyl side chains.

DNP Amino Acids Partition Coefficients. The theory related to the determination of the free energy of transfer of a methylene group between equilibrium phases has been previously explained and used by our group and others.^{55,60,61,64} When a solute, i , is transferred between two

phases in equilibrium, α and β , the free energy change, ΔG , is given by the difference between the chemical potentials of the solute, μ_i , in the two phases

$$\Delta G = \mu_i^\beta - \mu_i^\alpha \quad (1)$$

A contribution to ΔG results directly from the transfer of solute i from one phase to the other. This contribution is given by

$$\Delta G^* = \Delta G - RT \ln \left(\frac{C_i^\beta}{C_i^\alpha} \right) \quad (2)$$

where R is the universal gas constant, T is the temperature, and C_i is the concentration of component i in the indicated phase (α or β). At equilibrium, $\Delta G = 0$, and the concentration ratio on the right-hand side of eq 2 is the partition coefficient of solute, K_i

$$K_i = \frac{C_i^\beta}{C_i^\alpha} \quad (3)$$

Then ΔG^* can be written as a function of the partition coefficient

$$\Delta G^* = -RT \ln K_i \quad (4)$$

A more detailed explanation can be found elsewhere.^{65,66}

By taking into account the concept of group contributions, the free energy of transfer can be regarded as the combination of two different contributions, namely, the alkyl part of the molecule, constituted by n methylene groups, and the nonalkyl part of the molecule, as follows^{61–63}

$$\ln K = C + En(\text{CH}_2) \quad (5)$$

where $n(\text{CH}_2)$ is the number of methylene groups in the alkyl chain, the parameter C represents the contribution of the nonalkyl part of the molecule to the partition coefficient, and the parameter E represents the contribution of each methylene group to the partition coefficient. When considering a series of solutes that differ only in the length of the alkyl chain, a plot of the logarithm of the partition coefficients as a function of the number of equivalent methylene groups in the molecule will result in a straight line. The slope of this linear relation is equal to the parameter E , and the y intercept is equal to the parameter C . From eqs 4 and 5, the free energy of transfer of a methylene group can be calculated from parameter E as

$$\Delta G(\text{CH}_2) = -RTE \quad (6)$$

$\Delta G(\text{CH}_2)$ represents a measure of the difference between the affinities of the two phases for a CH_2 group. Because hydrocarbons are considered to be purely hydrophobic compounds, this property can be regarded as a measure of the relative hydrophobicity between phases α and β . In this work, the series of homologous solutes was a set of four dinitrophenylated (DNP) amino acids. The experimental partition coefficients of the DNP amino acids in the three systems studied are presented in Table 3.

All partition coefficients were found to be greater than unity. Because the partition coefficients were calculated as ratios between the concentrations in the top and bottom phases and the IL-rich phase was the bottom phase, this means that the DNP amino acids distributed preferentially to the water-rich phase. The partition coefficients decreased in the order $K_{\text{gly}} > K_{\text{ala}} > K_{\text{val}} > K_{\text{leu}}$. Therefore, the affinity of the solute for the IL-rich phase increased as the size of the alkyl chain in the amino

Table 3. Experimental Partition Coefficients of DNP Amino Acids for the Systems $[\text{C}_3\text{mpip}][\text{NTf}_2] + \text{Water}$, $[\text{C}_3\text{mpyr}][\text{NTf}_2] + \text{Water}$, and $[\text{C}_4\text{mpyr}][\text{NTf}_2] + \text{Water}$ at 293.15 K

solute	$[\text{C}_3\text{mpip}][\text{NTf}_2]$	$[\text{C}_3\text{mpyr}][\text{NTf}_2]$	$[\text{C}_4\text{mpyr}][\text{NTf}_2]$
DNP-Gly	526 ± 12	397.5 ± 2.1	413.4 ± 4.6
DNP-Ala	159.3 ± 2.2	150.4 ± 1.8	134.3 ± 3.4
DNP-Val	41.2 ± 1.1	40.31 ± 0.39	32.26 ± 0.77
DNP-Leu	18.24 ± 0.38	16.97 ± 0.11	11.40 ± 0.25

acid increased. Regarding the three IL + water systems, the partition of the DNP amino acids was more extreme for the system with the IL $[\text{C}_3\text{mpip}][\text{NTf}_2]$. Regarding the errors associated with the partition coefficients, the most significant are those for DNP-valine in the system with the IL $[\text{C}_3\text{mpip}][\text{NTf}_2]$ and DNP-alanine in the system with the IL $[\text{C}_4\text{mpyr}][\text{NTf}_2]$, which represent 2.54% and 2.53%, respectively, of the experimental value. All other errors were lower than 2.5%, and the average error was 1.62%.

In Figure 4, the linear relations between the logarithm of the partition coefficients and the equivalent number of methylene

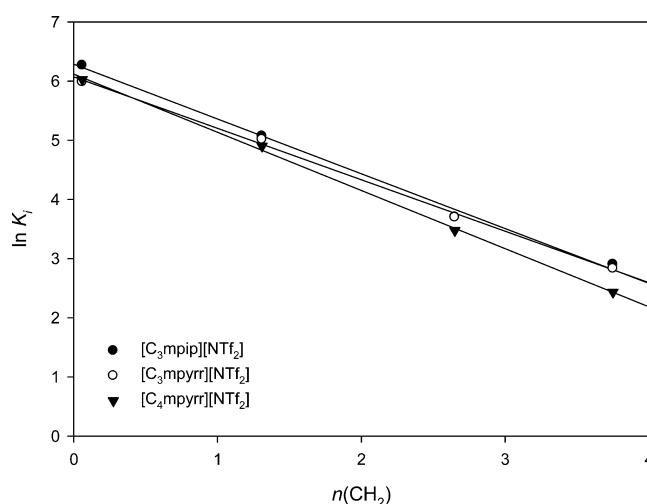


Figure 4. Natural logarithms of the experimental partition coefficients (K_i) as a function of the equivalent number of methylene groups in the alkyl chains of the DNP amino acids.

groups (CH_2) in the side chain of the solutes are shown. The number of equivalent methylene groups in the solutes was not exactly the same as the real number of methylene groups (0, 1, 3, and 4 for DNP-glycine, -alanine, -valine, and -leucine, respectively), and the reason for this was explained previously.⁶¹

As mentioned before, the relationships observed in Figure 4 are linear and can be described by eq 5. For this reason, parameters C and E can be calculated and are presented in Table 4 for all systems, together with regression coefficients (r^2) and the calculated free energies of transfer of a methylene group between the equilibrium phases, $\Delta G(\text{CH}_2)$.

Given that the IL-rich phase was the bottom phase, the free energy of transfer refers to the transfer of a methylene group from the IL-rich phase to the water-rich phase. As the calculated values of $\Delta G(\text{CH}_2)$ are positive, this transfer is not spontaneous. This shows that the IL-rich phases are more hydrophobic than the water-rich phases, as expected. The free energies of transfer of a methylene group and, thus, the relative

Table 4. Parameters *C* and *E*; Linear Regression Coefficients; Standard Deviations of Regressions; and Free Energies of Transfer of a Methylene Group, $\Delta G(\text{CH}_2)$

IL	<i>C</i>	<i>E</i>	r^2	standard deviation of regression	$\Delta G(\text{CH}_2)$ (kcal·mol ⁻¹)
[C ₃ mpip][NTf ₂]	6.28 ± 0.09	−0.92 ± 0.04	0.9961	0.1	0.544
[C ₃ mpyrr][NTf ₂]	6.07 ± 0.07	−0.87 ± 0.03	0.9977	0.08	0.512
[C ₄ mpyrr][NTf ₂]	6.12 ± 0.05	−0.98 ± 0.02	0.9990	0.06	0.585

hydrophobicities of the IL-rich phase follow the order [C₄mpyrr][NTf₂] > [C₃mpip][NTf₂] > [C₃mpyrr][NTf₂]. This variation is in accordance with the solubilities of the ILs in water observed in Figure 3; that is, the most hydrophobic IL is the least soluble in water. If we compare the relative hydrophobicities of the IL-rich phases, we are able to make several conclusions. First, the relative hydrophobicity of [C₄mpyrr][NTf₂] is greater than that of [C₃mpyrr][NTf₂]. Because these ILs differ only in the length of the alkyl side chain, this was expected: longer alkyl chains are associated with higher hydrophobicity. [C₃mpip][NTf₂] and [C₃mpyrr][NTf₂] differ only in the size of the ring. As can be seen in Table 1, [C₃mpip]⁺ has a ring with five carbon atoms, whereas [C₃mpyrr]⁺ has a ring with only four carbon atoms. It is again possible to correlate the higher relative hydrophobicity with the larger number of methylene groups (this time on the ring instead of the side chain). Finally, one can compare the relative hydrophobicities of the ILs [C₄mpyrr][NTf₂] and [C₃mpip][NTf₂]. The structures of these two ILs differ in both the size of the alkyl side chain and the size of the ring, but the total number of methylene groups is the same: [C₄mpyrr][NTf₂] has a longer alkyl side chain than [C₃mpip][NTf₂] (by one methylene group), but [C₃mpip][NTf₂] has a larger ring (also by one methylene group). It can be concluded that methylene groups in the alkyl chains provide a larger increase of hydrophobicity than methylene groups in (nonaromatic) rings. In our opinion, this can be explained by the fact that methylene groups in a ring (as in [C₃mpip]⁺) are in a more rigid position than methylene groups in alkyl chains (as in [C₄mpyrr]⁺), which have more freedom of movement.

When comparing these results to those obtained for usual aqueous two-phase systems (ATPSs), the calculated values of $\Delta G(\text{CH}_2)$ are similar to those obtained for polymer-salt ATPSs (0.2–0.7 kcal/mol).^{61,63,67}

CONCLUSIONS

The liquid–liquid phase diagrams for the systems [C₃mpyrr][NTf₂] + water and [C₃mpip][NTf₂] + water were experimentally determined for temperatures ranging from 278.15 K up to 343.15 K. The immiscibility gaps for these systems were found to be very large, and therefore, the UCSTs of the systems were not reached. The mutual solubilities are low (IL in water and vice versa). The two systems show similar immiscibility gaps, with that of cation [C₃mpip]⁺ being slightly larger.

The partition coefficients of the DNP amino acids in the two systems and in the system with the IL [C₄mpyrr][NTf₂] revealed that the solutes distribute preferentially to the water-rich phase. The partition coefficient decreased as the alkyl side chain in the amino acid increased. When comparing the three ILs, the partition was more extreme for the system [C₃mpip][NTf₂] + water, which means that higher concentrations of solutes were obtained for this system in the water-rich (top) phase. The free energy of transfer of a methylene group was found to be positive for all systems, which means that this

transfer is not spontaneous. $\Delta G(\text{CH}_2)$ was found to be higher for the system with the IL [C₄mpyrr][NTf₂]; therefore, the relative hydrophobicity between phases is higher for this system. When comparing the relative hydrophobicities of the IL-rich phases with the respective IL's structures, it was possible to conclude that a longer chain, both in the alkyl side chain and in the ring that forms the cation, is associated with a higher relative hydrophobicity. However, increasing the alkyl side chain was found to have a larger effect on hydrophobicity than increasing the ring.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Welton, T. Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis. *Chem. Rev.* **1999**, 99, 2071.
- (2) van Rantwijk, F.; Sheldon, R. A. Biocatalysis in Ionic Liquids. *Chem. Rev.* **2007**, 107, 2757.
- (3) Attri, P.; Reddy, P. M.; Venkatesu, P.; Kumar, A.; Hofman, T. Measurements and Molecular Interactions for *N,N*-Dimethylformamide with Ionic Liquid Mixed Solvents. *J. Phys. Chem. B* **2010**, 114, 6126.
- (4) Anderson, J. L.; Ding, J.; Welton, T.; Armstrong, D. W. Characterizing Ionic Liquids on the Basis of Multiple Solvation Interactions. *J. Am. Chem. Soc.* **2002**, 124, 14247.
- (5) Wasserscheid, P.; Welton, T. *Ionic Liquids in Synthesis*; Wiley-VCH: Weinheim, Germany, 2007; Vol. 2.
- (6) Favre, F.; Olivier-Bourbigou, H.; Commereuc, D.; Saussine, L. Hydroformylation of 1-hexene with rhodium in non-aqueous ionic liquids: How to design the solvent and the ligand to the reaction. *Chem. Commun.* **2001**, 1360.
- (7) Dupont, J.; Souza, R. F.; Suarez, P. A. Z. Ionic Liquid (Molten Salt) Phase Organometallic Catalysis. *Chem. Rev.* **2002**, 102, 3667.
- (8) Anderson, J. L.; Armstrong, D. W.; Wei, G. T. Ionic Liquids in Analytical Chemistry. *Anal. Chem.* **2006**, 78, 2892.
- (9) Gorlov, M.; Kloo, L. Ionic liquid electrolytes for dye-sensitized solar cells. *Dalton Trans.* **2008**, 37, 2655.
- (10) Wu, C. T.; Marsh, K. N.; Deev, A. V.; Boxall, J. A. Room temperature ionic liquids as novel media for 'clean' liquid–liquid extraction. *Chem. Commun.* **1998**, 1765.
- (11) Wei, G. T.; Chen, J. C.; Yang, Z. Studies on Liquid/Liquid Extraction of Copper Ion with Room Temperature Ionic Liquid. *J. Chin. Chem. Soc.* **2003**, 50, 1123.

- (12) Ohno, H.; Suzuki, C.; Fukumoto, K. K.; Yoshizawa, M.; Fujita, K. Electron transfer process of poly(ethylene oxide) modified cytochrome c in imidazolium type ionic liquid. *Chem. Lett.* **2003**, 32, 450.
- (13) Turner, M. B.; Spear, S. K.; Holbrey, J. D.; Daly, D. T.; Rogers, R. D. Ionic Liquid-Reconstituted Cellulose Composites as Solid Support Matrices for Biocatalyst Immobilization. *Biomacromolecules* **2005**, 6, 2497.
- (14) Soni, S. K.; Ramanathan, R.; Coloe, P. J.; Bansal, V.; Bhargava, S. K. Self-Assembled Enzyme Capsules in Ionic Liquid [BMIM][BF₄] as Templating Nanoreactors for Hollow Silica Nanocontainers. *Langmuir* **2010**, 26, 16020.
- (15) Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. Dissolution of Cellulose with Ionic Liquids. *J. Am. Chem. Soc.* **2002**, 124, 4974.
- (16) Leone, A. M.; Weatherly, S. C.; Williams, M. E.; Thorp, H. H.; Murray, R. W. An Ionic Liquid Form of DNA: Redox-Active Molten Salts of Nucleic Acids. *J. Am. Chem. Soc.* **2001**, 123, 218.
- (17) Fukumoto, K.; Yoshizawa, M.; Ohno, H. Room Temperature Ionic Liquids from 20 Natural Amino Acids. *J. Am. Chem. Soc.* **2005**, 127, 2398.
- (18) Cheng, D. H.; Chen, X. W.; Wang, J. H.; Fang, Z. L. An Abnormal Resonance Light Scattering Arising from Ionic-Liquid/DNA/Ethidium Interactions. *Chem.—Eur. J.* **2007**, 13, 4833.
- (19) Lu, X.; Hu, J.; Yao, X.; Wang, Z.; Li, J. Composite System Based on Chitosan and Room-Temperature Ionic Liquid: Direct Electrochemistry and Electrocatalysis of Hemoglobin. *Biomacromolecules* **2006**, 7, 975.
- (20) Li, L.; Bu, C.; Zhang, Y.; Du, J.; Lu, X.; Liu, X. Composite system based on biomolecules-functionalized multiwalled carbon nanotube and ionic liquid: Electrochemistry and electrocatalysis of tryptophane. *Electrochim. Acta* **2011**, 58, 105.
- (21) Cai, Y.; Li, H.; Du, B.; Yang, M.; Li, Y.; Wu, D.; Zhao, Y.; Dai, Y.; Wei, Q. Ultrasensitive electrochemical immunoassay for BRCA1 using BMIM-BF₄-coated SBA-15 as labels and functionalized graphene as enhancer. *Biomaterials* **2011**, 32, 2117.
- (22) Galonde, N.; Nott, K.; Debuigne, A.; Deleu, M.; Jérôme, C.; Paquoth, M.; Wathelata, J. P. Use of ionic liquids for biocatalytic synthesis of sugar derivatives. *J. Chem. Technol. Biotechnol.* **2012**, 87, 451.
- (23) Stahlberg, T.; Woodley, J. M.; Riisager, A. Enzymatic isomerization of glucose and xylose in ionic liquids. *Catal. Sci. Technol.* **2012**, 2, 291.
- (24) Li, X. F.; Lou, W. Y.; Smith, T. J.; Zong, M. H.; Wua, H.; Wang, J. F. Efficient regioselective acylation of 1- β -D-arabinofuranosylcytosine catalyzed by lipase in ionic liquid containing systems. *Green Chem.* **2006**, 8, 538.
- (25) Kragl, U.; Eckstein, M.; Kaftzik, N. Enzyme catalysis in ionic liquids. *Curr. Opin. Biotechnol.* **2002**, 13, 565.
- (26) Sheldon, R. A.; Lau, R. M.; Sorgedraeger, M. J.; van Rantwijk, F.; Seddon, K. R. Biocatalysis in ionic liquids. *Green Chem.* **2002**, 4, 147.
- (27) Park, S.; Kazlauskas, R. J. Biocatalysis in ionic liquids—Advantages beyond green technology. *Curr. Opin. Biotechnol.* **2003**, 14, 432.
- (28) van Rantwijk, F.; Lau, R. M.; Sheldon, R. A. Biocatalytic transformations in ionic liquids. *Trends Biotechnol.* **2003**, 21, 131.
- (29) Du, Z.; Yu, Y. L.; Wang, J. H. Extraction of Proteins from Biological Fluids by Use of an Ionic Liquid/Aqueous Two-Phase System. *Chem.—Eur. J.* **2007**, 13, 2130.
- (30) Dreyer, S.; Salim, P.; Kragl, U. Driving forces of protein partitioning in an ionic liquid-based aqueous two-phase system. *Biochem. Eng. J.* **2009**, 46, 176.
- (31) Absalan, G.; Akhond, M.; Sheikhan, L. Partitioning of acidic, basic and neutral amino acids into imidazolium-based ionic liquids. *Amino Acids* **2010**, 39, 167.
- (32) Wang, J.; Pei, Y.; Zhao, Y.; Hu, Z. Recovery of amino acids by imidazolium based ionic liquids from aqueous media. *Green Chem.* **2005**, 7, 196.
- (33) Zafarani-Moattar, M. T.; Hamzehzadeh, S.; Nasiri, S. A New Aqueous Biphasic System Containing Polypropylene Glycol and a Water-Miscible Ionic Liquid. *Biotechnol. Prog.* **2012**, 28, 146.
- (34) Han, J.; Wang, Y.; Yu, C.; Li, C.; Yan, Y.; Lui, Y.; Wang, L. Separation, concentration and determination of chloramphenicol in environment and food using an ionic liquid/salt aqueous two-phase flotation system coupled with high-performance liquid chromatography. *Anal. Chim. Acta* **2011**, 685, 138.
- (35) Han, J.; Wang, Y.; Kang, W.; Li, C.; Yan, Y.; Pan, J.; Xie, X. Phase equilibrium and macrolide antibiotics partitioning in real water samples using a two-phase system composed of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate and an aqueous solution of an inorganic salt. *Microchim. Acta* **2010**, 169, 15.
- (36) Liu, Q.; Yu, J.; Li, W.; Hu, X.; Xia, H.; Liu, H.; Yang, P. Partitioning Behavior of Penicillin G in Aqueous Two Phase System Formed by Ionic Liquids and Phosphate. *Sep. Sci. Technol.* **2006**, 41, 2849.
- (37) Soto, A.; Arce, A.; Khoshkbarchi, M. K. Partitioning of antibiotics in a two-liquid phase system formed by water and a room temperature ionic liquid. *Sep. Purif. Technol.* **2005**, 44, 242.
- (38) Li, C. X.; Han, J.; Wang, Y.; Yan, Y. S.; Xu, X. H.; Pan, J. M. Extraction and mechanism investigation of trace roxithromycin in real water samples by use of ionic liquid—salt aqueous two-phase system. *Anal. Chim. Acta* **2009**, 653, 178.
- (39) Han, J.; Wang, Y.; Yu, C.; Li, C.; Yan, Y.; Liu, Y.; Wang, L. Separation, concentration and determination of chloramphenicol in environment and food using an ionic liquid/salt aqueous two-phase flotation system coupled with high-performance liquid chromatography. *Anal. Chim. Acta* **2011**, 685, 138.
- (40) He, C.; Li, S.; Liu, H.; Li, K.; Liu, F. Extraction of testosterone and epitestosterone in human urine using aqueous two-phase systems of ionic liquid and salt. *J. Chromatogr. A* **2005**, 1082, 143.
- (41) Wang, J. H.; Cheng, D. H.; Chen, X. W.; Du, Z.; Fang, Z. L. Direct Extraction of Double-Stranded DNA into Ionic Liquid 1-Butyl-3-methylimidazolium Hexafluorophosphate and Its Quantification. *Anal. Chem.* **2007**, 79, 620.
- (42) Li, S.; He, C.; Liu, H.; Li, K.; Liu, F. Ionic liquid-based aqueous two-phase system, a sample pretreatment procedure prior to high-performance liquid chromatography of opium alkaloids. *J. Chromatogr. B* **2005**, 826, 58.
- (43) Attri, P.; Venkatesu, P.; Kumar, A. Activity and stability of α -chymotrypsin in biocompatible ionic liquids: Enzyme refolding by triethyl ammonium acetate. *Phys. Chem. Chem. Phys.* **2011**, 13, 2788.
- (44) Byrne, N.; Wang, L. M.; Belieres, J. P.; Angell, C. A. Reversible folding—unfolding, aggregation protection, and multi-year stabilization, in high concentration protein solutions, using ionic liquids. *Chem. Commun.* **2007**, 2714.
- (45) Lange, C.; Patil, G.; Rudolph, R. Ionic liquids as refolding additives: N'-Alkyl and N'-(ω -hydroxyalkyl) N-methylimidazolium chlorides. *Protein Sci.* **2005**, 14, 2693.
- (46) De Diego, T.; Lozano, P.; Gmouh, S.; Voulter, M.; Iborra, J. L. Fluorescence and CD Spectroscopic Analysis of the α -Chymotrypsin Stabilization by the Ionic Liquid, 1-Ethyl-3-methylimidazolium Bis[(trifluoromethyl)sulfonyl]amide. *Biotechnol. Bioeng.* **2004**, 88, 916.
- (47) Yang, Z.; Pan, W. Ionic liquids: Green solvents for nonaqueous biocatalysis. *Enzyme Microb. Technol.* **2005**, 37, 19.
- (48) Baker, S. N.; McCleskey, T. M.; Pandey, S.; Baker, G. A. Fluorescence studies of protein thermostability in ionic liquids. *Chem. Commun.* **2004**, 940.
- (49) Weingartner, H.; Cabrele, C.; Herrmann, C. How ionic liquids can help to stabilize native proteins. *Phys. Chem. Chem. Phys.* **2012**, 14, 415.
- (50) Lozano, P.; Diego, T.; Guegan, J. P.; Vaultier, M.; Iborra, J. L. Stabilization of α -Chymotrypsin by Ionic Liquids in Transesterification Reactions. *Biotechnol. Bioeng.* **2001**, 75, 563.
- (51) Attri, P.; Venkatesu, P.; Kumar, A. Activity and stability of α -chymotrypsin in biocompatible ionic liquids: Enzyme refolding by triethyl ammonium acetate. *Phys. Chem. Chem. Phys.* **2011**, 13, 2788.

- (52) Weaver, K. D.; Vrikkis, R. M.; Van Vorst, M. P.; Trullinger, J.; Vijayaraghavan, R.; Foureau, D. M.; McKillop, I. H.; MacFarlane, D. R.; Krueger, J. K.; Elliott, G. D. Structure and function of proteins in hydrated choline dihydrogen phosphate ionic liquid. *Phys. Chem. Chem. Phys.* **2012**, *14*, 790.
- (53) Summers, C. A.; Flowers, R. A., II. Protein renaturation by the liquid organic salt ethylammonium nitrate. *Protein Sci.* **2000**, *9*, 2001.
- (54) Maia, F. M.; Rodríguez, O.; Macedo, E. A. LLE for (water + ionic liquid) binary systems using $[C_x\text{mim}][\text{BF}_4]$ ($x = 6, 8$) ionic liquids. *Fluid Phase Equilib.* **2010**, *296*, 184.
- (55) Maia, F. M.; Rodríguez, O.; Macedo, E. A. Relative hydrophobicity of equilibrium phases in biphasic systems (ionic liquid + water). *J. Chem. Thermodyn.* **2012**, *49*, 221.
- (56) Freire, M. G.; Neves, C. M. S. S.; Carvalho, P. J.; Gardas, R. L.; Fernandes, A. M.; Marrucho, I. M.; Santos, L. M. N. B. F.; Coutinho, J. A. P. Mutual Solubilities of Water and Hydrophobic Ionic Liquids. *J. Phys. Chem. B* **2007**, *111*, 13082.
- (57) Freire, M. G.; Neves, C. M. S. S.; Ventura, S. P. M.; Pratas, M. J.; Marrucho, I. M.; Oliveira, J.; Coutinho, J. A. P.; Fernandes, A. M. Solubility of non-aromatic ionic liquids in water and correlation using a QSPR approach. *Fluid Phase Equilib.* **2010**, *294*, 234.
- (58) Wang, S.; Jacquemin, J.; Husson, P.; Hardacre, C.; Gomes, M. F. C. Liquid–liquid miscibility and volumetric properties of aqueous solutions of ionic liquids as a function of temperature. *J. Chem. Thermodyn.* **2009**, *41*, 1206.
- (59) Domanska, U.; Krolukowska, M.; Paduszynski, K. Physico-chemical properties and phase behaviour of piperidinium-based ionic liquids. *Fluid Phase Equilib.* **2011**, *303*, 1.
- (60) Rodríguez, O.; Madeira, P. P.; Macedo, E. A. Gibbs Free Energy of Transfer of a Methylene Group in Buffer + Ionic Liquid Biphasic Systems. *Ind. Eng. Chem. Res.* **2008**, *47*, 5165.
- (61) Zaslavsky, B. Y. *Aqueous Two-Phase Partitioning*; Marcel Dekker: New York, 1995.
- (62) Rogers, R. D.; Willauer, H. D.; Griffin, S. T.; Huddleston, J. G. Partitioning of small organic molecules in aqueous biphasic systems. *J. Chromatogr. B* **1998**, *711*, 255.
- (63) Moody, M. L.; Willauer, H. D.; Griffin, S. T.; Huddleston, J. G.; Rogers, R. D. Solvent Property Characterization of Poly(ethylene glycol)/Dextran Aqueous Biphasic Systems Using the Free Energy of Transfer of a Methylene Group and a Linear Solvation Energy Relationship. *Ind. Eng. Chem. Res.* **2005**, *44*, 3749.
- (64) Rodríguez, O.; Silvério, S. C.; Madeira, P. P.; Teixeira, J. A.; Macedo, E. A. Physicochemical Characterization of the PEG8000– Na_2SO_4 Aqueous Two-Phase System. *Ind. Eng. Chem. Res.* **2007**, *46*, 8199.
- (65) Koga, K.; Bhimalapuram, P.; Widom, B. Correlation between hydrophobic attraction and the free energy of hydrophobic hydration. *Mol. Phys.* **2002**, *100*, 3795.
- (66) Widom, B.; Bhimalapuram, P.; Koga, K. The hydrophobic effect. *Phys. Chem. Chem. Phys.* **2003**, *5*, 3085.
- (67) Silvério, S. C.; Rodríguez, O.; Teixeira, J. A.; Macedo, E. A. Gibbs free energy of transfer of a methylene group on {UCON + (sodium or potassium) phosphate salts} aqueous two-phase systems: Hydrophobicity effects. *J. Chem. Thermodyn.* **2010**, *42*, 1063.