GENERAL RESEARCH

Physicochemical Characterization of the PEG8000-Na_2SO_4 Aqueous Two-Phase System

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The polyethylene glycol−sodium sulfate aqueous two-phase system has been characterized at 23 °C. Tie-lines for the phase diagram were obtained experimentally. Phases in equilibrium were characterized by means of the solvatochromic parameters π^*, α, and β, which provide a measurement of the polarity/polarizability and the H-bond donor and acceptor abilities, respectively. The ability of the phases to participate in hydrophobic interactions was characterized by means of the free energy of transfer of a methylene group between the conjugated phases, using the partition of a homologous series of dinitrophenylated amino acids. The results show the effect of the presence of polymer and salt in the aqueous phase, and a comparison of both phases with pure water is made.

Introduction

The mixing of certain aqueous solutions of polymers (e.g., polyethylene glycol−dextran) or of a polymer and a salt (e.g., polyethylene glycol−Na_2SO_4) above certain critical conditions (e.g., concentration and temperature) leads to a system that splits into two immiscible phases. Both phases are composed predominantly of water, and each is richer in one of the other components. Furthermore, if a solute is added to this aqueous two-phase system (ATPS), it will distribute unevenly between the two phases.[1−3]

The aqueous two-phase extraction technique has a wide spectrum of applications. It is suitable for bioseparations,[1−4] separation of metal ion species,[5] and recovery of dyes, drug molecules, and small organic species (see ref 6 and references cited therein). In addition, the procedure is considered "environmentally friendly".[5] The low cost, high capacity, ease of scale-up, and possibility of direct application to fermentation broths are obvious advantages.

However, ATPS as a separation tool at the industrial level has not had a significant impact. The lack of a predictive tool describing the solute partition is one reason for the low level of employment in industry. More information concerning the physicochemical factors governing this partition is of paramount importance to the development of new approaches in phase modeling and partitioning.

Solute partitioning trends in ATPS are similar to those observed in organic solvent−water biphasic systems.[3,7−11] One important consequence is the application of the linear Collander relationship,[12]

$$\ln K_1 = a \ln K_2 + b \quad (1)$$

where K_1 and K_2 denote partition coefficients for any given solute in the two-solvent system, while a and b are constants characterizing the solvent system in question.[12] The physical meaning of parameters a and b are, however, not yet clearly understood. Notwithstanding, several solvent properties have been attributed to these parameters. These include the polarity of the phases,[13] the ability to participate in hydrogen bonds,[12] and the so-called solvophobic effect.[3] The study of these properties in polymer−salt ATPS is limited to only a few systems.[8,14,15] This information contributes to the development of new theories for the mechanisms involved in solute partition in ATPS.

Toward these ends, the solvent properties in PEG−Na_2SO_4 ATPS at room temperature (23 °C) are investigated in the present work. The solvatochromic shifts of the 4-nitroanisole, 4-nitrophenol, and Reichardt's dye were used as measures of the polarities and abilities of the phases to participate in hydrogen bonding. The free energy of transfer of a methylene group between the phases was used to describe the relative hydrophobicity of the phases.

Experimental Section

Chemicals. Polyethylene glycol, PEG (average molecular weight 8000), was obtained from Sigma. Sodium sulfate, Na_2SO_4, and sodium chloride, NaCl, were supplied by Merck (anhydrous GR for analysis, >99.0%, and GR for analysis, >99.5%, respectively). All dinitrophenilated (DNP) amino acids were obtained from Sigma: *N*-(2,4-dinitrophenyl)glycine, *N*-(2,4-dinitrophenyl)-L-alanine, *N*-(2,4-dinitrophenyl)-DL-*n*-valine, *N*-(2,4-dinitrophenyl)-DL-*n*-leucine, *N*-(2,4-dinitrophenyl)-DL-α-amino-*n*-caprylic acid. The solvatochromic probes 4-nitrophenol (reagent grade, >98%) and 4-nitroanisole (>97%, GC)

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were both supplied by Aldrich. Reichardt's carboxylated betaine dye was kindly provided by Prof. C. Reichardt (Philipps University, Marburg, Germany). All products were used as received without further purification, except PEG and Na_2SO_4, which were dried in a vacuum oven for at least 24 h (120 °C for PEG, 150 °C for Na_2SO_4). Stock solutions of each chemical were prepared (ca. 50 wt % for PEG, 17 wt % for Na_2SO_4, 0.2 wt % for DNP amino acids, 0.1 wt % for 4-nitrophenol and 4-nitroanisole, and 0.4 wt % for Reichardt's carboxylated betaine). Distilled water was used for all diluting purposes.

Phase Diagram. End points of the tie-lines were obtained experimentally at room temperature (23 ± 1 °C, controlled with air conditioning) by analysis of the conjugated phases at equilibrium. Samples with compositions in the heterogeneous region of the phase diagram were prepared by weight in decanting ampules. The ampules were thoroughly shaken and allowed to settle for at least 48 h. Samples of both top and bottom phases were withdrawn (top phase using a pipet, bottom phase through the valve of the ampule). Na_2SO_4 composition was measured using a flame atomic absorption spectrometer (FAAS) from GBC, model 932 plus. Calibration was done with NaCl for compositions in the range from 100 to 300 ppm Na. The samples from the top and bottom phases were diluted 65 and 150 times, respectively, and were both analyzed by FAAS five times. Highest errors in Na determination were 2 wt %. The PEG composition in the top phase was obtained by lyophilization and subtraction of the Na_2SO_4 composition. Lyophilization was carried out in triplicate for 48 h in a Christ lyophilizer, model Alpha 1-4, equipped with a FTS Systems vacuum pump, model VP-127D. Errors in the top-phase PEG composition were <5 wt %. The PEG composition in the bottom phase could not be accurately obtained by lyophilization, and thus, it was obtained by extending the line connecting the top phase and the feed compositions up to the Na_2SO_4 composition obtained for the bottom phase.

Solvatochromic Parameters. The solvatochromic probes 4-nitroanisole, 4-nitrophenol, and Reichardt's carboxylated betaine were used to measure the polarity/polarizability π^*, H-bond acceptor (HBA) basicities β, and H-bond donor (HBD) acidities α, for both phases of each tie-line. Three compositions of each probe were prepared for each analysis. Solvatochromic probe stock solution (10−40 μL) was added to 1 mL of the ATPS phase sample. NaOH (1M, 20 μL) was added to the samples containing the Reichardt's carboxylated betaine to ensure a basic pH.[15,16] The samples were mixed thoroughly in a vortex mixer and then held at 21 °C (which is below the equilibrium temperature of 23 °C) before analysis to prevent clouding. The solvatochromic experiments were carried out in a UV−vis spectrometer (Thermo Electron Corp., model UV1). A scan of the blank phase was performed to build the baseline, and then the different mixtures were scanned 1−3 times. The three different compositions examined for each probe allowed us to check that there were no aggregation effects. The absorbance was in the range 0.2−1.0 for all experiments, and the wavelength of maximum absorbance was calculated as the average between the wavelengths having 90% of the maximum absorbance (as suggested by Kamlet and co-workers for the solvatochromic comparison method, see ref 17, note 16). Average standard deviations of all measured wavelengths were 0.2 nm for 4-nitroanisole and 4-nitrophenol probes and 2.0 nm for the Reichardt's carboxylated betaine.

DNP Amino Acids Partition. The partition of five dinitrophenylated amino acids was measured experimentally for the tie-lines previously obtained. Six replicates of the feed composition of a given tie-line were prepared in eppendorf tubes containing 0−100 mg of a given DNP amino acid stock solution in order to assess aggregation effects. The tubes were thoroughly mixed on a vortex mixer for 2 min, and then the phases were resolved using centrifugation (10^4 rpm for 15 min). Samples of each phase were withdrawn and diluted conveniently, and the absorbances at 362 nm were measured. All absorbances were <0.8. The partition coefficient was calculated as the slope of the straight line obtained when comparing the absorbance in the top phase against that in the bottom phase and was corrected with the dilution factor, DF_{phase} (the final volume divided by the initial volume), thus,

$$K = \frac{\text{Abs(top)}\cdot DF_{top}}{\text{Abs(bottom)}\cdot DF_{bottom}} \quad (2)$$

The experimental error was <1% except for two cases that were <2%.

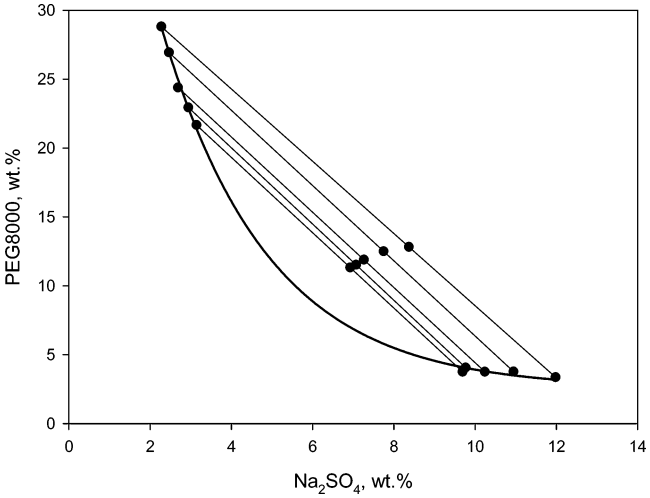

Figure 1. Phase diagram for the system PEG 8000 + Na_2SO_4 + water at 23 °C.

Table 1. Experimental Compositions (wt %) of Na_2SO_4 and PEG in Feed, Top, and Bottom Phases, Tie-line Length (TLL), Slope (STL)

feed		top phase		bottom phase			
Na_2SO_4	PEG	Na_2SO_4	PEG	Na_2SO_4	PEG	TLL	STL
8.38	12.79	2.29	28.77	11.99	3.33	27.23	−2.62
7.76	12.47	2.48	26.89	10.96	3.74	24.66	−2.73
7.27	11.86	2.70	24.36	10.25	3.73	21.97	−2.73
7.08	11.48	2.95	22.91	9.78	4.03	20.08	−2.77
6.94	11.29	3.15	21.64	9.70	3.73	19.07	−2.73

Results and Discussion

Phase Diagram. The end points, lengths, and slopes of the experimental tie-lines are given in Table 1. Figure 1 shows the phase diagram with the experimental tie-lines. The binodal curve is presented to facilitate visualization and was obtained by exponential decay regression of the ends of the tie-lines, using five parameters. This same ternary system has also been studied by other authors using PEG with similar molecular weights.[18,19] Despite the phase diagrams obtained by Snyder and co-workers[18] using PEG 3350 and 8000 being significantly different, those of Hammer and co-workers[19] using PEG 6000 concur with the present data. However, the slopes of the tie-lines (STL) are in good agreement in all cases, thus suggesting that differences in the phase diagram could be caused by differences in the polymer

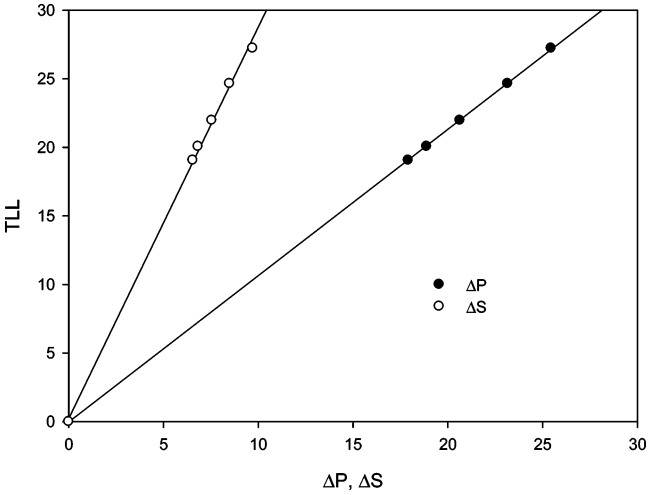

Figure 2. Linear relationship between tie-line length (TLL) and the difference in PEG (ΔP) or Na_2SO_4 (ΔS) composition in conjugated phases.

molecular weight, polydispersity, or end groups on the polymeric chains.

The STL and the tie-line length (TLL) are defined as

$$\mathrm{STL} = \frac{\Delta P}{\Delta S} \tag{3}$$

$$\mathrm{TLL} = (\Delta P^2 + \Delta S^2)^{1/2} \tag{4}$$

where ΔP and ΔS are the differences between the polymer and salt concentrations in the coexisting phases, respectively. According to eqs 3 and 4, it is straightforward to establish a relation between TLL and STL:

$$\mathrm{TLL} = \Delta P\left[1 + \left(\frac{1}{\mathrm{STL}}\right)^2\right]^{1/2} \tag{5a}$$

$$\mathrm{STL} = \Delta S(1 + \mathrm{STL}^2)^{1/2} \tag{5b}$$

This dependence was checked for the experimental data measured and is shown in Figure 2. The slopes of the lines are 1.067 ± 0.003 and 2.85 ± 0.06, for TLL vs ΔP and ΔS, respectively. These values are in good agreement with those obtained using the above equations and the experimental STL: 1.065 and 2.90, respectively.

Solvatochromic Parameters. π^*, β, and α were calculated from the experimental wave numbers of the solvatochromic probes (4-nitroanisole, 4-nitrophenol, and Reichardt's carboxylated betaine dye) using the equations given by Marcus,[20]

$$\pi^* = 0.427(34.12 - \upsilon) \tag{6}$$

$$\beta = 0.346(35.045 - \upsilon) - 0.57\pi^* - 0.12\delta \tag{7}$$

$$\alpha = 0.0649E_T(30) - 2.03 - 0.72\pi^* \tag{8}$$

where υ is the experimental wave number (in 10^3 cm^{-1}), δ is the polarizability correction (zero for the components used here), and $E_T{}^{30}$ is the Reichardt's solvent polarity index,[21] which is calculated from the wavelength of the Reichardt's carboxylated betaine using the following equation,[22]

$$E_T(30) = \frac{1}{0.932}\left(\frac{28591}{\lambda_{max}} - 3.335\right) \tag{9}$$

where λ_{max} is the wavelength of maximum absorbance of the probe. Figures 3−5 present π^*, β, and α, respectively, for top and bottom phases. The value of each parameter in pure water[20] is plotted as a solid line for comparison.

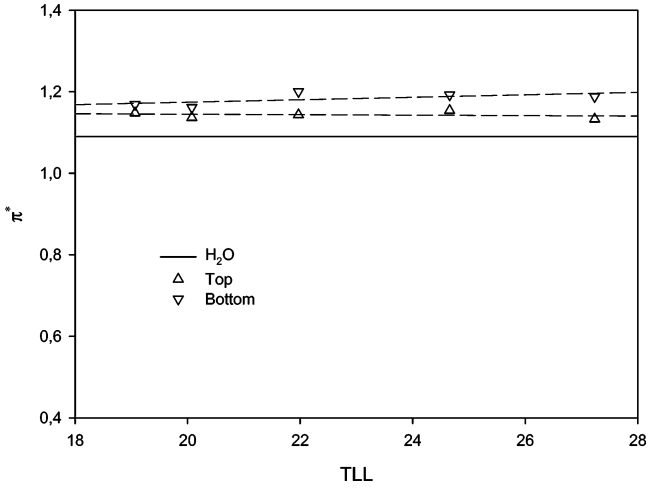

Figure 3. Polarity/polarizability (π^*) of top and bottom conjugated phases at different tie-line lengths (TLLs).

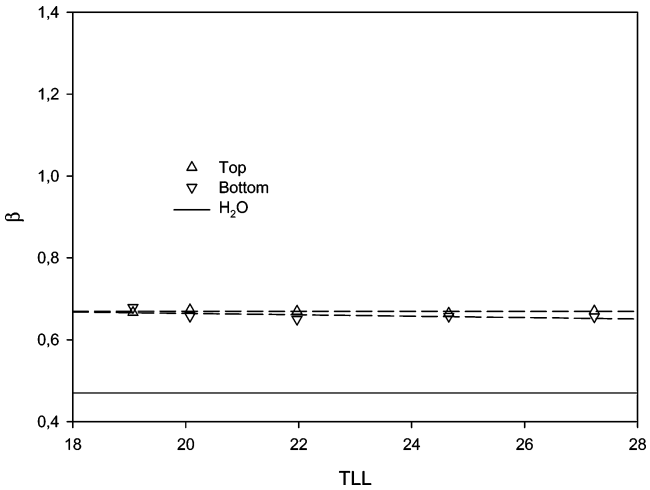

Figure 4. H-bond acceptance (β) of top and bottom conjugated phases at different tie-line lengths (TLLs).

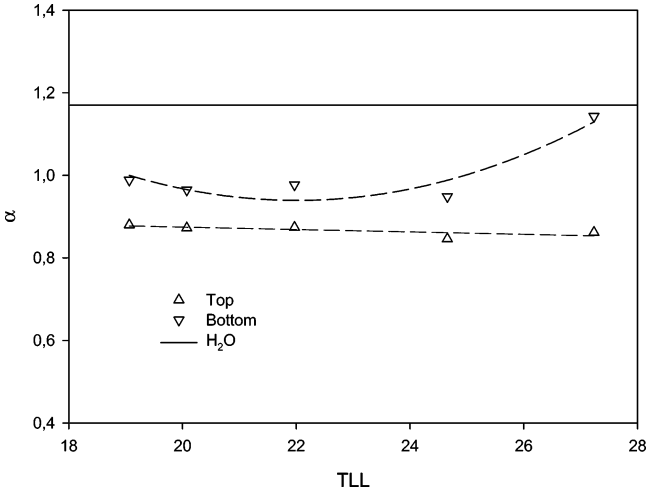

Figure 5. H-bond donor ability (α) of top and bottom conjugated phases at different tie-line lengths (TLLs).

The π^*, β, and α as calculated by Kamlet and co-workers are averaged values of the solvatochromic shifts of various probes.[17,20] The merits of using averaged values have been discussed elsewhere.[20] Single probes for each solvatochromic parameter were used in the present work, and the probes selected

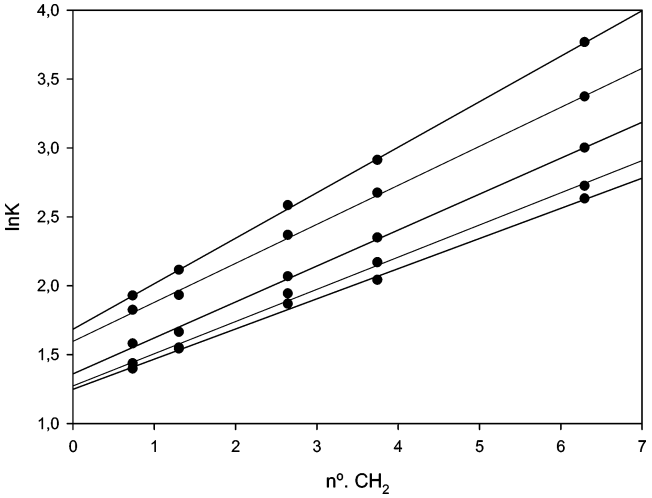

Figure 6. Logarithm of partition coefficients for homologous series of DNP amino acids as a function of the number of methylene (CH_2) groups: $\ln K = C + E{\cdot}n(CH_2)$.

are the same as used in previous publications involving polymer−salt ATPS[14−16] in order to facilitate comparisons of the data.

The polarity/polarizability of the conjugated phases (π^*) is virtually constant and independent of TLL, with average values of 1.14 (top phase) and 1.18 (bottom phase) (Figure 3). These values are higher than those of pure water (1.09).[20] Thus, the polarity/polarizability of water is enhanced in the PEG−Na_2SO_4 ATPS. Nevertheless, this effect is independent of the system concentration. The H-bond acceptance (HBA) shown in Figure 4 also gives an effectively constant and equal value of β for both phases (0.67 for top phase and 0.66 for bottom phase). These values are much higher than that of water (0.47),[20] indicating an important increase of solvent (water) basicity. Important differences arise from the H-bond donor (HBD) ability of the phases, as can be seen from Figure 5. The top phase gives a near composition-independent, constant value of α of 0.87, while in the bottom phase, the value increases significantly at high TLL, rising from approximately 0.96 to 1.14. Moreover, pure water gives an α value of 1.17,[20] thus showing an important lowering in the H-bond donor ability for ATPS. This effect is almost independent of the TLL for the top phase but rises when the TLL is reduced in the bottom phase. A similar behavior can also be found in other polymer−salt ATPS: PEG 2000−K_3PO_4 and PEG 2000−$(NH_4)_2SO_4$.[15]

DNP Amino Acids Partition. Figure 6 presents the relationships experimentally obtained for the logarithm of the partition coefficient K and the number of equivalent methylene groups in the aliphatic side chain of homologous DNP amino acids partitioned in the aqueous two-phase system examined. All the relationships observed are described as

$$\ln K = C + E{\cdot}n(CH_2) \qquad (10)$$

where $n(CH_2)$ is the average equivalent number of methylene (CH_2) groups in the amino acid aliphatic side chain and C and E are constants. The physical meaning of both parameters C and E has been considered previously.[3] Parameter E is related to the free energy of transfer of a methylene group between the phases, $\Delta G(CH_2)$, as

$$\Delta G(CH_2) = -RTE \qquad (11)$$

where R is the universal gas constant and T is the temperature. The parameter E is a measure of the difference between the

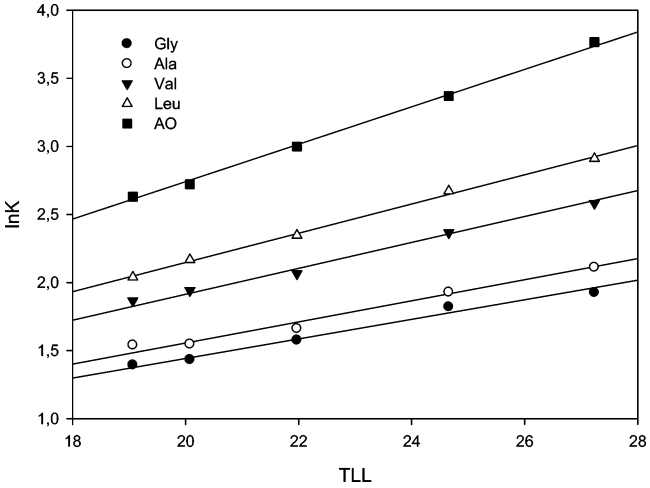

Figure 7. Logarithm of partition coefficients for homologous series of DNP amino acids as a function of the tie-line length (TLL).

Table 2. Parameters C and E (eq 10) and Free-Energy of Transfer for a Methylene Group between Conjugated Phases ($-\Delta G(CH_2)$)

TLL	C	E	$-\Delta G(CH_2)$, kcal/mol
27.23	1.684	0.3303	0.194
24.66	1.596	0.2832	0.167
21.97	1.360	0.2608	0.153
20.08	1.273	0.2336	0.137
19.07	1.248	0.2188	0.129

affinities of the two phases for a CH_2 group, i.e., it is a measure of the difference between the relative hydrophobicity of the phases. Parameter C gives the contribution of the non-alkyl part of the solute into the logarithm of the partition coefficient.[3]

Table 2 presents the values obtained for parameters C and E from linear regression of partition coefficients and the free energy of transfer, $\Delta G(CH_2)$, calculated with eq 11, for the tie-lines studied in the present work. The obtained values agree with previously reported ones for PEG/salt ATPS.[7,8] As can be seen from Figure 6, $n(CH_2)$ is different from the alkyl chain length (0, 1, 3, 4, and 6 for the dinitrophenylated amino acids glycine, alanine, valine, leucine, and aminocaprylic acid, respectively). The reason for such a difference has been considered previously,[3] and it was attributed to the influence of the water interactions with polar groups on those with nonpolar groups and to the possible difference between the intensities of the hydrophobic interactions for methylene and

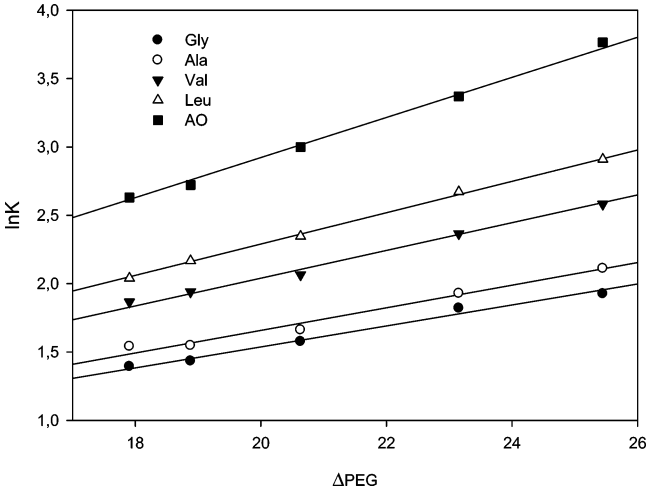

Figure 8. Logarithm of partition coefficients for homologous series of DNP amino acids as a function of the difference of PEG concentration in conjugated phases (ΔP).

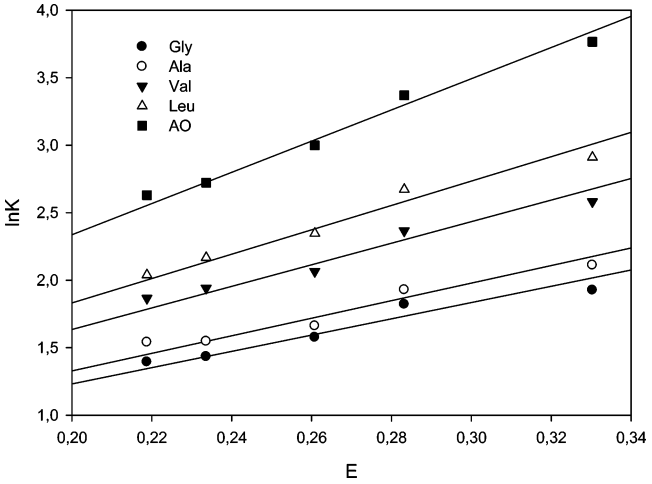

Figure 9. Logarithm of partition coefficients for homologous series of DNP amino acids as a function of the parameter *E*.

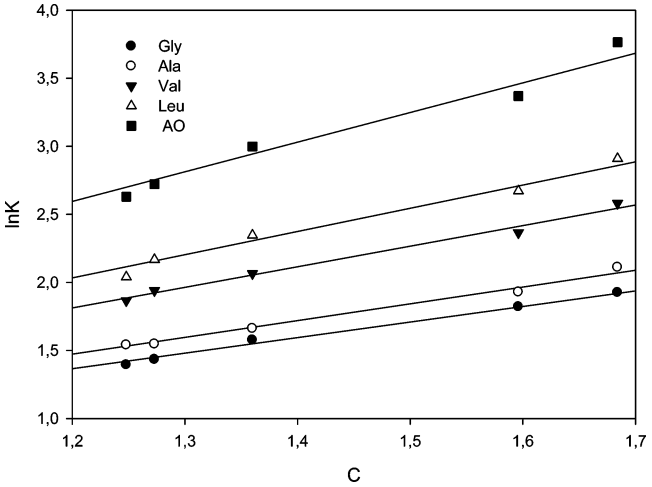

Figure 10. Logarithm of partition coefficients for homologous series of DNP amino acids as a function of the parameter *C*.

methyl groups. These discrepancies decay exponentially with the alkyl chain length. It is noteworthy that the "CH_2 equivalent numbers" used in the present work are equal to those usually employed in polymer/polymer ATPS except for DNP glycine. In this case, the value is over 10 times higher. Such effects might be due to the much higher $\Delta G(CH_2)$ values in polymer−salt with respect to polymer−polymer ATPS, which are also approximately a factor of 10 higher.[7]

Figures 7 and 8 present the obtained partition coefficients for the five DNP amino acids as a function of the TLL and the PEG concentration difference between conjugated phases ΔP, respectively. The linear trends are in accordance with others previously reported for polymer−salt and polymer−polymer ATPS.[3,23] It is noteworthy that parameters *E* (or $\Delta G(CH_2)$) and *C* are both linearly related to TLL (or ΔP or ΔS). Since TLL is linearly related to ln *K* (Figure 7), a similar relationship between *E* (or *C*) and ln *K* is expected. Such trends are shown in Figures 9 and 10, respectively. This relationship had already been presented for polymer−polymer ATPS.[3] The above suggests that the physicochemical factors governing partitioning in polymer/salt and polymer/polymer ATPS are similar, at least for the molecules studied herein.

Conclusions

The phase diagram for PEG 8000−Na_2SO_4 ATPS was obtained experimentally at 23 °C. Tie-lines are almost parallel and compare favorably with similar systems found in the literature.[21,22]

There are no clear differences in the H-bond acceptance of the aqueous phases (Figure 3) despite β being significantly higher than in pure water. Polarity/polarizability is slightly higher than that of water (Figure 2), but differences between conjugated phases remained small. The differences between the top and bottom phases arise from H-bond donor ability, α (Figure 4). Differences with respect to pure water indicate the structuring effects of polymer and salt in water: increase in its polarity/polarizability, important increase in the H-bond acceptance, and reduction of the H-bond donor ability.

In addition, $\Delta G(CH_2)$ values reported in the present work agree with others from the literature for different PEG−salt ATPS. They indicate that the PEG-rich phase (top phase) has greater affinity to participate in hydrophobic hydration interactions than the salt-rich phase (bottom phase).

The different solvent properties characterizing PEG−Na_2SO_4 ATPS reported here give insights into the physicochemical factors governing partitioning in such systems. As the basic purpose of ATPS is separation of biomolecules (and organelles), differences in the H-bond acceptance ability of the solutes and their relative hydrophobicity can be exploited in order to improve the separation capacity.

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