J. Chem. Thermodynamics 42 (2010) 1063–1069

Contents lists available at ScienceDirect

J. Chem. Thermodynamics

journal homepage: www.elsevier.com/locate/jct

Gibbs free energy of transfer of a methylene group on {UCON + (sodium or potassium) phosphate salts} aqueous two-phase systems: Hydrophobicity effects

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ARTICLE INFO

Article history:
Received 6 January 2010
Received in revised form 19 March 2010
Accepted 12 April 2010
Available online 18 April 2010

Keywords:
Aqueous two-phase systems
Gibbs free energy of transfer
Hydrophobicity

ABSTRACT

The Gibbs free energy of transfer of a suitable hydrophobic probe can be regarded as a measure of the relative hydrophobicity of the different phases. The methylene group (CH_2) can be considered hydrophobic, and thus be a suitable probe for hydrophobicity. In this work, the partition coefficients of a series of five dinitrophenylated-amino acids were experimentally determined, at 23 °C, in three different tie-lines of the biphasic systems: (UCON + K_2HPO_4), (UCON + potassium phosphate buffer, pH 7), (UCON + KH_2PO_4), (UCON + Na_2HPO_4), (UCON + sodium phosphate buffer, pH 7), and (UCON + NaH_2PO_4). The Gibbs free energy of transfer of CH_2 units were calculated from the partition coefficients and used to compare the relative hydrophobicity of the equilibrium phases. The largest relative hydrophobicity was found for the ATPS formed by dihydrogen phosphate salts.

1. Introduction

(Liquid + liquid) biphasic systems are the basis for solvent extraction, one of the most important separation units in chemical engineering. The separation occurs due to the different partitioning of the solutes present between the two different phases. Aqueous two-phase systems (ATPS) are a special case of biphasic systems in which both phases are composed mainly by water (*ca.* 80%). Together with liquid chromatography, ATPS are among the separation processes of choice for biotechnological application at laboratory scale. Nevertheless, the scale-up to industry has not gained much success, for different reasons: the complexity of ATPS, the poor understanding of the phenomena and, probably more important, the lack of trusted theoretical models to predict the separation of different target solutes.

The phase splitting of ATPS is found when two aqueous solutions of different constituents are mixed above some critical conditions (temperature and concentration). Most often, two polymers or a polymer and a salt are the components used to produce the ATPS, but also surfactants can be used [1,2], and recently salt–salt ATPS have been described combining inorganic salts with room-temperature ionic liquids [3–5]. The nature of the phenomena has been related to the effect of these components (frequently called phase-forming components: polymers, electrolytes, surfactants) on water supra-molecular structure [6].

ATPS have been widely used for extraction purposes, especially in biotechnology for the recovery of different types of biological solutes (proteins or even virus and whole cells) [7–9]. Other examples of application can also be found in the literature [10,11]. For proteins and other biological structures, hydrophobic interactions are of prime importance to maintain the 3D conformation (relevant for the biological function of the biomolecule). The simplest idea of hydrophobicity is associated to the demixing of non-polar compounds (like hydrocarbons and oil-like materials) from aqueous solutions. Nevertheless, hydrophobic effects are the result of a non-favorable (*i.e.*, positive) Gibbs free energy change produced when a non-polar compound is dissolved in water. This unfavorable Gibbs free energy, by its part, is due to a negative entropy change, despite the enthalpy change is rather favorable (the enthalpy change is also negative, and so favorable to the Gibbs free energy, but small):

$$\Delta G = \Delta H - T \cdot \Delta S, \tag{1}$$

where ΔG, ΔH, and ΔS are the Gibbs free energy, enthalpy, and entropy changes, respectively, and T is the absolute temperature. This important and negative entropic contribution is related to the energetic cost of building a cavity to account for the solute molecule, the re-arrangement needed in the water structure (with the hydrogen bonds associated) to fit it, and the restrictions imposed to water molecules' orientations. The term "hydrophobic hydration" refers to this re-arrangement of the water molecules around the non-polar solute molecule. Similarly, the term "hydrophobic attraction" refers to the attraction between non-polar molecules in aqueous media. But this

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doi:10.1016/j.jct.2010.04.005

phenomenon is related to the first, as the Gibbs free energy (and entropy) associated to account for two non-polar molecules is smaller when they are close together than when separated, and thus producing a solvent-mediated attraction between both non-polar molecules. All the above has been discussed in deep detail in the literature [12–15]. The implications and relevance of these hydrophobic attractions and hydration on the structure, stability, and function of biomolecules have also been discussed elsewhere [16].

As stated above, the phase splitting in ATPS is related to differences in water structure produced by the components added (polymers, electrolytes). These differences can be accounted by the relative hydrophobicity of the phases present in the ATPS, if it can be measured. An experimental measurement of relative hydrophobicity of different phases can be done through the Gibbs free energy of transfer of a suitable "*hydrophobic*" probe. Non-polar molecules such as methane and other hydrocarbons can be considered purely hydrophobic, and so be used towards this end. The methylene group (–CH_2–) can also be considered hydrophobic, and thus be a suitable probe for hydrophobicity (despite it is not a molecule). Using the "Group-Contribution" concept [17,18], the effect (*contribution*) of a methylene group to the Gibbs free energy of transfer of a solute can be calculated from the partition coefficient of a series of solutes which differ only in the number of methylene groups. Series of 1-alcohols, *n*-alkanes, and other molecules have been used for this in the literature. In this work, partition coefficients of five dinitrophenylated-amino acids (differing only in the number of methylene groups in their alkyl chain) were used for this purpose. The partitioning experiments were performed on three tie-lines of six different (polymer-salt) ATPS. Therefore, Gibbs free energy of transfer of methylene groups was then calculated using the thermodynamic framework detailed in the next section. The Gibbs free energy of transfer provides a quantitative measurement of the relative hydrophobicity of the phases in each tie-line of the different ATPS (each possible biphasic system). The ATPS studied here were obtained combining UCON (a random copolymer composed of ethylene oxide and propylene oxide) with sodium and potassium phosphate salts. The relative hydrophobicity (Gibbs free energy of transfer) can be used as a characteristic parameter of the biphasic system which provides some insights about the interactions governing the phase behavior. Moreover, it can also be used as a molecular descriptor in Linear Free Energy Relationships [6,19–22], similarly to solubility parameters (Hildebrand, Hansen) or McGowan volumes.

2. Theoretical background

The Gibbs free energy change accompanying the transfer of a solute from a phase α to a phase β in equilibrium, at a given temperature T and pressure P, is given by:

$$\Delta G = \mu_i^{\beta} - \mu_i^{\alpha}, \tag{2}$$

where μ_i is the chemical potential of the compound being transferred, *i*. From this Gibbs free energy ΔG, the part associated intrinsically to the transfer of the component *i* from one phase to another, ΔG^*, is:

$$\Delta G^* = \Delta G - R \cdot T \cdot \ln \frac{c_i^{\beta}}{c_i^{\alpha}}, \tag{3}$$

where R is the universal gas constant, and c_i is the molar concentration of the solute being transferred, *i*, between the phases indicated as superscripts. In equation (3), the second term on the right side subtracts the contributions to ΔG due to the differences on the concentration of the solute *i* in both phases. At equilibrium, $\Delta G = 0$ and the term in the logarithm becomes the partition coefficient of component *i*, K_i:

$$\Delta G^* = -R \cdot T \cdot \ln K_i. \tag{4}$$

This equation relates the Gibbs free energy of transfer of a component *i* from one phase to another with its partition coefficient between both phases. A deeper explanation can be found elsewhere [14,15]. Using a basic group-contribution concept, the logarithm of the partition coefficient $\ln K_i$ can be accounted as the summation of two contributions: the alkyl part of the molecule (constructed by the summation of *n* methylene groups) and the non-alkyl part of the molecule, as follows [6,21–24]:

$$\ln K_i = C + E \cdot n(CH_2), \tag{5}$$

where $n(CH_2)$ is the number of methylene groups in solute *i*, parameter C is the contribution of the non-alkyl part of the molecule to the partition coefficient, and parameter E is the contribution of each methylene group to the partition coefficient. When the partition coefficient of a series of solutes whose chemical structure differs only in the number of methylene groups on an alkyl chain is represented as a function of the number of these methylene groups, the figure so obtained is a straight line. From equations (4) and (5), it follows that the Gibbs free energy of transfer of each methylene group, $\Delta G^*(CH_2)$, is given by:

$$\Delta G^*(CH_2) = -R \cdot T \cdot E. \tag{6}$$

As previously stated, $\Delta G^*(CH_2)$ (or the E parameter) provides a comparison of the affinity of both phases in equilibrium for methylene groups. That can be used as a quantitative measurement of the relative hydrophobicity of phase β to phase α. $\Delta G^*(CH_2)$ can also be used as a molecular descriptor for the characterization of ATPS, suitable for Linear Free Energy Relationships (LFER) and other Quantitative Structure–Activity Relationships (QSAR) modeling [19,20].

3. Experimental section

3.1. Materials

UCON 50-HB-5100, a random copolymer (average molecular weight Mr = 3900) of 50% ethylene oxide and 50% propylene oxide, was obtained from Union Carbide (NY, USA). Potassium dihydrogen phosphate (KH_2PO_4) was provided by USB Corporation (anhydrous, $\geqslant$99.9%) and di-potassium hydrogen phosphate (K_2HPO_4) (anhydrous, 99.99 Suprapur), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) (p.a., Reag. Ph Eur), and di-sodium hydrogen phosphate (Na_2HPO_4) (anhydrous, GR for analysis, ACS, Reag. Ph Eur) were supplied by Merck. Stock solutions of each chemical were prepared in deionised water (*ca.* 50 wt% for UCON, 15.5 wt% for KH_2PO_4, 20 wt% for K_2HPO_4, 20 wt% for NaH_2PO_4, and 13 wt% for Na_2HPO_4) and all concentrations were obtained gravimetrically after evaporation on heating plate (Stuart hotplate SB300) for salts or after lyophilization (Scan Vac, model CoolSafe 55-4) for UCON. Potassium phosphate buffer (1 M, pH 7) was obtained combining the KH_2PO_4 and K_2HPO_4 salts. Sodium phosphate buffer (1 M, pH 7) was prepared combining the NaH_2PO_4 and Na_2HPO_4 salts. Buffer concentrations (14.3 wt% for potassium phosphate buffer and 13.2 wt% for sodium phosphate buffer) were obtained gravimetrically after evaporation on heating plate. The pH value was confirmed using a pH meter (VWR, SimpHony SB70P).

Dinitrophenylated (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, and *N*-(2,4-dinitrophenyl)-DL-R-amino-*n*-caprylic acid. Stock solutions of the five DNP-amino acids were prepared in deionised water (0.2 wt%).

All products were used as received without further purification. Deionised 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.

3.2. Methods

The procedure to obtain the partition coefficients has been explained in detail before [23,24]. Different amounts of a given DNP-amino acid stock solution (from 0 to 100 mg) were added to six replicates of a certain ATPS with the same feed composition. The corresponding amount of water (from 100 to 0 mg) was added to keep all compositions constant except for the DNP-amino acids. The components of the six replicates were vigorously vortex-mixed (VWR, model VV3) during 2 min and phase separation was accelerated by centrifugation (minispin, Eppendorf) at 10^4 rpm for 15 min. Then, samples of each phase were withdrawn, conveniently diluted with water, and their absorbance at 362 nm was measured in a UV–Vis spectrophotometer (Thermo electron corporation, UV1). Partition coefficients (K) for the five DNP-amino acids were determined as the slope of the straight line obtained when comparing the amino acid absorbance in the top phase against that in the bottom phase, both corrected with the corresponding dilution factors, DF (final volume divided by the initial volume):

$$K = \frac{Abs(top)\cdot DF_{top}}{Abs(bottom)\cdot DF_{bottom}}. \quad (7)$$

4. Results and discussion

The partition coefficients for the five DNP-amino acids were obtained in three different tie-lines of the six UCON-phosphate salt ATPS previously reported [25]. The feed composition of each tie-line and the corresponding partition coefficients are presented in table 1. For all DNP-amino acids, straight lines were obtained when the absorbance in the top phase was plotted against the absorbance in the bottom phase, for the six replicates with different solute concentration. The partition coefficients, K, were obtained after linear regression from the slope of the lines:

$$Abs(top) = K\cdot Abs(bottom) + b, \quad (8)$$

where b corresponds to the intercept and it was found to be very close to zero in all cases (average $b = 0.004$). The straight lines obtained have coefficients of determination $r^2 \geqslant 0.993$ with average $r^2 = 0.998$. This means that the partition coefficients determined are independent of the solute concentration and ensures there are no interactions affecting the solute partition behaviour, like solute self-association or dissociation [6,26].

Figure 1 shows the logarithm of the partition coefficients, ln K, as a function of the tie-line length (TLL) for the six ATPS. The TLL gives a measurement of the differences in phase composition and is defined as:

$$\mathrm{TLL} = \sqrt{(X_{top} - X_{bottom})^2 + (Y_{top} - Y_{bottom})^2}, \quad (9)$$

where X and Y stand for the salt and polymer mass fractions, respectively. Values of the TLL for each system are presented in table 1. For a given system, the increase of the TLL indicates a larger difference between the compositions of the equilibrium phases [27]. According to figure 1, the partition coefficients are a linear function of the TLL. As it was previously reported for other ATPS [21,23,28], ln K increases linearly with the TLL in all cases. Thus, the DNP-amino acids partition more preferentially to the top (UCON-rich) phase as the ATPS moves into the heterogeneous region (larger TLL). The lines represented in figure 1 are linear regressions of the experimental data and can be described by:

$$\ln K = \alpha\cdot \mathrm{TLL}, \quad (10)$$

where α is a constant that characterizes the effect of the composition of the equilibrium phases. The origin of coordinates corresponds to the critical point, where both phases have the same composition, thus TLL = 0 and the partition coefficient becomes unity (so ln $K = 0$).

In figure 2 the logarithms of the partition coefficients are plotted as a function of the average number of methylene groups present in the aliphatic side chain of the homologous DNP-amino acids, $n(CH_2)$. Note that the $n(CH_2)$ used do not match the alkyl chain length of the DNP-amino acids. This effect has been explained previously [6] and the reasons are attributed to the interactions of water with the polar group of the solute that can affect those with the non-polar part of the solute molecule. The effect decays exponentially with the increase of the alkyl chain length. An additional effect that can contribute to the discrepancies found for the $n(CH_2)$ and the alkyl chain length is the possible difference between the intensity of hydrophobic hydration interactions for methylene (CH_2) and methyl (CH_3) groups. The $n(CH_2)$ used in this work were optimized by Zaslavsky [6] and their suitability for polymer-salt ATPS has been proved before [23,24].

Applying the group-contribution concept, the linearity observed in figure 2 can be described by equation (5) (Section 2). Both parameters E and C in this equation were obtained by linear regression. Parameter E corresponds to the slope of the

TABLE 1
Partition coefficients obtained for the five DNP-amino acids at 23 °C. Tie-line data from reference [25].

Tie-line	Composition (% w/w)		TLL	K				
	Salt	Polymer		DNP-glycine	DNP-alanine	DNP-valine	DNP-leucine	DNP-Aca[a]
				(UCON + K_2HPO_4)				
I	3.80	18.02	0.230	4.947 ± 0.053	5.435 ± 0.040	9.069 ± 0.091	11.57 ± 0.19	31.91 ± 0.57
II	4.00	19.98	0.290	6.044 ± 0.033	7.555 ± 0.077	13.51 ± 0.15	16.15 ± 0.23	44.87 ± 0.56
III	4.20	22.00	0.332	8.586 ± 0.089	10.72 ± 0.18	16.55 ± 0.21	22.49 ± 0.30	67.4 ± 1.3
				(UCON + KPB)[b]				
I	5.30	13.01	0.215	4.354 ± 0.043	5.119 ± 0.072	7.88 ± 0.11	12.53 ± 0.11	38.56 ± 0.61
II	5.70	14.01	0.269	5.207 ± 0.069	7.136 ± 0.035	10.63 ± 0.10	18.68 ± 0.30	55.71 ± 0.72
III	6.10	15.02	0.308	7.21 ± 0.11	8.92 ± 0.16	17.64 ± 0.33	28.25 ± 0.41	82.36 ± 0.91
				(UCON + KH_2PO_4)				
I	7.00	15.00	0.258	5.933 ± 0.085	7.47 ± 0.12	15.38 ± 0.22	21.74 ± 0.28	79.2 ± 1.6
II	7.60	17.20	0.328	6.456 ± 0.056	8.43 ± 0.10	20.28 ± 0.21	32.48 ± 0.46	109.0 ± 2.3
III	8.50	18.99	0.387	14.17 ± 0.22	18.03 ± 0.19	43.22 ± 0.70	66.22 ± 0.59	294.6 ± 6.2
				(UCON + Na_2HPO_4)				
I	3.50	14.91	0.227	4.68 ± 0.11	5.405 ± 0.035	7.933 ± 0.044	11.55 ± 0.21	27.72 ± 0.45
II	3.90	16.01	0.283	7.200 ± 0.075	8.510 ± 0.092	12.50 ± 0.13	21.54 ± 0.17	60.89 ± 0.67
III	4.30	17.30	0.321	11.623 ± 0.085	13.02 ± 0.24	23.79 ± 0.44	33.56 ± 0.66	112.9 ± 1.4
				(UCON + NaPB)[c]				
I	3.91	15.97	0.259	6.153 ± 0.035	6.820 ± 0.067	11.376 ± 0.087	14.18 ± 0.11	39.10 ± 0.65
II	4.30	17.24	0.302	9.14 ± 0.10	10.186 ± 0.054	17.63 ± 0.20	25.96 ± 0.38	70.1 ± 1.0
III	4.70	18.59	0.344	12.15 ± 0.10	13.89 ± 0.16	21.12 ± 0.12	38.53 ± 0.36	104.4 ± 1.2
				(UCON + NaH_2PO_4)				
I	6.50	16.00	0.295	7.792 ± 0.068	10.72 ± 0.14	20.12 ± 0.25	29.11 ± 0.32	81.9 ± 2.5
II	6.99	18.26	0.351	8.95 ± 0.17	12.24 ± 0.21	25.13 ± 0.21	44.26 ± 0.33	116.3 ± 1.8
III	8.00	20.00	0.409	19.12 ± 0.24	30.0 ± 1.6	60.42 ± 0.82	98.1 ± 2.6	397 ± 12

[a] DNP-Aca: DNP-Amino caprylic acid.
[b] KPB: potassium phosphate buffer.
[c] NaPB: sodium phosphate buffer.

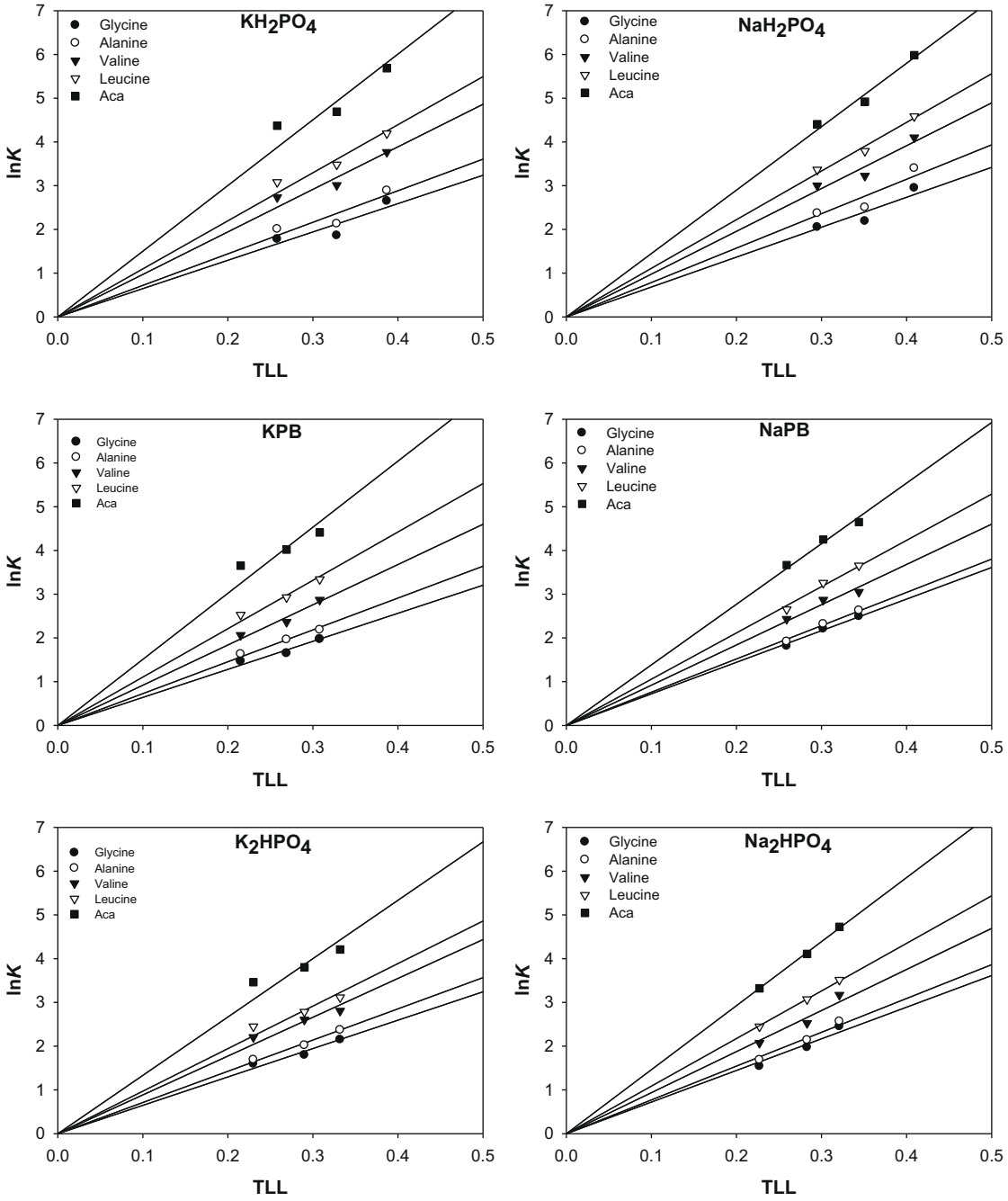

FIGURE 1. Logarithms of the partition coefficients of the DNP-amino acids as a function of the TLL for the six ATPS studied.

straight lines in figure 2, and parameter *C* corresponds to the intercept. Each parameter has a different meaning, as discussed in Section 2. Parameter *E* is related to the Gibbs free energy of transfer of a methylene group between the ATPS phases, $\Delta G^{*}(CH_2)$, through equation (6). $\Delta G^{*}(CH_2)$ values and parameters *C* and *E*, obtained from equations (5) and (6), are presented in table 2. There, it can be seen that both the *C* and *E* parameters depend on the system composition [29]. The biphasic systems composed by dihydrogen phosphate salts present the most negative $\Delta G^{*}(CH_2)$ values, thus these systems have a larger relative hydrophobicity. This larger relative hydrophobicity suggests a more efficient biphasic separation system, with lower cross-contamination between the equilibrium phases. $\Delta G^{*}(CH_2)$ values range from (−0.189 to −0.321) kcal/mol and are in agreement with those found in the literature for other polymer-salt ATPS (in the range −0.1 kcal/mol to −0.7 kcal/mol) [23,24,27].

The dependence of both *E* and *C* parameters with the TLL is shown in figure 3. The lines represented correspond to the linear regressions obtained. For parameter *C*, we can discriminate two tendencies: corresponding to the potassium and sodium salts. Therefore, *C* parameter, that represents the contribution of the polar groups present in the solute molecule to the partition coefficient, seems to be affected by the type of cation present in the biphasic systems. The anions used in this work are chemically similar, thus their effect in the *C* parameter are not identified in figure 3. On the other hand, the *E* parameter varies with the TLL, independently of the salt present in the system. A similar effect was reported in the literature for PEG–$(NH_4)_2SO_4$ ATPS formed by PEG of different molecular weights [29]: *E* parameter is independent of the molecular weight while *C* parameter is affected by it.

However, this independency is an oversimplification. When the representation is amplified, the *E* parameter shows different tendencies depending on the salt used in the ATPS formation. Figure 4 shows the $\Delta G^{*}(CH_2)$ values (proportional to *E* parameter through equation (6)) as a function of the TLL. The straight lines found indicate that $\Delta G^{*}(CH_2)$ increases linearly as the TLL increases, as previously reported [23]. Sodium phosphate salts provide higher slopes than the corresponding potassium salts, so $\Delta G^{*}(CH_2)$ for sodium phosphate salts ATPS is more affected by the differences between the compositions of the equilibrium phases. As it was pointed out before, the $\Delta G^{*}(CH_2)$ is a measure of the relative hydrophobicity of

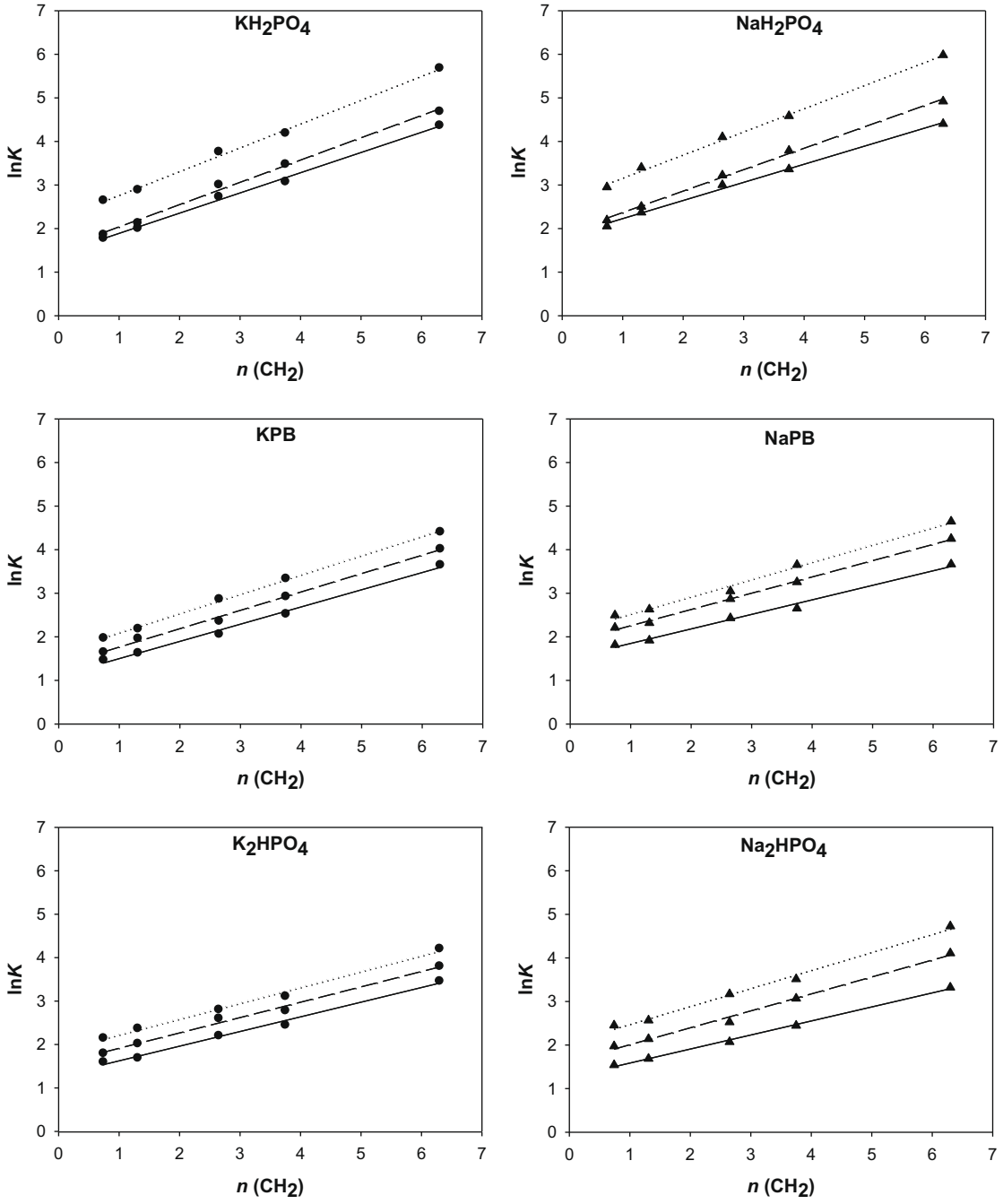

FIGURE 2. Logarithms of the partition coefficients of the DNP-amino acids as a function of the average number of methylene groups for the six ATPS studied.

the phases. The results shown herein confirm the expected idea that relative hydrophobicity increases with TLL: the larger a tie-line in the phase diagram, the larger the differences among the equilibrium phases, including their hydrophobicity. But the fact of being linearly related to the TLL allows to use $\Delta G^{*}(CH_2)$ as a parameter characteristic of the tie-line in a given ATPS. This can be used for comparison of different tie-lines on a given ATPS, or for comparison of different ATPS with a deeper understanding of the interactions involved in the phase splitting than just the length of the tie-lines.

The phosphate salt solutions used to prepare the biphasic systems have different pH: dihydrogen phosphate solutions have acidic pH, phosphate buffers have neutral pH, and hydrogen phosphate solutions have basic pH. Therefore, we can study the influence of the pH on the partition of the DNP-amino acids and, consequently, on the $\Delta G^{*}(CH_2)$. According to table 1 and comparing ATPS with similar TLL, the pH effect is more visible for the solutes with higher number of methylene groups, like DNP-leucine and DNP-amino caprylic acid. It is noted that the partition coefficient increases (more solute in the top phase) as the pH of the salt phase decreases. Consequently the $\Delta G^{*}(CH_2)$ becomes more negative as the pH of the salt solution decreases. When the $\Delta G^{*}(CH_2)$ values are plotted against the TLL and the pH of the salt phase used in the ATPS formation, a planar figure is obtained (figure 5).

For potassium salts the following equation was found:

– $\Delta G^{*}(CH_2) = (0.286 \pm 0.020) - (0.0165 \pm 0.0013) \cdot pH + (0.266 \pm 0.052) \cdot TLL$, $r^2 = 0.978$.

For sodium salts the equation found was:

– $\Delta G^{*}(CH_2) = (0.087 \pm 0.050) - (0.0036 \pm 0.0029) \cdot pH + (0.564 \pm 0.116) \cdot TLL$, $r^2 = 0.896$.

Thus, for these ATPS in the concentration range studied, the $\Delta G^{*}(CH_2)$ can be assumed as a linear function of both the TLL and the pH of the salt phase.

TABLE 2
$\Delta G^{*}(CH_2)$ values and parameters C and E obtained for the three tie-lines of the six UCON-phosphate ATPS.

Tie-line	C	E	r^2	$\Delta G^{*}(CH_2)$/(kcal/mol)
		(UCON + K_2HPO_4)		
I	1.286 ± 0.064	0.337 ± 0.018	0.992	−0.198
II	1.560 ± 0.070	0.353 ± 0.020	0.991	−0.208
III	1.856 ± 0.062	0.364 ± 0.017	0.993	−0.214
		(UCON + KPB)[a]		
I	1.105 ± 0.066	0.395 ± 0.019	0.993	−0.232
II	1.342 ± 0.056	0.422 ± 0.016	0.996	−0.248
III	1.653 ± 0.035	0.442 ± 0.010	0.999	−0.260
		(UCON + KH_2PO_4)		
I	1.427 ± 0.056	0.464 ± 0.016	0.997	−0.273
II	1.528 ± 0.076	0.511 ± 0.021	0.995	−0.301
III	2.226 ± 0.063	0.547 ± 0.018	0.997	−0.321
		(UCON + Na_2HPO_4)		
I	1.264 ± 0.034	0.322 ± 0.009	0.997	−0.189
II	1.617 ± 0.389	0.389 ± 0.019	0.993	−0.229
III	2.068 ± 0.069	0.413 ± 0.019	0.993	−0.243
		(UCON + NaPB)[b]		
I	1.514 ± 0.067	0.333 ± 0.019	0.991	−0.196
II	1.881 ± 0.037	0.373 ± 0.010	0.998	−0.219
III	2.126 ± 0.072	0.397 ± 0.020	0.992	−0.233
		(UCON + NaH_2PO_4)		
I	1.813 ± 0.053	0.416 ± 0.015	0.996	−0.245
II	1.926 ± 0.088	0.463 ± 0.025	0.992	−0.273
III	2.636 ± 0.059	0.532 ± 0.017	0.997	−0.313

[a] KPB: potassium phosphate buffer.
[b] NaPB: sodium phosphate buffer.

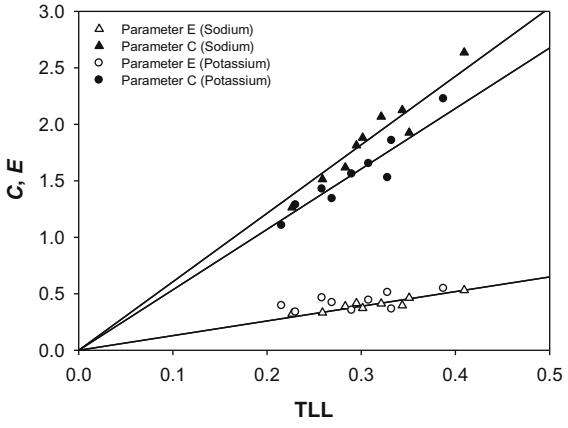

FIGURE 3. Parameters E and C as a function of the TLL for the six ATPS studied.

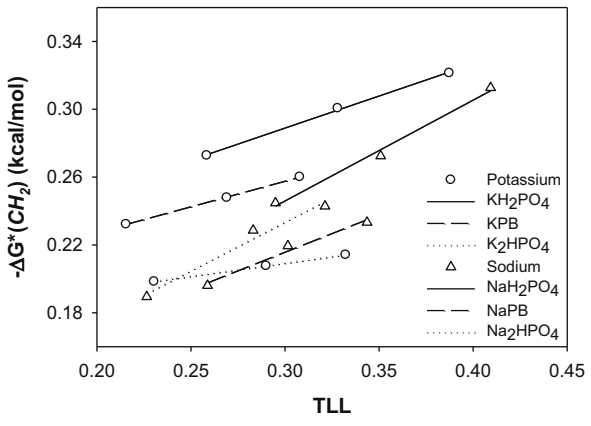

FIGURE 4. $\Delta G^{*}(CH_2)$ values as a function of the TLL for the six ATPS studied.

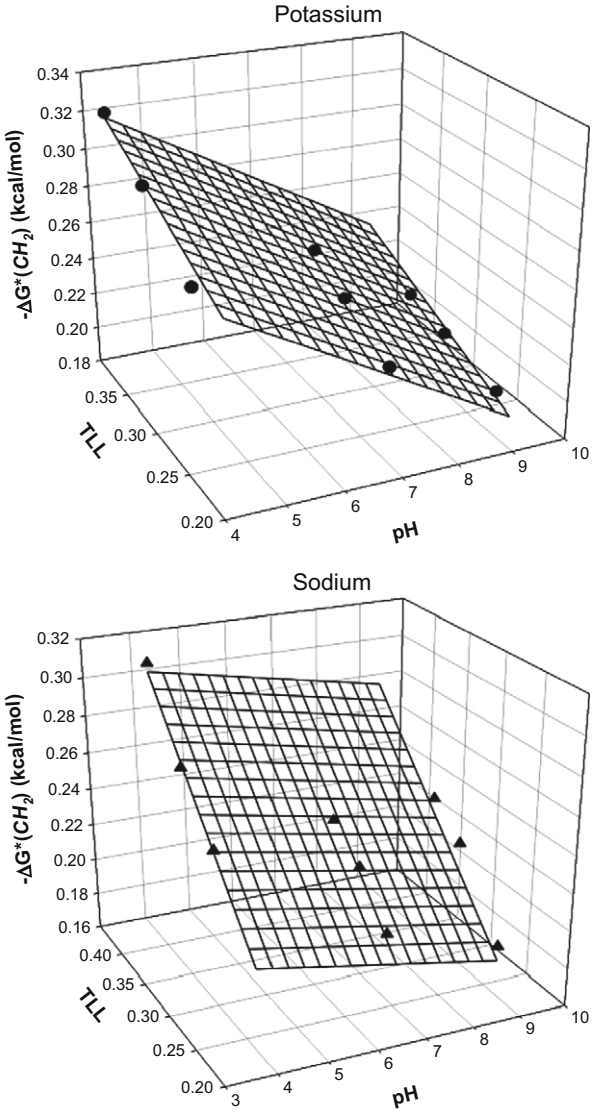

FIGURE 5. 3D representation of $\Delta G^{*}(CH_2)$ values as a function of the TLL and the pH of the salt for the potassium and sodium ATPS studied.

5. Conclusions

The partition coefficients experimentally obtained for five DNP-amino acids in three different tie-lines of the six UCON-phosphate salt ATPS allowed to determine the $\Delta G^{*}(CH_2)$. Using $\Delta G^{*}(CH_2)$ as a measure of the relative hydrophobicity of the equilibrium phases, ATPS formed by dihydrogen phosphate salts provide the largest relative hydrophobicity.

$\Delta G^{*}(CH_2)$ is dependent on the composition of the system and increases as the tie-line length increases. It was also found that, for the ATPS studied, $\Delta G^{*}(CH_2)$ can be considered a linear function of both the tie-line length and the pH of the salt phase.

Acknowledgements

Financial support for this work was in part provided by *Fundação para a Ciência e a Tecnologia* (FCT, Portugal) and by LSRE financing by FEDER/POCI/2010, for which the authors are thankful. Oscar Rodríguez acknowledges financial support of Programme Ciência 2007 (FCT). Sara Silvério acknowledges her Ph.D. grant of FCT (SFRH/BD/43439/2008).

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