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Salt effects on solvent features of coexisting phases in aqueous polymer/polymer two-phase systems☆

Pedro P. Madeira[a,b,*], Ana Bessa[a], Luís Álvares-Ribeiro[c], M. Raquel Aires-Barros[d,e], Celso A. Reis[b], Alírio E. Rodrigues[a], Boris Y. Zaslavsky[f]

[a] Laboratory of Separation and Reaction Engineering, Dpt. de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[b] IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[c] Requimte, Dep. Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre 687, 4169-007 Porto, Portugal
[d] IBB – Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Technical University of Lisbon, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
[e] Department of Bioengineering, Instituto Superior Técnico, Technical University of Lisbon, Lisboa, Portugal
[f] Analiza, Inc. 3615 Superior Ave., Suite 4407b, Cleveland, OH 44114, USA

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ABSTRACT

The solvatochromic parameters characterizing the solvent dipolarity/polarizability (π^*), solvent hydrogen-bond donor acidity (α), and solvent hydrogen-bond acceptor basicity (β) of aqueous media were measured in the coexisting phases of aqueous Dextran-Ficoll, Dextran-Ucon, Dextran-PEG, PEG-Ucon, Ficoll-Ucon, and Ficoll-PEG two-phase systems (ATPS). Ionic composition of each ATPS included 0.15 M KCl, 0.15 M KBr, 0.15 M NaBr, 0.1 M Na_2SO_4, and 0.1 M Li_2SO_4 in 0.01 M sodium phosphate buffer (NaPB), pH 7.4; and 0.01 M and 0.11 M sodium phosphate buffer, pH 7.4. Partition ratios of sodium salts of dinitrophenylated (DNP) amino acids with aliphatic side-chains (glycine, alanine, norvaline, norleucine, and α-amino-*n*-caprylic acid) were measured in all ATPSs, and the results were evaluated in terms of the differences between the relative hydrophobicity (parameter *E*) and the electrostatic properties (parameter *C*) of the aqueous media of the coexisting phases. It was established that parameter *E* is described by a linear combination of the differences between the solvent dipolarity/polarizability ($\Delta\pi^*$) and between the solvent hydrogen-bond acidity ($\Delta\alpha$) of the media in the coexisting phases. Parameter *C* depends on the phase forming polymer pair and is shown to be described by a linear combination of three parameters: the differences between the solvent hydrogen-bond acidity ($\Delta\alpha$) and between the solvent hydrogen-bond basicity ($\Delta\beta$) of the media in the coexisting phases, and a measure of the effect of a given salt additive on the hydrogen bonds in water. This effect was represented by a parameter (K_{b-l}), characterizing the equilibrium between populations of hydrogen bonds with a bent hydrogen bond conformation and with linear hydrogen bond conformation affected by a given salt additive.

1. Introduction

Aqueous two-phase systems arise in aqueous mixtures of different water-soluble polymers or a single polymer and a specific salt. When two specific polymers, for example, dextran and poly(ethylene glycol) (PEG), are mixed in water above certain concentrations, the mixture separates into two immiscible aqueous phases. There is a clear interfacial boundary separating two distinct aqueous-based phases, each preferentially rich in one of the polymers, with the aqueous solvent in both phases providing media suitable for biological products [1–4]. These systems are unique in that each of the phases contains over 80% water on a molal basis, yet they are immiscible and differ in their solvent properties [3,5–8], and therefore can be used for differential distribution of solutes and particles.

It has been shown previously [5–8] that the partition behavior of a solute in an aqueous two-phase system may be described within the framework of the so-called Abraham solvation parameter model [9–12] based on the assumption of the additivity of free energies of different types of solute–solvent interactions.

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* Corresponding author at: Laboratory of Separation and Reaction Engineering, Dpt. de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal. Tel.: +351 225081669; fax: +351 225081674.
E-mail address: ppalma@fe.up.pt (P.P. Madeira).

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The partition ratio[1] of a nonionic solute in an aqueous two-phase system may be expressed [5] as:

$$\log K_s = z_0 + s_s \Delta\pi^* + b_s \Delta\beta + a_s \Delta\alpha \quad (1)$$

where K is the solute partition ratio; π^* is the solvent dipolarity/polarizability, α is the solvent hydrogen-bond donor acidity, β is the solvent hydrogen-bond acceptor basicity; s_s, a_s, and b_s are constants (solute descriptors) that describe the complementary interactions of the solute with the solvent media in the coexisting phases; the subscript s designates the solute; and Δ denotes the difference for a given solvent property between the coexisting phases.

For description of the partition ratio of a charged solute, e.g. protein, an additional term must be added to account for the electrostatic interactions of such a solute with the solvent in the phases [6]:

$$\log K_s = z_0 + s_s \Delta\pi^* + b_s \Delta\beta + a_s \Delta\alpha + c_s C \quad (2)$$

where C is the contribution of a DNP-NH–CH–COO^-Na^+ group into $\log K$ (see below), coefficient c_s characterizes the electrostatic ion–ion, ion–dipole, and dipole–dipole interactions of the solute with the solvent media in the coexisting phases; and all the other terms are as defined above.

In this study the solvent features of aqueous media in the coexisting phases in aqueous two-phase systems (ATPSs) formed by various pairs of nonionic polymers in the presence of different salt additives were examined. The partitioning of a homologous series of sodium salts of dinitrophenylated amino acids with aliphatic side-chains was also investigated in each ATPS. The results obtained are analyzed from the viewpoint of the effect of salt additives on the aqueous media and the solute–solvent interactions in this media.

2. Material and methods

2.1. Material

2.1.1. Polymers

All polymers were used without further purification. Dextran 75 (lot 115195), weight-average molecular weight (M_w) ≅ 75 000 was purchased from USB (Cleveland, OH, USA). Polyethylene glycol 8000 (lot 69H00341), M_w = 8000 was purchased from Sigma–Aldrich (St. Louis, MO, USA). Ucon 50-HB-5100 (lot SJ1955S3D2), M_w = 3930 was purchased from Dow-Chemical (Midland, MI, USA). Ficoll 70 (lot 302970), M_w ≅ 70 000 was purchased from GE Healthcare Biosciences AB (Sweden).

2.1.2. Solvatochromic dyes

The solvatochromic probes 4-nitrophenol (reagent grade, >98%) and 4-nitroanisole (GC, >97%) were supplied by Aldrich, Milwaukee, WI, USA. Reichardt's carboxylated betaine dye was kindly provided by Professor C. Reichardt (Philipps University, Marburg, Germany).

2.1.3. Dinitrophenylated amino acids

Dinitrophenylated (DNP) amino acids – DNP-glycine, DNP-alanine, DNP-norvaline, DNP-norleucine, DNP-DL-α-amino-*n*-octanoic acid – were purchased from Sigma.

2.1.4. Other chemicals

All salts and other chemicals used were of analytical-reagent grade.

2.2. Methods

2.2.1. Solvatochromic studies

The ATPSs of the compositions listed in Table 1 were prepared as previously described [13,14]. The phases were separated and used for solvatochromic analysis. The solvatochromic probes 4-nitroanisole, 4-nitrophenol and Reichardt's carboxylated betaine (the carboxylated form of the dye of 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridinio)phenolate) were used to measure the polarity/polarizability π^*, H-bond acceptor (HBA) basicity β, and H-bond donor (HBD) acidity α in both phases of each particular ATPS. Three to four concentrations of each probe were prepared for each analysis in order to check for aggregation effects and specific interactions with the phase forming polymers by dissolving them and diluting in the phase sample. A strong base was added to the samples (~20 μL NaOH 1 M to 1.1 mL of the phase) containing the Reichardt's carboxylated betaine, to ensure a basic pH. The samples were mixed thoroughly in a vortex mixer and then scanned in a Sinergy-2 UV–vis microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) with a bandwidth of 2.0 nm, data interval of 1 nm, and high resolution scan (~0.5 nm/s). Pure phases containing no dye (blank) were scanned first to establish a baseline. The wavelength of maximum absorbance was determined as described by Huddleston et al. [15]. The maximum wavelength was the average between all these scans. Average standard deviations for each measured wavelength were ≤0.7 nm for all probes. A few drops of a strong acid (~20 μL HCl 1 M to 1.1 mL of the phase) were added to the samples containing 4-nitrophenol in order to eliminate charge transfer bands that were observed in some systems.

The behavior of the probes (4-nitrophenol, and Reichardt's carboxylated betaine dye) in several solvents (water, *n*-hexan, methanol and some phases of ATPSs) was tested in the presence and absence of HCl (for 4-nitrophenol) and NaOH (for the betaine dye) at different concentrations of the probes, acid or base, and the maximum shifts of the probes were compared to reference values found in the literature [15] and were within the experimental errors in all cases (data not shown).

The results of the solvatochromic studies were used to calculate π^*, β and α as described by Marcus [16].

2.2.1.1. Determination of the solvent dipolarity/polarizability π^*. π^* was determined from the wave number ($\nu_{(1)}$) of the longest wavelength absorption band of the 4-nitroanisole dye using the relationship:

$$\pi^* = 0.427(34.12 - \nu_{(1)}) \quad (3)$$

2.2.1.2. Determination of the solvent hydrogen-bond acceptor basicity β. β values were determined from the wave number ($\nu_{(2)}$) of the longest wavelength absorption band of the 4-nitrophenol dye using the relationship:

$$\beta = 0.346(35.045 - \nu_{(2)}) - 0.57\pi^* \quad (4)$$

2.2.1.3. Determination of the solvent hydrogen-bond donor acidity α. α values were determined from the longest wavelength absorption band of Reichardt's betaine dye using the relationship:

$$\alpha = 0.0649E_T(30) - 2.03 - 0.72\pi^* \quad (5)$$

The $E_T(30)$ values are based on the solvatochromic pyridinium N-phenolate betaine dye (Reichardt's dye) as probe, and are

[1] Partitioning ratio instead of partition coefficient as recommended by IUPAC terminology.

Table 1
Polymer composition of aqueous two-phase systems (ATPS) and solvatochromic solvent parameters[a] in the coexisting phases of ATPSs.

Polymer 1[b]	Polymer 2[b]	Total composition[b]		Top phase			Bottom phase		
		Polymer 1	Polymer 2	π^*	α	β	π^*	α	β
0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.114	1.014	0.689	1.166	1.022	0.636
Dextran	PEG	12.4	6.1	1.045	1.089	0.660	1.154	1.083	0.619
Dextran	Ucon	12.4	10.1	1.090	0.950	0.635	1.159	1.045	0.592
Ficoll	PEG	15.1	7.9	1.108	1.012	0.643	1.168	0.975	0.642
Ficoll	Ucon	13.0	9.9	1.095	0.944	0.590	1.138	1.009	0.654
PEG	Ucon	15.0	30.0	0.967	0.799	0.720	1.097	0.789	0.683
0.11 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.152	0.999	0.676	1.116	1.103	0.727
Dextran	PEG	12.4	6.1	1.084	1.038	0.598	1.294	0.993	0.583
Dextran	Ucon	12.4	10.1	1.098	0.878	0.681	1.153	1.045	0.666
Ficoll	PEG	15.1	7.9	1.119	0.984	0.647	1.171	0.995	0.656
Ficoll	Ucon	13.0	9.9	1.106	0.848	0.679	1.156	0.995	0.677
PEG	Ucon	15.0	30.0	–	–	–	–	–	–
0.15 M NaCl + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.188	0.984	0.633	1.150	1.048	0.678
Dextran	PEG	12.4	6.1	1.099	1.078	0.632	1.167	1.096	0.624
Dextran	Ucon	12.4	10.1	1.112	0.882	0.659	1.179	1.023	0.597
Ficoll	PEG	15.1	7.9	1.116	0.999	0.612	1.167	0.976	0.634
Ficoll	Ucon	13.0	9.9	1.146	0.850	0.667	1.031	1.063	0.644
PEG	Ucon	15.0	30.0	1.035	0.628	0.757	1.158	0.766	0.697
0.15 M KCl + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.141	1.046	0.633	1.142	1.073	0.618
Dextran	PEG	12.4	6.1	1.088	1.073	0.572	1.150	1.096	0.591
Dextran	Ucon	12.4	10.1	1.097	1.002	0.625	1.134	1.101	0.602
Ficoll	PEG	15.1	7.9	1.129	0.998	0.630	1.126	1.005	0.640
Ficoll	Ucon	13.0	9.9	1.087	0.957	0.649	1.114	1.048	0.660
PEG	Ucon	15.0	30.0	1.018	0.738	0.721	0.878	0.888	0.690
0.15 M NaBr + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.129	1.055	0.654	1.143	1.072	0.638
Dextran	PEG	12.4	6.1	1.076	1.083	0.606	1.139	1.115	0.609
Dextran	Ucon	12.4	10.1	1.082	0.937	0.641	1.081	1.147	0.631
Ficoll	PEG	15.1	7.9	1.379	0.799	0.619	1.263	0.890	0.682
Ficoll	Ucon	13.0	9.9	1.106	0.934	0.657	1.161	1.008	0.665
PEG	Ucon	15.0	30.0	1.040	0.706	0.762	1.086	0.820	0.698
0.15 M KBr + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.135	1.058	0.627	1.134	1.079	0.607
Dextran	PEG	12.4	6.1	1.082	1.083	0.569	1.124	1.130	0.588
Dextran	Ucon	12.4	10.1	1.083	0.963	0.624	1.138	1.104	0.607
Ficoll	PEG	15.1	7.9	1.194	0.950	0.697	1.168	0.991	0.712
Ficoll	Ucon	13.0	9.9	1.106	0.932	0.653	1.096	1.061	0.648
PEG	Ucon	15.0	30.0	1.073	0.632	0.761	1.089	0.789	0.670
0.10 M Na_2SO_4 + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.150	1.018	0.633	1.134	1.066	0.613
Dextran	PEG	12.4	6.1	1.099	1.035	0.577	1.134	1.110	0.594
Dextran	Ucon	12.4	10.1	1.079	0.894	0.644	1.129	1.098	0.608
Ficoll	PEG	15.1	7.9	1.136	0.961	0.689	1.153	1.004	0.686
Ficoll	Ucon	13.0	9.9	1.099	0.897	0.677	1.126	1.024	0.668
PEG	Ucon	15.0	30.0	–	–	–	–	–	–
0.10 M Li_2SO_4 + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	1.144	1.032	0.629	1.147	1.056	0.616
Dextran	PEG	12.4	6.1	1.075	1.065	0.578	1.128	1.117	0.589
Dextran	Ucon	12.4	10.1	1.084	0.920	0.634	1.118	1.119	0.619
Ficoll	PEG	15.1	7.9	1.147	0.987	0.698	1.131	1.031	0.668
Ficoll	Ucon	13.0	9.9	1.089	0.930	0.671	1.105	1.040	0.661
PEG	Ucon	15.0	30.0	1.024	0.580	0.820	1.121	1.008	0.708

[a] π^*, solvent dipolarity/polarizability; α, solvent hydrogen-bond donor acidity; β, solvent hydrogen-bond acceptor basicity.
[b] Polymer 1, predominant polymer in the bottom phase; polymer 2, predominant polymer in the top phase.

obtained directly from the wavelength (λ, nm) of the absorption band of the carboxylated form, as

$$E_T(30) = \frac{1}{0.932} \times \left(\frac{28591}{\lambda} - 3.335\right) \quad (6)$$

2.2.2. Partitioning

A semi-automated methodology for performing aqueous two-phase partitioning, was used to measure the partition of dinitrophenylated amino acids with aliphatic side-chains (glycine, alanine, norvaline, norleucine, and α-amino-*n*-caprylic acid) in all the ATPSs. Solutions of all compounds were prepared in water at concentrations of 1–5 mg/mL. Varied amounts (e.g. 0, 10, 20, 30, 40, and 50 μL) of a given compound solution and the complementary amounts (e.g. 100, 90, 80, 70, 60 and 50 μL) of water were added to a set of the same polymer/buffer/salt mixtures for a total mass of the system of 800 mg (~760 μL, volume ratio 1:1) using a Multipette Xstream pipette (Eppendorf, Hamburg, Germany). Systems were vortexed and centrifuged for 30–60 min at 10 000 × *g* in a minispin centrifuge (Eppendorf) to accelerate phase settling. Aliquots of 20–70 μL from the upper and lower phases were withdrawn with a Multipette Xstream pipette in duplicate for analysis.

Aliquots from both phases were diluted with water up to 250 μl in microplate wells. Following moderate shaking at room temperature (23 °C), a synergy-2 UV–vis plate reader (Bio-Tek Instruments) was used to measure optical absorbance at 360 nm. Phases of blank systems at corresponding dilutions were measured for comparison.

The partition ratio, *K*, is defined, in ATPS, as the ratio of the sample concentration in the upper phase to the sample concentration in the lower phase. Its value for each solute was determined as the slope of the plot of the concentration in the upper phase as a function of the concentration in the bottom phase obtained from six partition experiments carried out at different concentrations of the solute and at the fixed composition of the system. Deviation from the average *K* value was consistently below 5% and in most cases lower than 2%.

3. Results and discussion

3.1. Solvatochromic parameters

Each of the solvent parameters π^*, β, and α were obtained from a set of single solvatochromic probes as previously described [5]. The solvatochromic parameters measured in each phase of the ATPS are presented in Table 1. The differences between the values found for the top phases and for the corresponding bottom phases are shown in Table 2. The data reported earlier [5] for the same ATPSs with 0.15 M NaCl in 0.01 M sodium phosphate buffer (NaPB), pH 7.4 is also presented in Tables 1 and 2 for comparison.

The changes in the differences between solvatochromic parameters characterizing the coexisting phases due to the salt effects may be examined taking as reference the data obtained for ATPSs with 0.01 M NaPB, pH 7.4. This analysis shows that the changes under consideration are both parameter- and polymer combination-specific. As an example, all the salt additives decrease the difference between the solvent hydrogen-bond acidity ($\Delta\alpha$) in all the ATPSs under study and increase the difference between the solvent dipolarity/polarizability ($\Delta\pi^*$) of the coexisting phases in most of the ATPSs examined except in the Ficoll-Ucon ATPS where it is reduced in the presence of NaBr.

The effects observed are clearly polymer combination specific (effect of Na_2SO_4 on $\Delta\pi^*$ varies from 0.016 in Ficoll-Ucon ATPS up to 0.074 in Dex-PEG ATPS, and on $\Delta\alpha$ from −0.040 in Dex-Ficoll ATPS to −0.109 in Dex-Ucon ATPS). The salt effect on the difference between the solvent hydrogen-bond basicity ($\Delta\beta$) is even more dependent on the particular pair of polymers used to form an ATPS. As an example, all the salt additives examined increase the difference between the solvent hydrogen-bond basicity in the coexisting phases of Ficoll-Ucon ATPS and decrease this difference in the Dex-Ficoll and Dex-PEG ATPSs. In the Ficoll-PEG ATPS this difference increases in the presence of Na_2SO_4 and Li_2SO_4 and decreases in the presence of NaCl, KCl, NaBr, and KBr additives. These salt effects are likely to be related to their effect on the polymer composition of the coexisting phases in ATPS formed by different nonionic polymers as well as to the distribution of the salts between the phases, i.e. their particular ionic compositions. This issue remains to be explored and is beyond the scope of the present study.

It should be mentioned that the even very small differences between certain solvatochromic features of the coexisting phases, e.g. $\Delta\pi^* = -0.001$ in Dextran-Ficoll ATPS containing 0.15 M KCl in 0.01 M NaPB, pH 7.4 and in Dextran-Ucon ATPS in the presence of 0.15 M NaBr in 0.01 M NaPB, pH 7.4 are meaningful resulting from very different solvatochromic dipolarity/polariazability of the coexisting phases of 1.141 and 1.142 in the first case and 1.082 and 1.081 in the second one. Similarly the differences in the solvent hydrogen-bond donor acidity $\Delta\alpha = 0.006$ in Dextran-PEG ATPS containing 0.01 M NaPB, pH 7.4 results from the solvent H-bond acidity of the phases 1.089 and 1.083, while $\Delta\alpha = -0.007$ in Ficoll-PEG ATPS containing 0.15 M KCl in 0.01 M NaPB, pH 7.4 results from the solvent H-bond acidity of the phases 0.998 and 1.005.

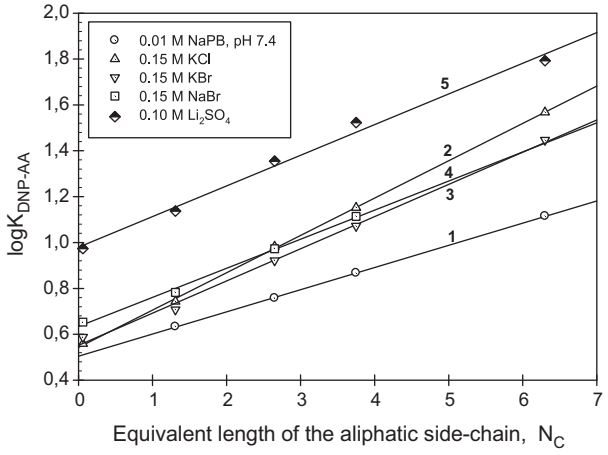

Fig. 1. Logarithm of the partition ratio, $\log K_{DNP\text{-}AA}$, value for sodium salts of DNP-amino acids with aliphatic side-chains in aqueous PEG-Ucon two-phase systems as a function of equivalent length of the side-chain, N_C, expressed in terms of equivalent number of CH_2 units. (1) 0.01 M sodium phosphate buffer, pH 7.4; (2) 0.15 M KCl in 0.01 M sodium phosphate buffer, pH 7.4; (3) 0.15 M KBr in 0.01 M sodium phosphate buffer, pH 7.4; (4) 0.15 M NaBr in 0.01 M sodium phosphate buffer, pH 7.4; (5) 0.10 M Li_2SO_4 in 0.01 M sodium phosphate buffer, pH 7.4.

The small value of the difference between any of the solvatochromic solvent feature of the phases should be viewed as representation of the difference between a given physical solvent property of aqueous media in the coexisting phases and treated as such.

3.2. Partition behavior of a homologous series of dinitrophenylated (DNP-) amino acids

Typical experimental data obtained for sodium salts of DNP-amino acids in different ATPSs are plotted in Figs. 1 and 2a–e, and the straight lines observed may be described as:

$$\log K^{(i)}_{DNP\text{-}AA} = C^{(i)} + E^{(i)} N_C \quad (7)$$

where $K_{DNP\text{-}AA}$ is the partition ratio of a DNP-amino acid with aliphatic side-chain; superscript (*i*) denotes the particular *i*th ATPS used for the partition experiments; N_C is the equivalent number of CH_2 groups in the aliphatic side-chain of a given DNP-amino acid; E is an average $\log K$ increment per CH_2 group; and *C* represents the total contribution of the non-alkyl part of the structure of a DNP-amino acid into $\log K_{DNP\text{-}AA}$.

The values determined for coefficients $E^{(i)}$ and $C^{(i)}$ in the ATPSs examined are listed in Table 2. It should be noted here that the partition ratio of a DNP-amino acid, $K_{DNP\text{-}AA}$, was determined in each ATPS as the ratio of the solute concentration in the top phase to that in the bottom phase. As the Gibbs energy of transfer of a solute between the coexisting phases is described as:

$$\Delta G^\circ = -RT \ln K \quad (8)$$

where *R* is the universal gas constant and *T* is the absolute temperature in Kelvin; it follows that

$$\Delta G(CH_2) = -RT \cdot E \quad (9)$$

where $\Delta G(CH_2)$ is the Gibbs energy of transfer of a methylene group from one coexisting phase to another, provided *E* is expressed in natural logarithmic units. $-\Delta G(CH_2)$ values calculated from the

Table 2
Polymer composition of aqueous two-phase systems (ATPS), solvatochromic solvent parameters[a] and their differences[a] in the coexisting phases of ATPSs.

Polymer 1[b]	Polymer 2[b]	Total composition[b]		Difference between phases[a]					
		Polymer 1	Polymer 2	$\Delta\pi^{*}$ [a]	$\Delta\alpha$[a]	$\Delta\beta$[b,a]	E[c]	C[c]	$\log K_{b-l}$[d]
0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	−0.051	−0.008	0.053	0.0105 ± 0.0002	0.1095 ± 0.0006	
Dextran	PEG	12.4	6.1	−0.109	0.006	0.040	0.0289 ± 0.0004	0.066 ± 0.001	
Dextran	Ucon	12.4	10.1	−0.069	−0.095	0.042	0.0632 ± 0.0006	0.214 ± 0.002	
Ficoll	PEG	15.1	7.9	−0.060	0.037	0.002	0.00748 ± 0.00003	−0.0424 ± 0.0001	
Ficoll	Ucon	13.0	9.9	−0.043	−0.065	−0.064	0.0396 ± 0.0004	0.0415 ± 0.0009	
PEG	Ucon	15.0	30.0	−0.130	0.010	0.036	0.0968 ± 0.0006	0.504 ± 0.002	
0.11 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	0.036	−0.104	−0.051	0.0195 ± 0.0001	0.1651 ± 0.0003	
Dextran	PEG	12.4	6.1	−0.210	0.045	0.015	0.0337 ± 0.0004	0.171 ± 0.002	
Dextran	Ucon	12.4	10.1	−0.056	−0.167	0.015	0.095 ± 0.006	0.404 ± 0.002	
Ficoll	PEG	15.1	7.9	−0.052	−0.011	−0.009	0.0220 ± 0.0003	−0.018 ± 0.001	
Ficoll	Ucon	13.0	9.9	−0.050	−0.147	0.002	0.0886 ± 0.0006	0.198 ± 0.002	
0.15 M NaCl + 0.01 M NaPB, pH 7.4[e]									
Dextran	Ficoll	12.9	18.1	0.038	−0.064	−0.045	0.0191 ± 0.0006	0.054 ± 0.002	0.01120
Dextran	PEG	12.4	6.1	−0.068	−0.018	0.008	0.0271 ± 0.001	−0.039 ± 0.003	0.01120
Dextran	Ucon	12.4	10.1	−0.068	−0.141	0.062	0.085 ± 0.002	0.0017 ± 0.007	0.01120
Ficoll	PEG	15.1	7.9	−0.051	0.023	−0.022	0.0097 ± 0.001	−0.092 ± 0.003	0.01120
Ficoll	Ucon	13.0	9.9	0.115	−0.213	0.023	0.054 ± 0.005	−0.03 ± 0.02	0.01120
PEG	Ucon	15.0	30.0	−0.123	−0.138	0.060	0.095 ± 0.007	0.61 ± 0.02	0.01120
0.15 M KCl + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	−0.001	−0.027	0.015	0.0102 ± 0.0001	0.0483 ± 0.0005	0.01094
Dextran	PEG	12.4	6.1	−0.062	−0.023	−0.019	0.0274 ± 0.0002	−0.0279 ± 0.0007	0.01094
Dextran	Ucon	12.4	10.1	−0.037	−0.099	0.023	0.063 ± 0.002	0.061 ± 0.004	0.01094
Ficoll	PEG	15.1	7.9	0.003	−0.007	−0.010	0.0079 ± 0.0002	−0.0513 ± 0.0007	0.01094
Ficoll	Ucon	13.0	9.9	−0.027	−0.091	−0.011	0.0405 ± 0.0003	0.028 ± 0.001	0.01094
PEG	Ucon	15.0	30.0	0.140	−0.150	0.031	0.112 ± 0.002	0.444 ± 0.009	0.01094
0.15 M NaBr + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	−0.014	−0.017	0.016	0.01448 ± 0.00009	0.0330 ± 0.0003	0.01252
Dextran	PEG	12.4	6.1	−0.063	−0.035	−0.003	0.0248 ± 0.0006	−0.051 ± 0.0002	0.01252
Dextran	Ucon	12.4	10.1	0.001	−0.210	0.010	0.0801 ± 0.0004	0.038 ± 0.001	0.01252
Ficoll	PEG	15.1	7.9	0.116	−0.091	−0.063	0.00882 ± 0.00004	−0.0644 ± 0.0002	0.01252
Ficoll	Ucon	13.0	9.9	−0.055	−0.074	−0.008	0.0462 ± 0.0005	−0.052 ± 0.002	0.01252
PEG	Ucon	15.0	30.0	−0.046	−0.114	0.064	0.095 ± 0.001	0.480 ± 0.004	0.01252
0.15 M KBr + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	0.001	−0.021	0.020	0.00779 ± 0.00008	0.0406 ± 0.0003	0.01369
Dextran	PEG	12.4	6.1	−0.042	−0.047	−0.019	0.0264 ± 0.0003	−0.038 ± 0.001	0.01369
Dextran	Ucon	12.4	10.1	−0.055	−0.141	0.017	0.0761 ± 0.0005	0.017 ± 0.002	0.01369
Ficoll	PEG	15.1	7.9	0.026	−0.041	−0.015	0.0080 ± 0.0002	−0.0439 ± 0.0008	0.01369
Ficoll	Ucon	13.0	9.9	0.010	−0.129	0.005	0.0477 ± 0.0005	−0.036 ± 0.002	0.01369
PEG	Ucon	15.0	30.0	−0.016	−0.157	0.091	0.090 ± 0.001	0.458 ± 0.003	0.01369
0.10 M Na_2SO_4 + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	0.016	−0.048	0.020	0.0194 ± 0.0004	0.139 ± 0.002	0.00352
Dextran	PEG	12.4	6.1	−0.035	−0.075	−0.017	0.0354 ± 0.0003	0.097 ± 0.001	0.00352
Dextran	Ucon	12.4	10.1	−0.050	−0.204	0.036	0.085 ± 0.003	0.357 ± 0.007	0.00352
Ficoll	PEG	15.1	7.9	−0.017	−0.043	0.003	0.016 ± 0.001	−0.047 ± 0.003	0.00352
Ficoll	Ucon	13.0	9.9	−0.027	−0.127	0.009	0.062 ± 0.001	0.177 ± 0.005	0.00352
0.10 M Li_2SO_4 + 0.01 M NaPB, pH 7.4									
Dextran	Ficoll	12.9	18.1	−0.003	−0.024	0.013	0.0138 ± 0.0003	0.093 ± 0.001	0.00038
Dextran	PEG	12.4	6.1	−0.053	−0.052	−0.011	0.0308 ± 0.0006	0.124 ± 0.002	0.00038
Dextran	Ucon	12.4	10.1	−0.034	−0.199	0.015	0.093 ± 0.004	0.37 ± 0.01	0.00038
Ficoll	PEG	15.1	7.9	0.016	−0.044	0.030	0.0104 ± 0.0006	−0.022 ± 0.002	0.00038
Ficoll	Ucon	13.0	9.9	−0.016	−0.11	0.010	0.055 ± 0.001	0.193 ± 0.002	0.00038
PEG	Ucon	15.0	30.0	−0.097	−0.428	0.112	0.088 ± 0.001	0.897 ± 0.004	0.00038

[a] π^{*}, solvent dipolarity/polarizability; α, solvent hydrogen-bond donor acidity; β, solvent hydrogen-bond acceptor basicity; all differences are calculated between values measured in the top phases and those measured in the bottom phases.
[b] Polymer 1, predominant polymer in the bottom phase; polymer 2, predominant polymer in the top phase; all concentrations of polymers are in wt.%.
[c] Parameter E and C obtained from Eq. (7).
[d] Salt effects on the hydrogen bond network of water [21] (for details see text).
[e] Data for ATPS with 0.15 M NaCl in 0.01 M NaPB, pH 7.4 were reported previously [5] and are presented here for comparison.

experimental data with Eqs. (7)–(9) are listed in Table 3 in decreasing order.

3.2.1. Parameter E

The values presented in Table 3 show that the difference between the relative hydrophobic character of the phases decreases in the following series: PEG-Ucon > Dex-Ucon > Ficoll-Ucon > Dex-PEG > Dex-Ficoll > Ficoll-PEG. The effects of various salt additives on the difference between the relative hydrophobic characters of the coexisting phases in ATPSs formed by different pairs of polymers vary according to the pair of polymers. For example, the difference in question decreases in the Dex-Ficoll ATPS as: 0.11 M NaPB ≥ Na_2SO_4 ≥ NaCl > NaBr > Li_2SO_4 > 0.01 M NaPB > KCl > KBr, while in the Dex-PEG ATPS it decreases as: Na_2SO_4 > 0.11 M NaPB > Li_2SO_4 > 0.01 M NaPB > KCl > NaCl > KBr > NaBr.

There are several factors likely involved in the effect of a given salt additive on the difference between the relative hydrophobic characters of the coexisting phases in a given ATPS. One factor is the salt additive effect on the phase diagram, i.e. on the polymer

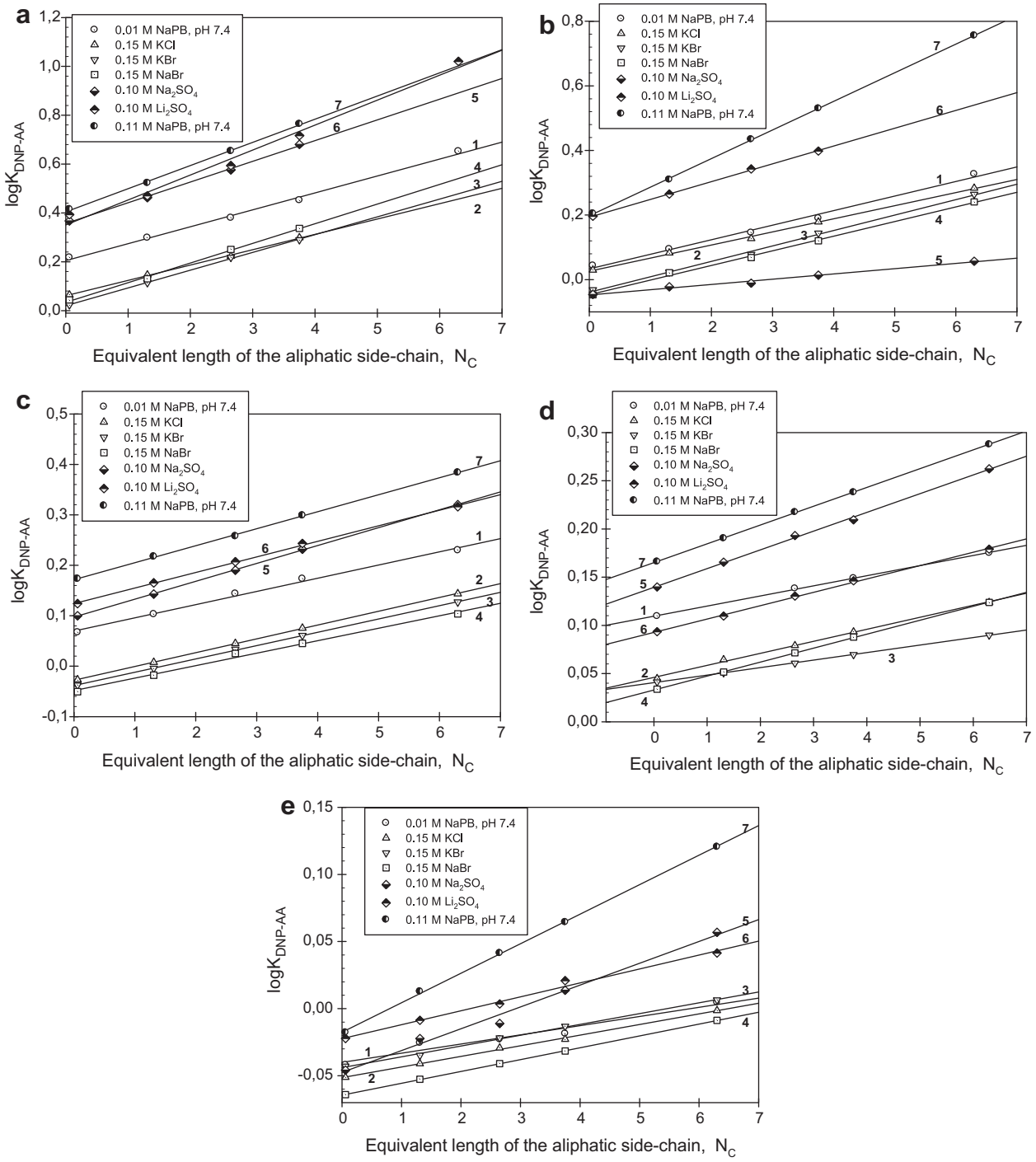

Fig. 2. Logarithm of the partition ratio, log $K_{DNP\text{-}AA}$, value for sodium salts of DNP-amino acids with aliphatic side-chains as a function of equivalent length of the side-chain, N_C, expressed in terms of equivalent number of CH_2 units. (1) 0.01 M sodium phosphate buffer, pH 7.4; (2) 0.15 M KCl in 0.01 M sodium phosphate buffer, pH 7.4; (3) 0.15 M KBr in 0.01 M sodium phosphate buffer, pH 7.4; (4) 0.15 M NaBr in 0.01 M sodium phosphate buffer, pH 7.4; (5) 0.10 M Na_2SO_4 in 0.01 M sodium phosphate buffer, pH 7.4; (6) 0.10 M Li_2SO_4 in 0.01 M sodium phosphate buffer, pH 7.4; (7) 0.11 M sodium phosphate buffer, pH 7.4. (a) In aqueous Dextran-Ucon two-phase systems. (b) In aqueous Ficoll-Ucon two-phase systems. (c) In aqueous Dextran-PEG two-phase systems. (d) In aqueous Dextran-Ficoll two-phase systems. (e) In aqueous Ficoll-PEG two-phase systems.

compositions of the coexisting phases. This factor is likely to be interrelated with the distribution of the salt additive in the phases, i.e. ionic compositions of the phases, and possibly with the influence of the salt additive on the features of the aqueous media.

The Gibbs energy of transfer of a methylene group between the phases is generally considered [3,5–8,17] to be equivalent to the difference in Gibbs energy of cavity formation in both phases of ATPSs. It was reported previously [5] that this energy difference (parameter E in Eq. (3)) is interrelated with solvatochromic parameters $\Delta\pi^*$ and $\Delta\alpha$. Analysis of these parameters (presented in Table 2) confirms this relationship which can be described as (for all ATPS except PEG-Ucon):

$$E = 0(\pm 0.001) - 0.27(\pm 0.02)\Delta\pi^* - 0.41(\pm 0.01)\Delta\alpha \quad (10)$$

$N = 43$; $R^2 = 0.9601$; SD = 0.006; $F = 481.4$

Table 3
Difference between the relative hydrophobic character of the coexisting phases of aqueous two-phase systems (ATPSs) of indicated polymer and ionic composition.

Polymer 1	Polymer 2	Total polymer composition[a]		Salt composition[b]	$-\Delta G(CH_2)$[c] cal/mol
		Polymer 1	Polymer 2		
PEG	Ucon	15.0	30.0	0.15 M KCl	220.7 ± 1.2
PEG	Ucon	15.0	30.0	0.15 M KBr	189.6 ± 2.9
PEG	Ucon	15.0	30.0	0.10 M Li_2SO_4	181.5 ± 4.1
PEG	Ucon	15.0	30.0	0.15 M NaBr	172.0 ± 3.5
PEG	Ucon	15.0	30.0	0.15 M NaCl	143.5 ± 0.6
Dextran	Ucon	12.4	10.1	0.10 M Li_2SO_4	138.1 ± 4.1
PEG	Ucon	15.0	30.0	0.01 M NaPB	131.1 ± 0.35
Dextran	Ucon	12.4	10.1	0.11 M NaPB	128.6 ± 3.5
Ficoll	Ucon	13.0	9.9	0.11 M NaPB	120.0 ± 0.35
Dextran	Ucon	12.4	10.1	0.15 M NaCl	115.1 ± 1.2
Dextran	Ucon	12.4	10.1	0.10 M Na_2SO_4	115.1 ± 1.8
Dextran	Ucon	12.4	10.1	0.15 M NaBr	107.5 ± 0.18
Dextran	Ucon	12.4	10.1	0.15 M KBr	97.7 ± 0.02
Dextran	Ucon	12.4	10.1	0.01 M NaPB	85.6 ± 0.35
Dextran	Ucon	12.4	10.1	0.15 M KCl	85.3 ± 1.2
Ficoll	Ucon	13.0	9.9	0.10 M Na_2SO_4	84.0 ± 0.59
Ficoll	Ucon	13.0	9.9	0.10 M Li_2SO_4	74.5 ± 0.59
Ficoll	Ucon	13.0	9.9	0.15 M NaCl	73.1 ± 2.9
Ficoll	Ucon	13.0	9.9	0.15 M KBr	64.6 ± 0.29
Ficoll	Ucon	13.0	9.9	0.15 M NaBr	62.6 ± 0.29
Ficoll	Ucon	13.0	9.9	0.15 M KCl	54.8 ± 0.18
Ficoll	Ucon	13.0	9.9	0.01 M NaPB	53.6 ± 0.24
Dextran	PEG	12.4	6.1	0.10 M Na_2SO_4	47.9 ± 0.18
Dextran	PEG	12.4	6.1	0.11 M NaPB	45.6 ± 0.24
Dextran	PEG	12.4	6.1	0.10 M Li_2SO_4	41.7 ± 0.35
Dextran	PEG	12.4	6.1	0.01 M NaPB	39.1 ± 0.24
Dextran	PEG	12.4	6.1	0.15 M KCl	37.1 ± 0.12
Dextran	PEG	12.4	6.1	0.15 M NaCl	36.7 ± 0.59
Dextran	PEG	12.4	6.1	0.15 M KBr	35.8 ± 0.18
Dextran	PEG	12.4	6.1	0.15 M NaBr	33.6 ± 0.35
Ficoll	PEG	15.1	7.9	0.11 M NaPB	29.8 ± 0.18
Dextran	Ficoll	12.9	18.1	0.11 M NaPB	26.4 ± 0.06
Dextran	Ficoll	12.9	18.1	0.10 M Na_2SO_4	26.3 ± 0.24
Dextran	Ficoll	12.9	18.1	0.15 M NaCl	25.9 ± 0.35
Ficoll	PEG	15.1	7.9	0.10 M Na_2SO_4	21.7 ± 0.59
Dextran	Ficoll	12.9	18.1	0.15 M NaBr	19.6 ± 0.05
Dextran	Ficoll	12.9	18.1	0.10 M Li_2SO_4	18.7 ± 0.18
Dextran	Ficoll	12.9	18.1	0.01 M NaPB	14.2 ± 0.12
Ficoll	PEG	15.1	7.9	0.10 M Li_2SO_4	14.1 ± 0.35
Dextran	Ficoll	12.9	18.1	0.15 M KCl	13.8 ± 0.06
Ficoll	PEG	15.1	7.9	0.15 M NaCl	13.1 ± 0.59
Ficoll	PEG	15.1	7.9	0.15 M NaBr	11.9 ± 0.02
Ficoll	PEG	15.1	7.9	0.15 M KBr	10.8 ± 0.12
Ficoll	PEG	15.1	7.9	0.15 M KCl	10.7 ± 0.12
Dextran	Ficoll	12.9	18.1	0.15 M KBr	10.5 ± 0.05
Ficoll	PEG	15.1	7.9	0.01 M NaPB	10.1 ± 0.02

[a] Polymer 1, predominant polymer in the bottom phase; polymer 2, predominant polymer in the top phase; all concentrations of polymers are in wt.%.
[b] Salt composition–indicated salt + 0.01 M NaPB, pH 7.4 or NaPB of indicated concentration.
[c] Gibbs energy of transfer of a CH_2 group from the bottom phase to the top phase of the ATPS indicated.

where E, $\Delta\pi^*$, and $\Delta\alpha$ are as defined above; N is number of ATPSs of different polymer and ionic composition; F is the ratio of variance; SD is the standard deviation, and R is the correlation coefficient.

For PEG-Ucon ATPS the above relationship is described as:

$$E = 0.1120(\pm 0.0006) + 0.034(\pm 0.003)\ \Delta\pi^* - 0.026(\pm 0.003)\ \Delta\alpha - 0.28(\pm 0.02)\,\Delta\beta \quad (11)$$

$$N = 6;\ R^2 = 0.9984;\ \mathrm{SD} = 0.0005;\ F = 407.5$$

The relationship described by Eq. (10) indicates that the relative Gibbs energy difference in the cavity formation between the phases of ATPS used (except PEG-Ucon) depends on both solvent dipolarity/polarizability, π^*, and solvent hydrogen-bond acidity, α, of the aqueous media in the coexisting phases. In the case of PEG-Ucon ATPSs the similar relationship includes additionally the β term describing the solvent hydrogen-bond basicity of the aqueous media in the phases. It seems reasonable that the Gibbs energy of cavity formation in an aqueous medium, resulting in rearrangement of the highly cooperative hydrogen-bonds network, would involve all types of solvent–solvent interactions. It is unclear yet why the hydrogen-bond basicity of aqueous media is not a significant factor in the Gibbs energy of cavity formation for the ATPSs examined except for those formed by PEG and Ucon. The different behavior of the ATPS PEG/Ucon may be due to the higher total polymer concentration (45 wt.%) which significantly exceeds the concentration used in the other ATPS tested (from 18.5 to 31 wt.%) and thus likely affects the properties of water–water interactions.

It should be noted that both equations are valid across all examined salt compositions, which seemingly agrees with the viewpoint that an ion affects only water molecules within its first hydration shell [18,19]. The salt effects were studied in aqueous polymer systems (not in water) where the presence of nonionic polymers appears to dominate over the other factors, such as the impact of salt additives on the solvent features of salt additives. The fact that the relative hydrophobic character of the phases of ATPSs is governed mainly by the corresponding pairs of

polymers and not so much influenced by the salts added, supports this assumption.

3.2.2. Parameter C

An important feature of the ATPSs used is the difference in concentrations of phosphate buffer and a salt additive in the coexisting phases. Buffers and salts are known to affect the polymer composition of the phases and their solvent properties so much that, for example, the same Dex–PEG systems with different concentrations of salt and/or buffer additives are to be considered as different ATPSs with different solvent properties of the phases [3, pp. 155–220]. The difference between the electrostatic properties of the coexisting phases translates to different ion–ion, ion–dipole and possibly dipole–dipole solute–solvent interactions. A linear solvation energy relationship (Eq. (1)) is applicable to the partitioning of nonionic solutes, but an added advantage of ATPS is the possibility to study the partitioning of ionizable compounds. It was shown [6] that an additional parameter capable of quantifying the difference between the electrostatic properties of the phases has to be included in Eq. (1) to describe partitioning of ionized solutes. Zaslavsky [3, pp. 208–216] proposed the use of the contribution of ionic group into the solute partition ratio as an empirical measure of the difference in question. The experimental results here were obtained with sodium salts of p-dinitrophenyl-amino acids, i.e. compounds possessing a DNP–NH–CH–COO^-Na^+ group. This moiety is bulky and contains a substituted aromatic ring. The use of this particular group as a probe for electrostatic ion–ion, ion–dipole and dipole–dipole interactions obviously has some limitations. Only to a first approximation the Gibbs energy of transfer of this group between the coexisting phases of an ATPS may be viewed as a measure of the ability of aqueous media to participate in a particular kind of molecular interactions. This parameter is represented by C in Eq. (7), and it is included in the modified linear solvation energy relationship Eq. (2) (used successfully for description of protein partitioning in ATPSs [6]).

Analysis of Table 2 indicates that the C values depend both on polymer and salt composition of the ATPS.

According to the C parameter values, the difference between the electrostatic ion–ion, ion–dipole and dipole–dipole interactions in the coexisting phases decreases in the following series: PEG-Ucon ($0.504 \le C \le 0.98$) > Dex-Ucon ($0.002 \le C \le 0.404$) > Ficoll-Ucon ($-0.052 \le C \le 0.198$) > Dex-PEG ($-0.051 \le C \le 0.171$) > Dex-Ficoll ($0.033 \le C \le 0.1651$) > Ficoll-PEG ($-0.092 \le C \le -0.018$).

In the presence of different salt additives the general order of decreasing parameter C values changes as 0.11 M NaPB, pH 7.4 > sulfate additives > 0.01 M NaPB, pH 7.4 > halides additives, with the order within a particular salt type depending on the pair of polymers forming a given ATPS.

It clearly follows from the suggested use of parameter C as a measure of the relative difference between the electrostatic properties of the aqueous media in the coexisting phases of ATPSs that this parameter might be expected to be influenced by the salt composition of an ATPS to a noticeable degree. There are several measures of the effects of anions (or cations) on the water structure [20] but rather limited information on the effects of salts. The salt effects on the hydrogen bond network of water were recently studied by Nucci and Vanderkooi [21], and the data reported is used here as a measure of the salt effect on the solvent properties of aqueous media. Infrared spectra of aqueous solutions of a variety of inorganic salts were examined [21] in terms of the line shape of the O—H stretch. The results obtained were analyzed in the framework of the two-state hydrogen bonding model (linear/bent), according to which the water hydrogen bond network exists as a continuous network of hydrogen bond distances and angles, naturally exhibiting a roughly bimodal distribution with respect to hydrogen bond angle. The spectra were examined in terms of the ratios of the areas assigned to populations of linear hydrogen bonds and bent hydrogen bonds, and these ratios were used to calculate the effective equilibrium constant K_{b-l} and an effective Gibbs energy of the transition from a bent hydrogen bond conformation to a linear conformation, $\Delta G_{b\to l}$ at the 4.4 molal ion concentration solution of a given salt in the 4% H_2O in D_2O mixture. The data reported in [21] for the salts used were back calculated in terms of $\log K_{b-l}$, normalized in regard to the salt concentration assuming the linear concentration dependence of $\log K_{b-l}$, and are presented in Table 2.

Parameter C was expressed in terms of the solvatochromic features of the coexisting phases and the salt composition of an ATPS represented by the $\log K_{b-l}$ parameter value. The results obtained are as follows.

For Dex-Ficoll ATPS:

$$C = 0.03(\pm0.01) - 2.0(\pm0.3)\ \Delta\alpha + 1.3(\pm0.2)\,\Delta\beta - 4.3(\pm0.6)\ \log K_{b-l} \quad (12)$$

$N = 6$; $r^2 = 0.9907$; SD = 0.006; $F = 71.1$

For Dex-PEG ATPS:

$$C = 0.09(\pm0.01) - 0.6(\pm0.2)\ \Delta\alpha - 0.4(\pm0.4)\,\Delta\beta - 12.4(\pm0.8)\ \log K_{b-l} \quad (13)$$

$N = 6$; $r^2 = 0.9968$; SD = 0.007; $F = 205.1$

For Dex-Ucon ATPS:

$$C = 0.4(\pm0.2) - 0.3(\pm0.7)\ \Delta\alpha - 1(\pm1)\,\Delta\beta - 30(\pm6)\log K_{b-l} \quad (14)$$

$N = 6$; $r^2 = 0.9571$; SD = 0.06; $F = 14.9$

For Ficoll-PEG ATPS:

$$C = -0.08(\pm0.01) - 0.6(\pm0.1)\ \Delta\alpha + 1.1(\pm0.2)\,\Delta\beta + 2(\pm1)\ \log K_{b-l} \quad (15)$$

$N = 6$; $r^2 = 0.9586$; SD = 0.008; $F = 15.4$

For Ficoll-Ucon ATPS:

$$C = 0.18(\pm0.09) - 1(\pm1)\ \Delta\alpha - 3(\pm5)\,\Delta\beta - 23(\pm5)\log K_{b-l} \quad (16)$$

$N = 6$; $r^2 = 0.9602$; SD = 0.03; $F = 16.1$

For PEG-Ucon ATPS:

$$C = 1.4(\pm0.6) + 2(\pm2)\ \Delta\alpha + 3(\pm2)\,\Delta\beta - 70(\pm39)\log K_{b-l} \quad (17)$$

$N = 5$; $r^2 = 0.9644$; SD = 0.07; $F = 9.0$

where N is the number of salt additives examined (ATPSs with 0.11 M NaPB, pH 7.4 were excluded, since there was no $\log K_{b\to l}$ data available); $\log K_{b\to l}$, r^2, SD, and F as defined above.

In order to explain the observed relationships described by Eqs. (12)–(17) it is necessary to emphasize that parameter C represents the average contribution of the charged polar DNP-NH–CH–COO^-Na^+ group into logarithm of the partition ratio of the dinitrophenylated amino acid, and as such represents all the specific interactions of this group with the solvent media in the coexisting phases. These interactions are clearly different in the phases of ATPSs formed by different pairs of polymers. Using parameter C in Eq. (2) for charged solutes (proteins) as a factor

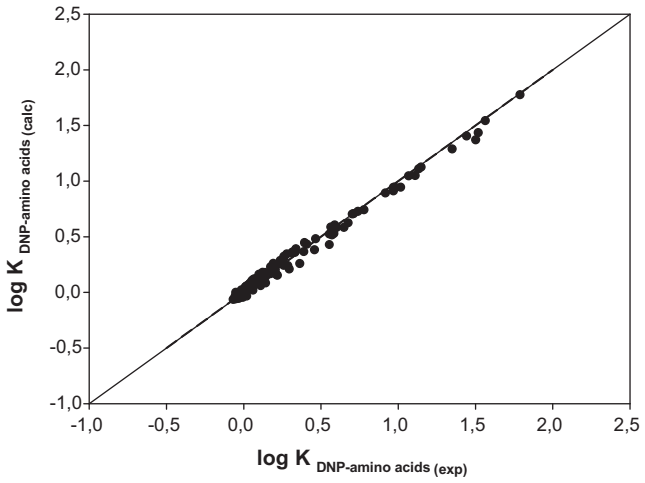

Fig. 3. Calculated vs. experimental values of logarithms of partition ratios for sodium salts of DNP-amino acids in all different ATPSs of different salt compositions.

representing the differences between the electrostatic properties of coexisting phases in ATPS formed by various pairs of polymers seems to take care of both specific polymer and ionic composition effects.

The large uncertainties associated to the coefficients for $\Delta\alpha$ and $\Delta\beta$ in Eqs. (14) and (16) and in the coefficient for $\Delta\beta$ in Eq. (13) should be noticed, the reasons remain unclear at this time. However, neglecting of one or both these parameters from the considered relationships does not improve the correlations to any significant degree. The coefficients for the parameter $\log K_{b-l}$ in Eqs. (12)–(17) change in agreement with the aforementioned sequence of the range of parameter *C*-values for ATPSs formed by different pairs of polymers. The salt additive effect on the relative difference between the electrostatic properties of the aqueous media in the coexisting phases of ATPSs appears to depend on the particular pair of phase forming polymers.

The relatively high values of coefficients for $\log K_{b-l}$ parameter in Eqs. (12)–(17) imply that the salt composition does affect the solvent properties of the aqueous media in the coexisting phases by influencing the hydrogen bond network [21]. These data, in seeming conflict with the aforementioned view that an ion affects only water molecules within its first hydration shell [18,19], support the viewpoint [21–27] that ions affect the water hydrogen bonds in the solution.

Combining Eqs. (7), (10) and (12) (for Dex-Ficoll ATPSs) or (7), (11) and (17) (for PEG-Ucon ATPSs), etc. it is possible to calculate $\log K_{\text{DNP-AA}}$ for each DNP-amino acid in every ATPS examined with a given salt additive. Near perfect agreement between the partition ratios directly measured and those *K*-values calculated using equations described above is shown in Fig. 3. These results imply that (a) partition behavior of charged solutes in two-polymer ATPS is governed by the interactions of the solutes with the aqueous media in the coexisting phases; and (b) the salt composition of the ATPS may be adequately represented by the effect of the salt on the properties of hydrogen bonds in the aqueous media.

The results obtained in this study allow to conclude that a given pair of polymers used to form an ATPS defines the space of the solvent features of the aqueous media in the coexisting phases. This space may overlap with the one defined by another pair of polymers as clearly observed (Table 3) in the Dex-Ficoll and Ficoll-PEG ATPSs, for example. Salt additives affect the position of the solvent properties of the phases within the space pre-defined by the combination of phase-forming polymers. Furthermore, the Gibbs energy of formation of a cavity in the aqueous media in the coexisting phases of two-polymer ATPSs is determined by a combination of the dipolarity/polarizability and hydrogen-bond acidity in most of the ATPSs examined (Eq. (10)) and additionally by the hydrogen-bond basicity of the solvent media in ATPSs formed by PEG and Ucon (Eq. (11)). The presence and type of salt additive do not affect the combinations in question. The solute–solvent interactions of a particular charged chemical group depend upon both polymer and ionic composition of two-polymer ATPS. The salt additive effect on the solute–solvent interactions of such a group may be represented by the effect of the salt upon the water hydrogen bond network. The final conclusion is that the solute partitioning in two-polymer ATPSs of different polymer and salt composition is governed by solute–solvent interactions in the aqueous media of the coexisting phases.

4. Conclusions

In this study the solvatochromic solvent parameters were measured in the coexisting phases of aqueous two-phase systems formed by different pairs of polymers and in the presence of several salts. The relative hydrophobicity and ability to participate in the electrostatic interactions of the aqueous media of the coexisting phases was also assessed by measuring the partition ratios of sodium salts of dinitrophenylated (DNP) amino acids with aliphatic side-chains in all ATPSs. From the performed studies two major conclusions can be drawn: (i) the solute partitioning in two-polymer ATPSs of different polymer and salt composition is governed by solute–solvent interactions in the aqueous media of the coexisting phases and (ii) the salt composition does affect the solvent properties of the aqueous media in the coexisting phases by influencing the hydrogen bond network.

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