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$\Delta G(CH_2)$ as solvent descriptor in polymer/polymer aqueous two-phase systems

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Abstract

Phase diagrams were determined for aqueous two-phase systems (ATPSs) formed by different paired combinations of Dextran (Dex-75), Ficoll-70, polyethylene glycol (PEG-8000), hydroxypropyl starch (PES-100), and Ucon50HB5100 (a random copolymer of ethylene glycol and propylene glycol) all containing 0.15 M NaCl in 0.01 M phosphate buffer, pH 7.4, at 23 °C. Partition coefficients of a series of dinitrophenylated (DNP) amino acids with aliphatic side-chains were studied in all the ATPSs at particular polymer concentrations. Free energies of transfer of a methylene group between the coexisting phases, $\Delta G(CH_2)$, were determined as measures of the difference between the hydrophobic character of the phases. Furthermore, partition coefficients of tryptophan (Trp) and its di- and tri-peptides and a set of *p*-nitrophenyl (NP)-monosaccharides were measured in all the two-phase systems, and the data obtained compared with the $\Delta G(CH_2)$ values obtained in the systems. It was established that for eight out of 10 of two-phase systems of different polymer compositions the partition coefficients for Trp peptides correlate well with the $\Delta G(CH_2)$ values. Similar correlations for NP-monosaccharides were valid for seven out of 10 two-phase systems. These observations indicate that the difference between the hydrophobic characters of the coexisting phases represented by the $\Delta G(CH_2)$ value cannot be used as a single universal measure for comparison of the ATPSs of different polymer compositions.

Keywords: Aqueous two-phase partitioning; Hydrophobicity; Amino acids; Tryptophan; Peptides; Monosaccharides

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 certain polymers, e.g., Dextran and polyethylene glycol, 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 with one of the polymers. The aqueous solvent in both phases was demonstrated to provide a media suitable for biological products [1–4]. These systems are unique because each of the phases contains over 80% water on a molal basis and yet the phases are immiscible and differ in their solvent properties [4,5]. Therefore these systems can be used for differential distribution of solutes and particles.

Extraction in ATPSs has been clearly demonstrated as an efficient method for large-scale recovery and purification of biological products [1–3,6,7]. Low cost, high capacity and easy scale-up are clear advantages of this technology. Partitioning in ATPSs may also be used as a bioanalytical tool for characterization of protein surface properties [4,8], changes in protein structure [9], conformation [10], ligand binding [1–3], etc. Successful application of partitioning in ATPSs can benefit from understanding the mechanisms of solute distribution in the systems and properties of the systems at molecular level.

From numerous studies reported in the literature [4,5,8,9,11–15] it seems to be clear that distribution of low molecular

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weight solutes as well as biomacromolecules in ATPSs is governed by the differences in the solvent features of aqueous media in the coexisting phases of a particular two-phase system. So far, however, the solvent properties of ATPSs were examined in the systems formed by only two pairs of polymers, Dex–PEG and Dex–Ficoll, [4,11] and those formed by PEG and inorganic salts [5,12–16]. One of the solvent features important for characterization of ATPSs of different polymer and salt compositions is the free energy of transfer of a methylene group between the coexisting phases [4,5,11–23].

In this work, we examined the free energy of transfer of a methylene group between the coexisting phases as a measure of the relative hydrophobic character of aqueous media in the phases of ATPSs formed by different pairs of Dextran, PEG, Ficoll, a random copolymer of 50% ethylene oxide and 50% propylene oxide, Ucon50HB5100 (Ucon), and hydroxypropyl starch (Reppal PES100). Phase diagrams for all the systems were analyzed, and partitioning of sodium salts of DNP-amino acids with aliphatic side-chains, a series of tryptophan and its di- and tri-peptides and a set of *p*-nitrophenyl-monosaccharides were studied in all the systems.

2. Experimental

2.1. Materials

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). Ucon50HB5100 (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 (Uppsala, Sweden). Reppal PES-100 (lot D702-09/01), M_w ≅ 100,000 was purchased from REPPE AB (Växjö, Sweden).

2.1.2. Amino acids

Dinitrophenylated (DNP) amino acids—DNP-glycine, DNP-alanine, DNP-norvaline, DNP-norleucine, DNP-DL-α-amino-*n*-octanoic acid, and L-tryptophan were purchased from Sigma. The sodium salts of the DNP-amino acids were prepared by titration.

2.1.3. Peptides

Di-tryptophan and tri-tryptophan were purchased from Bachem Bioscience (King of Prussia, PA, USA).

2.1.4. Monosaccharides

Nitrophenylated (NP) monosaccharides—4-NP-β-D-galactopyranoside, 4-NP-β-D-fucopyranoside, 4-NP-β-D-glucopyranoside and 4-NP-α-D-mannopyranoside were purchased from USB. 4-NP-α-D-glucopyranoside was purchased from Sigma.

2.1.5. Others

o-Phthaldialdehyde (OPA) reagent solution (complete) was purchased from Sigma. All salts and other chemicals used were of analytical-reagent grade.

2.2. Methods

2.2.1. Phase diagrams

The systems were prepared by adding appropriate amounts of the aqueous stock ca. 42% (w/w) Dex-75, 50% (w/w) PEG-8000, 70% (w/w) Ucon50HB5100, 48% (w/w) Ficoll-70 or 33% (w/w) PES-100 solution into a 50.0 mL separatory funnel. Appropriate amounts of 3.0 M NaCl, and 1.0 M sodium phosphate buffer, pH = 7.4 were added so as to give the required ionic and polymer composition. Water was finally added to obtain a 25 g amount of final system. After vigorous mixing the systems were allowed to settle for 24 h at room temperature (23 °C). Samples from both phases were collected for characterization. A pipette was used to remove the top phase, while the bottom phase was removed through the drain of the separatory funnel.

Dex, Ficoll or PES concentrations were measured by polarimetry (polarimeter AA-1000, Optical Activity, Ramsey, UK). Since Dex, Ficoll and PES are very hygroscopic, the stock solution concentration was determined gravimetrically following freeze-drying of aliquots of pre-determined weights. Concentrations of PEG or Ucon were assayed by refractive index measurements with an ABBEMAT refractometer (Dr. Kernchen, Hannover, Germany), taking into consideration contributions of salts (the experimental techniques used for sodium chloride and phosphate buffer quantification are given elsewhere [24]) and the other polymers. The refractive index measurements were performed at 25 °C. The relative uncertainty in polymer concentration determination was <5%. All gravimetric measurements were performed with Adam Equipment analytical balance model AAA 250 L with a 0.2 mg uncertainty. The PEG–Ucon phase diagram was determined by the cloud-point method [2] and described according to procedure presented elsewhere [12].

2.2.2. Partitioning

2.2.2.1. Phase systems. A mixture of polymers was prepared by dispensing appropriate amounts of the aqueous stock polymer solutions into a 1.2 mL microtube using a Hamilton (Reno, NV, USA) ML-4000 four-probe liquid-handling workstation. Appropriate amounts of stock buffer solutions were added to give the required ionic and polymer composition of the final system with total volume of 0.5 mL. All two-phase systems had the polymer composition indicated in Table 1 and salt composition of 0.15 M NaCl in 0.01 M sodium phosphate buffer (NaPB), pH 7.4.

2.2.2.2. Partitioning experiments. An automated instrument for performing aqueous two-phase partitioning, Automated Signature Workstation, ASW (Analiza, Cleveland, OH, USA) was used for the partition experiments. The ASW system is based on the liquid-handling workstation ML-4000 (Hamilton) inte-

Table 1
Polymer compositions[a] of the phases in the aqueous two-phase systems used for partitioning, tie-line lengths (TLL)[b] and average slopes of tie-lines $(STL)_{av}$[c]

No.	Polymer 1	Polymer 2	Total composition		Top phase		Bottom phase		TLL[b]	STL_{av}[c]
			Polymer 1	Polymer 2	Polymer 1	Polymer 2	Polymer 1	Polymer 2		
1	Dextran	Ficoll	12.94	18.06	3.23	28.31	21.57	9.03	26.61	−1.048 ± 0.019
2	Dextran	PEG	12.41	6.06	0.31	13.02	22.44	0.53	25.41	−0.566 ± 0.007
3	Dextran	Ucon	12.39	10.08	0.16	18.30	26.51	0.59	31.75	−0.682 ± 0.008
4	PES	Dextran	17.30	12.43	5.31	21.68	31.38	1.93	32.71	−0.741 ± 0.013
5	PEG	Ucon	15.00	29.97	0.36	50.25	35.46	1.58	60.01	−1.405 ± 0.020
6	Ficoll	PEG	15.06	7.90	9.55	11.65	23.97	1.83	17.45	−0.684 ± 0.003
7	Ficoll	Ucon	13.01	9.93	2.90	16.42	24.50	2.54	25.67	−0.638 ± 0.008
8	PES	Ficoll	17.31	14.86	10.31	20.20	25.35	7.80	19.49	−0.822 ± 0.008
9	PES	PEG	15.24	6.96	3.67	12.28	29.58	0.37	28.52	−0.467 ± 0.007
10	PES	Ucon	12.91	7.68	2.76	13.50	24.01	1.32	24.50	−0.554 ± 0.020

[a] Polymer concentrations are given in % (w/w).
[b] Tie-line length (TLL) is calculated as $TLL = (\Delta_{Polymer\text{-}1}{}^2 + \Delta_{Polymer\text{-}2}{}^2)^{1/2}$, where $\Delta_{Polymer}$ is the difference between the given polymer concentrations in the coexisting phases.
[c] Average slope of tie-line (STL) is calculated as the mean of slopes of tie-lines determined as the ratio $STL = \Delta_{Polymer\text{-}2}/\Delta_{Polymer\text{-}1}$ for four different polymer compositions of each two-phase system.

grated with a FL600 fluorescence microplate reader (Bio-Tek Instruments, Winooski, VT, USA) and a UV–Vis microplate spectrophotometer (SpectraMax Plus^{384}, Molecular Devices, Sunnyvale, CA, USA). Solutions of all compounds were prepared in water at concentrations of 1–5 mg/mL. Varied amounts (e.g., 0, 15, 30, 45, 60 and 75 μL) of a given compound solution and the corresponding amounts (e.g., 100, 85, 70, 55, 40 and 25 μL) of water were added to a set of the same polymer/buffer mixtures. The systems were vortexed in a Multi-pulse vortexer and centrifuged for 30–60 min at 1160 × *g* in a refrigerated centrifuge (Jouan, BR4i, Thermo Fisher Scientific, Waltham, MA, USA) to hasten phase settling. The upper phase in each system was removed, the interface discarded, and aliquots of 20–70 μL from the upper and lower phases were withdrawn in duplicate for analysis. Peptides and tryptophan samples were combined with 250 μL of *o*-phthaldialdehyde reagent solution (complete) in microplate wells. After moderate shaking for 2 min at room temperature fluorescence was determined with a fluorescence plate reader with a 360 nm excitation filter and a 460 nm emission filter, and with a 100–125 sensitivity setting. Aliquots from both phases in the partition experiments with DNP-amino acids and NP-carbohydrates were diluted with water up to 320 μL in microplate wells. Following moderate shaking for 20 min in an incubator (Vortemp 56EVC, Labnet International, Edison, NJ, USA) at room temperature (23 °C), optical absorbance was measured at 362 and 302 nm for DNP-amino acids and carbohydrates, respectively, with a UV–Vis plate reader. In all measurements the correspondingly diluted pure phases were used as blank solutions. The partition coefficient, *K*, is defined as the ratio of the sample concentration in the upper phase to the sample concentration in the lower phase. The *K* value for each solute was determined as the slope of the concentration in the upper phase plotted as a function of the concentration in the bottom phase averaged over the results obtained from two to four partition experiments carried out at the specified ionic composition of the system. The deviation from the average *K* value was always less than 5% and in most cases lower than 2%.

3. Results and discussion

Phase diagrams for ATPSs formed by Dextran with PEG, Ucon, Ficoll or PES are presented in Fig. 1. The phase diagram for each ATPS was obtained by analysis of polymer composition of coexisting phases at four different polymer concentrations as indicated above. For clarity, however, only particular compositions used for partitioning experiments are denoted in Fig. 1 with the tie-lines. If we assume that the polymer concentration required for phase separation in an aqueous mixture with Dextran is indicative of the polymer effect on the water structure, we might conclude from the data in Fig. 1 that the effect of a polymer on the water structure decreases in the series: Ucon ≈ PEG > PES > Ficoll.

Phase diagrams for the ATPSs formed by Ficoll with Dextran, PEG, Ucon or PES are presented in Fig. 2 in a manner described above. From these data we can conclude that the

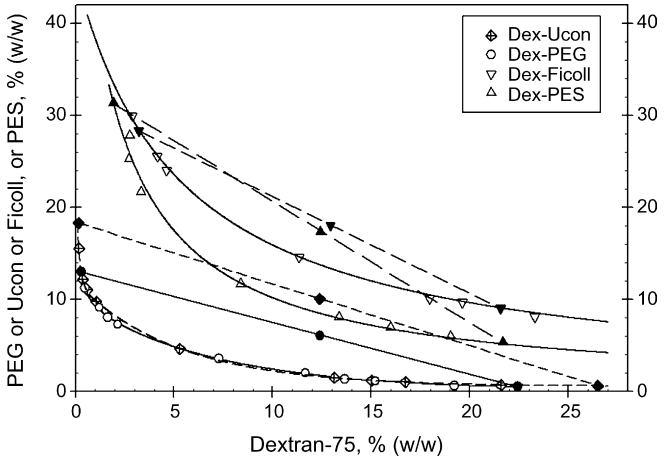

Fig. 1. Phase diagrams for Dex-75–PEG-8000 [24], Dex-75–Ucon50HB5100 [24], Dex-75–Ficoll-70 and Dex-75–PES-100 ATPSs at 23 °C. Tie-lines are shown for particular compositions used in partition experiments (for explanation see text). The standard deviation (SD) of the fitting curves to the experimental points was 0.20, 0.29, 0.22 and 0.19 for the Dex–PEG, Dex–Ucon, Dex–Ficoll and Dex–PES ATPSs, respectively.

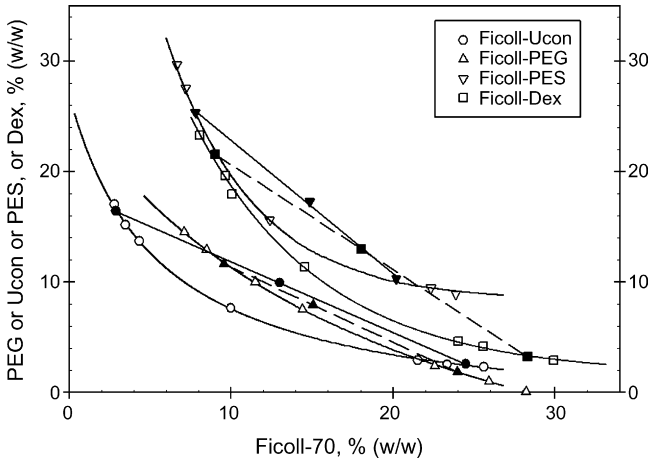

Fig. 2. Phase diagrams for Ficoll-70–Ucon50HB5100, Ficoll-70–PES-100, Ficoll-70–PEG-8000 and Ficoll-70–Dex-75 (same as in Fig. 1) ATPSs at 23 °C. Tie-lines are shown for particular compositions used in partition experiments (for explanation see text). The standard deviation (SD) of the fitting curves to the experimental points was 0.27, 0.26 and 0.08 for the Ficoll–Ucon, Ficoll–PES and Ficoll–PEG ATPSs, respectively.

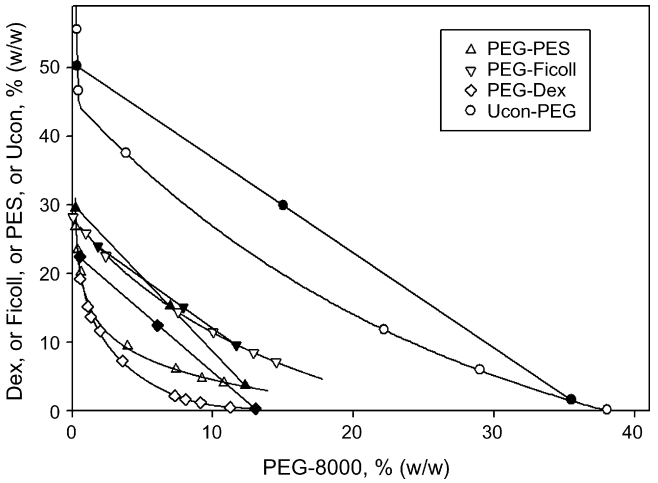

Fig. 4. Phase diagrams for PEG-8000–Ucon50HB5100, PEG-8000–PES-100, PEG-8000–Ficoll-70 and PEG-8000–Dex-75 ATPSs at 23 °C. Tie-lines are shown for particular compositions used in partition experiments (for explanation see text). The standard deviation (SD) of the fitting curve to the experimental points was 0.52 for the PEG–Ucon system.

effect of a polymer on the water structure decreases in the series: Ucon > PEG > Dex > PES.

Phase diagrams for the ATPSs formed by PES and either PEG or Ucon are shown in Fig. 3. Phase diagrams for the systems formed by PES with Dex and with Ficoll are already plotted in Figs. 1 and 2, correspondingly. It follows from all these data that in the presence of PES the effect of a polymer on the water structure decreases in the series: PEG >/< Ucon > Dex > Ficoll (where >/< denotes the observation that while Ucon seems to display stronger structural effect in the presence of relatively low PES concentrations in water, PEG appears to affect the water structure in a more pronounced manner once the PES concentration is above ~7.5% (w/w)).

Phase diagrams for the ATPSs formed by PEG with Dextran, Ficoll, Ucon and PES are presented in Fig. 4. From these data

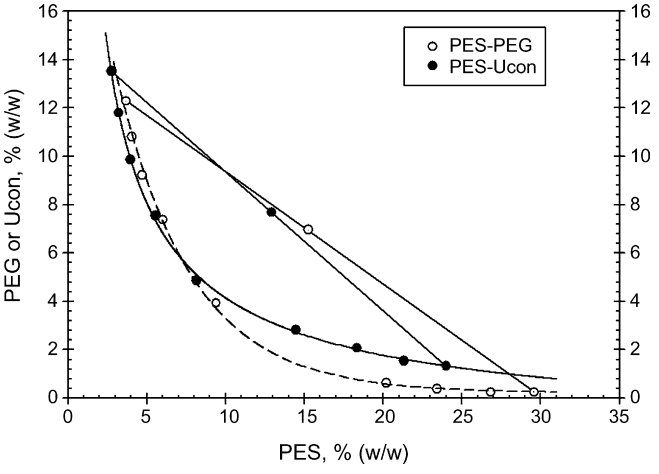

Fig. 3. Phase diagrams for PES-100–PEG-8000 and PES-100–Ucon50HB5100 ATPSs at 23 °C. Tie-lines are shown for particular compositions used in partition experiments (for explanation see text). The standard deviation (SD) of the fitting curves to the experimental points was 0.06 and 0.20 for the PES–PEG and PES–Ucon ATPSs, respectively.

it follows that the effect of a polymer on the water structure decreases in the series: Dex > PES > Ficoll > Ucon.

From similar analysis of the ATPSs formed by Ucon with Dextran, Ficoll, PEG and PES, it follows that the effect of a polymer on the water structure decreases in the series: Dex > PES > Ficoll > PEG.

If we classify all the polymers used here according to their chemical nature, i.e., as polysaccharides (Dex, Ficoll and PES) and poly(alkylene glycols) (PEG and Ucon), the series observed seems to agree with the above assumption that the polymer concentration required for phase separation in an aqueous mixture with another polymer is indicative of the polymer effect on the water structure. It seems reasonable to expect that the effects of polysaccharides on the water structure would be relatively similar, same as those of poly(alkylene glycols). Thus in the presence of, e.g., Dex, it is not surprising that PES and Ficoll aqueous solutions will give rise to phase separation at higher concentrations than PEG or Ucon aqueous solutions, resulting in the obtained sequence: Ucon ≈ PEG > PES > Ficoll. Also, the extremely high compatibility of PEG and Ucon aqueous solutions is in agreement with this view due to the structural similarity between these polymers.

Polymer compositions of the coexisting phases in the ATPSs used for partitioning of solutes in this study are listed in Table 1 together with the corresponding tie-line length (TLL) and slope of tie-line (STL) values.

Typical experimental data obtained for sodium salts of DNP-amino acids in different ATPSs are plotted in Fig. 5, and the linear curves observed may be described as:

$$\ln\ K^{(i)}_{\text{DNP-AA}} = C^{(i)} + E^{(i)} N_{\text{C}} \quad (1)$$

where $K_{\text{DNP-AA}}$ is the partition coefficient of a DNP-amino acid with aliphatic side-chain; superscript (i) denotes the particular ith ATPS used for the partition experiments; N_{C} is equivalent number of CH_2 groups in the aliphatic side-chain of a given

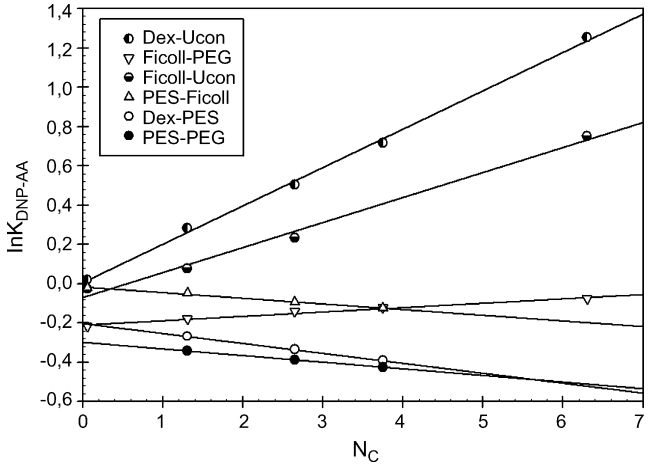

Fig. 5. Partition coefficients of sodium salts of DNP-amino acids with aliphatic side-chains as functions of side-chain lengths in different ATPSs as indicated.

DNP-amino acid; E is an average ln K increment per CH_2 group; C represents the total contribution of the non-alkyl part of the structure of a DNP-amino acid into ln $K_{DNP\text{-}AA}$.

The coefficients $E^{(i)}$ and $C^{(i)}$ values obtained in the ATPSs examined are listed in Table 2 (data for Dex–PEG and Dex–Ucon two-phase systems were reported previously [24] and are presented here for comparison). It should be noted here that the partition coefficient 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. Therefore coefficient E values listed in Table 2 may be positive or negative depending on which particular phase (upper or lower) is more hydrophobic than the coexisting phase. As the free energy of transfer of a solute between the coexisting phases is described by Eq. (2):

$$\Delta G = -RT \ \ln \ K \tag{2}$$

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

$$\Delta G(CH_2) = -RTE \tag{3}$$

where $\Delta G(CH_2)$ is the free energy of transfer of a methylene group from one coexisting phase to another. The $\Delta G(CH_2)$ values calculated from the experimental data with Eqs. (1)–(3) are listed in Table 2.

According to the $\Delta G(CH_2)$ values presented in Table 2, the relative hydrophobic character of the phases increases in the following series: Dex-rich phase < Ficoll-rich phase < PEG-rich phase < PES-rich phase < Ucon-rich phase. The particular position of each phase in this series may vary, however, depending upon the particular composition of the phase and that of the reference phase indicating that the particular compositions of the phases under comparison affect the difference between the hydrophobic characters of the phases. In other words, we may have a Dex–PEG composition in which $\Delta G(CH_2)$ is higher than in a particular Dex–Ucon ATPS. However, when we measure the same quantity in the system PEG–Ucon, the Ucon rich phase will always have more affinity for a CH_2 group. It should also be noticed here that the TLL which is commonly used as a convenient measure of the divergence of the properties of the phases

Table 2
Results of partitioning of sodium salts of DNP-amino acids with aliphatic side-chains (Gly, Ala, NVal, NLeu, α-amino-*n*-octanoic acid)[a] and series of homooligopeptides of tryptophan[b] in the aqueous two-phase systems indicated

No.	Polymer 1*	Polymer 2*	C[a]	E[a]	r^2**	F** ($\times 10^{-3}$)	SD** ($\times 10^3$)	$\Delta G(CH_2)$ (cal/mol)	a[b]	$\Delta \ln K_{Trp}$[b]	r^2**	F**	SD**
1	Dextran	Ficoll	0.125 ± 0.005	0.044 ± 0.001	0.997	1.00	6.6	−26.1 ± 0.6	−0.373 ± 0.113	0.378 ± 0.052	0.981	52	0.07
2	Dextran	PEG	−0.089 ± 0.007	0.062 ± 0.002	0.997	0.63	9.1	−36.7 ± 1.2	−0.579 ± 0.0001	0.559 ± 0.0001	1.000	>9000	<0.001
3	Dextran	Ucon	0.004 ± 0.017	0.196 ± 0.005	0.998	1.60	22	−116.1 ± 3.0	−1.602 ± 0.083	1.591 ± 0.038	0.999	1710	0.05
4	PES	Dextran	−0.203 ± 0.0002	−0.051 ± 0.0001	1.000	104	0.3	+30.2 ± 0.06	0.285 ± 0.006	−0.266 ± 0.003	1.000	9152	0.004
5	PEG	Ucon	1.402 ± 0.007	0.245 ± 0.003	1.000	6.09	8.4	−144.2 ± 11.0	−2.677 ± 0.0002	1.842 ± 0.0001	1.000	>9000	0.0002
6	Ficoll	PEG	−0.211 ± 0.007	0.022 ± 0.002	0.978	0.13	9.4	−13.0 ± 1.2	−0.211 ± 0.045	0.165 ± 0.021	0.984	62	0.03
7	Ficoll	Ucon	−0.072 ± 0.029	0.127 ± 0.008	0.991	0.23	4.0	−75.2 ± 4.7	−1.438 ± 0.0002	0.936 ± 0.0001	1.000	>9000	0.0001
8	PES	Ficoll	−0.017 ± 0.004	−0.029 ± 0.002	0.995	0.36	4.2	+17.2 ± 1.2	−0.077 ± 0.003	0.107 ± 0.002	1.000	4883	0.002
9	PES	PEG	−0.300 ± 0.0007	−0.034 ± 0.0003	1.000	5.10	0.8	+20.1 ± 0.2	0.442 ± 0.085	0.345 ± 0.039	0.987	77	0.06
10	PES	Ucon	−0.266 ± 0.023	0.082 ± 0.013	0.974	0.04	24.7	−48.6 ± 7.7	−0.533 ± 0.002	0.504 ± 0.001	1.000	>9000	0.002

*Polymer 1—predominant polymer in the bottom phase; polymer 2—predominant polymer in the top phase (see in Table 1). **r^2—correlation coefficient; F—ratio of variance and SD—standard deviation.
[a] Partitioning of sodium salts of DNP amino acids is described by Eq. (1); coefficients C and E are calculated from the experimental data using Eq. (1).
[b] Partitioning of homooligopeptides of Trp is described by Eq. (4); coefficients a and $\Delta \ln K_{Trp}$ were determined from experimental data using Eq. (4).

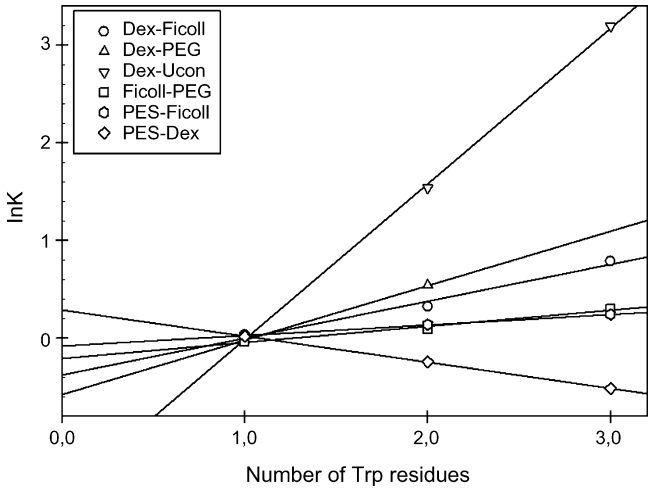

Fig. 6. Partition coefficients of tryptophan and its di- and tri-peptides as functions of the number of Trp residues in the molecule in different ATPSs as indicated.

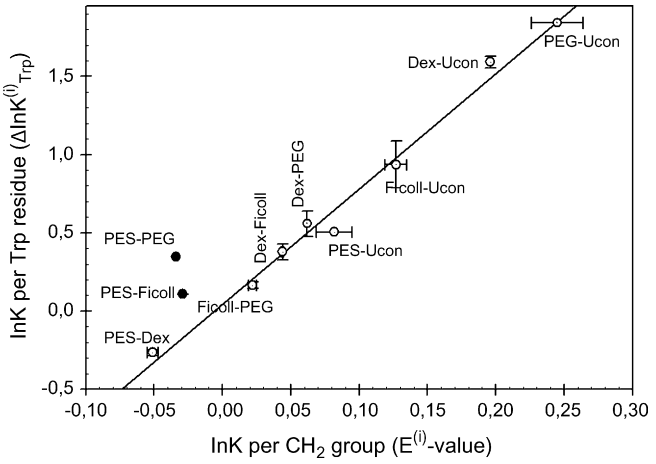

Fig. 7. Contribution of a Trp residue into ln K of a homooligopeptide versus coefficient E (contribution of a CH_2 group into ln K for DNP-amino acids with aliphatic side-chains) in different ATPSs as indicated. Filled symbols denote the ATPSs not fitting the correlation. (For explanation see text.)

for an ATPS of the qualitatively same polymer composition is invalid for comparison of the data obtained in the systems formed by different polymers. It seems that the difference between the hydrophobic characters of the coexisting phases may serve as a better general measure for the ATPSs comparison. In order to test this assumption, however, we need to consider the data obtained in the partition experiments with Trp and its di- and tri-peptides and monosaccharides.

Typical experimental data obtained for the series of homooligopeptides of Trp in different ATPSs are plotted in Fig. 6, and the linear curves observed may be described as:

$$\ln\ K^{(i)} = a^{(i)} + \Delta\ln\ K^{(i)}_{\mathrm{Trp}} n \tag{4}$$

where $K^{(i)}$ is the partition coefficient of a peptide or free Trp in the ith ATPS; n is the number of Trp residues in the solute molecule, $a^{(i)}$ is a constant, and $\Delta K^{(i)}_{\mathrm{Trp}}$ is the slope representing the total contribution of a nth Trp residue and newly formed peptide bond into the partition coefficient of a homooligopeptide.

The coefficient $a^{(i)}$ and $\Delta K^{(i)}_{\mathrm{Trp}}$ values obtained in the ATPSs examined are listed in Table 2 (data for Dex–PEG and Dex–Ucon two-phase systems were reported previously [24] and are presented here for comparison). For eight out of 10 ATPSs used here there is a clear correlation between the $\Delta\ln K^{(i)}_{\mathrm{Trp}}$ and $E^{(i)}$ values. It should be mentioned that when both $\Delta\ln K^{(i)}_{\mathrm{Trp}}$ and $E^{(i)}$ values are negative, as observed in the case of Dextran–PES two-phase system, the signs may be reversed, since it would mean only that the K values would be determined as the ratios of concentrations of a solute in the bottom (more hydrophobic) PES-rich phase to those in the top Dex-rich phase for both series of DNP-amino acids and Trp peptides. The correlation is presented in Fig. 7, and it is described as:

$$\Delta\ln\ K^{(i)}_{\mathrm{Trp}} = 0.043\ (\pm 0.037) + 7.38\ (\pm 0.30) E^{(i)}$$
$$N = 8;\ \ r^2 = 0.9879 \tag{5}$$

where N is the number of different ATPS, and r^2 is the correlation coefficient.

This correlation seems to indicate that the difference between the relative hydrophobic characters of the coexisting phases represented by coefficient $E^{(i)}$ (or the corresponding $\Delta G(CH_2)$ value) is a general measure enabling comparison of the partitioning of solutes in ATPSs formed by different polymers.

However, two ATPSs, formed by PES and PEG or PES and Ficoll do not fit the above correlation. While in all other ATPSs, partitioning of Trp and its peptides follows the same trend as displayed by DNP-amino acids with aliphatic side-chains, in these two systems Trp and its homooligopeptides distribute into the phase opposite to the one displaying higher affinity for a CH_2 group.

Typical experimental data obtained for p-nitrophenyl-monosaccharides in different ATPSs are plotted in Fig. 8, and

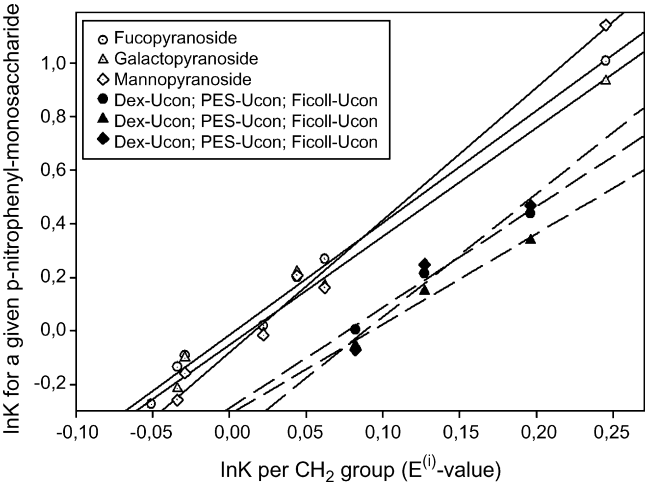

Fig. 8. Partition coefficients of 4-nitrophenyl-β-D-fucopyranoside, 4-nitrophenyl-β-D-galactopyranoside and 4-nitrophenyl-α-D-mannopyranoside versus coefficient E (contribution of a CH_2 group into ln K for DNP-amino acids with aliphatic side-chains) in different ATPSs. Filled symbols denote the aqueous Dex–Ucon, PES–Ucon and Ficoll–Ucon two-phase systems not fitting the correlation. (For explanation see text.)

Table 3
Results of partitioning of *p*-nitrophenyl-monosaccharides[a] in the aqueous two-phase systems

Carbohydrate	*a*	*b*	*N*	r^2*	*F**	SD*
Fucopyranoside	−0.013 ± 0.017	4.197 ± 0.165	7	0.992	581	0.04
Galactopyranoside	−0.052 ± 0.024	4.062 ± 0.213	7	0.981	254	0.06
β-Glucopyranoside	−0.018 ± 0.018	3.907 ± 0.155	7	0.989	440	0.05
α-Glucopyranoside	−0.022 ± 0.014	3.894 ± 0.121	7	0.993	721	0.04
α-Mannopyranoside	−0.080 ± 0.022	4.950 ± 0.192	7	0.989	462	0.06

N—number of ATPSs (see in Table 1); r^2—correlation coefficient; partition coefficients for each monosaccharide in Dex-Ucon, PES–Ucon and Ficoll–Ucon did not fit the correlations (see text); *r^2—correlation coefficient; F—ratio of variance and SD—standard deviation.

[a] Partitioning of each *p*-nitrophenyl-monosaccharide is described by Eq. (6); coefficients *a* and *b* are calculated from the experimental data using Eq. (6).

the linear curves observed may be described as:

$$\ln\ K_j^{(i)} = a_j + b_j E^{(i)} \quad (6)$$

where $K_j^{(i)}$ is the partition coefficient of a *j*th *p*-nitrophenyl-monosaccharide in the *i*th ATPS; a_j and b_j are constant coefficients and $E^{(i)}$ is as defined above, i.e., the measure of the difference between the relative hydrophobic characters of the coexisting phases in the *i*th ATPS.

The coefficients a_j and b_j values obtained for all the monosaccharides studied are listed in Table 3. For seven out of 10 ATPSs examined here there is a clear correlation between the $\ln\ K_j^{(i)}$ and $E^{(i)}$ values. It should be mentioned that when the $\ln\ K_j^{(i)}$ for each monosaccharide and $E^{(i)}$ values are negative, as observed in the case of PES–Ficoll two-phase system, the signs may be reversed, since it would mean only that the *K* values would be determined as the ratios of concentrations of a solute in the bottom (more hydrophobic) PES-rich phase to those in the top Ficoll-rich phase for both series of DNP-amino acids and monosaccharides under study. It should be noticed that partition coefficients for each monosaccharide studied do not fit the correlation described by Eq. (6) in three ATPSs—Dex–Ucon, PES–Ucon and Ficoll–Ucon. The partition coefficients for the monosaccharides in these systems follow the same trends as can be seen from Fig. 8 but they are down-shifted from the correlations observed in the other ATPSs.

More experimental studies are clearly necessary, but the unavoidable conclusion from this observation seems to be that the difference between the hydrophobic characters of the coexisting phases represented by the coefficient *E* or $\Delta G(CH_2)$ value alone cannot be used as a universal measure for comparison of the ATPSs of different polymer compositions. Properties of the phases are likely to be too complex to be characterized by a single measure. Further studies to gain better insight into this issue are in progress.

4. Conclusions

Partition coefficients of a series of DNP-amino acids, tryptophan and its di- and tri-peptides and a set of NP-monosaccharides were measured in 10 ATPSs formed by all possible paired combinations of Dex-75, Ficoll-70, PEG-8000, PES-100 and Ucon50HB5100, all containing 0.15 M NaCl in 0.01 M phosphate buffer, pH 7.4, at 23 °C. The data obtained were compared with the $\Delta G(CH_2)$ values determined in all the systems. For eight out of 10 of two-phase systems of different polymer compositions the partition coefficients for Trp peptides correlate well with the $\Delta G(CH_2)$-values. Similar correlations for NP-monosaccharides were valid for seven out of 10 two-phase systems. These observations indicate that the difference between the hydrophobic characters of the coexisting phases represented by the $\Delta G(CH_2)$ value is an adequate descriptor of the solvent properties of the phases. However, we concluded that this parameter alone cannot be used as a single universal measure for comparison of the ATPSs of different polymer compositions. It is suggested that additional solvent features of the aqueous media in the coexisting phases should be used to describe biomolecules partitioning in ATPSs.

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