ΔG(CH₂) 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, ΔG(CH₂), 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 ΔG(CH₂) 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 ΔG(CH₂) 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 ΔG(CH₂) value cannot be used as a single universal measure for comparison of the ATPSs of different polymer compositions.
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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
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-Phthalaldehyde (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 of the phases in the aqueous two-phase systems used for partitioning, tie-line lengths (TLL) and average slopes of tie-lines (STL).  

<table>
<thead>
<tr>
<th>No.</th>
<th>Polymer 1</th>
<th>Polymer 2</th>
<th>Total composition</th>
<th>Top phase</th>
<th>Bottom phase</th>
<th>TLL</th>
<th>STLavc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dextran</td>
<td>Ficoll</td>
<td>12.94</td>
<td>18.06</td>
<td>3.23</td>
<td>28.31</td>
<td>21.57</td>
</tr>
<tr>
<td>2</td>
<td>Dextran</td>
<td>PEG</td>
<td>12.41</td>
<td>6.06</td>
<td>0.31</td>
<td>13.02</td>
<td>22.44</td>
</tr>
<tr>
<td>3</td>
<td>Dextran</td>
<td>Ucon</td>
<td>13.29</td>
<td>10.08</td>
<td>0.16</td>
<td>18.30</td>
<td>26.51</td>
</tr>
<tr>
<td>4</td>
<td>Ficoll</td>
<td>Dextran</td>
<td>17.30</td>
<td>12.43</td>
<td>5.31</td>
<td>21.68</td>
<td>31.38</td>
</tr>
<tr>
<td>5</td>
<td>PEG</td>
<td>Ucon</td>
<td>15.00</td>
<td>29.97</td>
<td>0.36</td>
<td>50.25</td>
<td>35.46</td>
</tr>
<tr>
<td>6</td>
<td>Ficoll</td>
<td>PEG</td>
<td>15.06</td>
<td>7.90</td>
<td>9.55</td>
<td>11.65</td>
<td>23.97</td>
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<tr>
<td>7</td>
<td>Ficoll</td>
<td>Ucon</td>
<td>13.01</td>
<td>9.93</td>
<td>2.90</td>
<td>16.42</td>
<td>24.50</td>
</tr>
<tr>
<td>8</td>
<td>PES</td>
<td>Ficoll</td>
<td>17.31</td>
<td>14.86</td>
<td>10.31</td>
<td>20.20</td>
<td>25.35</td>
</tr>
<tr>
<td>9</td>
<td>PES</td>
<td>PEG</td>
<td>15.24</td>
<td>6.96</td>
<td>3.67</td>
<td>12.28</td>
<td>29.58</td>
</tr>
<tr>
<td>10</td>
<td>PES</td>
<td>Ucon</td>
<td>12.91</td>
<td>7.68</td>
<td>2.76</td>
<td>13.50</td>
<td>24.01</td>
</tr>
</tbody>
</table>

a Polymer concentrations are given in % (w/w).

b Tie-line length (TLL) is calculated as $TLL = (\Delta_{Polymer_1}^2 + \Delta_{Polymer_2}^2)^{1/2}$, 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 $STL = \Delta_{Polymer_2}/\Delta_{Polymer_1}$ for four different polymer compositions of each two-phase system.

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.](image-url)
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 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 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:

$$\ln K_{DNP-AA}^{(i)} = C^{(i)} + E^{(i)} N_C$$

(1)

where $K_{DNP-AA}$ is the partition coefficient 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 equivalent number of CH$_2$ groups in the aliphatic side-chain of a given
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 $\ln K_{\text{DNP-AA}}$.

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, $K_{\text{DNP-AA}}$, 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):

$$\Delta G = -RT \ln K$$

(2)

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

$$\Delta G(CH_2) = -RT E$$

(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 < PES-rich phase < PEG-rich phase < Ucon-rich phase. The particular composition 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Polymer 1*</th>
<th>Polymer 2*</th>
<th>$E^{(i)}$</th>
<th>$C^{(i)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dex</td>
<td>Ficoll</td>
<td>0.126 ± 0.005</td>
<td>1.09 ± 0.001</td>
</tr>
<tr>
<td>2</td>
<td>Dex</td>
<td>PEG</td>
<td>0.095 ± 0.007</td>
<td>0.98 ± 0.002</td>
</tr>
<tr>
<td>3</td>
<td>Dex</td>
<td>Ucon</td>
<td>0.004 ± 0.017</td>
<td>0.96 ± 0.005</td>
</tr>
<tr>
<td>4</td>
<td>PES</td>
<td>Ficoll</td>
<td>0.023 ± 0.002</td>
<td>0.99 ± 0.002</td>
</tr>
<tr>
<td>5</td>
<td>PES</td>
<td>PEG</td>
<td>0.152 ± 0.007</td>
<td>0.127 ± 0.008</td>
</tr>
<tr>
<td>6</td>
<td>PES</td>
<td>Ucon</td>
<td>0.017 ± 0.000</td>
<td>0.99 ± 0.002</td>
</tr>
<tr>
<td>7</td>
<td>Ficoll</td>
<td>PEG</td>
<td>0.012 ± 0.000</td>
<td>0.99 ± 0.002</td>
</tr>
<tr>
<td>8</td>
<td>Ficoll</td>
<td>Ucon</td>
<td>0.012 ± 0.000</td>
<td>0.99 ± 0.002</td>
</tr>
<tr>
<td>9</td>
<td>PES</td>
<td>PEG</td>
<td>0.012 ± 0.000</td>
<td>0.99 ± 0.002</td>
</tr>
<tr>
<td>10</td>
<td>PES</td>
<td>Ucon</td>
<td>0.012 ± 0.000</td>
<td>0.99 ± 0.002</td>
</tr>
</tbody>
</table>

*Polymer 1—predominant polymer in the bottom phase; polymer 2—predominant polymer in the top phase (see in Table 1). **SD—standard deviation.

The coefficients $F$ and $r^2$ are calculated from the 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.

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:

\[ \ln K_{(i)} = a_{(i)} + \Delta \ln K_{\text{Trp}}(n) \]

where \( K_{(i)} \) is the partition coefficient of a peptide or free Trp in the \( i \)th ATPS; \( n \) is the number of Trp residues in the solute molecule, \( a_{(i)} \) is a constant, and \( \Delta K_{\text{Trp}}(n) \) is the slope representing the total contribution of a \( n \)th Trp residue and newly formed peptide bond into the partition coefficient of a homooligopeptide.

The coefficient \( a_{(i)} \) and \( \Delta K_{\text{Trp}}(n) \) 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 \( \Delta \ln K_{\text{Trp}}(n) \) and \( E_{(i)} \) values. It should be mentioned that when both \( \Delta \ln K_{\text{Trp}}(n) \) 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:

\[ \Delta \ln K_{\text{Trp}}^{(i)} = 0.043 \pm 0.037 + 7.38 \pm 0.30 E_{(i)} \]

where \( N = 8 \); \( r^2 = 0.9879 \)

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 CH2 group.

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

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 CH2 group.

Typical experimental data obtained for \( p \)-nitrophenyl-monosaccharides in different ATPSs are plotted in Fig. 8, and
the linear curves observed may be described as:

$$\ln K^{(i)}_j = a_j + b_j E^{(i)}$$

(6)

where $K^{(i)}_j$ 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 $\ln K^{(i)}_j$ and $E^{(i)}$ values. It should be mentioned that when the $\ln K^{(i)}_j$ 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 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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