



Interrelationship between partition behavior of organic compounds and proteins in aqueous dextran-polyethylene glycol and polyethylene glycol-sodium sulfate two-phase systems



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ABSTRACT

Partition behavior of adenosine and guanine mononucleotides was examined in aqueous dextran-polyethylene glycol (PEG) and PEG-sodium sulfate two-phase systems. The partition coefficients for each series of mononucleotides were analyzed as a functions of the number of phosphate groups and found to be dependent on the nature of nucleic base and on the type of ATPS utilized. It was concluded that an average contribution of a phosphate group into logarithm of partition coefficient of a mononucleotide cannot be used to estimate the difference between the electrostatic properties of the coexisting phases of ATPS.

The data obtained in this study were considered together with those for other organic compounds and proteins reported previously, and the linear interrelationship between logarithms of partition coefficients in dextran-PEG, PEG-Na₂SO₄ and PEG-Na₂SO₄-0.215 M NaCl (all in 0.01 M Na- or K/Na-phosphate buffer, pH 7.4 or 6.8) was established. Similar relationship was found for the previously reported data for proteins in Dex-PEG, PEG-600-Na₂SO₄, and PEG-8000-Na₂SO₄ ATPS. It is suggested that the linear relationships of the kind established in ATPS may be observed for biological properties of compounds as well.

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1. Introduction

There are two main types of aqueous two-phase systems (ATPS) [1–5]. The first type includes those formed in aqueous mixtures of two different polymers, such as dextran and Ficoll or polyethylene glycol (PEG). These ATPS are used for separation of cells and other biological particles [1–4]. The second type of ATPS includes systems formed in aqueous mixtures of a single polymer, such as PEG or polypropylene glycol, and a salt, such as sodium sulfate, phosphate, citrate, and these ATPS are generally used for protein separation [6]. Both these types of ATPS may be used for analytical applications [7]. The third type of ATPS being recently developed

and characterized [8] is the ATPS formed in water by mixing ionic liquids and inorganic salts or polymers. This family of ATPS is used successfully for separation purposes but presents a particular challenge in regard to the mechanism of solute partitioning. It has been established [7] that solute partition behavior in polymer-polymer ATPS and to a lesser degree in polymer-salt ATPS is governed by the solute-water interactions without direct interactions of the solute with the phase-forming components of ATPS. The question of direct solute-ionic liquid interactions in the ionic liquid-salt ATPS, however, remains open.

It has been reported by us recently that various properties of polar organic compounds and proteins in aqueous solutions (solubility [9], lipophilicity (expressed as logD values in octanol-water system) [10], partition coefficients in aqueous two-phase systems (ATPS) [11]) in the presence of different salt additives, and optical rotation of enantiomeric amino acids and glucose in aqueous

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solutions in the presence of different salts [12,13] are interrelated as:

$$\log SP_{\text{salt}-1} = k_1 + k_2 \times \log SP_{\text{salt}-2} + k_3 \times \log SP_{\text{salt}-3} \quad (1)$$

where SP_{salt} is the property of a solute in the presence of a given salt additive, k_1 , k_2 , and k_3 are constants depending on the solute property under consideration and the salt compositions employed.

From a practical point of view, the most important aspect of the relationship described by Eq. (1) is that once the relationship is established it may be used to predict protein partition coefficient in a system with a certain ionic composition without need to perform experiments saving the necessary protein quantity to be used, time and labor. It may be important when the optimal separation conditions for a target protein are designed. From the theoretical viewpoint the relationship described by Eq. (1) if applicable to both polymer–polymer and polymer–salt ATPS may be used to gain better understanding in what respect these two different subfamilies of ATPS are similar or different and derive better insight into mechanisms of phase separation and fundamental properties of ATPS.

Analysis of the partition coefficients of small organic compounds and proteins in dextran-PEG and PEG–sodium sulfate ATPS was one of the aims of this study. In addition, the second aim of the study was to explore if the homologous series of mononucleotides may be used for characterization of ATPS. Partitioning of adenine and adenosine mono-, di-, and tri-phosphates and guanosine mono-, di-, and tri-phosphates in dextran-PEG and PEG- Na_2SO_4 with and without 0.215 M NaCl additive ATPS were examined to explore if contribution of a phosphate group into logarithm of partition coefficient of mononucleotide may be used for characterization of electrostatic properties of coexisting phases in ATPS.

2. Experimental

2.1. Material and methods

2.1.1. Materials

Polyethylene glycol 8000 (Lot 091M01372 V), Mw = 8000, adenine, adenosine, adenosine 5'-monophosphate disodium salt (AMP), adenosine 5'-diphosphate sodium salt (ADP), adenosine triphosphate sodium salt (ATP), guanosine, guanosine 5'-monophosphate disodium salt (GMP), guanosine 5'-diphosphate sodium salt (GDP), guanosine 5'-triphosphate sodium salt (GTP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dextran-75 (Lot 119945) with an average molecular weight (M_w) 75,000 by light scattering were purchased from USB Corporation (Cleveland, OH, USA).

2.2.1. Two-phase systems

Dextran-PEG systems of polymer composition of 6.0 wt.% PEG-8000 and 12.0 wt.% Dex-75 and ionic composition of 0.01 M K/NaPB, pH 7.4 and PEG-8000- Na_2SO_4 systems with composition of 11.10 wt.% PEG-8000, 6.33 wt.% Na_2SO_4 (~0.45 M) and 0.01 M NaPB, pH 6.8, with or without 0.215 M NaCl additive were prepared as described previously [11,14,15] by dispensing appropriate amounts of the aqueous stock polymer and salt solutions into a 1.2 mL microtube using a Hamilton Company (Reno, NV, USA) ML-4000 four-probe liquid-handling workstation. with total weight of 0.5 g (total volume 470 μL).

2.2.2. Partitioning

All partition experiments were performed with the Automated Signature Workstation, ASW (Analiza, Inc., Cleveland, OH, USA) as described elsewhere [11,14,15]. The ASW system is based on the ML-4000 liquid-handling workstation (Hamilton Company, Reno,

Table 1

Differences between the solvent properties of the phases and partition coefficients for indicated organic compounds in Dex-PEG-0.01 M K/NaPB, pH 7.4, PEG- Na_2SO_4 -0.01 M NaPB, pH 6.8, and PEG- Na_2SO_4 -0.215 M NaCl-0.01 M NaPB, pH 6.8 ATPS (NaPB–sodium phosphate buffer; K/NaPB–sodium/potassium phosphate buffer).

Solvent properties ^c	Dex-PEG 0.01 M NaPB ^a	PEG- Na_2SO_4 0.01 M K/NaPB ^b	PEG- Na_2SO_4 0.215 M NaCl ^b
$\Delta G(\text{CH}_2)$ (cal/mole)	-45 ± 1.2	-122 ± 1.8	-135 ± 0.6
E	0.033 ± 0.001	0.090 ± 0.002	0.102 ± 0.008
C	0.058 ± 0.003	0.445 ± 0.005	0.49 ± 0.031
Compound			
Adenine	1.220 ± 0.006 ^a	3.4 ± 0.12 ^b	3.7 ± 0.16 ^b
Adenosine	1.128 ± 0.004 ^a	2.63 ± 0.02 ^c	3.12 ± 0.04 ^c
AMP	0.714 ± 0.001	1.02 ± 0.05 ^b	0.83 ± 0.04 ^b
ADP	0.769 ± 0.002	0.77 ± 0.02 ^b	0.54 ± 0.03 ^b
ATP	0.826 ± 0.002	0.61 ± 0.01 ^b	0.40 ± 0.02 ^b
Guanosine	1.068 ± 0.007	1.673 ± 0.008 ^c	1.8 ± 0.03 ^c
GMP	0.709 ± 0.005	0.783 ± 0.007	0.606 ± 0.003
GDP	0.753 ± 0.004	0.577 ± 0.002	0.444 ± 0.003
GTP	0.789 ± 0.005	0.514 ± 0.003	0.410 ± 0.004

^a Reported in Ref. [16].

^b Reported in Ref. [17].

^c Reported in Ref. [18].

NV, USA) integrated with a UV–vis microplate spectrophotometer (SpectraMax Plus 384, Molecular Devices, Sunnyvale, CA). Solutions of all compounds were prepared in water at concentrations of 0.5–5 mg/mL depending on the compound solubility. Briefly, varied amounts of compound solution and water were added to a set of the same polymers/buffer or PEG and Na_2SO_4 mixtures. The systems were vortexed and centrifuged for 30–60 min at 23 °C to accelerate phase settling. The top phase in each system was removed, the interface discarded, and aliquots from the top and bottom phases were withdrawn in duplicate for analysis.

For the analysis of the compounds partitioning, aliquots from both phases were diluted with water, vortexed and centrifuged, aliquots were transferred into microplate wells, and the UV–vis plate reader was used to measure optical absorbance at of maximum absorption wavelengths.

The partition coefficient K -value defined as the ratio of the solute concentration in the top PEG-rich phase to the solute concentration in the bottom dextran-rich or salt-rich phase for each solute was determined as the slope of the concentration (absorbance) in the top phase plotted as a function of the concentration in the bottom phase averaged over the results obtained from two to four partition experiments [11,14,15]. Deviation from the average K -value was consistently below 3% and in most cases lower than 1%.

3. Results and discussion

The partition coefficients of AMP, ADP, ATP, GMP, GDP, and GTP in three ATPSs with previously characterized differences between the hydrophobic and electrostatic properties of the phases [14–16] are listed in Table 1. Some of the partition coefficients for adenine, adenosine, guanosine, AMP, ADP, and ATP in the same ATPSs were reported previously [17,18].

Commonly, the differences between the electrostatic and hydrophobic properties of the coexisting phases of ATPS are estimated by analysis of partitioning of a homologous series of dinitrophenylated (DNP-) amino acids Na salts with aliphatic alkyl side-chains of the increasing length [5,10,11,14–18]. The typical data in a given ATPS may be described as [3,8–13]:

$$\log K^i_{\text{DNP-AA}} = C^i + E^i \times N_c \quad (2)$$

where $K^i_{\text{DNP-AA}}$ is the partition coefficient of a DNP-amino acids Na-salt in i^{th} ATPS; N_c is the equivalent number of CH_2 groups in the side-chain [13]. Parameter E represents an average contribution of

a CH₂ group into $\log K$ and characterizes the difference between the hydrophobic properties of the phases [5,10,11,14–18]. Parameter C represents the contribution of the polar DNP-NH-CH-COONa moiety. The advantages and drawbacks of using this moiety as a probe for electrostatic properties of the phases were considered in detail in [5]. The obvious drawbacks of using the DNP-NH-CH-COONa moiety as a probe for electrostatic interactions are the volume of the moiety and the presence of substituted aromatic group. It would be preferable to replace this moiety with a moiety capable to participate solely in electrostatic interactions.

We used here the similar approach to analysis of partitioning of mononucleotides with the difference that the number of charged phosphate groups is varied. The data obtained in dextran-PEG ATPS are illustrated in Fig. 1a where logarithms of partition coefficients for the two series of mononucleotides are plotted against the number of phosphate groups. An increase in the number of phosphate groups results in the increase of the nucleotide partition coefficient for both series in dextran-PEG ATPS. The data plotted in Fig. 1a may be described as:

$$\log K_A = A_A + B_A \times N_P \text{ and } \log K_G = A_G + B_G \times N_P \quad (3)$$

where K_A and K_G are the partition coefficients of a given adenosine phosphate and guanine phosphate respectively; N_P is the number of phosphate groups; A_A , A_G , B_A , and B_G are coefficients with values dependent on the particular mononucleotides examined.

It can be seen in Fig. 1a, however, that the partition behavior of adenosine mononucleotides differs from that of guanine mononucleotides. The parameters B_A and B_G representing the average contribution of a phosphate group into $\log K$ of the corresponding nucleotides are 0.0316 ± 0.0004 and 0.023 ± 0.002 respectively. The observed dependence of the average contributions of a phosphate group on the nature of nucleobase makes it questionable whether contribution of a phosphate group may be used for evaluation of the difference between the electrostatic properties of the phases in ATPS.

The data obtained for the same two series of mononucleotides in PEG-Na₂SO₄ ATPS are illustrated graphically in Fig. 1b. The partition behavior of adenosine mononucleotides as a function of the number of phosphate groups may be described by Eq. (3), while the same dependence for guanine mononucleotides is clearly nonlinear and may be described as:

$$\log K_G = a_G + b_G \times e^{-N_P} \quad (4)$$

where a_G and b_G are constants; all the other parameters are as defined above.

Similar dependences in PEG-Na₂SO₄-0.215 M NaCl ATPS illustrated in Fig. 1c appear to be nonlinear for both adenosine and guanine mononucleotides series. An increase in the number of phosphate groups results in the decrease of the nucleotide partition coefficient for both series in PEG-Na₂SO₄ ATPS with and without NaCl additive.

If we assume that the partition behavior of adenosine mononucleotides as a function of the number of phosphate groups may be described by Eq. (3) under all conditions used here and plot the slope of the linear function (coefficient B_A) in all three ATPS versus the parameter C^1 (Eq. (2)), Fig. 2, used as the estimate of the difference between the electrostatic properties of the phases [5] we observe nonlinear relationship. This nonlinearity is not as important as the change of the sign of the difference between the electrostatic properties of the two phases between dextran-PEG and PEG-Na₂SO₄ ATPS observed with adenosine mononucleotides and not detected with DNP-amino acids Na-salts. The previously reported [14–20] studies of partition behavior of small organic compounds and proteins in PEG-Na₂SO₄ ATPS with and without NaCl additive showed that using the difference between the electrostatic properties of the coexisting phases characterized with

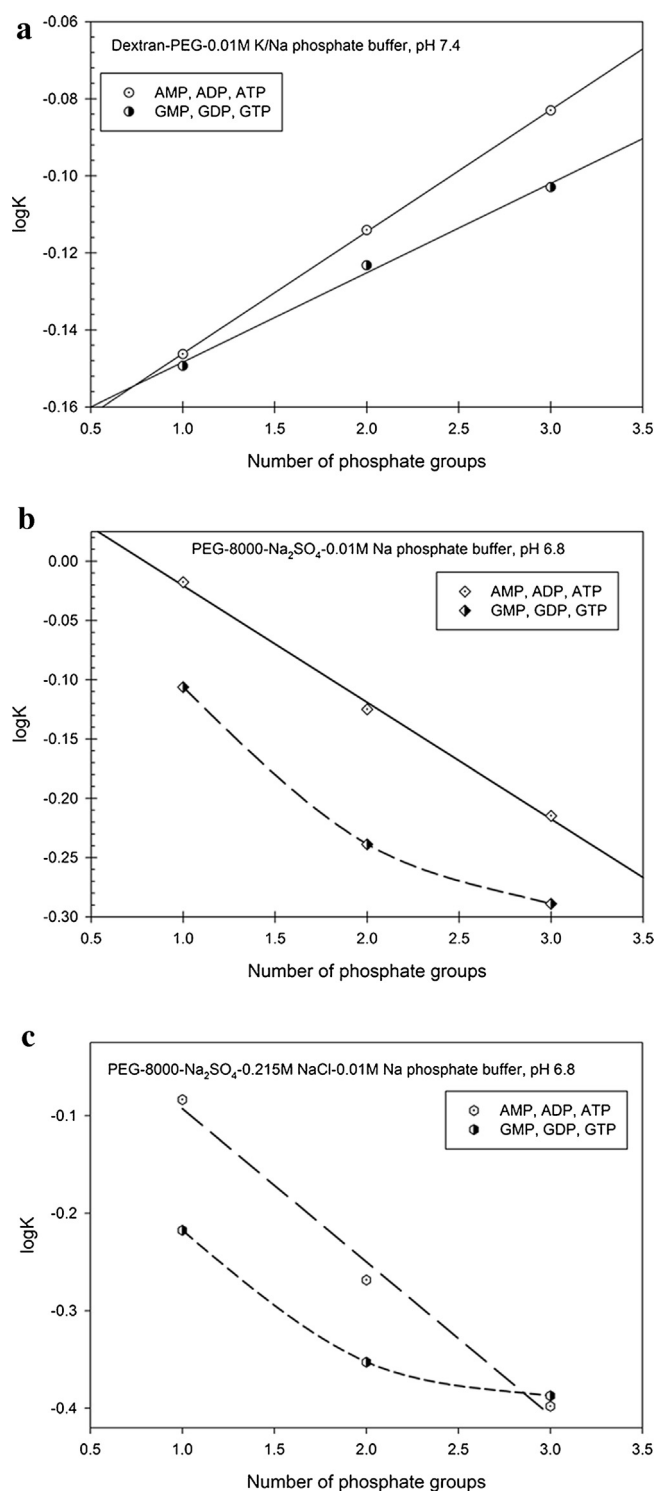


Fig. 1. (a) Logarithm of the partition coefficient value, $\log K$, for adenosine and guanine mononucleotides with different number of phosphate groups in aqueous dextran-PEG two-phase system as a function of number of phosphate groups; (b) logarithm of the partition coefficient value, $\log K$, for adenosine and guanine mononucleotides with different number of phosphate groups in aqueous PEG-Na₂SO₄ two-phase system as a function of number of phosphate groups; (c) logarithm of the partition coefficient value, $\log K$, for adenosine and guanine mononucleotides with different number of phosphate groups in aqueous PEG-Na₂SO₄-0.215 M NaCl two-phase system as a function of number of phosphate groups.

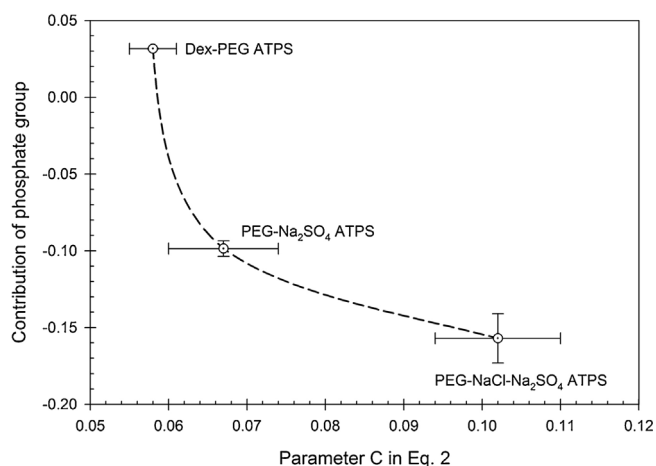


Fig. 2. An average contribution of phosphate group into logarithm of partition coefficient of adenosine mononucleotides versus parameter C in Eq. (2) characterizing the difference between electrostatic properties of two phases in ATPS indicated.

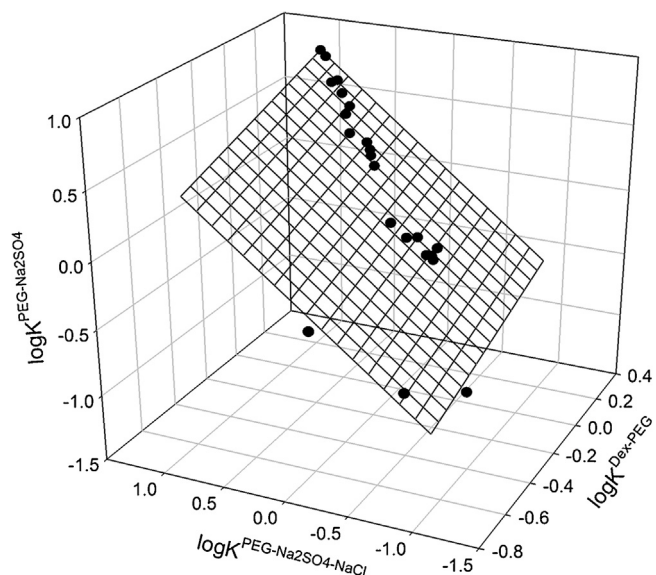


Fig. 3. Logarithms of partition coefficients for organic compounds and proteins in PEG-8000-Na₂SO₄-0.5 M sorbitol-0.01 M sodium phosphate buffer (NaPB), pH 6.8 ATPS versus those for the same compounds and proteins in PEG-8000-Na₂SO₄-0.01 M sodium phosphate buffer (NaPB), pH 6.8 ATPS and in PEG-8000-Na₂SO₄-0.5 M sucrose-0.01 M sodium phosphate buffer (NaPB), pH 6.8 ATPS.

parameter C^i (Eq. (2)) allowed to estimate the solute-solvent interactions. Hence it seems possible to conclude that the contribution of a phosphate group into partition coefficient of mononucleotide does not provide the reliable estimate of the difference between the electrostatic properties of the phases in PEG-salt ATPS.

All the partition coefficients for small organic compounds and proteins in the three ATPS under consideration reported previously [3,10–12] together with those obtained in this study are presented in Table 2. Analysis of these data shows that there is a linear relationship illustrated in Fig. 3 corresponding to Eq. (1) and described as:

$$\log K_i^{\text{PEG-Na}_2\text{SO}_4} = 0.05_{\pm 0.02} + 0.5_{\pm 0.12} \log K_i^{\text{Dex-PEG}} + 0.71_{\pm 0.05} \log K_i^{\text{PEG-Na}_2\text{SO}_4-0.215\text{M-NaCl}} \quad (5)$$

$N = 22$; $r^2 = 0.9775$; $SD = 0.079$; $F = 412.3$ where $K_i^{\text{PEG-Na}_2\text{SO}_4}$ —partition coefficient of the i -th compound in PEG-Na₂SO₄-0.01 M NaPB ATPS; $K_i^{\text{Dex-PEG}}$ —partition coefficient

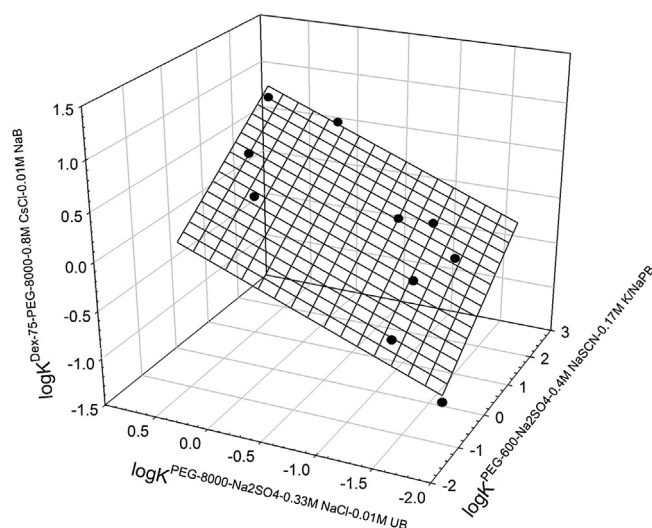


Fig. 4. Logarithms of partition coefficients for organic compounds and proteins in dextran-PEG-8000-0.8 M CsCl-0.01 M sodium phosphate buffer, pH 7.4 ATPS versus those for the same compounds and proteins in PEG-600-Na₂SO₄-0.4 M NaSCN-0.17 M sodium phosphate buffer, pH 7.4 ATPS and in PEG-8000-Na₂SO₄-0.33 M NaCl-0.01 M universal buffer, pH 7.4 ATPS.

of the i -th compound in dextran-PEG-0.01 M K/NaPB ATPS; $K_i^{\text{PEG-Na}_2\text{SO}_4-0.215\text{M NaCl}}$ —partition coefficient of the i -th compound in PEG-Na₂SO₄-0.215 M NaCl-0.01 M NaPB ATPS; N —number of compounds (including proteins); r^2 —correlation coefficient; SD —standard deviation; F —ratio of variance (K/NaPB —sodium/potassium phosphate buffer, pH 7.4; NaPB —sodium phosphate buffer, pH 6.8).

It should be noted that the above relationship holds for partition coefficients of proteins in PEG-Na₂SO₄-0.01 M NaPB and PEG-Na₂SO₄-0.215 M NaCl-0.01 M NaPB ATPS at pH 6.8 and in dextran-PEG-0.01 M K/NaPB at pH 7.4. This fact seems to imply that the pH differences as well as the differences in the buffer composition do not affect the nature and spatial arrangement of the solvent exposed groups in the proteins examined.

Previously the similar relationship was reported for partition coefficients of organic compounds and proteins in Dex-PEG ATPS with different ionic composition [11], and in PEG-Na₂SO₄ ATPS formed by PEG-8000 and PEG-600 with different salt additives [20]. Analysis of the data reported previously [16,20] shows also that the similar relationship for proteins may be found between polymer-polymer and polymer-salt ATPS. For example the relationship illustrated graphically in Fig. 4 may be described as:

$$\log K_i^{\text{Dex-PEG-0.8 M CsCl-0.01 M NaPB}} = 0.12_{\pm 0.10} + 0.30_{\pm 0.07} \log K_i^{\text{PEG-600-Na}_2\text{SO}_4-0.4\text{-NaSCN-0.17 M K/NaPB}} + 0.46_{\pm 0.08} \log K_i^{\text{PEG-8000-Na}_2\text{SO}_4-0.33\text{M NaCl-0.01M UB}} \quad (6)$$

$N = 10$; $r^2 = 0.9416$; $SD = 0.18$; $F = 56.4$

where $K_i^{\text{Dex-PEG-0.8 M CsCl-0.01 M NaPB}}$ —partition coefficient of the i -th compound in dextran-PEG-0.8 M CsCl-0.01 M NaPB ATPS; $\log K_i^{\text{PEG-600-Na}_2\text{SO}_4-0.4\text{ M NaSCN-0.17 M K/NaPB}}$ —partition coefficient of the i -th compound in PEG-600-0.4 M NaSCN-0.17 M K/NaPB ATPS; and $K_i^{\text{PEG-8000-Na}_2\text{SO}_4-0.33\text{ M NaCl-0.01 M UB}}$ —partition coefficient of the i -th compound in PEG-8000-Na₂SO₄-0.33 M NaCl-0.01 M UB ATPS; all the other parameters are as defined above (NaPB—sodium phosphate buffer, pH 7.4; UB—universal buffer, pH 7.4).

Table 2

Partition coefficients for organic compounds and proteins in Dex-PEG-0.01 M K/NaPB, pH 7.4 [16]; PEG- Na₂SO₄-0.01 M NaPB, pH 6.8 [14,17,18]; and PEG- Na₂SO₄-0.215 M NaCl-0.01 M NaPB, pH 6.8 ATPS [15,17] (NaPB—sodium phosphate buffer; K/NaPB—sodium/potassium phosphate buffer).

Compound	Dex-PEG 0.01 M K/NaPB	PEG-Na ₂ SO ₄ 0.01 M NaPB	PEG-Na ₂ SO ₄ 0.215 M NaCl
Adenine	1.220 ± 0.006	3.4 ± 0.12	3.7 ± 0.16
Adenosine	1.128 ± 0.004	2.63 ± 0.02	3.12 ± 0.04
AMP	0.714 ± 0.001	1.02 ± 0.05	0.83 ± 0.04
ADP	0.769 ± 0.002	0.77 ± 0.02	0.54 ± 0.03
ATP	0.826 ± 0.002	0.61 ± 0.01	0.40 ± 0.02
Benzyl alcohol	1.409 ± 0.009	3.500 ± 0.007	4.067 ± 0.008
Caffeine	1.154 ± 0.009	1.85 ± 0.039	2.11 ± 0.036
Coumarin	1.490 ± 0.009	4.86 ± 0.014	4.86 ± 0.014
Glucopyranoside ^a	1.232 ± 0.003	2.18 ± 0.023	2.47 ± 0.033
Guanosine	1.068 ± 0.007	1.673 ± 0.008	1.8 ± 0.03
GMP	0.709 ± 0.005	0.783 ± 0.007	0.606 ± 0.003
GDP	0.753 ± 0.004	0.577 ± 0.002	0.444 ± 0.003
GTP	0.789 ± 0.005	0.514 ± 0.003	0.410 ± 0.004
Methyl anthranilate	1.77 ± 0.01	7.28 ± 0.035	9.59 ± 0.027
Phenol	1.70 ± 0.02	4.60 ± 0.084	6.5 ± 0.17
2-Phenylethanol	1.469 ± 0.005	4.16 ± 0.020	4.98 ± 0.012
Vanillin	1.709 ± 0.009	6.78 ± 0.027	8.28 ± 0.020
α-Chymotrypsin	0.42 ± 0.01	0.117 ± 0.001	0.110 ± 0.001
α-Chymotrypsinogen A	1.00 ± 0.01	0.429 ± 0.003	0.543 ± 0.003
Concanavalin A	0.236 ± 0.003	0.192 ± 0.001	0.183 ± 0.001
Lysozyme	0.23 ± 0.003	0.406 ± 0.003	0.983 ± 0.003
Papain	1.05 ± 0.01	2.22 ± 0.011	1.92 ± 0.007

^a *p*-nitrophenyl-α-D-glucopyranoside.

The above relationships seem to indicate that Eq. (1) is rather universal. These correlations suggest that the properties and mechanism of phase separation in both types of polymer–polymer and polymer–salt ATPS are of the same nature.

We do not know yet the boundaries of its applicability to physicochemical and biological properties. The work in this regard is currently in progress in our laboratories.

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