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Effects of sodium chloride and sodium perchlorate on properties and partition behavior of solutes in aqueous dextran-polyethylene glycol and polyethylene glycol-sodium sulfate two-phase systems

Nuno R. da Silva[a], Luisa A. Ferreira[b], José A. Teixeira[a], Vladimir N. Uversky[c], Boris Y. Zaslavsky[a,*]

[a] IBB—Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Cleveland Diagnostics, 3615 Superior Ave., Cleveland, OH, 44114, USA
[c] Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida, 33612, USA

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ABSTRACT

Effects of two salt additives, NaCl and $NaClO_4$, at the fixed concentrations of 0.215 M on the properties of aqueous two-phase systems (ATPSs) formed by dextran (Dex) and polyethylene glycol (PEG), and the effects of $NaClO_4$ at the same concentration on the properties of ATPS formed by PEG and Na_2SO_4 were examined. The effects of these salt additives on partitioning of 12 small organic compounds and five proteins in the above ATPSs were studied. In each system with a given salt additive, 0.5 M sorbitol, 0.5 M sucrose, and 0.5 M and 1.5 M trimethylamine N-oxide (TMAO) were also used as additives. The results obtained were compared with those reported previously for the Dex-PEG ATPS without salt additives and PEG-Na_2SO_4 ATPS without salt additives and in the presence of 0.215 M NaCl. It is shown that the differences between the solvent properties of the phases in the systems formed by polymer and salt exceed those observed in the systems formed by two polymers. The three most significant solvent features of the systems are hydrophobic and electrostatic properties and hydrogen bonding donor acidity of the solvent media. Osmolyte additives were found to have a significant effect on the differences between the electrostatic properties of the phases. Analysis of the partition coefficients of 12 organic compounds and five proteins showed that the osmolyte additives may affect the partition behavior of compounds in a compound-specific manner. The relative contributions of different types of interactions of a given compound with aqueous media change in the presence of salt and osmolyte additives. Analysis of the variability ranges of partition coefficient, *K*, in the systems studied showed that for small organic compounds, the ranges of *K*-values observed in the PEG-Na_2SO_4 ATPSs exceed those determined in the Dex-PEG ATPSs quite significantly, whereas for proteins, the range of *K*-values in Dex-PEG ATPSs exceeded those in PEG-Na_2SO_4 ATPSs for three proteins, and were very similar for two proteins. This observation supported the notion that the ATPSs formed by two polymers are more suitable for protein analysis than those formed by a single polymer and a salt. The single polymer-salt ATPSs have an advantage for protein isolation/separation.

1. Introduction

Aqueous two-phase systems (ATPSs) are typically formed in mixtures of two compounds in water. The phase-forming compounds commonly include two polymers, a single polymer and a salt [1–5] or surfactant [6,7], two different surfactants [8], and ionic liquids [9–11]. The most thoroughly studied ATPSs include those formed by two polymers, such as dextran and poly(ethylene glycol) (PEG), or a single polymer and inorganic or organic salt, such as PEG and Na_2SO_4 [1–5]. These ATPSs are generally applicable for separation of various biological materials ranging from small biomolecules, proteins, and nucleic acids to cells and viruses [1–5], as well as for the analysis of proteins [5,12–14], discovery and monitoring of biomarkers in biological fluids [14,15], and for clinical diagnostics [16].

* Corresponding author at: Cleveland Diagnostics, 3615 Superior Avenue, Suite 4407B, Cleveland, OH, 44114, USA.
E-mail address: boris.zaslavsky@cleveland-diagnostics.com (B.Y. Zaslavsky).

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It is currently impossible to predict partition behavior of a protein in a given ATPS with a fixed composition. It is possible, however, and important in many cases to manipulate protein partitioning in order to increase/decrease its distribution into one of the phases (e.g., for improved separation or for analytical purposes). The partition behavior of proteins and other biomacromolecules may be manipulated by changing concentrations of phase-forming compounds or by introducing various additives in a given ATPS. Inorganic salts additives are well-known to affect the partition behavior of proteins by changing the properties of the coexisting phases on the one hand, and also modulating properties of proteins on the other hand. The other type of additives capable of manipulation of the partition behavior of solutes includes some nonionic compounds, such as osmolytes. These additives affect the solvent properties of phases, but not the properties of the solutes [17,18].

The purpose of this work was to study and compare the effects of two salt additives, NaCl and $NaClO_4$, at the fixed concentrations of ca. 0.2 M on the properties of two ATPSs formed by dextran and PEG and by PEG and Na_2SO_4, and on partition of 12 low molecular organic compounds and five proteins in these systems. In addition, in each system with a given salt additives, the osmolyte additives, such as 0.5 M sorbitol, 0.5 M sucrose, 0.5 M trehalose, and 0.5 and/or 1.5 M trimethylamine N-oxide (TMAO), were used. The obtained results were considered in terms of the solute-solvent interactions.

2. Materials

2.1. Polymers

Polyethylene glycol (PEG-8000, Lot 091M01372 V) with an average molecular weight (M_n) of 8000 and polyethylene glycol (PEG-10000, Lot 043K2522) with an average molecular weight (M_n) of 10,000 were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Dextran-75 (Dex-75, Lot 119945) with an average molecular weight (M_w) 75,000 by light scattering was purchased from USB Corporation (Cleveland, OH, USA).

2.2. Amino acids

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

2.3. Organic compounds

Benzyl alcohol, caffeine, coumarin, methyl anthranilate, p-nitrophenyl-α-D-glucopyranoside, sorbitol, sucrose, phenol, 2-phenylethanol, trimethylamine N-oxide (TMAO), and vanillin were purchased from Sigma-Aldrich and used without further purification. *o*-Phthaldialdehyde (OPA) reagent solution (complete) was purchased from Sigma.

2.4. Solvatochromic dyes

The solvatochromic probe 4-nitrophenol (spectrophotometric grade) was purchased from Sigma-Aldrich and 4-nitroanisole (GC, > 99%) was supplied by Acros Organic (New Jersey, USA). Reichardt's carboxylated betaine dye, sodium 2,6-diphenyl-4-[4-(4-carboxylato-phenyl)-2,6-diphenylpyridinium-1-yl]phenolate, was synthesized according to the procedure reported previously [19].

2.5. Proteins

α-Chymotrypsin, α-chymotrypsinogen A from bovine pancreas, and concanavalin A from *Canavalia ensiformis* (jack beans) were purchased from Sigma–Aldrich. Lysozyme (salt free) from chicken egg white was obtained from Worthington Biochemical Corp. (Lakewood, NJ, USA). Porcine pancreatic lipase was purchased from USB Corp. (Solon, OH, USA). Purity of all proteins was verified by electrophoresis.

2.6. Other chemicals

All salts and other chemicals used were of analytical-reagent grade and used without further purification.

3. Methods

3.1. Preparation of aqueous two-phase systems

Aqueous two-phase systems were prepared as described previously [20,21]. Stock solutions of PEG 8000 (50 wt.%), PEG-10000 (50 wt.%) and Na_2SO_4 (20.3 wt.%) were prepared in water. Sodium phosphate buffer (NaPB; 0.5 M, pH 6.8) was prepared by mixing appropriate amounts of NaH_2PO_4 and Na_2HPO_4. Stock solution of 2.0 M $NaClO_4$ was prepared in water. Stock solutions of osmolytes: sorbitol (2 M), sucrose (1.8 M), and TMAO (1.8 M and 5.0 M), were prepared in water. A mixture of PEG-8000 or PEG-10000, buffer, and $NaClO_4$ was prepared by dispensing appropriate amounts of the aqueous stock PEG-8000, Na_2SO_4, NaPB, and $NaClO_4$ solutions into a 1.2 ml microtube using a Hamilton (Reno, NV, USA) ML-4000 four-probe liquid-handling workstation. Appropriate amounts of water and/or stock solutions of osmolytes were added to give the required ionic, polymer, and osmolyte composition of the final system with total weight of 0.5 g (after addition of the solute sample, see below). All aqueous PEG-8000-Na_2SO_4-$NaClO_4$ two-phase systems had a fixed composition of 11.10 wt.% PEG-8000, 6.33 wt.% Na_2SO_4, 0.215 M $NaClO_4$, and 0.01 M NaPB, pH 6.8, with different 0.5 M osmolyte or 1.5 M TMAO additive. The aqueous PEG-10000–Na_2SO_4–$NaClO_4$ two-phase system had the same composition of 11.10 wt.% PEG-10000, 6.33 wt.% Na_2SO_4, 0.215 M $NaClO_4$, and 0.01 M NaPB, pH 6.8.

Similar protocol was used to prepare dextran-PEG ATPS. Stock solutions of PEG-8000 (50 wt.%), Dex-75 (42 wt.%), 1.5 M NaCl, 2.0 M $NaClO_4$ and osmolytes (as indicated above) were prepared in deionized (DI) water. Stock sodium/potassium phosphate buffer (K/NaPB; 0.5 M, pH 7.4) was prepared by mixing appropriate amounts of KH_2PO_4 and Na_2HPO_4. Using the Hamilton Company ML-4000 four-probe liquid-handling workstation, proper amounts of stock solutions of polymers, salt (NaCl or $NaClO_4$), osmolyte, stock buffer solutions, and water were added to give the ionic, polymer, and osmolyte composition required for the final system (after the sample addition – see below) with total weight of 0.5 g. All the two polymer-based aqueous two-phase systems used in this study had the same polymer composition of 6.0 wt.% PEG-8000 and 12.0 wt.% Dex-75 and same ionic composition of 0.01 M K/NaPB, pH 7.4, 0.215 M NaCl (or 0.215 M $NaClO_4$) with 0.5 M osmolyte or 1.5 M TMAO additive.

3.2. Partitioning

An automated instrument for performing aqueous two-phase partitioning, the Automated Signature Workstation, ASW (Analiza, Inc., Cleveland, OH, USA), was used for the partitioning experiments. The ASW system is based on the ML-4000 liquid-handling workstation (Hamilton Company, Reno, NV, USA) integrated 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). Solutions of all compounds were prepared in water at concentrations of 0.5–5 mg/mL depending on the compound solubility. Varied amounts (e.g. 0, 15, 30, 45, 60 and 75 μL) of compound solution and the corresponding amounts (e.g. 75, 60, 45, 30, 15 and 0 μL) of water were added to a set of the same polymers/buffer/salt mixtures with and without osmolyte additive. The systems were then vortexed in a Multipulse vortexer and centrifuged (Jouan, BR4i, Thermo Fisher Scientific, Waltham, MA, USA) for 30 min at 3500×g 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 proteins partitioning, aliquots of 30 μL from both phases were transferred and diluted with water up to 70 μL into microplate wells. Then, the microplate was sealed, shortly centrifuged (2 min at 1500 rpm) and following moderate shaking for 45 min in an incubator at 37 °C, 250 μL of o-phthaldialdehyde reagent was combined. After moderate shaking for 4 min at room temperature, fluorescence was determined using a fluorescence plate reader with a 360 nm excitation filter and a 460 nm emission filter, with a sensitivity setting of 100-125.

For the analysis of the other compounds partitioning, aliquots of 50–120 μL from both phases were diluted up to 600 μL in 1.2 mL microtubes. Water was used as diluent for all except phenol, and vanillin. 20 mM universal buffer with pH 12.4 was used as diluent (universal buffer is composed of 0.01 M each of phosphoric, boric, and acetic acids adjusted to pH 12.4 with NaOH). Following vortexing and a short centrifugation (12 min), aliquots of 250–300 μL were transferred into microplate wells, and the UV-VIS plate reader was used to measure optical absorbance at wavelengths previously determined to correspond to maximum absorption. The maximum absorption wavelength for each compound was determined in separate experiments by analysis of the absorption spectrum over the 240–500 nm range. In the case of the two aforementioned compounds the maximum absorption was found to be more concentration sensitive in the presence of the universal buffer at pH 12.4. In all measurements the dilution factors used for the upper and lower phases was taken into account and correspondingly diluted pure phases were used as blank solutions.

The partition coefficient, *K*, is defined as the ratio of the sample solute concentration in the top phase to that in the bottom phase. The *K*-value for each solute was determined as the slope of the concentration (fluorescence intensity or absorbance depending on the compound) 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 carried out at the specified composition of the system [22]. The deviation from the average *K* value was always less than 3% and in most cases lower than 1%.

3.3. Analysis of hydrophobic and electrostatic properties of the phases

Analysis of hydrophobic and electrostatic properties of the coexisting phases of all ATPSs used in this study was performed as described previously [5] using results of partitioning of DNP-amino acids sodium salts. The detailed description is provided in Supplementary Information (SI) A.

3.4. Solvatochromic measurements

All solvatochromic measurements in the phases of ATPSs used were performed as described previously [20,21]. The detailed description of the protocols used is provided in SI B.

4. Results and discussion

4.1. Solvent properties of ATPSs

Partitioning of the homologous series of Na-salts of dinitrophenylated (DNP-) amino acids was examined in order to estimate the difference between the relative hydrophobic and electrostatic properties of the phases as described previously [5,20,21]. Partition coefficients of Na-salts of DNP-amino acids with the aliphatic alkyl side-chains of the increasing length (alanine, norvaline, norleucine, and α-amino-*n*-octanoic acid) in the ATPS under study are listed below in Tables 1–3 and Tables A1–A3 and shown graphically as functions of the equivalent number of methylene groups representing the length of the alkyl side-chain in Fig. A1-A3. The data obtained may be described as:

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

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

Table 1
Partition coefficients for organic compounds and proteins in Dextran-PEG-0.215 M NaCl-0.01 M K/NaPB, pH 7.4 and Dextran-PEG-0.215 M NaCl-osmolyte-0.01 M K/NaPB, pH 7.4 ATPS (K/NaPB – sodium/potassium phosphate buffer).

Compound	0.01 M K/NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	1.5 M TMAO
DNP-Alanine Na	0.986 ± 0.003	1.100 ± 0.001	1.144 ± 0.002	1.000 ± 0.002	1.110 ± 0.001
DNP-Norvaline Na	1.077 ± 0.002	1.227 ± 0.001	1.251 ± 0.002	1.107 ± 0.001	1.271 ± 0.003
DNP-Norleucine Na	1.131 ± 0.003	1.300 ± 0.003	1.346 ± 0.003	1.170 ± 0.002	1.406 ± 0.002
DNP-Octanoic acid Na	1.308 ± 0.002	1.557 ± 0.002	1.624 ± 0.003	1.372 ± 0.004	1.722 ± 0.004
Benzyl alcohol	1.547 ± 0.002	1.630 ± 0.002	1.722 ± 0.003	1.563 ± 0.005	1.554 ± 0.004
Caffeine	1.154 ± 0.002	1.176 ± 0.003	1.184 ± 0.003	1.208 ± 0.004	1.311 ± 0.005
Coumarin	1.611 ± 0.003	1.742 ± 0.003	1.777 ± 0.004	1.660 ± 0.002	1.923 ± 0.006
Glucoside[a]	1.222 ± 0.002	1.310 ± 0.002	1.350 ± 0.002	1.260 ± 0.002	1.360 ± 0.004
Methyl anthranilate	1.929 ± 0.004	2.135 ± 0.004	2.218 ± 0.004	1.932 ± 0.005	2.467 ± 0.012
2-Phenylethanol	1.592 ± 0.003	1.779 ± 0.005	1.846 ± 0.005	1.667 ± 0.003	1.701 ± 0.003
Phenol	1.932 ± 0.003	2.116 ± 0.007	2.234 ± 0.006	1.906 ± 0.007	1.998 ± 0.008
Vanillin	1.702 ± 0.004	1.939 ± 0.005	1.987 ± 0.007	1.641 ± 0.004	1.818 ± 0.006
α-Chymotrypsin	0.927 ± 0.002	4.148 ± 0.024	5.184 ± 0.041	0.967 ± 0.004	1.560 ± 0.008
α-Chymotrypsinogen A	2.750 ± 0.007	1.177 ± 0.003	1.072 ± 0.002	3.100 ± 0.011	4.45 ± 0.021
Concanavalin A	1.481 ± 0.003	1.170 ± 0.002	1.156 ± 0.004	1.539 ± 0.004	0.928 ± 0.007
Lipase	0.801 ± 0.004	0.801 ± 0.003	0.817 ± 0.001	0.830 ± 0.002	0.757 ± 0.003
Lysozyme	2.380 ± 0.025	3.584 ± 0.017	3.766 ± 0.012	2.455 ± 0.005	3.848 ± 0.018

[a] p-Nitrophenyl-α-D-glucopyranoside.

Table 2
Partition coefficients for organic compounds and proteins in Dex-PEG-0.215 M $NaClO_4$-0.01 M K/NaPB, pH 7.4 and Dex-PEG-0.215 M $NaClO_4$-osmolyte-0.01 M K/NaPB, pH 7.4 ATPS (K/NaPB – sodium/potassium phosphate buffer).

Compound	0.01 M K/NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	1.5 M TMAO
DNP-Alanine Na	0.959 ± 0.004	0.966 ± 0.003	0.971 ± 0.002	0.965 ± 0.003	0.994 ± 0.002
DNP-Norvaline Na	1.029 ± 0.002	1.064 ± 0.002	1.074 ± 0.003	1.059 ± 0.004	1.175 ± 0.002
DNP-Norleucine Na	1.092 ± 0.002	1.154 ± 0.003	1.170 ± 0.003	1.142 ± 0.004	1.346 ± 0.001
DNP-Octanoic acid Na	1.249 ± 0.004	1.393 ± 0.005	1.419 ± 0.002	1.361 ± 0.003	1.854 ± 0.003
Benzyl alcohol	1.620 ± 0.003	1.767 ± 0.006	1.857 ± 0.004	1.586 ± 0.004	1.855 ± 0.006
Caffeine	1.197 ± 0.003	1.266 ± 0.002	1.246 ± 0.003	1.252 ± 0.004	1.432 ± 0.007
Coumarin	1.669 ± 0.005	1.974 ± 0.004	1.977 ± 0.003	1.781 ± 0.005	2.445 ± 0.008
Glucoside[a]	1.249 ± 0.007	1.396 ± 0.005	1.409 ± 0.004	1.303 ± 0.004	1.515 ± 0.007
Methyl anthranilate	1.985 ± 0.006	2.453 ± 0.007	2.581 ± 0.011	2.130 ± 0.008	3.099 ± 0.015
2-Phenylethanol	1.663 ± 0.005	1.895 ± 0.006	2.035 ± 0.009	1.726 ± 0.004	2.034 ± 0.008
Phenol	1.909 ± 0.011	1.233 ± 0.009	2.396 ± 0.006	1.939 ± 0.005	2.265 ± 0.007
Vanillin	1.653 ± 0.003	1.973 ± 0.007	2.047 ± 0.005	1.591 ± 0.003	1.778 ± 0.007
α-Chymotrypsin	1.220 ± 0.004	1.634 ± 0.006	1.845 ± 0.007	1.400 ± 0.003	1.603 ± 0.005
α-Chymotrypsinogen A	3.646 ± 0.014	6.519 ± 0.023	6.245 ± 0.032	4.550 ± 0.018	7.451 ± 0.041
Concanavalin A	0.213 ± 0.001	0.246 ± 0.003	0.267 ± 0.003	0.227 ± 0.002	0.235 ± 0.003
Lipase	0.727 ± 0.002	0.791 ± 0.003	0.849 ± 0.002	0.739 ± 0.003	0.670 ± 0.001
Lysozyme	20.3 ± 0.15	46.4 ± 0.22	49.10 ± 0.23	22.3 ± 0.13	38.9 ± 0.34

[a] p-Nitrophenyl-α-D-glucopyranoside.

Table 3
Partition coefficients for organic compounds and proteins in PEG-Na_2SO_4-0.215 M $NaClO_4$-0.01 M NaPB, pH 6.8 formed by PEG-8000 and PEG-10000 and PEG-Na_2SO_4-0.215 M $NaClO_4$-0.5 M osmolyte-0.01 M NaPB, pH 6.8 ATPS (NaPB – sodium phosphate buffer).

Compound	PEG-8000				PEG-10000
	0.01 M NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	0.01 M NaPB
DNP-Alanine Na	3.511 ± 0.009	4.023 ± 0.011	3.485 ± 0.008	3.908 ± 0.033	3.673 ± 0.029
DNP-Norvaline Na	5.188 ± 0.032	6.095 ± 0.044	5.398 ± 0.021	6.067 ± 0.064	5.358 ± 0.052
DNP-Norleucine Na	7.145 ± 0.047	8.610 ± 0.087	7.727 ± 0.053	8.166 ± 0.104	7.244 ± 0.081
DNP-Octanoic acid Na	13.932 ± 0.104	19.099 ± 0.145	17.730 ± 0.092	18.030 ± 0.213	14.997 ± 0.103
Benzyl alcohol	6.081 ± 0.058	7.989 ± 0.116	6.397 ± 0.014	6.237 ± 0.044	5.802 ± 0.026
Caffeine	2.582 ± 0.006	3.500 ± 0.032	2.938 ± 0.006	3.467 ± 0.028	2.985 ± 0.017
Coumarin	10.740 ± 0.087	15.171 ± 0.124	12.078 ± 0.023	12.794 ± 0.135	10.304 ± 0.108
Glucoside[a]	2.897 ± 0.007	4.150 ± 0.045	3.855 ± 0.014	3.639 ± 0.011	3.303 ± 0.027
Methyl anthranilate	18.576 ± 0.116	29.522 ± 0.178	21.627 ± 0.111	12.794 ± 0.128	15.922 ± 0.215
2-Phenylethanol	8.185 ± 0.035	9.452 ± 0.086	9.670 ± 0.071	7.690 ± 0.064	7.325 ± 0.068
Phenol	9.920 ± 0.048	11.780 ± 0.109	12.430 ± 0.086	8.854 ± 0.085	8.627 ± 0.097
Vanillin	12.471 ± 0.083	19.138 ± 0.127	14.859 ± 0.115	8.640 ± 0.073	12.372 ± 0.107
α-Chymotrypsin	0.070 ± 0.004	0.058 ± 0.003	0.094 ± 0.003	0.054 ± 0.002	0.064 ± 0.003
α-Chymotrypsinogen A	0.191 ± 0.003	0.171 ± 0.002	0.241 ± 0.002	0.159 ± 0.001	0.180 ± 0.041
Concanavalin A	0.136 ± 0.001	0.132 ± 0.002	0.139 ± 0.003	0.141 ± 0.002	0.154 ± 0.003
Lipase	0.512 ± 0.002	0.487 ± 0.003	0.468 ± 0.002	0.501 ± 0.003	0.550 ± 0.002
Lysozyme	28.710 ± 0.176	43.251 ± 0.370	27.290 ± 0.372	20.941 ± 0.251	22.92 ± 0.228

[a] p-Nitrophenyl-α-D-glucopyranoside.

acterize the difference between the electrostatic properties of the coexisting phases as described previously [5,20,21].

The differences between the hydrophobic and electrostatic properties of the phases characterized by the C and E values are listed for all the ATPSs under comparison in Table 4. The free energies of transfer of a CH_2 group from the lower to the upper phases determined from Eq. (1) as described in SI A are also present in Table 4. It has been shown previously [5,20,21] that partition behavior of solutes in any given ATPS is affected by the solvent features of the coexisting phases, such as the solvent dipolarity/polarizability (π^*) characterizing the ability of water to participate in dipole-dipole and dipole-induced dipole interactions with a solute, solvent hydrogen bond donor acidity (α), and hydrogen bond acceptor basicity (β). The differences between these solvent features of the phases determined as described in SI B are also listed in Table 4.

The differences between the solvent hydrogen bond donor acidity, $\Delta\alpha$, in the PEG-Na_2SO_4 ATPSs exceed those observed in Dextran-PEG ATPSs in the presence of all osmolytes additives used. The salts additives effects on $\Delta\alpha$ appears to be more pronounced in PEG-Na_2SO_4 ATPSs than in Dextran-PEG systems. In PEG-Na_2SO_4 ATPSs the salt additive effect depends on the presence of osmolyte additives. Addition of ~0.2 M NaCl increases the difference, while addition of $NaClO_4$ may reduce or increase it, depending on the particular osmolyte present. The differences between the solvent hydrogen bond acceptor basicity, $\Delta\beta$, in the PEG-Na_2SO_4 ATPSs generally exceed those observed in Dextran-PEG ATPSs in the presence of all osmolytes except in the presence of 0.5 M or 1.5 M TMAO in Dextran-PEG-NaCl ATPSs.

The differences between the solvent dipolarity/polarizability, $\Delta\pi^*$, vary in both types of ATPSs within the same range from −0.020 to −0.077 and the effects of salt and osmolyte additives do not display any noticeable trend.

The differences between electrostatic properties of the phases (parameter C) in PEG-Na_2SO_4 ATPSs exceed those observed in Dextran-PEG ATPSs as expected. Surprisingly, osmolyte additives affect the differences between electrostatic properties in both types of ATPSs rather strongly. In Dextran-PEG ATPSs, both NaCl and $NaClO_4$ additives reduce the difference. Both salts additives appear also to reduce the difference between the electrostatic properties of the phases in PEG-Na_2SO_4 ATPSs, and their effects seem to depend on the particular osmolyte additive present.

The differences between the relative hydrophobicity of the phases (parameter E) in PEG-Na_2SO_4 ATPSs exceed those observed in Dextran-PEG ATPSs in the presence of all additives. The effects of salt additives in these two types of ATPSs are different, however. In osmolyte free Dex-PEG ATPSs both NaCl and $NaClO_4$ additives

Table 4
Differences between the solvent properties of the coexisting phases Dextran-PEG-0.01 M K/NaPB, pH 7.4 and PEG-Na_2SO_4-0.01 M NaPB, pH 6.8 ATPS with indicated salts and osmolytes additives (K/NaPB – sodium/potassium phosphate buffer; NaPB – sodium phosphate buffer).

	Dextran-PEG (data from [20])				
	0.01 M K/NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	0.5 M Trehalose
$\Delta G(CH_2)$[a], cal/mole	−45 ± 1.3	−43 ± 1.1	−39.4 ± 0.44	−40.9 ± 0.6	−47.7 ± 0.6
E	0.033 ± 0.001	0.032 ± 0.002	0.029 ± 0.001	0.028 ± 0.001	0.035 ± 0.001
C	0.058 ± 0.003	0.090 ± 0.003	0.110 ± 0.002	0.083 ± 0.002	0.113 ± 0.002
$\Delta\pi^*$	−0.042 ± 0.002	−0.042 ± 0.004	−0.073 ± 0.004	−0.031 ± 0.002	−0.042 ± 0.003
$\Delta\alpha$	−0.051 ± 0.003	−0.066 ± 0.003	−0.046 ± 0.005	−0.074 ± 0.003	−0.081 ± 0.003
$\Delta\beta$	0.006 ± 0.004	0.006 ± 0.003	0.023 ± 0.006	0.009 ± 0.008	0.006 ± 0.005
	Dextran-PEG-0.215 M NaCl				
	0.01 M K/NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	1.5 M TMAO
$\Delta G(CH_2)$[a], cal/mole	−33 ± 1.2	−41 ± 1.8	−41 ± 2.9	−37 ± 1.2	−51 ± 2.4
E	0.024 ± 0.001	0.030 ± 0.001	0.031 ± 0.001	0.027 ± 0.001	0.038 ± 0.002
C	−0.036 ± 0.004	0.005 ± 0.005	0.017 ± 0.002	−0.032 ± 0.004	0.0006 ± 0.007
$\Delta\pi^*$	−0.037 ± 0.003	−0.041 ± 0.002	−0.040 ± 0.003	−0.033 ± 0.002	−0.026 ± 0.001
$\Delta\alpha$	−0.054 ± 0.002	−0.063 ± 0.002	−0.080 ± 0.002	−0.065 ± 0.002	−0.023 ± 0.002
$\Delta\beta$	0.002 ± 0.002	0.007 ± 0.002	0.004 ± 0.003	−0.019 ± 0.002	−0.105 ± 0.002
	Dextran-PEG-0.215 M $NaClO_4$				
	0.01 M K/NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	1.5 M TMAO
$\Delta G(CH_2)$[a], cal/mole	−31.2 ± 0.1	−43 ± 0.2	−44.7 ± 0.2	−40.6 ± 0.1	−73.5 ± 0.2
E	0.023 ± 0.003	0.032 ± 0.002	0.033 ± 0.001	0.067 ± 0.001	0.054 ± 0.001
C	−0.049 ± 0.001	−0.056 ± 0.009	−0.056 ± 0.003	0.0546 ± 0.0007	−0.074 ± 0.005
$\Delta\pi^*$	−0.037 ± 0.002	−0.040 ± 0.002	−0.044 ± 0.002	−0.031 ± 0.003	−0.020 ± 0.003
$\Delta\alpha$	−0.031 ± 0.003	−0.067 ± 0.003	−0.072 ± 0.003	−0.075 ± 0.003	−0.104 ± 0.003
$\Delta\beta$	0.004 ± 0.002	0.006 ± 0.002	0.008 ± 0.002	0.003 ± 0.004	−0.002 ± 0.003
.	PEG-Na_2SO_4 (Data from [23])				
	0.01 M NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	PEG-10000[b]
$\Delta G(CH_2)$[a], cal/mole	−122 ± 1.8	−144 ± 3.4	−178 ± 3.5	−146 ± 2.4	−137 ± 5.8
E	0.048 ± 0.002	0.102 ± 0.003	0.123 ± 0.006	0.108 ± 0.002	0.100 ± 0.004
C	0.445 ± 0.005	0.670 ± 0.011	0.670 ± 0.020	0.625 ± 0.007	0.440 ± 0.017
$\Delta\pi^*$	−0.029 ± 0.003	−0.046 ± 0.004	−0.077 ± 0.005	−0.010 ± 0.003	−0.020 ± 0.003
$\Delta\alpha$	−0.128 ± 0.004	−0.248 ± 0.005	−0.228 ± 0.007	−0.208 ± 0.004	−0.075 ± 0.004
$\Delta\beta$	0.015 ± 0.004	0.021 ± 0.008	0.028 ± 0.008	0.021 ± 0.009	0.013 ± 0.004
	PEG-Na_2SO_4-0.215 M NaCl (Data from [21])				
	0.01 M NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	PEG-10000[b]
$\Delta G(CH_2)$[a], cal/mole	−152.4 ± 0.6	−180 ± 5.3	−187 ± 2.9	−161 ± 1.8	−149 ± 4.1
E	0.112 ± 0.001	0.133 ± 0.004	0.138 ± 0.002	0.119 ± 0.001	0.110 ± 0.003
C	0.435 ± 0.002	0.530 ± 0.015	0.525 ± 0.008	0.571 ± 0.005	0.438 ± 0.011
$\Delta\pi^*$	−0.027 ± 0.003	−0.056 ± 0.002	−0.067 ± 0.003	−0.025 ± 0.002	−0.039 ± 0.001
$\Delta\alpha$	−0.189 ± 0.002	−0.259 ± 0.002	−0.253 ± 0.002	−0.271 ± 0.002	−0.183 ± 0.002
$\Delta\beta$	0.013 ± 0.002	0.025 ± 0.002	0.022 ± 0.003	0.015 ± 0.002	0.021 ± 0.002
	PEG-Na_2SO_4-0.215 M $NaClO_4$				
	0.01 M NaPB	0.5 M Sorbitol	0.5 M Sucrose	0.5 M TMAO	PEG-10000[b]
$\Delta G(CH_2)$[a], cal/mole	−162 ± 3.5	−184 ± 1.2	−191.9 ± 0.6	−179 ± 3.5	−166 ± 0.6
E	0.112 ± 0.003	0.136 ± 0.001	0.142 ± 0.001	0.132 ± 0.002	0.122 ± 0.001
C	0.395 ± 0.010	0.426 ± 0.001	0.357 ± 0.001	0.423 ± 0.010	0.404 ± 0.002
$\Delta\pi^*$	−0.042 ± 0.003	−0.053 ± 0.003	−0.068 ± 0.002	−0.018 ± 0.002	−0.030 ± 0.003
$\Delta\alpha$	−0.173 ± 0.004	−0.107 ± 0.012	−0.194 ± 0.002	−0.242 ± 0.003	−0.161 ± 0.019
$\Delta\beta$	0.006 ± 0.003	0.026 ± 0.002	0.023 ± 0.002	0.009 ± 0.002	0.018 ± 0.002

[a] $\Delta G(CH_2)$ - free energy of transfer of a CH_2 group from the bottom to the top phase of a given ATPS calculated as indicated in Supplementary Information (SI) A.
[b] PEG-10000-Na_2SO_4-0.01 M NaPB.

reduce it. In PEG-Na_2SO_4 ATPSs, both salt additives increase the difference between the relative hydrophobicity of the phases.

In order to simplify the comparison, we estimated the normalized Euclidean distances between all the ATPSs under consideration as described previously [21].

The solvent properties of each ATPS are represented by the set of differences between the solvent features of the coexisting phases: $\Delta\pi^*$, $\Delta\alpha$, $\Delta\beta$, C, and E values, listed in Table 4. The set of the $\Delta\pi^*$, $\Delta\alpha$, $\Delta\beta$, C, and E values for a given ATPS may be viewed as a point in a multi-dimensional space of solvent properties. To compare the properties of different ATPSs, we calculated the normalized Euclidian distance in the multi-dimensional space represented by the differences between the solvent features of the coexisting phases in different ATPSs:

$$d_{i,o} = \left[\sum_j \left(\frac{\partial_i - \partial_o}{\partial_o}\right)^2\right]^{0.5} \quad (2)$$

where $d_{i,o}$ is the distance between the solvent properties of i^{th} ATPS and solvent properties of the o^{th} ATPS chosen as a reference, ∂_i and ∂_o are the differences between the j^{th} solvent features in i^{th} and o^{th} ATPSs.

To compensate for differences in ∂-values measured for a given solvent property in different ATPSs, we normalized the experimental ∂-values to the reference ∂_o-value for each particular solvent property. Therefore, Eq. (2) represents the Euclidean distance between the points characterized by normalized differences between various solvent features in different ATPSs.

In order to compare the properties of the multiple ATPSs used here and previously [20–23], we selected the osmolyte and salt additive-free Dextran-PEG ATPS as the reference ATPS. The normalized Euclidean distances calculated with Eq. (2) are listed in Table 5.

In order to explore what solvent features dominate in the ATPS under comparison, we explored how the calculated distances vary with reducing the number of different solvent features included in calculation. The result illustrated graphically in Fig. 1 shows that

Table 5
Normalized Euclidean distances between the solvent properties of ATPSs calculated with Eq. (2) from the data in Table 4. (ATPSs compositions presented in Table 4).

ATPS	Distance, d_{io}[a]	Distance, d_{io}*[b]
Dex-PEG	0	0
Dex-PEG-0.5 M sorbitol	0.63	0.63
Dex-PEG-0.5 M TMAO	0.85	0.64
Dex-PEG-0.5 M trehalose	1.12	0.91
Dex-PEG-0.215 M NaCl-0.5 M sucrose	1.54	1.12
Dex-PEG-0.215 M NaCl-0.5 M sorbitol	1.61	1.51
Dex-PEG-0.215 M $NaClO_4$	1.91	1.60
Dex-PEG-0.215 M NaCl	1.93	1.80
Dex-PEG-0.215 M $NaClO_4$-0.5 M sorbitol	1.99	1.87
Dex-PEG-0.215 M $NaClO_4$-0.5 M TMAO	2.04	1.88
Dex-PEG-0.215 M $NaClO_4$-0.5 M sucrose	2.08	1.99
Dex-PEG-0.215 M $NaClO_4$-1.5 M TMAO	2.95	2.00
Dex-PEG-0.5 M sucrose	3.07	2.01
Dex-PEG-0.215 M NaCl-0.5 M TMAO	4.57	2.41
PEG-Na_2SO_4-0.215 M $NaClO_4$	6.75	2.58
PEG-Na_2SO_4	7.03	6.75
PEG-10000-Na_2SO_4	7.03	6.75
PEG-10000-Na_2SO_4-0.215 M $NaClO_4$	7.18	6.86
PEG-Na_2SO_4-0.215 M $NaClO_4$-0.5 M TMAO	7.35	6.89
PEG-Na_2SO_4-0.215 M $NaClO_4$-0.5 M sorbitol	7.89	6.91
PEG-Na_2SO_4-0.215 M $NaClO_4$-0.5 M sucrose	7.95	7.14
PEG-Na_2SO_4-0.215 M NaCl	8.29	7.91
PEG-Na_2SO_4-0.215 M NaCl-0.5 M sorbitol	8.69	8.09
PEG-Na_2SO_4-0.215 M NaCl-0.5 M TMAO	9.80	8.20
PEG-Na_2SO_4-0.215 M NaCl-0.5 M sucrose	9.93	9.54
PEG-10000-Na_2SO_4-0.215 M NaCl	9.94	9.62
PEG-Na_2SO_4-0.5 M TMAO	10.82	9.67
PEG-Na_2SO_4-0.5 M sorbitol	11.70	10.50
PEG-Na_2SO_4-0.5 M sucrose	12.04	11.43
PEG-Na_2SO_4-0.5 M trehalose	12.51	11.44
Dex-PEG-1.5 M TMAO	18.69	12.20

[a] Distances d_{io} calculated with Eq. (2) using Dex-PEG ATPS as a reference and all solvent properties of ATPS presented in Table 4.
[b] Distances d_{io}* calculated with Eq. (2) using Dex-PEG ATPS as a reference and only three solvent properties of ATPS (parameters C, E, and Δα).

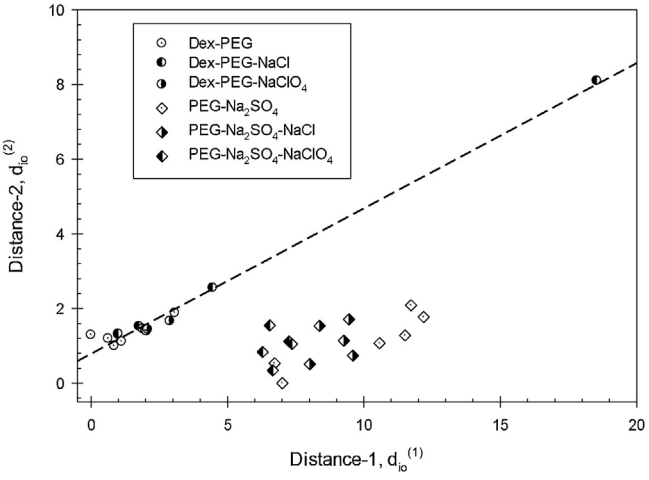

Fig. 1. Euclidian distance for aqueous two-phase systems, $d_{io}^{(2)}$, calculated using three solvent features (difference between the relative hydrophobicity of the two phases, E, difference between electrostatic properties, C, and difference between the solvent hydrogen bond donor acidity, Δα) plotted against Euclidian distance, $d_{io}^{(1)}$, calculated using all the solvent features (C, E, Δα, Δπ*, and Δβ).

there are three solvent features, the differences between electrostatic and hydrophobic properties (parameters C and E) and the difference between the solvent hydrogen bond donor acidity (Δα) sufficient for estimating the distance between the ATPS under comparison. Only three ATPSs (Dex-PEG-0.215 M NaCl-1.5 M TMAO, Dex-PEG-0.215 M NaCl-0.5 M TMAO, and Dex-PEG-0.5 M sucrose) are exceptions due to extraordinary Δβ of -0.105, negative value of Δβ -0.019, and high value of Δπ* -0.073, correspondingly.

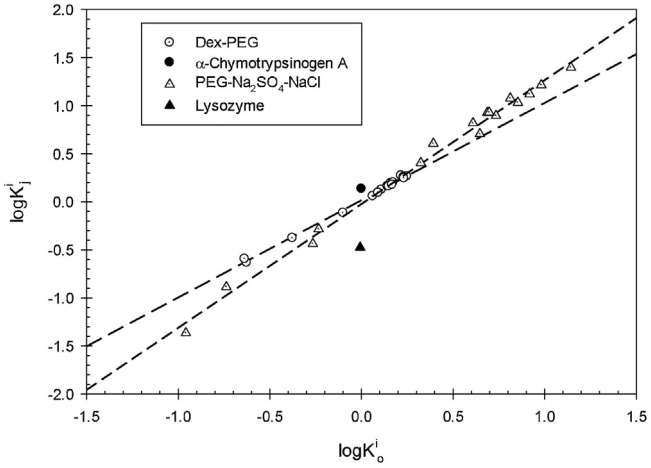

Fig. 2. Logarithms of partition coefficients for compounds (including proteins) in aqueous Dex-PEG and PEG-Na_2SO_4 two-phase systems with 0.5 M TMAO versus logarithms of partition coefficients for the same compounds in the corresponding systems without TMAO additive.

4.2. Partition behavior of organic compounds and proteins

Partition coefficients of 12 organic compounds and five proteins in Dex-PEG-0.215 M NaCl-0.01 M K/NaPB, pH 7.4, Dex-PEG-0.215 M $NaClO_4$-0.01 M K/NaPB, pH 7.4, and PEG-Na_2SO_4-0.215 M $NaClO_4$-0.01 M NaPB, pH 6.8 are presented in Tables 1–3. The partition coefficients for the same compounds and proteins in Dex-PEG-0.01 M K/NaPB, pH 7.4 reported previously [20,22], in PEG-Na_2SO_4-0.01 M NaPB, pH 6.8 [23] and in PEG-Na_2SO_4-0.215 M NaCl-0.01 M NaPB, pH 6.8 [21] are listed in Tables A1-A3 provided in the SI A. In order to compare partition behavior of the compounds utilized in the study, we used the so-called Collander linear relationship or solvent regression equation [5,24]. This equation describes the linear relationship between partition coefficients of various solutes in two different aqueous two-phase systems as:

$$\log K^i_j = a_{jo} \log K^i_o + b_{jo} \quad (3)$$

where K^i_j and K^i_o are partition coefficients for a solute *i* in the aqueous two-phase systems denoted by subscripts *j* and *o*; a_{jo} and b_{jo} are constants, the values of which depend on the particular composition of the two-phase systems under comparison. It should be emphasized that both ATPSs under comparison have the same ionic composition.

Typical Collander relationships for two ATPSs different in regard to the presence of nonionic osmolyte additive TMAO are shown in Fig. 2. Similar relationships for various ATPS are observed. These relationships are characterized by coefficients a_{jo} and b_{jo} listed in Table 6.

In order to explain the relationships observed and the fact that some of the compounds do not fit these relationships it is necessary to mention that as established previously [25,26] the partition coefficient of a solute in a given ATPS is governed by different types of interactions of the solute with the aqueous media in the coexisting phases and may be described as:

$$\log K^i_j = S_s{}^i \Delta\pi^*_j + B_s{}^i \Delta\alpha_j + A_s{}^i \Delta\beta_j + C_s{}^i c_j \quad (4)$$

where K^i_j is the solute *i* partition coefficient in j^{th} ATPS; $\Delta\pi^*_j$, $\Delta\alpha_j$, $\Delta\beta_j$ and c_j are the differences between the solvent properties of the top and bottom phases in the j^{th} ATPS (solvent dipolarity/polarizability, hydrogen-bond donor acidity, hydrogen-bond acceptor basicity, and electrostatic interactions, respectively; $S_s{}^i$, $B_s{}^i$, $A_s{}^i$, and $C_s{}^i$ are constants (solute-specific coefficients) that describe the contributions of the complementary interactions of

Table 6
Coefficients of Collander relationships (Eq. (3)) for compounds in ATPS with osmolytes.

ATPS	Osmolyte[a]	Salt	b_{jo}	a_{jo}	N	r^2	SD	F	Outliers[b]
Dex-PEG	TMAO	–	0.017 ± 0.005	1.01 ± 0.02	16	0.9963	0.019	3737	P2
	Sorbitol		0.032 ± 0.005	1.07 ± 0.02	14	0.9964	0.017	3307	P2, P5, 1
	Sucrose		0.041 ± 0.005	1.08 ± 0.02	14	0.9957	0.019	2764	P2, P5, 1
Dex-PEG	TMAO	NaCl	0.015 ± 0.004	0.96 ± 0.02	16	0.9945	0.010	2543	P2
	1.5 M TMAO		0.05 ± 0.02	1.32 ± 0.07	10	0.9758	0.034	323	2, 3, 4, 5, P1, P3. P4
	Sorbitol		0.03 ± 0.01	1.04 ± 0.05	13	0.9754	0.022	396	P1, P2, P3, P5
	Sucrose		0.06 ± 0.01	1.01 ± 0.06	12	0.9682	0.020	305	P1, P2, P3, P4, P5
Dex-PEG	TMAO	$NaClO_4$	0.018 ± 0.007	1.29 ± 0.02	14	0.9982	0.019	6794	P1, P3, P4
	1.5 M TMAO		0.07 ± 0.01	1.14 ± 0.04	16	0.9854	0.056	946	P2
	Sorbitol		0.02 ± 0.01	1.26 ± 0.04	15	0.9894	0.046	1211	4, P5
	Sucrose		0.017 ± 0.005	1.01 ± 0.01	16	0.9976	0.020	5881	P2
PEG-Na_2SO_4	TMAO	–	0.06 ± 0.02	1.11 ± 0.03	16	0.9872	0.077	1077	P5
	Sorbitol		0.04 ± 0.02	1.29 ± 0.02	14	0.9964	0.048	3343	1, P2, P5
	Sucrose		0.04 ± 0.02	1.36 ± 0.03	14	0.9938	0.066	1934	1, P2, P5
PEG-Na_2SO_4	TMAO	NaCl	0	1.29 ± 0.03	16	0.9925	0.073	1863	P5
	Sorbitol		−0.03 ± 0.01	1.04 ± 0.02	16	0.9966	0.040	4083	1
	Sucrose		0	1.26 ± 0.03	14	0.9934	0.069	1796	1, P2, P5
PEG-Na_2SO_4	TMAO	$NaClO_4$	0.06 ± 0.02	0.99 ± 0.02	17	0.9959	0.052	3626	–
	Sorbitol		0	0.96 ± 0.02	11	0.9929	0.067	1575	1, P5, 1a-4a
	Sucrose		0	1.00 ± 0.03	17	0.9858	0.098	1044	–

[a] Concentrations of each osmolyte – 0.5 M except in the indicated case of 1.5 M TMAO.
[b] Abbreviations: 1 – 2-phenylethanol, 2 - benzyl alcohol, 3 – p-nitrophenyl-glucopyranoside, 4 – phenol, 5 – vanillin, 1a – DNP-Ala Na, 2a – DNP-NVal Na, 3a – DNP-NLeu Na, 4a – DNP-AO Na, P1 – α-chymotrypsin, P2 - α-chymotrypsinogen A, P3 – concanavalin A, P4 – lipase, P5 –lysozyme.

the solute i with the solvent media in the coexisting phases; the subscript *s* designates the solute.

All the data in the set of ATPSs with the same ionic composition were used to determine solute-specific coefficients S_s, A_s, B_s, and C_s in Eq. (4) for each compound by the multiple linear regression analysis. The *p*-value was used to estimate the significance for each solute-specific coefficient in Eq. (4) for a given compound taking into account the small number of ATPSs with the same ionic composition utilized. If all four coefficients (S_s, A_s, B_s, and C_s) proved statistically significant ($p < 0.1$), then the correlation was accepted. If one or more values reveal a *p*-value > 0.1, then the equations contained different combinations of coefficients were examined. The equation with a set of coefficients providing *p*-values for all parameters below 0.1 was accepted. The solute-specific coefficients determined for each compound from the data obtained in the chosen ATPS set are presented in Table C1.

Taking Eq. (4) into account, it seems reasonable that the changes in the differences between the solvent properties of the phases brought by the presence of a given osmolyte may affect various compounds in a different manner. That is the likely reason why some compounds do not fit the Collander relationship under discussion (see in Table 6). As an example, the reason for α-chymotrypsinogen (P2) not to fit the linear relationship in the presence of 0.5 M TMAO in the salt additive free Dex-PEG ATPS seems to be the negative contribution of A_s solute-specific coefficient differing this protein from the other proteins examined (see in Table C1). The same protein does not fit the Collander relationship in Dex-PEG-0.215 M NaCl ATPS containing 0.5 M TMAO likely due to the relatively high negative A_s value together with the high negative value of the C_s solute-specific coefficient.

If a given salt additive does not interact directly with certain compounds, the partition coefficients of these compounds in the systems with different ionic composition may fit the linear Collander relationship [21,27]. Typical relationships for partition coefficients in Dex-PEG and Dex-PEG-0.215 M NaCl ATPSs and in PEG-Na_2SO_4 and PEG-Na_2SO_4-0.215 M NaCl ATPSs are illustrated graphically in Fig. 3a and b. The data presented in Fig. 3a and b show that proteins and Na-salts of DNP-amino acids do not fit the linear relationship shown for organic compounds. Vanillin is the only ionized compound among the other organic compounds examined, and therefore the effect of NaCl additive on its partition behavior might be expected. In order to explain why methyl

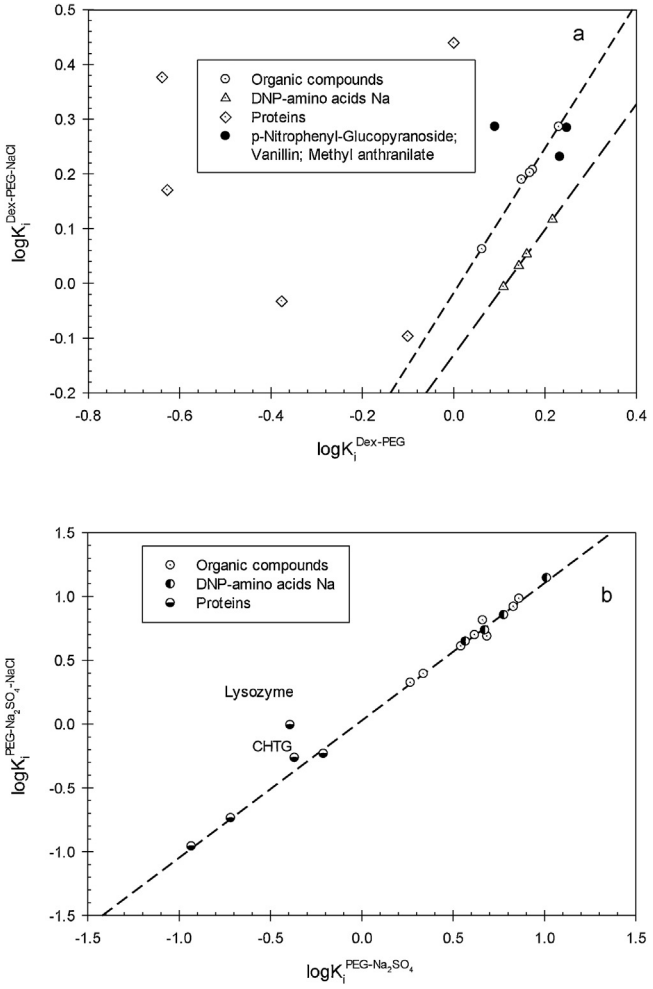

Fig. 3. **(a)** Logarithms of partition coefficients for compounds (including proteins) in aqueous Dex-PEG-0.215 M NaCl two-phase system versus logarithms of partition coefficients for the same compounds in the Dex-PEG system without NaCl additive. **(b)** Logarithms of partition coefficients for compounds (including proteins) in aqueous PEG-Na_2SO_4-0.215 M NaCl two-phase system versus logarithms of partition coefficients for the same compounds in the PEG-Na_2SO_4 system without NaCl additive.

anthranilate and p-nitrophenyl-α-D-glucopyranoside do not fit the relationship in question, it is necessary to explore the effect of NaCl additive on the solute-specific coefficients in Eq. (4) for these compounds relative to the other organic compounds examined. The data for all four solute-specific coefficients in the presence of 0.215 M NaCl for all organic compounds examine (except Na-salts of DNP-amino acids) are plotted against the data for same compounds in the absence of the NaCl additive in Figure C1a-d in SI C. It can be seen that in the presence of 0.215 M NaCl additive, the solute-water interactions of p-nitrophenyl-glucopyranoside deviates from the trends observed for other compounds in regard to dipole-dipole interactions (coefficient S_s), hydrogen bonding (coefficient A_s), and electrostatic interactions (coefficient C_s), whereas methyl anthranilate deviates from the trends observed in regard to hydrogen bonding (coefficients A_s and B_s). Other compounds, such as caffeine and coumarin, show changes in only one of the solute-specific coefficients only, B_s and A_s, correspondingly.

The effect of NaCl additive on partition behavior of compounds in PEG-Na_2SO_4 ATPSs causes the deviation from linear relationship only for two proteins, lysozyme (HEL) and α-chymotrypsinogen (CHTG), likely due to the direct interactions of these proteins with NaCl. Based on the obtained data, we explored the effects of salt additives on linear relationships for only organic compounds in Dex-PEG ATPSs and on those for all compounds in PEG-Na_2SO_4 ATPSs. The coefficients of Eq. (3) for the observed relationships are listed in Table 7. As aforementioned, the presence of salt additives affect partition behavior of solutes in the solute-specific manner for more compounds in the Dex-PEG ATPSs than in the PEG-Na_2SO_4 ATPSs likely because of the absence of excessive Na_2SO_4 concentration in the former ATPSs. It is important to note that the effects of salt additives appear to depend on the particular osmolyte present in a given ATPS. As an example, in Dex-PEG ATPSs, the effect of NaCl additive is observed on three compounds (p-nitrophenyl-α-D-glucopyranoside, vanillin, and methyl anthranilate) in the absence of any osmolyte, on p-nitrophenyl-α-D-glucopyranoside and vanillin in the presence of 0.5 M TMAO, and on 2-phenylethanol and p-nitrophenyl-α-D-glucopyranoside in the presence of 0.5 M sorbitol. Many other examples of the same effects of osmolytes may be found in Table 7. These osmolyte-specific effects are likely due to the different degrees of influence of various osmolytes on different solvent features of the phases in ATPSs.

It has been shown previously [27,28] that the logarithms of partition coefficients of proteins and organic compounds in three ATPSs with different ionic compositions may be linearly interrelated. Analysis of the partition coefficients presented in Tables 1–3 and Tables A1-A3 shows that the relationship between the logarithms of partition coefficients of compounds in Dex-PEG ATPSs illustrated in Fig. 4a may be described as:

$$\log K_i^{\text{Dex-PEG-NaCl}} = 0.22_{0.02}\log K_i^{\text{Dex-PEG-NaClO4}} + 0.77_{0.02}\log K_i^{\text{Dex-PEG}} \quad (5a)$$

$N = 14$; $r^2 = 0.9952$; $SD = 0.005$; $F = 1240$

where K_i is the partition coefficient of the i^{th} compound, superscripts denote the ATPS composition, N is the number of compounds fitting the relationship, r^2 is the correlation coefficient, SD is the standard deviation, and F is the ratio of variance. Three compounds (concanavalin A, lysozyme, and p-nitrophenyl-α-D-glucopyranoside) do not fit the relationship.

Similar relationship is observed for the logarithms of partition coefficients of compounds in PEG-Na_2SO_4 ATPSs. This relationship shown in Fig. 4b may be described as:

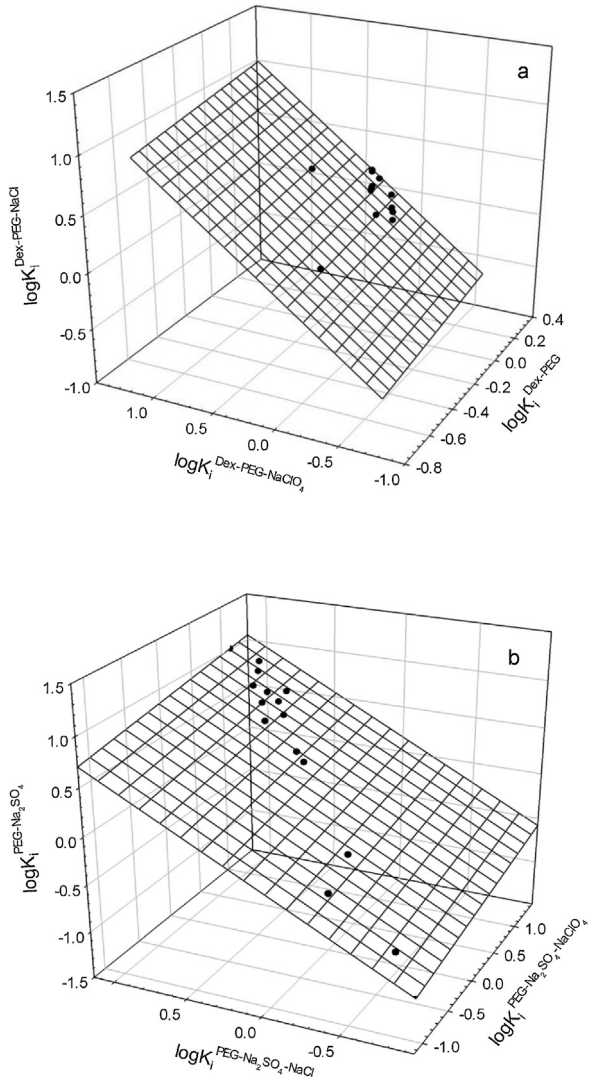

Fig. 4. **(a)** Interrelationship between logarithms of partition coefficients for organic compounds in aqueous Dex-PEG-0.215 M NaCl two-phase system, logarithms of partition coefficients for the same compounds in aqueous Dex-PEG-0.215 M $NaClO_4$ two-phase system, and logarithms of partition coefficients for the same compounds in aqueous Dex-PEG two-phase system without salt additive. **(b)** Interrelationship between logarithms of partition coefficients for organic compounds and proteins in aqueous PEG-Na_2SO_4 two-phase system, logarithms of partition coefficients for the same compounds in aqueous PEG-Na_2SO_4-0.215 M NaCl two-phase system and in PEG-Na_2SO_4-0.215 M $NaClO_4$ two-phase system.

$$\log K_i^{\text{PEG-Na2SO4}} = -0.03_{0.01} + 0.13_{0.06}\log K_i^{\text{PEG-Na2SO4-NaClO4}} + 0.78_{0.07}\log K_i^{\text{PEG-Na2SO4-NaCl}} \quad (5b)$$

$N = 16$; $r^2 = 0.9971$; $SD = 0.034$; $F = 2238$

where all parameters are as defined above. One compound (lysozyme) does not fit the relationship.

Analysis of the solute-specific coefficients for all organic compounds (including Na-salts of DNP-amino acids) examined in the Na_2SO_4-free aqueous media (i.e., in Dex-PEG ATPSs) shows the linear interrelationship illustrated graphically in Fig. 5 and described as:

$$C_s^i = -2.3_{0.3} - 0.45_{0.07}S_s^i - 1.26_{0.06}B_s^i \quad (6a)$$

$N = 48$; $r^2 = 0.9444$; $SD = 1.65$; $F = 382$

Table 7
Coefficients for Eq. (3) ($\log K_2 = b + a \log K_1$) for ATPS with different ionic composition (concentrations of NaCl and $NaClO_4$ additives – 0.215 M)[a].

ATPS-1	ATPS-2	b	a	N	r^2	SD	F	Outlier[b]	Osmolyte
Dex-PEG	Dex-PEG-NaCl	0	1.32 ± 0.05	5	0.9949	0.007	590	3, 5, 6	
Dex-PEG	Dex-PEG-NaCl	0.03 ± 0.01	0.98 ± 0.06	6	0.9848	0.010	259	3, 5	TMAO
Dex-PEG	Dex-PEG-NaCl	0	1.08 ± 0.05	6	0.9925	0.009	528	1, 3	Sorbitol
Dex-PEG	Dex-PEG-NaCl	0	1.03 ± 0.06	7	0.9832	0.013	293	3	Sucrose
Dex-PEG	Dex-PEG-$NaClO_4$	0.07 ± 0.01	0.92 ± 0.05	5	0.9926	0.004	405	3, 4, 7	
Dex-PEG	Dex-PEG-$NaClO_4$	0	1.47 ± 0.07	6	0.9914	0.013	461	1, 5	TMAO
Dex-PEG	Dex-PEG-$NaClO_4$	0	1.25 ± 0.06	6	0.9922	0.010	508	5, 4	Sorbitol
Dex-PEG	Dex-PEG-$NaClO_4$	0.03 ± 0.02	0.88 ± 0.08	7	0.9606	0.019	122	5	Sucrose
Dex-PEG	PEG-Na_2SO_4	0.06 ± 0.03	3.3 ± 0.2	7	0.9880	0.027	413	4	
Dex-PEG	PEG-Na_2SO_4	0.20 ± 0.04	2.7 ± 0.2	7	0.9676	0.041	150	4	TMAO
Dex-PEG	PEG-Na_2SO_4	0.16 ± 0.03	3.2 ± 0.1	7	0.9914	0.027	579	4	Sorbitol
Dex-PEG	PEG-Na_2SO_4	0.22 ± 0.03	2.9 ± 0.1	7	0.9925	0.025	658	4	Sucrose
PEG-Na_2SO_4	PEG-Na_2SO_4-NaCl	0.03 ± 0.01	1.08 ± 0.02	15	0.9972	0.034	4609	P2, P5	
PEG-Na_2SO_4	PEG-Na_2SO_4-NaCl	0	1.34 ± 0.03	8	0.9962	0.061	2101	P5, 7, 8;aa	TMAO
PEG-Na_2SO_4	PEG-Na_2SO_4-NaCl	−0.03 ± 0.01	0.87 ± 0.01	14	0.9968	0.039	3796	P2, P5, 8	Sorbitol
PEG-Na_2SO_4	PEG-Na_2SO_4-NaCl	0	0.97 ± 0.03	13	0.9926	0.055	1484	P1,P2,P5,7	Sucrose
PEG-Na_2SO_4	PEG-Na_2SO_4-$NaClO_4$	0.09 ± 0.01	1.34 ± 0.01	8	0.9993	0.026	9106	P2,P4,P5, 3, 5,aa	
PEG-Na_2SO_4	PEG-Na_2SO_4-$NaClO_4$	0.12 ± 0.01	1.25 ± 0.02	8	0.9987	0.038	4467	P2,P4,P5, 3,7, aa	TMAO
PEG-Na_2SO_4	PEG-Na_2SO_4-$NaClO_4$	0.08 ± 0.02	1.14 ± 0.03	11	0.9951	0.071	1835	P4, P5, aa	Sorbitol
PEG-Na_2SO_4	PEG-Na_2SO_4-$NaClO_4$	0	0.96 ± 0.01	9	0.9985	0.038	4766	P5, 1, 7, 8, aa	Sucrose
Dex-PEG-NaCl	PEG-Na_2SO_4-NaCl	0.20 ± 0.04	2.2 ± 0.2	5	0.9723	0.035	105	4, 5, 6	
Dex-PEG-NaCl	PEG-Na_2SO_4-NaCl	0.07 ± 0.03	3.9 ± 0.1	5	0.9971	0.018	1036	3, 4, 5	TMAO
Dex-PEG-NaCl	PEG-Na_2SO_4-NaCl	0.25 ± 0.02	1.75 ± 0.07	5	0.9958	0.012	713	3, 5, 6	Sorbitol
Dex-PEG-NaCl	PEG-Na_2SO_4-NaCl	0.25 ± 0.08	2.4 ± 0.3	6	0.9442	0.066	68	3, 5	Sucrose
Dex-PEG-$NaClO_4$	PEG- Na_2SO_4-$NaClO_4$	0	3.6 ± 0.3	5	0.9881	0.047	250	2, 4, 5	
Dex-PEG-$NaClO_4$	PEG- Na_2SO_4-$NaClO_4$	0.19 ± 0.07	2.8 ± 0.3	5	0.9750	0.066	117	2, 4, 5	TMAO
Dex-PEG-$NaClO_4$	PEG- Na_2SO_4-$NaClO_4$	0.16 ± 0.07	3.3 ± 0.3	5	0.9825	0.059	169	1, 4, 5	Sorbitol
Dex-PEG-$NaClO_4$	PEG- Na_2SO_4-$NaClO_4$	0.30 ± 0.03	2.4 ± 0.1	5	0.9892	0.029	275	1, 5, 8	Sucrose

[a] For the Dex-PEG ATPSs only small organic compounds (excluding DNP-amino acids Na salts) were considered, for PEG-Na_2SO_4 ATPSs all compounds (including proteins) were considered (for discussion see text).
[b] Abbreviations: 1 – 2-phenylethanol, 2 - benzyl alcohol, 3 – p-nitrophenyl-glucopyranoside, 4 – phenol, 5 – vanillin, 6 – Methyl anthranilate; 7 - Caffeine; 8 –Coumarin; aa – Na salts of DNP-amino acids (DNP-Ala Na, DNP-NVal Na, DNP-NLeu Na, DNP-amino-*n*-octanoic acid Na), P1 – α-chymotrypsin, P2 - α-chymotrypsinogen A, P3 – concanavalin A, P4 – lipase, P5 –lysozyme.

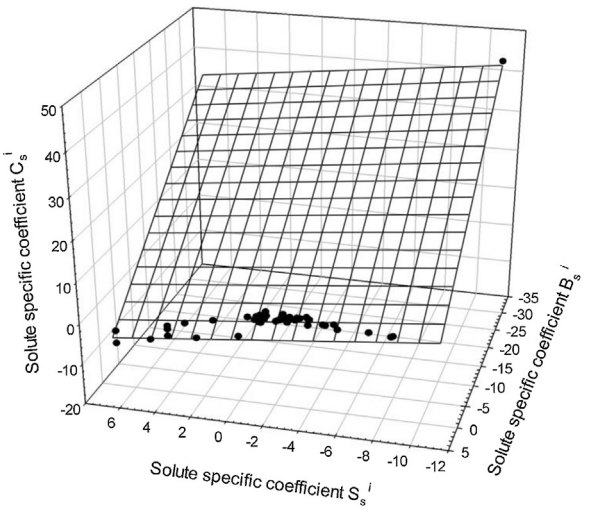

Fig. 5. Interrelationship between solute-specific coefficients C_s^i, B_s^i, and S_s^i for organic compounds calculated using Eq. (4) from the partition coefficients of the compounds in aqueous Dex-PEG two-phase systems with different salt additives (see data in Table C1).

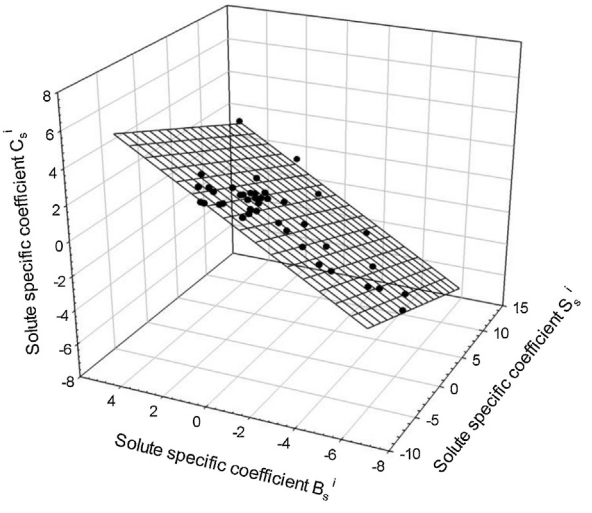

Fig. 6. Interrelationship between solute-specific coefficients C_s^i, B_s^i, and S_s^i for organic compounds and proteins calculated using Eq. (4) from the partition coefficients of the compounds in aqueous PEG-Na_2SO_4 two-phase systems with different salt additives (see data in Table C1).

where all parameters are as defined above. It should be emphasized that the solute-specific coefficients for proteins do not fit the relationship.

For solute-specific coefficients for the same compounds and certain proteins in aqueous media containing ~0.45 M Na_2SO_4 (i.e. in PEG-Na_2SO_4 ATPSs) is observed the similar linear interrelationship shown graphically in Fig. 6 and described as:

$$C_s^i = 0.76_{0.08} - 0.20_{0.02} S_s^i + 0.73_{0.03} B_s^i \quad (6b)$$

$$N = 41;\ r^2 = 0.9413;\ SD = 0.49;\ F = 305$$

where all parameters are as defined above. It should be noted that there is 10 outliers (Na-salts of DNP-Nval, DNP-Nleu, and DNP-AO, p-nitrophenyl-α-D-glucopyranoside, α-chymotrypsin, α-chymotrypsinogen, concanavalin A, and lipase (all in the presence of $NaClO_4$), and chymotrypsin and vanillin in the presence of NaCl. It should be noted that the direct interactions of salt additives with

Table 8
The overall range of partition coefficients for examined compounds in Dex-PEG-0.01 M K/NaPB and PEG-Na_2SO_4-0.01 M NaPB ATPSs with salt and osmolyte additives* (K/NaPB – sodium/potassium phosphate buffer, pH 7.4; NaPB – sodium phosphate buffer, pH 6.8).

	Dex-PEG ATPSs				PEG-Na_2SO_4 ATPSs			
Compound	K_{min}	Additives	K_{max}	Additives	K_{min}	Additives	K_{max}	Additives
DNP-Alanine Na	0.96	$NaClO_4$	1.44	w/o salt, trehalose	3.49	$NaClO_4$, sucrose	6.88	w/o salt, trehalose
DNP-Norvaline Na	1.03	$NaClO_4$	1.62	w/o salt, trehalose	4.73	w/o salt	10.7	w/o salt, trehalose
DNP-Norleucine Na	1.09	$NaClO_4$	1.77	w/o salt, trehalose	6.00	w/o salt	15.0	w/o salt, trehalose
DNP-Octanoic acid Na	1.25	$NaClO_4$	2.15	w/o salt, trehalose	10.3	w/o salt	32.1	w/o salt, trehalose
Benzyl alcohol	1.41	w/o salt	1.86	$NaClO_4$, TMAO[c]	3.50	w/o salt	7.99	$NaClO_4$, sorbitol
Caffeine	1.15	w/o salt[a]	1.43	$NaClO_4$, TMAO[c]	1.85	w/o salt	3.50	$NaClO_4$, sorbitol
Coumarin	1.49	w/o salt	2.45	$NaClO_4$, TMAO[c]	4.55	w/o salt[b]	15.2	$NaClO_4$, sorbitol
Glucoside	1.22	NaCl	1.52	NaCl, TMAO[c]	2.18	w/o salt	4.15	$NaClO_4$, sorbitol
Methyl anthranilate	1.77	w/o salt	3.10	$NaClO_4$, TMAO[c]	7.28	w/o salt	29.5	$NaClO_4$, sorbitol
2-Phenylethanol	1.47	w/o salt	2.03	$NaClO_4$, TMAO[c]	3.80	w/o salt[b]	9.67	$NaClO_4$, sucrose
Phenol	1.23	$NaClO_4$, sorbitol	2.40	$NaClO_4$, sucrose	4.60	w/o salt	12.4	$NaClO_4$, sucrose
Vanillin	1.59	$NaClO_4$, TMAO	2.11	w/o salt, trehalose	5.79	w/o salt[b]	19.1	$NaClO_4$, sorbitol
α-Chymotrypsin	0.41	w/o salt, trehalose	5.18	NaCl, sucrose	0.027	w/o salt, trehalose	0.117	w/o salt
α-Chymotrypsinogen A	1.00	w/o salt	7.45	$NaClO_4$, TMAO[c]	0.120	w/o salt, trehalose	0.60	NaCl,TMAO
Concanavalin A	0.21	$NaClO_4$	1.54	NaCl, TMAO	0.116	w/o salt, trehalose	0.195	w/o salt[b]
Lipase	0.67	$NaClO_4$, TMAO[c]	0.85	$NaClO_4$, sucrose	0.47	$NaClO_4$, sucrose	0.64	w/o salt[b]
Lysozyme	0.23	w/o salt	49.1	$NaClO_4$, sucrose	0.045	w/o salt, trehalose	43.3	$NaClO_4$, sorbitol

* Salt additives concentrations - 0.215 M; osmolyte additives concentrations – 0.5 M except for TMAO where indicated.
[a] Same K-value in Dex-PEG with NaCl additive.
[b] PEG-10000-Na_2SO_4 ATPS.
[c] TMAO at concentration 1.5 M.

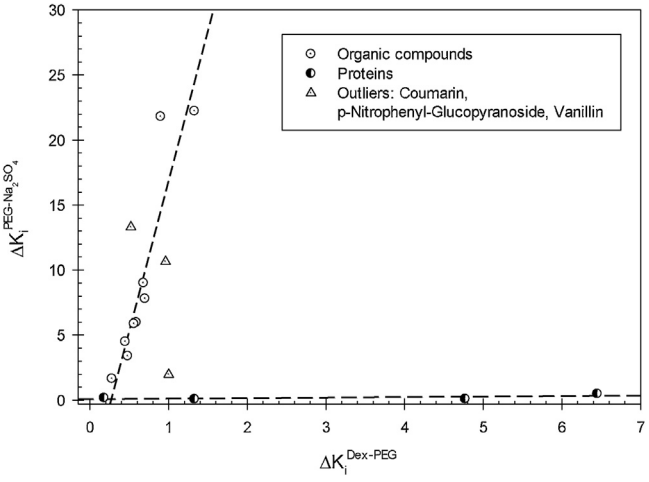

Fig. 7. The ranges of K-values variability for organic compounds and proteins in aqueous PEG-Na_2SO_4 two-phase systems with different salt additives, $\Delta K_i^{PEG-Na2SO4}$, versus ranges of K-values variability for the same compounds and proteins in aqueous Dex-PEG two-phase systems with different salt additives, $\Delta K_i^{Dex-PEG}$.

these compounds may be the reason for their solute-specific coefficients not fitting the relationship.

The number of compounds with determined solute-specific coefficients so far prevents one from drawing any general conclusion, and this issue is beyond the scope of the present study.

Finally, we examined the range of the partition coefficients values determined in the two types of ATPSs (Dex-PEG and PEG-Na_2SO_4). The highest (*Kmax*) and lowest (*Kmin*) *K*-values for each compound determined in the Dex-PEG ATPSs and in the PEG-Na_2SO_4 ATPSs with all different salt and osmolyte additives are listed in Table 8. The differences between these values (ΔK) represent the range of *K*-vales for a given compound in a given type of ATPSs. These differences observed in PEG-Na_2SO_4 ATPSs are plotted against those observed in Dex-PEG ATPSs for all studied compounds (except lysozyme) in Fig. 7. The data plotted in Fig. 7 show that for small organic compounds, the ranges of *K*-values observed in PEG-Na_2SO_4 ATPSs exceed those determined in Dex-PEG ATPSs quite significantly (the slope of the linear curve in the plot is ca. 23), while for proteins the range of *K*-values in Dex-PEG ATPSs exceed those in PEG-Na_2SO_4 ATPSs for three proteins (α-chymotrypsin, α-chymotrypsinogen, and concanavalin A) or are very similar (for lipase and lysozyme). This observation confirms the suggestion that the ATPSs formed by two polymers are more useful for protein analysis in comparison with the ATPSs formed by a single polymer and a salt. The ATPSs of the latter type have an advantage for protein isolation/separation.

5. Conclusions

Studies of solvent properties of aqueous Dex-PEG and PEG-Na_2SO_4 two-phase systems formed with (or without) 0.215 M NaCl and 0.215 M $NaClO_4$ in the presence (or absence) of 0.5 M sorbitol, sucrose, trehalose, and 0.5 M or 1.5 M trimethylamine N-oxide showed that the solvent properties of the systems vary in a wide range. The differences between the solvent properties of the systems formed by polymer and salt exceed those measured in the systems formed by two polymers. The three most significant solvent properties of the systems are hydrophobic and electrostatic properties and hydrogen bonding donor acidity of the solvent media. Osmolyte additives were found to have quite significant effects on the differences between the electrostatic properties of the phases. Furthermore, analysis of the partition coefficients of 12 organic compounds and five proteins showed that osmolyte additives may affect the partition behavior of compounds in a compound-specific manner. The relative contributions of different types of interactions of a given compound with aqueous media change in the presence of salt and osmolyte additives.

Analysis of the ranges of partition coefficients variability in the systems utilized shows that for small organic compounds, the ranges of *K*-values observed in PEG-Na_2SO_4 ATPSs exceed those determined in Dex-PEG ATPSs quite significantly. On the other hand, for proteins, the range of *K*-values in Dex-PEG ATPSs exceed those in PEG-Na_2SO_4 ATPSs for three proteins, and are very similar for two proteins. Therefore, the ATPS formed by two polymers can be more useful for the protein analysis, while ATPSs formed by a single polymer and a salt have an advantage for protein isolation/separation.

Declarations of interest

None

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.chroma.2018.11.015.

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