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Salt Effect on the Aqueous Two-Phase System PEG 8000−Sodium Sulfate

Luisa A. Ferreira* and José A. Teixeira

IBB−Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-054 Braga, Portugal

The effect of added salt (NaCl or KCl) on the polyethylene glycol 8000 (PEG)−sodium sulfate (Na_2SO_4) aqueous two-phase system (ATPS), containing 0.01 $mol \cdot L^{-1}$ of sodium phosphate buffer (NaPB), pH 7.4, has been investigated at 296.15 K. Phase diagrams determined by the cloud point method, including tie-lines assigned from mass phase ratios according to the lever arm rule, are presented for the different PEG 8000−Na_2SO_4 ATPSs, all containing 0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4, and increasing the concentration of a neutral salt, such as NaCl or KCl, up to 1.0 $mol \cdot L^{-1}$. Experimental binodal results were satisfactorily correlated with an empirical mathematical model, the Merchuk equation. The results indicate that the addition of both salts causes a depression of the binodal relatively to that for the salt-free system. The salting-out ability of the cations follows the Hofmeister series ($Na^+ > K^+$) and can be related to the ions Gibbs free energy of hydration (ΔG_{hyd}).

Introduction

Aqueous two-phase systems (ATPSs) are biphasic systems that appear, above certain conditions (e.g., temperature and composition), in aqueous mixtures of two polymers or of a single polymer and a specific salt.[1,2] The practical application (with special attention to the biotechnological applications) of ATPS has been receiving considerable attention. Aqueous two-phase extraction has emerged as one of the most important downstream processing techniques.[3−8] Therefore, the knowledge of the phase behavior of these systems is necessary for the design of the extraction process. Among others, Zaslavsky has been extensively studying phase separation, and ref 2 summarizes most of the experimental liquid−liquid data and equilibrium diagrams for systems formed by polymers, inorganic salts and water.

In recent studies there is one issue that seems to be one of the most interesting with regard to partitioning of biomolecules, from both theoretical and practical viewpoints: the effects of salt additives on partition. Farruggia et al.[9] studied the influence of chloride salts of Na^+, Rb^+, and Cs^+ at concentrations from (0.15 to 1.2) $mol \cdot L^{-1}$ on the partition of several proteins in an aqueous two-phase system formed by PEG 1500 and potassium phosphate, at pH 7.4. Aires-Barros and co-workers have reported the successful use of ATPSs for the extraction of antibodies from different cell culture supernatants adding NaCl to polymer/salt[4,10,11] and polymer/polymer[12−14] systems.

Zaslavsky[2] studied the effect of several salts on Dex-PEG, Dex-PVP, Dex-PVA, and Dex-Ficoll and stressed the role of “water structure” as an important factor controlling two-phase formation. Experimental evidence clearly dispells what was initially stated by Albertsson,[1] that only when used in very large quantities, inorganic salts additives would affect phase separation in aqueous two nonionic polymers. In polymer-salt ATPS, this issue has never been examined systematically; therefore, there are shortcomings in the phase behavior of these systems that need to be considered and improved.

In the present investigation, the effects of different salt additives (NaCl and KCl), with concentrations from (0 to 1.0) $mol \cdot L^{-1}$, on the ATPS formed by polyethylene glycol-8000 (PEG 8000) and sodium sulfate (Na_2SO_4), containing a fixed concentration of 0.01 $mol \cdot L^{-1}$ sodium phosphate buffer (NaPB), pH 7.4, have been investigated. The phase behavior in the presence of those inorganic salt additives has been studied in order to deliver new information important for understanding the mechanism controlling phase behavior.

Experimental Section

Materials. Polyethylene glycol (PEG) 8000 (batch# 059H0121), with an average molecular weight (M_w) of 8000, was purchased from Sigma-Aldrich. Sodium sulfate (Na_2SO_4; anhydrous, GR for analysis, ACS, Reag. Ph Eur), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$; p.a., Reag. Ph Eur), and disodium hydrogen phosphate (Na_2HPO_4; anhydrous, GR for analysis, ACS, Reag. Ph Eur) were supplied by Merck. Sodium chloride (NaCl), extra pure, was provided by Acros Organics and potassium chloride (KCl) was obtained from Panreac (purity ≥ 0.995). All products were used as received without further purification. Stock solutions of each chemical were prepared in deionized water and the concentrations were obtained after lyophilization (ca. 50 % by weight for PEG 8000 and 17 % by weight for Na_2SO_4). Sodium phosphate buffer (NaPB; 0.2 $mol \cdot L^{-1}$, pH 7.4) was prepared combining the Na_2HPO_4 and $NaH_2PO_4 \cdot 2H_2O$ salts. The pH value was confirmed using a pH meter (CRISON, microTT 2050, Spain). Stock solutions of 5 $mol \cdot L^{-1}$ NaCl and 3 $mol \cdot L^{-1}$ KCl were used. Deionized water was used for all diluting purposes. All weighing was carried out on an analytical balance precise to within ± 0.2 mg (OHAUS, Explorer Pro, model EP214DC, Switzerland).

Methods. Phase diagrams were characterized by the binodal curve and tie-lines. Binodal curves were determined experimentally at 296.15 K by the cloud point method. For the system without addition of neutral salt, two-phase systems with 0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4 and different compositions of PEG 8000 and Na_2SO_4 were prepared and titrated, dropwise, with 0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4, solution until one-phase systems was

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* Corresponding author. Tel: +351253604420. Fax: +351253678986. E-mail: laferreira@deb.uminho.pt.

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formed. For systems with neutral salt, appropriate amounts of polymer, Na_2SO_4 and of NaCl or KCl in 0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4 were added so as to give the required ionic concentration (increasing concentration up to 1.0 $mol \cdot L^{-1}$) and titrated, dropwise, with the NaCl or KCl in 0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4 solution necessary to keep the system ionic composition, until a homogeneous system was obtained. The system temperature was maintained constant and controlled to within ± 0.1 K by immersing the glass tubes and the stock solutions in a thermostatic bath (Grant Instruments, Cambridge, England, type GD100). The tubes were shaken and then placed back into the bath between each addition. With knowledge of the composition of starting PEG and Na_2SO_4 solutions and the added masses, the total system composition was calculated and provided a point on the binodal curve. This was continued until enough points were measured. The binodal curve was fitted by least-squares regression to an empirical relationship developed by Merchuk[15] and shown as eq 1:

$$Y_A = a \exp(bX_A^{0.5} - cX_A^{3}) \tag{1}$$

where Y_A and X_A are the concentrations of polymer and salt, respectively. The constants a, b, and c were obtained by least-squares regression.

For determination of the tie-lines, biphasic systems with different known total compositions of all the components were prepared by weight in 15 mL graduated tubes to obtain a 5 g amount of final system, vigorously vortexed, centrifuged for 30 min at approximately 2800×*g* (HERMLE, Z36HK, Germany) to speed the phase settling, and placed in a thermostatic bath for at least 24 h to equilibrate. The volume of each phase was marked in the tubes and samples from both phases and interface were carefully separated. The interface was discarded and the density of top and bottom phases measured using pycnometers. The volume of each phase was obtained from the mass of water which occupied the same volume as the corresponding phase.[16] Tie-line was determined by application of the lever arm rule to the relationship between the mass phase composition and the overall system composition.[15] The mathematical methods are shown in eqs 2 and 3

$$Y_T = (Y_F/R) - ((1 - R)/R)Y_B \tag{2}$$

$$X_T = (X_F/R) - ((1 - R)/R)X_B \tag{3}$$

where F, T, and B represent the feed, the top phase, and the bottom phase, respectively; Y and X are the concentration of polymer and salt; and R is the following measured ratio:

$$R = (\text{weight of the top phase})/(\text{weight of the mixture}) \tag{4}$$

The ends of the tie-lines were calculated solving this system of four equations (eqs 1 to 4) with four unknowns. The error in the determination of R was on the order of 10^{-3}.

The tie-line lengths (TLL) for the different compositions were calculated by the equivalent expression

$$\text{TLL} = [(Y_T - Y_B)^2 + (X_T - X_B)^2]^{1/2} \tag{5}$$

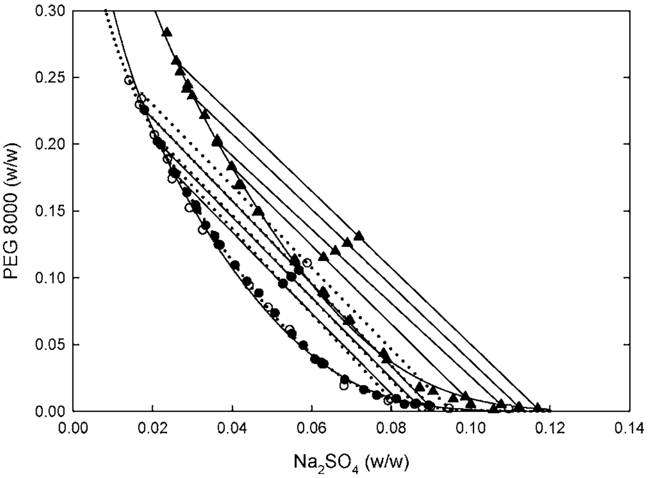

Figure 1. Phase diagram of PEG 8000−Na_2SO_4, −▲−; PEG 8000−Na_2SO_4−1 $mol \cdot L^{-1}$ NaCl,−●−; PEG 8000−Na_2SO_4−1 $mol \cdot L^{-1}$ KCl, ·○·, ATPSs, all containing 0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4. The binodal curve and tie-lines were obtained at 296.15 K.

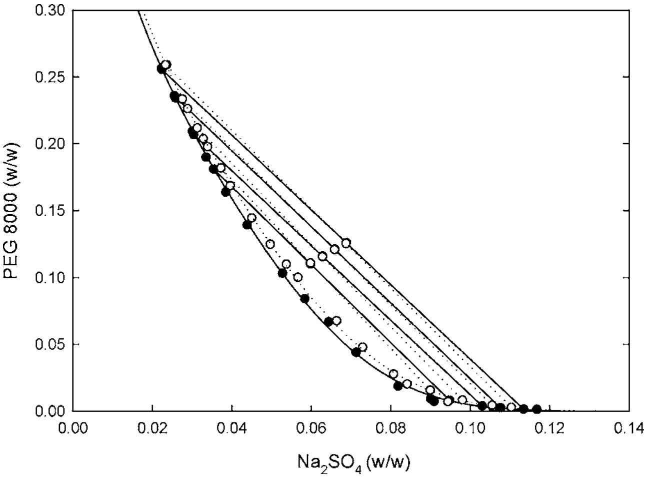

Figure 2. Phase diagram of PEG 8000−Na_2SO_4−0.10 $mol \cdot L^{-1}$ salt−0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4, ATPSs at 296.15 K: −●−, NaCl; ·○·, KCl.

and the slopes of the tie-lines (STL) were calculated according to

$$\text{STL} = \Delta P/\Delta S \tag{6}$$

where ΔP and ΔS are the differences in polymer and salt composition between the equilibrium phases, respectively.

Results and Discussion

The phase separation behavior of PEG 8000−Na_2SO_4, containing 0.01 $mol \cdot L^{-1}$ NaPB, pH 7.4, based ATPS has been studied and the binodal curve is shown in Figure 1. The concentration effect of inorganic salts (NaCl and KCl) on phase separation of this system, containing a fixed concentration of 0.01 $mol \cdot L^{-1}$ sodium phosphate buffer (NaPB), pH 7.4, was then investigated, and the binodal curves for the various systems studied presented in Figures 1 to 5. A rather good fit of the binodal data was given by the Merchuk equation,[15] an empirical relationship between the concentration of polymer and salt. The coefficients of eq 1 and the corresponding coefficients of determination (r^2) are given in Table 1.

Different cations were selected, and the concentration of a neutral salt increased up to 1.0 $mol \cdot L^{-1}$. Binodals for the

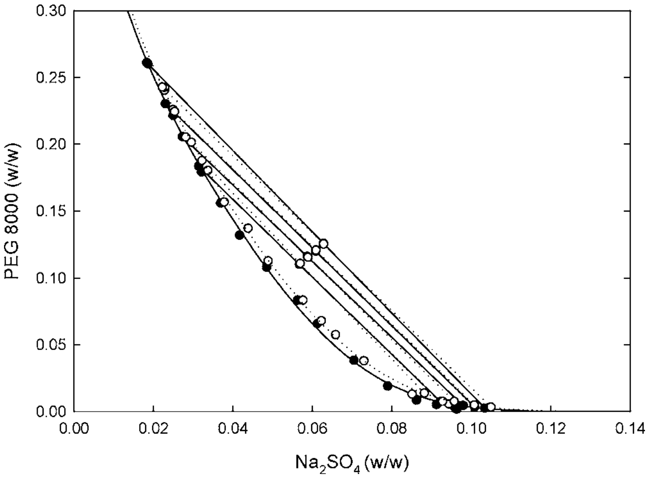

Figure 3. Phase diagram of PEG 8000−Na_2SO_4−0.25 mol·L^{-1} salt−0.01 mol·L^{-1} NaPB, pH 7.4, ATPSs at 296.15 K: —●—, NaCl; ·○·, KCl.

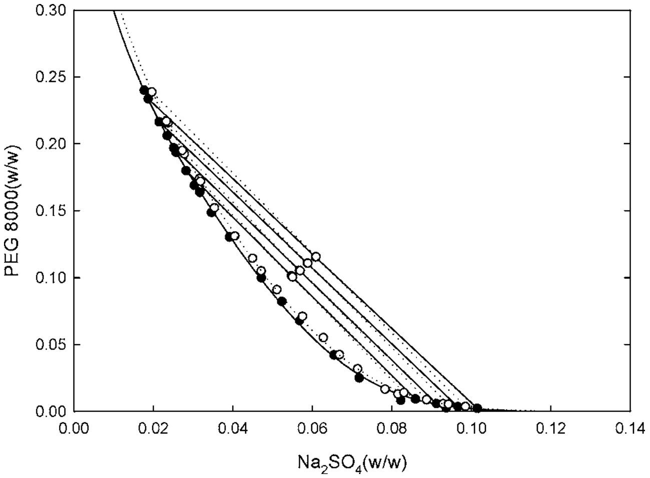

Figure 4. Phase diagram of PEG 8000−Na_2SO_4−0.50 mol·L^{-1} salt−0.01 mol·L^{-1} NaPB, pH 7.4, ATPSs at 296.15 K: —●—, NaCl; ·○·, KCl.

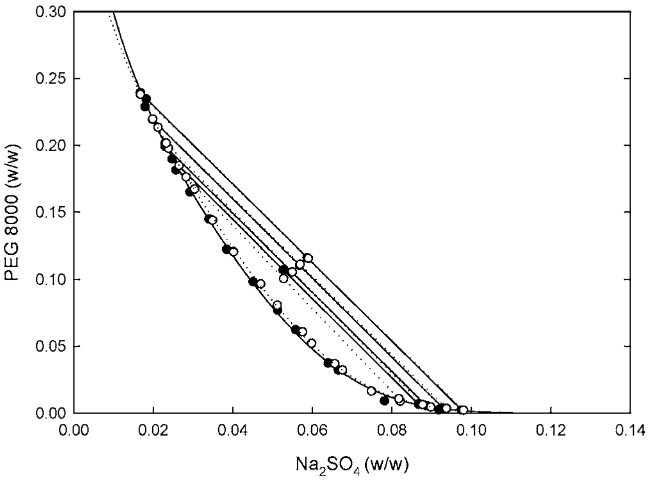

Figure 5. Phase diagram of PEG 8000−Na_2SO_4−0.75 mol·L^{-1} salt−0.01 mol·L^{-1} NaPB, pH 7.4, ATPSs at 296.15 K: —●—, NaCl; ·○·, KCl.

Table 1. Empirical Coefficients of eq 1 and the Coefficient of Determination (r^2) Obtained for the Systems PEG 8000−Na_2SO_4−Water, all Containing 0.01 mol·L^{-1} NaPB, pH 7.4 and Increasing Concentration of a Neutral Salt, NaCl or KCl, up to 1.0 mol·L^{-1} (T = 296.15 K)

[mol·L^{-1}]	a	b	c	r^2
		PEG 8000−Na_2SO_4−Water		
0.00	0.779 ± 0.052	−6.507 ± 0.403	2346 ± 146	0.9984
		PEG 8000−Na_2SO_4−NaCl−Water		
0.10	0.688 ± 0.044	−6.379 ± 0.401	2946 ± 173	0.9993
0.25	0.638 ± 0.042	−6.418 ± 0.428	3240 ± 224	0.9988
0.50	0.562 ± 0.027	−6.254 ± 0.319	3638 ± 191	0.9993
0.75	0.598 ± 0.036	−6.883 ± 0.419	3873 ± 277	0.9989
1.00	0.670 ± 0.069	−7.941 ± 0.699	3802 ± 469	0.9973
		PEG 8000−Na_2SO_4−KCl−Water		
0.10	0.691 ± 0.040	−6.180 ± 0.344	2674 ± 134	0.9993
0.25	0.649 ± 0.042	−6.362 ± 0.416	2891 ± 188	0.9991
0.50	0.603 ± 0.041	−6.402 ± 0.411	3382 ± 178	0.9992
0.75	0.522 ± 0.024	−5.898 ± 0.299	4053 ± 150	0.9996
1.00	0.534 ± 0.025	−6.345 ± 0.295	4469 ± 147	0.9995

Table 2. Phase Compositions for PEG 8000 (1)−Na_2SO_4 (2)−Water (3), 0.01 mol·L^{-1} NaPB, pH 7.4, ATPS[a]

	tie 1	tie 2	tie 3	tie 4
100 w_1 (feed)	11.53	11.97	12.56	13.06
100 w_2 (feed)	6.30	6.60	6.90	7.19
100 w_1 (top)	18.30	20.19	23.59	26.20
100 w_2 (top)	3.99	3.62	3.00	2.60
100 w_1 (bottom)	1.06	0.49	0.32	0.20
100 w_2 (bottom)	9.87	10.77	11.22	11.69
R	0.607	0.583	0.526	0.495
TLL	18.22	20.96	24.68	27.54
STL	−2.93	−2.76	−2.83	−2.86

[a] Composition of feed, top and bottom phases of the tie-line determined in 100w from eqs 1 to 3. Ratio (R), tie-line length (TLL), and slope (STL) calculated from eq 4, 5, and 6, respectively.

systems with higher concentration of added neutral salt and those without any salt added are shown in Figure 1 to illustrate the salt effect on the phase diagram. It is possible to observe that both cations depress the binodal line, meaning that less polymer and Na_2SO_4 is needed to form ATPS. For the higher concentration studied, the binodals of the phase diagrams are so close to each other that only one line could be drawn (Figure 1). For the other concentrations studied, NaCl proved to be the most effective in ATPS formation, providing the greatest heterogeneous region in the phase diagram (Figures 2 to 5). These results follow a similar order to the ones reported for the effectiveness of the cations on polymer-salt ATPS without any neutral salt added.[17,18] A thermodynamic approach using the Gibbs free energy of hydration (ΔG_{hyd}) has been used to quantify the Hoffmeister series and order the salts from kosmotropic to chaotropic. Comparing the cation ΔG_{hyd} (−89.6 kcal·mol^{-1} for Na^+ and −72.7 kcal·mol^{-1} for K^+)[19] it is possible to confirm that (Na^+) is the most kosmotropic ion. Kosmotropic ions have large negative ΔG_{hyd} due to the resulting structured water lattice around the ion, and therefore the salting-out effect of Na^+ is greater than K^+.

Additionally, the equilibrium compositions, tie-line data, for the investigated systems were calculated from eqs 1 to 4 and are shown in Figures 1 to 5. Numerical data for these tie-lines, respectively TLL and STL, are given in Tables 2 and 3. As may be seen from data, for all of the systems where KCl was added, the STL obtained is more negative. If for the system with 0.75 mol·L^{-1} NaCl the slope of the tie-lines is approximately constant, the STLs obtained for the other systems cannot be considered constant and, in general, the absolute value decreases with the increase of the TLL. As observed for the binodal curves for higher concentration of neutral salt (1.0 mol·L^{-1} NaCl or KCl) the tie-lines of both systems overlap and the STL become very similar.

Conclusions

New experimental data of liquid−liquid equilibrium for ATPSs containing PEG 8000, Na_2SO_4, 0.01 mol·L^{-1} NaPB,

Table 3. Phase Compositions for PEG 8000 (1)−Na_2SO_4 (2)−Water (3) ATPS, all Containing 0.01 mol·L^{-1} NaPB, pH 7.4, and Increasing Concentration of a Neutral Salt, NaCl or KCl, up to 1 mol·L^{-1}, at 296.15 K[a]

	PEG 8000 (1)−Na_2SO_4 (2)−Water (3)							
	0.10 mol·L^{-1} NaCl				0.10 mol·L^{-1} KCl			
	tie 1	tie 2	tie 3	tie 4	tie 1	tie 2	tie 3	tie 4
100 w_1 (feed)	10.97	11.55	12.06	12.54	11.04	11.52	12.04	12.48
100 w_2 (feed)	6.00	6.30	6.61	6.90	6.00	6.30	6.60	6.89
100 w_1 (top)	18.05	20.61	23.53	25.50	18.13	21.13	23.30	25.85
100 w_2 (top)	3.56	3.06	2.57	2.26	3.75	3.15	2.77	2.35
100 w_1 (bottom)	0.77	0.35	0.21	0.11	1.34	0.71	0.44	0.29
100 w_2 (bottom)	9.50	10.32	10.78	11.36	8.83	9.59	10.09	10.51
R	0.590	0.553	0.508	0.490	0.558	0.557	0.528	0.509
TLL	18.28	21.52	24.73	26.97	17.55	21.41	24.00	26.84
STL	−2.91	−2.79	−2.84	−2.79	−3.30	−3.17	−3.12	−3.13
	0.25 mol·L^{-1} NaCl				0.25 mol·L^{-1} KCl			
	tie 1	tie 2	tie 3	tie 4	tie 1	tie 2	tie 3	tie 4
100 w_1 (feed)	10.97	11.56	11.95	12.53	11.03	11.49	12.04	12.48
100 w_2 (feed)	5.69	5.89	6.10	6.30	5.71	5.91	6.11	6.30
100 w_1 (top)	18.30	20.50	22.98	26.05	18.71	20.08	22.40	24.22
100 w_2 (top)	3.16	2.75	2.32	1.85	3.24	2.97	2.55	2.24
100 w_1 (bottom)	0.70	0.40	0.30	0.22	1.34	0.71	0.44	0.29
100 w_2 (bottom)	9.24	9.81	10.10	10.36	8.83	9.59	10.09	10.51
R	0.584	0.555	0.514	0.477	0.558	0.557	0.528	0.509
TLL	18.61	21.30	23.98	27.20	18.26	20.47	23.21	25.32
STL	−2.90	−2.85	−2.92	−3.03	−3.11	−2.93	−2.91	−2.89
	0.50 mol·L^{-1} NaCl				0.50 mol·L^{-1} KCl			
	tie 1	tie 2	tie 3	tie 4	tie 1	tie 2	tie 3	tie 4
100 w_1 (feed)	10.09	10.50	11.03	11.49	10.01	10.47	11.03	11.52
100 w_2 (feed)	5.49	5.69	5.89	6.10	5.52	5.71	5.90	6.11
100 w_1 (top)	17.93	19.63	21.59	23.31	17.14	19.45	21.63	23.83
100 w_2 (top)	2.84	2.53	2.16	1.86	3.20	2.74	2.35	1.98
100 w_1 (bottom)	0.88	0.53	0.30	0.17	1.35	0.83	0.49	0.31
100 w_2 (bottom)	8.61	9.14	9.68	10.17	8.32	8.89	9.44	9.87
R	0.540	0.522	0.504	0.489	0.548	0.518	0.499	0.477
TLL	18.01	20.22	22.58	24.60	16.59	19.61	22.30	24.80
STL	−2.95	−2.89	−2.83	−2.78	−3.08	−3.03	−2.98	−2.98
	0.75 mol·L^{-1} NaCl				0.75 mol·L^{-1} KCl			
	tie 1	tie 2	tie 3	tie 4	tie 1	tie 2	tie 3	tie 4
100 w_1 (feed)	10.65	10.50	10.99	11.57	10.00	10.50	11.07	11.51
100 w_2 (feed)	5.29	5.50	5.70	5.89	5.30	5.51	5.71	5.92
100 w_1 (top)	18.92	19.86	21.88	23.88	17.58	20.10	21.92	23.75
100 w_2 (top)	2.49	2.32	2.00	1.69	2.85	2.34	2.01	1.70
100 w_1 (bottom)	0.62	0.50	0.32	0.19	1.04	0.58	0.30	0.18
100 w_2 (bottom)	8.69	8.89	9.31	9.77	8.19	8.79	9.39	9.82
R	0.548	0.516	0.495	0.480	0.542	0.508	0.498	0.481
TLL	19.32	20.44	22.76	25.03	17.38	20.57	22.85	24.94
STL	−2.95	−2.95	−2.95	−2.93	−3.09	−3.03	−2.93	−2.90
	1.00 mol·L^{-1} NaCl				1.00 mol·L^{-1} KCl			
	tie 1	tie 2	tie 3		tie 1	tie 2	tie 3	tie 4
100 w_1 (feed)	9.50	10.03	10.52		9.52	10.00	10.51	11.05
100 w_2 (feed)	5.29	5.50	5.69		5.30	5.52	5.70	5.91
100 w_1 (top)	17.85	20.14	22.50		18.82	20.64	22.90	24.72
100 w_2 (top)	2.53	2.14	1.81		2.39	2.06	1.69	1.42
100 w_1 (bottom)	0.89	0.55	0.40		0.90	0.48	0.30	0.17
100 w_2 (bottom)	8.15	8.65	8.97		8.01	8.61	9.01	9.48
R	0.508	0.484	0.458		0.481	0.472	0.452	0.443
TLL	17.87	20.64	23.23		18.78	21.20	23.76	25.84
STL	−3.02	−3.01	−3.09		−3.19	−3.08	−3.09	−3.05

[a] Composition of feed, top and bottom phases of the tie-line determined in 100*w* from eqs 1 to 3. Ratio (*R*), tie-line length (TLL), and slope (STL) calculated from eq 4, 5 and 6, respectively.

pH 7.4 and increasing concentration of a neutral salt (such as NaCl or KCl), up to 1.0 mol·L^{-1} have been determined at 296.15 K. Binodal data, determined by the cloud point method was satisfactorily correlated with the Merchuk equation. Furthermore, equilibrium compositions (tie-lines) were determined by application of the lever arm rule to the relationship between the mass phase composition and the overall system composition. Inorganic salts have a significant effect on the properties of ATPS. It was shown that increasing the concentration of both neutral salts, the enlargement of the two-phase area of ATPS can be observed being the binodal shift toward the origin. The salting-out ability of the cations follows the ordering $Na^+ > K^+$ and can be related to the ions Gibbs free energy of hydration (ΔG_{hyd}). With the increase of added salt to the PEG-Na_2SO_4

ATPS containing 0.01 mol·L^{-1} NaPB the position of the ATPS becomes more similar and the effect Na$^+$ effect becomes less pronounced.

Acknowledgment

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