Partitioning of Penicillin G Acylase in Aqueous Two-Phase Systems of Poly(ethylene glycol) 20000 or 35000 and Potassium Dihydrogen Phosphate or Sodium Citrate

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The partitioning of penicillin G acylase in aqueous two-phase systems (ATPS's) containing poly(ethylene glycol) (PEG) 20000 or 35000 and potassium dihydrogen phosphate (KH_2PO_4) or sodium citrate ($C_6H_5Na_3O_7 \cdot 5H_2O$) has been measured at three temperatures, (301.2, 307.2, and 310.2) K. The effects of temperature, polymer molecular weight, and polymer and salt concentrations on the partitioning of penicillin G in the ATPS were studied. The experimental data showed that the composition of salt has a large effect on partitioning of penicillin G in ATPS, and the temperature of the system has a small effect on the partitioning. The UNIFAC-FV group contribution model (Pazuki et al., *Ind. Eng. Chem. Res.* **2009**, *48*, 4109−4118) was used in correlating the partition coefficients of penicillin G in polymer + ATPS.

Introduction

Penicillin is a group of antibiotics obtained from *Penicillium* fungi. Penicillin is widely used today, though many types of bacteria are now resistant. Penicillin core structure is shown in Figure 1. In this figure, −R− is variable organic group.

All penicillins are β-lactam antibiotics and are used in the treatment of bacterial infections caused by a susceptible, usually Gram-positive organism. β-lactam antibiotics work by inhibiting the formation of peptidoglycan cross-links in the bacterial cell wall.[1] Separation and purification of penicillin are very important in pharmaceutical industries. There are many common methods for the separation of biomolecules, such as precipitation, the membrane system, and aqueous two-phase systems (ATPS's).

ATPS's are widely used for the separation and purification of biomolecule compounds such as proteins, amino acids, and enzymes. The ATPS's are formed by two different polymers or one polymer and one inorganic salt in pure water. The ATPS's have some advantages such as low viscosity and interfacial tension between two phases, selectivity of separation, low price, a scale of up to $2 \cdot 10^4$, and a short time required for equilibrium.[1] Beijerinck first formed ATPS's using agar and gelatin.[2] Albertsson used polymer−polymer ATPS's for separation and purification of some biological compounds.[3] It should be noted that the ATPS's were used for continuous and large-scale operations.[4,5] Zaslavsky et al. measured the partitioning of glycine, lysine, aspartic acid, and oligopeptides in an ATPS of dextran and PEG 8000.[6] Peng et al. studied the partitioning of lysozyme, bovine serum albumin (BSA), and DNA in an ATPS containing PEG 4000 and K_2HPO_4 + KH_2PO_4 at 298 K.[7] Grossmann et al. measured and correlated partition coefficients of some amino acids such as glycine, L-glutamic acid, L-phenylalanine, and L-lysine and some their low molecular weight peptides in ATPS's of K_2HPO_4 and PEG 6000 and 35000 at 293 K.[8] Liu et al. studied partitioning of penicillin G in ATPS's of ionic liquids and NaH_2PO_4.[9]

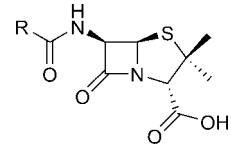

Figure 1. Penicillin core structure.

Gautam and Simon measured and modeled partition coefficients of β-glycosidase in ATPS's at various temperatures from (298 to 323) K and pH (6.5 to 8).[10] Recently, Khederlou et al. measured and modeled partition coefficients of cephalexin antibiotic in ATPS's of PEG 4000 or 10000 and K_2HPO_4 or Na_3 citrate at three temperatures (301.2, 307.2, and 310.2) K.[11]

In this work, the partition coefficients of penicillin G acylase are measured in polymer−salt ATPS's of PEG 20000 or 35000 and KH_2PO_4 or Na_3 citrate at three temperatures, (301.2, 307.2, and 310.2) K. The effects of temperature, polymer molecular weight, polymer concentration, and salt concentration on the partitioning of penicillin G were investigated. The UNIFAC-FV group contribution model[12] was used to compare and determine the accuracy of experimental results of the partition coefficients of penicillin G in ATPS's.

Experimental Section

Materials. Poly(ethylene glycol) with molecular weights of 20000 and 35000, citric acid monohydrate needed for pH control of the ATPS, sodium citrate, and potassium dihydrogen phosphate with 99.5 % purity were purchased from Merck (Germany, Darmstadt). Penicillin G acylase with 99.9 % purity was obtained from Antibiotic Sazi Company (Tehran, Iran). Double distilled and deionized water was used in all experiments.

Methods. ATPS's were prepared by a stock solution of polymer, salt, and penicillin in a glass vessel with 350 cm^3 volume. The glass vessel was connected to a thermostat. The ATPS was mixed using a magnetic stirrer for about 20 min. Then, the mixture was set to reach equilibrium for 24 h, and then samples of the upper and lower phases were removed by

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Table 1. Parameters of the Refractive Index Equation (eq 1)

component	a_0	a_1	a_2	100 σ^a
water	1.3324			
PEG 20000		0.1345		0.1206
PEG 35000		0.1356		0.0605
Na_3 citrate			0.1424	0.0872
KH_2PO_4			0.1175	0.1216

[a] $\sigma = [\sum_{i=1}^{N}((n_D^{calc} - n_D)/n_D)^2]^{0.5}$, where N = number of experimental data points.

a plastic syringe. The mass fraction of salt (KH_2PO_4 or $Na_3C_6H_5O_7$) was determined using Flame Photometry (Coring 410, from Jenway, England). The mass fraction of PEG was determined by refractive index measurements using an Erma optical refractometer (model 17101, from Erma Optical, Japan). The relation between the refractive index (n_D) and the mass fractions of polymer (w_P) and salt (w_S) can be obtained from the following equation

$$n_D = a_0 + a_1 w_P + a_2 w_S \quad (1)$$

The parameters of eq 1 as well as the standard deviation for each system are reported in Table 1 for different systems.

The mass fraction of penicillin G was determined by UV−vis spectrophotometry at 227 nm by a spectrophotometer (M501, from CamSpec, England).

The aqueous solutions containing polymer, salt, and water without penicillin G were used as blank solutions. The absorbance of penicillin G was determined using the blank solutions.

The pH of the ATPS's was measured with a pH meter (model 744, from Deutsche Metrohm, Filderstadt, Germany).

The relative uncertainty in the mass fractions of salt and polymer is less than 3 %, while the relative uncertainty in the mass fractions of penicillin G is less than 7.5 %.

Results and Discussion

Experimental Section. The experimental results for the mass fractions of poly (ethylene glycol), salt and penicillin G in the upper and lower phases as well as the partition coefficients of penicillin G at three temperatures (301.2, 307.2 and 310.2) K are reported in Tables 2 and 3. The experimental data for the partitioning of penicillin G in PEG + Na_3 citrate + water at various pH values are reported in Table 2. The experimental data for the partitioning of penicillin G in PEG + KH_2PO_4 + water at various pH values are reported in Table 3. As can be seen from these results, the partitioning of penicillin G in PEG + Na_3 citrate + water systems decreases with increasing salt concentration and decreasing polymer concentration. Also, the partitioning of penicillin G in PEG + KH_2PO_4 + water systems decreases with decreasing salt concentration and increasing polymer concentration. It can be found from Tables 2 and 3 that the partition coefficients of penicillin G increase as PEG molecular weight increases. It is concluded that penicillin G prefers to stay in the polymer-rich phase in most experiments. The experimental results also showed that the influence of temperature on the partitioning of penicillin G is small. As the temperature increases, the partitioning of penicillin G increases.

The effect of temperature on the binodal curve for the PEG 35000 + KH_2PO_4 + water ATPS is shown in Figure 2. It can be seen from Figure 2 that the two-phase systems are formed in the lowest temperature of system (T = 301.2 K). A comparison of the PEG + KH_2PO_4 + water systems is plotted in Figure 3. The results showed that the ATPS's are formed in a lower concentration of polymer and salt in PEG 35000 + KH_2PO_4 + water. Also, the binodal curves for the PEG 20000 + KH_2PO_4 + water system are presented in Figure 4 at pH (4.5 and 4.8). The results presented in Figure 4 indicated that the ATPS's are formed in a lower concentration of PEG and salt at higher pH. The phase diagrams of the PEG 35000 + Na_3 citrate + water system at 307.2 K and PEG 20000 + KH_2PO_4 + water at 301.2 K are shown in Figures 5 and 6. It can be seen from these figures that the two-phase region for system containing Na_3 citrate is greater than the system containing KH_2PO_4. It should be pointed out that the PEG 35000 + KH_2PO_4 + water ATPS is the best system for partitioning of penicillin G. The experimental partition coefficients of penicillin G are compared with the results obtained by Marcos et al.[13] in Table 4.

The phase separation curves showed that as the molecular weight of polymer increases, the binodal curve is formed at lower concentrations. Temperature is indirectly affected on the partitioning of penicillin G. The variations of temperature can be changed with phase compositions and binodal curves. The ATPS's are shifted to lower concentrations at low temperature, and the tie-line length (TLL) is increased. Also, the experimental partition coefficients showed that penicillin G is uniformly distributed in the ATPS.

Thermodynamic Modeling. In this section, the partition coefficients of penicillin G in the ATPS's were correlated using the corresponding experimental data. The partition coefficient of penicillin G in ATPS can be defined as[12]

$$K_{Pen} = \frac{w_{Pen}^{I}}{w_{Pen}^{II}} \quad (2)$$

where in eq 2 superscripts I and II refer to the upper and lower phases and w_{Pen} is mass fraction of penicillin G, respectively. The activity for penicillin G is equal in the upper and lower phases at equilibrium state

$$a_{Pen}^{I} = a_{Pen}^{II} \quad (3)$$

or

$$w_{Pen}^{I}\gamma_{Pen}^{I} = w_{Pen}^{II}\gamma_{Pen}^{II} \quad (4)$$

According to eq 3, partition coefficient of penicillin G can be obtained from the activity coefficients of penicillin G in the upper and lower phases as below[12]

$$K_{Pen} = \frac{\gamma_{Pen}^{II}}{\gamma_{Pen}^{I}} \quad (5)$$

The UNIFAC-FV group contribution model proposed by Pazuki et al. was used to correlate the partition coefficients of penicillin G in polymer−salt ATPS's.[12] It is assumed that water and penicillin G are considered as single groups, and salts (KH_2PO_4 and Na_3 citrate) are assumed to dissociate completely into ionic groups. The PEG molecule is divided into two PEG end groups (−CH_2−OH−) and PEG middle groups (−CH_2−O−CH_2−). The UNIFAC-FV model has six binary interaction parameters among anion−cation (E_{AC} and E_{CA}), anion−water (E_{AW} and E_{WA}), and cation−water (E_{CW} and E_{WC}) groups in correlating mean ionic activity coefficient data. The model considers two binary interaction parameters between the middle group and the water (E_{PW} and E_{WP}). It should be pointed out that the interaction parameters between the middle group/water pair as well as the end group/middle group are set to stand at zero. Also, the proposed model needs 10 binary interaction parameters between the anion/middle group (E_{AP} and E_{PA}), cation/middle group (E_{CP} and E_{PC}), penicillin G/middle group

Table 2. Mass Fractions of PEG (1), Na_3 Citrate (2), and Penicillin G (3) in the Upper and Lower Phases as well as Partition Coefficients of Penicillin G in ATPS

T		feed			upper phase			lower phase			
K	pH	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	K_{Pen}
					PEG 20000 + Na_3 Citrate						
301.2	4.80	26.469	11.396	0.018	42.958	6.195	0.0115	12.293	23.220	0.0147	0.782
301.2	4.78	26.471	11.397	0.009	42.380	7.092	0.0118	13.811	20.312	0.0134	0.885
301.2	4.60	27.059	9.419	0.019	37.199	8.264	0.0116	12.988	20.878	0.0136	0.852
301.2	4.81	16.613	12.923	0.021	32.008	8.391	0.0114	6.888	20.882	0.0206	0.551
307.2	5.00	26.468	11.396	0.018	29.723	18.134	0.0113	13.616	19.162	0.0122	0.926
307.2	5.20	26.471	11.397	0.009	42.765	3.358	0.01230	11.085	20.148	0.0117	1.048
307.2	4.80	27.059	9.419	0.019	41.317	3.532	0.0118	9.633	19.062	0.0115	1.019
307.2	5.00	16.613	12.924	0.021	37.451	3.250	0.0113	6.387	17.352	0.0131	0.860
310.2	4.60	26.469	11.396	0.018	43.985	5.225	0.0115	14.985	17.798	0.0116	0.984
310.2	4.50	26.471	11.397	0.009	44.473	5.326	0.0117	9.752	23.865	0.0122	0.964
310.2	4.40	27.059	9.419	0.019	39.839	6.332	0.0114	11.482	20.124	0.0121	0.938
310.2	4.60	16.613	12.924	0.021	35.316	6.391	0.0116	12.255	14.830	0.0117	0.993
					PEG 35000 + Na_3 Citrate						
301.2	4.80	26.469	11.396	0.018	43.121	7.111	0.0120	27.367	22.184	0.0121	0.995
301.2	4.82	26.471	11.397	0.009	42.645	7.635	0.0117	24.204	20.912	0.0127	0.924
301.2	4.73	27.059	9.419	0.019	36.808	8.207	0.0118	22.237	21.030	0.0126	0.938
301.2	4.78	16.613	12.923	0.021	35.265	7.850	0.0115	24.302	18.360	0.0122	0.941
307.2	4.80	26.468	11.396	0.018	42.332	6.879	0.0132	15.053	19.795	0.0121	1.093
307.2	4.77	26.471	11.397	0.009	40.874	7.355	0.0119	14.439	20.520	0.0116	1.020
307.2	4.80	27.059	9.419	0.019	37.469	7.367	0.0128	11.288	18.043	0.0126	1.011
307.2	4.71	16.613	12.924	0.021	32.435	8.719	0.0123	9.554	19.694	0.0117	1.050
310.2	4.80	26.469	11.396	0.018	50.138	5.344	0.0124	10.929	25.337	0.0151	0.822
310.2	4.80	26.471	11.397	0.009	47.454	4.529	0.0132	7.914	24.486	0.0155	0.851
310.2	4.90	27.059	9.419	0.019	34.938	15.465	0.0128	14.204	17.794	0.0151	0.848
310.2	4.80	16.613	12.924	0.021	31.220	10.719	0.0119	8.725	18.658	0.0120	0.997

Table 3. Mass Fractions of PEG (1), KH_2PO_4 (2), and Penicillin G (3) in the Upper and Lower Phases as well as Partition Coefficients of Penicillin G in ATPS

T		feed			upper phase			lower phase			
K	pH	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	K_{Pen}
					PEG 20000 + KH_2PO_4						
301.2	4.56	18.524	10.008	0.021	31.781	5.834	0.0116	1.576	16.920	0.0120	0.972
301.2	4.58	18.526	10.009	0.010	28.373	6.756	0.0113	1.440	17.161	0.0119	0.954
301.2	4.56	18.041	12.356	0.020	33.196	5.491	0.0113	1.167	17.643	0.0116	0.980
301.2	4.51	14.716	10.475	0.022	23.923	8.105	0.0114	2.105	16.229	0.0114	1.007
307.2	4.80	26.469	11.396	0.018	40.303	3.484	0.0113	3.163	15.698	0.0116	0.977
307.2	4.80	26.471	11.397	0.009	40.137	3.673	0.0114	1.320	17.808	0.0113	1.000
307.2	4.80	27.059	9.419	0.019	40.754	4.584	0.0114	1.045	21.153	0.0120	0.951
307.2	4.80	16.613	12.924	0.021	33.885	4.787	0.0115	3.269	14.641	0.0118	0.971
310.2	4.74	18.524	10.008	0.021	39.737	3.961	0.0117	2.673	15.749	0.0114	1.023
310.2	4.69	18.526	10.009	0.010	33.707	5.331	0.0114	4.755	14.642	0.0114	0.997
310.2	4.40	18.041	12.356	0.020	32.996	4.869	0.0116	7.050	10.738	0.0114	1.019
310.2	4.46	14.716	10.475	0.022	32.829	3.868	0.0117	8.150	10.756	0.0115	1.012
					PEG 35000 + KH_2PO_4						
301.2	4.63	18.524	10.008	0.021	29.042	6.143	0.0113	5.768	13.258	0.0118	0.961
301.2	4.65	18.526	10.009	0.010	29.373	5.421	0.0114	3.119	15.805	0.0119	0.955
301.2	4.60	18.041	12.356	0.020	30.583	4.790	0.0117	3.892	13.891	0.0116	1.015
301.2	4.56	14.716	10.476	0.022	25.143	6.728	0.0116	3.806	14.926	0.0118	0.988
307.2	4.80	26.469	11.396	0.018	38.594	4.140	0.0115	4.222	16.064	0.0114	1.005
307.2	4.80	26.471	11.397	0.009	38.614	5.053	0.0114	5.034	16.319	0.0113	1.013
307.2	4.80	27.059	9.419	0.019	37.794	4.979	0.0116	3.976	17.794	0.0114	1.023
307.2	4.80	16.611	12.924	0.021	36.065	5.272	0.0116	2.918	17.569	0.0114	1.020
310.2	4.60	26.469	11.396	0.019	43.057	3.160	0.0116	4.996	16.447	0.0114	1.011
310.2	4.70	26.471	11.397	0.009	40.104	4.185	0.0115	2.326	19.529	0.0114	1.004
310.2	4.70	27.059	9.420	0.019	45.127	2.899	0.0115	4.260	16.700	0.0115	0.999
310.2	4.70	16.613	12.924	0.021	42.372	4.375	0.0120	4.391	16.295	0.0113	1.054

(E_{PG-P} and E_{P-PG}), penicillin G/anion (E_{PG-A} and E_{A-PG}), and penicillin G/cation (E_{PG-C} and E_{C-PG}) to correlate the partition coefficients of penicillin G in polymer−salt ATPS's.

The experimental data of mean ionic activity coefficients for KH_2PO_4 and activity of water in Na_3 citrate solution were obtained from the literature.[14,15]

The interaction parameters between ionic groups and water of the UNIFAC-FV model are reported in Table 5.

The experimental data of the activity of water in PEG aqueous solutions for PEG 20000 and 35000 were obtained from the literature.[16,17]

The interaction parameters between the middle group and the water are reported in Table 6. The partition coefficients of penicillin G in polymer−salt ATPS's were used in obtaining the interaction parameters for the pairs penicillin G/middle group, penicillin G/anion, and penicillin G/cation. The interaction parameters between middle group and ionic species are presented in Table 6. Also, the interaction parameters for the pairs penicillin G/middle group, penicillin G/anion, and penicillin G/cation and the average absolute relative deviation (ARD) values of the UNIFAC-FV model are presented in Table 7. The experimental data of the partition coefficients of penicillin G

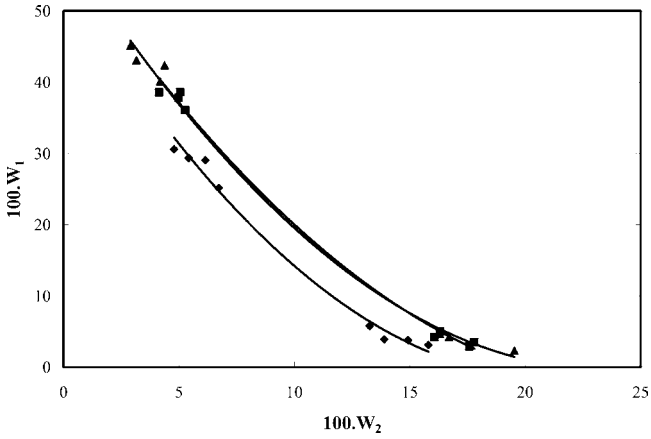

Figure 2. Effect of temperature on two-phase separation in PEG 35000 (1) + KH_2PO_4 (2) ATPS: ◆, $T = 301.2$ K; ■, $T = 307.2$ K; ▲, $T = 310.2$ K; solid line, binodal curves.

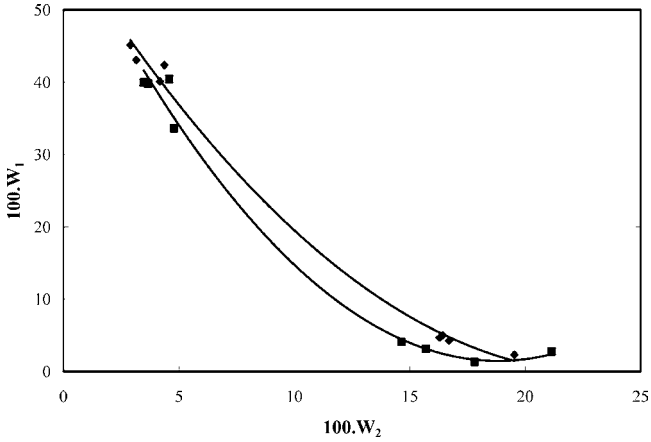

Figure 3. Effect of PEG molecular weight on two-phase separation in PEG (1) + KH_2PO_4 (2): ◆, PEG 20000; ■, PEG 35000; solid line, binodal curves.

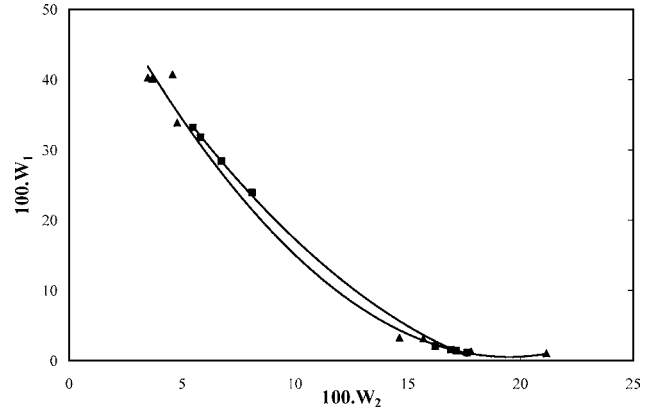

Figure 4. Effect of pH of ATPS on two-phase separation in PEG (1) + KH_2PO_4 (2):▲, pH = 4.8; ■, pH = 4.5; solid line, binodal curves.

in ATPS of PEG 35000 + Na_3 citrate + water and PEG 20000 + KH_2PO_4 + water were compared with those obtained from the model in Table 8.

The results indicated that the UNIFAC-FV model can accurately correlate the partition coefficients of penicillin G in polymer−salt ATPS's.

Conclusion

In this research, the partition coefficients of penicillin G acylase were measured in ATPS's of PEG + Na_3 citrate + water and PEG + KH_2PO_4 + water. The partitioning of penicillin G is depended on temperature, polymer molecular weight, and salt and polymer concentrations in feed. The UNIFAC-FV group contribution model was used to correlate the experimental data. The results of the UNIFAC-FV model showed that the proposed model can accurately correlate the partition coefficients of penicillin G in polymer−salt ATPS's. Also, the experimental data showed that the partitioning of penicillin G is strongly dependent on concentration of salt in feed. The variations of

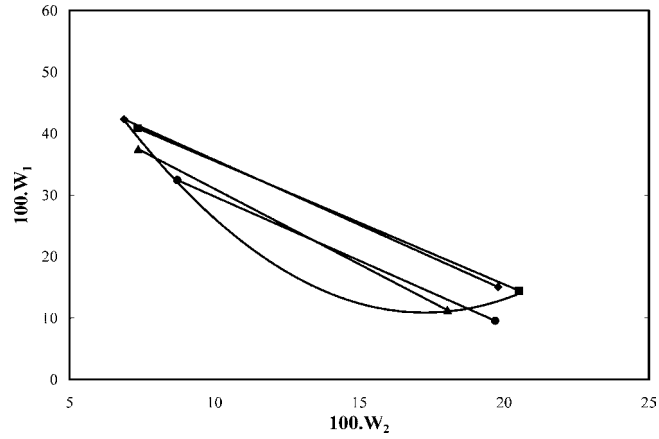

Figure 5. Phase diagram and tie lines of PEG 35000 (1) + Na_3 citrate (2) ATPS at 307.2 K.

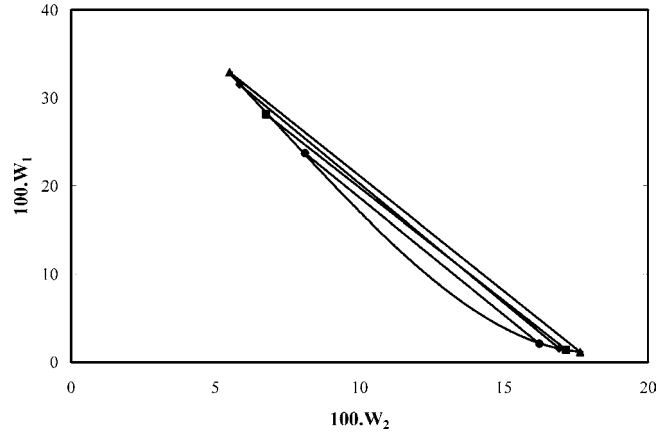

Figure 6. Phase diagram and tie lines of PEG 20000 (1) + KH_2PO_4 (2) ATPS at 301.2 K.

Table 4. Comparison of the Experimental Data of Partition Coefficients for PEG (1) + Na_3 Citrate (2) + Water (3) with the Results Reported by Marcos et al.[13]

	PEG 3350[a]			PEG 20000[b]			PEG 35000[b]		
100 w_1	100 w_2	K_{Pen}	100 w_1	100 w_2	K_{Pen}	100 w_1	100 w_2	K_{Pen}
19	8.71	0.58	16.61	12.92	0.51	27.06	9.42	0.85
9	12.81	1.27	26.47	11.39	1.04	26.47	11.40	1.10
14	10.76	1.22	27.06	9.42	1.02	16.61	12.92	1.05

[a] Marcos et al. [b] This work.

Table 5. Values of the Parameters of the UNIFAC-FV Model Obtained Using the Mean Ionic Activity Coefficient and Activity of Water of Electrolyte Solutions[a,b]

system	N	E_{AC}/R	E_{CA}/R	E_{AW}/R	E_{WA}/R	E_{CW}/R	E_{WC}/R
KH_2PO_4 + water	14	88.960	−954.382	15.563	298.389	736.767	−824.761
Na_3 citrate + water	11	468.658	−1184.633	690.293	823.747	621.477	−847.747

[a] N: number of experimental data points; A: anion, C: cation, W: water. [b] E/R [≡] T.

Table 6. Values of the Parameters of the UNIFAC-FV Obtained Using PEG + Salt ATPS[a,b]

PEG	salt	E_{AP}/R	E_{PA}/R	E_{CP}/R	E_{PC}/R	E_{PW}/R	E_{WP}/R
20000	KH_2PO_4	45.175	1258.545	−67.937	−5.693	−1868.362	−1056.148
20000	Na_3 citrate	1.473	−18083.907	−2.070	−0.035	−1868.362	−1056.148
35000	KH_2PO_4	45.141	1258.543	−67.986	−4.763	−2029.130	−877.055
35000	Na_3 citrate	45.273	1258.557	−67.841	−5.595	−2029.130	−877.055

[a] P: PEG. [b] E/R [≡] T.

Table 7. Values of the Parameters and the AAD % of the UNIFAC-FV Model Obtained Using Partition Coefficients of Penicillin G in PEG + Salt ATPS[a,b,c]

PEG	salt	$E_{A\text{-}PG}/R$	$E_{PG\text{-}A}/R$	$E_{C\text{-}PG}/R$	$E_{PG\text{-}C}/R$	$E_{P\text{-}PG}/R$	$E_{PG\text{-}P}/R$	100 ARD
20000	KH_2PO_4	−71.596	2328.407	334.514	−1855.577	−1838.745	68889.049	1.502
20000	Na3 citrate	152.434	−16158.810	125.762	19435.860	890.456	4898.573	12.373
35000	KH_2PO_4	333.813	2363.250	472.364	−1598.360	−1869.880	68894.368	1.671
35000	Na3 citrate	−122.676	2024.852	948.452	−1628.375	−180.263	67919.880	6.780
overall								5.581

[a] PG: penicillin G. [b] ARD = $[\sum_{i=1}^{N}((K_{Pen}^{expt} - K_{Pen}^{calc})/K_{Pen}^{expt})]/N$. [c] E/R [≡] T.

Table 8. Comparison between the Experimental Partition Coefficients and the Results Obtained from the UNIFAC-FV Model for Penicillin G in Polymer + Salt ATPS[a]

	PEG 20000 + KH_2PO_4 + water			PEG 35000 + Na_3 citrate + water	
K_{Pen}(calc)	K_{Pen}(expt)	100 RD	K_{Pen}(calc)	K_{Pen}(expt)	100 RD
0.972	0.972	0.000	0.938	0.995	5.729
0.966	0.954	1.258	0.931	0.924	0.758
0.966	0.980	1.429	0.943	0.938	0.533
0.975	1.007	3.178	0.957	0.941	1.700
0.993	0.977	1.638	0.901	1.093	17.566
0.974	1.000	2.600	0.903	1.020	11.471
0.950	0.951	0.105	0.906	1.011	10.386
0.990	0.971	1.957	0.915	1.050	12.857
0.999	1.023	2.346	0.853	0.822	3.771
0.993	0.997	0.401	0.851	0.851	0.000
1.024	1.019	0.491	0.927	0.848	9.316
1.002	1.012	0.988	0.917	0.997	8.024

[a] RD = $(|K_{Pen}^{expt} - K_{Pen}^{calc}|)/K_{Pen}^{expt}$.

temperature have small effect on the partitioning of penicillin G in ATPS's. Also, it is concluded that with increasing the PEG molecular weight, the partitioning of penicillin G increases.

Appendix A

Group Contribution Model. According to the previous work,[12] the activity coefficient of each component can be written as:

$$\ln \gamma_i = \ln \gamma_i^{LR} + \ln \gamma_i^{COMB-FV} + \ln \gamma_i^{SR} \qquad \text{(A-1)}$$

The Debye−Hückel equation was used to calculate the long-range effect as:

$$\ln \gamma_{\pm}^{LR} = -\frac{A|Z_A Z_C| I^{1/2}}{(1 + BI^{1/2})} \quad \text{for salt} \qquad \text{(A-2)}$$

$$\ln \gamma_i^{LR} = \frac{2AM_W}{(10B)^3}\left[(1 + BI^{1/2}) - \frac{1}{(1 + BI^{1/2})} - 2\ln(1 + BI^{1/2})\right] \quad i = \text{water, polymer, and biomolecule} \qquad \text{(A-3)}$$

where:

$$I = \frac{1}{2}\sum_k m_k Z_k^2 \qquad \text{(A-4)}$$

In the above equations, A and B are Debye−Hückel constants, and also Z and m are charge and molality of each ion in solution, respectively.

The activity coefficient obtained from eqs A-2 and A-3 must be converted from molality scale to mole fraction scale by using the following equation:

$$\ln \gamma_{i,x}^{LR} = \ln \gamma_{i,m}^{LR} + \ln(1 + \nu m M_W/1000) \qquad \text{(A-5)}$$

The combinatorial term can be obtained by the Freed−FV model as:

$$\ln \gamma_i^{COMB-FV} = \ln\left(\frac{\phi_i^{FV}}{x_i}\right) + 1 - \left(\frac{\phi_i^{FV}}{x_i}\right) + f_i^{Freed-FV} \qquad \text{(A-6)}$$

$$f_i^{Freed-FV} = R_i^{FV}\left[\sum_j \beta_{ji}^{FV}\phi_j^{FV}(1 - \phi_j^{FV}) - 0.5\sum_{j\neq i}\sum_{k\neq i}\beta_{jk}^{FV}\phi_j^{FV}\phi_k^{FV}\right] \qquad \text{(A-7)}$$

where:

$$\beta_{ji}^{FV} = \alpha_{ji}\left(\frac{1}{R_j^{FV}} - \frac{1}{R_i^{FV}}\right) \qquad \text{(A-8)}$$

$$\phi_i^{FV} = \frac{x_i v_i^{FV}}{\sum_j x_j v_j^{FV}} \qquad \text{(A-9)}$$

In eq A-8 α_{ij} is the nonrandomness factor ($\alpha_{ij} = 0.2$), and R_j^{FV} is the ratio of v_j^{FV} to v_i^{FV}.v_i^{FV} is free volume defined as:

$$v_i^{FV} = v_i - v_i^{vdW} \qquad \text{(A-10)}$$

where v_i is the molar liquid volume of component i and v_i^{vdW} is the van der Waals volume ($cm^3 \cdot mol^{-1}$) calculated via by the following equations:

$$v_i = (0.3 + 4.5\cdot 10^{-4}T)v_i^{vdW} \qquad \text{(A-11)}$$

$$v_i^{vdW} = 15.17R_i \qquad \text{(A-12)}$$

The residual term of activity coefficient for each group was calculated by:

$$\ln \Gamma_m = 1 - \ln\left(\sum_j \Theta_j H_{mj}\right) - \sum_j \frac{\Theta_j H_{mj}}{\sum_k \Theta_k H_{kj}} - \sum_j \Theta_j\left[\sum_k \Theta_k \ln\left(\frac{H_{kj}}{H_{mj}H_{jm}}\right)\right] \qquad \text{(A-13)}$$

H_{ij} is the Boltzmann factor which is defined as:

$$H_{ij} = \exp\left(-\frac{E_{ij}}{10 \cdot RT}\right) \tag{A-14}$$

where $(E_{ij})/(R)$ is the adjustable parameter between groups i and j. Also, Θ_k is surface of group k which is defined as follows:

$$\Theta_k = \frac{X_k Q_k}{\sum_j X_j Q_j} \tag{A-15}$$

In eq A-15, X_m, the group fraction can be obtained as:

$$X_m = \frac{\sum_{i=1}^{n} x_i \nu_{mi}}{\sum_{i=1}^{n} x_i \sum_{k=1}^{s} \nu_{ki}} \tag{A-16}$$

where ν_{mi} is the number of group m in molecules, n is the number of components, and s is number of various groups in mixture.

The residual activity coefficient of each component can be determined as the following relation:

$$\ln \gamma_i^{SR} = \sum_{m=1}^{s} \nu_{mi}\left(\ln \Gamma_m - \ln \Gamma_m^{(i)}\right) \tag{A-17}$$

where Γ_m is the activity coefficient of group m in solution and $\Gamma_m^{(i)}$ is the activity coefficient of group m in pure component i.

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