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The Effect of pH on the Liquid-liquid Equilibrium for a System Containing Polyethylene Glycol Di-methyl Ether and Tri-potassium Citrate and its Application for Acetaminophen Separation

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In this work, liquid-liquid equilibrium for an aqueous two phase system composed of polyethylene glycol di-methyl ether and tri-potassium citrate at different medium pH values (6.00, 7.00 and 8.00) and 298.15 K was studied. The results showed that the two-phase area expands with increasing pH values. The performances of the Merchuk and semi-empirical equations were examined in correlating the obtained binodal data. The reliability of the experimental tie-lines was verified using the Hand and Bachman equations. Then, this two-phase system was used to investigate partitioning of acetaminophen by determining the composition of coexisting phases in the presence of acetaminophen. Experimental results showed that 60-80% of the drug could be extracted into the polymer-rich phase in a single-step separation. The partitioning coefficient of acetaminophen was changed slightly with the increase of tie-line length and also with the increase of pH values. Thermodynamic studies indicated that hydrophobic interactions were the main driving force, although electrostatic interactions and salting-out effects were also important for the drug transformation. Finally, the equation proposed by Diamond-Hsu was used to the correlating of the experimental partition coefficients of acetaminophen in the studied polymer- salt aqueous two-phase system.

Keywords: Aqueous two-phase system, Partitioning coefficient, Poly ethylene glycol di-methyl ether, Tri-potassium citrate, Acetaminophen

INTRODUCTION

Acetaminophen is a well-known analgesic and antipyretic drug which is a major ingredient in cold and flu medications. Recently, developments made on generic drugs have motivated pharmaceutical researchers to find a way to minimize operational costs by optimizing the lenitive of drug productions and their purification processes. For separation and purification of biological materials such as nucleic acids, proteins and enzymes, aqueous two-phase systems (ATPSs) can be used. ATPS is a non-chromatographic and unit operation method which is more economical than the other existing separation processes of drugs [1,2]. There are different types of ATPs. For separation and purification of biomaterials, ATPs containing of water soluble polymer and salt have been frequently used. By mixing a single polymer and a salt at appropriate concentrations or particular temperature, these ATPSs can be formed [3,4]. Aqueous polymer-salt two-phase systems have gained interest due to their lower viscosity, higher density and lower cost of salt phase in comparison with traditional polyethylene glycol (PEG)-dextran system [5]. Separation and extraction of biomaterials by ATPSs have been the subject of many recent studies [6-12]. Vernau and Kula [13] have investigated the possible use of citrates as a substitute for inorganic salts. They found that sodium and potassium citrates also form aqueous two-phase systems with PEG which are suitable for protein extraction. Unlike phosphate or sulfate, citrate is biodegradable and also nontoxic; therefore, citrate can be discharged into biological

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waste water treatment plants. More recently, the application of PEG/citrate systems to the recovery of biomolecules of alpha-amylase [13], penicillin acylase [14-18], hexokinase [19], insulin [20] cephalexin [21] has been studied. Poly ethylene glycol di-methyl ether (PEGDME) is a polymer that has a similar structure to PEG, thus it can be used to form ATPSs with kosmotropic (i.e. water-structuring) salts. The partitioning of a biomolecule in ATPSs is affected by the molar mass of polymer, system temperature, polymer structure, size of biomolecule, tie-line length (*TLL*), pH of the solution and other factors.

In this research, at first, the phase diagrams for the aqueous two-phase system containing polyethylene glycol di-methyl ether and tri-potassium citrate at different pH values (6.00, 7.00 and 8.00) and 298.15 K are determined. Then, the partition coefficients of acetaminophen in the mentioned ATPSs are measured. In addition, the effects of tie-line length and pH of solution are considered on partition of acetaminophen in ATPS. Furthermore, the obtained binodal curves are correlated with Merchuk [22] equation and an empirical equation [23]. Also, to check the reliability of obtained tie-lines, the Hand and Bachman equations are used. For correlating experimental acetaminophen partition coefficient values, the Diamond-Hsu [24] equation is used.

EXPERIMENTAL

Materials

Polyethylene glycol di-methyl ether 2000, tri-potassium citrate and citric acid were supplied by Merck. Acetaminophen was provided from Temad. The polymers and salt were used without further purification and double distilled deionized water was used to prepare the solutions in all of the experiments. The purity of the materials is shown in Table 1.

Methods

First, the polymer stock solution of 50% by mass was prepared. Then, by mixing appropriate amounts of tri-potassium citrate and citric acid, the concentrated salt stock solutions at the desired pH values were prepared with the required mass fractions reported in Table 2. The pH was controlled with a precision pH meter (Model: 692 pH/Ion Meter-Metrohm, Herisau, Switzerland). To measure the compositions of solutions, an analytical balance (Model: 321-34553, Shimatzu, Japan) with an uncertainty of ± 1 × 10^{-7} kg was used. The calculated pH values based on acidity constants are also given in Table 2.

Determination of binodal curves. The experimental apparatus used in this work has been described in previous works [25-27]. A glass vessel with an external jacket was used to obtain binodal curves. In order to control the vessel temperature, water at constant temperature was circulated in external jacket using a thermostat with an uncertainty 0.05 K. Clouding point titration method was employed to determine the binodal curves. In this method, the composition of the mixture for each point on the binodal curve was determined out of the amount of titrant added until turbidity was observed using an analytical balance. Using this method, the maximum uncertainty was found to be 0.005 in determining the mass fraction of both polymer and salt.

Determination of the partition coefficient of the acetaminophen. The experimental method for the measurement of biomolecule partition coefficient in aqueous two-phase systems has been described previously [25]. For each experiment, the ATPS was prepared in a glass vessel with volume about 2 × 10^{-5} m^3 by mixing deionized water, polymer, salt and acetaminophen with specific mass fractions. The prepared ATPSs were used to evaluate the acetaminophen partitioning at T = 298.15 K and different aqueous medium pH values (6.00, 7.00 and 8.00). The samples were stirred for 15 min, then, to obtain two clear phases at a constant temperature and achieve a complete acetaminophen partitioning between the two phases, samples placed in the water bath at a constant temperature for overnight. The temperature of water bath was controlled with a thermostat (JULABO model MB, Germany) with an accuracy of ±0.02 K. The concentration of acetaminophen in each vessel was about w (mass fraction) = 0.0037 by mass. After separation of samples in two phases, each phase in a sample was withdrawn and analyzed to determine the acetaminophen content. UV spectroscopy method was employed for quantification of acetaminophen in both phases using a spectrophotometer (Model: Shimadzu UV-1700-Pharma) at the wavelengths of 253 nm. To avoid interference from the phase components, the samples were diluted and analyzed against the blanks

Table 1. A Brief Summary of the Purity of the Used Materials

Material	Source	Molecular formula	Mass fraction purity
Polyethylene glycol di-methyl ether 2000	Merck	$CH_3O(CH_2CH_2O)_nCH_3$	>0.99 w/w
Tri-potassium citrate	Merck	$K_3C_6H_5O_7$	>0.99 w/w
Citric acid	Merck	$C_6H_8O_7$	>0.99 w/w
Acetaminophen	Temad	$C_8H_9NO_2$	>0.98 w/w

Table 2. Mole Percents for the Mixed Tri-potassium Citrate/citric Acid at Different pH Values and T = 298.15 K

Material	pH = 6.00 (6.14)	pH = 7.00 (7.05)	pH = 8.00 (8.30)
M[a]	281.8	299.5	305.6
K_3citrate/(mol%)	78.44[§]	94.00[§]	99.26[§]
Citric acid/(mol%)	21.56[§]	6.00[§]	0.74[§]

[a]Average molecular weight for the mixed K_3cite and citric acid. [§]Estimated mole percent values for the components of the conjugated acid-base systems at different pH values.

Table 3. Values of the Parameters of the Eq. (1), a_i, for {$PEGDME_{2000}$ (1) + Tri-potassium Citrate/citric Acid (2) + Water (3)} System at pH = (6.00, 7.00, and 8.00) and T = 298.15 K

Material	Constant	Value	C range (w/w)	R^2
$PEGDME_{2000}$	a_1	0.1311	0-0.10	0.9995
K_3citrate/Citric acid (pH = 6.00)	a_2	0.1428	0-0.09	0.9998
K_3citrate/Citric acid (pH = 7.00)	a_2	0.1510	0-0.09	0.9998
K_3citrate/Citric acid (pH = 8.00)	a_2	0.1521	0-0.09	0.9998

containing the same phase components but without acetaminophen. The polymer and salt concentrations in both phases were determined with the procedure given in our previous work [25]. Using a refractometer (ATAGO DR-A_1, Japan) with a precision of ± 0.0001, the concentration of $PEGDME_{2000}$ in both phases was determined by refractive index measurements at T = 298.15 K. The uncertainty in refractive index measurement is 0.0002. The following relation between the refractive index, n_D, and the mass fractions of polymer, w_1, and salt, w_2 was used for aqueous

solutions containing a polymer and a salt:

$$n_D = n_0 + a_1 w_1 + a_2 w_2 \quad (1)$$

here, n_0 is the refractive index of pure water for which we obtained the value of 1.3325 at T = 298.15 K. Two constants a_1 and a_2 corresponding to polymer and salt respectively are obtained from the linear calibration plots of refractive index of the solution. Since Eq. (1) is valid only for dilute solutions, before refractive index measurements, the samples were diluted to be in the C mass fraction range as given in Table 3. The uncertainty for mass fraction of $PEGDME_{2000}$ achieved by this method and Eq. (1) was better than 0.002. The values of these constants and respective correlation coefficient values, R, are reported in Table 3. The potassium content in the top and bottom phases was determined using a flame photometer (Model: PFP 7, Jenway, England). The salt content was subsequently determined by mass balance, considering the proportion in which tri-potassium citrate and citric acid were added to the system.

Partition coefficients of acetaminophen, K_{acet}, between the two phases were calculated by using the following relation:

$$K_{acet} = \frac{w_{acet}^{top}}{w_{acet}^{bot}} \quad (2)$$

In addition, the extraction percent, E, is defined by:

$$E = \frac{k_{acet}}{1 + k_{acet}} \times 100\% \quad (3)$$

where, w_{acet}^{top} and w_{acet}^{bot} are the mass fraction of the partitioned acetaminophen, acet, in the $PEGDME_{2000}$-rich top, top, phase and the salt-rich bottom, bot, phase, respectively. The Eqs. (4) and (5) can be used to calculate the tie-line length, TLL, and the slope of the tie-line, S, which are two important characteristics of the tie-lines, respectively;

$$TLL = \left[\left(w_1^{top} - w_1^{bot}\right)^2 + \left(w^{top}_2 - w_2^{bot}\right)^2\right]^{0.5} \quad (4)$$

$$S = \left(w_1^{top} - w_1^{bot} / \left(w_2^{top} - w_2^{bot}\right)\right) \quad (5)$$

where, w_1 and w_2 represent the equilibrium compositions (in mass fraction) of polymer (1) and salt (2), respectively. The results obtained from the above equations are reported in Tables 5 and 6.

RESULTS AND DISCUSSION

Experimental Results

To design aqueous two-phase extraction process and develop models for predicting of partitioning of biomolecules, phase diagram data are required. The experimental data for the binodal curves and the equilibrium distribution of acetaminophen together with the mass fractions of K_3citrate, $PEGDME_{2000}$ and acetaminophen in the top and bottom phases at pH = (6.00, 7.00 and 8.00) and T = 298.15 K are given in Tables 4 and 5, respectively. As can be seen from Table 6, the mass fraction of acetaminophen in the $PEGDME_{2000}$-rich top phase is more than that of the citrate-rich bottom phase; therefore, the partition coefficients are greater than unity ($K_{acet} > 1$).

Effect of pH

The effect of medium pH values on the phase-forming ability and tie-lines of the studied system is also illustrated in Figs. 1 and 2, respectively. The locus for the experimental binodals shown in Fig. 1 demonstrates that the two-phase area is expanded with an increase in pH value. This observation can be analyzed based on the hydration theories [28-30]. These theories were briefly stated in previous work [26]. From the thermodynamic viewpoint [31], utilizing the Gibbs free energy of hydration, ΔG_{hyd}, of the constitutive ions of the salts makes it possible to analyze the water structure-promoting capability (kosmotropicity) of inorganic salts in a polymer-based ATPS with the certain polymer. ΔG_{hyd} is the change in free energy from an isolated naked ion in the gas phase to the aqueous solvated ion in solution. The ions with the higher kosmotropicity have a more negative ΔG_{hyd}, due to the resulting more structured water 'lattice' around the ion [32]. In the present case, we have unaffected cation (K^+); and therefore, by increasing the aqueous medium pH, the capability of the citrate anions to promote the water structure and the consequent phase

Table 4. Binodal Data for the {[PEGDME2000 (1) + Tri-potassium Citrate/Citric Acid (2) + H_2O (3)} System at pH = (6.00, 7.00, and 8.00) and T = 298.15 K[a]

pH = 6.00		pH = 7.00		pH = 8.00	
100 w_1[b]	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
39.91	8.51	33.15	8.98	34.03	8.01
39.46	8.65	32.75	9.08	32.28	8.31
35.56	9.49	31.91	9.21	31.07	8.66
34.29	9.82	31.09	9.32	28.89	9.10
31.74	10.26	30.13	9.55	27.13	9.57
29.63	10.66	29.26	9.77	24.90	10.30
26.92	11.27	28.00	10.15	23.11	10.97
24.88	11.90	26.73	10.44	20.84	11.91
22.55	12.81	25.05	10.88	19.09	12.72
20.41	13.43	23.74	11.43	16.99	13.84
18.05	14.36	22.03	12.11	15.15	14.62
16.22	15.11	20.52	12.67	13.12	15.45
14.37	15.90	18.76	13.49	11.36	16.62
12.93	16.57	17.33	14.22		
11.41	17.29	15.73	14.96		
		14.29	15.65		
		12.99	16.43		

[a]Standard uncertainties for mass fraction and temperature are 0.002 and 0.05 K, respectively. [b]w_1 and w_2 represented mass fractions of polymer and salt, respectively

separation is increased as illustrated in Fig. 1. The reason for this observation is that the degree of protonation of the citrate ions is varied at different pH medium. Table 2 shows that as the aqueous medium pH increase the citrate ions are less protonated and consequently have higher valency. The results obtained from this study are consistent with our previous work in regard with the effect of pH on the phase forming ability of aqueous 1-butyl-3-methylimidazolium bromide + potassium citrate system [26].

Effect of pH on the Partition Coefficient and the Extraction Percent

It was found that in partitioning of drugs in an ATPS, the pH of solution can play an important role by affecting the characteristics of drug and also the phase forming compounds. It is also well known that the ability of pH in partitioning of drug depends on its pK_a of isoelectric point. Other factors such as the hydrophilicity/hydrophobicity character, electrostatic characteristics of drug and salting-

Table 5. Experimental Tie-line Data and Partition Coefficient in Mass Fraction, w_i, for {$PEGDME_{2000}$ (1) + Tri-potassium Citrate/Citric Acid (2) + Water (3)} System at pH = (6.00, 7.00 and 8.00) and T = 298.15 K[a]

Feed sample[b]		Top phase		Bottom phase		TLL	Slope
100 w_1'	100 w_2'	100 w_1	100 w_2	100 w_1	100 w_2		
			pH = 6.00				
19.77	17.98	33.07	9.90	5.182	27.29	32.87	-1.60
19.81	20.64	36.55	9.23	3.489	32.09	40.19	-1.45
19.78	23.27	40.59	8.33	2.486	36.25	47.24	-1.37
19.74	25.96	42.68	7.94	1.269	41.36	53.21	-1.24
19.70	30.04	46.45	7.51	0.714	46.61	60.17	-1.17
			pH = 7.00				
19.77	17.98	31.71	9.23	1.43	30.08	36.73	-1.46
19.81	20.64	34.99	8.80	0.81	34.41	42.72	-1.34
19.78	23.27	37.97	8.33	0.87	38.08	47.55	-1.25
19.74	25.96	41.02	7.63	0.47	42.27	53.33	-1.17
19.70	30.04	45.58	6.98	0.53	46.09	59.66	-1.15
			pH = 8.00				
19.77	17.98	29.08	9.00	2.23	26.89	32.26	-1.50
19.81	20.64	32.56	8.48	1.42	32.22	39.16	-1.31
19.78	23.27	35.87	7.99	0.99	37.46	45.66	-1.18
19.74	25.96	40.45	7.65	0.48	43.50	53.70	-1.11
19.70	30.04	44.15	7.36	0.44	46.55	58.70	-1.12

[a]Standard uncertainties for mass fraction and temperature are 0.002 and 0.05 K, respectively. [b]w_1 and w_2 are total mass fraction of polymer and salt in its feed samples, respectively; and also tie-line length (TLL) and Slope at different concentrations calculated from Eqs. (4) and (5).

out effect are also important on the partitioning. Depending on the pH of the aqueous phase, the hydrophilic groups of drug can be ionized and lead to different equilibrium forms: a cationic form, a zwitterionic form, and an anionic form. In our investigated system, since pH of solutions (= 6.00, 7.00 and 8.00) are less than pK_a of the acetaminophen (= 9.38) reported in Ref. [33], there exist only cationic form of the drug. The experimental results for the partition coefficients

Table 6. Experimental Partition Coefficient Data, K_{acet}, and the Extraction Percent, E, for {$PEGDME_{2000}$ (1) + Tri-potassium Citrate (2) + Water (3)} System at pH = (6.00, 7.00 and 8.00) and T = 298.15 K

TL	pH = 6.00		pH = 7.00		pH = 8.00	
	K_{acet}[a]	E	K_{acet}	E	K_{acet}	E
1	1.3	55.52	1.6	61.54	2.1	67.74
2	1.5	60.00	1.9	65.52	2.3	69.69
3	1.6	61.54	2.1	67.74	2.8	73.68
4	2.1	67.74	2.6	72.22	3.2	76.19
5	2.4	70.59	3.2	76.19	3.9	79.59

[a]The standard uncertainty σ for partitioning coefficient is: σ (K_{acet}) = 0.1.

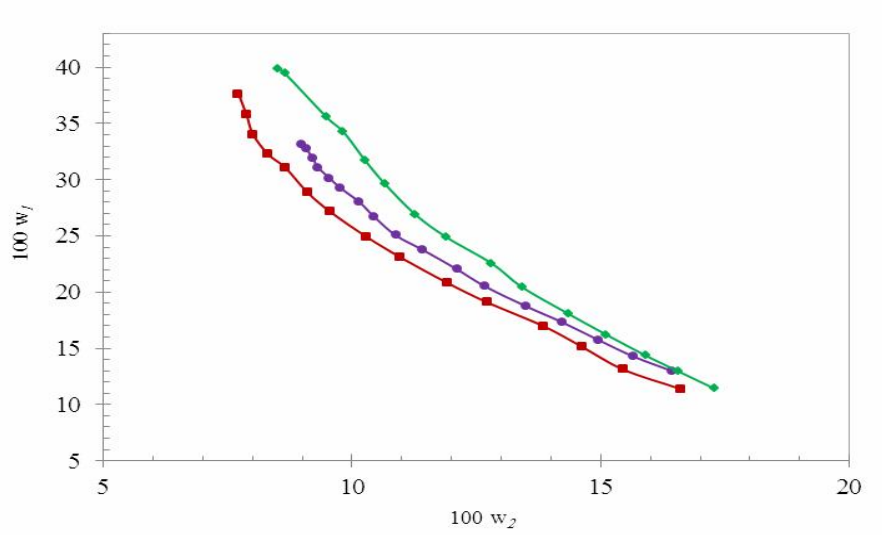

Fig. 1. Binodal curves for the {$PEGDME_{2000}$ (1) + potassium citrate/citric acid (2) + H_2O (3)} two-phase system at different medium pH and T = 298.15 K: (—♦—) pH = 6.00, (—●—) pH = 7.00, (—■—) pH = 8.00.

of the acetaminophen and the extraction percentage in the {$PEGDME_{2000}$ + K_3citrate + H_2O} ATPS are presented in Table 6. Figures 3 and 4 show the effect of pH on the partitioning and the extraction percentage of the acetaminophen in the studied polymer-based ATPS, respectively. As can be seen, partitioning coefficient of acetaminophen is increased with increasing the pH values. Similar behavior has also been observed in the case of the percentage of extraction. Since the studied drug in the working pH values are in their cationic forms, the medium pH values have no effect on the equilibrium form of the drug. In other words, partitioning of acetaminophen is not affected by its pK_a of isoelectric point. Therefore, in the present case, the partitioning of acetaminophen in

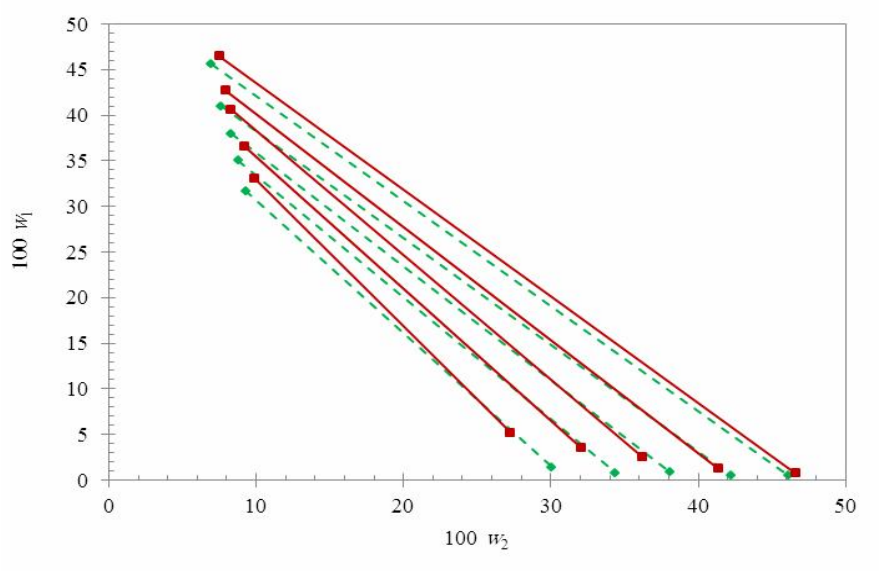

Fig. 2. Tie-line data for the {$PEGDME_{2000}$ (1) + potassium citrate/citric acid (2) + H_2O (3)} two-phase system at different medium pH and T = 298.15 K: (--♦--) pH = 6.00, (--●--) pH = 8.00.

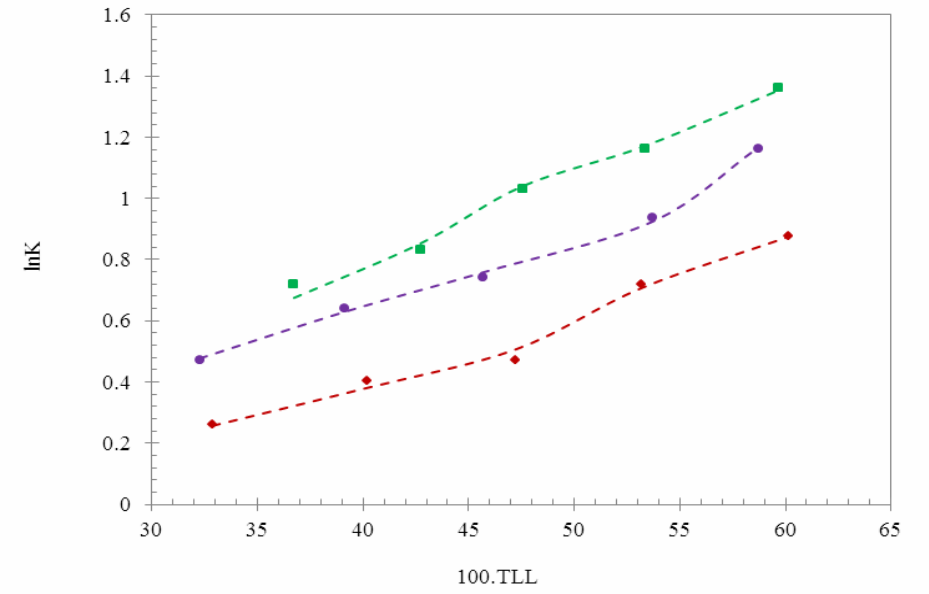

Fig. 3. Plots of partition coefficients of acetaminophen vs. tie-line length in the {$PEGDME_{2000}$ (1) + potassium citrate/citric acid (2) H_2O (3)} ATPS at T = 298.15 K: (--■--) pH = 6.00, (--●--) pH = 7.00, and (--♦--) pH = 8.00.

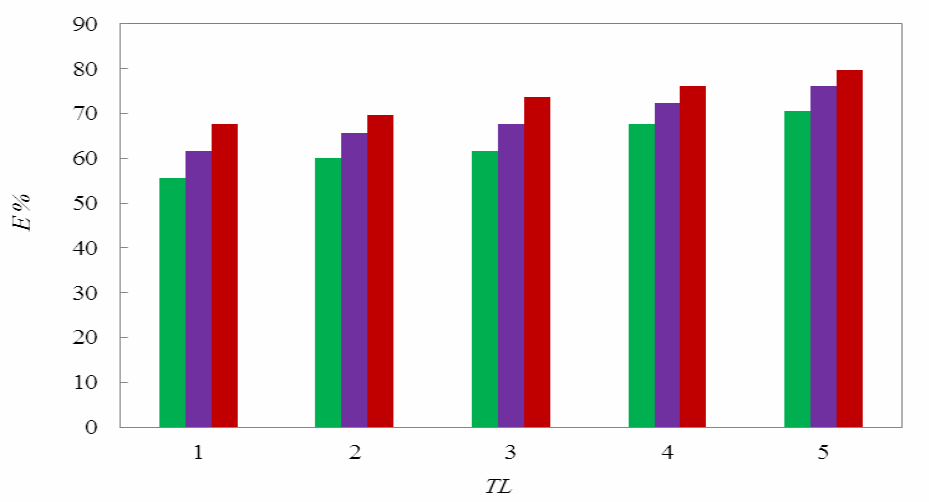

Fig. 4. Plots of the acetaminophen extraction percent *vs.* tie-line number (TL) in the {$PEGDME_{2000}$ (1) + potassium citrate/citric acid (2) H_2O (3)} ATPS at $T = 298.15$ K: (green column) pH = 6.00, (purple column) pH = 7.00, and (brown column) pH = 8.00.

$PEGDME_{2000}$-citrate is driven by a combination of hydrophobic/hydrophilic interaction, electrostatic interactions and salting-out effect.

CORRELATION

Binodal Curve Correlation

We examined the performances of the Merchuk [22] equation (Eq. (6)) and empirical equation (Eq. (7)) [23] for correlating binodal data of {$PEGDME_{2000}$ + K_3citrate + water} system at different pH values,

$$w_1 = a.\exp[b.(w_2)^{0.5}] - c.(w_2)^3 \quad (6)$$

$$w_1 = \alpha + \beta \ln(w_2) + \gamma w_2 \quad (7)$$

where w_1 and w_2 are the mass percentages of the polymer and salt, respectively. Also a, b, c are fitting parameters of Eq. (6) and α, β, γ are fitting parameters of Eq. (7). The fitting parameters for these equations along with the corresponding standard deviation for each pH and T = 298.15 K are given in Table 7. The standard deviations (*sd*) reported in Table 7 indicate that both of Eqs. (6) and (7) are suitable for representing the binodal data; however, smaller *sd* values obtained with Eq. (7) show that this equation has a better performance than the Merchuk equation (Eq. (6)).

Correlation of Tie-lines with Bachman and Hand Equations

The Bachman (Eq. (8)) [34] and Hand (Eq. (9)) [35] equations have been used to correlate and also to check the reliability of experimental tie-lines compositions:

$$w_2^{top} = A + B.\left(\frac{w_2^{top}}{w_3^{bot}}\right) \quad (8)$$

$$\ln\left(\frac{w_2^{bot}}{w_3^{bot}}\right) = A' + B'.\ln\left(\frac{w_s^{top}}{w_3^{top}}\right) \quad (9)$$

where, A, B, A' and B' represent fit parameters. Superscripts "*top*" and "*bot*" indicate top and bottom phases, respectively. By linear fitting, the experimental tie-line data listed in Table 5 with Eqs. (8), and (9), the parameters of Bachman and Hand equations together with the correlation coefficients (R^2) were obtained and given in Table 8. The obtained R^2 values indicate that the measured tie-line data have acceptable consistency.

Partitioning Coefficient Correlation

We adopted the equation proposed by Diamond-Hsu for

Table 7. Values of Parameters of Eq. (6) (a_i, b_i, c_i) and Eq. (7) (α_i, β_i, γ_i), for { $PEGDME_{2000}$ (1) + Tri-potassium Citrate/Citric Acid (2) + H_2O (3)} System at pH = (6.00, 7.00, and 8.00) and T = 298.15 K

pH	a	b	$10^5 \times$ c	sd[a]
		Merchuk equation (Eq.(6))		
6.00	326.1263	-0.7627	-0.7627	0.19
7.00	56.8442	-0.1639	12.619	0.53
8.00	339.88	-0.8102	0.81599	0.43
		Empirical equation (Eq. (7))		
	α	β	γ	sd
6.00	33.3071	-6.3907	-0.03	0.09
7.00	40.3252	-9.6184	0.0675	0.08
8.00	35.0619	-7.3882	-0.0343	0.14

[a] $sd = \left(\sum_{i=1}^{N} (w_{1i}^{cal} - w_{1i}^{\exp})^2\right)^{0.5}$, where w_1 and N represented mass fraction of $PEGDME_{2000}$ and number of binodal data, respectively.

Table 8. Parameters of the Bachman Equation (Eq. (8)) and Hand Equation (Eq. (9)) for {$PEGDME_{2000}$ (1) + Tri-potassium Citrate/Citric Acid (2) + H_2O (3)} System at pH = (6.00, 7.00 and 8.00) and T = 298.15 K

	Bachman equation (Eq. (8))			Hand equation (Eq. (9))		
	A	B	R^2	A′	B′	R^2
pH = 6	0.1586	0.3504	0.997	2.1069	1.0599	0.873
pH = 7	0.1505	0.3521	0.998	2.5959	1.3938	0.981
pH = 8	0.3392	0.1717	0.983	2.4104	1.2337	0.952

modeling of experimental partition coefficients of acetaminophen [24]. Originally, Diamond and Hsu have used this equation [24] for modeling of protein partitioning coefficient. Later, for a drug (cephalexin) partition coefficient correlation, this equation was also used by Shahriari *et al*. [21]. In the studied two-phase system, for partitioning of acetaminophen, the Diamond and Hsu equation can be written as follows:

$$\frac{\ln K_{acet}}{\Delta w(PEGDME_{2000})} = A_1 + A_2.\Delta w(PEGDME_{2000}) \quad (10)$$

where, the parameters A_1 and A_2 are functions of

acetaminophen molar mass, amount of $PEGDME_{2000}$ and the concentration of tri-potassium citrate. $\Delta w(PEGDME_{2000})$ is the mass fraction difference of $PEGDME_{2000}$ in the top and bottom phase. In fitting the experimental partition coefficient data, the Eq. (10) together with the following objective function (Eq. (11)) were used to obtain the A_1 and A_2 and corresponding standard deviations values.

$$of = \frac{\sqrt{\sum_{i=1}^{N}(K_{acet}^{cal} - K_{acet}^{\exp})}}{N}, (N \text{ is the number of tie-lines}) \quad (11)$$

The results are collected in Table 9. From the standard deviations reported in Table 9, we conclude that the Eq. (9) has a good performance in representing the partitioning coefficients of acetaminophen in the aqueous two-phase system containing $PEGDME_{2000}$ and tri-potassium citrate at different pH values and 298.15 K.

CONCLUSIONS

The phase diagram for an aqueous two-phase system composed of $PEGDME_{2000}$ + potassium citrate was determined at different medium pH values (6.00, 7.00 and 8.00) and T = 298.15 K. Then, the efficiency of this system was evaluated for partitioning of acetaminophen at different aqueous medium pH values and different phase compositions. On the basis of the results, two-phase area is increased with an increase in aqueous medium pH value of 6.00 to 8.00. According to the partition coefficient of drug obtained, partition of acetaminophen is increased with increasing the pH values. Furthermore, it was found that the extraction percentage of acetaminophen is increased with an increase in aqueous medium pH value and tie-line length. This study indicated that hydrophobic interactions were the main driving force, although salting-out effect and electrostatic characteristics were also important for the transfer of the acetaminophen from the salt-rich bottom phase to polymer-rich top phase. The Merchuk and semi-empirical equations were successfully used for correlating the experimental data of binodal curve at different pH values. The correlating of binodal curves displayed that the semi-empirical equation has more efficiency than the Merchuk equation. Also, the results of correlation of obtained tie-lines with the Hand and Bachman equations indicated the reliability of experimental data. Finally, the equation proposed by Diamond-Hsu was used for modeling the experimental data of the partition coefficients of drug. The results indicated that the proposed equation has good performance for modeling of experimental partition coefficients.

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