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Partitioning of Cephalexin in Aqueous Two-Phase Systems Containing Poly(ethylene glycol) and Sodium Citrate Salt at Different Temperatures

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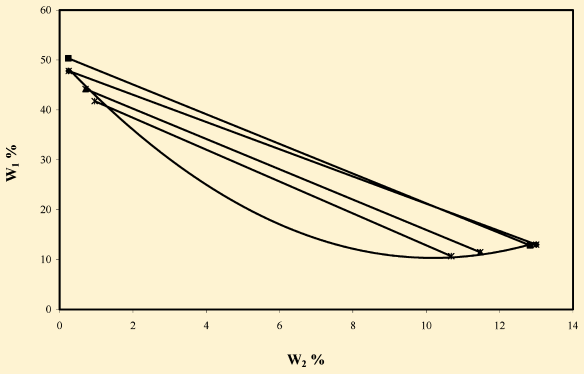
ABSTRACT: In this study, the partitioning process of cephalexin antibiotic in aqueous two-phase systems (ATPS's) containing poly(ethylene glycol) (PEG) with molar masses of (1500 and 6000) g·mol^{-1} and sodium citrate salt has been measured. The experimental partition coefficients were measured at three temperatures: (298, 303, and 308) K. The effects of temperature, polymer mass fraction, polymer molar mass, and salt mass fraction on the experimental partition coefficient of cephalexin were also studied. The results showed that the polymer molar mass has a large effect on the partition coefficient and the temperature influence is almost negligible. The experimental data of the partition coefficients of cephalexin in polymer−salt ATPS's were correlated using the equation proposed by Diamond−Hsu.



INTRODUCTION

Cephalexin is one of the most important antibiotics and has many applications in medicine. Cephalexin is a member of the first generation cephalosporin class of antibiotics. This drug substance is soluble in water, and it is relatively resistant to changes in pH and temperature. For the production and commercialization of antibiotics, the purification value of antibiotics has to be confirmed by pharmaceutical sectors such as the Food and Drug Administration (FDA). Nowadays, the production of antibiotics is increasing rapidly, but separation and purification are the cause of existing problems. This process includes a large part of the final cost of drug. In most cases, antibiotics separation is based on methods which emphasize obtaining an integrated product with a high purity. Nevertheless, finding a purification method that decreases the cost of production has a specific importance as well. Recycling and purifying antibiotics using aqueous two-phase systems (ATPS's) can be an important method for separating such biological materials. Economically, antibiotics extraction and separation by using ATPS's can be more economical than the processes such as chromatography or deposition. In addition, wasting antibiotics in these systems is very low. Lee and Sandler performed research in the field of antibiotics separation in the systems of poly(ethylene glycol)−dextran and poly(ethylene glycol)−phosphate.[1] Some studies were also performed on antibiotics purification in the poly(ethylene glycol)−sodium phosphate ATPS's by Yixin et al.[2] Wei et al. performed research on cephalexin synthesis and the synthesis separation in the poly(ethylene glycol)−sulfate magnesium−water ATPS's.[3] The research conducted on this issue showed that a large number of antibiotics are separable by ATPS's. In addition, these systems provide an appropriate environment for antibiotics synthesis cultivation, and they finally provide a suitable environment for synthesis separation from the environment of production because there is enough water in these systems in all steps of the process which will allow antibiotic activity. By using this method, not only the antibiotics can be produced but also this method is useful for separating valuable materials which exist in the effluent coming from pharmaceutical factories.

The ATPS's containing polymer and salt are widely used in the separation and purification of biological compounds such as amino acids, proteins, and enzymes.[4] It should be noted that the ATPS's consist of two different polymers such as poly(vinyl alcohol) (PVA) and dextran (DEX), or these systems include one polymer and one salt such as poly(ethylene glycol) (PEG) and sodium sulfate (Na_2SO_4). In ATPS's, both phases are water-rich. In these systems, the densities of both phases are approximately equal, and the interfacial tension between two phases is very low.[5]

Grossman et al. studied the partitioning of small amounts of amino acids, simple peptides in the ATPS's of dipotassium hydrogen phosphate, and PEG with molar masses of (6000 and 35000) g·mol^{-1} at 293 K.[6]

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Liu et al. measured the partition coefficients of penicillin G in the ATPS's containing ionic liquids and phosphate.[7]

Salabat et al. measured the partitioning of some amino acids in the PEG−salt ATPS's in the absence and presence of surfactant at 298 K.[8,9]

In addition, Louros et al.[10] and Claudio et al.[11] studied the extraction of biomolecules, for example, vanillin, using the ionic liquid-based ATPS's.

Recently, our group has studied successfully the partitioning process of enzymes (β-amylase and amyloglucosidase) and amino acid (L-lysine). In addition, the pharmaceutical compound (penicillin G−asylase and cephalexin) in the PEG−salt ATPS's was measured.[12−16]

The partitioning of a biomolecule in ATPS's is determined by the type of salt, the molar mass of polymer, the temperature of system, the polymer structure, the size of biomolecule, tie line length (TLL), and other factors.

In this research, the partition coefficients of cephalexin in PEG and the sodium citrate ATPS's were measured. In addition, the effect of polymer and salt concentrations, the molar mass of polymer, the temperature, and the TLL were considered on partition of cephalexin in ATPS.

EXPERIMENTAL SECTION

Materials. Poly(ethylene glycol) ($C_{2n}H_{4n+2}O_{n+1}$; CAS Registry No.: 25322-68-3) with molar masses of (1500 and 6000) g·mol^{-1} was purchased from Sigma-Aldrich (St. Louis, MO), and sodium citrate ($Na_3C_6H_5O_7$; CAS Registry No.: 6858-44-2) with a purity of 99 % was purchased from Merck (Darmstadt, Germany). Cephalexin ($C_{16}H_{17}N_3O_4S$; CAS Registry No.: 15686-71-2) was provided from Jaber Ebne Hayyan Company (Tehran, Iran). Distilled, deionized water with a conductivity of 0.055 μS·cm^{-1} was used to prepare the solutions in all of the experiments.

Method. The experimental method for the measurement of the biomolecule partition coefficients in PEG−salt ATPS's was explained in previous works.[12−15] For each experiment the ATPS's were prepared by mixing deionized water, polymer, salt, and antibiotic with a specific mass fraction in a beaker with 100 cm^3 volume. A magnetic stirrer was used to shorten the equilibrium time. After 30 min the mixtures were left without stirring for almost 24 h to make sure that the equilibrium condition was attained. The temperature of the beaker was controlled in an incubator (Memmert, Germany) with an accuracy of ± 0.01 °C. The temperature of the mixture was controlled in an incubator. The incubator maintains an optimal temperature. The incubator is equipped with a temperature control system with an accuracy of ± 0.01 °C. Incubator applications are diverse because of the number industries and protocols they are required for, such as biochemical studies and bacteriology. Three design features differentiate laboratory incubators: temperature range, temperature uniformity, and chamber size. Temperature uniformity in incubators is calculated by using a multiprobe temperature reader, mapping the temperature of the chamber from top to bottom simultaneously, and then calculating the range.

Then after 24 h the solution reached final phase equilibrium, and samples of the top and bottom phases were removed by a Pasteur pipettes. The glass Pasteur pipet was used to separate the phases. They are usually glass tubes tapered to a narrow point and fitted with a rubber bulb at the top.

Quantitative Analysis. Flame photometry (Sherwood 410, from Cambridge, England) was used for measuring the mass fraction of Na_3 citrate.

The mass fraction of PEG was assessed by refractive index measurements using a digital ABBE refractometer (model DR-A1, from Atago, Japan). The correlation between the refractive index (n_D) and the mass fractions of polymer (w_p) and salt (w_s) can be expressed as the following relation:

$$n_D = \alpha_0 + \alpha_1 w_p + \alpha_2 w_s \quad (1)$$

The parameters of eq 1 and standard deviations are shown in Table 1 for different molar masses of PEG.

Table 1. Parameters of Refractive Index Introduced into Equation 1

component	α_0	α_1	α_2	$100\sigma^a$
water	1.3325			
PEG 1500		0.1430		0.004
PEG 6000		0.1471		0.005
Na_3 citrate			0.1424	0.008

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

The mass fractions of cephalexin in the top and bottom phases were obtained by using a UV−vis spectrophotometer (model: Cary 300, Varian, Santa Clara, CA, USA). The concentration of cephalexin was measured at 262 nm against the blanks with the same composition as the samples, but without any antibiotic, to avoid the interface of PEG and salt.

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

Modeling. The experimental data of the partition coefficients produced in this study were modeled with the equation proposed by Diamond and Hsu.[17]

The partition coefficient (K_{ceph}) of cephalexin in ATPS's can be obtained as follows:[12−16]

$$K_{ceph} = \frac{w_{ceph}^{top}}{w_{ceph}^{bottom}} \quad (2)$$

where, in eq 2, w_{ceph} is the mass fraction of cephalexin.

A linear correlation between the mass fractions of polymers in the two phases and the natural logarithm of the partition coefficients was first proposed by Diamond and Hsu based on the lattice theory of Flory−Huggins.[17] The equation takes the following form:

$$\frac{\ln K_{ceph}}{\Delta w(PEG)} = A + B\cdot\Delta w(PEG) \quad (3)$$

In this equation, A is a function of protein molar mass, phase-forming polymers, protein−water interaction parameters, protein−polymer, polymer−water, pH, the type of salt, and the concentration of salt. Similarly, B is a function of protein molar mass, water−polymer interaction parameters, pH, the salt type, and the salt concentration.

From eq 1 a plot of $(\ln K_{ceph})/(\Delta w(PEG))$ vs $\Delta w(PEG)$ gives a straight line. The slope and intercept gives A and B.

The mass fraction difference of PEG in the top and bottom phase was shown by $\Delta w(PEG)$.

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Table 2. Mass Fractions of PEG 1500 (1) + Na_3 Citrate (2) + Cephalexin (3) in the Top and Bottom Phases and Partition Coefficients of Cephalexin in ATPS's

	feed			top phase			bottom phase				
T/K	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$	100TLL	K
					PEG 1500 + Na_3 Citrate						
298 ± 0.01	26.467	11.395	0.024	43.864	1.598	0.0242	21.850	11.906	0.0239	24.308	1.011
298 ± 0.01	26.468	11.396	0.012	43.984	1.617	0.0245	21.676	11.730	0.0086	24.493	2.856
298 ± 0.01	27.057	9.418	0.024	40.755	2.051	0.0430	19.997	11.309	0.0142	22.729	3.027
298 ± 0.01	26.611	12.923	0.026	33.923	3.223	0.0440	16.425	11.245	0.0258	19.249	1.705
303 ± 0.01	26.467	11.395	0.024	44.102	1.499	0.0246	21.002	11.915	0.0238	25.340	1.033
303 ± 0.01	26.468	11.396	0.012	44.070	1.601	0.0250	21.082	11.905	0.0080	25.192	3.104
303 ± 0.01	27.057	9.418	0.024	40.840	2.035	0.0435	19.443	11.374	0.0132	23.347	3.288
303 ± 0.01	26.611	12.923	0.026	34.207	3.219	0.0475	16.311	11.218	0.0256	19.602	1.853
308 ± 0.01	26.467	11.395	0.024	45.256	1.393	0.0300	20.431	11.997	0.0221	26.995	1.359
308 ± 0.01	26.468	11.396	0.012	45.322	1.468	0.0275	20.408	12.020	0.0070	27.056	3.918
308 ± 0.01	27.057	9.418	0.024	42.020	1.974	0.0466	18.646	11.402	0.0113	25.204	4.124
308 ± 0.01	26.611	12.923	0.026	35.694	2.990	0.0495	15.752	11.288	0.0249	21.600	1.985

Table 3. Mass Fractions of PEG 6000 (1) + Na_3 Citrate (2) + Cephalexin (3) in the Top and Bottom Phases and Partition Coefficients of Cephalexin in ATPS's

	feed			top phase			bottom phase				
T/K	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$	100TLL	K
					PEG 6000 + Na_3 Citrate						
298 ± 0.01	26.467	11.395	0.024	47.178	0.352	0.0240	20.669	11.514	0.0240	28.763	1.000
298 ± 0.01	26.468	11.396	0.012	46.436	0.486	0.0230	21.133	10.894	0.0091	27.360	2.538
298 ± 0.01	27.057	9.418	0.024	44.147	0.604	0.0400	18.125	11.473	0.0156	28.200	2.558
298 ± 0.01	26.611	12.923	0.026	39.002	1.424	0.0415	16.024	10.273	0.0256	24.623	1.621
303 ± 0.01	26.467	11.395	0.024	47.822	0.249	0.0242	18.960	12.998	0.0239	31.552	1.008
303 ± 0.01	26.468	11.396	0.012	50.346	0.240	0.0240	21.567	12.833	0.0095	31.413	2.509
303 ± 0.01	27.057	9.418	0.024	44.181	0.709	0.0405	17.378	11.472	0.0147	28.883	2.760
303 ± 0.01	26.611	12.923	0.026	41.766	0.957	0.0435	15.902	10.680	0.0255	27.631	1.705
308 ± 0.01	26.467	11.395	0.024	50.729	0.125	0.0265	19.673	13.035	0.0233	33.633	1.137
308 ± 0.01	26.468	11.396	0.012	50.731	0.123	0.0248	19.808	12.966	0.0085	33.484	2.912
308 ± 0.01	27.057	9.418	0.024	47.226	0.372	0.0450	17.321	12.374	0.0139	32.224	3.237
308 ± 0.01	26.611	12.923	0.026	44.742	0.481	0.0450	15.732	11.487	0.0254	31.027	1.771

The following objective function was used to obtain the parameters of eq 3:

$$\text{rmsd} = \frac{\sqrt{\sum_{i=1}^{N} \left(K_{\text{ceph}}^{\text{expt}} - K_{\text{ceph}}^{\text{calc}}\right)_i^2}}{N} \quad (4)$$

Notably, in eq 4, N is the number of tie lines.

RESULTS AND DISCUSSION

The experimental data for the partition coefficients of antibiotics and the mass fractions of Na_3 citrate, PEG(1500 and 6000), and cephalexin in the top and bottom phases at temperatures T = (298, 303, and 308 ± 0.01) K are shown in Tables 2 and 3. As can be seen, the mass fraction of cephalexin in the top phase (polymer rich) is more than that of cephalexin in the bottom phase (salt rich). It can be seen in Tables 2 and 3 that cephalexin tends toward the top phase; therefore, the partition coefficients are greater than unity ($K_{\text{ceph}} > 1$).

In addition, the results presented in Tables 2 and 3 emphasized that the mass fractions of compounds in feed have an important effect on the partitioning of cephalexin. Therefore, it can be concluded that an increase in the molar mass of PEG can decrease the partition coefficients of cephalexin. The reason for such a decrease in the partition coefficients can be attributed to decrease in the free volume available for antibiotic molecules in phase.

The results reported in Tables 2 and 3 indicated that the partition coefficients of cephalexin increased with increasing temperature of the ATPS's. The experimental data of the partition coefficients were correlated with the equation proposed by Diamond and Hsu. The results in Table 4 show that the Diamond and Hsu equation which is based on the lattice theory of Flory–Huggins model can accurately correlate the experimental data collected in this work.

Table 4. Values for the Constant Parameters A and B Introduced in Equation 3 along with the rmsd of the Model from the Experimental Data of Partition Coefficients

system	A	B	rmsd
PEG 1500 + Na_3 citrate	3.486	0.609	0.297
PEG 6000 + Na_3 citrate	4.023	−6.033	0.220

The binodal curve and tie line of PEG 6000 + Na_3 citrate + water at 303 K are shown in Figure 1.

The effect of molar mass of PEG on the separation of the ATPS's containing PEG + Na_3 citrate + water is represented in Figure 2. According to Figure 2, by increasing the PEG molar

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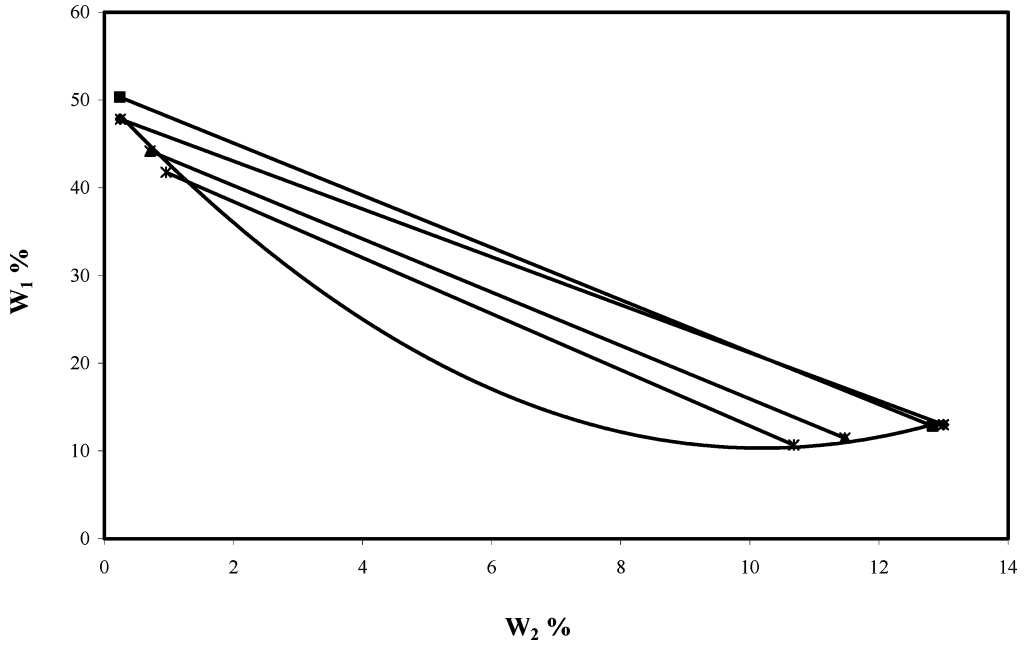

Figure 1. Binodal curve and tie lines of PEG 6000 (1) + Na_3 citrate (2) ATPS's at 303 ± 0.01 K.

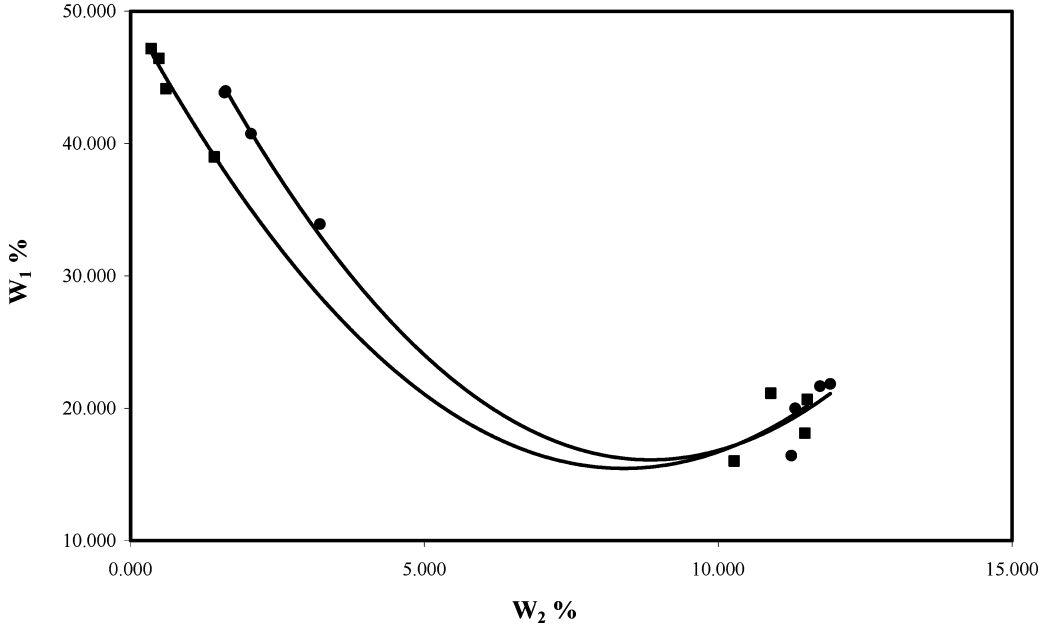

Figure 2. Effect of PEG molar mass on the two-phase separation in PEG (1) + Na_3 citrate (2): ●, PEG 1500; ■, PEG 6000; —, binodal curves at 298 ± 0.01 K.

mass, the binodal curves shift toward a lower mass fraction of PEG and salt.

By comparing the results reported in the present study and our previous work,[12] it is concluded that the partitioning of cephalexin in the ATPS containing a low molar mass PEG (1500) is better than the ATPS containing a high molar mass PEG (4000 and 10000).

The effect of temperature on binodal curves for the ATPS of PEG 6000 + Na_3 citrate + water is presented in Figure 3. As shown in Figure 3, the slope of equilibrium tie lines increases with an increase in temperature. Voros et al. have observed this behavior previously for the similar ATPS's.[18] Also, it should be pointed out that the temperature can change the mass fractions of each component in the top and bottom phases. Thus, the slope of the tie line can be changed with temperature.

The variations of the mass fractions of PEG in the top phase in connection with the variations in the TLL are reported in Figure 4. As shown, the mass fraction of polymer increased with an increase in temperature and, in turn, decreased in the bottom phase.[18]

The mass fraction of salt in the top phase based on the TLL is shown in Figure 5. As it is observed, the mass fraction of salt decreases in the top phase as temperature increases.[18]

The partition coefficient of cephalexin was studied as a function of TLL in PEG and Na_3 citrate ATPS's, which contained the different concentrations of PEG and Na_3 citrate.

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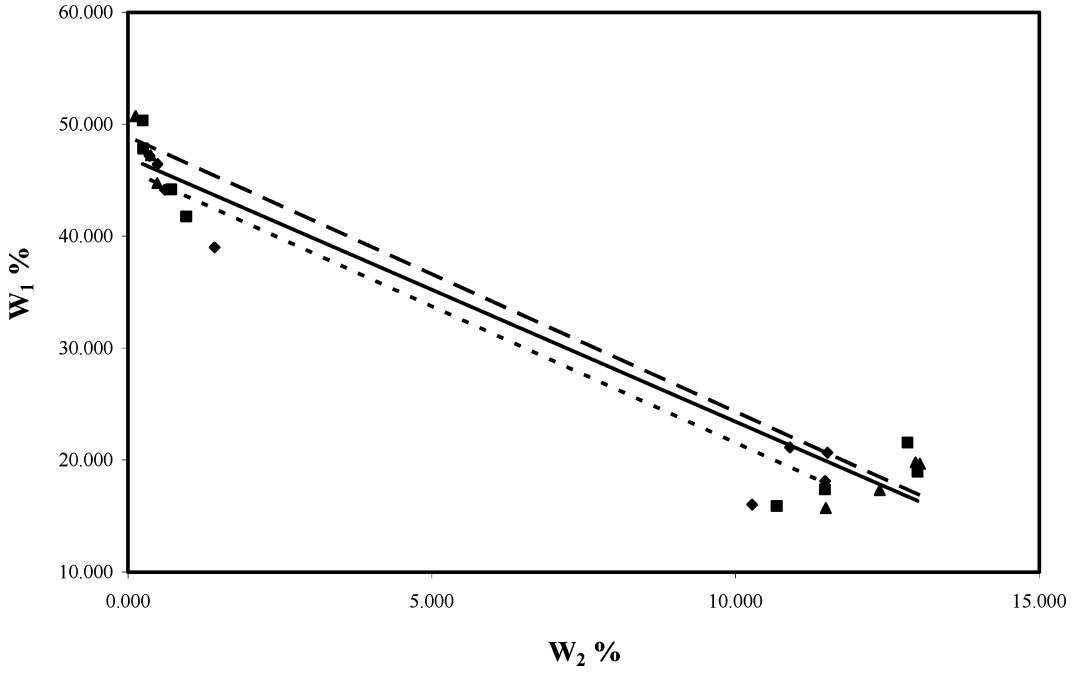

Figure 3. Effect of temperature on two-phase separation in PEG 6000 (1) + Na_3 citrate (2) ATPS's: ♦, T = 298 ± 0.01 K; ■, T = 303 ± 0.01 K; ▲, T = 308 ± 0.01 K; —, ---, binodal curves.

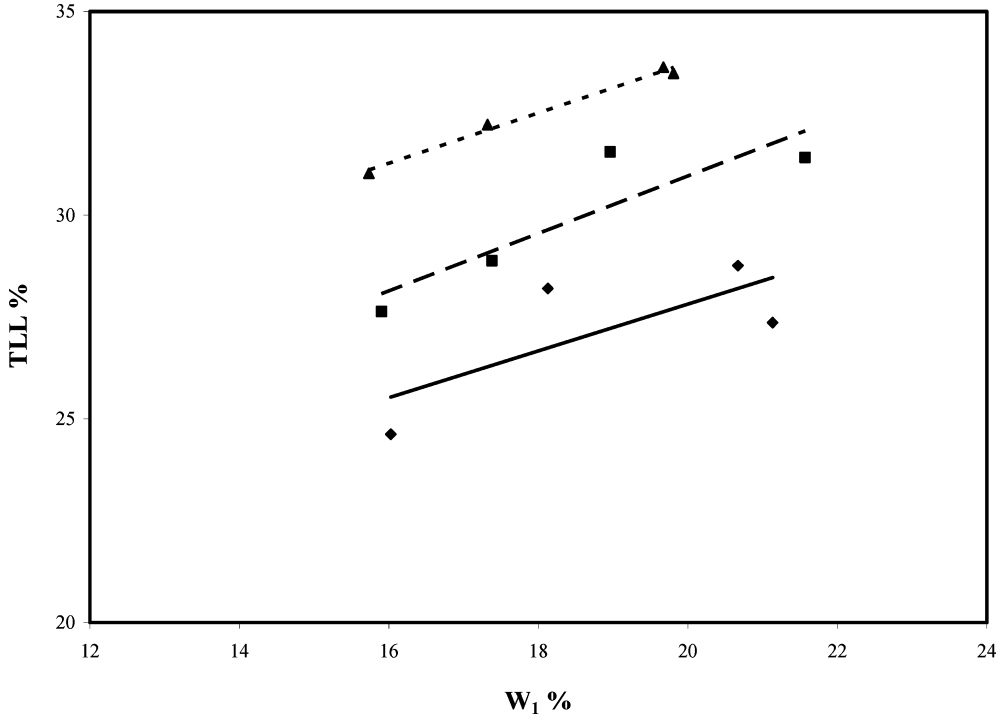

Figure 4. Effect of temperature on the TLL (TLL %) in PEG 6000 (1) + Na_3 citrate (2): ♦, T = 298 ± 0.01 K; ■, T = 303 K; ▲, T = 308 ± 0.01 K.

Figure 6 shows the variation of partition coefficients versus the TLL. The TLL can be given according to the following equation:

$$\mathrm{TLL} = [(w_1^{\mathrm{top}} - w_1^{\mathrm{bottom}})^2 + (w_2^{\mathrm{top}} - w_2^{\mathrm{bottom}})^2]^{1/2} \quad (5)$$

wherein subscripts 1 and 2 refer to polymer and salt, respectively. As it is observed from this figure, by increasing the TLL, the partition coefficient increases. When increasing the TLL, the two-phase region is widened, so that the recovery of antibiotics can increase.

■ CONCLUSIONS

In this research, the partitioning of cephalexin was evaluated in the polymer−salt ATPS's containing PEG with different molar masses in the presence of Na_3 citrate at different temperatures. The experimental data showed that the partition coefficients of cephalexin decreased by increasing the molar mass of PEG. In

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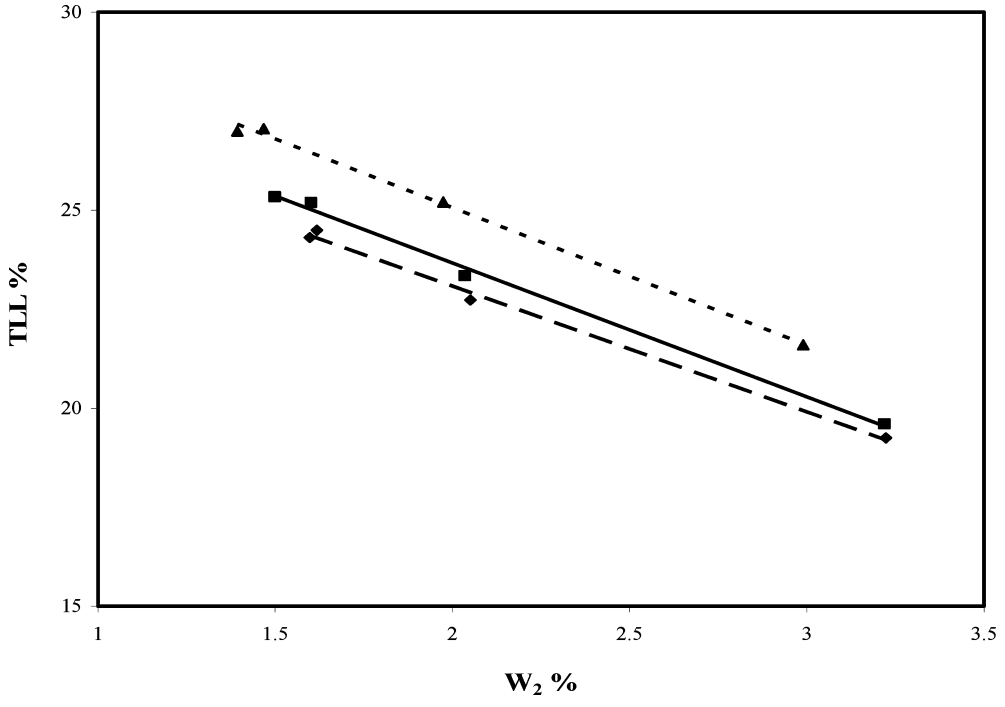

Figure 5. Effect of temperature on the TLL (TLL %) in PEG 1500 (1) + Na_3 citrate (2): ♦, T = 298 ± 0.01 K; ■, T = 303 ± 0.01 K; ▲, T = 308 ± 0.01 K.

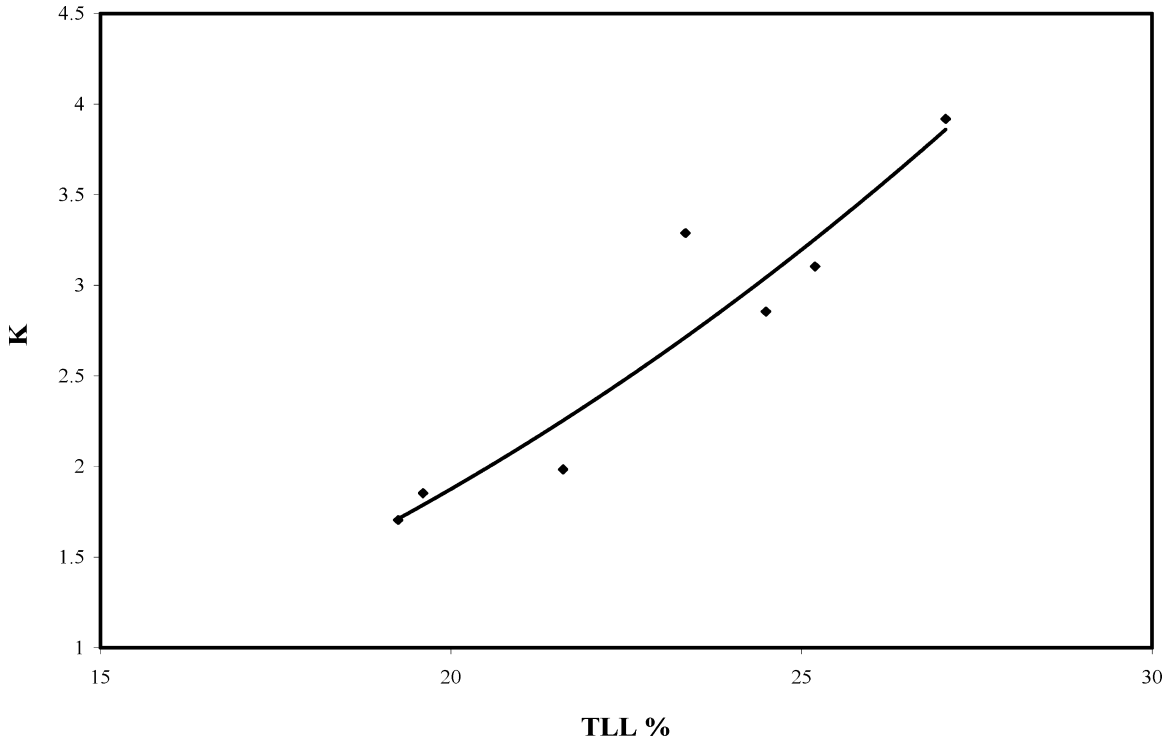

Figure 6. Partitioning of cephalexin in PEG 1500 + Na_3 citrate ATPS's versus the TLL.

addition, the mass fraction of salt in initial feed had a significant effect on the partitioning of cephalexin. Also, the partition coefficients of cephalexin increased by increasing the length of tie line. In addition, the experimental results were modeled using the Diamond and Hsu equation. The results showed that the proposed model had a good agreement with the experimental data of partition coefficients.

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