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Aqueous two-phase systems for cephalexin monohydrate partitioning using poly ethylene glycol and sodium tartrate dihydrate: Experimental and thermodynamic modeling

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Abstract−The partitioning of cephalexin monohydrate in aqueous two-phase system (ATPS) of polyethylene glycol (PEG) with different molecular weights (1000, 2000, 6000, and 8000) and sodium tartrate dihydrate salt was investigated. Equilibrium compositions of two liquid phases were obtained in each system. The experimental data were correlated using the extended NRTL and a modified UNIQUAC (UNIQUAC+DH) models by adding a Debye-Hückel term (DH) to original models for taking into account the long range forces term of activity coefficient of components. The results show that the molecular weight of the polymer has a considerable effect on the partition coefficient of cephalexin monohydrate. The comparison of results for presented models with the experimental data demonstrates that the investigated thermodynamic models can correlate the equilibrium composition very well. The extended NRTL model with AARD of 0.099 is superior to UNIQUAC+DH.

Keywords: Cephalexin Monohydrate, Aqueous Two Phase System, Thermodynamic Modeling, Partition Coefficient

INTRODUCTION

Downstream processing of biomolecules usually encompasses recovery, isolation, purification and polishing (RIPP). Among these, purification is the most expensive and important one [1]. In the past half century, researchers have focused special attention on the recovery and purification of biomolecules by liquid-liquid extraction. Liquid-liquid extraction, which usually is done by an organic solvents, is not suitable for biological processes. Since organic materials are not compatible solvents with the biomolecules and may change or denature them, aqueous two-phase system (ATPS) is proposed as a valuable method for separation and purification of such molecules [2-6]. Usually, ATPS was constructed by mixing two different water-soluble polymers [7] or one water-soluble polymer and a salt [8]. However, some new biphasic systems have been constructed using ionic liquids, and the salt [9] or ionic liquid and organic solvents [10] have been categorized as ATPS. For example, Li et al. determined phase diagrams of aqueous two-phase systems composed of N-butylpyridinium nitrate as IL, ammonium sulfate and sodium sulfate salt and water at different temperatures. Yu et al. studied the conditions for separation and purifying papain in ATPS consisting of IL [11]. Some IL are toxic, and organic solvents usually have denaturing properties that may be inappropriate for processing of biological products. So, traditional ATPS with biocompatible PEG and salt seems to be preferable. Also, surface-active substances prepared in certain conditions were applied to form ATPS [12-14]. For example, ATPS composed of Tween 20 surfactant and three different salts at 298.15 K was studied by Madadi et al. [12]. Aqueous two-phase system is a special case of liquid-liquid extraction. When the limiting concentrations of components are exceeded, two immiscible aqueous phases could be formed [15]. This system makes the transfer of a solute from one aqueous phase to another possible. Electrostatic characteristics and construction of components in each phase lead to association and selection of a phase for target molecule and separation of impurities [16].

Aqueous two-phase system was found for the first time in 1896 by the German microbiologist, Byjrynk [17]. He mixed appropriate amounts of agar and gelatin solution and observed that two separated phases were formed eventually. In 1958, Albertson used these systems for separation of plant cells. This discovery provided the development of this technique. So, separation of different materials such as plant, animal cells and viruses [15,18] was performed. ATPS is a potentially effective technique that has been studied widely in the field of biotechnology. Separation and purification of biological materials, such as proteins [19], enzymes [20], amino acids [21] and virus, were studied using ATPS. The simplicity of the process and low cost of materials for formation of phases allow this method to be applicable in large-scale purification [22]. Many studies have been done in aqueous two-phase systems, and the influence of various factors on phase formation and partitioning of materials in phases were investigated. Different factors, such as molecular weight of polymer [23], pH [24,25], temperature [10,26-29], Salt concentration [30], biomolecule size and its surface properties [31], affect the partitioning of biomolecules in ATPS. Partitioning of some biomolecules such as lysozyme, bovine serum and albumin in systems of PEG (1500, 4000) and dipotassium phosphate or sodium

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sulfate was studied by Haghtalab et al. [32]. Duran et al. studied the partition coefficient of Molybdate ion at different pH and temperatures for a system containing copper sulfate, PEG 4000 and water [33].

Antibiotics are a group of drugs used in the medical and veterinary treatment of infections [34,35]. Cephalexin monohydrate, one of the most important antibiotics, has many applications in medicine. Also, it can treat a number of bacterial infections. Cephalexin monohydrate is a beta-lactam antibiotic and is counted as member of the first generation cephalosporin class of antibiotics. It kills gram- positive and some gram-negative bacteria by disrupting the growth of the bacterial cell walls [36]. This drug is relatively resistant with respect to changes of the pH and temperature. Process of purification of antibiotics is very important because it includes a large part of the final cost of the drug [37]. The production of antibiotics is increasing rapidly. Nevertheless, finding a purification method that decreases the cost of production has a specific importance as well. The aqueous two-phase system could be an important method for separating such biological materials from impurities that exist in the solution during the biological production [38]. In fact this process as a liquid-liquid extraction method causes the separation and concentration of the antibiotic in one aqueous phase [39]. In many cases of antibiotic production, ATPS could be applied to separate undesirable materials from the culture and concentrate the target product at the same time. The process seems to be less expensive than alternative methods such as ultrafiltration [40]. The benefit of ATPS is that it does not contain any organic solvent that is usually toxic and incompatible with biological product. Also, this system (ATPS) could be an important method for separating such biological materials, and it can make the following process of chromatography more effective [41].

While many ternary equilibrium data have been published for various polymer-salt-water ATPS, quaternary aqueous two-phase systems for partitioning of cephalexin as fourth component are limited. Doozandeh et al. investigated the partitioning of cephalexin in system of PEG (1500 and 6000) and potassium phosphate at three temperatures: 301.15, 306.15 and 311.15 K [42]. Khederlou et al. studied the partition coefficients of cephalexin in systems containing poly ethylene glycol 4,000, 10,000 and potassium phosphate and sodium citrate [43]. Moreover, the effect of nanoparticle additives on partitioning of cephalexin in aqueous two-phase systems containing poly (ethylene glycol) and organic salts was studied by Shoushtari et al. [44].

Beside the experimental efforts, thermodynamic models have been used to predict the phase behavior of ATPS. They are important in order to reduce time, cost, and the need for experimental data in industrial units. Also, thermodynamic models were used to predict behavior of ATPS. While phase equilibria calculations of ternary systems of ATPS have been studied widely by verity of activity coefficient models [26,29,45-49], thermodynamic modeling of quaternary ATPS, containing fourth components such as proteins or antibiotics, are limited.

Polyethylene glycol (PEG) is used as one of the phase forming polymers in ATPS because it is inexpensive, nontoxic, and biodegradable and forms a two-phase system with other neutral polymers as well as salts. On the other hand, quaternary ATPS, containing fourth components such as antibiotics with sodium tartrate and these molecular weights of PEG are not studied yet. Since sodium tartrate is a biodegradable and non-toxic salt, it could be in competition with other salts and may help a better biphasic separation.

In present work, the studied aqueous two-phase systems are quaternary mixtures of sodium tartrate dihydrate salt, polyethylene glycol with different molecular weight of 1000, 2000, 6000 and 8000, water and cephalexin monohydrate at 25 °C. The partition coefficients of cephalexin were reported. In addition, thermodynamic study of this system helps for better understanding and prediction of cephalexin behavior in extraction, concentration and purification after biological production process. Thermodynamic models based on the local composition activity coefficient equations (UNIQUAC+ DH and extended NRTL) were applied by adding a Debye-Hückel term (DH) to calculate partition coefficient of antibiotic in two phases. Comparison of experimental results and thermodynamic modeling has been done.

EXPERIMENTAL

1. Materials

Polyethylene glycol (with molecular weights of 1000, 2000, 6000 and 8000) and sodium tartrate dihydrate ($C_4H_4Na_2O_6 \cdot 2H_2O$) with purity of 99% were purchased from Merck (Germany, Darmstadt). Cephalexin monohydrate with 99% was obtained from Jaber-Ebne-Hayyan Company (Tehran, Iran). All materials were dried in oven to ensure that any absorbed water was eliminated. No further purification was applied for materials. Double distilled deionized water with conductivity of 0.055 μS/cm was used in all of the experiments. Table 1 shows the material used in present study beside their purities and sources.

2. Methods

The cloud-point method was used for determination of the binodal curve data to obtain the region in which two phases can be formed. A concentrated stock of PEG solution is added dropwise to a known amount of a concentrated stock of second component solution (salt). The mixture is clear while one phase is available. At a critical point (cloud point) the solution becomes turbid, which shows the second phase is formed. The composition, just prior to two-phase formation, could be calculated by knowing the amount of added second solution, which provides a point on the binodal curve. The mixture is then diluted to below the cloud point and the procedure is repeated to obtain the next point of the binodal curve data.

Table 1. Source and purity of materials used in present work

Material	Source	Purity
PEG 1000	Merck	0.99
PEG 2000	Merck	0.99
PEG 6000	Merck	0.99
PEG 8000	SIGMA	0.99
Sodium Tartrate dihydrate	Merck	0.99
Cephalexin monohydrate CAS No: 15686-71-2	Jaber-Ebne-Hayyan	0.99

Aqueous two-phase systems were prepared by mixing known amounts of polymer and salt and cephalexin in a beaker. The concentration of materials was chosen in the range which forms two phases based on binodal data. Materials were measured using a digital balance (Sartorius Expert, Germany) with a standard uncertainty of 0.0001 g. Stock aqueous solutions were prepared in th concentration range of 14-24% mass for PEG, 9-15% mass for salt and 0.02-0.07% mass of Cephalexin. Concentration of the biomolecule was chosen extremely low so does not affect the formation of two phases. For example, one of these systems consists of 20.10 g PEG 1000, 14.01 g sodium tartrate and 0.020 g cephalexin plus 65 mL H_2O. In the mentioned range, the cephalexin is completely soluble in water, especially in the presence of polymer and salt. The solution was mixed using a magnetic stirrer for about 30 minutes. Then the stirring of the mixture stopped and the solution was centrifuged for 15 min at 3,600 rpm. Then the solutions were put into a water bath (Water bath WNB 29, Memmert, Germany) with an accuracy ±0.1 °C for 24 hours to maintain the mixture at a fixed temperature of 25 °C. The experiments were performed at 101 kPa. After this time, equilibrium was achieved and samples of the top and bottom phases were removed by a syringe. After removal of samples, it is necessary to determine the mass fraction of each phase that contains polymer, salt, cephalexin and water. A flame photometer (PFP-7 model, Jenway, England) was used to determine the concentration of sodium tartrate dihydrate. Mass fractions of PEG were evaluated by refractive index measurements using a refractometer (model ABBE, from Erma Optical). Since the refractive index of a ternary mixture containing polymer, salt and water depends on the mass fractions of polymer and salt and independent with respect to cephalexin (because of is low concentration), the following linear relation was applied to obtain such relationship:

$$N_D = a_0 + a_1 w_p + a_2 w_s \quad (1)$$

where w_p and w_s are mass fractions of polymer and salt, respectively. Experimental measurement of refractive index at different concentrations showed that a linear relationship exists between refractive index and concentration of salt and polymer. To obtain a_0, a_1 and a_2 as a constant of Eq. (1), a least square method and minimization of absolute average relative deviation (AARD) of calculated refractive indexes (as objective function) were applied. Optimized values of constants of Eq. (1) are listed in Table 2. Eq. (1) can be used as a regression to obtain the unknown concentration of polymer in a sample after determination of its salt concentration by flame photometric method. As can be seen in Table 2 the AARD % of this equation is about 0.05%.

The mass fraction of Cephalexin monohydrate was determined using a UV/vis spectrophotometer (SQ2800, from UNICO, USA) at 262 nm. The Cephalexin monohydrate absorbance was measured based on the blank solutions of salt and PEG. The blanks have the same composition as the samples but without any cephalexin. Also, each test was repeated for two times and the reported data are average values of results. Moreover, the standard uncertainty of the obtained parameters was reported.

Table 2. Parameters of refractive index equation

a_0	a_1	a_2	AARD %
1.3325	0.1403	0.1362	0.0507

$$\text{AARD \%} = \frac{100}{n}\Sigma_1^n \left| (N_D^{exp} - N_D^{cal})/N_D^{exp} \right|$$

n is the number of experimental data points

THERMODYNAMIC FRAMEWORK

Since salt dissociates into ions in ATPS, it should be considered as an electrolyte solution. Positive and negative charges of anion and cation cause electrostatic forces. Besides, the forces between non-ionic species in the mixture should be taken into account. So at least two terms are necessary for calculation of activity coefficient of each component in the mixture. One term includes long range interaction of components which considers electrostatic forces and second term contributes to short range forces in the mixture. Consequently, the activity coefficient of each component could be the sum of two contributions as shown below [50]:

$$\ln \gamma_i = \ln \gamma_i^{LR} + \ln \gamma_i^{SR} \quad (2)$$

where in Eq. (2) subscripts "LR", and "SR" stand for the long-range and the short-range effects, respectively.

In present study, the long-range contribution is described by Debye-Hückel equation. The mean ionic activity coefficient of an electrolyte solution for considering the long range electrostatic forces between ions could be written as [50]:

$$\ln \gamma_{\pm}^{LR} = \frac{|Z_a Z_c| A I^{0.5}}{1 + b I^{0.5}} \quad (3)$$

where Z_a and Z_c are absolute charge numbers of the anions and cations respectively. I is the ionic strength of the solution on the molality scale, given as:

$$I = \frac{1}{2}\Sigma Z_i^2 m_i \quad (4)$$

In the above equation, m_i is molality of the ion i. A and b are Debye-Hückel parameters calculated by the following equations:

$$A = 1.32775 \times 10^5 \left(\frac{d^{0.5}}{(DT)^{1.5}} \right) \quad (5)$$

$$b = 6.369696 \frac{d^{0.5}}{(DT)^{0.5}} \quad (6)$$

where d and D are the density and dielectric constant of the mixture, respectively. These parameters could be related to density and dielectric constants of constituents as follows:

$$D = \Sigma \phi_k D_k \quad (7)$$

$$d = \Sigma \phi_k d_k \quad (8)$$

where, ϕ is salt-free volume fraction of non-ionic species, defined as:

$$\phi_k = \frac{n_k V_k}{\Sigma_{i \neq ion} n_i V_i} \quad (9)$$

PEG is considered as a pseudo-solvent. Dielectric constants of PEG and water are set equal to 2.2 and 78.34, respectively, as reported

in the literature [51]. V_k is molar volume of pure non-ionic species k and n_i is mole number of each non-ionic component in the solution.

Also, the long range term of activity coefficient of non-ionic component is calculated by the following equation [52]:

$$\ln\gamma_i^{LR}=\frac{2AdV_i}{b^3}[1+bI^{0.5}-(1+bI^{0.5})^{-1}-2\ln(1+bI^{0.5})] \quad (10)$$

Calculated values of long-range activity coefficient were converted from molality scale to the mole fraction using the following equation:

$$\ln\gamma_x^{LR}=\ln\gamma_m^{LR}+\ln(1+\upsilon mM_w/1000) \quad (11)$$

M_w is molecular weight of water. m is molality of each ion in the solution and υ shows stoichiometric number of the electrolyte.

Two models were used to calculate short range term of the activity coefficient. UNIQUAC and NRTL equations were applied to take into account the short-range interaction of species. UNIQUAC model could be written as:

$$\ln\gamma_i^{SR}=\ln\frac{\phi_i}{x_i}+\frac{z}{2}q_i\ln\frac{\theta_i}{\phi_i}+l_i-\frac{\phi_i}{x_i}\Sigma_{j=1}^{m}x_jl_j+q_i\left[1-\ln\Sigma_{j=1}^{m}\theta_j\tau_{ji}-\Sigma_{j=1}^{m}\frac{\theta_j\tau_{ij}}{\Sigma_{k=1}^{m}\theta_k\tau_{kj}}\right] \quad (12)$$

$$l_i=\frac{z}{2}(r_i-q_i)-(r_i-1) \quad (13)$$

z is the coordination number and is taken equal to 10. r_i and q_i are the volume and surface parameters of component i respectively. These parameters can be obtained by structural parameters of functional group for each molecules. Available volumetric and surface parameters for materials used in current research were obtained from literature and listed in Table 3. Mentioned parameters for PEG 8000 and sodium tartrate were calculated by the authors using group contribution method. ϕ_i and θ_i are the volume fraction and the surface area fraction of component i respectively, defined as follows:

$$\phi_i=\frac{x_ir_i}{\Sigma_i x_ir_i} \quad (14)$$

$$\theta_i=\frac{x_iq_i}{\Sigma_i x_iq_i} \quad (15)$$

τ_{ij} is called the Boltzmann factor was given as:

Table 3. The volume and surface parameters of material used in this study

Component	r	q	Reference
H_2O	0.92	1.4	[53]
PEG 1000	37.26	31.59	[53]
PEG 2000	73.42	61.55	[53]
PEG 6000	218.7	181.4	[53]
PEG 8000	290.58	241.2	
Cephalexin monohydrate	12.455	10.288	[54]
Sodium tartrate	7.7762	7.3368	

$$\tau_{ij}=\exp\left(\frac{-a_{ij}}{T}\right) \quad (16)$$

where, a_{ij} is adjustable binary interaction parameter of i-j pair and shows energy of interaction between components i and j.

The NRTL activity coefficient model was used for evaluation of liquid-liquid equilibrium in the studied aqueous two phase systems [55]. According to NRTL model, the short-range contribution of activity coefficient is:

$$\ln\gamma_i^{SR}=\frac{\Sigma_{j=1}^{n}x_j\tau_{ji}G_{ji}}{\Sigma_{k=1}^{n}x_kG_{kj}}+\Sigma_{j=1}^{n}\frac{x_jG_{ij}}{\Sigma_{k=1}^{n}x_kG_{kj}}\left(\tau_{ij}-\frac{\Sigma_{m=1}^{n}x_m\tau_{mj}G_{mj}}{\Sigma_{k=1}^{n}x_kG_{kj}}\right) \quad (17)$$

where x_j is mole fraction of the component j and α_{ij} is the parameter related to the nonrandom behavior of the mixture and could vary between 0.20 to 0.47 [50]. Also, it could be considered as an adjustable parameter. Different values were tested for non-randomness parameter. Finally, its value was set equal to 0.38 because this value led to the best results. τ_{ij} is the binary interaction parameter of i-j pair and could be optimized as adjustable parameter of the model for each pair.

$$G_{ij}=\exp(-\alpha_{ij}\tau_{ij}) \quad (18)$$

RESULTS AND DISCUSSION

The effect of PEG molecular weight on (liquid+liquid) equilibrium of PEG and sodium tartrate dihydrate systems is shown in Fig. 1. It can be seen that by increasing the molecular weight of PEG, slight displacements of binodal curves to the left is observable. This happens because the PEG chain length increases. According to the Flory-Huggins' theory for polymeric solutions, an increase in the macromolecular chain of the polymer causes a decrease in configurational entropy of the solution [56,57]. Thus, lower concentrations of polymer are required for phase separation. This may be caused by the increase in the incompatibility between the system components, due to the more hydrophobic character of PEGs of higher molecular weight. Binodal curve can be used to choose

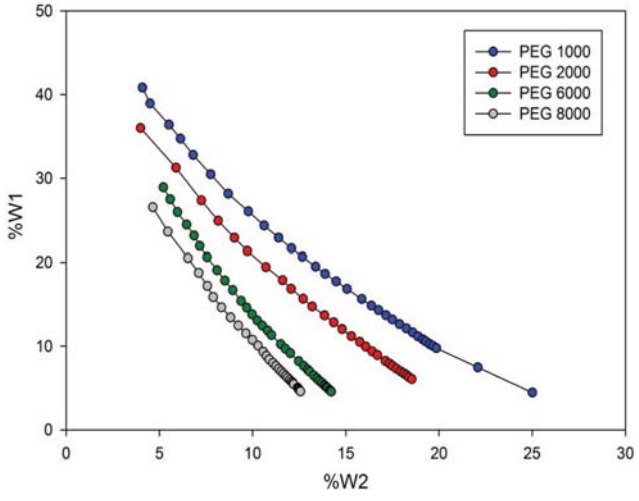

Fig. 1. Binodal curves (polyethylene glycol (1)+sodium tartrate dehydrate (2)+water) ternary system at different molecular weight of PEG at 25 °C.

Table 4. The mass fractions of PEG (1)+sodium tartrate (2)+cephalexin (3) in the top and bottom phases in ATPS as well as the partitioning coefficients, TLL, STL of cephalexin in ATPS at 25 °C

Feed			Top phase			Bottom phase			k	TLL	STL
w_1%	w_2%	w_3%	w_1%	w_2%	w_3%	w_1%	w_2%	w_3%			
PEG 1000											
19.801	13.500	0.024	28.493	6.901	0.025	10.441	20.221	0.016	1.536	0.224	−1.355
20.360	15.026	0.054	32.451	5.934	0.059	10.138	22.317	0.031	1.919	0.277	−1.362
21.141	15.011	0.052	34.277	5.052	0.063	10.289	22.986	0.026	2.454	0.299	−1.337
21.108	14.061	0.042	31.127	6.755	0.046	9.900	21.991	0.025	1.841	0.262	−1.393
22.114	15.047	0.038	35.743	5.025	0.037	8.319	25.707	0.015	2.404	0.343	−1.326
PEG 2000											
20.986	11.007	0.050	25.600	8.166	0.055	3.225	22.297	0.034	1.636	0.265	−1.584
21.075	11.036	0.032	27.702	7.056	0.028	4.545	21.007	0.019	1.435	0.271	−1.659
22.092	10.048	0.021	26.685	7.003	0.025	5.900	20.001	0.014	1.802	0.245	−1.599
22.992	9.9361	0.039	27.502	7.330	0.041	4.135	21.724	0.023	1.774	0.274	−1.623
24.104	10.068	0.043	28.806	7.386	0.043	3.138	23.192	0.023	1.895	0.301	−1.624
PEG 6000											
18.925	10.300	0.046	24.200	7.350	0.044	0.772	21.025	0.042	1.045	0.272	−1.713
19.200	9.150	0.077	22.010	7.680	0.091	0.739	19.056	0.074	1.245	0.242	−1.869
20.049	9.120	0.024	23.500	7.350	0.027	0.988	19.900	0.024	1.140	0.257	−1.794
20.960	10.750	0.036	27.374	7.112	0.058	1.325	22.320	0.041	1.402	0.302	−1.713
21.200	9.501	0.054	25.368	7.056	0.054	1.182	21.780	0.035	1.508	0.283	−1.642
PEG 8000											
14.808	10.082	0.032	18.900	8.280	0.031	3.350	15.021	0.020	1.506	0.169	−2.307
15.637	9.987	0.019	20.102	7.900	0.025	4.039	15.301	0.016	1.527	0.177	−2.170
16.901	9.801	0.034	21.301	7.903	0.035	4.041	15.728	0.026	1.322	0.189	−2.206
17.900	9.740	0.016	22.952	7.546	0.017	3.237	16.483	0.012	1.441	0.216	−2.206
17.501	10.658	0.021	25.200	7.501	0.020	2.889	17.012	0.013	1.546	0.243	−2.346

Standard uncertainties are: u (w_1%)=0.002, u (w_2%)=0.003, u (w_3%)=0.005, u(T)=0.1 K, u(P)=5 kPa

the proper composition of the feed. The feed composition should be chosen at such concentration which forms two phases. Binodal data helps to find what are the proper concentrations of each component in the feed.

Experimental partition coefficients of cephalexin and equilibrium composition of each tie-line for quaternary systems of PEG (1000, 2000, 6000, and 8000), sodium tartrate, water and cephalexin are presented in Table 4. Also, the length of each tie line (TLL) that shows the difference of mass fraction between top and bottom phases was calculated and reported in Table 4. TLL can be obtained by the following equation:

$$TLL = \sqrt{\left(w^{T}_{PEG} - w^{B}_{PEG}\right)^2 + \left(w^{T}_{Salt} - w^{B}_{Salt}\right)^2} \quad (19)$$

According to Table 4, for all systems, an increase in the global mass fractions of PEG and salt leads to an increase in TLL values, which can be mainly attributed to the increase in the mass fraction of PEG in the top phase and the mass fraction of salt in the bottom phase. Also, the obtained experimental results show that the slope of tie line changes with molecular weight of PEG. Slope of each tie line is defined as:

$$STL = \frac{w^{T}_{PEG} - w^{B}_{PEG}}{w^{T}_{Salt} - w^{B}_{Salt}} \quad (20)$$

According to Table 4, the slope of the tie lines (STL) is practically constant for each PEG, which means that tie lines are parallel to each other, thus allowing us to know the coexisting phase compositions for any given total polymer phase-forming composition. The higher STL values were obtained by increasing the molecular weights of PEG. This phenomenon indicates an increase in the difference between the polymer concentrations at a given difference in the salt concentrations. This shows a decrease in the mutual solubility of the aqueous polymer- and salt.

The partition coefficient of cephalexin in aqueous two-phase systems (k) has been defined as [58]:

$$k = \frac{w^{t}_{cep}}{w^{b}_{cep}} \quad (21)$$

where w^{t}_{cep} and w^{b}_{cep} are cephalexin mass fractions in the top and bottom phases, respectively. According to Table 4, the mass fraction of cephalexin in the polymer rich phase (top phase) is more than the salt rich phase (bottom phase). So the partition coefficient of cephalexin is greater than unity; that is interesting when the aim is to extract cephalexin from fermentation, because other particulate matter typically remains in the bottom phase (salt-rich phase).

It is concluded that cephalexin prefers to move into the poly-

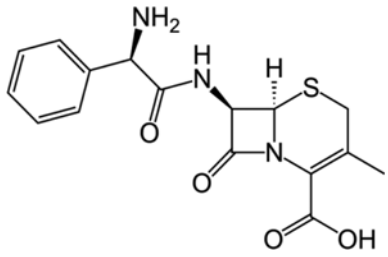

Fig. 2. Chemical structure of cephalexin.

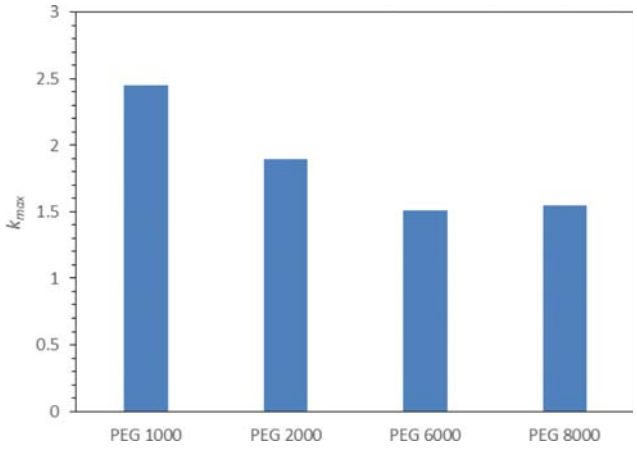

Fig. 3. The maximum values of partition coefficient of cephalexin (k_{max}) using different molecular weight of PEGs.

mer-rich phase in all concentration ranges of the feed. Chemical structure of cephalexin is shown in Fig. 2. There are two NH groups in the cephalexin structure in which can develop hydrogen bonding between the hydrogen and the oxygen atoms. On the other hand, PEG chemical structure has OH groups that are susceptible for hydrogen bonding. Therefore, cephalexin tends to transfer to the top phase. Also, cephalexin has a pI value of about 4.5 to 5 [59]. In this study, pH values of the systems were 6.5-7. Then cephalexin would have a negative surface charge and tend to be in polymer-richer phase. Because, the salt rich phase (bottom phase) has a dominant negative charge (tartrate ion charge 2-) and causes a repulsive effect between the target molecule (cephalexin) and the salt.

Fig. 3 shows the maximum values of partition coefficients of cephalexin between all tie lines have been determined at different molecular weight of PEGs (1000, 2000, 6000 and 8000). To compare the partition coefficients, it is better that the compositions of the feed be the same for different PEGs. However, according to Table 5, the feed compositions of PEG 1000, 2000 and 6000 are closer to each other in contradiction to PEG 8000, which the feed has more different composition. So. the comparison of k_{max} for three former PEGs is more reasonable. It can be seen in three molecular weights of PEG (1000, 2000, and 6000), the partition coefficients of cephalexin decreased by increasing of PEG molecular weight. It can be explained that by increasing the PEG molecular weight, its chain length increases. This effect causes the reduction of the excluded volume, meaning less space available for the cephalexin in top phase and consequent descending the tendency of cephalexin to polymeric phase. Therefore, the partition coefficient of cephalexin decreases with increasing the molecular weight of PEG.

Table 5. Refractive index of PEG+sodium tartrate+cephalexin+water in the top and bottom phases in ATPS at 25 °C

N_D		Top phase	Bottom phase
PEG 1000+salt+water	Tie 1	1.3819	1.3742
+cephalexin	Tie 2	1.3850	1.3770
	Tie 3	1.3875	1.3780
	Tie 4	1.3854	1.3765
	Tie 5	1.3900	1.3792
PEG 2000+salt+water	Tie 1	1.3810	1.3660
+cephalexin	Tie 2	1.3810	1.3675
	Tie 3	1.3795	1.3685
	Tie 4	1.3818	1.3679
	Tie 5	1.3830	1.3685
PEG 6000+salt+water	Tie 1	1.3780	1.3628
+cephalexin	Tie 2	1.3750	1.3595
	Tie 3	1.3770	1.3610
	Tie 4	1.3820	1.3645
	Tie 5	1.3800	1.3645
PEG 8000+salt+water	Tie 1	1.3710	1.3590
+cephalexin	Tie 2	1.3735	1.3585
	Tie 3	1.3735	1.3594
	Tie 4	1.3750	1.3595
	Tie 5	1.3715	1.3580

Standard uncertainty is $u(N_D)=0.0004$, $u(T)=0.1$ K, $u(P)=5$ kPa

The refractive index of polymer, salt and water ternary system depends on the salt and PEG concentrations. Refractive indexes of the mentioned quaternary system for different molecular weight of PEG were determined experimentally and are reported in Table 5. It can be seen that the refractive index in the top phase is higher than bottom phase, because the polymer content in top phase is greater.

Equality of frugalities for each component in top and bottom phases as criteria of equilibrium can be written as follows:

$$(x_i\gamma_i)^t=(x_i\gamma_i)^b \tag{22}$$

The partition coefficients of cephalexin can be calculated as:

$$k=\frac{w_{cep}^t}{w_{cep}^b} \tag{23}$$

where w_{cep}^t and w_{cep}^b show mass fractions of cephalexin in top and bottom phases, respectively. The calculated partition coefficients of cephalexin by extended NRTL and UNIQUAC+DH models along with experimental values are reported in Table 6. To compare the experimental data with results of the model absolute relative deviation (ARD) is used:

$$ARD=\left|\frac{k^{exp}-k^{cal}}{k^{exp}}\right| \tag{24}$$

Absolute average relative deviation of cephalexin partition coefficient was calculated for each model and is reported in Table 6. The results indicate that the extended NRTL model with AARD

Table 6. Experimental and calculated partitioning coefficient (k) of cephalexin by extended NRTL and UNIQUAC+DH models

Molecular weight PEG	k^{exp}	$k^{DH+nrtl}$	$k^{DH+uniquac}$	ARD (NRTL+DH)	ARD (UNIQUAC+DH)
1000	1.5365	1.5477	1.6551	0.00728	0.07718
	2.4542	2.2425	2.1419	0.08626	0.12725
	1.9195	1.9992	2.0653	0.04152	0.07595
	1.841	1.8149	1.8504	0.01417	0.00510
	2.4045	2.1334	1.9699	0.11274	0.18074
2000	1.8018	1.7656	1.7023	0.02010	0.05523
	1.4355	1.7497	1.7341	0.21890	0.20803
	1.7739	1.6558	1.7077	0.06659	0.03733
	1.8947	1.7976	1.7714	0.05124	0.06507
	1.6363	1.6151	1.6139	0.01296	0.01370
6000	1.2450	0.9883	1.0119	0.20618	0.18722
	1.1402	0.9649	0.9963	0.15374	0.12620
	1.4021	0.9815	1.0005	0.29997	0.28642
	1.0456	1.0562	1.0247	0.01013	0.01998
	1.5083	0.9962	1.0080	0.33952	0.33169
8000	1.506	1.3165	1.3662	0.12583	0.09282
	1.5273	1.3536	1.3317	0.11373	0.12806
	1.4413	1.4372	1.4390	0.00284	0.00159
	1.3222	1.3512	1.3485	0.02193	0.01989
	1.5466	1.4099	1.3935	0.08838	0.09899
				AARD=0.0997	AARD=0.1062

$$AARD = \frac{1}{N}\sum_{i=1}^{N}\left|\frac{k_i^{exp} - k_i^{model}}{k_i^{exp}}\right|$$

N is the number of experimental data points

Table 7. The NRTL binary interaction parameters in the PEG (1)+sodium tartrate (2)+water (3)+cephalexin (4) quaternary system

τ_{12}	τ_{21}	τ_{14}	τ_{41}	τ_{23}	τ_{32}	τ_{24}	τ_{42}	τ_{34}	τ_{43}
PEG 1000									
20.2652	14.9845	8.7523	26.6333	−147.5665	−3.3281	13.3617	−4.9954	15.1866	91.2688
PEG 2000									
7.17750	−6.2223	0.1283	1.6617	5.9808	13.6792	−16.5838	2.3656	9.9121	−0.7671
PEG 6000									
0.9183	38.4900	23.8870	−6.8562	−3.9295	12.8234	−13.1905	−0.1837	−6.8614	3.2625
PEG 8000									
−236.5423	15.0419	−14.8549	−9.0293	−47.4340	0.6714	−13.6407	138.9749	−16.3361	1212.4623

equal to 0.0997 could correlate the partition coefficients of cephalexin in studied aqueous two-phase system better than UNIQUAC+DH model with AARD=0.1062.

Since both studied models contain binary interaction parameters, the objective function (RMSD) was minimized to estimate the best set of parameters:

$$RMSD = \sqrt{\frac{1}{N}\Sigma_j\Sigma_i[(w_{ij}^{exp,\,t} - w_{ij}^{cal,\,t})^2 + (w_{ij}^{exp,\,b} - w_{ij}^{cal,\,b})^2]} \quad (25)$$

Rachford-Rice method was used for calculation of LLE. Also, MATLAB software was applied for performing the modeling step. Simplex method was used to optimize interaction parameters of the models.

Note that the binary interaction parameter between water and PEG for NRTL model was set equal τ_{31}=1.8018 and τ_{13}=−1.1787 according to the values reported in the literature [52]. Also, interaction parameter between water and cephalexin for UNIQUAC model was reported by Yousefi et al. [54]. Remaining parameters of the models optimized using the experimental data and are shown in Tables 7 and 8.

A comparison between the experimental data and calculated values of tie-line compositions by UNIQUAC+DH and extended NRTL models at different molecular weight of PEG (1000 and 2000) is depicted in Figs. 4 and 5. Results of modeling show that the extended NRTL model could correlate the equilibrium composition of the studied ATPS better than UNIQUAC+DH model. The RMSD values of NRTL and UNIQUAC models were 0.0299 and 0.0319, respectively. It seems that since in the NRTL model

Table 8. The UNIQUAC binary interaction parameters in (K) for the PEG (1)+sodium tartrate (2)+water (3)+cephalexin (4) quaternary system

a_{14}	a_{24}	a_{41}	a_{42}	a_{31}	a_{13}
		PEG 1000			
250.8181	2229.8453	503.2127	644.8834		
		PEG 2000			
−30.5715	460.3859	355.1219	−176.2255	4.6427	−267.3141
		PEG 6000			
123.2191	20659.3712	14435.3202	2560.1640		
		PEG 8000			
1714.9980	22680.2727	39.2129	16660.6385		

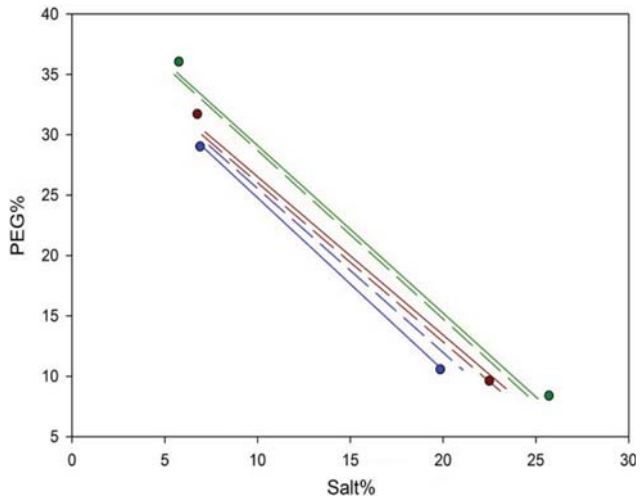

Fig. 4. Mass fraction of the experimental tie line data (●) with the calculated values using UNIQUAC+DH (- -) and extended NRTL model (—) for PEG 1000-sodium tartrate-water-cephalexin quaternary system.

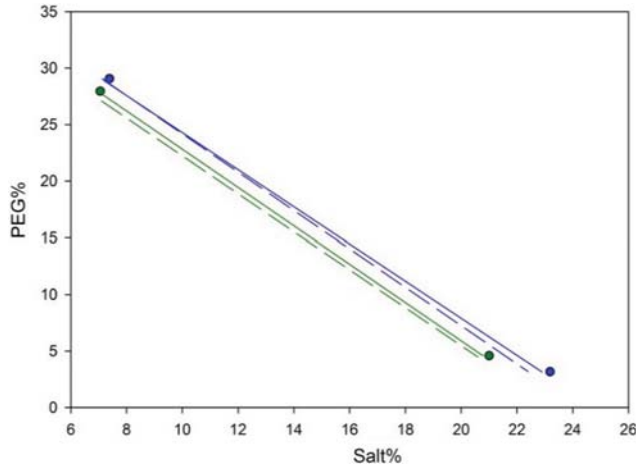

Fig. 5. Mass fraction of the experimental tie line data (●) with the calculated values using UNIQUAC+DH (- -) and extended NRTL model (—) for PEG 2000-sodium tartrate-water-cephalexin quaternary system.

more adjustable parameters were fitted using obtained experimental data, the value of RMSD was lower than that of UNIQUAC model. However, more adjustable parameters means that more calculations are needed and reduces the prediction ability of the model.

CONCLUSIONS

The liquid-liquid equilibrium data of PEG+sodium tartrate+water+cephalexin systems were measured. Experimental data show that partition coefficient of cephalexin in ATPS depends on polymer molecular weight. In all concentration ranges of the feed, cephalexin preferred to move in the polymer-rich phase. The result shows that with increasing the PEG molecular weight, the cephalexin partition coefficient decreases.

Two models, extended UNIQUAC and extended NRTL, were applied to fit the partition coefficients of cephalexin. The proposed models have a good agreement with the experimental data. The extended NRTL model is better than the extended UNIQUAC with AARD=0.0997.

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