Partitioning of Ciprofloxacin in aqueous two-phase system of poly(ethylene glycol) and sodium sulphate

Babak Mokhtarani^b, Ramin Karimzadeh^{a,*}, Mohammad Hassan Amini^b,
Siavash Darvish Manesh^a

^a Department of Chemical Engineering, Tarbiat Modares University, P.O. Box 14115-111, Tehran, Iran

^b Chemistry and Chemical Engineering Research Center of Iran, P.O. Box 14335-186, Tehran, Iran

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Abstract

Extraction by an aqueous two-phase system is a powerful technique for separation, concentration, and purification of biomolecules and pharmaceutical products. In the present study, the partitioning of Ciprofloxacin in aqueous two-phase system of PEG–Na₂SO₄–water has been investigated. The influences of temperature, salt concentration, polymer concentration and polymer molecular weight on the partitioning of Ciprofloxacin were studied. The experiments were designed using an orthogonal central composite design (orthogonal CCD) based on 2³ full factorial and a second-order model was used to determine the effects of different factors on Ciprofloxacin partitioning. The results of the model indicate that the Ciprofloxacin partitioning is highly dependent on salt concentration. Temperature and PEG concentration have moderate effects on partitioning but PEG molecular weight has no significant effect on the antibiotic partitioning.

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1. Introduction

Extraction by aqueous two-phase systems (ATPS) has become an important emerging technique for separation, concentration and purification of proteins, biomolecules and pharmaceutical products. These systems consists of two immiscible aqueous solutions containing different polymers, such as poly(ethyleneglycol) (PEG) and Dextran or one polymer and one salt, such as PEG and ammonium sulfate. Aqueous two-phase systems have some advantages in comparison with other commonly used separation and purification techniques. Advantages can be summarized as the high water content of both phases (70–85%, w/w), high biocompatibility and low interfacial tension, low degradation of biomolecules, good resolution and high separation yield, relatively high capacity, ease of scale-up, low material costs and the possibility of polymer and salt recycling.

Beijernick introduced this technique in 1896 [1]. Albertson reported that some polymers and electrolytes form a two-phase system in a definite concentration [2]. He found when two water-

soluble polymers or a polymer and a strong electrolyte were dissolved in water, a two-phase system is formed, in which one-phase was rich in polymer and the other phase, rich in electrolyte. Albertson investigated the application of these systems for partitioning of cells and macromolecules. In recent years, the separation of proteins and other biomolecules were studied by many researchers as reported by Walter and Johansson [3].

The application of these systems to recovery or purification of antibiotics has been addressed by different researchers. The effects of pH, salt and ligand on partitioning of Vancomycin have been studied by Lee and Sandler [4]. Yang et al. investigated Cephalosporin C partitioning in ATPS [5]. Yixin et al. studied the recovery of antibiotics by these systems [6]. Marcos et al. purified Penicillin acylase by ATPS [7]. Liao et al. studied the extraction of Penicillin acylase by using these systems [8]. The extraction of Cephalexin in ATPS was conducted by Wei et al. [9]. Mahesh and Vilas worked on the Penicillin G partitioning [10]. According to the results of these investigations, the ATPS is clearly a suitable method for antibiotic recovery and purification.

In this research, the partitioning of Ciprofloxacin antibiotic in ATPS of PEG–Na₂SO₄–water has been investigated. Ciprofloxacin is an antibiotic used to treat certain infections caused by a wide spectrum of bacteria. It is in a class of antibi-

* Corresponding author. Tel.: +98 21 88011001; fax: +98 21 88005040.
E-mail address: Ramin@modares.ac.ir (R. Karimzadeh).

Nomenclature

B_i	linear coefficient in the model
B_{ii}	squared coefficient in the model
B_{ij}	interaction coefficient in the model
B_0	constant coefficients in the model
Exp	experimental value
K	the partitioning coefficients of Ciprofloxacin
Model	modeling value
R	The model accuracy
W_i	weight percent of species
x_1	temperature
x_2	the polymer to salt weight percent in feed
x_3	salt weight percent in feed
x_4	Ciprofloxacin weight percent in feed
x_5	the PEG molecular weight
x_{ci}	value of x_i at the center point
Δx	step change
X_i	dimensionless coded value of the variable, x_i

otics called fluoroquinolones. Ciprofloxacin has been used since 1987 for a variety of indications and is the most widely used fluoroquinolone in humans and animals. As far as we know, there is no report on partitioning of Ciprofloxacin using ATPS. There are a number of factors, such as temperature and polymer molecular weight, which influence partitioning of Ciprofloxacin in ATPS. The experimental design is a useful tool for interpreting the effects of these factors. In this research the orthogonal CCD was used to study the effects of temperature, PEG molecular weight, and salt and polymer concentrations on partitioning of Ciprofloxacin in ATPS. From the results of the experimental design, a regression analysis was carried out and a new model for antibiotic partitioning was proposed. The comparison of the experimental data with this model shows that the model can correlate well with the liquid–liquid equilibria in this system.

2. Experiments

2.1. Materials and methods

Poly(ethyleneglycol) with molecular weights of 1500, 2000 and 4000, and sodium sulfate were purchased from Merck. Ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$) with molecular weight of 385.82 and 99.9% purity was provided from TEMAD Company (Tehran, Iran).

Flame photometry (Jenway PFP7) was used to determine Na_2SO_4 concentration. The relative experimental uncertainty in the salt concentration measurement was less than 3%. PEG concentration was determined by refractive index measurements at 298 K using a Kruss Abbe refractometer AR3D. Similar to a previous work, since the refractive index of an aqueous phase in our experiments depends not only on the concentration of PEG but also on the salt concentration, extensive calibrations were performed to determine the influence of PEG and salt on the refractive index of an aqueous solution [11]. The relative

experimental uncertainty for the PEG concentration measurement was less than 3% with a minimum absolute uncertainty of 0.05 mass%. The concentration of Ciprofloxacin in each phase was determined by using a UV (CARY WIN 50) ultraviolet spectrometer at wavelength of 275 nm [12]. As the aqueous solution of salts and PEG absorb some ultraviolet light, so the absorbance coefficient of salts and PEG should be measured in a free antibiotic solution. Furthermore, the Ciprofloxacin absorbance was measured based on the blank solutions of salts and PEG. The relative experimental uncertainty for the antibiotic concentration was less than 7.5%.

2.2. Experimental procedure

The aqueous two-phase systems, in different salt concentrations were prepared by stock solutions of PEG and salt. The concentrations of PEG and Na_2SO_4 in stock solutions were 40 and 15% by mass, respectively. To speed up phase separation, these solutions were mixed using a magnetic stirrer for about 5 min. In order to reach the equilibrium, the solution containers were placed in a water bath at the experimental temperature for 24 h. The samples of the top phases of aqueous two-phase systems were carefully withdrawn at right above the interface. The samples of the bottom phase were withdrawn by using a plastic syringe with long needle. Samples were analyzed to determine the salt, the polymer, and the antibiotic concentrations.

3. Experimental results

Ciprofloxacin partitioning depends on temperature, polymer and salt concentrations and PEG molecular weight. The effect of these factors and their interactions on ATPS is investigated using the employed experimental design. In this research, the orthogonal CCD is applied for ATPS [13]. To simplify the recording and processing of the experimental data, the factor levels have been selected so that the upper level, the lower level and the basic level correspond, respectively, to +1.414, −1.414 and zero. Independent variables, experimental ranges and statistical levels for this system are given in Table 1. The parameters of temperature (x_1), the polymer to salt weight percent in feed (x_2), salt weight percent in feed (x_3) and Ciprofloxacin weight percent in feed (x_4) were chosen as independent variables and the weight percent of PEG, Na_2SO_4 , and Ciprofloxacin in the upper and lower phases were selected as dependent variables. Based on preliminary experiments, the range of independent variables were determined, in a safe region, where the two-phase system was formed. The orthogonal CCD based on 2^3 full factorial experimental designs were performed for three available different molecular weights of PEG 1500, 2000, and 4000, respectively. The response variables (dependent variables) are the weight percent of PEG, Na_2SO_4 , and Ciprofloxacin in the upper and the lower phases. Experimental scheme showing the real values of the independent variables together with equilibrium compositions in upper and lower phases are given in Tables 2–4. In order to calculate the experimental error, three central replicate experiments were carried out for each system. For statistical calculations, the variable x_i was coded as X_i according to the

Table 1
The experimental range and levels for temperature X_1

Levels	−1.414	−1	0	1	1.414
X_1 (temperature)	10.0	13.7	22.5	31.3	35.0
X_2 (PEG per salt weight percent)	1.5	1.6	2.0	2.4	2.5
X_3 (salt weight percent)	8.5	8.9	9.8	10.6	11.0
X_4 (Ciprofloxacin weight percent)	0.005	0.007	0.013	0.018	0.020

PEG per salt weight percent in feed X_2 , salt weight percent in feed X_3 , and Ciprofloxacin weight percent in feed X_4 .

following equation [14]:

$$X_i = \frac{x_i - x_{ci}}{\Delta x_i} \quad (1)$$

where x_{ci} stands for the value of x_i at the center point and the step change Δx_i is the real deviation of x_i from this point. In order to investigate the effect of important parameters on Ciprofloxacin partitioning, a model based on the experimental results were proposed. The behavior of the system was assumed to follow the quadratic equation as below:

$$W = B_0 + \sum B_i X_i + \sum B_{ii} X_i^2 + \sum_i \sum_j B_{ij} X_i X_j \quad (2)$$

where W stands for the dependent variables, X stands for the independent variables and B_0 , B_i , B_{ii} , and B_{ij} are constant coefficients of different variables in the model. In order to investigate the effect of polymer molecular weight on Ciprofloxacin partitioning, the PEG molecular weight was considered as fifth independent variable (x_5) in the model. The results of the experimental design (Tables 2–4) were studied and interpreted by SPSS

11.2 statistical software to estimate the responses of the dependent variables. The levels of PEG molecular weight variable were assumed to be −1.414, −0.850, and 1.414 corresponding with real values of 1500, 2000, and 4000, respectively.

4. Results and discussion

The experimental results show that Ciprofloxacin prefers to stay in the PEG rich phase (the upper phase). Due to the electrostatic effects, the tendency of Ciprofloxacin to stay in the top phase is more than the lower phase. With increasing the salt concentration in feed the concentration of Ciprofloxacin in the lower phase is increases.

Based on Eq. (2), the coefficients of assumed polynomial model and related statistical tests were calculated. The independent variables and their interactions which have been found to be non-significant were removed from the equation by a stepwise regression method, and hence, the Eqs. (3)–(8) were proposed for the weight percent of PEG, salt and Ciprofloxacin in top and bottom phases of the ATPS. As these equations show, the weight

Table 2
The experimental design for equilibrium composition as weight percent w in PEG 1500 (1)– Na_2SO_4 (2)–water (3)–Ciprofloxacin (4) aqueous two-phase system

Number	Temperature (°C)	Feed			Upper phase			Lower phase		
		w_1	w_2	w_4	w_1	w_2	w_4	w_1	w_2	w_4
1	31.3	25.44	10.60	0.018	66.10	0.56	0.0190	5.49	19.68	0.0126
2	31.3	25.44	10.60	0.007	65.86	0.46	0.0080	5.38	19.59	0.0053
3	31.3	25.44	8.90	0.018	46.31	1.30	0.0200	6.20	18.05	0.0078
4	31.3	25.44	8.90	0.007	47.29	1.26	0.0090	6.08	17.89	0.0034
5	31.3	14.24	10.60	0.018	47.93	0.56	0.0170	4.82	18.66	0.0111
6	31.3	14.24	10.60	0.007	47.80	0.49	0.0070	4.71	18.51	0.0028
7	31.3	14.24	8.90	0.018	37.02	2.41	0.0178	7.53	14.65	0.0054
8	31.3	14.24	8.90	0.007	36.74	2.34	0.0079	7.48	14.50	0.0019
9	13.7	25.44	10.60	0.018	66.21	0.65	0.0185	5.70	19.74	0.0125
10	13.7	25.44	10.60	0.007	66.17	0.59	0.0076	5.52	19.65	0.0048
11	13.7	25.44	8.90	0.018	46.86	1.36	0.0195	6.34	18.17	0.0076
12	13.7	25.44	8.90	0.007	46.75	1.33	0.0086	6.17	18.08	0.0033
13	13.7	14.24	10.60	0.018	48.05	0.59	0.0165	4.90	18.79	0.0109
14	13.7	14.24	10.60	0.007	47.97	0.52	0.0066	4.86	18.70	0.0028
15	13.7	14.24	8.90	0.018	37.12	2.44	0.0173	7.67	14.78	0.0050
16	13.7	14.24	8.90	0.007	37.05	2.38	0.0073	7.56	14.68	0.0023
17	22.5	19.60	9.80	0.013	47.93	1.17	0.0140	5.23	17.52	0.0077
18	10	19.60	9.80	0.013	47.97	1.26	0.0139	5.31	17.58	0.0071
19	35	19.60	9.80	0.013	47.82	1.08	0.0144	5.19	17.43	0.0077
20	22.5	12.75	9.80	0.013	41.30	1.20	0.0160	5.59	16.97	0.0065
21	22.5	27.5	9.80	0.013	55.37	0.74	0.0165	4.58	18.97	0.0088
22	22.5	19.60	8.50	0.013	39.16	1.97	0.0145	8.62	15.12	0.0047
23	22.5	19.60	11	0.013	55.68	0.43	0.0140	6.18	19.74	0.0098
24	22.5	19.60	9.80	0.005	47.86	1.17	0.0060	5.30	17.52	0.0035
25	22.5	19.60	9.80	0.020	47.93	1.23	0.0190	5.34	17.55	0.0113

Table 3

The experimental design for equilibrium composition as weight percent w in PEG 2000 (1)–Na₂SO₄ (2)–water (3)–Ciprofloxacin (4) aqueous two-phase system

Number	Temperature (°C)	Feed			Upper phase			Lower phase		
		w_1	w_2	w_4	w_1	w_2	w_4	w_1	w_2	w_4
1	31.3	25.44	10.60	0.018	64.81	0.49	0.0193	4.08	19.07	0.0128
2	31.3	25.44	10.60	0.007	64.56	0.40	0.0082	3.97	18.97	0.0055
3	31.3	25.44	8.90	0.018	45.01	1.23	0.0203	4.78	17.43	0.0080
4	31.3	25.44	8.90	0.007	45.99	1.20	0.0093	4.67	17.28	0.0036
5	31.3	14.24	10.60	0.018	46.64	0.49	0.0173	3.41	18.05	0.0113
6	31.3	14.24	10.60	0.007	46.50	0.43	0.0073	3.29	17.89	0.0030
7	31.3	14.24	8.90	0.018	35.72	2.34	0.0181	6.11	14.04	0.0056
8	31.3	14.24	8.90	0.007	35.44	2.28	0.0082	6.06	13.88	0.0022
9	13.7	25.44	10.60	0.018	64.92	0.59	0.0188	4.29	19.13	0.0127
10	13.7	25.44	10.60	0.007	64.88	0.52	0.0079	4.11	19.03	0.0050
11	13.7	25.44	8.90	0.018	45.56	1.30	0.0198	4.93	17.55	0.0078
12	13.7	25.44	8.90	0.007	45.45	1.26	0.0089	4.75	17.46	0.0035
13	13.7	14.24	10.60	0.018	46.75	0.52	0.0168	3.49	18.17	0.0111
14	13.7	14.24	10.60	0.007	46.67	0.46	0.0069	3.44	18.08	0.0030
15	13.7	14.24	8.90	0.018	35.82	2.38	0.0176	6.26	14.16	0.0052
16	13.7	14.24	8.90	0.007	35.75	2.31	0.0076	6.15	14.07	0.0025
17	22.5	19.60	9.80	0.013	46.63	1.11	0.0143	3.82	16.91	0.0077
18	10	19.60	9.80	0.013	46.67	1.20	0.0140	3.89	16.97	0.0075
19	35	19.60	9.80	0.013	46.52	1.02	0.0148	3.77	16.81	0.0081
20	22.5	12.75	9.80	0.013	40.00	1.14	0.0163	4.18	16.35	0.0067
21	22.5	27.5	9.80	0.013	54.07	0.68	0.0168	3.16	18.36	0.0090
22	22.5	19.60	8.50	0.013	37.86	1.91	0.0148	7.21	14.50	0.0049
23	22.5	19.60	11	0.013	54.39	0.37	0.0143	4.76	19.13	0.0100
24	22.5	19.60	9.80	0.005	46.56	1.11	0.0063	3.88	16.91	0.0037
25	22.5	19.60	9.80	0.020	46.63	1.17	0.0193	3.92	16.94	0.0115

Table 4

The experimental design for equilibrium composition as weight percent w in PEG 4000 (1)–Na₂SO₄ (2)–water (3)–Ciprofloxacin (4) aqueous two-phase system

Number	Temperature (°C)	Feed			Upper phase			Lower phase		
		w_1	w_2	w_4	w_1	w_2	w_4	w_1	w_2	w_4
1	31.3	25.44	10.60	0.018	59.55	0.31	0.0196	1.14	16.60	0.0132
2	31.3	25.44	10.60	0.007	59.30	0.22	0.0085	1.03	16.50	0.0059
3	31.3	25.44	8.90	0.018	39.75	1.05	0.0206	1.84	14.96	0.0084
4	31.3	25.44	8.90	0.007	40.74	1.02	0.0096	1.73	14.81	0.0040
5	31.3	14.24	10.60	0.018	41.39	0.31	0.0176	0.47	15.58	0.0117
6	31.3	14.24	10.60	0.007	41.25	0.25	0.0076	0.35	15.43	0.0034
7	31.3	14.24	8.90	0.018	30.46	2.16	0.0184	3.17	11.57	0.0060
8	31.3	14.24	8.90	0.007	30.19	2.10	0.0085	3.12	11.41	0.0025
9	13.7	25.44	10.60	0.018	59.66	0.40	0.0191	1.35	16.66	0.0128
10	13.7	25.44	10.60	0.007	59.62	0.34	0.0080	1.17	16.57	0.0055
11	13.7	25.44	8.90	0.018	40.30	1.11	0.0200	1.99	15.09	0.0080
12	13.7	25.44	8.90	0.007	40.20	1.08	0.0091	1.81	14.99	0.0036
13	13.7	14.24	10.60	0.018	41.49	0.34	0.0171	0.55	15.70	0.0113
14	13.7	14.24	10.60	0.007	41.42	0.28	0.0071	0.50	15.61	0.0030
15	13.7	14.24	8.90	0.018	30.57	2.19	0.0176	3.32	11.69	0.0051
16	13.7	14.24	8.90	0.007	30.50	2.13	0.0080	3.21	11.60	0.0021
17	22.5	19.60	9.80	0.013	41.37	0.93	0.0146	0.88	14.44	0.0082
18	10	19.60	9.80	0.013	41.42	1.02	0.0144	0.95	14.50	0.0076
19	35	19.60	9.80	0.013	41.26	0.83	0.0150	0.83	14.35	0.0088
20	22.5	12.75	9.80	0.013	34.74	0.96	0.0145	1.23	13.88	0.0070
21	22.5	27.5	9.80	0.013	48.82	0.49	0.0172	0.22	15.89	0.0094
22	22.5	19.60	8.50	0.013	32.60	1.73	0.0152	4.27	12.03	0.0051
23	22.5	19.60	11	0.013	49.13	0.19	0.0146	1.82	16.66	0.0104
24	22.5	19.60	9.80	0.005	41.31	0.93	0.0066	0.94	14.44	0.0041
25	22.5	19.60	9.80	0.020	41.38	0.99	0.0197	0.98	14.47	0.0119

percent of PEG, salt and Ciprofloxacin in top and bottom phases depend on the temperature, the weight percents of PEG, salt, and Ciprofloxacin in feed, and also the PEG molecular weight.

$$W_1^{\text{TOP}} = 43.936 + 6.589X_2 + 7.218X_3 - 2.318X_5 + 0.627X_1^2 + 0.847X_2^2 + 0.627X_4^2 + 2.082X_2X_3 \quad (3)$$

$$W_2^{\text{TOP}} = 0.982 - 0.036X_1 - 0.244X_2 - 0.629X_3 + 0.028X_4 - 0.085X_5 + 0.039X_1^2 - 0.061X_2^2 + 0.055X_3^2 + 0.055X_4^2 + 0.276X_2X_3 \quad (4)$$

$$W_4^{\text{TOP}} = 0.01535 + 0.00025X_1 + 0.00068X_2 - 0.0004X_3 + 0.00511X_4 + 0.0002X_5 - 0.0006X_1^2 + 0.00051X_2^2 - 0.0005X_3^2 - 0.0014X_4^2 + 0.00025X_2X_4 \quad (5)$$

where W_1^{TOP} , W_2^{TOP} and W_4^{TOP} are the weight percent of PEG, salt and Ciprofloxacin in top phase, respectively.

$$W_1^{\text{BOT}} = 2.369 - 0.061X_1 - 0.203X_2 - 0.855X_3 + 0.048X_4 - 1.54X_5 - 0.099X_1^2 - 0.181X_2^2 + 0.978X_3^2 - 0.064X_4^2 + 0.428X_5^2 + 0.516X_2X_3 \quad (6)$$

$$W_2^{\text{BOT}} = 15.962 - 0.063X_1 + 1.021X_2 + 1.453X_3 + 0.049X_4 - 1.09X_5 + 0.247X_2^2 - 0.598X_2X_3 \quad (7)$$

$$W_4^{\text{BOT}} = 0.008582 + 0.000251X_1 + 0.000958X_2 + 0.001699X_3 + 0.002888X_4 + 0.000179X_5 - 0.00055X_1^2 - 0.00043X_2^2 - 0.00064X_3^2 - 0.00054X_4^2 + 0.000975X_3X_4 + 0.000975X_3X_4 \quad (8)$$

where W_1^{BOT} , W_2^{BOT} and W_4^{BOT} are the weight percent of PEG, salt and Ciprofloxacin in the lower phase, respectively.

The results of the experimental design show that the salt weight percent in feed (X_3) has the highest influence on PEG and salt concentrations in the upper phase (Eqs. (3)–(4)). Effect of polymer to salt weight percent ratio in feed (X_2) is observed in terms of linear and/or quadratic interaction with the salt weight percent in feed and the salt concentration in the upper phase. The weight percent of Ciprofloxacin in feed (X_4) has the most important effect on the Ciprofloxacin partitioning in upper or lower phases as shown in Eqs. (5) and (8). for the upper phase, the effect of Ciprofloxacin weight percent in feed (X_4), is expressed as the interaction with the polymer to salt weight percent ratio in feed (Eq. (5)), while the interaction with the salt concentration of feed is regarded as the controlling phenomenon of the bottom phase (see Eq. (8)).

In order to check the accuracy of the model, the results of each dependent variable with their coefficients of determination R^2 were calculated. The statistical analysis indicates that the model can correlate the experimental data. The R^2 values for Ciprofloxacin, PEG, and Na_2SO_4 in the upper and lower phases are reported in Table 5. The close value of R^2 to unity means that

Table 5

The overall goodness of fit evaluation for all phases

Equation	R^2	F test
Ciprofloxacin in upper phase	0.991	794
Polymer in upper phase	0.988	807
Salt in upper phase	0.991	811
Ciprofloxacin in lower phase	0.989	647
Polymer in lower phase	0.998	3436
Salt in lower phase	0.993	1589

the empirical model fits the actual data well. The F test shows the power of analysis. In general the larger magnitude of F test, the better accuracy the analysis has.

To compare the experimental data with results of the model, the following relation is used:

$$\text{Deviation} = \left| \frac{K^{\text{Exp}} - K^{\text{Model}}}{K^{\text{Exp}}} \right| \times 100 \quad (9)$$

where K^{Exp} and K^{Model} are the partitioning coefficients of Ciprofloxacin obtained from experiments and the model, respectively. The Ciprofloxacin partitioning coefficient is defined as the ratio of weight percents of Ciprofloxacin in the upper and lower phases.

The comparison of the experimental data with the results of the model for Ciprofloxacin partitioning coefficient in ATPS of PEG (1) + Na_2SO_4 (2) + water (3) is given in Table 6. As one can observe the results of the model agree with the experimental data.

Table 6

The comparison of Ciprofloxacin partitioning coefficient (K) of experimental data and model for ATPS of PEG 1500 (1) + Na_2SO_4 (2) + water (3)

K (Experiment)	K (Model)	Deviation (%)
1.51	1.47	2.79
1.51	1.58	4.79
2.56	2.60	1.55
2.65	2.40	9.24
1.53	1.55	1.33
2.50	2.24	10.40
3.30	3.15	4.29
4.16	4.43	6.44
1.48	1.48	0.32
1.58	1.64	3.84
2.57	2.72	5.92
2.61	2.62	0.49
1.51	1.58	4.27
2.36	2.46	4.50
3.46	3.36	2.76
3.17	3.70	16.58
1.82	1.81	0.50
1.96	1.97	0.42
1.87	1.88	0.27
2.46	2.47	0.49
1.87	1.93	3.05
3.09	3.15	2.07
1.43	1.43	0.00
1.71	1.59	7.08
1.68	1.72	2.30

Fig. 1. The effect of Na₂SO₄ (2) weight percent in feed on Ciprofloxacin partition coefficient (K) in PEG (1) + Na₂SO₄ (2) + water (3) aqueous two-phase system at 35 °C and 25.4 wt.% of PEG: (■) PEG 1500; (▲) PEG 4000.

The results of the model show that the salt concentration in feed has a great effect on antibiotic partitioning. Fig. 1 shows the effect of salt concentration in feed on Ciprofloxacin partitioning in ATPS of PEG (1) + Na₂SO₄ (2) + water (3). By increasing the salt concentration in feed, the ionic strength of system increases and consequently the tendency of Ciprofloxacin toward the lower phase (salt rich phase) increases.

The effect of PEG concentration in feed for two PEG molecular weights is shown in Fig. 2. As PEG weight percent increases, the partitioning coefficient reduces at low concentrations of PEG, while an increase is observed at high PEG concentrations. This behavior may be related to the interaction of the PEG and Ciprofloxacin molecules. At low polymer concentrations, the PEG molecules tend to reject the ciprofloxacin to the lower phase, but at high concentrations of the polymer, this exclusion decreases.

The results of the model for the effect of temperature on Ciprofloxacin partitioning are shown in Fig. 3, indicating only a small influence of temperature on antibiotic partitioning. The effect of antibiotic concentration in feed on Ciprofloxacin partition coefficient is shown in Fig. 4. By enhancing the Ciprofloxacin concentration in feed the partitioning coefficient increased in low concentrations, while the vice versa holds for high concentrations. The influence of PEG molecular weight on antibiotic partitioning is trivial. The results of the model show that by increasing the polymer molecular weight

Fig. 2. The effect of PEG weight percent in feed on Ciprofloxacin partition coefficient (K) in PEG (1) + Na₂SO₄ (2) + water (3) aqueous two-phase system at 10 °C and 10.6 wt.% of Na₂SO₄: (■) PEG 1500; (▲) PEG 4000.

Fig. 3. The effect of temperature (T °C) on Ciprofloxacin partition coefficient (K) in PEG (1) + Na₂SO₄ (2) + water (3) aqueous two-phase system at 9.8 wt.% of Na₂SO₄ and 19.6 wt.% of PEG: (■) PEG 1500; (▲) PEG 4000.

Fig. 4. The effect of Ciprofloxacin weight percent (4) in feed on partition coefficient (K) in PEG (1) + Na₂SO₄ (2) + water (3) aqueous two-phase system at 35 °C with 9.8 and 19.6 wt.% of Na₂SO₄ and PEG, respectively: (■) PEG 1500; (▲) PEG 4000.

from 1500 to 4000, the partitioning coefficient changes only by 0.01.

5. Conclusion

New experimental data for partitioning of Ciprofloxacin within an ATPS of PEG–Na₂SO₄–water have been presented. The experimental data show that the partitioning of antibiotic is dependent on the temperature, the polymer molecular weight, as well as the salt, polymer and Ciprofloxacin concentration in feed. Orthogonal CCD based on full factorial experimental designs was applied to fit a polynomial model for the ATPS. The proposed model has a good agreement with the experimental data. The results of the model show that the salt concentration in feed is the most important factor in antibiotic partitioning. This factor reduces the partitioning coefficient of antibiotic significantly. The PEG concentration in feed decreased the Ciprofloxacin partitioning coefficient at low concentrations, while the opposite effect was observed in higher concentrations. The effect of temperature on antibiotic partitioning is similar to that of the polymer concentration. The results of the model show that the Ciprofloxacin weight percent in feed has a relatively small effect on antibiotic partitioning. Also, the influence of the PEG molecular weight on Ciprofloxacin partitioning coefficient is almost negligible.

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