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Extraction of Cephalexin Using Aqueous Two-Phase Systems Composed of Cholinium Chloride and K_3PO_4

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Abstract

Cephalexin is an important antibiotic. It is very significant to determine an appropriate method for the extraction of this valuable antibiotic for industrial applications. Aiming at developing an efficient method for the extraction of cephalexin, the partitioning of cephalexin has been evaluated in aqueous two-phase system, including cholinium chloride and potassium phosphate. The effect of three main and independent variables on the partition coefficient of cephalexin has been estimated. These variables are as follows: equilibrium temperature, different weight percent of salt, and cholinium chloride. The results showed that by increasing the salt weight percent and the temperature, the partition coefficient of cephalexin will increase, while by increasing the weight percent of cholinium chloride, its partition coefficient will decrease.

***Keywords*:** *Aqueous two-phase system, Choliniumchloride, Cephalexin, Partitioning.*

Introduction

Cephalexin is one of the most common antibiotics. This medicine has been classified in the category of the first generation cephalosporin, and it is soluble in water. Cephalexin is utilized in the infections of the upper and lower respiratory system, the skin, the urinary system, and the middle ear [1, 2]. The antibiotics separation and purification in the downstream stage are the important processes in production. The final cost of product depends a lot on the selected method for the separation and purification. Also, this separation method should be selected in such a way that provides a high purity product in order to be utilized in the pharmaceutical industries [3].

The prevalent methods in the pharmaceutical industry are the solvent extraction methods for extracting an antibiotic in two different liquids

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which are usually water and an organic solvent [4]. These methods are based on the liquid-liquid extraction methods which are usually performed using the organic solvents such as ethyl acetate, acetonitrile, methanol, hexane, etc. Although the economic costs of these methods are low, but these organic solvents have disadvantages. On the other hand, a number of the organic solvents can have a negative effect on the structure and activity of antibiotic. The pharmaceutical industry is facing the growth of antibiotics production and their purification steps. There is an important requirement for searching the optimum methods. With respect to the biocompatibility of extraction processes, aqueous two-phase systems (ATPSs) can represent a more suitable option [4].

The aqueous two-phase systems have been reported for the first time by Beijerinck in 1896 [5].The aqueous two-phase systems have much more advantages than the traditional extraction using the organic solvents. Since a large part of both phases consists of water, they form an appropriate environment for biomaterials. The aqueous two-phase systems, with the features such as non-volatility, sustainability in a wide range of pH, low interface surface tension between two phases, and high efficiency can be an appropriate and a pro-environment option [6, 7].

According to the literature, it was observed that much research in the field of the separation and purification of the antibiotics such as cephalexin [3], ciprofloxacin [8], tetracycline [4] and penicillin [9] in the aqueous two-phase systems had been performed. One of the challenges available in the separation of the biological products using ATPSs is the determination of the best system for separating a specified antibiotic. In recent years, the researchers have considered ionic liquid-based aqueous two-phase systems which were compatible with environment [10].

Perrira et al. considered a specific group of ionic liquids (ILs) for the separation and purification of vital biomolecules[11]. Domínguez-Pérez et al. evaluated the capability of aqueous two phase systems containing ionic liquids forthe extraction of three biomolecules (caffeine, ciprofloxacin and ciprofloxacin.HCl) [12].Ventura et al. evaluated the ability of ionic liquid-based aqueous biphasic systems for extracting an essential amino acid, L-tryptophan [13].

The ionic liquids are a new group of the chemical compounds, which with their extraordinary properties and features, have the ability to create green chemical processes. The ability to use ionic liquid as a solvent for a wide range of usages has been increasingly proven [14-17]. The ionic liquids are used as appropriate substitutes for volatile solvents in the separation processes [18].At present, the number of the ionic liquids applications in the pilot and commercial units is expanding [19].

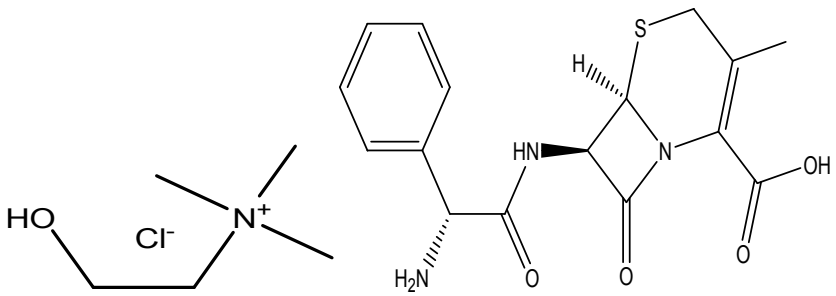
Nowadays the ionic liquids due to their unique physical-chemical properties have attracted the attention of many researchers [20, 21].

The recent research has shown the production of a new category of cholinium-based ionic liquids. The cholinium-based ionic liquids have properties such as non-flammability, negligible vapor pressure, and liquefaction in a wide range of temperatures [8].These ILs were synthesized using the choline cation and different anions. The nonvolatile nature of cholinium-based ionic liquids has created an opportunity, so that the diffusion of pollutants into the atmosphere decreases. Therefore, ionic liquids are compatible with environment. Cholinium chloride (choline) is an essential nutrient soluble in water, and its source is vitamin b which has abundant biological applications. This ionic liquid has attracted the attention of many researchers to themselves [8]. In this research, the partitioning of cephalexin in aqueous two-phase system, including cholinium chloride+ K_3PO_4+H_2O, and the effects of change of the different parameters such as the equilibrium temperature, the weight percent of ionic liquid, and the weight percent of salt on the cephalexin partitioning have been considered.

Experimental

Materials

In this study, potassium phosphate salt (K_3PO_4) with high purity of 98% manufactured by Sigma company and cholinium chloride ($HOCH_2CH_2N$ $(CH_3)_3Cl$) with high purity of 99% manufactured by Daejung company have been utilized. Cephalexin ($C_{16}H_{17}N_3O_4S$) with the molecular weight of 41.365 $g.mol^{-1}$ is in the form of powder, and its color is yellow, which has been purchased from Loghman pharmaceutical company (Iran). The double distillation water was utilized for manufacturing all solutions. The molecular structure of the cholinium chloride and cephalexin has been shown in Figure 1.



(a) (b)

Figure1. The molecular structure of (a) choline chloride and (b) cephalexin.

Methods

Phase diagram

In order to consider the phase behavior of aqueous two-phase systems, the phase diagram is used, so that the data containing the concentration of the components of two phases in equilibrium are selected using this diagram. The cloud point titration method was utilized for determining the phase diagram at the temperature of 298±1 K and ambient pressure. The K_3PO_4 solution with the weight percent of about 20 wt% and the cholinium chloride solution with the weight percent in the range of 80-90 wt% for determining the experimental points of binodal curve were used. In the cloud point titration method, the solution droplets of the mineral salt was added to the ionic liquid solution up to the time that the turbid solution was observed, and then the drops of water was added until the monophasic region (the clear solution) was obtained. Adding the drops was accomplished under the steady stirring, and the ternary systems compositions were estimated by measuring the weight of all the added components with the uncertainty of ± 0.001g. According to previous laboratory reports, the points for forming the phase diagram was determined visually [22-25]. Roger et al. for the first time offered a series of mathematical equations in order to create relations between the results and the experimental data based on the equation which had been previously offered for the polymer systems by Merchuk et al [26]. The binodal curves are correlated well with a three-parameter equation which is as follows [26]:

$$Y = A\exp[(BX^{0.5}) - (CX^3)] \qquad (1)$$

Where Y and X are the weight percent of the cholinium chloride and salt, respectively. The parameters A,B and C, were obtained by the regression. The researchers use this equation to describe the experimental data of the binodal curve [10, 16, 20]. In general, this equation has provided an appropriate definition for the coexistence curves of ionic liquid-based aqueous two-phase systems.

Tie lines

At first, Merchuk et al. proposed the gravimetric method for determining the tie lines of polymer-based aqueous two-phase systems, which the results were available by placing the binodal curve data in the Equation(1), and also by establishing a mass balance between the top and bottom phases [26]. Roger et al. successfully utilized this opportunity for ionic liquid-based aqueous two-phase systems [27]. In order to find the tie line, a ternary mixture composed of K_3PO_4+ [Ch]Cl + $H_2O$was prepared in the two-phase region, so that both phases had been completely separated in the equilibrium temperature of 298±1 K for 12 hours. The top and bottom phases were accurately separated by the Pasteur pipette.

Each tie line is obtained by solving the following four-variable equations [28]:

$$Y_T = A\exp[(BX_T^{0.5}) - (CX_T^3)] \quad (2)$$

$$Y_B = A\exp[(BX_B^{0.5}) - (CX_B^3)] \quad (3)$$

$$Y_T = \frac{Y_M}{\alpha} - \frac{1-\alpha}{\alpha} \times Y_B \quad (4)$$

$$X_T = \frac{X_M}{\alpha} - \frac{1-\alpha}{\alpha} \times X_B \quad (5)$$

In the Equations (2 –5), the letter "*T*", "*B*", and "*M*" show the top phase, the bottom phase, and the mixture. Also, *X* and *Y* show the weight percent of salt, and the weight percent of cholinium chloride, respectively, and α represent the ratio between the mass of choline-rich phase and the total mass of the mixture. A, B and C are the constants in the Equation (1).

For determining the tie line length, the related equation is as follows [28-33]:

$$TLL = \sqrt{(X^{top} - X^{bottom})^2 + (Y^{top} - Y^{bottom})^2} \quad (6)$$

Partitioning of cephalexin

The experimental method for the determination of the partition coefficients in ATPS was reported in the previous works [4, 8].In order to evaluate the partitioning of antibiotic at the temperature of 298 ±1 K, the weight percent of the mixture components in the two-phase region was determined, and each sample was prepared by mixing salt, IL, and the aqueous solution of cephalexin (0.001mol. dm^{-3}). The two-phase solution was stirred well for achieving the partitioning of cephalexin between the two phases. The optimum time needed in order to achieve the equilibrium of the two phases is based on the previous reported studies[3,8].The temperature of each sample was controlled in an incubator (Fater electric, Iran) with accuracy of ± 0.01˚C. The top and bottom phases were separated by a plastic syringe, and they were placed into two separate containers. The weight fraction of cephalexin in both phases was determined by a UV/V spectrophotometer (Lambada 25, Perkin Elmer Company). The weight fraction of cephalexin was calculated by drawing a calibration curve at the maximum absorption wavelength of 266 nm. The partition coefficients of antibiotic are determined based on the triplicate mean accomplished for each sample. The partition coefficient of cephalexin in an ATPS can be written as [10]:

$$K_{Ceph.} = \frac{W_{Ceph.}^{top}}{W_{Ceph.}^{bottom}} \quad (7)$$

Where in Equation (7) $w_{Ceph.}^{top}$ and $w_{Ceph.}^{bottom}$ are the weight fraction of cephalexin in choline and in salt aqueous-rich phases, respectively.

The Standard Deviation (σ) is a statistical measure that is closely linked to the Variance. Both measures are commonly used across a set of values, to identify the amount that the values differ (or deviate) from the average value. The standard deviation was calculated in order to determine the data accuracy and authenticity. The following equation has been used in order to calculate the standard deviation:

$$\sigma = \sqrt{\sum \frac{(x - \bar{x})^2}{(n-1)}} \quad (8)$$

Where, x takes each value in the set, $\bar{x}$ is the average (statistical mean) of the set of values, and is the number of values.

Results and discussion

One of the challenges available in the separation of the bio-molecular medicines using aqueous two-phase systems is the determination of an optimum system for separating the intended medicines. The separation of bio-molecular medicines in these systems depends on various parameters, which their mechanism and the accurate behavior of them are not predictable. Therefore, in order to determine the best system for separating antibiotics, the stages such as the determination of the types of the system components, the determination of the two-phase region, and the consideration of the impact of the parameters such as the temperature and the weight percent of the two-phase system components on the partitioning of the antibiotics are examined.

Phase diagrams and tie-lines

At the temperature of 298 K and atmosphere pressure, the phase diagram for the system, including the salt of K_3PO_4, cholinium chloride, and water was determined and the results have been shown in Figure 2. The basis of forming ATPS consists of ionic liquids and salts because of the phenomenon of the effect of salting-out. This phenomenon is done due to the creation of the water-ion complexes that causes the loss of water in the soluble materials and an increase in the surface tension of the cavity in the aqueous ambient [10].Therefore, when a high density salt such as K_3PO_4 is added to an aqueous ambient, including an ionic liquid, both solutes compete with each other for achieving the solvent molecules. Those groups of ions that have more ability for forming the hydration compound will be the conquerors of this competition, and as a result, the solvent molecules migrate towards them. This remoteness of the ionic liquid ions from the ions of the salts leads to the liquid-liquid mix, and then it causes the formation of two separated phases [10].

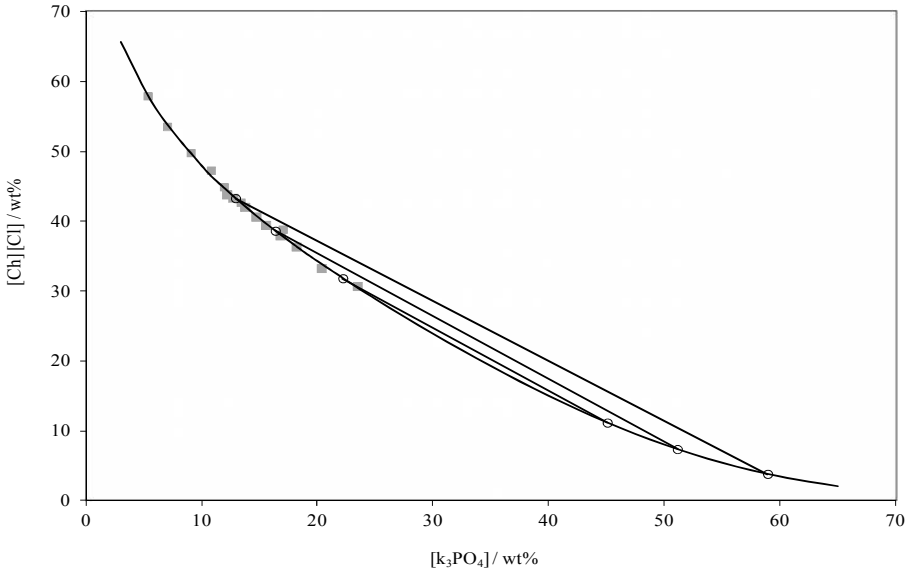


Figure 2. Phase diagram of the ATPS containing [Ch]Cl+ K_3PO_4+H_2O at 298 K:(○) TL data ; (■) binodal curve data; (-) adjusted data through Equation (1).

In aqueous two-phase system, including cholinium chloride and K_3PO_4, the top phase is related to the ionic liquid-rich phase and the bottom phase is related to the salt-rich phase [8]. The experimental binodal curves are fitted by the least squares regression using the non-linear Equation (1). The parameters *A,B* and *C* the standard deviation (σ), and the correlation coefficient (R^2) have been reported in Table 1. The statistical measurement of the correlation coefficient (R^2) confirms a good correlation between the experimental data and the Merchuk equation. In accordance with Figure 2, this equation has an acceptable adaptation with the binodal curve of the considered system. The experimental results for the tie lines length and the weight percents of salt and choline in the top phase and the bottom phase have been reported in Table 2. It should be noted that and indicate the top phase *T* and *B* the bottom phase.

Table 1. The Correlation parameters of Equation (1), the standard deviation, and the correlation coefficientat 298K.

System	A ± σ[a]	B ± σ[a]	10^6C ± σ[a]	(R^2) [b]
[Ch]Cl + K_3PO_4 + Water	95.2973±1.01	-0.2149±0.003	7.660±0.02	0.9965

a:Standard deviation
b:Correlation coefficient

Table 2. Experimental weight percent (wt%) for the TLL,andcholin chloride and K_3PO_4 at the top phase (T), initial mixture (M) and bottom phase (B) at 298 K.

Weight fraction composition/ wt %							
$[IL]_T$	$[K_3PO_4]_T$	$[IL]_M$	$[K_3PO_4]_M$	$[IL]_B$	$[K_3PO_4]_B$	α^a	TLL^b
31.82	22.21	20.27	34.99	45.08	11.16	0.41	30.82
38.61	16.37	19.96	37.12	7.36	51.14	0.39	46.76
43.28	12.93	19.96	40.12	3.81	58.95	0.34	60.62

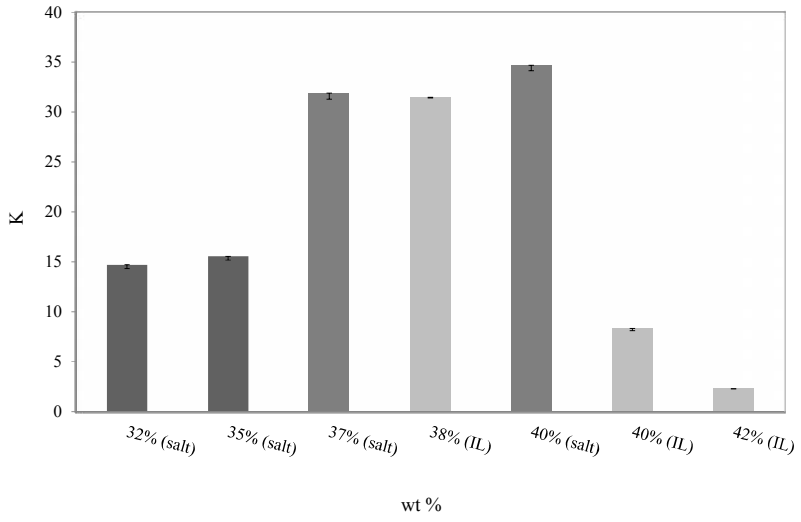
a: Ratio between the mass of the choline rich phaseto the total mass of the mixture
b: Tie Line Length

Partitioning of cephalexin

The basis of the antibiotic separation in aqueous two- phase system depends on its partition coefficient. It indicates that antibiotic tends to select one of the two available phases. The parameters of the weight percent of the phase's compounds such as salt, ionic liquid, and the equilibrium temperature of the system have an impact on the partitioning of the antibiotic particles between the phases.

Effect of the weight percent of salt in the cephalexin partitioning

Figure 3a shows the changes of the weight percent of salt on the cephalexin partitioning in the aqueous two-phase system along with the constant value of cholinium chloride. By considering the results of the experiments conducted on aqueous two-phase systems containing 20 wt% cholinium chloride and the different weight percents (32, 35, 37, and 40 wt%) of potassium phosphate at the temperature of 298±1 K, and room pressure, it was specified that a change in the weight percent of salt had a significant effect on the partition coefficient of cephalexin. The increase in the weight percent of salt causes an increase in the partition coefficient of cephalexin, and this is because of the salting-out effect. By increasing the weight percent of salt in the feed, the salt value in the bottom phase increases. The number of water available in the salt-rich phase (the bottom phase) decreases, and as a result, the solubility of cephalexin in the bottom phase decreases and the salting-out phenomenon occurs, and then cephalexin tends towards the top phase [8].

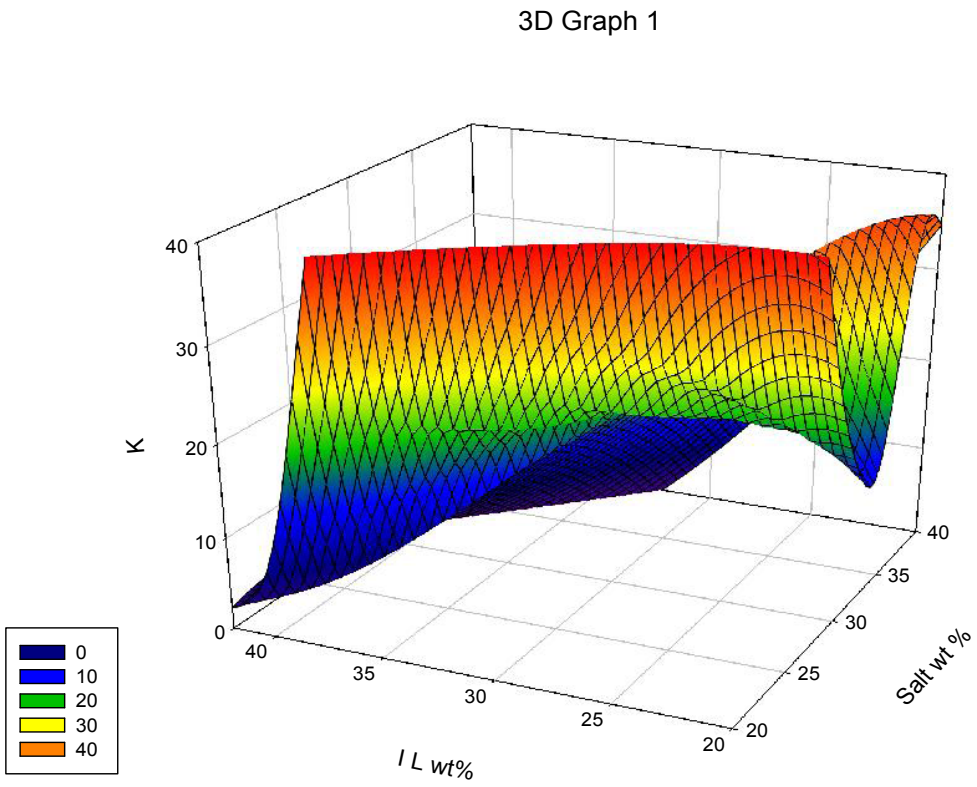


(a)

Figure 3. a) The effect of the weight percent of salt and IL on the cephalexin partitioning.

Effect of the weight percent of [Ch]Cl in the cephalexin partitioning

In order to consider the effect of the weight percent of the cholinium chloride on the cephalexin partitioning, the experiments on the two-phase systems with 20 wt% potassium phosphate and the different weight percents (38, 40, and 42 wt %) of cholinium chloride were accomplished. The results showed that by increasing the weight percent of cholinium chloride, the partition coefficient of cephalexin decreased. The reason for this behavior can be related to the decrease in the available free water molecule and the effect of salting-out by ionic liquid. Figure3arepresents the effect of increasing the weight percent of cholinium chloride on the cephalexin partitioning at the temperature of 298 K. Figure 3b shows a three-dimensional curve on the partition coefficient of cephalexin with combined effects of salt and IL.



(b)

Figure 3.b) Three-dimensional curve on the partition coefficient of cephalexin with combined effects of salt and IL.

Effect of temperature in the cephalexin partitioning

In order to determine the effect of temperature on the partition coefficient of cephalexin in aqueous two-phase system of [Ch] Cl + K_3PO_4 + H_2O, the systems containing the weight percent of constant salt and the different weight percents (38,40,and 42 wt%) of cholinium chloride at three temperatures (295,298, and 303) K have been considered. The Figure 4 shows the results obtained by the effect of the temperature in the [Ch] Cl + K_3PO_4 + H_2O system. In each three weight percent, the effect of the temperature was observable, so that by increasing the temperature, the cephalexin partitioning increased. The appropriate interactions of hydrophilic ionic liquids with water increase by increasing the temperature, and the mutual solubility of them increases. The increase of the appropriate interactions between cholinium chloride and salt has led to an increase in the temperature and the partition coefficients of cephalexin. It should be noted that the effect of the temperature on the antibiotics and biomolecules partitioning is complex, because the change of temperature

has an effect on the parameters such as the phase compounds, the electrostatic impacts, and the hydrophobic phenomenon. Also, the temperature has an indirect effect on the entropy of water molecules in contact with ionic liquid, which has an effect on the partitioning of antibiotics [33].

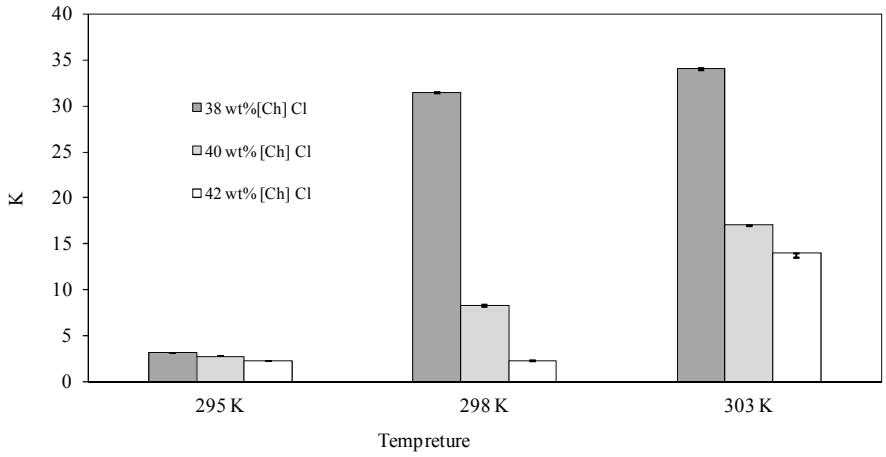


Figure 4. The effect of the temperature on the cephalexin partitioning in the aqueous two-phase system of [Ch]Cl+ K_3PO_4+H_2O.

Conclusion

The main purpose of the separation and purification of biomedicines is to achieve a high quality, high speed extraction, and minimum investment amount for product. The separation in the aqueous two-phase systems is utilized as a valuable method in the purification of biomedicines. In this study, the partition coefficient of cephalexin in aqueous two-phase system containing K_3PO_4 and cholinium chloride was studied. In order to consider the partition coefficient, the experiments were conducted, so that by keeping the other conditions constant, the effect of the salt weight percent, cholinium chloride weight percent, and three different temperatures (295,298, and 305) K on the cephalexin partitioning can be studied. The experimental results showed that in all the studied systems, cephalexin had the partition coefficient more than 1, and the cholinium chloride -rich phase was preferred. Also, the experimental data of the partition coefficients of cephalexin certificate that by increasing the temperature and the weight percent of salt, a large amount of cephalexin migrates towards the cholinium chloride -rich phase, so that it causes an increase in its partition coefficient. By increasing the amount of cholinium chloride, cephalexin has more tendency to migrate towards the bottom phase (the salt-rich phase), and consequently the partition coefficient gradually decreases.

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