Effect of Organic Solutions on the Stability and Extraction Equilibrium of Penicillin G

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The effects of organic solutions on the stability and extraction equilibrium of penicillin G were investigated. *N*-Butyl acetate, methyl isobutyl ketone, 2-ethyl hexanol, kerosene, and *n*-heptane were used for physical extraction; di-*N*-octylamine, tri-*N*-octylamine, N235 (a mixture of tertiary amines), tributyl phosphate, and di-(2-ethylhexyl)phosphoric acid were used as the extractants, with *N*-butyl acetate, kerosene, and *n*-heptane as diluents for reactive extraction. The degradation efficiency of penicillin G increases with increasing temperature. The stability of penicillin G was better in the presence of an amine-based extractant, while it was worse in the presence of a phosphorus acid extractant. The effect of neutral extractant on the stability of penicillin G was mainly dependent on the temperature. The degradation of penicillin G in alkali solution was governed by pH. The efficiency of physical extraction of penicillin G increased with decreasing pH in the aqueous solution. The efficiency of reactive extraction with an amine-based extractant was highest under the range studied. The extractant of phosphorus acid was better than neutral phosphorus extractant for the extraction of penicillin G. The distribution coefficient decreases with increasing pH, temperature, and initial penicillin G concentration in the aqueous solution under the studied conditions. The mechanism of degradation and extraction was also discussed.

Introduction

Penicillin G, a weak monocarboxylic acid ($pK_a = 2.75$), is the most widely used β-lactam antibacterial agent and the raw material of the semisynthesis penicillin. The recovery of penicillin G from the fermentation broth is an important step in the production. Solvent extraction is a widely used recovery technique.

In a commercial process, penicillin G is usually recovered from the fermentation broth by physical extraction with *N*-butyl acetate at 278.15 K and pH of 2.0 to 2.5 in a centrifugal extractor to avoid degradation. Back-extraction is carried out at pH of 6.8 to 8.0 by a carbonate or phosphate buffer. Esters of phosphoric acid or quaternary ammonium salts are used as additives to avoid the formation of stable emulsions due to proteins flocculated at low pH values.[1−4] In spite of the low temperature and short contact time, a considerable amount of penicillin G (10 % to 15 %) is lost as a result of degradation.[1−6] On the other hand, because of the high solubility of *N*-butyl acetate in aqueous solution, the recovery of solvent from raffinate solution is a critical problem for environmental concerns and process economics.[7]

The reactive extraction of penicillin G with a complexometric extractant can minimize the loss of penicillin G during the recovery process, in which the operation can be carried out at moderate pH and temperature. Reschke and Schügerl[1−3] first presented the reactive extraction of penicillin G under a moderate pH condition with an amine-based extractant, such as Amberlite LA-2, di-*N*-octylamine (DOA), tri-*N*-octylamine (TOA), and so forth.[4−6,8] Yang et al.[9] studied the extraction of penicillin G with neutral phosphorus esters, such as tributyl phosphate (TBP), triethyl phosphate (TEP), tributylphosphine oxide (TBPO), trialkylphosphine oxide (TRPO), and so forth. Wang et al.[10] and Yang et al.[11] used aliphatic alcohols as extractants, such as hexan-1-ol, octan-1-ol, and so forth. Among these reactive extractants, the solubility in the aqueous solution is lower, and the efficiency of extraction is higher. Now, the recovery of penicillin G from aqueous solutions by the liquid membrane technique has received increasing attention, which is based on the mechanism of carrier-facilitated transport and can perform the extraction and stripping process in the same stage with combined characteristics of nonequilibrium mass transfer, uphill effect, and low consumption of solvent, and so forth.[12−16] The concept of reactive extraction can be applied in the liquid membrane technique.

The fundamental data of extraction equilibrium are the most important and basic thermodynamics parameters in many chemical engineering processes and equipment designs, especially in solvent extraction and liquid membrane processes. However, most of studies were focused on the characterization of the complexes and the improvement of solvent extraction technology. Only very less fundamental data of extraction equilibrium of penicillin G between various organic phase and aqueous phase are available.

On the other hand, the penicillin G is not stable in the presence of acid, alkaline, or penicillinases, because the basic structure of penicillin G consists of a thiazolidine ring, an attached β-lactam ring, and a side chain. The dominating reactions of penicillin G are at the highly labile β-lactam ring carbonyl at the 7-position.[17] A few studies on the stability of penicillin G in aqueous solution have been reported, including

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the effects of pH, temperature, buffer media, surfactants, ionic strength, heavy metals (Cu^{2+}, Zn^{2+}), and so forth, on the degradation of penicillin G.[1,17] However, the stability of penicillin G in the presence of various extractants are seldom reported and considered, which have great influences on the reactive extraction process.

The main objective of this paper is to study the stability of penicillin G in the presence of organic solutions and the extraction equilibrium of penicillin G with various extractants and diluents. The effects of organic composition, pH, temperature, and initial penicillin G concentration in the feed solution, stripping phase, and so forth, on the stability and extraction equilibrium of penicillin G are investigated. The mechanism of degradation and extraction is also discussed.

Experimental Section

Reagents. The sodium salt of penicillin G with an activity of 1667 units·mg^{-1} and a molecular weight of 356.4 is purchased from Huabei Medicine Producing Plant. *N*-Butyl acetate (BA) with a purity > 99.0 % and TBP with a purity > 98.5 % are analytical grade reagents from Beijing Beihua Refined Chemical Co., Ltd. Di-(2-ethylhexyl)phosphoric acid (D2EHPA) with a purity > 96.0 % is an analytical grade reagent from Tianjin Jinke Refined Chemical Engineering Institute. Methyl isobutyl ketone (MIBK), from Tianjin Fuchen Chemical Reagent Plant, is of analytical grade quality with a purity > 99.0 %. 2-Ethyl hexanol, from Beijing Yili Refined Chemical Co., Ltd., is of analytical grade quality with a purity > 99.0 %. DOA from Hunan Shaoyang Chemical Engineering Institute and TOA, from Shanghai Darui Refined Chemical Reagent Plant, are both analytical grade with a purity > 98 %. N235 (trialkyl amine, $[CH_3(CH_2)_6CH_2]_3N$), from Shanghai Laiyashi Chemical Reagent Plant, is of technical grade. Kerosene, from Tianjin Fuchen Chemical Reagent Plant, is of laboratory reagent grade. *n*-Heptane, from Beijing Beihua Refined Chemical Co., Ltd. is an analytical grade reagent with a purity > 95 %. Anhydrous sodium carbonate with a purity > 99.8 %, potassium dihydrogen phosphate with a purity > 99.5 %, and sodium hydroxide with a purity > 96 %, are analytical grade reagents from Beijing Beihua Refined Chemical Co., Ltd. Methanol is high-performance liquid chromatography (HPLC) grade from Tianjin Xihua Chemical Reagent Plant. All chemical reagents are used without further purification.

Degradation Experiments. The penicillin G solutions are prepared by dissolving a known mass of penicillin G in the aqueous solution saturated by various organic phases. The pH is adjusted at 6.0 by phosphate buffer solution for the media of deionized water. For the media of an alkali solution of Na_2CO_3, K_2CO_3, $NaHCO_3$, and so forth, the pH is adjusted by dilute NaOH solution. The solutions are transferred into a 250 mL volumetric flask, which is set in an air thermostat at a given temperature. The sample is taken for pH and penicillin G concentration analysis at present time intervals.

Extraction Experiments. The aqueous solution is prepared by dissolving a known mass of sodium salt of penicillin G in the phosphate buffer media, in which the pH is adjusted. All of the extraction experiments are conducted with 100 mL stoppered flasks at a given constant temperature in a thermostatted bath shaker. Equal volumes (25 mL) of organic solution and aqueous solution are added to each flask. The mixture in the flask is vigorously mixed by a shaker for about 5 min, and then the mixture is settled for (10 to 20) min. It is observed that this time is sufficient to establish the equilibrium between these two phases. The mixture is then transferred to a separating funnel and allowed to settle for at least (8 to 15) min, which is shown in previous experiments to be sufficient for a complete phase separation. If the phase separation takes longer, a centrifuge will be needed. For the physical extraction process, the rapid operation of each step is preferred; less extraction time can prevent the degradation of penicillin G. After phase separation, the aqueous phase sample is taken for pH and penicillin G concentration analysis.

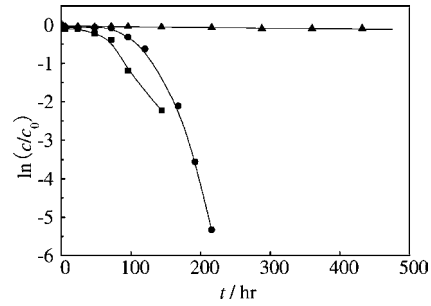


Figure 1. Influence of temperature on the stability of penicillin G in phosphate buffer media at pH of 6.0, where c is the concentration of penicillin G in aqueous solution at time t, c_0 is the initial concentration of penicillin G in aqueous solution. ▲, $T = 283.2$ K; ●, $T = 293.2$ K; ■, $T = 301.2$ K.

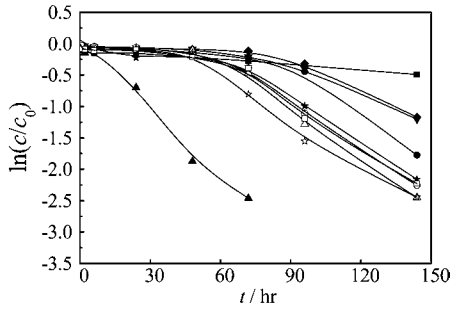
Sample Analysis. The penicillin G concentration in aqueous solution is immediately analyzed by HPLC (Shimadzu LC-20A, Japan) with an octadecyl silane chemically bonded to silica gel (ODS) C18 column (Shimadzu, 5 μm) and an UV detector at 225 nm. The mobile phase is a mixture of 0.02 mol·L^{-1} KH_2PO_4 solution and methanol in a volume ratio of 38:62, and the flow rate is 1.0 mL·min^{-1}.[18] The concentrations of penicillin G in the organic phase are calculated by a mass balance. A digital precision ionometer model PXS-450 (Shanghai Dapu Co., Ltd.) with a combined glass electrode is used for pH measurements (± 0.01 pH). The meter is calibrated against 4.01, 6.85, and 9.14 standard buffer solutions.

Results and Discussion

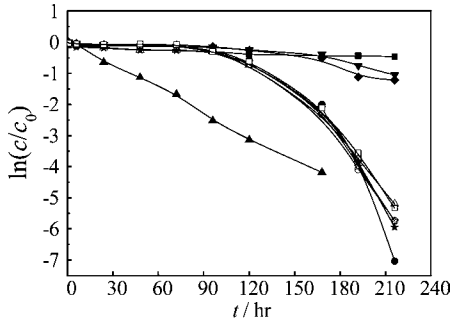
All of the experiments are carried out three or five times, and the reproducibility is found to be ± 3 %.

Stability of Penicillin G. Penicillin G is stable at a pH range from 5.0 to 8.0 in aqueous solution,[1,4] in which the penicillin acids are present in dissociated form. In this work, the effects of organic solution on the stability of penicillin G in aqueous feed solution are studied at the pH 6.0 adjusted by the phosphate buffer. The effects of temperature on the stability of penicillin G are conducted at a range from (283.2 to 303.2) K (nearly room temperature). The results are shown in Figures 1 to 3. The degradation rate of penicillin G increases sharply with increasing temperature either in phosphate buffer media at pH 6.0 or alkali media in stripping phase or in the presence of organic phase. The penicillin G is more stable at a temperature of 283.2 K. The degradation rate of penicillin G is sharply increased with time at high temperatures, because the degradation rate of penicillin G will continue to accelerate in presence of penicillin degradation products until all of the penicillin G has been exhausted.

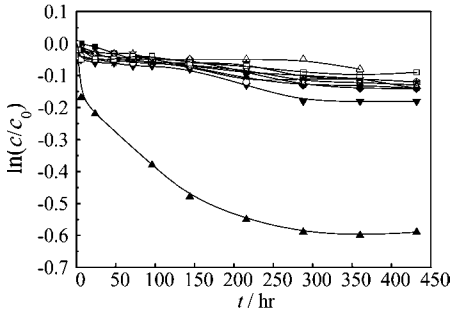
Figure 2 gives the stability of penicillin G in the presence of various organic components, including BA, DOA, TBP, N235, MIBK, *n*-heptane, kerosene, D2EHPA, 2-ethyl hexanol, and so forth. The stability of penicillin G in the presence of amine-based extractant, DOA or N235, is better than that in phosphate buffer media. Because they are weak organic bases, the penicillin G is stable at pH 5.0 to 8.0 as mentioned above. The stability



a: $T = 301.2$ K



b: $T = 293.2$ K



c: $T = 283.2$ K

Figure 2. Influences of various organic solutions on the stability of penicillin G in phosphate buffer media at pH 6.0 and temperatures from (283.2 to 301.2 K). □, blank; ○, BA; ■, DOA; ♦, TBP; ▼, N235; ★, MIBK; ●, *n*-heptane; △, kerosene; ▲, D2EHPA; ☆, 2-ethyl hexanol.

of penicillin G is the worst in the presence of D2EHPA, whereas the degradation rate of penicillin G is highest. Because D2EHPA is a Lewis acid agent (organophosphorus acid) with a pK_a of 3.01, the penicillin G is not stable in the presence of acid. The degradation of penicillin G is catalyzed by a hydrogen ion, and penicillin G is thought to be degraded to penicillenic acid initially and then further to a range of products.[17] The effects of neutral extractant on the stability of penicillin G mainly depends on the temperature, such as alkanes of *n*-heptane and kerosene, alcohols of 2-ethyl hexanol, esters of TBP and BA, ketones of MIBK, and so forth.

In the recovery process of solvent extraction, penicillin G is extracted from the fermentation broth by organic solvents and then re-extracted by alkali solutions to get products. Therefore, the stability of penicillin G in the stripping media is studied as a function of the type of alkali solution (Na_2CO_3, K_2CO_3, and $NaHCO_3$), the concentration, and the pH in the stripping phase, and so forth. As shown in Figure 3, the degradation of penicillin G mainly depends on the pH in the stripping alkali solution. Because the degradation of penicillin G in alkali media is also catalyzed by the hydroxide ion, penicillin G is thought to be degraded to penicilloic acid initially and then further to a range of products.[17] The degradation rate of penicillin G decreases with a decreasing concentration of alkali in solution. The stripping phase of $NaHCO_3$ solution is better for the stability of penicillin G than Na_2CO_3 or K_2CO_3. For the stripping phases of Na_2CO_3 and K_2CO_3, the degradation behavior of penicillin G is similar at identical pH values.

Extraction Equilibrium of Penicillin G. On the basis of the results of stability, the important thermodynamics parameters, that is, extraction equilibria of penicillin G between aqueous solutions and various organic solutions, are studied. Under the extraction experimental conditions in this paper, the degradation of penicillin G can be neglected because of the short operation time.

The dissociation equilibrium of penicillin G in the aqueous solutions is:

$$HP \Leftrightarrow H^+ + P^- \tag{1}$$

Physical Extraction of Penicillin G. All of the physical extraction experiments are carried out at a temperature of 278.2 K and pH range from 2.0 to 7.0 adjusted by phosphate buffer solution, to avoid degradation of penicillin G and obtain favorable results. Five kinds of extractant, BA, MIBK, 2-ethyl hexanol, kerosene, and *n*-heptane, are used for this purpose. The distribution coefficient D_{Phy} of penicillin G (HP) extraction is defined as follows:

$$D_{Phy} = \frac{\overline{[HP]}}{[HP_{aq}] + [P_{aq}]} = \Lambda \frac{1}{1 + 10^{pH\text{-}pK_a}} \tag{2}$$

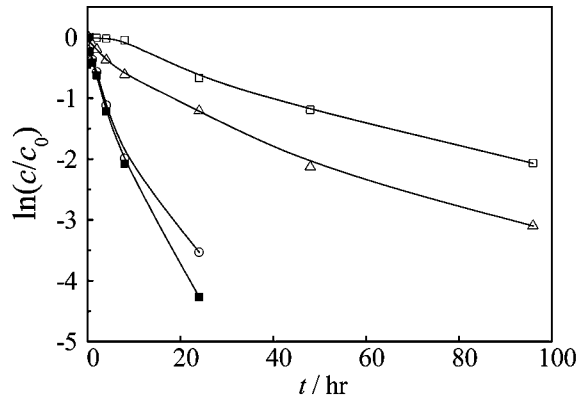
where the charge of the ions is omitted for simplicity, the bar indicates the species in the organic phase, aq represents the aqueous phase, and Phy is the physical extraction process. Λ is the partition coefficient, that is, the ratio of the free acid concentration between the two phases.

$$\Lambda = \frac{\overline{[HP]}}{[HP_{aq}]} \tag{3}$$

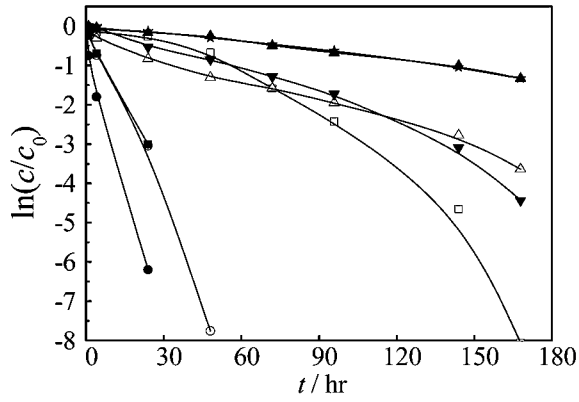
As shown in Table 1, when the BA, MIBK, or 2-ethyl hexanol is used as the solvent, the distribution coefficient sharply decreased with increasing initial pH in the aqueous solutions because of the dissociation equilibrium of penicillin G as described by eq 1. When the initial pH in the aqueous solution is greater than its pK_a value (2.75), penicillin G exists mainly in its dissociation form. This does not benefit the physical extraction process. Then, the initial pH in the aqueous solution is usually set at 2.0 to 2.5 for the physical extraction process to remain in the acid anion into an undissociated free state. For the extractants of BA, MIBK and 2-ethyl hexanol, the pH in the stripping solution for back-extraction process can be set at 6.0 or larger. The distribution coefficient also decreases with increasing initial penicillin G concentration in the aqueous solutions as shown in Table 2. Unfortunately, the kerosene and *n*-heptane cannot extract penicillin G from aqueous solutions without the extractant.

The value of partition coefficient Λ is also evaluated from experimental data in this work: 55 for BA at 5 °C, which agrees well with the data given in the literature.[1] The value of Λ is obtained as 65 for MIBK and 45 for 2-ethyl hexanol. The distribution coefficient of physical extraction can be calculated by eq 2.

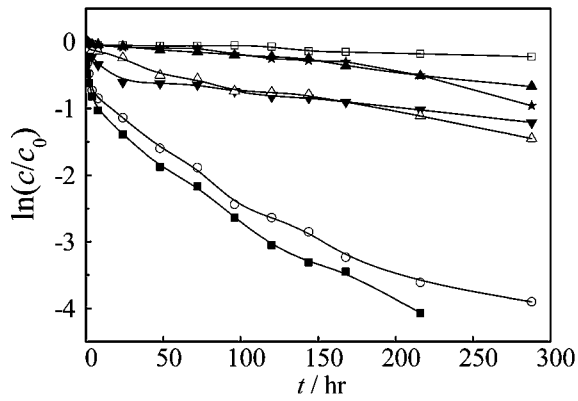
Reactive Extraction of Penicillin G. In reactive extraction experiments, aliphatic amine extractants of DOA and N235 and phosphorus extractants of TBP and D2EHPA are used as extractants; BA, kerosene, and *n*-heptane are selected as diluents.



a: T = 301.2 K. □ , deionzied water; ○ , 0.5 mol·L^{-1} K_2CO_3, pH = 11.78; Δ, 0.1 mol·L^{-1} K_2CO_3, pH = 11.54; ■ , 0.5 mol·L^{-1} Na_2CO_3, pH = 11.81.



b: T = 293.2 K. □ , deionzied water; ○ , 0.5 mol·L^{-1} K_2CO_3, pH = 11.99; Δ, 0.1 mol·L^{-1} K_2CO_3, pH = 11.71; ▼ , 0.5 mol·L^{-1} K_2CO_3, pH = 10.01; ▲ , 0.5 mol·L^{-1} K_2CO_3, pH = 9.01; ● , 1.2 mol·L^{-1} K_2CO_3, pH = 12.01. ■ , 0.5 mol·L^{-1} Na_2CO_3, pH = 11.87; ★ , 0.5 mol·L^{-1} $NaHCO_3$, pH = 8.27;



c: T = 283.2 K. □ , deionzied water; ○ , 0.5 mol·L^{-1} K_2CO_3, pH = 11.81; Δ, 0.1 mol·L^{-1} K_2CO_3, pH = 11.54; ▼ , 0.5 mol·L^{-1} K_2CO_3, pH = 10.01; ▲ , 0.5 mol·L^{-1} K_2CO_3, pH = 9.10; ■ , 0.5 mol·L^{-1} Na_2CO_3, pH = 11.97; ★ , 0.5 mol·L^{-1} $NaHCO_3$, pH = 8.44;

Figure 3. Influences of stripping solutions on the stability of penicillin G at temperatures from (283.2 to 301.2) K.

Although a quaternary amine presents the nature of ion exchange extraction and rapid kinetics, the pH in the aqueous solution has no influence on the efficiency of the extraction. The back-extraction of penicillin G is very difficult.[2,16] The secondary and tertiary amines have a higher distribution coefficient than primary amines.[1−3] Then these two types of amine-based extractants are considered in our studies. A neutral phosphorus extractant has the nature of demulsification, and the extraction capability increases in the following order:[9] $(RO)_3PO < R(RO)_2PO < R_2(RO)PO < R_3PO$. Then the TBP is studied in this work. As a phosphorus acidic extractant, D2EHPA has been extensively used as the solvent for the extraction of organic and inorganic compounds; however, there is no report on the extraction of penicillin G with D2EHPA as the solvent yet.

The mechanism of reactive extraction of an acid with DOA, TOA, N235, or TBP as the extractant corresponds to a neutralization reaction.[1,3,9,19] The extractant A dissolved in the

Table 1. Influence of the Initial/Equilibrium pH in Aqueous Solution on the Physical Extraction of Penicillin G at 278.2 K[a]

pH	organic phase	c_0 / mg·L^{-1}	c_1 / mg·L^{-1}	D_{Phy}
2.02/1.96	BA	3.633	0.063	56.67
2.99/3.66	BA	3.633	0.310	10.72
4.04/5.13	BA	3.633	2.884	0.26
5.04/5.41	BA	3.633	2.964	0.23
5.96/5.92	BA	3.633	3.532	0.03
7.03/7.02	BA	3.633	3.568	0.02
2.23/2.26	PA	3.519	0.115	29.60
3.05/3.40	PA	3.519	0.826	3.26
4.05/3.79	PA	3.519	1.129	2.12
5.18/3.87	PA	3.519	1.218	1.89
5.96/4.01	PA	3.519	1.415	1.49
6.94/4.83	PA	3.519	2.735	0.29
2.09/2.37	MIBK	3.729	0.090	40.43
3.02/4.01	MIBK	3.729	0.726	4.14
4.95/5.37	MIBK	3.729	2.986	0.25
6.04/5.94	MIBK	3.729	3.472	0.07
6.91/6.92	MIBK	3.729	3.582	0.04
1.96/1.99	2-ethyl hexanol	3.753	0.107	34.07
3.05/3.67	2-ethyl hexanol	3.753	1.036	2.62
4.11/5.01	2-ethyl hexanol	3.753	3.311	0.13
5.01/5.27	2-ethyl hexanol	3.753	3.415	0.10
5.99/5.91	2-ethyl hexanol	3.753	3.635	0.03
6.99/7.03	2-ethyl hexanol	3.753	3.627	0.03

[a] c_0 is initial concentration in the feed solution; c_1 is final equilibrium concentration; D_{Phy} is the distribution coefficient of physical extraction calculated from eq 2.

Table 2. Influence of the Initial Penicillin G Concentration in Aqueous Solution on the Physical Extraction at the Initial/Equilibrium pH and 278.2 K

c_0 / mg·L^{-1}	c_1 / mg·L^{-1}	organic phase	pH	D_{Phy}
1.914	0.033	BA	2.34 /2.52	57.00
3.868	0.150	BA	2.35/2.76	24.79
7.001	0.402	BA	2.24/2.86	16.42
10.445	1.854	BA	2.36/3.43	4.63
13.767	2.503	BA	2.42/3.45	4.50
30.279	14.79	BA	2.90/3.87	1.05
1.914	0.081	PA	2.34 /2.40	22.63
3.868	0.214	PA	2.35/2.65	17.07
7.001	0.507	PA	2.24/2.70	12.81
10.445	1.660	PA	2.36/3.07	5.29
13.767	2.399	PA	2.42/3.01	4.74
30.279	12.037	PA	2.90/3.64	1.52
1.769	0.028	MIBK	2.51/2.79	62.18
2.957	0.086	MIBK	2.34/2.58	33.38
3.800	0.167	MIBK	2.46/2.61	21.75
4.746	0.322	MIBK	2.58/3.28	13.74
14.865	3.632	MIBK	2.69/4.11	3.09
20.635	8.295	MIBK	2.68/4.67	1.49
1.914	0.082	2-ethyl hexanol	2.34/2.48	22.34
3.868	0.183	2-ethyl hexanol	2.35/2.70	20.14
7.001	0.415	2-ethyl hexanol	2.24/2.78	15.87
10.445	1.936	2-ethyl hexanol	2.36/3.33	4.40
13.767	2.63	2-ethyl hexanol	2.42/3.30	4.23
30.279	9.948	2-ethyl hexanol	2.90/4.05	2.04

organic phase reacts with the penicillin acid anion P^- and proton H^+ in the aqueous phase as below:

$$nA_{org} + mP^-_{aq} + mH^+_{aq} \Leftrightarrow [A_n(HP)_m]_{org} \quad (4)$$

As done previously for the reactive extraction of penicillin G, the physical extraction of penicillin G by dilute and the coextraction of buffer, water, and anions (Cl^-, etc.) are not taken into account. Then the extraction distribution coefficient D_E of penicillin G is defined as follows:

$$D_E = \frac{\overline{[A_n(HP)_m]}}{[HP_{aq}] + [P_{aq}]} \quad (5)$$

while the extraction of penicillin G (HP) with D2EHPA is an ion-exchanger reaction, which can be expressed by the following stoichiometric relation equations:[19]

$$HP_{aq} + H^+_{aq} \Leftrightarrow (H_2P)^+_{aq} \quad (6)$$

$$H_2P^+_{aq} + 2H_2A_{2org} \Leftrightarrow H_2PA\cdot 3HA_{org} + H^+_{aq} \quad (7)$$

Then, on the basis of the same simplification addressed above, the extraction distribution coefficient D_E of penicillin G is defined as:

$$D_E = \frac{\overline{[H_2PA\cdot 3HA]}}{[HP_{aq}] + [H_2P_{aq}] + [P_{aq}]} \quad (8)$$

The influences of organic composition on the distribution equilibria of penicillin G are shown in Tables 3 to 5. The distribution coefficient increases with increasing extractant concentration in the organic phase. For the amine-based extractant, the distribution coefficient between aqueous phase and DOA in BA is greater than that between aqueous solution and N235 or TOA in BA. Unfortunately, there is almost no occurrence of extraction when the kerosene or *n*-heptane is used

Table 3. Influence of Solvent Concentration (c_A) in BA on the Distribution Equilibrium of Penicillin G among DOA, TOA, N235, TBP, or D2EHPA in BA and Aqueous Solution at the Initial/Equilibrium pH and 287.2 K[a]

solvent	c_A / mmol·L^{-1}	pH	c_0 / mg·L^{-1}	c_1 / mg·L^{-1}	D_E
DOA	10.0	5.06/6.26	3.583	1.274	1.81
DOA	20.0	5.06/6.27	3.583	0.938	2.82
DOA	50.0	5.06/6.47	3.583	0.478	6.50
DOA	100.0	5.06/6.61	3.583	0.341	9.51
DOA	200.0	5.06/6.81	3.583	0.261	12.73
DOA	300.0	5.06/6.93	3.583	0.236	14.18
DOA	400.0	5.06/7.02	3.583	0.230	14.58
TOA	50.0	5.00/6.13	3.640	1.623	1.24
TOA	100.0	5.00/6.25	3.642	1.244	1.93
TOA	150.0	5.00/6.26	3.518	0.957	2.68
TOA	200.0	5.00/6.31	3.623	0.856	3.23
TOA	250.0	5.00/6.33	3.641	0.807	3.51
TOA	300.0	5.00/6.35	3.651	0.732	3.99
TOA	400.0	4.99/6.33	3.624	0.708	4.12
TOA	500.0	4.99/6.34	3.624	0.686	4.28
N235	50.0	5.03/6.15	3.514	1.623	1.17
N235	100.0	5.03/6.21	3.514	1.206	1.91
N235	150.0	5.03/6.23	3.720	0.957	2.89
N235	200.0	5.03/6.27	3.514	0.860	3.09
N235	300.0	5.03/6.33	3.514	0.693	4.07
N235	400.0	5.03/6.35	3.514	0.640	4.49
N235	500.0	5.03/6.33	3.720	0.644	4.78
N235	600.0	5.03/6.35	3.720	0.592	5.28
TBP	50.0	3.05/4.35	3.536	1.420	1.49
TBP	100.0	3.05/4.42	3.536	1.313	1.69
TBP	200.0	3.05/4.53	3.536	1.340	1.64
TBP	300.0	3.05/4.60	3.536	1.221	1.90
TBP	400.0	3.05/4.73	3.536	0.995	2.55
TBP	500.0	3.05/4.78	3.536	0.969	2.65
TBP	600.0	3.05/4.83	3.536	0.981	2.60
D2EHPA	50.0	2.98/4.03	3.952	0.734	4.38
D2EHPA	100.0	2.98/3.75	3.952	0.419	8.43
D2EHPA	200.0	2.98/3.52	3.952	0.243	15.26
D2EHPA	300.0	2.98/3.39	3.952	0.148	25.70
D2EHPA	400.0	2.98/3.30	3.952	0.081	47.79
D2EHPA	500.0	2.98/3.21	3.952	0.052	75.00
D2EHPA	600.0	2.98/3.13	3.952	0.05	78.04

[a] DE is the distribution coefficient of reactive extraction calculated by eqs 5 and 8.

Table 4. Influence of Solvent Concentration (c_A) in *n*-Heptane on the Distribution Equilibrium of Penicillin G among DOA, TOA, N235, TBP, or D2EHPA in *n*-Heptane and Aqueous Solution at the Initial/Equilibrium pH and 287.2 K

	c_A		c_0	c_1	
solvent	mmol·L^{-1}	pH	mg·L^{-1}	mg·L^{-1}	D_E
DOA	50.0	5.06/5.91	3.692	3.472	0.06
DOA	100.0	5.06/6.07	3.692	3.626	0.02
DOA	200.0	5.06/6.28	3.692	3.614	0.02
DOA	300.0	5.06/6.43	3.692	3.613	0.02
DOA	400.0	5.06/6.54	3.692	3.539	0.04
TOA	50.0	5.00/5.12	3.640	3.599	0.01
TOA	100.0	5.00/5.23	3.642	3.567	0.02
TOA	150.0	5.00/5.26	3.518	3.517	0.00
TOA	200.0	5.00/5.35	3.623	3.526	0.03
TOA	250.0	5.00/5.38	3.641	3.541	0.03
TOA	300.0	5.00/5.44	3.651	3.363	0.09
N235	50.0	5.03/5.14	3.720	3.718	0.00
N235	100.0	5.03/5.19	3.720	3.720	0.00
N235	200.0	5.03/5.30	3.720	3.720	0.00
N235	300.0	5.03/5.44	3.720	3.707	0.00
N235	400.0	5.03/5.51	3.720	3.523	0.06
TBP	50.0	3.06/3.35	3.503	3.203	0.09
TBP	100.0	3.06/3.37	3.503	2.751	0.27
TBP	200.0	3.06/3.57	3.503	2.260	0.55
TBP	300.0	3.06/3.76	3.503	1.877	0.87
TBP	400.0	3.06/3.87	3.503	1.619	1.16
D2EHPA	50.0	3.05/2.62	3.566	2.989	0.19
D2EHPA	100.0	3.05/2.43	3.566	2.716	0.31
D2EHPA	200.0	3.05/2.31	3.566	2.310	0.54
D2EHPA	300.0	3.05/3.66	3.566	0.967	2.69
D2EHPA	400.0	3.05/3.38	3.566	0.587	5.60

Table 5. Influence of Solvent Concentration (c_A) in Kerosene on the Distribution Equilibrium of Penicillin G among DOA, TOA, N235, TBP or D2EHPA in Kerosene and Aqueous Solution at the Initial/Equilibrium pH and 287.2 K

	c_A		c_0	c_1	
solvent	mmol·L^{-1}	pH	mg·L^{-1}	mg·L^{-1}	D_E
DOA	50.0	5.06/5.97	3.600	3.600	0.00
DOA	100.0	5.06/6.12	3.600	3.600	0.00
DOA	200.0	5.06/6.31	3.607	3.600	0.00
DOA	300.0	5.06/6.43	3.607	3.557	0.01
DOA	400.0	5.06/6.55	3.607	3.509	0.03
TOA	50.0	5.00/5.24	3.640	3.594	0.01
TOA	100.0	5.00/5.40	3.642	3.588	0.02
TOA	150.0	5.00/5.35	3.618	3.610	0.00
TOA	200.0	5.00/5.43	3.623	3.572	0.01
TOA	250.0	5.00/5.42	3.641	3.512	0.04
TOA	300.0	5.00/5.47	3.651	3.349	0.09
N235	50.0	5.03/5.13	3.720	3.711	0.00
N235	100.0	5.03/5.21	3.720	3.684	0.01
N235	200.0	5.03/5.31	3.720	3.719	0.00
N235	300.0	5.03/5.43	3.720	3.596	0.03
N235	400.0	5.03/5.56	3.720	3.536	0.05
TBP	50.0	3.05/3.33	3.536	2.819	0.25
TBP	100.0	3.05/3.36	3.536	2.584	0.37
TBP	200.0	3.05/3.57	3.536	1.984	0.78
TBP	300.0	3.05/3.73	3.536	1.568	1.26
TBP	400.0	3.05/3.89	3.536	1.288	1.75
TBP	500.0	3.05/4.04	3.536	1.102	2.21
TBP	600.0	3.05/4.17	3.536	0.978	2.62
D2EHPA	50.0	3.05/3.51	3.566	1.136	2.14
D2EHPA	100.0	3.05/3.24	3.566	0.925	2.86
D2EHPA	200.0	3.05/2.99	3.566	0.775	3.60
D2EHPA	300.0	3.05/2.83	3.566	0.526	5.78
D2EHPA	400.0	3.05/2.74	3.566	0.445	7.01

as diluent. For the phosphorus extractant, the reactive extraction efficiency of penicillin G with D2EHPA is higher than that with TBP as solvent, mainly because of the different mechanism of extraction as shown in eqs 4 and 7. The order of dilute with phosphorus extractant as the extractant for the reactive extraction of penicillin G is as follows: BA > kerosene > *n*-heptane.

Table 6. Influence of Initial Penicillin G Concentration (c_0) in the Aqueous Solution on the Distribution Equilibrium of Penicillin G between Aqueous Solution and Various Organic Solutions at the Initial/Equilibrium pH and Temperature

c_0	c_1		C_A		T	
mg·L^{-1}	mg·L^{-1}	organic phase	mmol·L^{-1}	pH	K	D_E
1.454	0.114	DOA/BA	100.0	5.00/6.63	287.2	11.75
3.273	0.306	DOA/BA	100.0	5.00/6.66	287.2	9.70
5.656	0.987	DOA/BA	100.0	5.05/6.89	287.2	4.73
10.282	2.049	DOA/BA	100.0	5.00/7.12	287.2	4.02
16.637	5.920	DOA/BA	100.0	5.00/7.43	287.2	1.81
33.731	17.698	DOA/BA	100.0	5.00/7.81	287.2	0.91
1.812	0.350	TOA/BA	150.0	4.99/6.03	290.2	4.18
3.624	1.067	TOA/BA	150.0	4.99/6.26	290.2	2.40
6.889	2.793	TOA/BA	150.0	4.99/6.48	290.2	1.47
11.713	5.307	TOA/BA	150.0	4.99/6.63	290.2	1.21
19.467	10.891	TOA/BA	150.0	4.99/6.79	290.2	0.79
36.178	25.867	TOA/BA	155.0	4.99/7.00	290.2	0.40
1.938	0.192	N235/BA	200.0	5.02/6.05	287.2	9.09
3.754	0.748	N235/BA	200.0	5.02/6.28	287.2	4.02
7.316	2.510	N235/BA	200.0	5.02/6.52	287.2	1.91
11.013	4.886	N235/BA	200.0	5.02/6.65	287.2	1.25
18.697	10.058	N235/BA	200.0	5.02/6.81	287.2	0.86
36.281	25.417	N235/BA	200.0	5.02/7.05	287.2	0.43
1.660	0.097	TBP/BA	400.0	3.06/3.73	283.2	16.11
3.383	1.059	TBP/BA	400.0	3.06/4.77	283.2	2.19
6.856	4.081	TBP/BA	400.0	3.06/5.29	283.2	0.68
10.022	7.485	TBP/BA	400.0	3.06/5.50	283.2	0.34
17.709	15.078	TBP/BA	400.0	3.06/5.71	283.2	0.17
34.759	28.880	TBP/BA	400.0	3.06/5.91	283.2	0.20
1.758	0.105	D2EHPA/BA	50.0	3.07/3.65	283.2	15.74
3.386	0.636	D2EHPA/BA	50.0	2.94/3.18	283.2	4.32
5.682	1.777	D2EHPA/BA	50.0	3.09/4.22	283.2	2.20
10.212	2.572	D2EHPA/BA	50.0	3.00/4.26	283.2	2.97
13.315	7.493	D2EHPA/BA	50.0	2.97/4.43	283.2	0.78
25.685	15.883	D2EHPA/BA	50.0	3.17/4.55	283.2	0.62

The distribution coefficient of penicillin G between aqueous solution and extractant in BA decreases with increasing initial penicillin G concentration in the aqueous solution as shown in Table 6. As shown in Table 7, the distribution coefficient decreases with the increase in initial pH in the aqueous solution as expected, because the concentration of proton will decrease with the increase in pH, which does not benefit the extraction process as shown in eqs 4 and 7. In contrast to physical extraction of penicillin G, the distribution coefficient of reactive extraction can remain at a higher value at a relative higher pH. For the amine-based extractant, the pH in the aqueous solution for the extraction process can be set at 5.0 to 6.0; the pH in the stripping solution for the back-extraction process can be set at a higher pH of 7.0. However, for the phosphorus extractant, the pH in the aqueous feed solution for the extraction process will be set at about 3.0; the pH in the stripping solution for the back-extraction process can be set at lower values, such as 5.0. The effect of temperature on the reactive extraction equilibria of penicillin G is listed in Table 8. The distribution coefficient decreases with increasing temperature. As reported for the extraction of aliphatic carboxylic acids with the amine-based extractant, the complexation reaction between the acid and the amine in the organic phase involves proton transfer or hydrogen bond formation and is thus expected to be exothermic. Also, formation of a complex makes the system more ordered and therefore decreases the entropy.[8]

Conclusions

In this work, the effects of organic solution on the stability and extraction equilibrium of penicillin G are studied. BA, MIBK, 2-ethyl hexanol, kerosene, and *n*-heptane are used for physical extraction; DOA, TOA, N235, TBP, and D2EHPA are

Table 7. Influence of the Initial/Equilibrium pH in the Aqueous Solution on the Distribution Equilibrium of Penicillin G between Aqueous Solution and Various Organic Solutions

		C_A	c_0	c_1	T	
pH	organic phase	mmol·L^{-1}	mg·L^{-1}	mg·L^{-1}	K	D_E
2.06/5.02	DOA/BA	100.0	3.446	0.027	288.2	126.63
3.05/6.43	DOA/BA	100.0	3.446	0.200	288.2	16.23
4.03/6.66	DOA/BA	100.0	3.446	0.277	288.2	11.44
5.07/6.69	DOA/BA	100.0	3.446	0.324	288.2	9.64
5.97/6.81	DOA/BA	100.0	3.446	0.439	288.2	6.85
6.94/7.45	DOA/BA	100.0	3.446	1.316	288.2	1.62
2.25/3.57	TOA/BA	150.0	3.580	0.046	290.2	76.83
3.11/5.27	TOA/BA	150.0	3.580	0.176	290.2	19.34
4.09/6.09	TOA/BA	150.0	3.580	0.957	290.2	2.74
5.07/6.19	TOA/BA	150.0	3.580	1.200	290.2	1.98
6.09/6.46	TOA/BA	150.0	3.580	1.589	290.2	1.25
7.01/7.14	TOA/BA	150.0	3.580	2.994	290.2	0.20
2.02/3.67	N235/BA	200.0	3.566	0.034	287.2	103.88
3.03/5.21	N235/BA	200.0	3.566	0.102	287.2	33.96
4.01/6.16	N235/BA	200.0	3.566	0.563	287.2	5.33
5.06/6.26	N235/BA	200.0	3.566	0.733	287.2	3.86
5.98/6.46	N235/BA	200.0	3.566	1.061	287.2	2.36
7.01/7.16	N235/BA	200.0	3.566	2.431	287.2	0.47
2.04/2.16	TBP/BA	400.0	3.638	0.037	283.2	97.32
3.06/4.41	TBP/BA	400.0	3.638	0.572	283.2	5.36
4.06/5.42	TBP/BA	400.0	3.638	2.421	283.2	0.50
5.01/5.67	TBP/BA	400.0	3.638	2.854	283.2	0.27
6.04/6.06	TBP/BA	400.0	3.638	3.250	283.2	0.12
6.96/6.96	TBP/BA	400.0	3.638	3.501	283.2	0.04
2.09/2.10	D2EHPA/BA	50.0	3.707	0.056	283.2	65.20
3.06/3.76	D2EHPA/BA	50.0	3.707	0.656	283.2	4.65
3.87/4.61	D2EHPA/BA	50.0	3.707	2.110	283.2	0.76
5.04/4.82	D2EHPA/BA	50.0	3.707	2.429	283.2	0.53
5.95/5.23	D2EHPA/BA	50.0	3.707	2.997	283.2	0.24
6.95/6.02	D2EHPA/BA	50.0	3.707	3.385	283.2	0.10

Table 8. Influence of Temperature from (274.2 to 303.2 K) on the Distribution Equilibrium of Penicillin G between Aqueous Solution and Various Organic Solutions at the Initial/Equilibrium pH

T		C_A	c_0	c_1		
K	organic phase	mmol·L^{-1}	mg·L^{-1}	mg·L^{-1}	pH	D_E
274.2	DOA/BA	100.0	3.867	0.224	5.00/6.77	16.26
278.2	DOA/BA	100.0	3.867	0.234	5.00/6.71	15.53
283.2	DOA/BA	100.0	3.867	0.292	5.00/6.67	12.24
287.2	DOA/BA	100.0	3.867	0.390	5.00/6.70	8.92
293.2	DOA/BA	100.0	3.867	0.439	5.00/6.67	7.81
297.2	DOA/BA	100.0	3.867	0.554	5.00/6.65	5.98
303.2	DOA/BA	100.0	3.867	0.714	5.00/6.65	4.42
274.2	TOA/BA	150.0	3.527	0.766	5.02/6.29	3.60
278.2	TOA/BA	150.0	3.527	0.807	5.02/6.30	3.37
283.2	TOA/BA	150.0	3.735	0.889	5.00/6.27	3.20
287.2	TOA/BA	150.0	3.527	1.148	5.02/6.25	2.07
293.2	TOA/BA	150.0	3.527	1.342	5.02/6.23	1.63
297.2	TOA/BA	150.0	3.527	1.340	5.02/6.22	1.63
303.2	TOA/BA	150.0	3.527	1.341	5.02/6.19	1.63
274.2	N235/BA	200.0	3.620	0.614	5.02/6.31	4.90
278.2	N235/BA	200.0	3.620	0.668	5.02/6.31	4.42
283.2	N235/BA	200.0	3.620	0.729	5.02/6.32	3.97
287.2	N235/BA	200.0	3.620	0.856	5.02/6.26	3.23
293.2	N235/BA	200.0	3.620	1.002	5.02/6.24	2.61
297.2	N235/BA	200.0	3.620	1.089	5.02/6.22	2.32
303.2	N235/BA	200.0	3.620	1.192	5.02/6.18	2.04
274.2	TBP/BA	400.0	3.739	0.800	2.35/2.69	3.67
278.2	TBP/BA	400.0	3.739	0.731	2.35/2.69	4.11
283.2	TBP/BA	400.0	3.739	0.710	2.35/2.68	4.27
287.2	TBP/BA	400.0	3.739	0.702	2.35/2.67	4.33
297.2	TBP/BA	400.0	3.739	0.706	2.35/2.64	4.30
303.2	TBP/BA	400.0	3.739	0.700	2.35/2.61	4.34
274.2	D2EHPA/BA	50.0	3.329	0.113	2.94/3.60	28.46
278.2	D2EHPA/BA	50.0	3.329	0.165	2.94/3.71	19.18
287.2	D2EHPA/BA	50.0	3.329	0.240	2.94/3.74	12.87
293.2	D2EHPA/BA	50.0	3.329	0.488	2.94/3.86	5.82
297.2	D2EHPA/BA	50.0	3.329	0.614	2.94/3.92	4.42
303.2	D2EHPA/BA	50.0	3.329	0.729	2.94/3.94	3.57

used as extractants, with BA, kerosene, and *n*-heptane as diluents for the reactive extraction.

Results show that the degradation rate of penicillin G increases with an increase in temperature. As a weak organic base, the stability of penicillin G in the presence of the amine-based extractant is better, while as an organophosphorus acid, the stability of penicillin G in the presence of phosphorus acid extractant, D2EHPA, is the worst, because the degradation of penicillin G is catalyzed by a hydrogen ion. The effect of neutral extractant on the stability of penicillin G mainly depends on the temperature. For the back-extraction process by alkali solution, the degradation of penicillin G is mainly governed by the pH.

For the physical extraction processes with BA, MIBK, and 2-ethyl hexanol, the distribution coefficient sharply decreases with increasing pH and initial penicillin G concentration in the aqueous feed solution. Kerosene and *n*-heptane almost cannot extract penicillin G from aqueous solution without an extractant.

For the reactive extraction, the distribution coefficient of penicillin G is higher than that of physical extraction at a higher pH in the feed solution. The distribution coefficient for the amine-based extractant is higher. The extraction capacity of phosphorus acid extractant is higher than that of neutral phosphorus extractant under the range studied because of the different mechanism of the extraction process. When the kerosene or *n*-heptane is used as diluent, almost no extraction occurs for the amine-based extractant. The distribution coefficient increases with the increase in extractant concentration in the organic solution and decreases with the increase in pH, temperature, and initial penicillin G concentration in the aqueous solution.

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